



## Effect of flock age, egg size, and incubator type on embryo development and hatchability in the Indian River Broiler breeders in South Africa

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### Abstract

This study investigated the effects of flock age, egg size, and incubator type on embryo development and hatchability traits in the Indian River broiler breed in South Africa. A total of 4752 hatchable eggs from the breeder flocks at 25, 40, and 55 weeks of age were incubated in Linco and Buckeye incubators. Before incubation, eggs were graded into small (49 g), medium (50-59 g), and large (60-69 g) size groups. The general linear model procedure in Minitab 17 was used to analyse data and the means were separated using Fisher's LSD test ( $p < 0.05$ ). The breeder flocks at 40 weeks of age had higher ( $p < 0.05$ ) hatchability of total eggs set (91.06%), hatchability of fertile eggs set (92.52%), and first-grade chicks (90.93%) followed by 55 week flocks (72.69, 77.37, and 71.18%, respectively) and 25 week flocks (56.39, 64.07, and 55.61%, respectively). The higher proportion ( $p < 0.05$ ) of cull chicks was observed in flocks at 25 (0.78%) and 55 weeks of age (1.52%) compared to 40 weeks of age (0.13%). Flocks at 55 weeks yielded heavier ( $p < 0.05$ ) chicks (44.14g) at hatching. The highest ( $p < 0.05$ ) fertility (98.52%) and lowest total embryo mortality (7.41%) were observed in flocks with 40 weeks of age. Small-sized eggs had the highest ( $p < 0.05$ ) fertility (94.21%) compared to medium-sized eggs (92.80%). Large-sized eggs hatched heavier ( $p < 0.05$ ) chicks (44.21g) compared to medium (39.69g) and small-sized (33.86g) eggs, whereas the other hatchability traits were similar ( $p > 0.05$ ). Incubator type had no significant effect on the incubation and hatchability traits. In conclusion, flock age and egg size were the main determinants of Indian River broiler breed incubation and hatchability traits and should be considered when incubating eggs.

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## 1. Introduction

The poultry industry is an important subsector of animal husbandry worldwide, sustaining the livelihoods of millions of people and supplying high-quality protein (Mphephya et al. 2019; Idowu et al. 2021; Attia et al. 2022; Franzo et al. 2023). Commercial poultry production, particularly broiler chicken, plays a significant role in contributing to food and nutritional security and meeting Sustainable Development Goals (SDGs) in Southern Africa, primarily focusing on SDG 2 (Zero Hunger), SDG 1 (No Poverty), and SDG 5 (Gender Equality). Beyond commercial production, most of the village poultry farming is managed by women, empowering them economically and enhancing their role in household decision-making (Galiè et al. 2019; Tenza et al. 2024). In South Africa, the poultry industry is a R65 billion strategic national asset and the largest employer and contributor to the agricultural sector and supporting emerging and commercial producers alike, in addition to the food security in the country (Ramaphala and Mbajiorgu 2013; SAPA 2020). Approximately 75% of the poultry production is associated with meat production and 25% with egg production (SAPA 2019). It is well noted that the consumption and demand of poultry meat have been growing exponentially over the years, attributed to population growth and greater per-capita consumption (Mottet and Tempio 2017; Connolly et al. 2022; Castro et al. 2023).

To cater the growing demand for broiler chicken meat, new broiler breeds, such as Ross, Cobb, Hubbard, and Arbor Acres, have been developed with the aim of improving crucial traits such as growth rate, feed efficiency, and disease resistance. However, all the operations of poultry industry depend on the supply of day-old chicks (Ogbu and Oguike 2019; SAPA 2020). Incubation techniques such as the use of different incubator types (Mesquita et al. 2021), modifying the incubation temperatures (Avsar et al. 2022; Agyekum et al. 2022; Masia et al. 2024), and ventilation techniques (Bilalissi et al. 2022) have been used to improve and maximize hatchery output, to support the growing demand for day-old chicks. The Indian River broiler breed, developed by Aviagen, has adapted to challenging climatic conditions (Indian River Broiler Management Handbook 2025) and has recently been introduced to South Africa (SAPA 2021). Despite its growing prominence in South Africa, there is a significant paucity of research done on this breed to date, specifically on reproductive performance, embryo development, hatchability, and chick quality. With the existing body of literature on other breeds, such as Ross 308, Cobb 500, and Arbor Acres, making direct extrapolation of the findings to the Indian River broiler breed can be potentially inaccurate or insufficient. Therefore, investigating the effect of the flock age, egg size, and incubator type on the embryo development, hatchability traits, and chick quality in the Indian River broiler breed in South Africa is essential for optimising production efficiency and economic

sustainability. The findings of this study are expected to provide valuable, breed-specific data to broiler producers, hatcheries, and genetic companies, enabling them to optimise incubation management practices for Indian River broiler flocks.

2. Materials and Methods

2.1 Study Site and Experimental Design

The study was carried out at a hatchery farm in Mpumalanga province, South Africa. The study was used a 3 x 3 x 2 factorial design, with 3 flock ages (25, 40 and 55 weeks of age), 3 egg sizes (small 49 g, medium 50-59 g, and large 60-69 g), and 2 single-stage incubator types (Linco and Buckeye incubators). Eggs of these flock age groups were incubated separately, and the effects of flock age, egg size, and incubator type on incubation performance and hatchability were assessed.

2.2 Flock Management

The broiler chicken breeders were housed in an open-sided, curtain-ventilated house, covered with wood shavings to a depth of 3-5 cm. The stocking density was 4.97 birds/m<sup>2</sup> and the male-to-female mating ratio was 1:10, following the Indian River Broiler Management Handbook (2025) recommended standards. From placement at 21 weeks of age till disposal of birds, feeding was done once daily in the morning, following the guidelines provided in the Indian River Broiler Management Handbook (2025):

- **Pre-breeder meal (mash):** placement to 22 weeks of age
- **Phase 1 meal (mash):** 25–39 weeks of age
- **Phase 2 meal (mash):** 40–50 weeks of age
- **Phase 3 meal (mash):** 50 weeks of age to flock disposal

Water was provided ad libitum. Each house was provided with 32 nest boxes, each with 12 holes filled with wood shavings, two chain feeder lines, and one male feeder. The flocks were vaccinated against salmonella and treated for roundworms and mites. At 24, 48, and 56 weeks of age, Ostridose was used for control of roundworms (2.5 L in 10 L of water). At 26, 32, 38, 44, 50, 56, and 62 weeks of age, Servin (44 mL in 10 L of water) was sprayed next in the nest boxes to prevent mite infestation of birds. Furthermore, at 23, 28, 33, 38, 43, 48, 53, 58, and 63 weeks of age, Terminal Fogging Solution (TFS) 40 disinfectant (1.5 L of T.F.S 40 with 6 L of water mix) was sprayed on the wood shavings before transfer to poultry houses.

2.3 Incubation Performance Measurements

A total of 4752 hatchable eggs from Indian River broiler breeder flocks aged 25, 40, and 55 were used in this study. After collection from the breeder house, the eggs were graded into small (49 g), medium (50-59 g), and large (60-69 g) sizes. After grading, the eggs were placed on setter trays and fumigated at the central egg room of the farm with paraformaldehyde. Following fumigation, the eggs were kept at 18–19°C and 60-80% relative humidity (RH) overnight before being moved to the hatchery the next day. Within each egg size category, eggs were further randomly allocated to one of the incubators (Buckeye or Linco). The features of the incubators used are presented in Table 1. Eggs were weighed before incubation to determine the setting weight. Eggs were preheated at 19.5 °C and 17% RH for 9 hours and the incubation at 37.2 °C and 29.0% RH started automatically and immediately after preheating. On day 18, eggshell temperature (EST) was taken using Braun Thermoscan, eggs were re-weighed to determine the transfer weight, and candled. The eggs with evidence of living embryos were

Table 1. Features of the Linco and Buckeye incubators		
Feature	Linco Incubator	Buckeye Incubator
Trolley structure	6 trolleys: 3 columns/ trolley, 15 spaces/ column, 45 trays max	6 trolleys: 2 columns/ trolley, 18 spaces/ column, 36 trays max
Total tray capacity	270	216
Eggs per tray	132	132
Total egg capacity	35 640	28 512
Operation	Automatic	Manual
Data recording	Yes (records incubation data)	No (does not record incubation data)
Temperature	Fahrenheit (°F)	Degrees Celsius (°C)
User interface	Screen display	No screen
Ventilation	Damper, automatic control	Damper, manual control
Humidity control	Defencer and electrolyte to heat water	Cold water with wet wick

transferred into hatchers fogged with a mixture of 125 mL of water and 125 mL of formalin at a temperature of 36.9 °C and 29.0% RH. The clear eggs were removed and kept for breakout. Chicks hatched on day 21 were weighed using a Snowrex scale, evaluated for physical quality by Pasgar@score system to categorise them as first-grade or culls, and their cloaca temperature was recorded as well. The unhatched eggs were kept for breakout. Chicks with good, dry and clean feathers, completely healed navel, smooth stomach, hocks without blemishes, flexibility (chick should flip over in less than 3 seconds), free from defects such as injuries, blindness, one eye, or crossed beak were considered as first-grade chicks and those without these characteristics were considered as culls. For the clear and unhatched eggs, the breakout procedure was conducted to determine the embryo mortality stage (early 1-9 days, mid 10-17 days, and late 18-21 days), and conditions (contaminated, eggs with early cracks, pips, exposed brain, crossed beak, and malposition). The embryo mortality stage determination was performed following the earlier protocol description by Reijrink et al. (2009) and Masia et al. (2024). Briefly, early-stage embryonic mortality occurs during the first 9 days of incubation and at this point, the embryo's eye pigment is visible, but it has not yet developed feathers. Middle-stage embryonic mortality takes place from day 10 to day 17 of incubation. During this period, the embryo is still small but has started to develop feathers. Lastly, late-stage embryonic mortality occurs from day 18 to day 21 of incubation and involves a fully developed embryo, where death may occur either with the yolk sac still external to the body, or with the yolk sac already absorbed, regardless of whether the embryo was alive or dead at the time of examination.

The following incubation and hatchability parameters were determined as described by Masia et al. (2024):

Day 0 – 18 Incubation moisture loss (%) =  $\frac{\text{Egg weight at setting} - \text{Egg weight at candling}}{\text{Egg weight at setting}} \times 100$

Fertile eggs (%) =  $\frac{\text{Number of fertile eggs}}{\text{Total eggs per tray}} \times 100$

Infertile eggs (%) =  $\frac{\text{Number of infertile eggs}}{\text{Total eggs per tray}} \times 100$

Hatch of total egg set (%) =  $\frac{\text{Total number of chicks hatched}}{\text{Total number of eggs set}} \times 100$

Hatch of fertile egg set (%) =  $\frac{\text{Total number of chicks hatched}}{\text{Total fertile eggs}} \times 100$

Embryo mortality rate (%) =  $\frac{\text{Total number of the dead embryos}}{\text{Total number of fertile eggs}} \times 100$

## 2.4 Statistical Analysis

The General Linear Model procedure of the MiniTab 17 (MiniTab 17 Statistical Software 2017) was used to analyse the data, whereas the mean differences were evaluated using Fisher's LSD test ( $p < 0.05$ ).

The following statistical model was used:

$$Y_{ij} = \mu + T_i + B_j + S_k + TB_{ij} + TS_{ik} + BS_{jk} + TBS_{ijk} + \varepsilon_{ijk}$$

Where  $Y_{ij}$  = measurement of response (incubation, hatchability, and mortality),  $\mu$  = overall mean,  $T_i$  = fixed effect of the flock age (25, 40, and 55 weeks),  $B_j$  = fixed effect of egg sizes (small, medium, and large),  $S_k$  = fixed effect of incubator types (Linco and Buckeye incubators), = interaction effects of the  $i^{\text{th}}$  flock age and  $j^{\text{th}}$  egg size, = interaction effects of the  $i^{\text{th}}$  flock age and  $k^{\text{th}}$  incubator type, = interaction effects of the  $j^{\text{th}}$  egg size and  $k^{\text{th}}$  incubator type, = interaction effects of the  $i^{\text{th}}$  flock age,  $j^{\text{th}}$  egg size, and  $k^{\text{th}}$  incubator type, and  $\varepsilon_{ijk}$  = random error.

Noteworthy, the results showed that two-way and three-way interactions were insignificant ( $p > 0.05$ ), therefore, the model was redefined to include only main effects as follows:

$$Y_{ij} = \mu + T_i + B_j + S_k + \varepsilon_{ijk}$$

## 3. Results

The least-squares means (LSM) for incubation and hatchability parameters, embryo mortality traits, and mortality breakdown across different age groups of Indian River broiler breeder flocks are presented in Table 2. Flock age groups significantly affected ( $p < 0.05$ ) all the incubation, hatchability, and embryo mortality traits except the cloaca temperature ( $p > 0.05$ ). For the incubation traits, flocks at 55 weeks of age had higher ( $p < 0.05$ ) set weight (62.27 g), transfer weight (54.49 g), and day 0 -18 moisture loss (12.46%) followed by flocks at 40 weeks of age (set weight: 59.59 g, transfer weight: 52.66 g, day 0 -18 moisture loss: 11.62%) and flocks at 25 weeks of age (set weight: 50.47 g, transfer weight: 45.03 g, day 0 -18 moisture loss: 10.76%). Notably, day-18 eggshell temperature was statistically different ( $p < 0.05$ ) across flock age groups, with older flocks having higher values (38.25 °C) compared to flocks at 25 weeks of age (37.82 °C).

For hatchability traits, the flocks with 40 weeks of age had higher ( $p < 0.05$ ) hatch of total egg set (91.06%), hatch of fertile egg set (92.52%), and first grade chicks (90.93%) followed by flocks with 55 weeks of age (hatch of total egg set: 72.69%, hatch of fertile egg set: 77.37%, first grade chicks: 71.18%) and lower mean values were observed in flocks with 25 weeks of age (hatch of all egg set: 56.39%, hatch of fertile egg set: 64.07%, first grade chicks 55.61%). The higher proportion ( $p < 0.05$ ) of cull chicks was observed in flocks with 55 weeks of age (1.52%) compared to flocks with 40 weeks of age (0.13%) and intermediate values were observed in flocks with 25 weeks of age (0.78%). Flocks with 55 weeks of age yielded chicks with higher ( $p < 0.05$ ) hatch weight (44.14 g), followed by flocks with 40 weeks of age (39.57 g) and low weight was observed in flocks with 25 weeks of age (33.93 g). However, cloaca temperature of hatched chicks was statistically similar ( $p > 0.05$ ) across all flock ages.

All three age group breeder flocks exhibited significant differences from each other in terms of embryo mortality traits. A higher proportion ( $p < 0.05$ ) of infertile eggs (12.05%), early dead (20.10%), mid-dead (3.58%), late dead (10.36%), and overall embryo mortality (34.03%) was observed in flocks with 25 weeks of age followed by the

**Table 2. Least square means and their standard errors (SE) for incubation, hatchability, embryo mortality, and mortality breakdown across different flock ages**

Traits	25 weeks	Flock age 40 weeks	55 weeks
<b>Incubation traits</b>			
Set weight (g)	50.47 <sup>c</sup> ±0.16	59.59 <sup>b</sup> ±0.12	62.27 <sup>a</sup> ±0.15
Transfer weight (g)	45.03 <sup>c</sup> ±0.10	52.66 <sup>b</sup> ±0.12	54.49 <sup>a</sup> ±0.15
day 0 -18 moisture loss (%)	10.76 <sup>c</sup> ±0.05	11.62 <sup>b</sup> ±0.06	12.46 <sup>a</sup> ±0.08
Day-18 eggshell temperature (°C)	37.82 <sup>b</sup> ±0.01	37.90 <sup>ab</sup> ±0.01	38.25 <sup>a</sup> ±0.01
<b>Hatchability traits</b>			
Hatch of total egg set (%)	56.39 <sup>c</sup> ±0.79	91.06 <sup>a</sup> ±0.57	72.69 <sup>b</sup> ±0.73
Hatch of a fertile (%)	64.07 <sup>c</sup> ±0.50	92.52 <sup>a</sup> ±0.58	77.37 <sup>b</sup> ±0.73
Culls (%)	0.78 <sup>b</sup> ±0.15	0.13 <sup>c</sup> ±0.17	1.52 <sup>a</sup> ±0.23
First grade chicks (%)	55.61 <sup>c</sup> ±0.50	90.93 <sup>a</sup> ±0.58	71.18 <sup>b</sup> ±0.75
Chick-hatch weight (g)	33.93 <sup>c</sup> ±0.10	39.57 <sup>b</sup> ±0.10	44.14 <sup>a</sup> ±0.10
Cloaca temperature (°C)	39.58±0.08	39.69±0.08	39.83±0.08
<b>Embryo mortality traits (%)</b>			
Infertile	12.05 <sup>a</sup> ±0.25	1.48 <sup>c</sup> ±0.28	5.92 <sup>b</sup> ±0.42
Fertile	87.95 <sup>c</sup> ±0.25	98.52 <sup>a</sup> ±0.29	94.08 <sup>b</sup> ±0.37
Early dead	20.10 <sup>a</sup> ±0.35	3.42 <sup>c</sup> ±0.41	11.62 <sup>b</sup> ±0.53
Mid dead	3.58 <sup>a</sup> ±0.20	0.46 <sup>c</sup> ±0.24	2.08 <sup>b</sup> ±0.30
Late dead	10.36 <sup>a</sup> ±0.31	3.52 <sup>c</sup> ±0.35	8.46 <sup>b</sup> ±0.53
Total embryo mortality	34.03 <sup>a</sup> ±0.49	7.41 <sup>c</sup> ±0.56	22.01 <sup>b</sup> ±0.43
<b>Embryo mortality breakdown (%)</b>			
Pipped	33.53 <sup>a</sup> ±3.29	8.33 <sup>c</sup> ±3.58	20.91 <sup>b</sup> ±3.82
Early crack	2.87 <sup>a</sup> ±1.36	0.75 <sup>b</sup> ±1.49	3.35 <sup>a</sup> ±1.58
Contaminated	2.88 <sup>c</sup> ±2.39	6.00 <sup>b</sup> ±2.60	22.78 <sup>a</sup> ±2.77
Chick upside-down	1.17 <sup>b</sup> ±1.14	5.61 <sup>a</sup> ±1.24	6.10 <sup>a</sup> ±1.32
Malposition	46.16±4.25	56.68±4.63	42.07±4.93
Exposed brain	0.13 <sup>b</sup> ±0.47	1.33 <sup>a</sup> ±0.51	1.55 <sup>a</sup> ±0.55
Crossed beak	6.17 <sup>a</sup> ±1.01	0.91 <sup>b</sup> ±1.10	2.21 <sup>b</sup> ±1.17
Abnormality	1.86±0.78	1.85±0.85	3.29±0.90

a, b, c Row means with different superscripts differ significantly ( $p < 0.05$ ); Row means without superscripts are not significantly different ( $p > 0.05$ )

breeder flock group of 55 weeks of age and lower values were observed in breeder flocks of 40 weeks of age. Fertility ranged between 98.52 – 87.95%, with the highest value ( $p < 0.05$ ) observed in flocks with 40 weeks of age (98.52%) followed by flocks with 55 weeks of age (94.08%) and 25 weeks of age (87.95%). Flock age affected ( $p < 0.05$ ) all the embryo mortality parameters except malposition and abnormality. The pipped egg percentage was higher in flocks with 25 weeks of age (33.53%) followed by flocks with 55 weeks of age (20.91%) and 40 weeks of age (8.33%). Eggs with early cracks ranged between 0.75 – 3.35% with significantly lowers values observed in flocks with 40 weeks



of age (0.75%) compared to 25 weeks of age (2.87%) and 55 weeks of age (3.35%) which did not differ statistically from each other. Flocks with 55 weeks of age had higher ( $p < 0.05$ ) contaminated eggs (22.78%) followed by flocks with 40 weeks of age (6.00%) and lower values were observed in flocks with 25 weeks of age (2.88%). The chick upside-down and exposed brain conditions were significantly lower in flocks with 25 weeks of age (1.17%, 0.13%) compared to flocks with 40 weeks of age (6.61%, 1.33%) and 55 weeks of age (6.10%, 1.55%) which were statistically similar. The unhatched chicks with higher ( $p < 0.05$ ) crossed beak were observed in flocks with 25 weeks of age (6.16%) compared to flocks with 40 weeks of age (0.91%) and 55 weeks of age (2.21%) which did not differ from each other. However, malposition and abnormality mortality conditions were statistically similar ( $p > 0.05$ ) in all flock ages.

The least-squares means (LSM) for incubation and hatchability parameters, embryo mortality traits, and mortality breakdown across different egg sizes are presented in Table 3. All the incubation traits were significantly affected ( $p < 0.05$ ) by the egg size. The large-sized eggs had higher ( $p < 0.05$ ) set weight (58.59 g) and transfer weight (51.68 g) followed by medium-sized eggs (set weight: 57.38 g, transfer weight: 50.62 g) and small-sized eggs (set weight: 56.37 g, transfer weight: 49.87 g). The day 0 -18 moisture loss was lower ( $p < 0.05$ ) in small-sized eggs (11.41%) compared to medium (11.72%) and large-sized eggs (11.73%) which did not differ significantly from each other. Day-18 eggshell temperature was statistically higher in large-sized eggs (38.29 °C) compared to the small sized eggs (37.74 °C), whereas day-18 eggshell temperature of the medium-sized eggs (37.82 °C) was statistically similar to both small and large-sized eggs.

Among the hatchability traits only chick-hatch weight was significantly ( $p < 0.05$ ) affected by the chick weight. Large-sized eggs hatched into high weight chicks (44.21 g) followed by medium-sized eggs (39.69 g) and lower weights were observed in small-sized eggs (33.86 g). Among the embryo mortality traits the egg size significantly affected ( $p < 0.05$ ) percentage infertile, fertile, and late dead chicks. Small-sized eggs revealed lower ( $p < 0.05$ ) infertile eggs (5.79%) compared to medium-sized (7.20%) and large-sized (6.46%) eggs which were statistically similar to each other. The small-sized eggs revealed higher fertility (94.21%) and late dead (8.46%) compared to medium-sized eggs (92.80%, 6.76%), whereas large-sized eggs were not statistically different from either sizes. For embryo mortality breakdown, egg sizes significantly affected ( $p < 0.05$ ) mortality due to contaminated eggs, chick upside-down, exposed brain, and crossed beak. The proportion of contaminated eggs was higher in medium-sized eggs (13.16%) compared to small-sized eggs (7.13%), whereas contaminated proportion of large-size eggs (11.37%) was statistically similar to other two egg sizes. The lower proportion of chick upside-down (0.93%) and crossed beak condition (1.28%) was observed in large-sized eggs compared to small-sized eggs (chick upside-down: 6.64%, crossed beak: 3.99%) and medium-sized eggs (chick upside-down: 5.31%, crossed beak: 4.02%) which did not differ significantly from each other. The higher proportion of unhatched chicks with exposed brains were observed in medium-sized eggs (2.33%) compared to large-sized eggs (0.06%) and proportion of small-sized eggs (0.51%) was statistically similar to other two sizes. Pipped eggs, eggs with early cracks, malposition, and abnormality were statistically similar ( $p > 0.05$ ) in all egg sizes.

The least-squares means (LSM) for incubation and hatchability parameters, embryo mortality traits and mortality breakdown in two

**Table 3. Least square means and their standard errors (SE) for incubation, hatchability, embryo mortality, and mortality breakdown across different egg sizes**

Traits	Egg size		
	Small	Medium	Large
<b>Incubation traits</b>			
Set weight (g)	56.37 <sup>c</sup> ±0.26	57.38 <sup>b</sup> ±0.18	58.59 <sup>a</sup> ±0.14
Transfer weight (g)	49.87 <sup>c</sup> ±0.17	50.62 <sup>b</sup> ±0.11	51.68 <sup>a</sup> ±0.09
day 0 -18 moisture loss (%)	11.41 <sup>b</sup> ±0.09	11.72 <sup>a</sup> ±0.06	11.73 <sup>a</sup> ±0.05
Day-18 eggshell temperature (°C)	37.74 <sup>b</sup> ±0.01	37.82 <sup>ab</sup> ±0.02	38.29 <sup>a</sup> ±0.01
<b>Hatchability traits</b>			
Hatch of total egg set (%)	73.26±0.84	73.31±0.55	73.58±0.44
Hatch of a fertile (%)	77.40±0.86	78.48±0.56	78.08±0.45
Culls (%)	0.62±0.26	0.98±0.17	0.83±0.14
First grade chicks (%)	72.64±0.86	72.32±0.56	72.76±0.45
Chick-hatch weight (g)	33.86 <sup>c</sup> ±0.10	39.69 <sup>b</sup> ±0.10	44.21 <sup>a</sup> ±0.10
Cloaca temperature (°C)	39.56±0.08	39.58±0.08	39.75±0.08
<b>Embryo mortality traits (%)</b>			
Infertile	5.79 <sup>b</sup> ±0.42	7.20 <sup>a</sup> ±0.23	6.46 <sup>ab</sup> ±0.22
Fertile	94.21 <sup>a</sup> ±0.42	92.80 <sup>b</sup> ±0.27	93.54 <sup>ab</sup> ±0.22
Early dead	10.93±0.61	12.17±0.40	12.04±0.32
Mid dead	2.32±0.35	1.73±0.23	2.04±0.18
Late dead	8.46 <sup>a</sup> ±0.53	6.76 <sup>b</sup> ±0.34	6.97 <sup>ab</sup> ±0.28
Total embryo mortality	22.73±0.83	20.66±0.55	21.06±0.44
<b>Embryo mortality breakdown (%)</b>			
Pipped	26.78±5.13	13.89±3.68	22.10±2.54
Early crack	0.81±2.13	3.94±1.53	2.22±1.06
Contaminated	7.13 <sup>b</sup> ±3.73	13.16 <sup>a</sup> ±2.67	11.37 <sup>ab</sup> ±1.85
Chick upside-down	6.64 <sup>a</sup> ±1.78	5.31 <sup>a</sup> ±1.28	0.93 <sup>b</sup> ±0.88
Malposition	50.14±6.63	47.84±4.76	46.93±3.29
Exposed brain	0.51 <sup>ab</sup> ±0.74	2.33 <sup>a</sup> ±0.53	0.06 <sup>b</sup> ±0.37
Crossed beak	3.99 <sup>a</sup> ±1.57	4.02 <sup>a</sup> ±1.13	1.28 <sup>b</sup> ±0.78
Abnormality	4.73±1.21	1.22±0.87	1.05±0.60

a, b, c Row means with different superscripts differ significantly ( $p < 0.05$ ); Row means without superscripts are not significantly different ( $p > 0.05$ )

different incubators are presented in Table 4. All the incubation, hatchability, and embryo mortality traits were statistically similar ( $p > 0.05$ ) across the two incubators except for the day-18 eggshell temperature which was higher ( $p < 0.05$ ) in the Linco incubator (38.19°C) than the Buckeye incubator (37.78 °C). However, among the embryo mortality breakdown incubator type significantly affected ( $p < 0.05$ ) percentage of pipped, eggs with early cracks, contaminated eggs, chick upside-down, malposition, and crossed beak conditions. Buckeye incubator yielded higher ( $p < 0.05$ ) proportion of pipped (22.97%) and contaminated (11.83%) eggs compared to Linco incubator (18.87%,

**Table 3. Least square means and their standard errors (SE) for incubation, hatchability, embryo mortality, and mortality breakdown in different incubators**

Traits	Incubator	
	Buckeye	Linco
<b>Incubation traits</b>		
Set weight (g)	57.33±0.08	57.56±0.08
Transfer weight (g)	50.60±0.08	50.85±0.08
day 0 -18 moisture loss (%)	11.66±0.04	11.57±0.04
Day-18 eggshell temperature (°C)	37.78 <sup>b</sup> ±0.01	38.19 <sup>a</sup> ±0.01
<b>Hatchability traits</b>		
Hatch of total egg set (%)	73.65±0.40	73.16±0.40
Hatch of a fertile (%)	78.32±0.41	77.63±0.41
Culls (%)	0.69±0.12	0.92±0.12
First grade chicks (%)	72.96±0.40	72.19±0.41
Chick-hatch weight (g)	39.25±0.09	39.18±0.09
Cloaca temperature (°C)	39.68±0.06	39.73±0.06
<b>Embryo mortality traits (%)</b>		
Infertile	6.58±0.20	6.35±0.20
Fertile	93.42±0.20	93.65±0.20
Early dead	11.56±0.29	11.86±0.29
Mid dead	2.02±0.16	2.06±0.16
Late dead	7.16±0.25	7.63±0.25
Total embryo mortality	20.72±0.39	21.55±0.39
<b>Embryo mortality breakdown (%)</b>		
Pipped	22.97 <sup>a</sup> ±3.00	18.87 <sup>b</sup> ±2.84
Early crack	0.83 <sup>b</sup> ±1.25	3.82 <sup>a</sup> ±1.18
Contaminated	11.83 <sup>a</sup> ±2.18	9.28 <sup>b</sup> ±2.06
Chick upside-down	3.35 <sup>b</sup> ±1.04	5.24 <sup>a</sup> ±0.99
Malposition	44.02 <sup>b</sup> ±3.88	52.59 <sup>a</sup> ±3.67
Exposed brain	1.03±0.43	0.90±0.41
Crossed beak	1.86 <sup>b</sup> ±0.92	4.33 <sup>a</sup> ±0.87
Abnormality	2.70±0.71	1.87±0.67

<sup>a, b, c</sup> Row means with different superscripts differ significantly ( $p < 0.05$ ); Row means without superscripts are not significantly different ( $p > 0.05$ )

9.28%), whereas Linco incubator yielded higher early crack eggs (3.82%), unhatched eggs with chick upside-down (5.24%), malposition (52.59%), and crossed beak (4.33%) compared to the Buckeye incubator (0.83%, 3.35%, 44.02%, 1.86%, respectively). The percentage of exposed brain and abnormality were statistically similar ( $p > 0.05$ ) between Buckeye and Linco incubators.

#### 4. Discussion

The effects of flock age, egg size, and incubator type are well documented in most of the broiler chicken breeds; however, to the knowledge of the authors, no such research is available for the Indian

River broiler breeder. Most of the previous studies focussed on the effect of age in Ross 308 (Damaziak et al. 2021; Darmus et al. 2021), Hubbard Flex (Damaziak et al. 2021), egg weight/size in Cobb 500 (Ramaphala and Mbajorgu 2013), Potchefstroom Koekoek chickens (Molapo and Motselisi 2020), Indigenous Venda chickens (Alabi et al. 2012; Ng'ambi et al. 2013) and Indonesian local chickens (Rahardja et al. 2020), and incubator type in Emu (*Dromaius novaehollandiae*) (Adewumi et al. 2008), Cobb and Avian breeders (El-Shhat et al. 2017), Ross 308 (Yousaf et al. 2021), and Cobb 500 (Araujo et al. 2016; Mesquita et al. 2021; Kalaba et al. 2023). However, no such focused studies on the Indian River broiler breeders have been carried out. It is well established in the literature that flock age significantly influences the incubation traits and fertility (Koppenol et al. 2015; Abudabos et al. 2017), hatchability, and embryo mortality (Ipek and Soczu 2015; Nangsuay et al. 2016; Mitrovic et al. 2017; Nowak et al. 2019). The association between flock age and chick quality is also well established in the literature (Nowak et al. 2019; Durmus et al. 2021; Dassi et al. 2022; Wlzlak et al. 2023; Agbehadzi et al. 2024; Cam et al. 2024).

The present study revealed that eggs produced by older flocks (55 weeks of age) exhibited higher moisture loss by day 18 of incubation compared to medium-age (40 weeks) and young flocks (25 weeks of age). An increasing trend of moisture loss was observed with increasing age of breeder flocks. This could be attributed to the fact that thinner eggshell in older flocks facilitates more evaporative loss compared to younger flocks (Molapo and Motselisi 2020). A similar moisture loss trend across flock ages was observed in Cobb 500 and Ross 308 breeds (Jabba and Ditta 2017; Yousaf et al. 2024). On the contrary, Jabba and Yousaf (2017) reported the higher moisture loss in the prime flock (medium-aged flocks) compared to young and old flocks in the Ross 308 breed. This proves that flocks of different ages do not lose the same amount of moisture during incubation, irrespective of the breed. The normal moisture loss of the eggs at day 18 of the incubation ranges between 9.5-12.5% (Cormic 2019). The moisture loss in all flock ages for the present study ranged from 10.76 to 12.46% which is within an acceptable range. Eggs that lose insufficient water during incubation will increase the costs of maintaining chicks at farms due to poor post-hatch performance (Maatjens et al. 2016; Abera et al. 2017; Souza da Silva et al. 2021). The explained phenomenon of poor post-hatch performance in this study would not be expected because the moisture loss was within the acceptable range.

The intrinsic characteristics of a newly hatched chick are critical determinants of its ability to survive, grow efficiently, and obtain optimal health throughout its productive life. The finding that medium-aged Indian River broiler breeder had a higher hatchability of eggs set than young (25 weeks of age) and older flocks (55 weeks of age) could be attributed to the fact that older flocks lay eggs with poor firmness, low albumin and yolk quality (Mendes et al. 2012; Feddern et al. 2017), and poor shell thickness, which have adverse impact on the survival of embryo (Sujata et al. 2019). More importantly, young flock fails to transmit enough antibodies to the egg and there is a decline in absorption of macronutrients in older flock (Wlzlak et al. 2023) which affect the life of the developing embryo and consequently affect the hatchability of eggs. The hatchability trend with respect to the age of breeder flock in the present study is supported by earlier researchers as well in Ross 308 and Cobb 500 (Jabbar and Ditta 2017; Jabbar and Yousaf 2017; Mitrovic et al. 2017). Older flocks (55 weeks of age) in the present study produced chicks with higher hatch weight compared to

young (25 weeks of age) and medium-age (40 weeks) flocks, which could be attributed to the fact that the egg weight increases with the advancing age of the hens due to physiological ageing, providing more yolk and albumen crucial for embryonic development (El Sabry et al. 2013; Araujo et al. 2016; Iqbal et al. 2016; Rahardja et al. 2020). Consequently, chicks hatching from these heavier eggs tend to have a higher initial body weight at hatch, as their initial size directly correlates with the nutrients available in the egg. In addition, the yolk sac is absorbed into the stomach of the chick at the last stage of embryo development or the incubation process. This observation is supported by the previous studies, which reported the higher Cobb 500 and Ross 308 chick-hatch weight in older flocks compared to young flocks and medium-aged flocks (Ipek and Sozcu 2015; Jabbar and Ditta 2017; Yousaf et al. 2024).

The present study revealed a greater proportion of cull chicks in older flocks, which is consistent with the previous reports in Cobb 500 breed (Ipek and Sozcu 2015; Nowak et al. 2019) and Hubbard classic (Iqbal et al. 2016), whereas it is contrary to previous reports in Ross 308 (Nowak et al. 2019). It is well documented that older flocks produce thin shell eggs (Molapo and Motselisi 2020) and subsequently result in more culls (Onasanya and Ikeobi 2013). Higher culling rates could be caused by a longer hatch window (Ipek and Sozcu 2013), where the thin shell eggs tend to hatch early and consequently spend a longer time under high temperature in the hatchers, which can lead to poor chick quality. Ideally, chicks should be removed from the hatcher within 24 hours to the chick holding room, since the temperature in the hatcher is higher than that in the chick processing area. Moreover, thin eggshells in older laying flocks increase the risk of egg fracture, moisture loss, and bacterial penetration (Cheng and Ning 2023), which can severely impact the developing embryo, leading to poor chick quality and higher culling rates. This decline in shell quality is primarily due to age-related physiological changes in hens, affecting calcium utilisation and deposition, and is a major concern for poultry producers due to its substantial economic implications for hatchability and overall productivity.

Though higher fertility was observed in 40 week old flocks, followed by 55 week old flocks compared to young 25 week old flocks, the fertility range was within an acceptable range across all flock ages in the present study. Earlier studies have reported normal fertility across all ages in Ross, Hubbard Classic, Arbor Acre, and Cobb breeds (Jabbar and Ditta 2017; Nowak et al. 2019). This could be attributed to enough male-to-female mating ratio in the poultry house. The present study observed higher embryo mortality in the young flocks (25 weeks of age) compared to old flocks (55 weeks of age) and lowest mortality at medium age (40 weeks of age). This could be attributed to the fact that eggs from older flocks contain a large yolk (Nangsuay et al. 2016; Araujo et al. 2017; Molapo and Motselisi 2020), which provides sufficient nutrients for the growth and survival of the developing embryo compared to the eggs from young flocks (Araujo et al. 2017; Van der Wagt et al. 2020; Yang et al. 2020; Alkubaisy et al. 2021). In addition, better shell quality at 40 weeks of age further enhance the egg fertility. Another reason of low fertility might be the immature gametes and inexperienced mating in the young flocks compared to mature and older flocks. Conversely, several studies suggest a consistent pattern where advanced maternal age in broiler breeders correlates with a greater incidence of embryonic death during the incubation period (Salahi et al. 2012; Fikremariam and Tilahun 2016; Yousaf et al. 2024).

However, several other researchers have reported that flock age does not affect embryo mortality during incubation (Alsobayel and Albadry 2012; Sujata et al. 2019). Hence, there is no consensus amongst researchers on the effect of flock age on embryo mortality during incubation.

The present study shows that large and medium-sized eggs lost greater moisture during incubation compared to small-sized eggs. This could be due to the large number of egg pores on large and medium-sized eggs compared to small-sized eggs. The results are in line with those reported by Kumar et al. (2024) in the Himachal Pradesh native breed. Contrary to the present findings, in Cobb strain (Ulmer-Franco et al. 2010), CARI-BRO broiler breed (Sudhir et al. 2016), and Ross 308 strain (Duman and Sekereglu 2017), higher moisture loss in small eggs compared to medium and large eggs was observed. The higher amount of water lost might be due to the higher surface-to-volume ratio of small-sized eggs (Sudhir et al. 2016). In the present study, it was observed that the eggshell temperature varies with the breeder age and egg size. There is consensus among researchers that older flocks produce larger eggs compared to younger flocks. This can be attributed to the fact that embryos from larger eggs have higher metabolic heat production, and these eggs have a lower surface-area-to-volume ratio for heat exchange (Lourens et al. 2006; Hamidu et al. 2007; Gualhanone et al. 2012; Boonkum et al. 2025). Consequently, heat exchange may be reduced among eggs in the incubator.

In the present study, the hatchability traits except chick-hatch weight remained similar across egg sizes. This means that egg sizes have no impact on hatchability, and this could be attributed to the non-significant total embryo mortality across egg sizes observed in the current study. These results are similar to those reported in Cobb 500 chickens (Ramaphala and Mbajorgu 2013). However, significant differences in hatchability traits were observed in Indigenous Venda chickens (Ng'ambi et al. 2013), Hubbard classic broiler (Iqbal et al. 2016), Fayoumi chickens (Senbeta 2017), CARI-BRO Vishal (Sudhir 2016), Potchefstroom Koekoek chickens (Molapo and Motselisi 2020), Indonesian local chickens (Rahardja 2020), Rhodes Island Red X Fayoumi chickens (Bassareh and Razaiepour 2021), and Himachal Pradesh Native chickens (Kumar et al. 2024). There is a consensus among researchers that medium-size eggs result in the production of good-quality day-old chicks (Ulmer-Franco et al. 2010; Alabi et al. 2012; Rahardja et al. 2020; Kruent et al. 2022). Similar to the results of the present study, increase in egg size increases the chick-hatch weight (Ipek and Sozcu 2015; Bassareh and Razaiepour 2021; Muhammed et al. 2023). This could be due to the higher set and transfer weight of large-sized eggs compared to small and medium-sized eggs, as observed during the incubation in the present study. Contrary to the observation of present study, higher proportion of cull chicks were observed in large-sized eggs compared to small and medium-sized eggs in Fayoumi x Dominant breed (Fikremariam and Tilahun 2016) and Hubbard classic broiler breeders (Iqbal et al. 2016).

The present study observed that small-sized eggs had higher fertility compared to medium-sized eggs which was reflected in an earlier study on Cobb 500 broiler chicken as well (Ramaphala and Mbajorgu 2013). The observed higher fertility in small-sized eggs could be attributed to the fact that small-sized eggs have a thick eggshell, which protects and shields the internal egg quality from easily deteriorating. In contrast, Bassareh and Razaiepour (2021) observed that medium-sized eggs had higher fertility, followed by large-sized



eggs and lastly by small-sized eggs in Ross 308 broiler breed. Furthermore, the variation in fertility in the present study and those in the literature could be attributed to the breed difference, male and female factors, and nutritional and environmental factors. Though, no differences in early and mid-embryonic deaths was observed, the results of the present study revealed higher late dead embryonic mortality in small-sized eggs. This could be attributed to the fact that embryos have increased metabolic demands during late stages of embryonic development and need to remove increasing levels of carbon dioxide (Givisiez et al. 2020; Wang et al. 2025). However, small-sized eggs have a less number of egg pores, which can lead to insufficient gaseous exchange and could consequently increase embryonic mortality (Muhammedhujar and Negassi 2023). On the contrary, several researchers reported higher total embryo mortality in large-sized eggs in Indigenous Venda chickens, Fayoumi, Desi, Crossbred, and Ross 308 breeders (Ng'ambi et al. 2013; Rashid et al. 2013; Iqbal et al. 2016; Duman and Sekeroglu 2017). This could be attributed to the fact that pH is higher in large-sized eggs (Nasri et al. 2020), which is conducive to bacterial contamination and can terminate fertility on the yolk or end the life of the developing embryo during incubation. Furthermore, Sudhir et al. (2016) and Molapo and Motselisi (2020) reported higher embryonic mortality in small-sized eggs on CARI-BRO Vishal and Potchefstroom Koekoek broiler chickens.

The mortality breakdown in the present study revealed a lower mortality due to contaminated eggs in young flocks and small-sized eggs. This could be attributed to thin eggshells in older laying flocks, which could increase the risk of egg cracks and bacterial penetration (Cheng and Ning 2023). Furthermore, higher proportion of large-size contaminated eggs could be attributed to their high pH that promotes bacterial infection or penetration and old flocks lay eggs with high pH (Nasri et al. 2020). In line with the results of present study high egg contamination was observed in Isa Label Naked Neck older flocks (Machado et al. 2020; Araujo et al. 2016). However, contrasting reports have revealed higher contamination of eggs in young flocks (Jabbar and Yousaf 2017; Yousaf et al. 2024). Higher proportion of cracked eggs in older flocks was observed in the present study and similar observations have been reported in earlier studies as well (Jabbar and Yousaf 2017; Yousaf et al. 2024). This could be due to thin eggshells (Molapo and Motselisi 2020) or poor eggshell quality (Mendes et al. 2012; Feddern et al. 2017; Wlazlak et al. 2023) in older flocks. Similarly, higher proportion of unhatched pipped eggs was observed in young flocks compared to medium and older flocks. This could be attributed to the fact that young flocks produce eggs with a thick eggshell (Molapo and Motselisi, 2020). However, egg size did not affect the pipped unhatched eggs, early crack, malposition, and abnormality across eggs in the present study. Most of these embryo mortality conditions are caused by poor nutrition or human error at setting and transfer, such as mistakes during egg grading, rough placing of eggs on the trollies, and rough transfer. Contrary to the present study, large-sized eggs were reported to have higher unhatched pipped eggs compared to small and medium-sized eggs (Iqbal et al. 2016).

Incubator type did not affect the incubation, embryo mortality traits, and hatchability, with the exception of a higher day-18 eggshell temperature observed in the Linco single-stage incubator. This could be attributed to the structural and operational design of the incubator. Previous studies reported that design and operational profiles, such as multi-stage or single stage, can affect the incubation process (Jabbar and

Ditta 2017). The present study used only single-stage incubators. High incubation temperatures are generally associated with early hatching and deformities, while lower temperatures lead to poor embryo development (Hamidu et al. 2018; Agyekum et al. 2022). However, this was not the case in this study, as Linco single-stage incubator yielded chicks with lower deformities compared to the Buckeye single-stage incubator. An optimum day 18 eggshell temperature is 37.0°C, which supports ideal embryo metabolism, mitigating possible heat stress, and consequently results to an appropriate hatch window, which increases chick quality (Hamidu et al. 2018; Agyekum et al. 2022).

## 5. Conclusions

Flock age and egg size are the main determinants of Indian River broiler breed incubation, hatchability traits, and embryonic mortality, whereas the type of single-stage incubator had an insignificant effect. Better results were observed in flocks at 40 weeks of age and in medium-sized eggs. Future studies on this breed can be designed towards dynamic incubation profiles, adjusting incubation environment based on the egg characteristics, such as flock laying age and egg sizes, rather than a one-size-fits-all approach.

## Declarations

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