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Corticosterone *in ovo* Injection Effects on the Development of Iraqi Native Chicken Embryos

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ABSTRACT

Background: Corticosterone is a major glucocorticoid hormone in the plasma of birds. It is produced in the adrenal gland and transferred to the eggs after 1-2 days of circulation in females by distributing within the yolk. So, this study aimed to evaluate Iraqi native chicken hatching eggs with different levels of corticosterone hormone on embryonic development.

Methods: Three hundred Iraqi native hatching eggs from 30 weeks old breeds were utilized in this research. Eggs were divided into five treatments and three replicates (20 eggs for each). Eggs were injected with different levels of corticosterone hormone as follows: Control group without injection, Sesame oil group: Injected with 5 µl of sterile sesame oil only, Group 1: Injected with 0.25, Group 2: Injected with 0.5 and Group 3: Injected with one ng of corticosterone hormone dissolved with sterile sesame oil. The embryonic test was checked after 72 hours, 7 days, 14 days and 18 days of egg incubation. After hatching, hatchability from fertile eggs, early, intermediate and late embryonic mortality, life and dead pipped eggs as a percentage of fertile eggs were measured.

Result: Results showed a significant increase ($P \leq 0.05$) in embryo length for Group 1 and a significant decrease ($P \leq 0.05$) in amniotic fluid and amniotic weight percentage for Group 2, a significant decrease ($P \leq 0.05$) in amniotic fluid, amniotic sac membrane percentage, hatchability early embryonic mortality percentage for Group 3 compared with a control group. In conclusion, a high concentration of corticosterone hormone in Iraqi native chicken hatching eggs causes an increase in embryo length, amniotic fluid and amniotic weight percentage. Also, it causes a decrease in amniotic fluid, amniotic sac membrane percentage, hatchability and early embryonic mortality percentage.

Key words: Corticosterone, Embryonic development, Hatching eggs, Iraqi native chicken.

INTRODUCTION

Corticosterone is a major glucocorticoid hormone in the plasma of birds (Pu *et al*., 2019). It is produced in the adrenal gland and transferred to the eggs after 1-2 days of circulation in females by distributing within the yolk (Almasi, 2012). It has been found that avian eggs contain a different maternal level of corticosterone as reported in domestic fowl (Groothuis *et al*., 2005; Rettenbacher *et al*., 2005) and Japanese quail (Hayward *et al*., 2006). This concentration is modified by different factors such as hen physiological status (Reed and Clark, 2011) and environmental conditions (Hayward *et al.,* 2004). It is found that corticosterone serum levels increased with high environmental temperature via increasing levels of adrenal steroidogenic enzymes that led to an increase in the yolk (Pu *et al*., 2019). In general, glucocorticoid concentrations in blood plasma are widely used to monitor stress response in different species (Von Holst, 1988).

The levels of parents' blood plasma corticosterone concentration in the yolk are influenced via passive diffusion (Hayward and Wingfielde, 2004). Corticosterone concentration in egg yolk and albumin differ between eggs produced from slow or fast-growing broiler chicks (Ahmed, 2013). Yolk corticosterone level also differs between breeds. Navara and Pinsion (2010) reported that corticosterone concentration levels in yolk were twice as higher in white hens than in brown hen's eggs. Also, Ahmed (2016) recorded significantly differing yolk corticosterone levels between white Leghorn and Hy-Line brown eggs. Corticosterone high concentration orally gives a significant increase in yolk corticosterone (Almasi *et al*., 2012).

In contrast, parents' injection of ACTH failed to induce detectable corticosterone deposition in chicken eggs (Rettenbacher *et al*., 2005). Early life experience or poor environments form a risk factor for post-hatch stress life disturbance by elevation of egg corticosterone that influences offspring growth rate, behavior and gene expression (Ahmed, 2016). The elevation of yolk corticosterone

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concentration makes a growth decrease in males but not female chicks with the reduced HPA axis responsiveness of adult females compared to males (Hayward *et al*., 2006). Few articles have focused on the effects of egg corticosterone levels in Iraqi native chick embryonic development; therefore, we conducted this study to evaluate the role of corticosterone hormone in the embryonic development of Iraqi native chickens.

MATERIALS AND METHODS

In this study, we used 300 Iraqi native chicken eggs from 30-week-old breeds reared in the research poultry station, Abu Ghraib, Baghdad. Eggs were divided into five treatments and three replicates (20 eggs each). The injection surface was sterilized with an antiseptic (Dettol). Eggs were injected from the wide side with a micropipette by puncture (13 m/m) in the air sac. Then every egg was injected with corticosterone hormone (Sigma-Aldrich, 98.5%) dissolved with sesame oil as follows: Control group eggs were placed in the incubator without injection. Sesame oil group: Injected with 5 µl of sterile sesame oil only. Group 1: Injected with 0.25 ng corticosterone dissolved with 5 µl of sterile sesame oil. Group 2: Injected with 0.5 ng corticosterone dissolved with 5 µl of sterile sesame oil. Group 3: Injected one ng corticosterone dissolved with 5 µl of sterile sesame oil.

Punctures were closed by using dye pedicures. Eggs were incubated in an egg incubator (Weiqian 1050 brand) by distributed the groups randomly. The embryonic test was checked per the method described by Abdulateef (2010) described. After 72 hours of egg incubation, the egg was horizontally placed for 20 minutes, then checked for shell cutoff and test embryo length, vascular region and pairs of somites. The second test was conducted after seven days of incubation by taking out the egg contents after the shell breakout to study new plasma weight, allantois membrane and fluid weight, amnion fluid and amniotic sac albumin, yolk weight, embryo weight and shell weight. The third embryonic test was conducted after 14 days of incubation by breaking the eggs shell, wherein the contents of the egg were taken out. The weights of the shell, embryo, yolk and yolk sac, amniotic fluid and amniotic membrane, allantois fluid and allantois membrane and albumin were recorded. The fourth embryonic test was conducted at 17 days of incubation and the weight of the egg, shell, embryo, yolk and yolk sac, amniotic fluid and amniotic sac and allantois and chorion membrane with fluids were recorded. Hatchability from fertile eggs was measured as the number of hatched eggs/numbers of fertile eggs set in the incubator.

After hatching, early embryonic mortality (before seven days embryo mortality during incubation), intermediate embryo mortality (7-14 days embryo mortality during incubation), late embryonic mortality (14-17 days embryo mortality during incubation), life and dead pipped eggs were determined as a percentage of fertile eggs.

Statistical analysis

Complete random design (CRD) inside five medicines and three repeats were utilized in this analysis. Information was examined by utilizing the GLM model strategy of SAS (Statistical analysis system) (Fernandez, 2010). Means for treatments are thought about by utilizing Duncan's polynomial utilizing different significance levels to decide massive contrasts between the averages (Duncan, 1955).

RESULTS AND DISCUSSION

Table 1 shows a significant increase ($P \leq 0.05$) in embryo length for (0.5 ng/5 µL sesame oil) bunch contrasted and control gathering and there are no massive contrasts in other embryonic development parameters at 72 hours of incubation.

Table 2 shows a significant decrease ($P \leq 0.05$) in amniotic fluid and amniotic weight percentage for (0.5 ng/ 5 µL sesame oil) in the gathering contrasted and the benchmark group and there are no huge contrasts in other embryonic development parameters at seven days of incubation.

Table 3 shows no significant differences in all embryonic development parameters between all treatments and the control group at 14 days of incubation.

Table 4 shows a significant decrease ($P \leq 0.05$) in amniotic fluid and amniotic sac membrane percentage for (1 ng/5 µL sesame oil) in the gathering contrasted and the benchmark group. The results showed no significant

Table 1: Effect of *in ovo* injection with different levels of corticosterone in embryonic development at 72 hours of incubation.

Treatment	Embryo length/mm	Blood vessels diameter/mm	Transparent area/mm	Somite number
Control	6.5	6*	3.50*	16*
Sesame oil injection	8	12*	4.50*	14
0.25 ng/5 µL sesame oil	6	8.50*	4.50*	20*
0.5 ng/5 µL sesame oil	11.5*	10*	4.00*	12.50
1 ng/5 µL sesame oil	9.5	10.50*	4.00*	12.50
Mean	10	9.33	4.11	15
SEM	0.74	0.91	0.20	1.08
Significant	0.05	NS	NS	0.09

SEM: standard error of means.

NS: No significant differences between columns.

differences in all embryonic development parameters between all treatments and the control group in 17 days of incubation.

Fig 1 shows a significant decrease (P≤0.05) in hatchability percentage for corticosterone (1 ng/5 µL sesame oil) compared with the control group.

In Table 5, the results indicate that significant decrease (P≤0.05) in the early embryonic mortality percentage of the (1 ng/5 µL sesame oil) group compared with the control group. The results showed no significant differences between all treatments and control groups in intermediate and last embryo mortality stage and dead and life pipped eggs.

Table 2: Effect of *in ovo* injection with different levels of corticosterone in embryonic development at seven days of incubation (% of egg weight).

Treatment	New plasma	Allantois membrane and fluid	Amnion fluid and amniotic sac	Albumin weight	Yolk weight	Embryo weight	Shell weight
Control	12.84	9.40	3.07	18.98	22.43	2.56	12.84
Sesame oilinjection	13.37	6.03	2.85*	13.68	19.25	8.36	13.37
0.25 ng/5 µL sesame oil	12.80	4.46	3.15*	15.02	16.44	2.85	12.80
0.5 ng/5 µL sesame oil	13.36*	4.43*	2.59	17.06*	15.12*	3.43*	13.36*
1 ng/5 µL sesame oil	13.22	6.84	4.22	18.05	19.68	3.39	13.22
Mean	13.09	6.17	3.07	16.55	18.42	3.93	13.09
SEM	1.46	0.84	0.20	0.81	1.78	0.98	0.199
Significant	NS	NS	0.05	NS	NS	NS	NS

SEM: Standard error of means.

NS.: No significant differences between columns.

Table 3: Effect of *in ovo* injection with different levels of corticosterone in embryonic development at 14 days of incubation (% of egg weight).

Treatment	Egg shell weight	Allantois fluid weight	Albumin weight	Allantoic membrane weight	Yolk and yolk sac weight	Amniotic membrane weight	Amniotic fluid weight	Embryo weight
Control	11.59	10.94	2.71	9.260	20.08	7.27	5.84	33.147*
Sesame oilInjection	10.95*	18.85*	4.59*	4.96*	10.80*	10.49*	10.49*	16.18
0.25 ng/5 µL sesame oil	11.65	16.99	2.81	3.34	12.08	1.08	5.07	40.58
0.5 ng/5 µL sesame oil	12.43	16.97	2.57	2.55	14.27	1.17	7.96	32.99*
1 ng/5 µL sesame oil	12.27	21.40	6.44	3.66	12.72	1.24	4.50	34.06*
Mean	11.85	16.89	3.84	4.84	14.38	4.01	6.61	31.85
SEM	0.43	1.61	0.81	1.47	1.40	1.53	0.94	2.93
Significant	NS	NS	NS	NS	NS	NS	NS	0.05

SEM: Standard error of means.

NS: Non significant differences between columns.

Table 4: Effect of *in ovo* injection with different levels of corticosterone in embryonic development at 17 days of incubation (% from egg weight).

Treatment	Egg weight	Shell weight	Embryo weight	Yolk and yolk sac weight	Amniotic fluid and amniotic sac membrane	Allantois and chorion membrane with fluids
Control	42.29	11.44	42.29	22.14	8.82	10.06
Sesame oilinjection	51.61	10.63	51.61	20.87	3.83	9.20
0.25 ng/5 µL sesame oil	51.35*	11.66*	51.35*	19.50*	7.07	5.97*
0.5 ng/5 µL sesame oil	46.50*	11.20*	46.50*	19.73*	5.60	10.00*
1 ng/5 µL sesame oil	53.30*	12.27*	53.30*	16.52*	5.84	7.46*
Mean	49.15	11.49	49.15	19.39	6.14	8.57
SEM	2.85	0.30	2.85	1.73	0.55	0.73
Significant	NS	NS	NS	NS	0.05	NS

SEM: Standard error of means.

NS: No significant differences between columns.

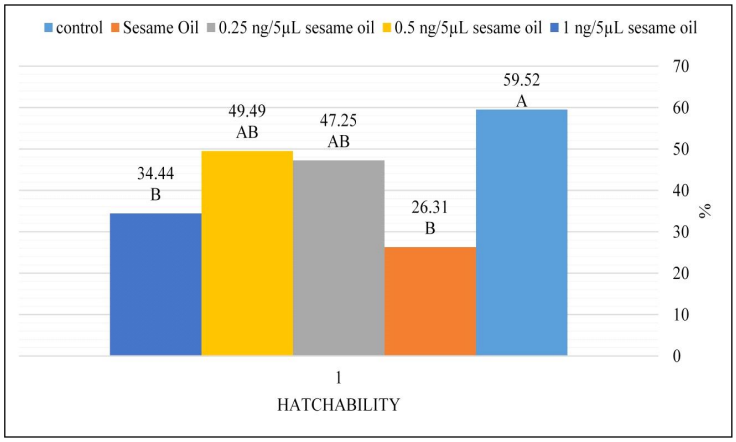


Fig 1: Effect of *in ovo* injection with different levels of corticosterone in hatchability percentage.

Table 5: Effect of *in ovo* injection with different levels of corticosterone on embryonic mortality (percentage of the fertile non-hatching egg).

Treatment	Embryonic mortality stage			Egg pipped	
	Early	Intermediate	Late	Life	dead
Control	21.43*	2.38	16.67	0.00	0.00
Sesame oilinjection	39.59	6.11	24.64	3.33	0.00
0.25 ng/5 µL sesame oil	27.65*	5.12	17.39	0.00	2.56
0.5 ng/5 µL sesame oil	23.48*	3.03	14.89	3.03	3.03
1 ng/5 µL sesame oil	26.11*	5.55	21.11	0.00	2.77
Mean	29.65	4.44	18.94	1.27	1.67
SEM	2.44	1.12	1.57	0.86	0.89
Significant	0.05	NS	NS	NS	NS

SEM: Standard error of means.

NS: No significant differences between columns.

Vassallo *et al*. (2014) showed that only 0.4% of corticosterone injected in quail *Coturnix japonica* eggs reached embryos, with the first being metabolized. Offspring can be exposed to maternal glucocorticoid deposition into the yolk or through placental transfer, then make interaction with embryonic tissues (Hayward *et al*., 2006; Love and Williams, 2008). In addition, maternal stress effects can arise without direct exposure to glucocorticoids in embryos (Carter *et al*., 2018). In this study, a decrease in the weight of allantoic membrane fluid, hatchability percentage and increase in the percentage of embryonic mortality for the high doses of corticosterone groups is a result of corticosterone affection in the chicken embryo. The embryos are capable of metabolizing corticosterone with less than 1% of the original dose accumulated in embryos (Carter *et al.,* 2018).

In addition, the results indicate that corticosterone injection increases with embryos' height compared with the control group. This increase may be due to the role of increasing growth hormone concentration in embryos. These results agree with Yu *et al*. (2018), who showed that low doses of corticosterone in eggs significantly induce goose embryos' somatotroph differentiation. In addition, corticosterone makes for stimulates and differentiates somatotroph differentiation of chicken embryonic development (Sato and Watanabe, 1998). *In vitro* corticosterone increases the number of cells that secrete growth hormones in chicken embryo pituitary cell cultures (Bossis *et al*., 2004). In addition, the high concentration of corticosterone makes to induce growth hormone secretion by e14 of chicken embryos, as it turns out that a single *inovo* injection of corticosterone increased the level of GH*mrna* and plasma growth hormone significantly in pituitary somatotrophs and blood (Yu *et al*., 2018).

But it seems that a high dose of corticosterone concentration in an egg may cause embryonic abnormality and death in the early and late stages of embryonic development (Al-Bayar, 2016). Because the high concentration of corticosterone in eggs has poisoning effects on avian embryos and may negatively affect embryonic biological systems (Pavlik *et al*., 1986; Mashaly, 1991; Kaltner *et al.,* 1993), among breeds may there is a different ability to bear difficult and stressful situations and may be differences in the embryonic ability to corticosterone metabolism because low concentrations of maternal corticosterone in unmanipulated yolks can metabolize maternal corticosterone in natural systems to avoid fitness consequences (Carter *et al*., 2018). So, we need to do more research to study the ability of Iraqi native chicken embryos to arrive at safe levels of egg corticosterone concentration.

CONCLUSION

In conclusion, a high concentration of corticosterone hormone in Iraqi native chicken hatching eggs causes an increase in embryo length, amniotic fluid and amniotic sac weight percentage and decreasing in amniotic fluid, amniotic sac membrane percentage, hatchability and early embryonic mortality percentage.

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Conflict of interest

I confirm that there is no conflict of interest to declare.

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