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A Study on the Effects of Prolactin and Its Abnormalities on Semen Parameters of Male White Rats

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Abstract**—**Male factor infertility due to endocrine disturbances such as abnormalities in prolactin levels are encountered in a significant proportion. This case control study was carried out to determine the effects of prolactin on the male reproductive tract, using 200 male white rats. The rats were maintained as the control group (G1), hypoprolactinaemic group (G2), 3 hyperprolactinaemic groups induced using oral largactil (G3), low dose fluphenazine (G4) and high dose fluphenazine (G5). After 100 days, rats were subjected to serum prolactin (PRL) level measurements and for basic seminal fluid analysis (BSA). The difference between serum PRL concentrations of rats in G2, G3, G4 and G5 as compared to the control group were highly significant by Student's t-test ($p<0.001$). There were statistically significant differences in seminal fluid characteristics of rats with induced prolactin abnormalities when compared with those of control group (p value <0.05), effects were more marked as the PRL levels rise.

Keywords**—**Male factor infertility, Prolactin, Seminal fluid analysis, animal studies.

I. Introduction

INFERTILITY is defined as the failure of a couple to conceive after at least 12 months of unprotected intercourse [1]. Infertility in a couple can be described as male factors, female factors or issues in both partners, while the male reproductive capacity was found to be deficient in more than 50% of infertile couples [1]. Primary male factor infertility is when the man has never impregnated a woman whereas secondary male factor infertility is irrespective of the outcome of the pregnancy; man has impregnated a woman not necessarily the current partner [1].

It is publicized that infertility affects 10-15% of the world's population with two million new couples with infertility per year leading to immense psychosocial and personality disputes in most cases [2]-[5]. This implies the burden of the problem and the need to broaden the understanding of the pathophysiology of infertility in order to develop efficient intervention and treatment.

The variety of causes of male factor infertility can be classified arbitrarily into pre testicular, testicular and post testicular causes [6]. Other important causes are idiopathic spermatozoan abnormalities (40%), infection of the male accessory reproductive glands namely the prostate, seminal vesicles, iatrogenic insults to the testicles such as testicular irradiation, antimitotic medication, androgen therapy, use of anabolic steroids and antihypertensive medication, antibiotics and antipsychotic medication and autoimmune causes [7]. A link between male factor infertility and low sperm count has been described but the exact mechanism of which is unknown [7].

The pre testicular causes account for up to 10% of male factor infertility and mainly include hormonal factors. This signifies the role of follicular stimulating hormone (FSH), leutinizing hormone (LH) and prolactin (PRL). Elevated levels of PRL have been shown to result in drastic inhibition of sperm production and its quality [7]. The hypothalamo-pituitary hypofunction contributes to about 1% of cases [2].

Prolactin abnormality can result from trauma, tumours in the pituitary gland, malfunction of the pituitary gland, chronic liver disease, thyroid dysfunction and genetic and chromosomal defects such as the Klinefelter syndrome [8].

Hyperprolactinaemia has been known to cause male factor infertility resulting in decreased libido and impotence. Treatment with bromocriptine to suppress the elevated PRL level has been very successful in reversing the condition and achieving a pregnancy. The role of PRL on the male reproductive system has been shown in only a few studies and the exact role in male factor infertility remains unclear. Hence this study was carried out to fill this void to a certain extent.

II. Methodology

The study was a case control type and was carried out in the animal house of the Faculty of Medicine, University of Ruhuna, Sri Lanka. The objective of this study was to determine the effects of prolactin on the male reproductive tract in otherwise normal rats and thereby to determine whether abnormality of PRL levels is a contributory factor to infertility in males. Ethical consent for this study was obtained from Ethical Review Committee, Faculty of Medicine, University of Ruhuna, Sri Lanka.

Male white rats of the Wistar strain were obtained from the Medical Research Institute, Borella, Colombo and also from breeding carried out at the animal house, Faculty of Medicine, University of Ruhuna. 10 ± 2 week old rats weighing 200 ± 10 g were maintained at a room temperature of 28 ± 4 degrees Celsius and fed with animal feed made of pellets obtained from Messers Moosajees Ltd, Colombo, for a period of 2 weeks. The quantity of feed and volume of water consumed by the rats was measured and recorded on a daily basis. 200 rats were selected and grouped from G1 to G6. 30 rats were included in each group and maintained in separately labelled

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cages. These groups were subjected to the following procedures.

Group 1 (G1) – The 30 rats in this group were maintained under normal conditions at room temperature, in order to obtain a control value for the normal serum PRL level of rats.

Group 2 (G2) – The 30 rats in this group were fed with oral bromocriptine 4.65 mg per kg body weight per day in a divided dose twice a day, dissolved in 2ml of distilled water. Another lot of 30 male white rats, age and weight matched, were fed with an equal volume of distilled water and served as a control. A daily chart of food intake, drugs intake, and fluid intake and body weights was maintained.

Group 3 (G3) – The 30 rats in this group were fed with oral largactil 10mg per kg body weight per day in a divided dose given twice a day, dissolved in 2ml of distilled water. Another 30 male white rats, age and weight matched, were treated exactly as described in the control of the G2.

Group 4 (G4) – The 30 rats in this group were treated with daily subcutaneous injections of fluphenazine in sesame oil in a dose of 0.42 mg per kg body weight per day in a single dose given in the morning. A daily chart of food intake, drugs intake, and fluid intake and body weights was maintained.

Group 5 (G5) – The 30 rats in this group were treated identically as with group 4 except that the dose of fluphenazine was increased to 0.84 mg per kg body weight per day.

Group 6 (G6) – The 30 rats in this group served as control for the rats treated with subcutaneous injections of fluphenazine in groups 4 and 5. They were given an equal volume of sesame oil as injections.

Hyperprolactinaemia was induced in the rats using oral largactil and subcutaneous injections of fluphenazine. Hypoprolactinaemia was induced in the rats using bromocriptine. The dosages of drugs used in the induction of experimental variations in serum PRL concentrations were obtained from the British National Formulary. The oral drugs were dissolved in measured volumes of distilled water and administered to the rats using a feeding tube. The feeding was done over a period of 100 days.

A. Assessment of Prolactin Levels in Rat Serum

At the end of 100 days 20 rats from each group were subjected to serum PRL assays by drawing 2ml of blood using sterile plastic disposable syringes under aseptic conditions. The PRL concentrations of rats were measured using the immulite random access chemiluminescent immunoassay method machine. The machine used in the study has sensitivity of 0.5ng/ml for PRL measurements. Many samples of rat serum would have PRL concentrations below this amount and would therefore not be read by the machine. In order to overcome this difficulty the procedure adapted was modified as follows.

100µl of rat serum was mixed with an equal volume of serum obtained from a male human volunteer with previously estimated PRL concentration. The blood samples from the donor were obtained and the 4 samples mixed. The mean value for serum PRL concentration of the donor sample obtained from 4 assays done on different days was 7.3ng/ml. Following the assays the concentration of PRL in the rat serum was calculated by difference from the value for the human serum alone.

A strict parallelism test involving recovery of added known quantities of rat serum PRL was not possible in this study due to the unavailability of the necessary rat hormone in pure form. To compensate for this, studies were carried out utilizing different volumes of rat serum (spiking recovery test).

B. Analysis of Epididymal Semen of Experimental Rats

Epididymal semen was analysed in order to study the effects of PRL abnormalities on semen quality in rats. Four rats from each of the experimental groups were subjected to semen analysis. Semen was collected from the cauda of the epididymis of each rat after dissection. Pieces of 1cm length of the distal cauda of the epididymis were identified and sectioned. Each piece was blotted on blotting paper. Thereafter each piece was introduced into a sterile plastic bottle containing 5ml of Earle's balance salt solution (EBSS medium), and minced well. Each sample was left for 15 minutes. 3ml of the supernatant was collected using a clean sterile glass pipette and centrifuged for 10 minutes at a speed of 1750 revolutions per minute. Thereafter 0.5ml of the pellet from each centrifuged sample was collected and subjected to semen analysis. Following aspects were analyzed.

Macroscopic analysis - colour, consistency, pH value, volume, turbidity, coagulation, odour

Microscopic analysis – Motility, presence of other cells, agglutination and aggregation, viability, concentration (using the improved Neuber haemocytometer), morphological abnormalities in the head, mid piece and tails of the spermatozoa

III. Results

A. Results of the Serum Prolactin Studies of Rats

As shown in Table I, the difference between the obtained values and corrected values for the serum PRL concentrations in the control group of rats was found to be highly significant by Student's t-test ($p<0.001$).

The differences between the experimentally obtained values as well as the corrected values for the serum PRL concentrations of the rats treated with bromocriptine as compared to the control group are highly significant by Student's t-test ($p<0.001$). (Table II).

The differences between the experimentally obtained values as well as the corrected values for the serum PRL concentrations of the rats treated with largactil as compared to the control group are highly significant by Student's t-test ($p<0.001$). (Table III)

TABLE I
RESULTS OF PRL CONCENTRATIONS OF CONTROL GROUP OF RATS

Date of Birth	Body weight (g)	Average food intake per day(g)	Average fluid intake per day (ml)	Dosage of Drug per day distilled water (ml)	Duration of Drug therapy (days)	Total serum PRL conc. (Obtained value (ng/ml)	Serum PRL conc. (Corrected value) (ng/ml)
16.4.2002	300	38	28	2	100 days	9.8	2.5
16.4.2002	300	40	30	2	100 days	10.6	3.3
16.4.2002	285	40	33	2	100 days	8.2	0.9
16.4.2002	280	44	35	2	100 days	10.6	3.3
16.4.2002	285	45	28	2	100 days	10.2	2.9
16.4.2002	275	38	30	2	100 days	8.2	0.9
16.4.2002	290	44	33	2	100 days	10.8	3.5
16.4.2002	295	45	30	2	100 days	9.8	2.5
16.4.2002	285	40	29	2	100 days	11.6	4.3
16.4.2002	280	38	30	2	100 days	11.6	4.3
16.4.2002	290	35	29	2	100 days	10.8	3.5
16.4.2002	295	37	33	2	100 days	10.4	3.1
16.4.2002	285	40	32	2	100 days	9.8	2.5
16.4.2002	290	35	34	2	100 days	10.2	2.9
16.4.2002	285	37	29	2	100 days	11.4	4.1
16.4.2002	280	45	30	2	100 days	11.8	4.5
16.4.2002	285	39	29	2	100 days	10.6	3.3
16.4.2002	300	35	32	2	100 days	9.8	2.5
16.4.2002	275	40	31	2	100 days	9.4	2.1
16.4.2002	280	39	33	2	100 days	11.2	3.9
Mean	**287**	**39.7**	**30.9**	-	-	**10.34**	**3.04**
SD	**7.8472**	**3.3419**	**2.0749**	-	-	**1.0013**	**1.0013**

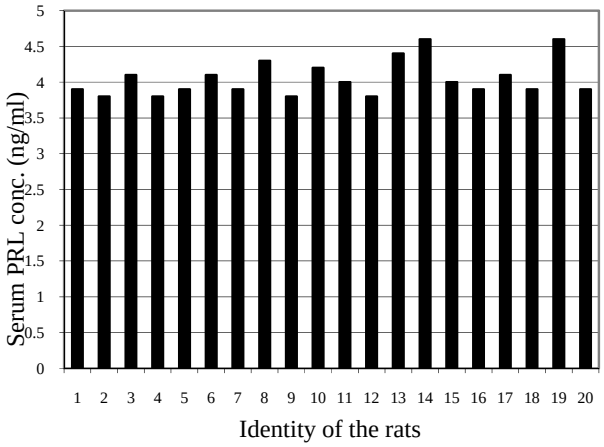

Fig. 1 (a) Results of obtained (total) values of PRL concentrations of control group of rats, PRL – prolactin

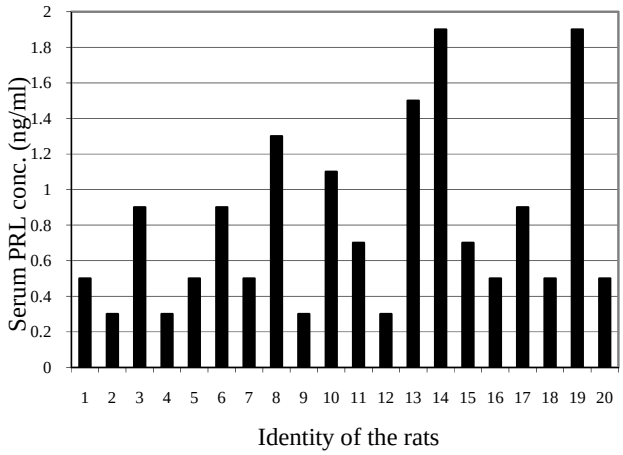

Fig. 1 (b) Results of corrected values of PRL concentrations of control group of rats, PRL – prolactin

TABLE II
RESULTS OF PRL CONCENTRATIONS OF RATS TREATED WITH BROMOCRIPTINE

Date of Birth	Body Weight (g)	Average food intake per day (g)	Average fluid intake per day (ml)	Dosage of drug (mg/kg body weight per day)	Duration of Drug therapy (days)	Total serum PRL conc. (Obtained value) (ng/ml)	Serum PRL conc. (Corrected value) (ng/ml)
01.04.2002	292	35	30	0.083	100 days	7.8	0.5
01.04.2002	276	40	28	0.083	100 days	7.6	0.3
01.04.2002	281	40	28	0.083	100 days	8.2	0.9
01.04.2002	277	42	32	0.083	100 days	7.6	0.3
01.04.2002	280	38	25	0.083	100 days	7.8	0.5
01.04.2002	285	45	30	0.083	100 days	8.2	0.9
01.04.2002	272	37	28	0.083	100 days	7.8	0.5
01.04.2002	300	40	30	0.083	100 days	8.6	1.3
01.04.2002	278	38	25	0.083	100 days	7.6	0.3
01.04.2002	270	35	25	0.083	100 days	8.4	1.1
01.04.2002	275	40	31	0.083	100 days	8.0	0.7
01.04.2002	290	40	27	0.083	100 days	7.6	0.3
01.04.2002	285	38	31	0.083	100 days	8.8	1.5
01.04.2002	295	39	25	0.083	100 days	9.2	1.9
01.04.2002	270	40	28	0.083	100 days	8.0	0.7
01.04.2002	285	44	27	0.083	100 days	7.8	0.5
01.04.2002	280	38	29	0.083	100 days	8.2	0.9
01.04.2002	288	35	30	0.083	100 days	7.8	0.5
01.04.2002	285	38	29	0.083	100 days	9.2	1.9
01.04.2002	290	40	25	0.083	100 days	7.8	0.5
Mean	**282.7**	**39.1**	**28.15**	**-**	**-**	**8.1**	**0.8**
SD	**8.2914**	**2.6537**	**2.2775**	**-**	**-**	**0.5047**	**0.5047**

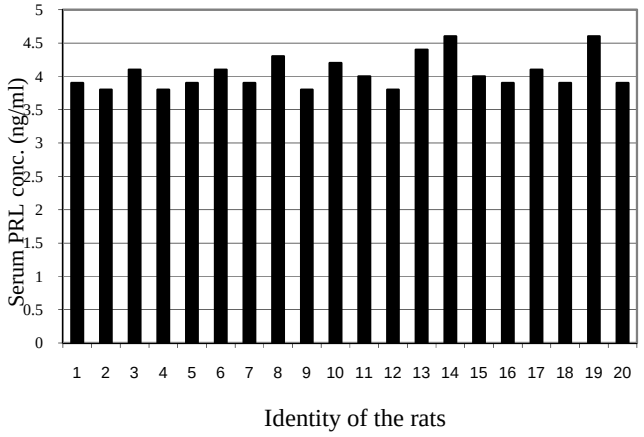

Fig. 2 (a) Results obtained (total) values of PRL concentrations of rats treated with bromocriptine, PRL – prolactin

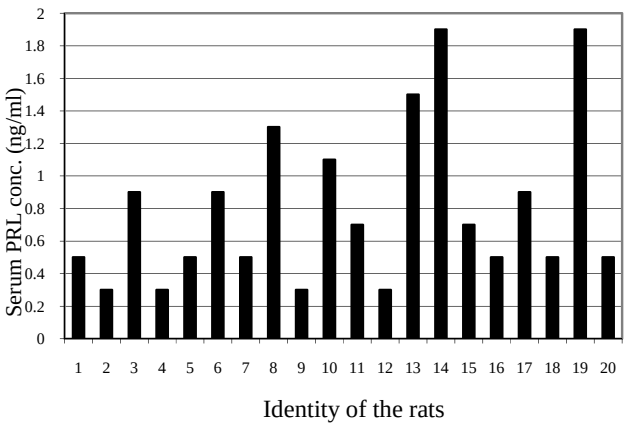

Fig. 2 (b) Results of corrected values of PRL concentrations of rats treated with bromocriptine, PRL - prolactin

TABLE III
RESULTS OF PRL CONCENTRATIONS OF RATS TREATED WITH LARGACTIL

Date of Birth	Body weight (g)	Average food intake per day (g)	Average fluid intake per day (ml)	Dosage of Drug (mg/kg body weight per day)	Duration of Drug therapy (days)	Total serum PRL conc. (Obtained value) (ng/ml)	Serum PRL conc. (Corrected value) (ng/ml)
02.04.2002	285	37	33	2	100 days	13.2	5.9
02.04.2002	290	40	29	2	100 days	11.8	4.5
02.04.2002	295	38	27	2	100 days	12.2	4.9
02.04.2002	285	41	26	2	100 days	13.4	6.1
02.04.2002	290	39	25	2	100 days	15.8	8.5
02.04.2002	275	38	30	2	100 days	12.2	4.9
02.04.2002	300	41	35	2	100 days	13.0	5.7
02.04.2002	285	40	28	2	100 days	15.2	7.9
02.04.2002	290	42	27	2	100 days	12.6	5.3
02.04.2002	295	38	28	2	100 days	15.4	8.1
02.04.2002	285	41	25	2	100 days	12.0	4.7
02.04.2002	275	37	29	2	100 days	12.8	5.5
02.04.2002	300	36	30	2	100 days	12.6	5.3
02.04.2002	300	37	31	2	100 days	12.8	5.5
02.04.2002	295	44	28	2	100 days	12.0	4.7
02.04.2002	270	32	26	2	100 days	14.0	6.7
02.04.2002	272	45	28	2	100 days	13.4	6.1
02.04.2002	285	39	27	2	100 days	16.4	9.1
02.04.2002	280	37	28	2	100 days	13.0	5.7
02.04.2002	300	35	30	2	100 days	14.2	6.9
Mean	**287.6**	**38.85**	**28.5**	**-**	**-**	**13.4**	**6.1**
SD	**9.6212**	**3.0483**	**2.5236**	**-**	**-**	**1.3518**	**1.3518**

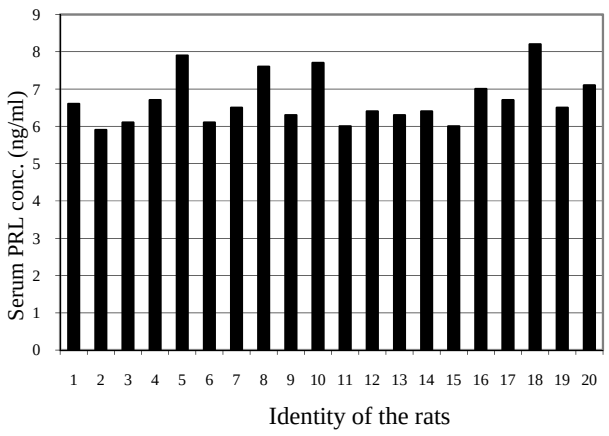

Fig. 3 (a) Results of obtained (total) values of PRL concentrations of rats treated with largactil, PRL - prolactin

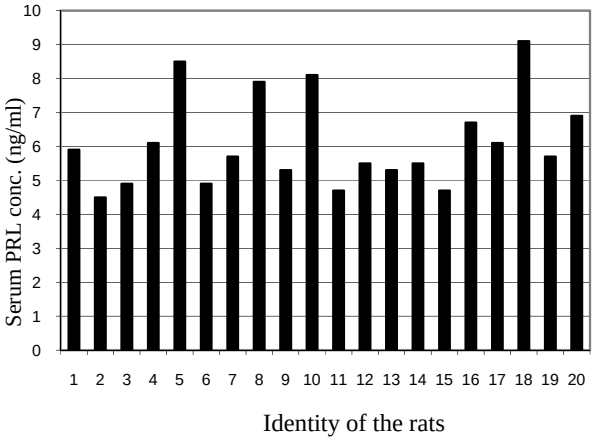

Fig. 3 (b) Results of corrected values of PRL concentrations of rats treated with largactil, PRL - prolactin

The differences between the experimentally obtained values as well as the corrected values for the serum PRL concentrations of the rats treated with a low dose of fluphenazine as compared to the control group are highly significant by Student's t-test ($p<0.001$). (Table IV)

TABLE IV
RESULTS OF PRL CONCENTRATIONS OF RATS TREATED WITH A LOW DOSE OF FLUPHENAZINE

Date of birth	Body weight (g)	Average food intake per day (g)	Average fluid intake per day (ml)	Dosage of Drug (mg/kg body weight per day)	Duration of Drug therapy (days)	Total serum PRL conc. (Obtained value) (ng/ml)	Serum PRL conc. (Corrected value) (ng/ml)
02.04.2002	270	35	25	0.42	100 days	16.2	8.9
02.04.2002	282	37	27	0.42	100 days	24.0	16.7
02.04.2002	275	38	23	0.42	100 days	23.2	15.9
02.04.2002	290	39	21	0.42	100 days	21.4	14.1
02.04.2002	285	40	26	0.42	100 days	19.4	12.1
02.04.2002	280	42	25	0.42	100 days	20.6	13.3
02.04.2002	277	38	25	0.42	100 days	20.0	12.7
02.04.2002	275	37	26	0.42	100 days	23.2	15.9
02.04.2002	290	39	27	0.42	100 days	16.2	8.9
02.04.2002	300	42	28	0.42	100 days	18.4	11.1
02.04.2002	285	40	27	0.42	100 days	17.2	9.9
02.04.2002	290	38	25	0.42	100 days	17.4	10.1
02.04.2002	300	41	23	0.42	100 days	18.4	11.1
02.04.2002	295	39	25	0.42	100 days	23.4	16.1
02.04.2002	285	40	25	0.42	100 days	16.2	8.9
02.04.2002	287	46	26	0.42	100 days	16.2	8.9
02.04.2002	295	43	28	0.42	100 days	18.4	11.1
02.04.2002	293	37	29	0.42	100 days	19.4	12.1
02.04.2002	285	40	27	0.42	100 days	18.6	11.3
02.04.2002	280	38	26	0.42	100 days	18.4	11.1
Mean	**285.95**	**39.45**	**25.7**	**-**	**-**	**19.31**	**12.005**
SD	**8.3444**	**2.5021**	**1.8945**	**-**	**-**	**2.5764**	**2.5804**

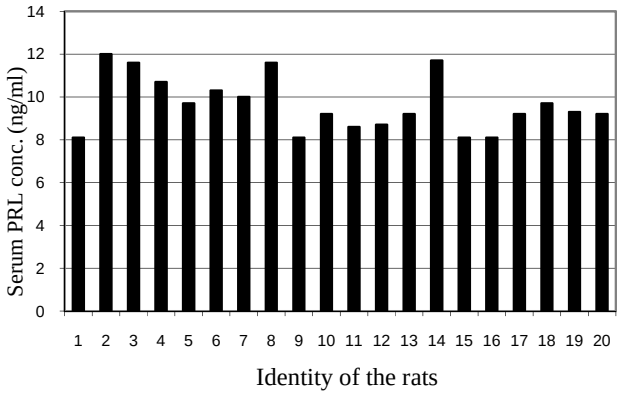

Fig. 4 (a) Results of obtained (total) values of PRL concentrations of rats treated with a low dose of fluphenazine, PRL – prolactin

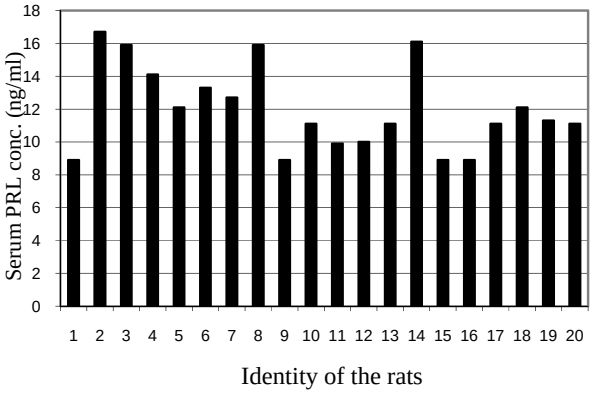

Fig. 4 (b) Results of corrected values of PRL concentrations of rats treated with a low dose of fluphenazine, PRL – prolactin

The differences between the experimentally obtained values as well as the corrected values for the serum PRL concentrations of the rats treated with a high dose of fluphenazine as compared to the control group are highly significant by Student's t-test ($p<0.001$). (Table V)

TABLE V
RESULTS OF PRL CONCENTRATIONS OF RATS TREATED WITH A HIGH DOSE OF FLUPHENAZINE

Date of Birth	Body Weight (g)	Average food intake per day (g)	Average fluid intake per day (ml)	Dosage of Drug (mg/kg body weight per day)	Duration of drug therapy (days)	Total serum PRL conc. (Obtained value) (ng/ml)	Serum PRL conc. (Corrected value) (ng/ml)
09.04.2002	287	40	26	0.84	100 days	24.8	17.5
09.04.2002	290	41	23	0.84	100 days	26.0	18.7
09.04.2002	293	39	27	0.84	100 days	19.4	12.1
09.04.2002	295	40	27	0.84	100 days	18.6	11.3
09.04.2002	280	38	25	0.84	100 days	21.2	13.9
09.04.2002	270	40	26	0.84	100 days	25.8	18.5
09.04.2002	275	37	29	0.84	100 days	18.2	10.9
09.04.2002	300	39	27	0.84	100 days	25.2	17.9
09.04.2002	290	38	25	0.84	100 days	25.8	18.5
09.04.2002	295	40	31	0.84	100 days	19.6	12.3
09.04.2002	300	39	26	0.84	100 days	18.76	11.46
09.04.2002	295	38	25	0.84	100 days	20.8	13.5
09.04.2002	285	40	25	0.84	100 days	19.2	11.9
09.04.2002	290	40	24	0.84	100 days	25.8	18.5
09.04.2002	275	40	27	0.84	100 days	21.4	14.1
09.04.2002	285	45	27	0.84	100 days	22.4	15.1
09.04.2002	280	38	26	0.84	100 days	22.2	14.9
09.04.2002	295	40	31	0.84	100 days	25.8	18.5
09.04.2002	275	49	25	0.84	100 days	24.8	17.5
09.04.2002	280	39	31	0.84	100 days	19.2	11.9
Mean	**286.75**	**40**	**26.65**	**-**	**-**	**22.248**	**14.948**
SD	**8.9788**	**2.6754**	**2.2775**	**-**	**-**	**2.9539**	**2.9539**

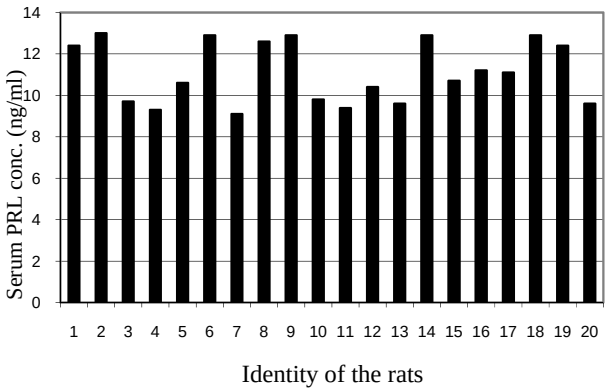

Fig. 5 (a) Results of obtained (total) values of PRL concentrations of rats treated with a high dose of fluphenazine, PRL – prolactin

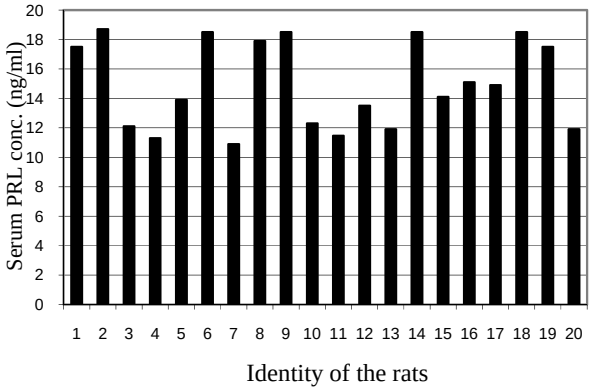

Fig. 5 (b) Results of obtained (total) values of PRL concentrations of rats treated with a low dose of fluphenazine, PRL – prolactin

B. Results of Basic Seminal Fluid Analysis

On comparison of the BSA figures of the group of rats treated with bromocriptine with those of the control group by applying Student's t-test: (Tables VI and VII)

The differences in the percentages of non-progressively motile forms, the motile forms, those with head abnormalities and tail abnormalities were statistically significant ($p < 0.05$) and the difference in the percentages with normal morphology was also significant at 95% probability ($p<0.05$). But the difference in the percentage of progressively motile forms was not significant at a p value of >0.05 nor the differences in the cells per field after dilution and sperm concentrations ($p > 0.05$).

On comparison of the BSA figures of the group of rats treated with largactil with those of the control group by applying Student's t-test: (Tables VI and VIII)

The differences in the percentages of non-progressively motile forms, the motile forms, those with head abnormalities and tail abnormalities were significant at 95% probability with a p value of <0.05 as well as the differences in the cell counts per field after dilution and sperm concentrations ($p<0.05$). But the difference in the percentage of progressively motile forms was not significant at a p value of >0.05. The difference in the percentage with normal morphology was also statistically not significant ($p > 0.05$).

On comparison of the BSA figures of the group of rats treated with a low dose of fluphenazine with those of the control group by applying t-test: (Tables VI and IX)

The differences in the percentages of non-progressively motile forms, the motile forms, and of those with head abnormalities were significant at 95% probability with a p value of <0.05.

The differences in the cell counts per field after dilution and sperm concentrations were also significant at 95% probability ($p<0.05$). The difference in the percentage of progressively motile forms was not significant at a p value of >0.05. The differences in the percentages with tail abnormalities and normal morphology were statistically not significant at a p value of >0.05.

On comparison of the BSA figures of the group of rats treated with a high dose of fluphenazine with those of the control group by applying Student's t-test: (Tables VI and X)

The differences in the percentages of non-progressively motile forms and the motile forms were significant at 95% probability with a p value of <0.05; the difference in the percentage with tail abnormalities, and the differences in the cell counts per field after dilution and the sperm concentration were also significant at 95% probability ($p<0.05$). The difference in the percentage of rats with head abnormalities was not statistically significant at >0.05. The difference in the percentage of progressively motile forms was not significant ($p>0.05$). The difference in the percentage with normal morphology were statistically not significant at a p value of >0.05.

TABLE VI
RESULTS OF BSA OF RATS IN CONTROL GROUP

Identity of the Rat	MOTILITY			MORPHOLOGY			Cells per Field after Dilution (200x)	CONCENTRATION (millions per ml) (N.B. approximately)
	Progressively Motile %	Non-prog Motile %	% Motility	% Head Abnormalities	% Tail Abnormalities	% Normal Morphology		
C1	7	51	58	12	9	79	95.33	17.159
C2	3	48	51	16	21	63	109.6	19.728
C3	1	39	40	11	13	76	112.6	16.02
C4	9	49	58	8	11	81	105	20.279
Mean	**5**	**46.75**	**51.75**	**11.75**	**13.5**	**75.75**	**105.6325**	**18.2965**
SD	**3.6514**	**5.3151**	**8.5**	**3.304**	**5.2599**	**8.0984**	**7.5461**	**2.0376**

TABLE VII
RESULTS OF BSA OF RATS TREATED WITH BROMOCRIPTINE

Identity of the Rat	MOTILITY			MORPHOLOGY			Cells per Field after Dilution (200x)	CONCENTRATION (millions per ml) (N.B. approximately)
	Progressively Motile %	Non-prog Motile %	% Motility	% Head Abnormalities	% Tail Abnormalities	% Normal Morphology		
B1	2	23	25	16	22	62	59	18.9
B2	1	36	37	13	23	64	89	10.62
B3	1	27	28	27	16	57	92	16.56
B4	3	29	32	21	22	53	107	19.26
Mean	**1.75**	**28.75**	**30.5**	**19.25**	**20.75**	**59**	**86.75**	**16.335**
SD	**0.9574**	**5.4391**	**5.1961**	**6.1305**	**3.2016**	**4.9666**	**20.106**	**3.9936**

TABLE VIII
RESULTS OF BSA OF RATS TREATED WITH LARGACTIL

Identity of the Rat	MOTILITY			MORPHOLOGY			Cells per Field after Dilution (200x)	CONCENTRATION (millions per ml) (N.B. approximately)
	Progressively Motile %	Non-prog Motile %	% Motility	% Head Abnormalities	% Tail Abnormalities	% Normal Morphology		
L1	0	23	23	21	28	51	50.5	9.09
L2	2	31	33	28	23	69	71.5	12.87
L3	1	26	27	27	21	52	87	15.66
L4	1	31	32	21	26	53	79	14.22
Mean	**1**	**27.75**	**28.75**	**24.25**	**24.5**	**56.25**	**72**	**12.96**
SD	**0.8165**	**3.9476**	**4.6458**	**3.7749**	**3.1091**	**8.5391**	**15.6684**	**2.8203**

TABLE IX
RESULTS OF BSA OF RATS TREATED WITH A LOW DOSE OF FLUPHENAZINE

Identity of the Rat	MOTILITY			MORPHOLOGY			Cells per Field after Dilution (200x)	CONCENTRATION (millions per ml) (N.B. approximately)
	Progressively Motile %	Non-prog Motile %	% Motility	% Head Abnormalities	% Tail Abnormalities	% Normal Morphology		
F1	0	16	16	26	31	43	12.8	2.295
F2	0	8	8	31	28	41	9.3	9.3
Mean	**0**	**12**	**12**	**28.5**	**29.5**	**42**	**11.05**	**5.798**
SD	**0**	**5.6569**	**5.6569**	**3.5355**	**2.1213**	**1.4142**	**2.4749**	**4.9533**

TABLE X
RESULTS OF BSA OF RATS TREATED WITH A HIGH DOSE OF FLUPHENAZINE

Identity of the Rat	MOTILITY			MORPHOLOGY			Cells per Field after Dilution (200x)	CONCENTRATION (millions per ml) (N.B. approximately)
	Progressively Motile %	Non-prog Motile %	% Motility	% Head Abnormalities	% Tail Abnormalities	% Normal Morphology		
F3	0	10	10	29	33	38	10	1.8
F4	0	5	5	22	39	39	22	3.96
Mean	**0**	**7.5**	**7.5**	**25.5**	**36**	**38.5**	**16**	**2.88**
SD	**0**	**3.5355**	**3.5355**	**4.9497**	**4.2426**	**0.7071**	**8.4853**	**1.5274**

IV. DISCUSSION

From the results (Tables II to V) it is evident that the rats subjected to experimental variations in their serum PRL do not differ in their average body weight gain, average daily food intake or fluid intake when compared with those in the control group, showing that the drugs used to induce artificial variations in the serum PRL did not have any effect on the metabolism of the rats in the study ($p>0.05$).

Oral bromocriptine induced a state of hypoprolactinaemia in the rats whereas oral largactil and subcutaneous injections of fluphenazine induced a state of hyperprolactinaemia. The state of hyperprolactinaemia appears to be affected by the dose of fluphenazine administered, the serum PRL levels attained being higher with high doses of fluphenazine and vice versa.

Higher levels of serum PRL with mean percentage values of 22.248 ± 2.9539 (SD) and 14.948 ± 2.9539 (SD) respectively have been achieved with a higher dose of fluphenazine of 0.84 mg/kg body weight per day. These results are illustrated in Table V and also in Figs. 5 (a) and (b).

The difference between serum PRL concentrations of rats in G2, G3, G4 and G5 as compared to the control group are highly significant by Student's t-test ($p<0.001$).

When the serum analysis studies are considered the percentage of progressively motile sperms is low in all the test groups of rats as compared to the control, with those treated with fluphenazine exhibiting zero progressive motility while those in the control group showing better results with a mean value of 5 ± 3.6514 (SD). This is due to the fact that sperms in the distal cauda (from where the samples were obtained and analysed) usually are in a state of quiescence under physiological states. These sperms are known to acquire total motility only on ejaculation.

The non progressively motile forms were found to be lowest in the rats treated with low and high doses of fluphenazine with mean percentage values of 12 ± 5.6569 (SD) and 7.5 ± 3.5355 (SD) respectively. The control group had the highest value with a mean percentage value of 46.75 ± 5.3151 (SD). The control group also had a high percentage in total motility with a mean value of 51.75 ± 8.5 (SD) when compared with the other groups. Total motility is lowest in the two groups of rats treated with fluphenazine with mean percentage values of 12 ± 5.6569 (SD) and 7.5 ± 3.5355 (SD) respectively.

The results of semen morphology as shown in Tables VI to X indicate that the percentage with abnormal morphology (both head abnormalities and tail abnormalities) is highest in the group treated with fluphenazine and lowest in the control group, the latter group having the highest percentage of those with normal morphology with a mean value of 75.75 ± 8.0984 (SD).

The rats treated with different doses of fluphenazine show the lowest cell counts per field with mean values of 11.05 ± 2.4749 (SD) and 16 ± 8.4853 (SD) respectively whereas the highest figures have been seen in the control group with values of 72 ± 15.6684 (SD).

The total count (concentration) is lowest with mean values of 5.798 ± 4.9533 (SD) and 2.88 ± 1.5274 (SD) respectively in rats treated with fluphenazine, the hypoprolactinaemic rats showing much better concentration readings with a value of 16.335 ±3.9936 (SD). The concentration is highest in the control group with a mean value of 18.2965 ± 2.0376 (SD).

The above results show that the level of serum PRL in rats has an effect on the sperm parameters of the rats in the experimental study in a PRL level dependent fashion. Motility, morphology, cell counts per field and concentration seem to be affected by the serum PRL level with the effects being more marked as the PRL levels rise. The most adverse results are seen in the group treated with high doses of fluphenazine levels (0.84 mg/ kg body weight) with those treated with a lower dose of fluphenazine (0.42 mg/ kg body weight), oral largactil and oral bromocriptine showing improved results.

The above results show a good correlation to the results of the semen analysis of the experimental rats and the serum PRL levels. Those with very high PRL levels show more abnormal BSA findings while those with moderate rises in serum PRL levels and the hypoprolactinaemic rats show better BSA results. Thus it is conclusive that abnormal PRL levels appear to exert an effect on the spermatogenic cycle and thus the sperm parameters of rats.

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