HISTOMORPHOMETRICAL STUDIES ON THE SPLEEN OF LOHMANN BROWN LAYERS TREATED WITH CRYSTALLINE PROGESTERONE

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ABSTRACT
The study determined the effect of Crystalline Progesterone (CP) on eighteen Lohmann Brown layers. Completely randomized design was used with each treatment (0, 5, 10, 15, 20 and 25 mg per bird) administered intramuscularly via the breast muscle and replicated thrice for six weeks. Data were analyzed using GraphPad InStat®package. Results revealed non-significant (P>0.05) effect of CP proportion of spleen vascular skeleton and B follicles were not significantly different and proportion of spleen red pulp (0 mg vs. 15 mg = 80% vs. 40%) and ellipsoids and peri-arteriolar lymphoid sheath (PALS) (0 mg vs. 15 mg = 10% *vs*. 50%). CP affected proportion of spleen red pulp and ellipsoids and PALS.
Key words: Crystalline Progesterone, layers

INTRODUCTION
Spleen in avian species is a round or oval structure lying dorsal to and on the left side of the proventriculus (Payne, 1971). Eerola *et al*. (1987) observed that splenic development occurs after hatching, following exposure to antigens. Spleen is a principal organ of systemic immunity and its importance in disease resistance is accentuated by the scarcity of avian lymph nodes (John, 1994). It is also used as primary organ of systemic body defense and plays important role against invasion of pathogenic organisms into the animal's body (Kannan *et al*., 2015). The main function of the spleen is filtering of blood and production of immunoglobulins and lymphocytes (T-help) involved in body defense (Kopp, 1990).
Spleen in birds has attracted the interest of many scientists (Corbin *et al*., 2008). Thus, the spleen is a greater asset in the study of immune response as producer and a store for lymphocytes (Smith and Hunt, 2004). The objective of the study is to evaluate spleen histomorphometry in Lohmann Brown layers treated with different levels of Crystalline Progesterone.

MATERIALS AND METHODS
Experimental Birds and their Management
A total of eighteen 24-week old Lohmann Brown strain of layers were used for the experiment at Poultry unit of the Teaching and Research Farm of the Department of Animal Science, Bayero University Kano (GPS Coordinates: 11.97643^{o}N, 008.42995^{o}E). Birds were managed in battery cages. Multivitamins and Oxytetracycline were given as prophylaxis for stress and secondary bacterial infections, respectively before the commencement of the study. They were allowed to acclimatize to the new environment for two weeks before commencement of the experiment and fed layer mash (Super Layer®), water was provided *ad libitum.*
Experimental Design and Progesterone administration
The experiment was laid in a single factor completely randomized design with six treatment groups replicated three times.
Crystalline Progesterone (Gesteron-25®) was purchased in 1 ml ampoules of 25 mg per ml from Wellcare Pharmaceuticals, Kano. The treatment groups received Crystalline Progesterone injections intramuscularly via the breast muscle at 0, 5, 10, 15, 20 and 25 mg/bird. The control group (0) mg/bird designated treatment (A) was injected with 1 ml normal saline. Injections were given twice per week at an interval of two days apart in the morning between 10.00 am and 11:00 am throughout the experimental period of six weeks.
Spleen harvesting and histological processing

At the end of the experimental period, spleen samples were harvested using the procedure of Thierry (2000). After harvesting, organs were immersed in 10% Neutral Buffered Formalin. Fixed samples were taken to the laboratory for histological processing using standard histological techniques as described by Bancroft and Gamble (2008) and histological slides were prepared.

Slides produced were subjected to histomorphometry following the method of Maas and Orthel (1976) with modifications by the use of an Olympus CX21 microscope at x100 total magnification (eyepiece + nosepiece) for visual percentage analysis. Five fields were examined and observer-dependent percent composition of each component visible on hematoxylin and eosin only stained slides were noted and the averages were recorded as percentages. Vascular skeleton was identified as medium- and small-sized arterioles with their branches visible at x100 magnification. Red pulp includes all red pulp areas including capillary and sinusoids. Ellipsoids and peri-arteriolar lymphatic sheath (PALS) were evaluated as ellipsoids merged with PALS without added histochemical staining (reticulin stain) to separate them. B follicles were identified as circular aggregates of small-sized lymphocytes.

Data analysis

Data generated were subjected to analysis using GraphPad InStat Statistical. Kruskal-Wallis test was used to determine the effect of different levels of Crystalline Progesterone on proportion of splenic red pulp, and proportion of ellipsoids and periarteriolar lymphoid sheath. Significant differences in mean rank differences across Crystalline Progesterone treatment groups were separated using the Dunn's Multiple Comparisons test. One-Way Analysis of Variance was used to determine the effect of different levels of Crystalline Progesterone on the proportion of splenic vascular skeleton and B follicles. Significant mean across treatments were separated using Tukey's test.

RESULTS

Table 1: Selected Pairs Dunn's Multiple Comparisons for Proportion of Red Pulp in the Spleen across Crystalline Progesterone Treatment Levels in Lohmann Brown Hens

Comparison	Mean Rank Difference	Level of Significance
0 mg *vs*. 15 mg	17.900	*
5 mg *vs*. 15 mg	22.100	***
5 mg *vs*. 25 mg	17.000	*

*P<0.05; ***P<0.001

Table 2:Selected Pairs Dunn's Multiple Comparisons on Proportion of Ellipsoids and PALS[1] across Crystalline Progesterone Treatment Levels in Lohmann Brown Hens

Comparison	Mean Rank Difference	Level of Significance
0 mg *vs*. 15 mg	-19.700	**
5 mg *vs*. 15 mg	-19.700	**
10 mg *vs*. 15 mg	-16.800	*

*P<0.05, **P<0.01, PALS [1]= periarteriolar lymphatic sheath

Table 3: Effect of Crystalline Progesterone on Proportion of B Follicles in the Spleen of Lohmann Brown Hens

Crystalline Progesterone (mg)	Mean Proportion (%)	Standard Error of the Mean
0	0.0	0.000
5	0.0	0.000
10	1.6	1.030
15	0.0	0.000
20	0.0	0.000
25	2.6	1.939

P>0.05

Table 4: Effect of Crystalline Progesterone on Proportion of Vascular Skeleton in the Spleen of Lohmann Brown Hens

Crystalline Progesterone (mg)	Mean Proportion (%)	Standard Error of the Mean

0	10.4	1.631
5	6.0	1.000
10	8.0	1.225
15	11.0	1.000
20	11.0	2.449
25	7.0	1.225

P>0.05

DISCUSSION

Progesterone receptor (PR) has been described in tissues where the action of progesterone is less well defined, including vascular endothelium (Perrot-Applanat*et al*., 1995) and rat thymus (Pearce, Khalid & Funder, 1983). The lack of a well-defined action in the splenic vascular skeleton and B follicles could be responsible for the non-significant effect of Crystalline Progesterone (CP) in the present study. This trend could also be due to non-significant individual variability in hens across CP treatment groups.

Balika *et al*. (1976) reported that the percentage of all splenic erythroid cells increased in newborn rats that received antenatal doses of progesterone. They also reported that the relative percentage of splenic myeloid cells in young rats fell by half under the influence of exogenous progesterone administered during the antenatal period. As the effects of progesterone are mediated by its receptor and PR is induced by estrogen in most target tissues, the delineation of specific progesterone effects, as distinct from those of estrogen, is similarly not clear (Graham & Clarke, 1997). This could be responsible for the general decrease in red pulp proportion and the corresponding increase in splenic white pulp (ellipsoid and peri-arteriolar lymphatic sheath as well as B follicles) proportion recorded in the current work. Moreover, the present experiment took place when the birds were actively ovulating; thus presenting an opportunity for adequate estrogen priming prior to administration of Crystalline Progesterone. According to Sasaki and Ito (1981), splenic white pulp showed a slight but significant increase in volume in estrogen-treated and estrogen-progesterone treated mouse. In the progesterone-treated group, however, the red and white pulps did not undergo any significant change in volume (Sasaki and Ito, 1981).

CONCLUSIONAND RECOMMENDATION

In conclusion, CP affected proportion of spleen red pulp and ellipsoids and PALS. Effect of CP on other lymphoid organs should be study.

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