EFFECT OF ASPIRIN, INDOMETHACIN AND $PGF_{2\alpha}$ ON SPERMATOGENESIS IN RATS

DISSERTATION

SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF

Master of Science

IN

DAIRYING

(ANIMAL BIOCHEMISTRY)
TO THE KURUKSHETRA UNIVERSITY,
KURUKSHETRA

BY

Hari Pal Singh

DIVISION OF DAIRY CHEMISTRY
NATIONAL DAIRY RESEARCH INSTITUTE
(Indian Council of Agricultural Research)
KARNAL (Haryana)

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Registration No. 76-DK-56

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DEDICATED TO MY GRANDFATHER

LATE SHRI DAULAT SINGH

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Dated the 5th May,1978

CERTIFICATE OF ORIGINAL WORK

This is to certify that Shri Hari Pal Singh of the National Dairy Research Institute, Karnal participated in the planning of this study, carried out the experimental work involved, analysed the data and prepared this report on "Effect of aspirin, indomethacin and $PGF_{2\alpha}$ on spermatogenesis in rats". He did these in partial fulfilment of the requirements for the degree of Master of Science in Animal Biochemistry, Faculty of Dairying, Animal Husbandry and Agriculture of the Kurukshetra University, under my supervision. Help and assistance given by individuals as well as institutions in the prosecution of the work has been suita-bly acknowledged.

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(N.C.GANGULI)

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HPingh

(HARI PAL SINGH)

C O N T E N T S

CHAPTER I

INTRODUCTION

INTRODUCTION

Spermatogenesis is a biological process involving a number of complex, diverse and sequential steps leading to the differentiation of a highly specialized cell, the spermatozoa. It consists of two major processes; spermatocytogenesis which leads to the formation of haploid spermatids from the gonocytes through a series of mitotic and two meiotic divisions, and spermiogenesis which involves the metamorphosis of the spermatids into the spermatozoa. The spermatozoa are then released into the lumen of the seminiferous tubules and transported. Due to the periodic renewal of the germinal epitheliol cells, the evolution of the spermatogenic series has often been termed as spermatogenic cycle. The duration of this cycle is 74 days in man, 60 days in bull, 49 days in ram and 14 days in rats.

Spermatogenesis is controlled by a number of factors. Multiplication and growth of gonocytes occuring prenatally, during first few days after birth, formation of prespermatogenia and spermatids are known to be controlled by testosterone. FSH and LH regulate spermatogenesis indirectly.

Since the discovery of prostaglandins in human semen, much work has been done to elucidate their role in female reproduction. In male reproduction, however, little work has been done. Prostaglandins are known to be present in almost all, if not all, body cells. Excess prostaglandin production in body creates symptoms such as headache, body ache, inflammation, vomiting,etc. These symptoms subside on administration of analgesic and antipyretic drugs such as aspirin and indomethacin. Perhaps they act through inhibition of prostaglandin synthesis and have thus antogonistic effects.

Recently it has been reported that genes coding for some testicular enzymes are expressed or suppressed at a specific stage in spermatogenesis and thus may serve as biological markers of the various stages of spermatogenesis. A change in activity of these enzymes produced by a treatment may reflect upon the spermatogenesis. Thus using some of the testicular enzymes as 'markers' the effect of prostaglandin $F_{2\alpha}$ on spermatogenesis in rats has been sought for in this investigation. To achieve this goal, two approaches have been tried, namely, administration of exogenous $PGF_{2\alpha}$ and by depriving the animals of the endogenous prostaglandins by administration of aspirin and indomethacin.

CHAPTER II

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1 SPERMATOGENESIS

The complex process of spermatogenesis results in the production of spermatozoa. The earliest observations were defining of certain qualitative morphological parameters relating to the development of the germinal epithelium. Several methods have been used for studying the cell kinetics of spermatogenesis. Quantitative histological techniques of Leblond and Clermont (1) were based on the development of the acrosomic system of the spermatids and affiliated transitions. Those of Roosen-Runge and Giesel (2) and Ortavant (3) were based on the morphological changes of the germ nuclei.

Cell differentiation during spermatogenesis in rats has been analysed in terms of formation of specific 'marker' enzymes. Many of these enzymes and their variations during spermatogenesis have been reviewed (4-6).

The activity of hyaluronidase during the hormone dependent cell differentiation of the germinal epithelium in rats testis has been studied (7). The activity was undetectable until 33 to 34 days of age, at which time it appears and subsequently increase 400-fold. This rapid increase is correlated with acrosome formation in the newly formed spermatids.

The activity of β-glucuronidase was detectable only in Sertoli and spermatogonia (8). The specific activity of this enzyme was high in the immature rat, and decreased markedly with the development of spermatocytes.

Acid phosphatase was detectable in all the germinal cells (8). Its specific activity increased with the development of the spermatocytes and with the formation of the acid phosphatase containing Golgi and pro-acrosomal structures of the spermatids. Parvinen _et al._ (9) identified two isozymes of acid phosphatase in testes. The tubular AP IV showed a high activity in stages corresponding to the final maturation process of the spermatid. AP III was evenly active in the different stages. Vanha and Nikkanen (10) detected four isozymes of acid phosphatase. API & II were of interstitial origin while AP III and AP IV were of tubular origin and their activities varied according to the pattern observed by Parvinen _et al._ (9).

The activity of uridine diphosphatase markedly increased with the formation of spermatogonia and spermatocytes (upto 26 days of age) and then sharply decreased with the formation of spermatids (11). It was also observed that the activity of 5'-nucleotidase was low at four days, increased and reached maximum at the age of 26 days. Thereafter it remained constant (11).

Ornithine decarboxylase activity was first detectable by 21st day (12). The activity increased ten-fold in both the head and tail of epididymis prior to their rapid growth responses. In contrast the activity of S-adenosyl methionine decarboxylase changed very little during development.

Low activity of N-acetyl- β -D-glucosaminidase was observed in testes of rat four days of age (13). It increased with the formation of spermatogonia and spermatocytes, became maximum at 25 days and then decreased rapidly with the formation of spermatid. β -Galactosidase activity was high on day four when only Sertoli cells and gonocytes appear prominent in the seminiferous tubules. With the appearance of spermatogonia and spermatocytes the activity reduced.

Tracking of the morphological changes of the germ nuclei has been used as a method for studying cell kinetics of spermatogenesis (2,3). But as the duration of the division is not always accurately known, this method may not be a true representative of the kinetics. However, one activity of nuclei known to be associated with spermatogenesis is the replication of DNA (14). The rate of synthesis of DNA in somatic cells (Sertoli cells included) is low as compared to that in spermatogonia. In a recent study(15) the incorporation of ^{3}H-thymidine in testicular DNA was used as an index of spermatogenesis in guinea-pigs. The incorporation increased almost linearly upto about 24 hrs after intracardiac injections of ^{3}H-thymidine. After 24 hrs, the incorporation showed little change, if any.

2.2 PROSTAGLANDINS

The biological activity of seminal fluid and prostate gland extracts has been recognized for many years. Von Euler (16,17) and Goldblatt (18,19) observed the smooth muscle stimulating activity of human seminal fluid. Later on Von Euler (20) firmly established that the pharmacological effect of the human seminal fluid was due to a new substance which he named prostaglandin assuming it to be secreted from prostate gland. Bergstrom(21) confirmed this observation. Eliasson (22), however, showed that human seminal fluid prostaglandins originate from the seminal vesicles and not from prostate gland. In the following years several prostaglandins (PGs) were isolated from human seminal plasma and from sheep vesicular glands and their chemi--cal structures, metabolites and nomenclature elucidated (23,24).

Samuelsson (25) identified chromatographically the presence of PGE_1, PGE_2, PGE_3, $PGF_{1\alpha}$ and $PGF_{2\alpha}$ in human seminal plasma. In addition to these 19-hydroxy derivatives of the natural prostaglandins were also detected, the concentration of which was four times higher than that of PGE_1 compounds (26). Carpenter (27) detected PGE_1, PGE_2, $PGF_{1\alpha}$, $PGF_{2\alpha}$, PGB_1 and PGB_2 in rat testes.

Synthesis of prostaglandins from their fatty acid pre--cursors is under the control of prostaglandin synthetase, the activity of which has been demonstrated in a large number of organs in addition to testes.

Aspirin, indomethacin and other antipyretic and analgesic drugs inhibit prostaglandin synthetase. This has been proved beyond doubt by a wide variety of experiments in various tissues both <u>in vitro</u> and <u>in vivo</u>. Ferriera <u>et al</u>. (28) per--fused dog spleen in Kreb's solution through the splenic artery. The effluent of splenic vein was bioassayed for prostaglandins.

Infusion of indomethacin resulted in complete inhibition of prostaglandin production. Aspirin was less potent an inhibitor than indomethacin. Similar inhibition of PG synthesis was also observed by other workers (29-36).

2.3 PROSTAGLANDINS AND SPERMATOGENESIS

Based on several investigations it was shown that there is no correlation between the prostaglandin content of the seminal fluid and the number and motility of spermatozoa. Addition of PGE_1 to sperms did not affect their motility or metabolism (22). However, the amount of PGE has been correlated with male inferti-lity (37). Semen samples of men in infertile marriages contained less prostaglandins as compared with samples from men with recently proven fertility. The effect of exogenous prostaglandins on spermatogenesis has been revealed in recent studies. Prolonged daily administration of PGE_2 or $PGF_{2\alpha}$ to male rabbits did not affect their fertility (38). The quality of semen, the percentage of motile spermatozoa, the percent of live spermatozoa or the measurable sperm output were also not affected. But the pattern of radioactive sperm cells in the ejaculates indicated that PGE_2 and $PGF_{2\alpha}$ caused a decrease of 2 days in the interval from the injection of ^3H-thymidine to the arrival of labelled spermatozoa. Hafs <u>et al.</u> (39) and later on Marshall and Hafs (40) reported an increase in sperm concentration in semen on treat-ment of bulls with $PGF_{2\alpha}$ prior to ejaculation. The seminal volume was, however, not affected significantly nor was the initial and post-thaw motility of sperm and acrosomal integrity before and after freezing.

Several histological, histochemical and ultrastructural studies have been conducted in recent years which have pin-pointed the steps in spermatogenesis which were affected by prostaglandins. Inhibition of spermatogenesis and disruption of seminiferous tubules on prolonged subcutaneous administration of PGE_1 and PGE_2 in rats was reported (41). The epididymis from both groups contained sperm with decreased motility, immature germinal cells and sperm separated into head and tail segments.

A decrease was also observed in weights of testes and accessory sex glands, blood testosterone levels. These observations are converse to those observed later on (38,40). Memon (42,43) and Tierney (44) also confirmed these results. A decrease in testes and accessory sex glands weights, plasma testosterone levels on bilateral intratesticular injection of PGE_2 and $PGF_{2\alpha}$ in rats was obtained. PGE_1 and PGE_2 decreased spermatogenesis with a decrease in the step 7 spermatids (45). This inhibition was during the meiotic phase of spermatogenesis (46). $PGF_{2\alpha}$ was more potent an inhibitor than $PGF_{1\alpha}$. Histo-pathological examination revealed increased numbers of exfoliated immature germ cells in the seminiferous tubules and epididymis of PG treated rats. When compared to PGE_1 and PGE_2, PGE_2 produced strongest inhibition with $PGF_{2\alpha}$, PGE_1 and $PGF_{1\alpha}$ following in decreasing order.

2.4 ASPIRIN, INDOMETHACIN AND SPERMATOGENESIS

Depriving the testes of their natural prostaglandins by administration of prostaglandin synthetase inhibitors, aspirin and indomethacin has also been one of the approaches. The affect of aspirin on the fertility was investigated by Cenedella and Crouthamel (47). Administration of aspirin increased fertility in mice initially judged as subfertile. Aspirin produced a reduction in seminal fluid in adult male rats (48). The consistency of the seminal fluid was thicker. Aspirin and indomethacin registered an increase in spermatogenesis accompanied by an increase in the number of step 7 spermatid. Testicular weight was, however, not affected (45).

CHAPTER III

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Chemicals

Hyaluronic acid, p-nitrophenyl- β -D-galactoside, p-nitrophenyl-N-acetyl- β -D-glucosaminide, uridine-5'-diphosphate and adenosine-5'-monophosphate which were used as substrates for the assay of hyaluronidase, β -galactosidase, N-acetyl- β -glucosaminidase, uridine diphosphatase and 5'-nucleotidase, respectively were obtained from Sigma Chemical Company, Missouri, U.S.A. p-Nitrophenyl phosphate used as sub--strate for acid phosphatase was from Patel Chest Institute, New Delhi. Aspirin and indomethacin used for treatments were from Sigma Chemical Company, Missouri, U.S.A., and $PGF_{2\alpha}$ (tromethamine salt) also used for treatment was a gift from the Upjohn Company, U.S.A. Other chemicals used were of analytical grade.

3.1.2 Animals

Male albino rats, 57 days-old and weighing between 110-150 gm were obtained from the Institute's Small Animal House.

3.2 METHODS

3.2.1 Treatments

Male albino rats 57 days-old and weighing between 110-150 gm were randomly assigned to 5 groups. Prior to this the rats had been maintained under uniform conditions for about 15 days during which time they were given cow milk, rat feed pellets and water *ad lib*.

The treatment period of 15 days was chosen so as to study their effect on one complete spermatogenic cycle.

Group I consisting of 5 animals received aspirin 300 mg/kg body wt/rat through gastric intubation once a day. Group II (5 rats) received indomethacin 3 mg/kg body wt/rat through gastric intubation once a day. The III group (5 rats) served as control and was normally fed. Group IV (3 rats) was administered $PGF_{2\alpha}$ (tromethamine salt) 3 mg/kg body wt/rat intraperitoneally dissolved in normal saline once a day. The Group V (4 animals) served as control and received normal saline intraperitoneally. The treatments were carried out for 15 days.

On the 16th day the rats were weighed and sacrificed by decapitation.

3.2.2 Preparation of tissue extract

The rats were slaughtered by decapitation and allowed to bleed. The testes were excised, stripped of the tunica albuginea and chilled quickly prior to weighing. The epididymis was also freed from the adipose tissue and weighed.

The chilled testes were then homogenized in ice-cold 0.25M sucrose solution (1:10 w/v) containing 0.2% Triton X-100 in a glass homogenizer fitted with a power-driven teflon pestle at 4°C. The homogenate was centrifuged at 35,000 r.p.m. for 30 min under refrigeration in an ultracentrifuge. The supernatant was decanted and used for enzyme assay and the pellet was used for estimation of DNA and RNA.

3.2.3 Enzyme assay

3.2.3.1 Acid phosphatase

The property to hydrolyze p-nitrophenyl phosphate was used for the assay of this enzyme. The assay system consiting of 0.8 ml of 5.5×10^{-3} M p-nitrophenyl phosphate in 0.05 M citrate buffer (pH 4.8), 0.18 ml of the same buffer and 0.02 ml of the sample was incubated at 37°C for 30 min. The reaction was termi--nated by the addition of 5 ml of 2% Na_2CO_3. The intensity of the colour developed was determined in Klett-Summerson Photo-electric colorimeter using blue filter. One enzyme unit was defined as the amount which catalyzes the release of 1 µmole of p-nitrophenol in 30 min at 37°C.

3.2.3.2. Hyaluronidase

The activity of the enzyme was measured by the rate of release of free N-acetyl-glucosamine from hyaluronic acid with a modification of the method of Rhodes et al.(49). The assay system contained (a) 0.20 ml of 2 mg/ml hyaluronic acid in 0.05 M citrate buffer pH 4.5, containing 0.1 ml of 9% NaCl, (b) 0.10 ml of the same buffer, and (c) 0.10 ml of sample. This mixture was incubated at 37°C for 30 min. The reaction was terminated by 0.20 ml of 0.16 M potassium tetraborate pH 8.9, followed by boiling for exactly three min. After cooling the N-acetyl-glucosamine released was estimated by the method of

Reissig *et al.*(50). 3.0 ml of dimethylamino benzaldehyde solution (10 gm of dimethylamino benzaldehyde was dissolved in a mixture of 37.5 ml glacial acetic acid and 12.5 ml of 10 N HCl, diluted 1:9 with glacial acetic acid just prior to use) was added and incubated at 37°C for 20 min. The intensity of the colour developed was estimated in Klett-Summerson photo-electric colorimeter using a green filter. Enzyme unit was defined as the amount of enzyme which catalyzes the production of 1 μ mole of N-acetyl-glucosamine in 30 min at 37°C.

3.2.3.3 5'-nucleotidase

The enzyme was assayed by a modification of the method of Haung and Keenan (51) in a reaction medium consisting of (a) 1.40 ml of 7.5×10^{-3} M AMP in 0.05 M Tris-HCl buffer pH 7.5 containing 3.5×10^{-3} M $MgCl_2$, (b) 0.40 ml of the same buffer and (c) 0.20 ml of the sample, was incubated at 37°C for 15 min. with occasional shaking with air as the gas phase. The reaction was stopped by the addition of an equal volume (2.0 ml) of 10% TCA solution. After centrifugation, inorganic phosphorus liberated was estimated in the clear supernatant by the method of Chen *et al.*(52). 0.2 ml of the supernatant was taken. The volume was made to 2.0 ml with distilled water. 2.0 ml of freshly prepared ascorbic acid reagent (mix one volume of 6 N H_2SO_4 with 2.5% ammonium molybdate and one volume of 10% ascorbic acid solution and mix well) was added. After capping with parafilm and mixing, the tubes were incubated at 37°C for one hr. The intensity of the colour was measured in Klett-

Summerson photoelectric colorimeter using a red filter. Enzyme unit was defined as the amount of enzyme which catalyzes the release of 1 μ mole of inorganic phosphorus (Pi) in 15 min at 37°C.

3.2.3.4. N-acetyl- β -D-Glucosaminidase

The activity of the enzyme was determined by the modification of the method of Conchie (53). 0.8 ml of 5×10^{-3}M solution of p-nitrophenyl-N-acetyl- β -D-glucosaminide in 0.1M citrate buffer, pH 4.0 was incubated with 0.20 ml of enzyme preparation at 37°C for 30 min. 5.0 ml of 0.2 N sodium hydroxide was added to stop the reaction. The colour developed was read in a Klett-Summerson photoelectric colorimeter using a blue filter. Enzyme unit was defined as the amount which catalyzes the release of 1 μ mole of p-nitrophenol in 30 min at 37°C.

3.2.3.5. β -Galactosidase

A modification of the method of Lederberg (54) was used for the assay of β -galactosidase. The assay system contained 0.80 ml of 5×10^{-3}M solution of p-nitrophenyl- β D-galactoside in 0.1 M citrate buffer, pH 3.5 and 0.20 ml of the enzyme preparation. The mixture was incubated at 37°C for 30 min. The reaction was terminated by adding 5.0 ml of 0.2 N sodium hydroxide. p-Nitrophenol released was measured in Klett-Summerson photo-electric colorimeter using blue filter. Enzyme unit was defined as the amount of enzyme which catalyzes the release of 1 μ mole of p-nitrophenol in 30 min at 37°C.

3.2.3.6. Uridine diphosphatase

UDPase activity was assayed by modification of the method described by Kuma and Turkington (11) by the estimation of the inorganic phosphorus released by enzymatic hydrolysis of UDP. The standard assay system contained 0.90 ml of 0.25 mg/ml solution of UDP in 0.05 M Tris-HCl buffer, pH 7.0 containing 40 u moles of $MgCl_2$ and 0.10 ml of the sample. The mixture was incubated at 37°C for 30 min, after which the reaction was terminated by the addition of 0.30 ml of ice-cold 10% TCA. After centrifugation, inorganic phosphorus was estimated in the supernatant by the method of Chen et al. (52) as described earlier using 0.2 ml of the supernatant. Enzyme unit was defined as the amount which catalyzes the release of 1 μ mole of inorganic phosphorus in 30 min at 37°C.

3.2.3.7. Protein Estimation

Protein was estimated by the method of Lowry et al. (55) using bovine serum albumin as standard.

3.2.4. Preparations of Nucleic acid extract

Nucleic acid extract was prepared according to the method of Schneider (56). The pellet obtained on centrifugation of testicular homogenate was suspended in 2.0 ml of 0.25 M sucrose solution. 2.5 ml of cold 10% TCA was added followed by centrifugation. The pellet was washed once more with 2.5 ml of cold 10% TCA and recovered by centrifugation. It was then washed twice with 5.0 ml of 95% ethanol and recovered each time by centrifugation. The final sediment was suspended in 5.0 ml of 5% TCA, heated at 90°C for 15 min with occasional stirring, cooled to room temperature and centrifuged. Supernatant so obtained was used for the estimation of DNA and RNA.

3.2.4.1 Estimation of DNA

DNA was estimated by the method of Burton (57) by the diphenylamine reaction. One ml of the extract, 0.25 ml of 60% perchloric acid, 1.50 ml of 5% TCA and 2.50 ml of diphenylamine reagent (1.5 gm of steam distilled diphenylamine dissolved in 100 ml of redistilled glacial acetic acid followed by 1.5 ml of concentrated sulphuric acid and mixing) were mixed and heated in boiling water bath for 10 min. The intensity of colour developed was read in Klett-Summerson photoelectric colorimeter using a green filter.

3.2.4.2. Estimation of RNA

RNA was estimated by a modification of the method of Golovanni and Ceriotti (58). To 1.0 ml of the extract 2.0 ml of distilled water and 2.0 ml of orcinol reagent (1 gm of orcinol and 0.5 gm $FeCl_3$ are dissolved in conc. HCl and the volume made to 100 ml with conc. HCl) were added. The mixture was mixed well and heated in boiling water bath for 10 min. The colour thus obtained was measured in Klett-Summerson photoelectric colorimeter using red filter.

3.2.5. Estimation of Total Nitrogen

The method used was as described by Hawk *et al.* (59).

To 1.0 ml of homogenate in the Kjeldahl flask 1.0 gm potassium sulphate, 0.1 gm copper sulphate and 2 ml of concentrated sulphuric acid were added. The contents were digested for sufficient time till the whole mixture became clear. This digested material was then transferred to a micro-kjeldahl distillation assembly. The digestion flask was washed twice or thrice to transfer the rest of the digested material. 15.0 ml of 50% NaOH was added and the contents boiled. The ammonium produced was absorbed in 10 ml of 2% boric acid containing mixed indicator (2 drops). About 40-50 ml of the distillate was collected and titrated with 0.023 N HCl.

CHAPTER IV

RESULTS AND DISCUSSION

4. RESULTS AND DISCUSSION

Rats randomly assigned to different groups and treated with aspirin, indomethacin and $PGF_{2\alpha}$ (tromethamine salt) for 15 days were subsequently sacrificed and marker enzymes in the testes, were studied. The enzyme assayed were acid phosphatase, hyaluronidase, 5'-nucleotidase, β-galactosidase, N-acetyl-β-D-glucosaminidase and UDPase. Other parameters studied were DNA, RNA and total nitrogen contents of testes and body weight, testes weight and weight of the epididymis.

4.1 BODY AND SEX ORGAN WEIGHTS

Whole body weights and weights of the testes and epididymis were recorded. The results obtained are delineated in Table 1. The increase in body weight was observed to be less in case of aspirin and more in indomethacin and $PGF_{2\alpha}$ treated rats. But this difference in increase in body weight was statistically non-significant. These results are in contra--diction to those of Tierney (44) and Daly (60) who observed a decrease in body weight on treatment of adult male rats with $PGF_{2\alpha}$.

A decrease in testes weight was observed in rats treated with indomethacin and $PGF_{2\alpha}$. The decrease observed was non-significant. Aspirin treated rats showed little increase in testes weight over those of controls but this

Table 1. Effect of aspirin, indomethacin and $PGF_{2\alpha}$ on body weight, testes weight and epididymi weight

TREATMENTS	Number of rats	Body weight (gm) before treatment	Body weight (gm) after treatment	% increase in body weight	Weight of testes (gm)	Weight of epididymis (gm)
Control	5	131.00± 8.72	170.6±17.21	30.23	1.8232±0.1121	0.3704±0.01937
Aspirin	5	132.00± 7.65	161.40±19.11	22.27	1.8986±0.4783	0.37864±0.04638
Indomethacin	5	128.2 ±10.98	175.80±18.52	37.19	1.7650±0.2334	0.39840±0.0221
Normal saline	4	137.75± 9.71	175.0 ± 8.37	27.04	1.7000±0.1234	0.36500±0.05155
$PGF_{2\alpha}$	3	134.33± 9.71	175.67±23.01	30.77	1.4763±0.2168	0.3437 ±0.0729

Values are expressed as Mean

± indicates the standard deviation of mean.

increase was also insignificant statistically. These results are in concordance with those of Daly(60), Tierney(44), Ericsson (41) and Lau (61) in rats and Memon (42,43) in hamsters and rats. Saksena *et al*.(62), however, failed to observe any effect of indomethacin on weight of testes in rats.

The weight of epididymis of rats treated with aspirin and indomethacin was observed to be more than those of controls, whereas in rats treated with $PGF_{2\alpha}$, the weight of epididymis was less than the controls. These differences, however, were statistically non-significant. These results are similar to those reported earlier (41,42,45,46,61).

4.2 ENZYMES

4.2.1 Acid phosphatase

The activity of acid phosphatase in the supernatant of testicular homogenate of control and rats treated with aspirin, indomethacin and $PGF_{2\alpha}$ was determined, the results of which are shown in Table2. On treatment of rats with aspiring and indomethacin, the activity of acid phosphatase decreased. This decrease was found to be statistically signi--ficant ($P < 0.01$) in case of aspirin and non-significant in case of indomethacin, whereas a significant increase($P < 0.05$) in the activity was observed in $PGF_{2\alpha}$ treated rats. The level of enzyme was, however, low in all rats when compared to the data of Males and Turkington (8).

4.2.2. Hyaluronidase

The results in Table 2 show the activity of hyaluronidase in the rat testes of different groups. Aspirin and indomethacin treated rats showed a low activity of hyaluronidase than the controls. The decrease in activity was statistically non-significant. $PGF_{2\alpha}$, on the other hand, caused a significant ($P < .01$) decrease in the activity. From this change in activity it appears that $PGF_{2\alpha}$ causes a reduction in the number of mature spermatids and spermatozoa as this enzyme is present in the acrosomal cap of the developing spermatids and sperms. Indomethacin treatment has an inhibitory effect on hyaluronidase activity (63) in addition to the inhibition of prostaglandin synthetase enzyme system. The decrease in activity of testicular hyaluronidase in indomethacin treated rats may be due to this effect. Aspirin, it appears, also has a similar effect.

4.2.3. 5'-Nucleotidase

The results of the effect of the treatments on activity of 5'-nucleotidase are summarised in Table 2. Both aspirin and indomethacin and $PGF_{2\alpha}$ decreased the enzyme activity. The decrease produced was statistically insignificant.

4.2.4. N-acetyl- β -D-Glucosaminidase

Aspirin and indomethacin treatments caused an insignificant reduction in the activity of the enzyme. $PGF_{2\alpha}$ treated rats showed an insignificant increase in the activity of the enzyme (Table 2). The pattern of variation of activity of this enzyme (64) suggested that aspirin and indomethacin either inhibit spermatogenesis at the spermatogonia stage or they stimulate spermatogenesis as either case would result in a decrease in the activity of the enzyme. $PGF_{2\alpha}$ appears to inhibit spermatogenesis by blocking the development of mature spermatozoa due to the fact that the activity of the enzyme is high in the premature stages of spermatogenesis.

4.2.5 <u>β -Galactosidase</u>

The data in Table 2 show that the activity of β-galactosidase was low in case of rats treated with aspirin and indomethacin when compared with controls. The difference was found to be non-significant. $PGF_{2\alpha}$ treatment registered an insignificant increase in the activity. However, the slight increase observed suggests that $PGF_{2\alpha}$ might decrease spermatogenesis as the activity of the enzyme is high in immature germ cells. Aspirin and indomethacin act in an antagonistic manner.

4.2.6 <u>Uridine Diphosphatase</u>

Results in Table 2 indicate that aspirin and indomethacin treatment reduced the activity of Uridine Diphosphatase (UDPase). Though the decrease was statistically insignificant, it appears that both the drugs stimulate spermatogenesis. This was evident from the pattern of variation of the enzyme activity which was reported (11) to be low in the gonocytes, increases and reaches a maximum in spermatocytes, decreases in spermatids

Table 2. Effect of aspirin, indomethacin and $PGF_{2\alpha}$ on the activity of marker enzymes in rat testes

TREATMENT	Number of rats	Acid phosphatase	Hyaluronidase	5'-Nucleotidase	N-acetyl-β D-Glucosamidase	β-Galactosidase	Uridine-Diphosphatase
		Specific Activity					
Control	5	1.03956 ±0.66364	0.22776 ±0.007235	1.29474 ±0.309738	0.22948 ±0.032816	0.09000 ±0.005286	1.3479 ±0.1582
Aspirin	5	0.87666** ±0.0367	0.21724 ±0.033588	1.26194 ±0.145349	0.21538 ±0.011202	0.07682 ±0.00765	1.2086 ±0.10913
Indomethacin	5	0.97378 ±0.03092	0.2276 ±0.009421	1.11288 ±0.078947	0.18854 ±0.006217	0.07830 ±0.004113	1.2390 ±0.2480
Normal Saline	4	0.96927 ±0.04871	0.23135 ±0.01175	1.06795 ±0.054754	0.20457 ±0.014430	0.0813 ±0.002562	1.1690 ±0.1210
$PGF_{2\alpha}$	3	1.0847* ±0.06317	0.2111** ±0.010822	1.06293 ±0.09324	0.20943 ±0.024679	0.08463 ±0.004465	1.2250 ±0.0958

Values are expressed in Mean
± indicates the standard deviation of mean

* Significant at $P < 0.05$
** Significant at $P < 0.01$

and then levels out with the appearance of mature sperms. $PGF_{2\alpha}$ treatment increased the activity of the enzyme sugges-ting that the treatment may induce an increase in the number of spermatids and spermatocytes with high enzyme activity.

4.3 DNA

From the results in Table 3 it is evident that DNA content in the testes of rats treated with aspirin and indomethacin was significantly low ($P < 0.05$). $PGF_{2\alpha}$ in contrast increased the DNA content of the testes though not significantly. The rate of synthesis of DNA in spermatogonia being more than in other cells (15) a change in the concentration of DNA in testes is expected to reflect on the spermatogenesis. From low DNA concentration in case of aspirin and indomethacin it may be predicted the number of cells with low DNA content were predominent. In $PGF_{2\alpha}$ treated rats, the increased DNA suggested an increase in the number of spermatogonia, spermatocytes and spermatids.

4.4 RNA

RNA content in the testes of the rats of all groups was determined. The data in Table 3 indicate that all three treat-ments produced an insignificant increase in RNA content of the testes. The observations for $PGF_{2\alpha}$ treated animals correlated with the increased DNA contents in this group as high RNA concentration may be secondary to high DNA concentration. However, the increased RNA concentration along with decreased DNA concentrations in case of aspirin and indomethacin treated groups cannot be accounted for in the light of the present observations.

4.5 Total Nitrogen

The observations in Table 3 indicate that the total nitrogen in the testicular homogenate of treated rats was not significantly different from those of the controls. $PGF_{2\alpha}$ treatment increased the total nitrogen which follows from the high levels of DNA and RNA, and may be due to increase in the number of cells with higher DNA, RNA and hence total nitrogen.

Table 3. Effect of aspirin, indomethacin and $PGF_{2\alpha}$ on DNA, RNA and Total Nitrogen content in rat testes

TREATMENT	Number of rats	DNA concentration	RNA concentration	Total Nitrogen
		mg/ml homogenate		
Control	5	0.2191±0.0293	0.1527±0.0167	1.5584±0.0668
Aspirin	5	0.1721±0.0258*	0.1946±0.0981	1.6583±0.1428
Indomethacin	5	0.1689±0.0243*	0.1758±0.0561	1.5134±0.1018
Normal Saline	4	0.1785±0.0133	0.1812±0.0357	1.6207±0.0186
$PGF_{2\alpha}$	3	0.2136±0.0284	0.2159±0.0286	1.6529±0.0670

Values are expressed as Mean

± Indicates the standard deviation of mean

* Significantly at $P < 0.05$

CHAPTER V

SUMMARY AND CONCLUSION

5. SUMMARY AND CONCLUSION

1. An attempt was made in this investigation to study the effect of aspirin, indomethacin and $PGF_{2\alpha}$ on spermatogenesis in rats. The parameters studies were body weight, testes weight and epididymis weight, DNA, RNA and total nitrogen contents of the testes. Some marker enzymes like acid phosphatase, hyaluro--nidase, 5'-nucleotidase, β -galactosidase, N-acetyl- β -D-glucosaminidase and uridine diphosphatase of the testes were also assayed.

2. Increase in body weight during the treatment period was less in case of treated rats than in the controls. Testes wt. was less in indomethacin and $PGF_{2\alpha}$ treated rats, and slightly more in those treated with aspirin. Indomethacin and aspirin increased the weight of epididymis while $PGF_{2\alpha}$ decreased the wt.of epididymis.

3. Aspirin treatment resulted in a decrease in the activi-ties of all the enzymes studied. The decrease was significant only in acid phosphatase activity. Indomethacin also had a simi--lar effect on the activity of all the enzymes. $PGF_{2\alpha}$ treated rats showed a significant increase in acid phosphatase, a signi--ficant decrease in hyaluronidase and an insignificant decrease in 5'-nucleotidase activity. The activity of other enzymes was higher than those of control.

4. $PGF_{2\alpha}$ treatment resulted in an increase in DNA concen--tration with a corresponding increase in RNA and total nitrogen content of the testes. Such a synchrony was not achieved with aspirin and indomethacin treatments. Both the treatments produced a significant decrease in DNA. An insigni--ficant increase in RNA and total nitrogen in case of aspirin and an increase in RNA, and a decrease in total nitrogen in case of indomethacin was observed.

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