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EXPLORING THE BIOLOGICAL EFFECTS OF PROGESTERONE ON REPRODUCTIVE ORGANS AND DEVELOPMENT OF CHICK EMBRYOS

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Abstract

Progesterone is an endogenous hormone that is involved in the production of steroid hormones such as corticosteroids, sex hormones, oocyte formation, maturation and development of the endometrium. The aim of this study was to explore the effects of progesterone affected the sex cell-producing organs and the development of chick embryos. The experiment was designed into three trials based on various injection days at 0 hours, 7 days, double dosage administered one on 7 days and the other on 14 days of incubation. Five groups were designed as A, B, C, D and E. Progesterone 0.04 ml, 0.06 ml, and 0.08 ml were administered to experimental groups A, B, and C. Group D received 0.04 ml of physiological saline (0.9% NaCl), whereas Group E did not receive any injections, both serving as control groups. Effects of progesterone on different body parameters were accesed. Results showed that body weight, body length, beak length, right shank weight, and right shank length were highly significant ($p<0.01$) in phase I of trial 1. Phase 2 result showed all parameters were highly statistically significant ($p<0.01$) except gonad weight, percentage of the right shank length and percentage of gonad weight that showed non-significance ($p>0.05$). Trial 3 result of both phases showed a highly significant difference ($p<0.01$) in all parameters, while gonad weight, percentage of right shank weight, and percentage of gonad weight showed non-significance ($p>0.05$) difference. Progesterone had negatively affected the growth of different body parts as it reduced the sex-producing cells or gonad weight, while a double dose had increased its weight.

Keywords

Progesterone; Chick embryos; Sex-cell producing organs/Gonads; Mortality

1. Introduction

Progesterone (C21 H30 O2) is an endogenous steroid and progestogen sex hormone (De Groot & jameson, 2013). Progesterone plays a vital role in our body they perform an intermediate step in the production of steroid hormones such as corticosteroids, and sex hormones. Its function occurs in the oocyte formation, maturation, development of the endometrium, and the uterus of living organisms (Roman *et al*., 2000). Notwithstanding the upkeep of the endometrium during pregnancy and the vascularization of the endometrium during ovulation, progesterone additionally assumes a part in bone development (Arab *et al*., 2019). The interaction of resorption and the formation of the new bone requires chemicals like estradiol and progesterone, as well as bone-shaping cells, like osteoblasts. Progesterone expands the interaction of bone development by animating P-4-receptor-mediated osteoblastic development during the commencement of bone display (Prior, 2018). Progesterone plays a crucial part in the support of the uterus during pregnancy. When compared to non-pregnant women, pregnant women have roughly 10 times more progesterone in their blood and their levels rise gradually throughout the pregnancy. Thusly, a significant issue of concern in regards to the deficiency of this steroid chemical relates to premature delivery and pre-term work (Cunningham, 1993). Progesterone likewise impacts the creation of fiery middle people, for example, human lymphocytes inside the uterine hole. In this way, a deficiency of progesterone prompts an increment in myometrial contractility combined with an abatement in warding off immunologic dangers, finally prompting a higher danger of unsuccessful labour and early conveyance of the hatchling (Arab *et al*., 2019). In addition to its role as a natural hormone, progesterone is also used as a medication, such as in combination with estrogen for contraception, to reduce the risk of uterine or cervical cancer (Prior, 2019). Progesterone's pharmacokinetics and pharmacodynamics have been researched extensively since it was first synthesized in 1935 (Di Renzo *et al*., 2012). Historically, the most common delivery method of progesterone has been through intramuscular injection. The major advantages of intramuscular administration are that it results in optimum progesterone plasma concentrations (Abo-EL-Sooud *et al*., 2011). During pregnancy, progesterone has advantageous consequences for the improvement of the undeveloped organism. There are a few confirmations on the unfavorable effects of these medications (Ludwig & Diedrich, 2001). Progesterone injections, whether chronic or acute, can have various effects on birds (Liu *et al.,* 2001). In normal laying chicken hens, an acute dosage of exogenous progesterone induces mature follicles to premature ovulation at a specified moment throughout ovulatory cycles (Ahmad & Zamenhof, 1979). A single injection of progesterone during the preovulatory open phase in laying hens has been found to have a favorable effect on promoting fertilization. However, chronic progesterone injection has been demonstrated to elevate baseline progesterone concentrations, causing turkeys to stop laying and having their hierarchical follicle

distribution disturbed (Bacon & Liu, 2004). The chick embryo's molecular, cellular and anatomical similarities to the human embryo, as well as its rapid development, accessibility for visualization and experimental manipulation and its relatively large size and planar structure during early development, all contributed to its acceptance as a research model (Vergara & Canto-soler, 2012).This study was aimed to investigate the effect of progesterone on the sex cell-producing organs and the development of chicks along with how high doses of progesterone affect the growth of chick embryos. To find its effect, different parameters such as body weight, body length, beak length, right shank weight, right shank length, gonad weight, percentage of right shank weight, percentage of the right shank length and percentage of gonad weight were evaluated on different days. The mortality rate was also observed in different phases of the experiment.

2. Material and Methods

2.1 Sample collection and Chemicals

150 pathogen free non-incubated fertilized chicken eggs were taken and an experiment was conducted at Lab-II, Department of Zoology, Ghazi University, D. G Khan. Progesterone injection of 50ml and physiological saline (0.9% NaCl) were purchased commercially from the local market.

2.2 Method of injection

The eggs were washed with 70% ethanol and properly labelled on the outer shell with a permanent marker. Candling was performed at 0 hours of incubation to check the fertility and identity of air sacs of eggs. Then the blunt side of the eggs having an air sac was marked with the permanent marker and holes were made with the help of a sharp and thick needle. After doing that, a 25-gauge aseptic syringe was used for the injection of a dose. Five groups of eggs were formed: A, B, C, D and E. Different dosages of progesterone were injected. Experimental groups A, B, and C received 0.04ml, 0.06ml, 0.08ml progesterone respectively. 0.04ml physiological saline (0.9% NaCl) was injected in group D, and group E did not get any injection. Group D and E act as control groups. Melted paraffin wax was used to seal the pores in the eggs and incubated at 37℃ and 75% relative humidity.

2.3 Experimental design

The experiment was conducted in 3 different trials based on different days of dose administration. Each trial was divided into 2 phases based on different days of embryo observation.

2.3.1 Trial No.1

50 fertilized pathogen-free eggs were taken for the first trial. The trial was further divided into 2 phases based on observation days. Both phases of trial 1 contained 25 eggs. Different dosages of progesterone and physiological saline as mentioned above were given on the 0 days of incubation. In Phase I, embryos were observed on the 18th day of incubation and in Phase II, embryos were observed on the 21st day of incubation.

2.3.2 Trial No.2

50 fertilized pathogen-free eggs were taken for trial 2. This trial was further divided into 2 phases based on observation days. Each phase had 25 eggs. Different dosages of progesterone and physiological saline were injected within eggs on the 7th day of incubation. In Phase I, embryos were examined on

the 18th day of incubation and in Phase II, embryos were examined on the 21st day of incubation.

2.3.3 Trial No. 3

50 fertilized pathogen-free eggs were taken for trial. The trial was further divided into 2 phases based on observation days. 25 eggs were present in each phase. In trial 3, a double dose was injected. Different dosages of progesterone and physiological saline were injected within eggs on the 7th and 14th days of incubation. In Phase I, embryos were examined on the 18th day of incubation and in Phase II, embryos were observed on the 21st day of incubation.

2.4 Embryo Observation

On the observation day, eggs were taken from the incubator, cracked with forceps, and carefully placed in a petri dish and examined the different parameters. Body length (mm), beak length (mm) and right shank length (mm) were measured by measuring scales. Body weight (mg), right shank weight (mg), and gonad weight (mg) were weighed by an electronic weight balance machine (Model no. FA2204). Percentage (%) of right shank length of chick embryos =Right shank length/Body length × 100 Percentage (%) of gonad weight of chick embryos =Gonad weight/Body weight × 100. The percentage of mortality was calculated by=Dead embryos/Total embryos × 100

2.5 Statistical Analysis

Microsoft Excel was used for the compiling of the statistical data. All the data were analyzed by using SPSS version 20.0. Data were examined using one-way ANOVA, and significant means were separated using Duncan's multiple range tests. Percentage data were obtained by using Arcsin % transformed data.

3. Results and Discussion

3.1 Trial no. 1

Table 1 shows the results of trial 1 with effect of progesterone of various concentrations administered within eggs at day 0 of incubation and examined different parameters on 18th (phase I) and 21st day (phase II) Phase I: Results showed a highly statistically significant ($p<0.01$) difference in body weight, body length, beak length, right shank weight, and right shank length. The percentage of the right shank length was significant ($p<0.05$), while gonad weight, percentage of right shank weight, and percentage of gonad weight showed a non-significant ($p>0.05$) difference between experimental and control groups as shown in Table 1. In experimental group A, 0.04ml of progesterone caused a reduction in body weight, body length, beak length, right shank weight, right shank length, gonad weight and percentage of the right shank length. And their values increased in control group E. But 0.04ml progesterone had increased the percentage of right shank weight, and percentage of gonad weight. And a reduction in their values occurs in control group D. Phase 2: Table 1 depicts the reduction in body weight, body length, beak length, right shank weight, and right shank length, gonad weight, percentage of right shank weight, percentage of the right shank length in experimental groups, while a reduction of the percentage of gonad weight was in control group D. However, values of all parameters were increased in control group E. All parameters were revealed highly statistically significant ($p<0.01$) difference except gonad weight, percentage

of the right shank length and percentage of gonad weight that showed non-significance (p>0.05).

Table 1: Effect of progesterone of various concentrations administered within eggs at day 0 of incubation and examined different parameters on 18th (phase I) and 21st day (phase II)

Parameters	Trial 1	Experimental groups			Control groups		p-value
		A	B	C	D	E	
Body weight (mg)	Phase I	1303±577.54[a]	3742.2±2166.78[ab]	9287.4±5130.2b[c]	16606.8±2199.3[c]	19203.2±5[c]	0**
	Phase II	3117.40±928.51[b]	2042.80±187.07[b]	7632.20±4702.32[b]	21474.80±2847.77[a]	27286.40±3125.4[a]	0**
Body length (mm)	Phase I	36.4±2.97[a]	42.0±8.50[ab]	56.0±13.75[bc]	77.4±2.67[c]	91.6±3.07[c]	0**
	Phase II	41.80±2.22[b]	37.80±1.88[b]	44.80±8.21[b]	75.20±3.18[a]	85.60±3.82[a]	0**
Beak length (mm)	Phase I	2.00±0.44[a]	3.00±0.70[a]	3.60±1.20[b]	5.80±0.49[b]	6.40±0.24[b]	0.00**
	Phase II	2.80±0.49[b]	2.81±0.49[b]	3.20±1.11[b]	6.00±0.31[a]	6.40±0.24[a]	0**
Right shank weight (mg)	Phase I	8.88±0.62[a]	39.45±30.22[b]	67.5±35.77[c]	162.6±33.37[c]	244.8±16.43[c]	0**
	Phase II	18.40±9.17[b]	7.56±1.50[b]	47.14±31.27[b]	154.26±17.57[a]	207.08±25.82[a]	0**
Right shank length (mm)	Phase I	4.60±0.81[a]	6.75±2.05[ab]	8.40±2.78[bc]	12.8±0.37[c]	17.2±0.66[c]	0**
	Phase II	7.20±0.49[b]	6.00±0.70[b]	7.60±2.33[b]	14.00±0.54[a]	16.60±1.20[a]	0**
Gonad weight (mg)	Phase I	2.90±0.10[a]	15.7±13.1[a]	21.63±12.9[a]	8.68±3.50[a]	19.78±14.3[a]	0.82
	Phase II	1.44±0.39[a]	1.10±0.30[a]	12.23±10.40[a]	4.78±1.63[a]	23.14±13.2[a]	0.29
Percentage (%) of right shank weight	Phase I	5.26±3.05[a]	3.16±2.61[a]	1.33±0.68[a]	0.93±0.08[a]	1.24±0.04[a]	0.37
	Phase II	0.49±0.08[b]	0.35±0.42[b]	0.43±0.11[b]	0.72±0.06[a]	0.75±0.21[a]	0.00**
Percentage (%) of right shank length	Phase I	12.12±1.19[a]	13.05±2.43[ab]	13.4±1.50[b]	16.2±1.01[b]	18.4±1.16[b]	0.03*
	Phase II	16.80±0.37[a]	15.40±1.16[a]	15.20±1.98[a]	18.20±0.49[a]	18.80±0.80[a]	0.12
Percentage (%) of gonad weight	Phase I	1.58±1.49[a]	1.23±1.03[a]	0.15±0.10[a]	0.05±0.02[a]	0.10±0.07[a]	0.19
	Phase II	0.04±0.002[a]	0.04±0.01[a]	0.06±0.02[a]	0.02±0.00[a]	0.08±0.04[a]	0.69

[a, b, c] Values (Mean±SE) having different superscript letters in a row indicate the significant difference (p<0.01**, p<0.05*)

3.2 Trial No. 2

Tables 2 shows the results of experimental trial 2 with effects of different progesterone. In this trial, all parameters body weight, body length, beak length, right shank weight, and right shank length showed a highly significant difference (p<0.01). Gonad's weight was significant (p<0.05).

Table 2: Effect of progesterone of various concentrations administered within eggs on day 7th of incubation and examined different parameters on 18th (phase I) and 21st day (phase II)

Parameters	Trial 2	Experimental groups			Control groups		p-value
		A	B	C	D	E	
Body weight (mg)	Phase I	3750.2±10 64.17^{b}	3120±1069.9 0^{b}	1834.2±882.2 6^{b}	21728.8±981 .67^{a}	20900.6±400 6.26^{a}	0**
	Phase II	3068.6±13 48.08^{b}	5561.8±3241 .73^{b}	3250.6±1439. 24^{b}	30174±1439. 44^{a}	28498.4±199 7.08^{a}	0**
Body length (mm)	Phase I	38.4±3.90^{b}	37.8±3.61^{b}	32.8±3.05^{b}	82±2.07^{a}	73.2±5.97^{a}	0**
	Phase II	38.6±6.14^{b}	38.4±10.10^{b}	39.2±6.25^{b}	96.6±4.72^{a}	86.6±2.81^{a}	0**
Beak length (mm)	Phase I	3.75±0.62^{b}	3.75±0.62^{b}	3.33±0.66^{b}	7.00 ±0.31^{a}	6.00±0.31^{a}	0**
	Phase II	3.50±0.28^{b}	4.33±0.66^{c}	3.20±0.49 ^{c}	6.40±0.4^{ab}	6.40±0.24^{a}	0**
Right shank weight (mg)	Phase I	44.3±17.87 ^{b}	40.025±17.5 3^{b}	49.833±20.21^{b}	207±24.45^{a}	137.22±32.4 4^{a}	0**
	Phase II	26.55±8.59 ^{b}	76.833±22.5 5^{b}	26.52±7.24^{b}	175.22±21.7 0^{a}	199.74±32.7 9^{a}	0**
Right shank length (mm)	Phase I	7.25±0.85^{b}	6.25±0.47^{b}	700±1.00^{b}	13.6±0.24^{a}	11.2±1.39^{a}	0**
	Phase II	6.50±0.50^{b} ^{c}	8.67±1.85^{d}	5.20±0.49^{cd}	15.0±0.63^{a}	16.0±1.78^{ab}	0**
Gonad weight (mg)	Phase I	5.32±1.36^{b}	4.05±1.15^{b}	4.16±1.46^{b}	10.16±2.03^{ab}	12.5±2.97^{a}	0.03*
	Phase II	1.97±0.43^{a}	4.10±1.15^{a}	1.72±0.36^{a}	7.66±3.53^{a}	23±13.32^{a}	0.20
Percentage (%) of right shank weight	Phase I	1.1±0.60^{a}	2.33±1.88^{a}	5.34±4.52^{a}	0.92±0.07^{a}	0.61±0.09^{a}	0.34
	Phase II	2.37±1.94^{a}	3.15±2.42^{b}	2.89±1.55^{b}	0.56±0.05^{ab}	0.67±0.08^{ab}	0.49
Percentage (%) of right shank length	Phase I	16.75±1.03 ^{ab}	15.12±0.92^{ab}	18.33±1.76^{a}	15.96±0.61^{ab}	14.6±0.87^{b}	0.15
	Phase II	14.75±0.94 ^{a}	16.0±0.00^{c}	13.36±1.17^{bc}	15.54±1.06^{ab}	18.08±1.63^{bc}	0.11
Percentage (%) of gonad weight	Phase I	0.13±0.05^{a}	0.19±0.13^{a}	0.41±0.34^{a}	0.04±0.01^{a}	0.05±0.01^{a}	0.31
	Phase II	0.145±0.10 ^{a}	0.17±0.14^{ab}	0.14±0.07^{ab}	0.01±0.003^{b}	0.08±0.05^{ab}	0.58

$^{a, b, c}$ Values (Mean±SE) having different superscript letters in a row indicate the significant difference ($p<0.01$**, $p<0.05$*)

Phase II: As shown in Table 2, body weight, body length, beak length, right shank weight, and right shank length showed a highly significant difference ($p<0.01$). Gonad weight, percentage of right shank weight, percentage of the right shank length and percentage of gonad weight showed non-significant differences ($p>0.05$).

3.2 Trial No. 3

Table 3 shows the results of trial 3 with effects of different progesterone. It showed that a double dose of 0.04ml of progesterone had decreased the body weight, body length, beak length, right shank weight, and right shank length, its values increased in control group D given physiological saline. While the percentage of the right shank length was reduced in group B given 0.06ml progesterone. As progesterone had negatively affected the above-mentioned parameters, it had increased gonad weight, percentage of right shank weight and percentage of gonad weight in experimental groups,

and decreased in control groups. Double dose of progesterone had decreased the body weight, body length, beak length, right shank weight, right shank length, percentage of right shank weight and percentage of the right shank length in experimental group B, while their values increased in control groups. Gonad weight and percentage of gonad weight were not affected by the double dosage of progesterone and showed non-significant differences ($p>0.05$) between experimental and control groups as depicted in table 3.

Table 3: Effect of a double dose of progesterone of various concentrations administered within eggs on day 7th and 14th of incubation and examined different parameters on 18th (phase I) and 21st day (phase II)

Parameters	Trial 3	Experimental groups			Control groups		p-value
		A	B	C	D	E	
Body weight (mg)	Phase I	2039.2±1024.38^{b}	2337.6±770.92^{b}	14899.8±5406.15^{a}	21411.6±847.19^{a}	18642.6±2373.56^{a}	0**
	Phase II	4570.8±1908.56^{b}	2344±1184.32^{b}	4704±1310.0^{b}	22157.75±3244.64^{a}	21599.4±1902.90^{a}	0**
Body length (mm)	Phase I	35.6±5.25^{b}	37.2±3.98^{b}	65.6±12.28^{a}	80.2±0.8^{a}	72.8±5.52^{a}	0**
	Phase II	44.6±5.21^{b}	37.8±4.70^{b}	46.2±3.83^{b}	75.75±3.25^{a}	80.8±3.15^{a}	0**
Beak length (mm)	Phase I	2.00±0.97^{b}	2.40±0.6^{b}	4.80±1.24^{b}	7.00±0.31^{a}	6.20±0.37^{a}	0.00**
	Phase II	4.50±0.64^{bc}	3.25±0.85^{c}	4.00±0.54^{c}	6.00±0.40^{b}	7.00±0.31^{a}	0.00**
Right shank weight (mg)	Phase I	14.48±5.54^{c}	16.96±4.43^{c}	107.5±32.37^{b}	246.6±32.76^{b}	153.26±41.59^{a}	0**
	Phase II	55.5±34.36^{b}	14.22±8.24^{b}	22.24±8.08^{b}	157.3±21.52^{a}	134.5±18.94^{a}	0**
Right shank length (mm)	Phase I	5.00±0.74^{a}	5.20±0.58^{b}	11.80±2.39^{a}	13.0±0.31^{a}	11.80±0.58^{a}	0**
	Phase II	10.00±1.78^{bc}	5.25±1.10^{d}	7.00±1.14^{cd}	14.00±0.707^{a}	12.6±1.12^{a}^{b}	0**
Gonad weight (mg)	Phase I	7.40±0.20^{a}	35.13±32.93^{a}	24.75±9.36^{a}	7.06±1.58^{a}	12.48±1.34^{a}	0.42
	Phase II	6.72±1.74^{a}	4.56±2.53^{a}	4.76±1.22^{a}	4.27±1.70^{a}	9.38±1.42a	0.16
Percentage (%) of right shank weight	Phase I	1.28±0.41^{a}	1.00±0.25^{a}	1.28±0.30^{a}	1.10±0.11^{a}	0.72±0.16^{a}	0.58
	Phase II	0.79±0.20^{a}	0.43±0.05^{b}	0.43±0.04^{b}	0.71±0.05^{ab}	0.62±0.07^{a}^{b}	0.07
Percentage (%) of right shank length	Phase I	15.06±0.25^{b}	13.92±0.35^{b}	17.46±1.09^{a}	15.66±0.38^{ab}	16±0.83^{ab}	0.01*
	Phase II	20.94±1.92^{a}	13.09±0.93^{c}	14.82±1.33^{bc}	18.49±0.56^{ab}	15.56±1.20^{bc}	0.00**
Percentage (%) of gonad weight	Phase I	0.61±0.44^{a}	0.94±0.88^{a}	0.13±0.05^{a}	0.03±0.00^{a}	0.06±0.01^{a}	0.26
	Phase II	0.16±0.07^{a}	0.12±0.01^{ab}	0.12±0.04^{ab}	0.01±0.00^{b}	0.04±±0.00^{ab}	0.09

a, b, c Values (Mean±SE) having different superscript letters in a row indicate the significant difference ($p<0.01$**, $p<0.05$*)

3.4 Mortality percentage (%)

The mortality percentage of chick embryos showed fluctuation between different phases of all trials as shown in Figure 1. In both phases of Trial 1, an inverse relationship was present between progesterone dose and mortality rate of chick embryos. In experimental groups, high progesterone doses had decreased mortality. This result showed that a high dosage of progesterone assists in preventing mortality when given at 0 hr's incubation. In trial 2, chick embryos showed 100% mortality in progesterone-treated groups. This showed that progesterone now released by the gonads of chick embryos and administration of progesterone from an external source on the 7th day during the development stage had adversely affected the chick embryos. Trial 3 results showed increased progesterone dose decreased mortality in phase 1. All experimental groups revealed 100% mortality in phase 2. This showed double dosage of progesterone had also affected the mortality. Control groups of all trials showed no mortality.

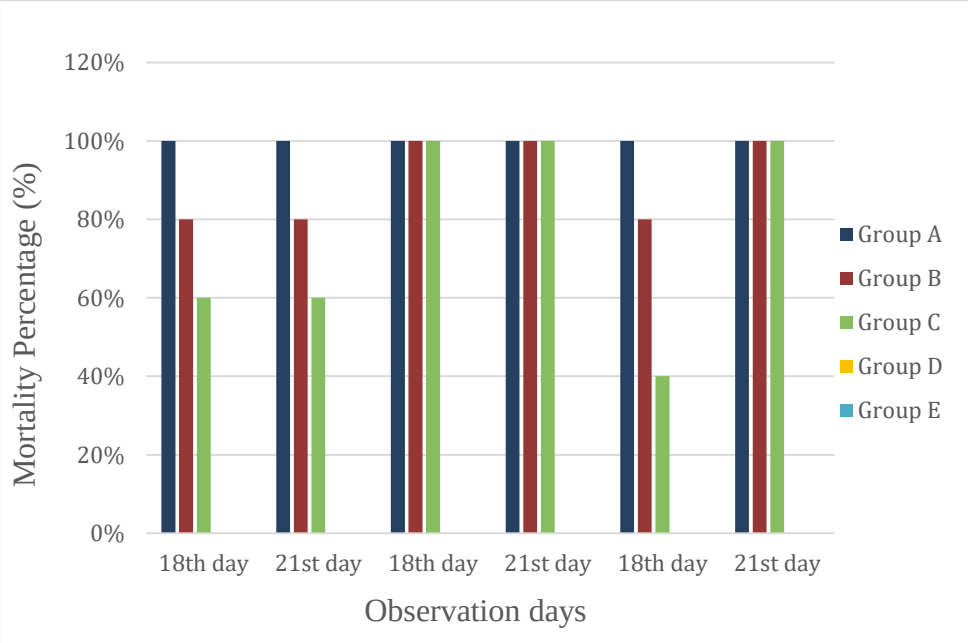

Figure 1: Mortality percentage (%) in different experimental and control group

Mortality percentage (%) in different experimental and control groups administered various concentrations of progesterone on different days (at 0, 7 and double dose on 7th and 14th day of incubation) and observed. Our results are agreed with the previous studies. Zhou *et al.,* (2020) have shown treating the eggs with different concentrations of progesterone did cause a reduction in body weight. The result was also significant. Besides decreasing body weight, progesterone did not cause any gross modifications in fetal development. Our study also consistent with Huang et al., (2021) study that administered steroid hormones directly to the embryo and discovered that the progesterone (0.2mg to 1.0mg) had harmful and teratogenic effects. Because progesterone is present

in the adrenal glands of 9th-day-old chick embryos and acts as a precursor of corticosteroids. In a previous study, Guo *et al.,* (2021) described the conversion of a small amount of progesterone into corticosterone. Corticosterone is a growth inhibition factor that retards embryonic growth due to progesterone. Also, Cui et al. (2020) described the role of corticosterone in the growth reduction of chick embryos. Progesterone, disrupts normal skeletal growth by interfering with chondrocyte metabolic functions. It blocks normal steroid-cell interactions, thus disrupting normal RNA and protein synthesis. In the current study, the body length (both male and female) of the chick embryos examined on the 18th and 21st day of the incubation period was significantly reduced in those groups treated with different concentrations of progesterone as compared to the control groups and showed highly significant difference. It was supported by the findings of Li *et al*., (2019) that revealed a decrease in body length of the chick embryo injected with progesterone and examined on the 18th day. It was significantly different from the control groups. In the current study, the right shank length (mm) and right shank weight (mg) of the chick embryo examined on the 18th and 21st day of the incubation period was significantly reduced in those groups treated with different concentrations of progesterone as compared to the control groups. The progesterone-treated groups were statistically significant. It was consistent with Licein *et al*., (2021) findings that found the steroid hormone progesterone directly affected the development of chick bones, their joints and especially the hind limbs. The current study was also highly correlated with Renden and Mouton et al., (2020) conducted experiment on chick embryos, in embryos treated with 0.5mg progesterone on the 4th day of the incubation period examined on the 18th day and then compared to control embryos. Right shank length and weight were reduced in embryos and found the right shank length and weight of progesterone-treated groups were significantly different from the control groups. The present study revealed no significant difference in gonad weight of the chick embryo examined on the 18th and 21st day of the incubation period among those groups treated with different concentrations of progesterone as compared to control and saline groups. But gonad weight showed a significant difference in phase I of trial two, in which progesterone was injected on day 7 and examined on day 18. The gonad weight was not affected by the progesterone and the results were statistically non-significant. The present finding was consistent with Orozco (2019) findings which reported that the gonad weight was not affected by progesterone, and revealed a non-significant difference. They described it might be due to progesterone didn't alter typical morphology nor the separation of male or female sex organs or related ductal frameworks. Testicles of male incipient organisms treated with progesterone showed typical advancement of the medulla and possible seminiferous tubules. The left ovary of treated females showed typical advancement of the cortex and optional sex strings, while the right ovary went through an ordinary relapse. Mouton et al., (2020) also found no significant difference in the percentage of shank weight. but opposite of the result of phase

II of trial I which showed a highly significant difference. Shank length expressed as a percent of body length treated with different concentrations of progesterone was not significantly different from the control groups. The present study showed similarities to the Ewuola (2020) findings to the result of phase I of trial 1 of the present finding.

4. Conclusion

This study aimed to investigate the effect of progesterone on sex cell-producing organs and the development of chicks. Also how high doses of progesterone had an impact on the growth of early chick embryos. For the study, chick embryos were used that correspond to the initial stage of embryogenesis in mammals, providing them suitable for the study of biotechnology. In the present study, it was found progesterone did not help in growth, instead of reducing embryonic growth and its size as compared to control groups. But it did not change the typical morphology of the embryo's gonads structure. Progesterone had reduced the gonad weight in experimental groups given a single injection of progesterone. But the double dose of progesterone had increased the gonad weight. It was concluded that progesterone had negatively affected the growth of different body parts. And progesterone had reduced the sex-producing cells/ gonad weight, while a double dose had increased its weight.

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