Nigerian Veterinary Journal Vol. 28(2) 2007:12-17

THE EFFECTS OF PROGESTERONE, STILBESTEROL AND ITS COMBINATION ON THE HAEMATOLOGICAL PARAMETERS IN FEMALE ALBINO RATS.

SANDABE[1]*, U. K. and TIMOTHY[2], W. Y.

[1]Department of Veterinary Physiology and Pharmacology, University of Maiduguri, P.M.B. 1069, Maiduguri. Borno State. Nigeria. [2]National Veterinary Research Institute, Vom, Jos, Plateau State, Nigeria.

***Correspondence: E-mail:** usandabe@yahoo.com

SUMMARY

The effects of different hormonal treatments on blood parameters in 24 female Albino rats were studied. The rats were kept in four clean cages and given feeds and water *ad libitum*. Progesterone, stilbesterol (estrogen preparation) and progesterone-stilbesterol combination were injected intramuscularly daily for three weeks and withdrawn for two weeks. Blood (0.2-0.3 ml) was collected from the tail on weekly basis and analyzed for the values of red blood cell (RBC), white blood cell (WBC), differential leucocytes count (DLC), packed cell volume (PCV) and haemoglobin concentration (Hb). The results showed that RBC counts decreased ($P<0.05$) when the rats were treated with progesterone and stilbesterol, while after withdrawal of treatment stilbesterol showed no change ($P>0.05$). Progesterone administration did not show any change in total WBC counts ($P>0.05$) while stilbesterol and progesterone-stilbesterol combination showed changes ($P<0.05$) across the weeks. All the three treatment regimens did not affect the DLC counts ($P>0.05$) across the weeks. Progesterone alone and its combination with stilbesterol did not alter the PCV of the rats across the weeks. Stilbesterol showed decreasing effect on the PCV value on the third week ($P<0.05$), but after the withdrawal of the treatment, it did not show any change ($P>0.05$). Only stilbesterol treatment showed changes in Hb concentration across the weeks, but the effect reversed after the withdrawal of the treatment. In general the results showed that the three different hormonal treatments did not cause any severe untoward effects on the blood; however it was concluded that progesterone, stilbesterol or their combination may be employed for usage, up to two weeks in rats.

KEY WORDS: Progesterone, Stilbesterol and Blood Parameters

INTRODUCTION

Progesterone and estrogen are steroid reproductive hormones. Progesterone is produced by the lutein cells of the Corpus Luteum (CL) and converts the uterine epithelium from the proliferative to secretory phase. It is necessary for successful implantation of the ovum and is essential throughout the last two third of pregnancy during which it is secreted in large amounts by the placenta. Progesterone and its synthetic analogues are used for suppression or delay of estrus in bitches and cats. They have also been used in behaviour modification and for the treatment of dermatologic disorders. Progesterone supplementation is used to support pregnancies considered to be at risk (Schalm *et al.*, 1975; Hafez, 1980 and Labored *et al.*, 1999). Long-, medium- and short-term treatments with progesterone and progestational agents have been used to synchronize estrus in cattle. In humans, progesterone has been prescribed for contraception, menopause and post-menopause hormone replacement therapy, menstrual disorders, breast and endometrial cancers (Williams and Stancel, 1996 and Laurence *et al.*, 1997).

In the follicular phase of estrous or menstruation cycles, the follicular stimulating hormone (FSH) causes the ovarian follicles to mature and secrete estrogen from the theca

interna and granulosa cells (Sprott *et al.*, 1984 and Williams and Stancel, 1996). Estrogens have also been secreted minimally from adrenals, placenta and testes of the male (Hafez, 1980 and McDonald, 1980). This endogenous estrogen is responsible for the development of female secondary sex characteristics, and it acts on the female genitalia to produce an environment suitable for fertilization, implantation and nutrition of the early embryo (Hafez, 1980 and Blood and Studdert, 1999). It also creates a state of sexual receptivity in the female (Swenson, 1997 and Williams and Stancel, 1996). Exogenously administered estrogen and its preparations are used as abortifacients in some species (e.g. bovine) and to prevent pregnancy after unwanted mating in canine. Estrogens are used in induction of estrus and for anti-tumor activity in prostatic and peri-anal tumours. Estrogen and oxytocin are the best non-antibiotic agents for the treatment of pyometra, retained placenta and endometritis (Tennant, 2000). Orally active estrogen such as ethinyl estradiol is often combined with a synthetic progestin such as norethindrone and administered as contraceptive (Ganong, 1985).

It is important to find out the effects of these hormones on blood parameters. Blood parameters are of immense value in establishing tentative and confirmatory diagnosis of many diseases and also in determining the extent of damage to blood cells and evaluating the response of animals to therapy (Kaneko, 1980 and Woerpel and Rosskopt, 1984). Therefore, the aim of this work was to find out the effect of progesterone, estrogen and progesterone-estrogen combination on blood parameters of the female rats.

MATERIALS AND METHODS

Animals

Twenty four (24) female Sprague-Dawley albino rats weighing between 190 and 310 grams were used. They were randomly separated and kept in four groups of six rats each and were housed in a room exposed to a natural light cycle. They were fed grower's mash diet produced by SEEPC Nigeria Ltd, Lagos and provided with clean water *ad-libitum*. The rats were acclimatized to the laboratory conditions for two weeks before the commencement of the experiment. All animals were handled according to international guiding principles for biochemical research involving animals (C.I.O.M.S., 1985)

Hormonal treatment

Group A rats were treated with distilled water and served as control group. Group B rats were treated with 25 mg/ml of progesterone (Aldrich Chemical Company, Inc. Milwauke, Wisconsin, USA) at a dose of 2 mg/kg intramuscularly. Group C rats were treated with a preparation of estrogen (stilbesterol, 0.5 mg/ml, Aldrich Chemical Company, Inc. Milwauke, Wisconsin, USA) at a dose of 0.1 mg/kg orally. Group D rats were treated with combination of progesterone (25 mg/ml) at a dose of 2 mg/kg intramuscularly and stilbesterol (0.5 mg/ml) at a dose of 0.1 mg/kg orally. Administration of the drugs was done 21 days. Observations were made during the treatment and withdrawal period of 14 days for reversal effects.

Blood collection

Blood was collected from the rats every week at day 7, 14, 21 of administration of the hormones at and for two weeks after the withdrawal of the administration at day 28 and 35. The blood samples (0.2-0.3 ml) were taken from the dorsotarsal vein (tail) by cutting the tip after injection of Xylocaine-Pfizer Nigeria PLC (local anesthetic) to stop pain (Lane Peter, 1976). The blood samples were collected directly into four different commercially prepared EDTA vacutainer tubes for red blood cells (RBC) counts, white blood cells (WBC) counts, Haemoglobin (Hb) estimation and packed cell volume (PCV). A drop was also obtained on a clean glass slide and Leishman stain made for differential leucocytes counts (DLC).

Determination of blood parameters

Red blood cells (RBC), WBC and DLC were determined according to methods described by Coles (1980). Hemoglobin concentration (Hb) and PCV determination were according to Schalm *et al.* (1975).

Statistical analysis

All data obtained were expressed as mean and standard deviation (Mean ± SD). Differences within and between groups were tested for significance using analysis of variance (ANOVA). GraphPad InStat Version 3.0 Windows, USA, computer statistical software was used.

RESULTS

Table I shows effects of different hormonal treatments on RBC counts in female rats. Progesterone and stilbesterol showed changes on the RBC counts across the weeks (P<0.001), but after withdrawal of the treatment stilbesterol showed no change on the RBC (P>0.05). The combination of these two hormones did not affect the RBC counts (P>0.05), but the differences in RBC changes among the different hormonal treatments within weeks were significant (P<0.001).

Progesterone administration did not induce any change in total WBC counts (P>0.05), while stilbesterol and progesterone and stilbesterol combination showed changes (P<0.05) across the weeks (Table II).

Table III shows different hormonal treatments did not affect the differential leucocytes counts (DLC) across the weeks (P>0.05), but lymphocyte values showed changes within weeks due to progesterone and stilbesterol combination at the 1st and 3rd weeks (P<0.05).

Effects of different hormonal treatments on PCV indicate that progesterone alone and its combination with stilbesterol did not affect the PCV of the rats across the weeks, but stilbesterol showed changes up to 3rd week (P<0.05). There were difference in PCV changes among the different hormonal treatments within weeks (P<0.001) up to 3rd week and 1st and 2nd week (P< 0.05) after withdrawal of treatment (Table IV).

Stilbesterol treatment showed changes in Hb levels across the weeks, but the changes began to reverse after the withdrawal of the treatment. All the hormonal treatments showed changes within weeks, starting from the 1st week (Table V).

TABLE I: Effect of different hormonal treatments on red blood cell counts (RBC 10^6/L) in female rats (Mean ± SD)

Treatment group	Number of Rats (n)	Weeks of treatment 1+	2+	3+	Weeks post treatment 1+	2+
Control (Distilled water)	6	5.96 ± 0.06	5.97 ± 0.03	5.96 ± 0.07	5.90 ± 0.06	5.93 ± 0.06
Progesterone (2 mg/kg)	6	5.57 ± 0.38	4.88 ± 0.20	4.30 ± 0.22	4.57 ± 0.19	4.89 ± 0.16*
Stilbesterol (0.1 mg/kg)	6	3.76 ± 0.19	3.69 ± 0.01	3.18 ± 0.43*	3.74 ± 0.09	4.02 ± 0.05
Progesterone (2 mg/kg) + Stilbesterol (0.1 mg/kg)	6	4.83 ± 1.63	3.99 ± 0.02	3.35 ± 0.18	3.43 ± 0.16	3.60 ± 0.13
		P <0.05	P <0.05	P <0.05	P <0.05	P <0.05

+ Statistical difference within each week is shown under each column
*** P<0.05 across the weeks**

TABLE II: Effect of different hormonal treatments on total white blood cell counts (WBC 10^3/L) in female rats (Mean ± SD)

Treatment group	Number of Rats (n)	Weeks of treatment 1+	2+	3+	Weeks post treatment 1+	2+
Control (Distilled water)	6	6.04 ± 0.35	6.11 ± 0.13	6.14 ± 0.17	6.12 ± 0.19	6.01 ± 0.30
Progesterone (2 mg/kg)	6	5.90 ± 0.13	6.00 ± 0.53	5.65 ± 0.37	5.90 ± 0.42	6.09 ± 0.38
Stilbesterol (0.1 mg/kg)	6	6.07 ± 0.50	5.45 ± 0.26	4.78 ± 0.68	5.58 ± 0.32	5.85 ± 0.17*
Progesterone (2 mg/kg) + Stilbesterol (0.1 mg/kg)	6	6.19 ± 0.16	5.56 ± 0.29	5.81 ± 0.39	5.98 ± 0.33	6.09 ± 0.21*
		P>0.05	**P<0.05**	**P<0.05**	**P>0.05**	**P>0.05**

+ Statistical difference within each week is shown under each column
***P<0.05 across the weeks**

TABLE III: Effect of different hormonal treatments on differential leucocyte counts (DLC %) in female rats (Mean ± SD) *

Leucocytes	Treatment groups (6 rats in each treatment)	Weeks of treatment 1+	2+	3+	Weeks post-treatment 1+	2+
Neutrophils	Control	10.90 ± 0.60	11.10 ± 0.90	11.30 ± 0.30	10.90 ± 0.10	10.60 ± 0.57
	Progesterone (2mg/kg)	18.50 ± 1.87	20.70 ± 1.68	20.30 ± 2.03	20.00 ± 2.74	20.70 ± 2.16
	Stilbesterol (0.1mg/kg)	19.50 ± 1.87	18.50 ± 1.87	21.30 ± 2.39	20.70 ± 1.97	20.30 ± 1.16
	Progesterone (2mg/kg) + Stilbesterol(0.1mg/kg)	20.20 ± 1.18 P<0.05	21.30 ± 1.05 P<0.05	20.00 ± 1.61 P<0.05	21.20 ± 1.96 P<0.05	20.30 ± 1.08 P<0.05
Lymphocytes	Control	72.60 ± 1.27	73.01 ± 1.24	72.80 ± 1.46	72.90 ± 1.73	73.04 ± 1.57
	Progesterone (2mg/kg)	60.30 ± 2.42	58.00 ± 2.61	55.00 ± 3.22	56.50 ± 3.33	57.80 ± 3.06
	Stilbesterol (0.1mg/kg)	67.80 ± 3.43	62.80 ± 4.26	56.50 ± 4.59	58.20 ± 3.06	66.80 ± 3.17
	Progesterone (2mg/kg) + Stilbesterol(0.1mg/kg)	67.70 ± 5.65 P<0.05	66.50 ± 5.36 P>0.05	64.00 ± 5.18 P<0.05	69.00 ± 5.33 P<0.05	71.50 ± 3.51 P<0.05
Monocytes	Control	03.29 ± 0.76	03.20 ± 1.10	03.31 ± 0.80	03.15 ± 1.10	02.81 ± 0.81
	Progesterone (2mg/kg)	04.17 ± 1.86	04.00 ± 1.14	04.67 ± 1.73	05.00 ± 1.50	04.67 ± 1.51
	Stilbesterol (0.1mg/kg)	03.06 ± 0.17	03.00 ± 0.09	03.00 ± 0.59	02.80 ± 0.94	02.00 ± 0.00
	Progesterone (2mg/kg) + Stilbesterol(0.1mg/kg)	04.14 ± 0.56 P>0.05	04.30 ± 1.01 P>0.05	04.50 ± 1.00 P>0.05	04.41 ± 0.41 P>0.05	03.83 ± 1.01 P>0.05
Eosinophils	Control	01.57 ± 0.53	01.57 ± 1.10	01.55 ± 1.00	01.54 ± 1.07	01.55 ± 1.15
	Progesterone (2mg/kg)	01.60 ± 1.26	01.63 ± 1.34	01.60 ± 1.41	01.67 ± 1.37	01.83 ± 1.17
	Stilbesterol (0.1mg/kg)	01.66 ± 0.75	01.20 ± 0.59	01.33 ± 0.75	01.33 ± 0.93	01.06 ± 0.98
	Progesterone (2mg/kg) + Stilbesterol(0.1mg/kg)	01.63 ± 0.51 P>0.05	01.00 ± 0.67 P>0.05	01.67 ± 0.51 P>0.05	01.00 ± 0.03 P>0.05	01.33 ± 0.03 P>0.05

+ Statistical difference within each week is shown under each column for each parameter
**** P>0.05 across the week***

TABLE IV: Effect of different hormonal treatments on packed cell volume (%) in female rats (Mean ± SD)

Treatment group	Number of Rats (n)	Weeks of treatment 1+	2+	3+	Weeks post treatment 1+	2+
Control (Distilled water)	6	46.10 ± 0.69	45.60 ± 1.35	45.70 ± 1.01	46.10 ± 0.99	45.90 ± 1.08
Progesterone (2mg/kg)	6	44.00 ± 1.28	44.10 ± 1.05	44.20 ± 0.75	45.20 ± 0.75	45.20 ± 0.75
Stilbesterol (0.1mg/kg)	6	42.00 ± 0.07	42.30 ± 0.05	41.70 ± 0.03*	42.80 ± 1.17	43.10 ± 0.75
Progesterone (2mg/kg) + Stilbesterol (0.1mg/kg)	6	45.00 ± 0.90	45.00 ± 0.89	44.80 ± 0.47	44.50 ± 1.05	44.80 ± 0.98
		P<0.05	P<0.05	P<0.05	P<0.05	P<0.05

+ Statistical difference within each week is shown under each column
****P<0.05 on 3rd week***

TABLE V: Effect of different hormonal treatments on hemoglobin (Hb gm/dl) concentration in female rats (Mean ± SD)

Treatment group	Number of Rats (n)	Weeks of treatment 1+	2+	3+	Weeks post treatment 1+	2+
Control (Distilled water)	6	17.70 ± 0.56	17.70 ± 0.58	17.60 ± 0.33	17.70 ± 0.42	17.80 ± 0.24
Progesterone (2mg/kg)	6	14.53 ± 1.07	14.55 ± 0.81	14.22 ± 0.45	14.42 ± 0.63	15.60 ± 0.42
Stilbesterol (0.1mg/kg)	6	13.43 ± 0.83	12.58 ± 0.39	12.10 ± 0.62*	14.45 ± 0.83	14.92 ± 0.66
Progesterone (2mg/kg) + Stilbesterol (0.1mg/kg)	6	13.10 ± 0.90	13.10 ± 0.45	13.30 ± 0.90*	13.30 ± 0.60	13.70 ± 0.48
		P<0.05	P<0.05	P<0.05	P<0.05	P<0.05

+ Statistical difference within each week is shown under each column
*** P<0.05 across weeks**

DISCUSSION

Progesterone and stilbesterol combination has no effect on the RBC values while the effect of stilbesterol was apparently reversible as evidenced in the increase in RBC values. However combined treatment with all the hormones reduced the RBC values each week. This clearly showed that reproductive hormones have tendency to reduce RBC values.

It was observed that erythrocytes declined during last part of gestation (Jain, 1993), which was attributed to haemodilution effect resulting from an increased plasma volume. Since progesterone and stilbesterol have similar effects, it therefore implies that these hormones increase plasma volume. Stilbesterol decreased slightly the PCV on the third week indicating that prolonged treatment with this drug could cause a decrease in PCV. This was also confirmed by the decreased Hb concentration up to the third week period by the same drug. McDonald (1980) observed that large doses of an estrogen administration to a dog caused anaemia in various forms. In this work the effect was reversible, perhaps due to the fact that the normal dosages were administered to the rats. Progesterone did not affect the total WBC values while stilbesterol alone and with combination with progesterone showed decreasing values. This was due to the decrease in lymphocytes values within weeks. Jain (1993) clearly showed that treatment of dogs with glucocorsteroids induce lymphopenia and eosinopenia. Therefore, reproductive steroid hormones have similar effect.

CONCLUSION

The results of the three different hormonal treatments could not establish any severe untoward effects on blood, therefore it was concluded that progesterone, stilbesterol or their combinations (Progestrone and stilbesterol) could be employed for the usage of these agents for up to two weeks in rats.

REFERENCES

BLOOD, D.C. and STUDDERT, V.P. (1999): Saunders Comprehensive Veterinary Dictionary. 2nd Edn. W.B. Saunders Company Philadelphia, USA.

COLE, E.H. (1980): Veterinary Clinical Pathology. 3rd Ed. W.B. Saunder, London; 83-89.

COUNCIL FOR INTERNATIONAL ORGANIZATIONS OF MEDICAL SCIENCES (C.I.O.M.S, 1985): International Guiding Principles for Biomedical Research Involving Animals, c/o WHO 1211, Geneva 27, Switzerland.

GANONG, W.F. (1985): Review of Medical Physiology. 12th Ed. Lange medical publications, Los Altos, California, 94023. 371.

HAFEZ, E.S.E. (1980): Reproduction in Farm Animals. 3rd Ed. Bailliere Tindall, London; 248-568.

JAIN, N.C. (1993): Essentials of Veterinary Hematology. Lea and Febiger, Philadelphia; 19-53.

KANEKO, J.J. (1980): Clinical Biochemistry of Domestic Animals. 3rd Ed. Academy Press, New York; 63-70.

LABORED, J.B., WALL, K.S., BOLON, B., KUMPE, T.S., PATTON., R., ZHENG, Q., KODELL, R.
and YOUNG, J.F. (1999): Hematology and serum chemistry parameters. Inter. J. Lab. Anim. Sci. and Welfare, **33**: 46-49.

LANE PETER, W. (1976): The Laboratory Rat. In: The UFAW Handbook on the Care and Management of Laboratory Animals. 5th Ed. Churchill Livingstone; 193-217.

LAURENCE, D.R. BENNETT, P.N. and BROWN, M.J. (1997): Clinical Pharmacology. 8th Ed. Churchill Livingstone Edinburgh.

McDONALD, L.E. (1980): Veterinary Endocrinology and Reproduction. 3rd Ed. Lea and Febiger, Philadelphia, 201-497.

SCHALM, O.W., JAIN, N.C. and CARROLL, E.J. (1975): Veterinary Hematology. 3rd Ed. Lea and Febiger, Philadelphia; 54-470.

SPROTT, L.R., WILTBANK, J.N., SONGSTER, W.N. and WEBEL, S. (1984): Oestrous and Ovulation In Beef Cows Following Use Of Progesterone-Releasing Devices, Progesterone And Estradiol Valerate. Therio., **21**: 349-356.

SWENSON, M.J. (1997): Duke's Physiology of Domestic Animals. 9th Ed. Cornell University Press Ltd, London.

TENNANT, B. (2000): Small Animal Formulary. 3rd Ed. British Small Animal Veterinary Association
Publisher.

WILLIAMS, L.C. and STANCEL, M.G. (1996): Estrogens and Progestins. In: Goodman and Gilman's
Pharmacological Basis of Therapeutics. 9th Ed. McGraw Hill; 1411-1437.

WOERPEL, R.W and ROSSKOPT, W.J.R. (1984): Avian Hematology. Veterinary Clinics North Am. Small Anim. Pract.., **14**: 249-285.