# CHAPTER 3

## Effect of Deltamethrin 1% + Triazophos 35% EC on Reproductive System of Wistar Rats

The toxic effect of chemicals on the human reproductive system has become a major health concern due to several factors. First is the number of reproductive hazards to which we are rarely exposed in the environment that is rising, secondly, relatively large number of individuals get exposed more directly because of occupational exposure in work place through manufacturing, packaging, or handling. Exposure of male or non-pregnant females may cause abnormalities that may lead to reproductive failure. Thus, reproductive toxicity studies are designed for the characterization of effects of the test chemicals upon multi-dose exposure to experimental animals.

Reproductive toxicity can be defined as any adverse effect seen on any aspect of male and female gonads and on the conception or on lactation, which would interferes with the development of a normal offspring, which could be reared to sexual maturity of the parent **(Schardein, 1999).** The safety evaluation of pesticide for reproductive toxicity has to take into consideration several factors, which are not relevant for drug testing.

Overview of the potential health risks associated with the exposure of chemicals or physical agent which evokes a prominent concern that these agents may interfere with the ability of individuals to produce normal, healthy children. Numerous reviews have classified a myriad of environmental agents as male reproductive toxicant. The out come of such experiment includes reduced fertility, embryo/fetal loss, birth defects, childhood cancer, and other postnatal, structural or functional deficits **(Zenick *et al.*, 1994).**

Several industrial and environmental chemicals are spermatotoxic in man and numerous additional chemicals are known to affect the spermatogenic process in experimental animals. Experimental data on this subject are largely derived from the repeated dose studies commonly used to support regulatory decision **(Linder *et al.*, 1992).**

The relation between the exposure and reproductive dysfunction is highly complex because the mother, father or both may have influence on the reproductive out-come. In addition,

reproductive dysfunction might increase, had the subject been exposed to the xenobiotic some time in the past or immediately prior to conception or during gestation. The relevant period of exposure can be identified in some cases e.g. chromosomal abnormalities detected in the embryo can arise from mutation in the germ cells of either parent prior to conception or at fertilization or from direct exposure of embryo during gestation. Major malformation however, usually occurs with the exposure during a discrete period during pregnancy **(Manson and Kang, 1994)**.

With the ever increasing production and application of pesticide, chances of getting exposed to critical levels of toxicants by nontargeted species are also on the rise. Therefore it is pertinent to speculate that many of the abnormalities that become prominently noticed in the recent time could be a product of exposure to a combination of toxicants. Hence, an effort was made to study the effect of a combination pyrethroids and organophosphorus pesticide (deltamethrin 1% + triazophos 35% EC) on the reproductive performance and function of albino rats.

REVIEW OF LITERATURE

A survey through literature reveals the presence of a plethora of reports on the reproductive toxic manifestation(s) of synthetic pyrethroids and organophosphorus compounds but not in combination. Few relevant ones are cited hereafter.

The primary toxicity associated with the acute exposure to organophosphate insecticide is cholinergic crisis resulting from acetylcholinesterase inhibition. However, these compounds have been numerous specific effects such as delayed neuropathy, immunotoxicity, carcinogenesis as well as endocrine, developmental and reproductive toxicity. Assessment of reproductive toxicity of organophosphorus insecticides is an important public health issue.

Pregnant rats orally treated with deltamethrin at the dose levels of 1, 2.5 and 5-mg/kg body weight during day 6 – 15 of gestation resulted in higher early embryonic deaths, retardation of growth and increase in prenatal weight at 5 mg/kg body weight **(Abd El-Khalik *et al.*, 1993).** Effects on reproductive organs and fertility of male rats were evaluated with the oral administration of diazinon and deltamethrin for consecutive 65 days. Decreased weight of most genital organs, sperm motility and an increase in the percentage of dead and morphologically abnormal spermatozoa, decrease in the plasma testosterone levels and decreased conception rate were observed in the treated rats **(Abd el-Aziz *et al.*, 1994).**

Oral administration of deltamethrin to pregnant female Sprague Dawley rats at the dose level of 0.1 or 10 mg/kg body weight during 6-18 day of pregnancy revealed no effects on reproductive or teratogenic parameters **(Glomot and Vannier, 1977).** Another investigation revealed dose related reduction ($P \leq 0.01$) in maternal weight gain during pregnancy in rats treated with deltamethrin at 5 mg/kg body weight administered during day 7-20 of gestation **(Kavlock *et al.*, 1979).** Lower mean fetal weight was observed in the litters of New Zealand white rabbit exposed to deltamethrin orally during organogenesis period. **(Glomot and Vannier, 1977).** A three-generation reproduction study was carried out in Charles River rats treated with deltamethrin in diet at the dose level of 0, 2, 20 or 50 mg/kg. Decrease in body weight of $F_0$ male, and decrease in feed consumption of $F_1$ male parent rats was observed at high dose group **(Wrenn *et al.*, 1980).** Treatment of deltamethrin in Sprague Dawley rats during $7^{th}$ day of gestation to $15^{th}$ day of lactation did not affect parturition, litter size, or pup viability **(Kavlock *et al.*, 1979).**

Embryotoxicity and fetotoxicity induced by organophosphate insecticide was evaluated in pregnant mice treated with chlorpyriphos at the dose level of 01, 10 and 25-mg/kg body weight during 6-$15^{th}$ day of gestation. Cholinesterase level was significantly reduced at all the dose levels. Body weight gain of dams during gestation was significantly less at 25-mg/kg-dose level when compared with the control. The amount of feed and water consumed by pregnant mice at the dose level of 25-mg/kg-body weight was significantly lower than the control. Fetotoxicity was also noted among litters of mice dosed 25 mg/kg of chlorpyriphos **(Deacon *et al.*, 1980).**

**Phillips *et al.*, (1997)** assessed developmental neurotoxicity of chlorpyrifos in Long-Evans rats following late gestational exposure (days 14-18). No overt neurological signs were detected in the dams, but there was a trend towards lower open-field activity in the high-dose group. Male rats in the high-dose group showed decreased handling reactivity and decreased activity and rearing in the open field throughout the course of testing. On the other hand, female rats showed increased reactivity before weaning. These data suggest qualitative sex-related differences associated with chlorpyrifos exposure, but the effects were small. Thus there were few persistent neurobehavioral consequences of chlorpyrifos following late gestational exposure.

Embryotoxic and teratogenic effect in rats which were orally treated with an organophosphate insecticide (monocrotophos) at the dose level of 2 and 4-mg/kg body weight during whole gestation was evaluated by **Singh and Sharma (1998).** They observed significant reduction in gestation period in animals of high dose group. Treatment caused a significant diminution in dam weight, litter size and weight and average pup weight at both

dose levels. Reduction in gestation period, average dams weight, litter size and average pup weight and live birth indices were also observed in the experimental group.

A one-generation reproductive toxicity study was carried out in Sprague Dawley in rats with fenitrothion administered in the diet at concentrations of 10, 20, and 60 ppm. Parental animals (P) were fed *ad libitum* for 10 weeks prior to mating and throughout gestation and lactation until euthanasia. Their offspring (F1) were fed *ad libitum* from weaning until maturation at the age of 10 weeks There were no effects on reproductive performance, parturition, or lactation. In F1 generation, no effects were observed on systemic and anti-androgenic endpoints **(Okahashi *et al.*, 2003)**.

A two-generation toxicity study was carried out to assess the potential reproductive and neonatal toxicity of organophosphate insecticide. Chlorpyrifos administered to Sprague-Dawley rats in diet for a week. The parental toxicity observed at 5.0 mg/kg/day was accompanied by decreased neonatal body weights and survival in the F1 litters only **(Quast *et al.*, 1993).**

Multiple end points of spermatotoxicity in short duration test were investigated in a number of chemical those are reported to produce mild to severe reproductive effect in Sprague Dawley rats. Based on the results of the experiment it has been concluded that testicular histology, testicular sperm head counts, cauda sperm counts, sperm morphology and sperm velocity are found to be most toxicologically sensitive end points and histopathology of testis and epididymis is most consistent indicator of reproductive damage **(Linder *et al.*, 1992).**

Studies by **Okamura *et al.*, (2004)** on Wistar rats subjected to dichlorvos (0,1,2,4 mg/kg) subcutaneously for 9 weeks showed definite impairment in sperm motility. However no significant difference was observed in the reproductive organ weights. Treatment of malathion (100 mg/kg body weight) and dichlorvos (10 mg/kg body weight) to Wistar rats for a duration of 48 days revealed 60 –95% reduction in sperm motility **(Akbarsha *et al.*, 2000).**

The above literature clearly revealed that no reproductive toxicological experiments were carried out using the present combination (pyrethroids and organophosphorus), which is very much in use because of their greater success in controlling insect pest due to possible 'potentiation' and hence, the present experiment was planned to evaluate the adverse effect of this combination on reproductive system of male rats which include effects on spermatogenesis and fertility while in female rats the study was extended to estrous cycle and fertility which also included the study of litter size, litter weight etc. Dose related effects

on survival, body weight, feed consumption and gross and microscopic organ changes in the parent generation were also analyzed in the current study.

## MATERIAL AND METHODS

Four groups of animals comprising 5 males and 5 females were given one time daily dose of deltamethrin 1% + triazophos 35% EC through oral gavage at the dose levels of 0 (G1: control), 10 (G2: Low dose), 20 (G3: Mid dose) and 30 (G4: high dose) mg /kg body weight. All the animals were starved for overnight prior to dosing and 3 - 4 hours post dosing except females during gestation and lactation period. Deltamethrin 1% + triazophos 35% EC was dissolved in distilled water and administered at the dose volume of 10-mL/kg-body weight. Control group animals were maintained in similar condition with the treatment of distilled water.

Male animals were dosed for 15 weeks (70 days prior to mating, during mating and thereafter till sacrifice). Female animals were dosed 14 days prior to mating (at least two cycles), during mating, throughout gestation and lactation period. Treated male and female rats from the same dose group were allowed for cohabitation. Each litter was examined after parturition to establish the number and sex of pups born, stillbirth, live birth, runts and the presence of gross abnormalities, if any. Live pups were counted, sexed and weighed along with dam on day 1, 4, 7, 14 and 21 postpartum.

At the termination of study different sperm parameter such as motility, Testicular Sperm Head Count, epididymal count and morphological evaluation were carried out according to standard procedure **(Blazak *et al.*, 1993; Chinoy *et al.*, 1993; Ron Filler, 1993; Williams, 1993).**

Absolute weight of uterus, ovaries, testes, epididymis, spleen and thymus were recorded. Relative weight of these organs was calculated later. The organ was dissected out and was cleared from the adhering fat, and blotted free from the blood. At the end of experiment, after blood collection, all the animals were euthanised by carbon dioxide asphyxiation and subjected to a complete necropsy. The organs *viz.,* liver, kidney, spleen, thymus and lymph node and reproductive organs like testes, seminal vesicle, coagulation gland prostate, epididymis, ovaries, uterus, vagina, cervix and mammary gland of the animals were examined microscopically **(Godkar, 1994)**.

Male fertility index, Female fertility index, gestation index, lactation index live birth index, survival index, live litter size, dead litter size, sex ratio were calculated using standard formulae **(Thomas, 1996).**

## RESULTS

Body weight of male rats at the dose level of 20 and 30-mg/kg-body weight was significantly ($p \leq 0.01$) lower than the control group. The percentage reduction observed in body weight of male rats was 4, 18 and 27 in low, mid and high groups, respectively **(Table 3.1, Figure3.1).** Female rats in the high dose group too registered a drop in mean body weight compared to the controls **(Table 3.2, Figure 3.6).** The pregnant females of high dose group failed to register optimal body weight gain during gestation **(Table 3.2, Figure 3.6).** Further, the dams treated with the test substance showed dose dependent reduction in body weight during lactation. However the alteration in body weight of low and mid dose group dams during lactation was not statistically significant except for day 7 wherein the body weight of dams from high dose group was found significantly lower compared to controls **(Table 3.2, Figure 3.6).**

With increase in dose level a concomitant decrease was observed in food consumption in male rats. **(Table 3.1, Figure 3.2).** Female rats treated with deltamethrin 1% + triazophos 35 % EC too showed a significant ($p \leq 0.01$) reduction in feed consumption at high dose group. During gestation, feed consumed by dams was found to be dose dependent. However, statistically significant ($p \leq 0.01$) reduction in feed consumption during lactation was found only in high dose group animals **(Table 3.3; Figure 3.7).**

In the present investigation definite alterations were observed in sperm motility, epididymal counts, testicular sperm head count and sperm morphology of rats subjected to deltamethrin 1% + triazophos 35% EC. Result showed concomitant inhibition of sperm motility with the increase in dose levels. Percentage of motile sperm inhibited at high dose group was significantly ($p \leq 0.01$) lower than the control group value. **(Table 3.1; Figure 3.3).** Decrease in number of sperms counted in cauda epididymus of male rats treated with deltamethrin 1% + triazophos 35% EC was found to be statistically significant at 20-mg/kg body weight ($p \leq 0.05$) and 30 mg/kg body weight ($p \leq 0.01$) when compared with control. A treatment related decrease in number of testicular sperm head count was observed in treated groups when compared to control group. The decrease observed was significant at all the dose levels **(Table 3.1; Figure 3.3 and 3.4).** Morphological examination of sperms revealed various abnormalities of sperms related to head and tail such as no head, no hook,

blunt hook, broken tail, no tail, etc. Percentage of abnormal sperm observed in various treated groups however, does not show any statistical significance **(Table 3.1; Figure 3.5).**

Absolute and relative weight of reproductive organs such as testis, epididymis, ovaries, uterus, of control and various treatment groups were recorded and presented **(Table 3.4 and 3.5; Figure 3.8 and 3.9).** Absolute and relative organ weights of male and female rats from treated groups were found to be comparable with that of the control group except for female rats of high dose group, which showed significant increase ($p \leq 0.05$) in absolute and relative weights of uterus.

Treatment of deltamethrin 1% + triazophos 35 % EC at high dose (30mg/ kg body weight) produced significant reduction in litter size, total pup weight ($p \leq 0.01$) and average pup weight ($p \leq 0.01$) compared to control group. Mean value of litter size, total litter weight and average pup weight (total and either sex) in groups treated with the test article at the dose levels of 10 and 20 mg/kg body weight were comparable with that of the control group. No significant change in sex ratio was found in pups due to treatment of deltamethrin 1% + triazophos 35 % EC **(Table 3.6 and 3.7; Figure 3.10-3.13).**

Dams of group G1, G2, G3 and G4 delivered a total of 53, 50, 48 and 29 pups respectively showing a certain reduction in the number of pups at the time of parturation. Moreover, on the day 21 of lactation only 18 pups survived from high dose group. Hence, various indices *viz.*, Mortality, survival and live birth were also reduced significantly in the high dose group **(Table 3.8).** However, other reproductive indices such as fertility, gestation, lactation, parturition and prenatal loss were not altered significantly **(Table 3.9).**

At the termination of experiment (on day 21 of lactation), all the pups from control and various treatment groups were subjected to gross pathological examination. Visceral examination of pups revealed gross changes such as pneumonic changes/lesions in lung such as congestion, consolidation, emphysema and hepatisation (G1: 3/53, G2: 5/46,G3: 6/43 and G4: 1/18); liver-congestion/mottling/pallor (G2: 5/46 and G3: 7/43); spleen enlargement (G4: 1/18) and kidney cyst (1/18) in high dose group **(Table 3.11).** These changes appear nonspecific, spontaneous and treatment independent as they were not overtly/exclusively expressed by the experimental groups. External and visceral examination of the pups did not reveal any treatment related pathological changes.

Gross and histopathological evaluation of the reproductive organ of male and female rats treated with delatmethrin1% + triazophos 35% EC was carried out at the termination of the

experiment. Gross pathological evaluation revealed changes like testis-atrophy and uterus-hydrometra. Histopathological assessment of male rats showed lesions in prostate (hyperplasia), focal and diffuse degenerative changes in testis, degenerative/atrophic changes in seminiferous tubules **(Figure 3.17and 3.18).**

Lumina of ductus epididymis was completely devoid of spermatozoa and epithelial lining showed hyperplasia (2 - 4 layers). The lumina of sum of the ductus epididymis contain tissue detritus, focal vacuolation of living epithelium of ductus epididymis **(Figure 3.14-3.16).**

The histopathological evaluation of female rats revealed lesions in ovary (angiectasis). Uterus showed endometrial glandular hyperplasia/luminal dilation. Mammary glands were undeveloped **(Figure 3.19-3.21).**

## DISCUSSION

The safety evaluation of pesticide for reproductive toxicity has to take into consideration several factors, which are not normally relevant for drug testing. Exposure to a toxic agent may affect the reproductive system at various stages of life prenatal as well as postnatal **(Ratcliffe *et al.*, 1995).** In the current investigation therefore, the effect(s) of a combination pesticide on the reproductive system of male and female rats were evaluated by conducting a single generation toxicity test.

The current treatment of deltamethrin 1% + triazophos 30% EC elicited clinical signs *viz.* salivation, tremor, lacrimation and nasal discharge in the male rats of high dose group **(Table 3.10).** One male rat from the high dose group i.e. 30mg/kg body weight was found dead during 5$^{th}$ week of the treatment. The others however, showed mild clinical symptoms. These toxic symptoms were indicative of CNS involvement and form part of choreoathetosis salivation (CS) syndrome normally associated with alpha-cyano pyrethroid compound. Similar symptoms were also noticed by **Shiva Kumar and Coworkers (2002)** in rats administered with deltamethrin. Therefore it is logical to surmise that the CS syndrome observed in the present study could be a result of pyrethroids component of the combination pesticide.

The exposure to test article evoked a dose dependent and significant reduction in body weight in male and female rats that is well correlated with the reduced feed intake observed in the treated rats. Similar loss in body weight of rats was reported due to treatment of monocrotophos **(Janardan and Sisodia, 1990).** Chronic exposure to deltamethrin is known to reduce the body weight **(Shaker *et al.* 1998).** Rats treated with deltamethrin at the dose

level of 2.5 and 10 mg/kg body weight /day for a period of 13 weeks showed lower body weight gain **(Hunter *et al.*, 1977).** A three-generation study of deltamethrin in Charles River rats revealed decrease in body weight of $F_0$ male, and also in feed consumption of $F_1$ male parent rats at 50mg/kg body weight **(Wrenn *et al.*, 1980). Singh *et al.*, (2001)** suggested that malabsorption and hepatic dysfunction might be responsible for loss of bodyweight in pyrethroids poisoned animals. The present test article appears to be a potent anorexic agent, together with the reported malabsorption and digestion associated with the active principle would account for the reduced bodyweight gain in treated animals.

The functions of male reproductive system are to produce and transfer spermatozoa to the female for fertilization of the mature egg cell. It also synthesizes male sex hormone – testosterone which influences the development of secondary sexual characters. There are two critical points of concern in male reproductive toxicity. The first is the production of sufficient sperms, which are capable of fertilizing an egg and second is the production of sperm with normal chromosome number, structure and genetic material. A toxic agent may produce alteration in spermatogenesis via an effect on the hypothalamus, pituitary, testicular axis or effect on accessory sex gland function or sexual function (libido, potency, ejaculation), which may result into failure in fertilization. However, a toxin may cause genetic or chromosomal damage to the germ cell, so that if a sperm carrying damaged genetic material fertilizes an egg, fetal death or structural/functional abnormality in the newborn occurs **(Raticliff *et al.*, 1995).**

Multiple end points of spermatotoxicity were investigated in a number of chemicals those are reported to produce mild to severe reproductive effect in Sprague Dawley rats. Most toxicologically sensitive end points used for the assessment of a potential male reproductive toxicant are testicular histology; testicular sperm head counts, cauda sperm counts, sperm morphology and sperm velocity. Apart from the above parameters histopathology of testis and epididymis is also considered as the most consistent indicator of reproductive damage **(Linder *et al.*, 1992).**

In the present experiment, rats treated with deltamethrin 1% + triazophos 35 % EC revealed significant inhibition of motile sperm at high dose group. The finding is in agreement with the observations of **Okamura *et al.*, (2004)** who has reported significant decrease in sperm motility in Wistar rats treated with organophosphorus insecticide (dichlorvos) at 2 mg/kg subcutaneously for a period of 9 weeks. **Buttar *et al.* (1997)** observed decrease in motility in rats treated with Alpha-chlorohydrin (ACH) and suggested that this could be most likely due to its direct toxicity on developing spermatocytes/spermatids and epididymis or both. One of the obvious effects of epididymal maturation is that spermatozoa display changes in

the pattern and effectiveness of their flagellar motion. The alteration in sperm motility during epididymal maturation might be due to changes in ATP metabolism, ion concentration and enzymatic activity in the spermatozoon **(Eddyy and O'Brienda, 1989). Knobil *et al.*, (1994)** also suggested the decline in sperm motility due to altered epididymal physiology since epididymis is responsible for sperm maturation, which is controlled by circulating androgen.

It has been clearly established that mammalian spermatozoa leaving the testis contain a cytoplasmic droplet. Generally, a greater proportion of spermatozoa loose their cytoplasmic droplet during their transit from carpus to cauda epididymus, since loss of cytoplasmic droplets is considered as an index of spermatozoa maturation in mammals **(Bedford, 1975).** On leaving the testis, the cytoplasmic droplet is located in the region near to head of spermatozoon. Subsequently it moves posteriorly along the midpiece until it reaches the point of annulus and then is shed when spermatozoon leaves the corpus epididymidis **(Hermo *et al.*, 1988).** The loss of the cytoplasmic droplet from the spermatozoon represent an additional reduction in cell volume occurring extra testicularly and also after a considerable amount of the time once the spermatozoa is released from the Sertoli cells **(Gist *et al.*, 1992).** The cytoplasmic droplet plays a role in estrogen biosynthesis in the luman of male reproductive tract. **Janulis *et al.*, (1996, 1998)** demonstrated the activity of P450 aromatase, the enzyme that converts androgen to estrogen in the cytoplasmic droplet.

**Akbarsha *et al.*, (2000)** found that 60 – 95% of spermatozoa residing in the lumen of cauda epididymidis of Wistar rats treated with organophosphate insecticide for 48 days retained cytoplasmic droplet and hence the motility of spermatozoa released from cauda epidimidis was inhibited. Therefore, it is logistic to conclude that one of the mechanisms of action of this toxicant on male reproductive function may be the retention of the cytoplasmic droplet and the resultants impairment of sperm motility.

Testicular homogenization resistant spermatids data are particularly useful for confirming reductions in sperm production. Deltamethrin 1% + triazophos 35% EC administered to male rats significantly reduced testicular homogenization resistant spermatids and epididymal sperm counted. Decrease in testicular homogenization resistant spermatids indicates reduced number of elongated spermatids. This could be due to direct effect of test article on these cells or due to maturation depletion following effects on an earlier cell type. Exposure of deltamethrin 1% + triazophos 35% EC also exhibited significant reduction in epididymal sperm count in this experiment which may be due to antispermatogenic nature of this combination. **Lanning *et al.*, (2002)** suggested that a reduction in testicular homogenization resistant spermatids coincides with a decrease in epididymis sperm count.

Results of the current study therefore suggest that the pesticide combination of deltamethrin1% + triazophos 35% EC is inhibiting the sperm production and motility either directly or indirectly. However the exact mechanism through which this is achieved needs to be further evaluated.

In an attempt to identify potential male reproductive toxicant, various end points have been proposed as a screen. **Wyrobek and Bruce (1975)** have suggested that epididymal sperm morphology may be useful in assessing the effects of spermatotoxicants. **Meistrich (1993)** suggested that the morphogenesis of the spermatid nucleus is important in production of a spermatozoon that is transported to the ovum effectively and is capable of achieving successful fertilization. Defects in sperm nuclear morphogenesis, for example abnormal shape or incomplete nuclear condensation, results in lowered fertility. Moreover, sperm morphology is a useful end point for providing insight on certain toxic manifestations. For example mouse sperm head morphology test is widely used in screening for mutagenic chemical. Increasing attention has been given to sperm head morphology as endpoints of spermatotoxicity and even fewer studies have included sperm morphology as a tool for the assessment of flagellar changes **(Linder *et al.*, 1992).** Exposure of deltamethrin 1% + triazophos 35% EC revealed a progressive increase in various morphological abnormalities in sperm. These morphological changes might be considered due to the toxicant induced germ cells mutagens on the differentiation of spermatogenic stem cells as suggested by **Ron Filler (1993).** It has been demonstrated that the administration of a wide variety of chemicals to male rats has been associated with an increase in abnormal sperm morphology. Moreover, adverse effects on other male reproductive system such as reproductive organ weight, testis histopathology, sperm number, and sperm motility may accompany these morphological alterations **(Ron Filler, 1993).**

In present investigation, histopathological observation in epididymis revealed definite alterations such epithelial vacuolation, complete absence of spermatozoa etc. Examination of epididymal epithelium, histological examination of longitudinal section of the epididymus, provides further information regarding testicular damage. Increased incidences of immature germ cell, residual bodies, cycloplasmic debris, or reduced sperm number in the epididymal lumen are indication of possible treatment related testicular damage **(Linder *et al.*, 1992).** Microvacuolation of the epididymal epithelium can be treated as a specific chemically induced toxic manifestation **(Creasy, 2001).** Microvacuolation and cribriform changes (infolding of the epithelium within itself) is often seen accompanied by concentration of the atrophic aspermic epididymis. This may represent a normal mechanism of surface area reduction but also has been reported as a toxicological change **(Foley, 2001).** Fluid

absorption and secretion are both major functions of the epididymal epithelium; vacuolation is a likely sequel to disturbance of either function **(Lanning *et al.*, 2002).**

Histopathological changes found in testis were focal and diffuse degenerative changes in seminiferous tubules with interstitial hyperplasia. The most common degenerative change in rats' testis is degeneration and loss of germinal epithelium with seminiferous tubules. Sertoli cells with few or no spermatogenic cells, particularly mature spermatid, line the affected seminiferous tubules. Cell debris and multinucleated giant cells that represent fused spermatic nuclei are present in the affected tubules particularly in the early stages of degeneration. These giant cells, immature germ cells, and cell debris are present in the duct of epididymis when there is testicular degeneration with marked generation and loss of spermatogonial epithelium, diameter of the tubules is often decreased, the wall is slightly thickened, at this stage when testis is grossly reduced in size and germ cells are rarely present and the term atrophy is often used to characterize these lesion **(Boorman *et al.*, 1990).**

Hyperplasia is characterized by aggregates of interstitial cells between the seminiferous tubules. The cells typically have abundant vacuolated or granular eosinophilic cytoplasm. An aggregate of interstitial cells smaller than diameter of seminiferous tubules is a focal hyperplasia. A mass of interstitial cells with a diameter greater than that of seminiferous tubules is considered an adenoma. Some imbalance or lack of negative feedback inhibition may allow pituitary stimulation of the interstitial cells which eventually results in incidence of proliferative lesion **(Boorman *et al.*, 1990).**

The currently observed male reproductive anomalies in the treated animals *viz.* reduced cauda epididymal sperm motility, testicular sperm head count, epididymal sperm count and induced morphological abnormalities might have been the reason for the reduced fertility index and smaller litter size observed in high dose group animals. The mating efficiency of male rats was also reduced due to this combination insecticide. It is known that the insecticides are able to bind to the biochemical component of the cells to exert these toxic effects. Many xenobiotics are known to inhibit spermatogenic activity in testis of the animals including man **(Zenick *et al.*, 1994).** Thus, testis and sperm parameters affected by this treatment were finally contributory to a loss of fertility potential of treated rats. Hence a decrease in the fertility index could be explained.

On the other hand when it comes to the female reproductive system, successful pregnancy depends on two sets of physiological events. Transport of gametes through the reproductive tract (so that fertilization can be effected) and establishment of an appropriate hormonal

environment through cervical stimulation, so that the fertilized egg can implant in the uterus and be maintained during subsequent gestation **Chapin and Heindel (1993).**

Compound that disrupts ovarian cycle and thus the fertility potential could induce a pattern of constant vaginal estrous, repetitive pseudo-pregnancies, or anestrous condition. In present investigation, during mating period regular examination of the mated females for the presence of cervical plug or sperm in vaginal smear revealed disturbance in the estrous cycle in female rats (one each from the dose level of 20 and 30 mg/kg body weight). Stages of estrous cycle and interconversion of their sub stages (proestrous, estrous, metaestrous and diestrous) are governed by neuroendocrine control **(Freeman, 1994).** Disturbed estrous cycle might be due to disturbed hypothalamic pituitary - ovarian axis where by both estrogen and progesterone in combination interfere with pituitary gonadotropic function through reduction of FSH and suppression of LH peak as suggested by **Singh *et al.*, (1998).** Constant vaginal estrous usually indicates that the female cannot achieve an ovulatory surge of LH. The ovaries of such females are polyfollicular and contain no corpora lutea. Anestrous or prolonged vaginal diestrous, may be indicative of compounds that interfere with follicular development or deplete the pool of primordial follicles. Irregular cycles may reflect impaired ovulations, as delayed ovulation may extend the period of vaginal cornification (**Cooper *et al.*, 1993).** During histopathological evaluation two female rats (one each from 20 and 30 mg/kg body weight) showed undeveloped mammary gland, which might be correlated with the findings of estrous cycle since these rats never got pregnant.

In the present study, treatment of deltamethrin 1% + triazophos 35% EC at 30 mg/kg bodyweight revealed reduction in body weight and feed consumption in dam during gestation and lactation and has caused significant diminution in litter size, total litter weight, live birth index at high dose group.

Significant reduction in average dam's weight and feed consumption may be probably due to malnutrition arising from indigestibility and malabsorption, while litter size, average pup weight and live birth indices may be reduced because of direct maternal toxicity. Treatment related reduction in the total litter weights was found to be significant at high dose group and was a result of reduced litter size.

Present findings are in agreement with the findings reported during the evaluation of embryotoxic and teratogenic effect of organophosphate (monocrotophos) following daily oral administration at the dose level of 2 and 4 mg/kg for whole gestation period in female albino rats by **Singh and Sharma (1998).** Dose related reduction ($P \leq 0.01$) in maternal

weight gain during pregnancy was observed in rats treated with deltamethrin at 5-mg/kg-body weight during day 7-20 of gestation, although treatment did not affect number of implantation site and fatal mortality weight **(Kavlock *et al.*, 1979).** A three-generation reproduction of deltamethrin in rats evoked slight decrease in mean pup weight at high dose group of 50mg/kg body weight **(Wrenn *et al.*, 1980). Chapin and Heindel (1993)** had suggested that the most satisfying demonstration of reproductive toxicity is decrease in pup number which supports the data observed in high dose group in the present study, while fewer live pups in treated female indicate a female reproductive toxicity probably induced due to altered estrous cyclicity and/or altered mating behavior.

The histopatholical evaluation revealed alterations in ovary (inflamatic changes/ angiectasis), uterus (endometrial glandular hyperplasia/luminal dilation) and Mammary gland (undeveloped). The changes observed in ovary and uterus was found to be inconsistent and hence, may not be treatment related. However undeveloped mammary gland might be correlated with the disturbance observed in the estrous cycle of one female each from mid dose and high dose. It can thus be deduced that xenobiotic induced disturbed estrous cycle may lead to infertility in females.

Results of the current study therefore suggest that the pesticide combination of deltamethrin1% + triazophos 35% EC is inhibiting the sperm production and motility either directly or indirectly. Further the pesticide combination is also a potent maternal toxicant as evidenced by altered histological profile of the female reproductive organs and litter size in the treated dams. However the exact mechanism through which these changes are achieved needs to be further evaluated

**TABLE 3.1 Body weight, feed consumption and sperm evaluation in male rats at the termination of Study**

| Parameter | Group Number and Dose (mg/kg body weight) | | | |
|---|---|---|---|---|
| | G1 (0) | G2 (10) | G3 (20) | G4 (30) |
| Body Weight (g) | 397 ± 5.85[@] | 381 ± 3.20 | 326 ± 6.15**↓ | 291 ± 4.11**↓ |
| Feed Consumption (g) | 151.6 ± 2.87 | 130.8 ± 7 52*↓ | 121.0 ± 4.94**↓ | 99.0 ± 1.79**↓ |
| Sperm Motility (%) | 87.12 ± 0.93 | 85 72 ± 0.44 | 84 72 ± 1.14 | 81.92 ± 0.37**↓ |
| Epididymal count ($10^6$/g) | 1458 8 ± 8.11 | 1382.5 ± 10.70 | 1354.00 ± 27.78*↓ | 869.80 ± 52.79**↓ |
| Sperm head Count ($10^6$/g) | 135.50 ± 2.57 | 123 00 ± 3.23*↓ | 114 25 ± 1 16**↓ | 99.63 ± 4 43**↓ |
| Sperm abnormality (%) | 5 9 ± 1.53 | 6.18 ± 1 31 | 6.20 ± 1 8 | 7 00 ± 1.58 |

[@] Mean ± SE, * p≤0.05, ** p≤0.01

**TABLE 3.2 Body weight (g) of female rats at the end of pre-mating, gestation and lactation period**

| Duration | Group Number and Dose (mg/kg body weight) | | | |
|---|---|---|---|---|
| | G1 (0) | G2 (10) | G3 (20) | G4 (30) |
| Pre-mating (Week 0) | 230 ± 3.69[@] | 229 ± 3.39 | 229 ± 3.43 | 228 ±3.02 |
| Pre-mating (Week 2) | 233 ± 3.46 | 227 ± 2.93 | 224 ± 4.64 | 215 ± 2.95**↓ |
| Gestation (Day 0) | 227 ± 2.18 | 226 ± 3.12 | 216 ± 7.18 | 225 ± 3.42. |
| Gestation (Day 20) | 376 ± 5.16 | 364 ± 9.45 | 344 ± 17.73 | 312 ± 7.64**↓ |
| Lactation (Day 1). | 287 ± 13.15 | 292 ± 10.33 | 275 ± 16.09 | 251 ± 323 |
| Lactation (Day 7). | 290 ± 13.13 | 293 ± 7.46 | 278 ± 16.83 | 247 ± 4.37*↓ |
| Lactation (Day 21). | 276 ± 11.46 | 274 ± 13.05 | 254 ± 16.25 | 252 ± 5.87 |

[@] Mean ± SE, * p≤0.05, ** p≤0.01

**TABLE 3.3 Feed consumption (g) of female rats during pre-mating, gestation and lactation period**

| Duration | Group Number and Dose (mg/kg body weight) | | | |
|---|---|---|---|---|
| | G1 (0) | G2 (10) | G3 (20) | G4 (30) |
| Pre-mating (week 1) | 115 40 ± 4 51@ | 114.40 ± 3 76 | 104.40 ± 6.19 | 58.6 ± 3.33**↓ |
| Pre-mating (week 2) | 126.40 ± 3 09 | 121.40 ± 1.75 | 113.36 ± 1.89 | 81.20 ± 7.93**↓ |
| Gestation (day 0-6) | 152 50 ± 3.23 | 142.00 ± 5 97 | 137 00 ± 1.91 | 129.25 ± 4.714 |
| Gestation (day 7-14) | 180 25 ± 8 39 | 177.5 ± 9.89 | 176 25 ± 6.25 | 169.75 ± 2.32 |
| Gestation (day 15-20) | 183.50 ± 7.05 | 180.00 ± 8 66 | 176.75 ± 7 73 | 167 25 ± 3.42 |
| Lactation (day 1-4) | 154 ± 14 38 | 132.75 ± 6.18 | 129.75 ± 5 95 | 114.75 ± 5.69*↓ |
| Lactation (day 5-7) | 153 50 ± 5.95 | 141 25 ± 6 48 | 136.25 ± 112.25 | 112 25 ± 7.157**↓ |
| Lactation (day 7-14) | 207.00 ± 16.02 | 194.25 ± 9 70 | 172.00 ± 8.63 | 137.5 ± 6.40**↓ |
| Lactation (day 15-21) | 233.75 ± 14 38 | 230 00 ±11 68 | 221.50 ± 19.78 | 153 75 ± 3.12**↓ |

@ Mean ± SE, * $p \leq 0.05$, ** $p \leq 0.01$

**TABLE 3.4 Absolute organ weight (g)**

| Organ | Group Number and Dose (mg/kg body weight) | | | |
|---|---|---|---|---|
| | G1 (0) | G2 (10) | G3 (20) | G4 (30) |
| Testes | 3.24 ± 0.13@ | 3.22 ± 0.58 | 3.65 ± 0.18 | 3.27 ± 0.16 |
| Epididymus | 0.613 ±0.03 | 0.626 ± 0.08 | 0.705 ± 0.09 | 0.64 ± 0.053 |
| Ovaries | 0.102 ± 0.012 | 0.080 ± 0.023 | 0.092 ± 0.015 | 0.140 ± 0.013 |
| Uterus | 0.350 ± 0.036 | 0.352 ± 0.079 | 0.410 ± 0.061 | 0.618 ± 0.088*↑ |

@ Mean ± SE, * $p \leq 0.05$

**TABLE 3.5 Relative organ weight (g)**

| Organ | Group Number and Dose (mg/kg body weight) | | | |
|---|---|---|---|---|
| | G1 (0) | G2 (10) | G3 (20) | G4 (30) |
| Testes | 0.816 ± 0.03@ | 0.843 ± 0.15 | 1.120 ± 0.06 | 1.12 ± 0.06 |
| Epididymus | 0.15 ± 0.01 | 0.16 ± 0.02 | 0 22 ± 0 03 | 0.22 ± 0.02 |
| Ovaries | 0.037 ± 0 004 | 0.028 ± 0.006 | 0.037 ± 0.007 | 0.055 ± 0.004 |
| Uterus | 0.128 ± 0.017 | 0.125 ± 0.022 | 0.164 ± 0.027 | 0.244 ± 0.032*↑ |

@ Mean ± SE, * $p \leq 0.05$, ** $p \leq 0.01$

**TABLE 3.6 Litter size during lactation**

| Day of Lactation | Group Number and Dose (mg/kg body weight) | | | |
|---|---|---|---|---|
| | G1 (0) | G2 (10) | G3 (20) | G4 (30) |
| 1 | 10.60 ± 0.93[@] | 10 00 ± 0.89 | 12.00 ± 1.22 | 7.25 ± 2.02 |
| 4 | 10.60 ± 0.93 | 10.00 ± 0.89 | 10.75 ± 1.11 | 4.50 ± 2.53*↓ |
| 7 | 10.60 ± 0.93 | 9.80 ± 0.92 | 10.75 ± 1.11 | 4.50 ± 2.53 *↓ |
| 14 | 10.60 ± 0.93 | 9 40 ± 1.12 | 10.75 ± 1.11 | 4.50 ± 2.53*↓ |
| 21 | 10.60 ± 0.93 | 9.20 ± 1.16 | 10.50 ± 1.19 | 4.50 ± 2.53*↓ |

[@] Mean ± SE, * p≤0.05

**TABLE 3.7 Litter size, litter weight, average litter weight and sex ratio on day 21 of lactation**

| Parameters | Group Number and Dose (mg/kg body weight) | | | |
|---|---|---|---|---|
| | G1 (0) | G2 (10) | G3 (20) | G4 (30) |
| Litter size | 10 60 ± 0 93 | 9 20 ± 1.16 | 10.50 ± 1.19 | 4.50 ± 2.53**↓ |
| Total litter wt. (g) | 348 40 ± 24.82 | 328.60 ± 20 54 | 296.50 ± 30.45 | 116 00 ± 53.86**↓ |
| Average litter wt (g) | 32.87 ± 0 42 | 35 72 ± 0.93 | 28.24 ± 0 67 | 25.78 ± 1.17**↓ |
| Number of male litter | 5.20 ± 0 66 | 5 00 ± 1.34 | 6.25 ± 1 03 | 2 00 ± 0 71*↓ |
| Male litter wt. (g) | 173.00 ± 19 93 | 174 40 ± 35.90 | 177.50 ± 35.33 | 56.00 ± 15.67**↓ |
| Ave. male litter wt. (g) | 33.27 ± 0 51 | 34 88 ± 1 10 | 28.40 ± 0.94 | 28.00 ± 1.95*↓ |
| Number of female litter | 5 40 ± 0.68 | 4.20 ± 0 86 | 4.25 ± 1.11 | 2.50 ± 1.85*↓ |
| Female litter wt. (g) | 175.40 ± 20 02 | 154.20 ± 31 79 | 119 00 ± 28.64 | 60.00 ± 40.01**↓ |
| Ave female litter wt. (g) | 32 48 ± 0 67 | 36 91 ± 1 56 | 28.00 ± 0.92 | 24 00 ± 1.22**↓ |
| Male/female sex ratio | 1 05 ± 0 21 | 1.69 ± 0 84 | 2 78 ± 1.74 | 0.88 ± 0.43 |

[@] Mean ± SE, * p≤0.05, ** p≤0.01

**TABLE 3.8 Pup mortality, survival index, lactation index, live birth index and postnatal loss**

| Parameter | | Group Number and Dose (mg/kg body weight) | | | |
|---|---|---|---|---|---|
| | | G1 (0) | G2 (10) | G3 (20) | G4 (30) |
| Survival Index (%) | On day 4 | 100.00 | 100.00 | 97.78 | 62.07**↓ |
| | On day 21 | 100.00 | 92.00 | 95.45 | 100.00 |
| Mortality Index (%) | days (0-4) | 0 | 0 | 2.22 | 37.93**↓ |
| | days (14-21) | 0 | 2.13 | 2.33 | 0 |
| Lactation Index (%) | | 100 00 | 92.00 | 95.45 | 100.00 |
| Live birth index (%) | | 100 00 | 100.00 | 100.00 | 62.07**↓ |
| Post natal loss (%) | | 0.00 | 0.00 | 1.00 | 0.00 |

[@] Mean ± SE, ** p≤0.01

**TABLE 3.9 Fertility data**

| Parameters | Group Number and Dose (mg/kg body weight) | | | |
|---|---|---|---|---|
| | G1 (0) | G2 (10) | G3 (20) | G4 (30) |
| Male Fertility Index (%) | 100 | 100 | 80 | 80 |
| Female Fertility Index (%) | 100 | 100 | 80 | 80 |
| Gestation Index (%) | 100 | 100 | 100 | 100 |
| Parturition Index (%) | 100 | 100 | 100 | 100 |
| Pregnancy Rate (%) | 100 | 100 | 80 | 80 |

**TABLE 3.10 Summary of clinical signs**

| Clinical Sign | Number of Animals showed Clinical Sign | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Male (N =5) | | | | Female (N=5) | | | |
| | G1 | G2 | G3 | G4 | G1 | G2 | G3 | G4 |
| Normal | 5 | 5 | 5 | 2 | 5 | 5 | 5 | 5 |
| Tremor | - | - | - | 1 | - | - | - | - |
| Lacrimation | - | - | - | 3 | - | - | - | 2 |
| Salivation | - | - | - | 3 | - | - | - | 2 |
| Nasal discharge | - | - | - | 4 | - | - | - | 3 |
| Death | - | - | - | 1 | - | - | - | - |

(Numerals indicate number of animals showing lesion)

**TABLE 3.11 Summary of lesions observed during gross pathological examination of pups**

| Organs and Lesion | Group | G1 | G2 | G3 | G4 |
|---|---|---|---|---|---|
| | Dose (mg/kg body weight) | 0 | 10 | 20 | 30 |
| | No. of Pups | 53 | 46 | 42 | 18 |
| **Lung:**Congestion/consolidation/emphysema/hepatisation | | 3 | 5 | 6 | 1 |
| **Liver:** Congestion/mottling/pallor | | - | 5 | 7 | - |
| **Spleen:** Enlargement | | - | - | - | 1 |
| **Kidney** : Cyst | | - | - | - | 1 |

Numerals indicate number of pups showing lesions

Figure 3.1: Terminal Body Weight of Male Rats

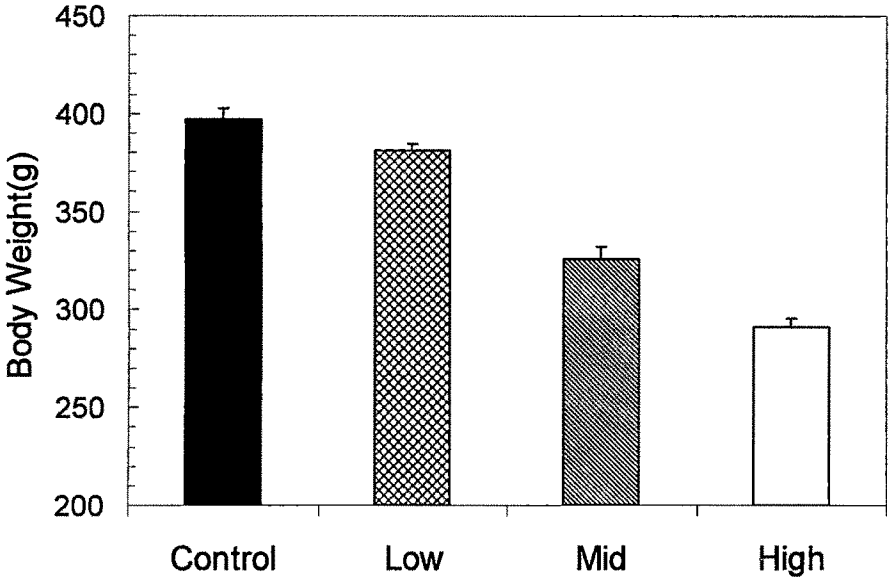
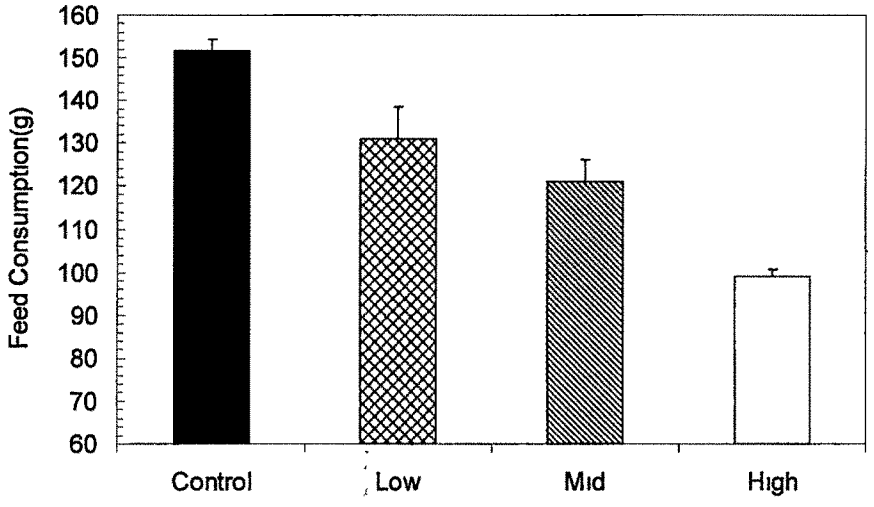



Figure 3.2 Terminal Feed Consumption In Male Rats



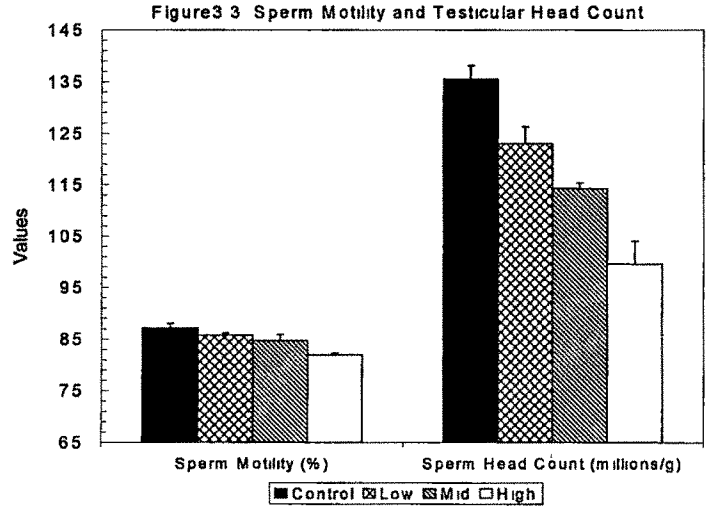



Figure 3.4: Epididymal Sperm Count

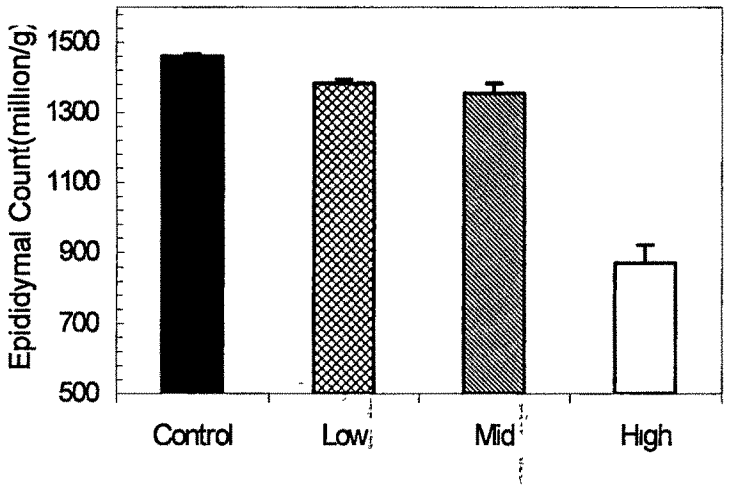



Figure 3.5:Sperm Abnormality (%)

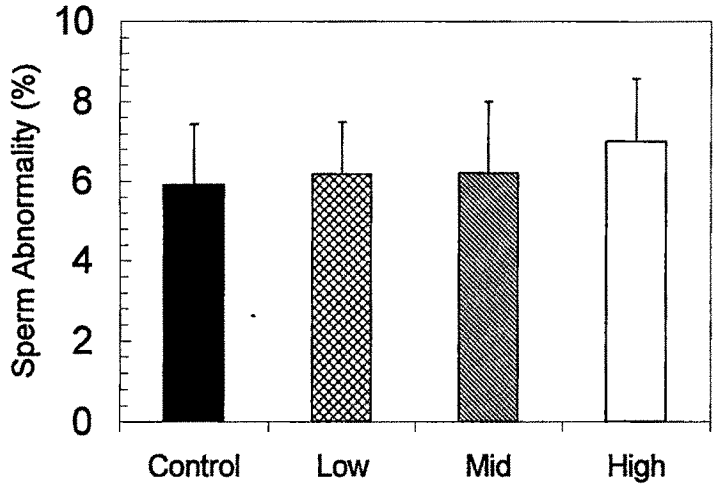


Figure3 6: Body Weight of Female Rats

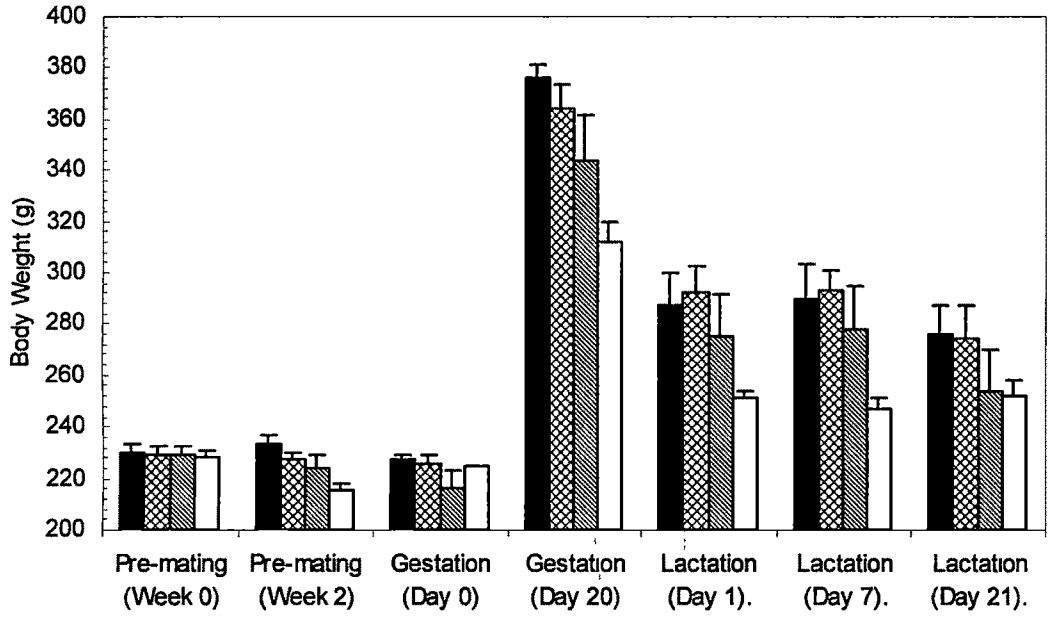



FIGURE 3.7: Feed Consumption in Female Rats

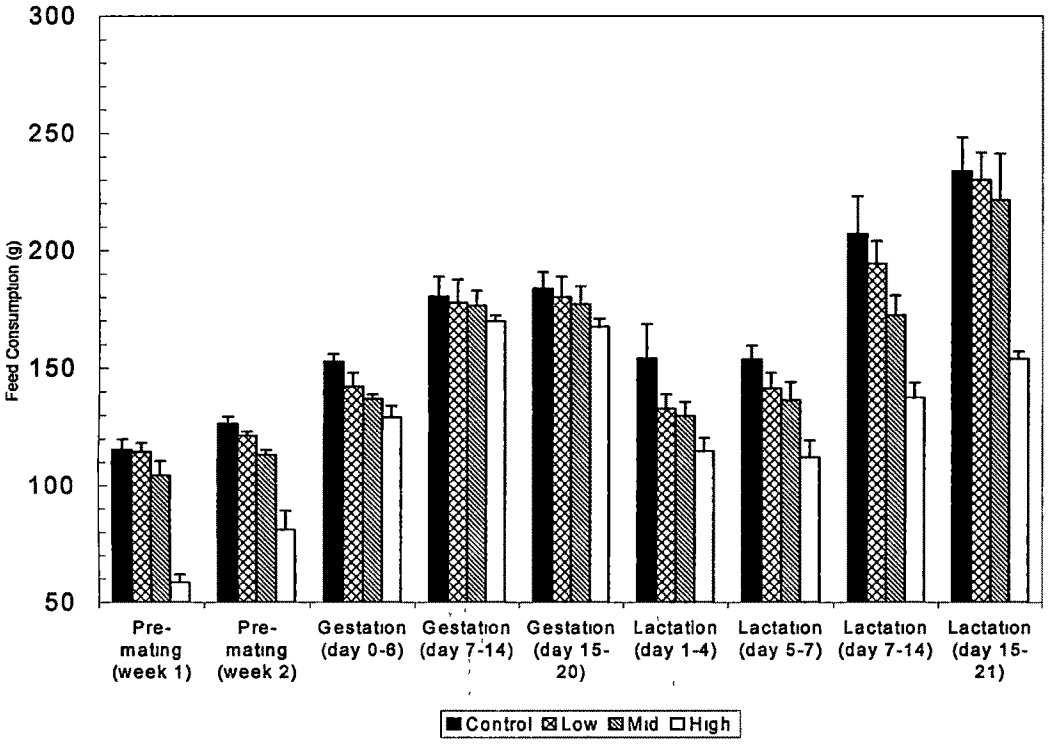


Figure 3.8:Absolute Organ Weights

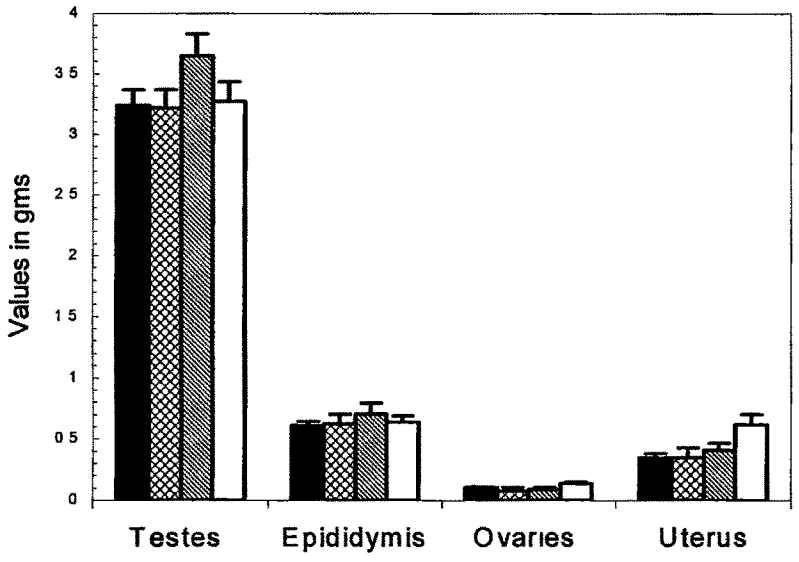



Figure 3.9: Realtive Organ Weights

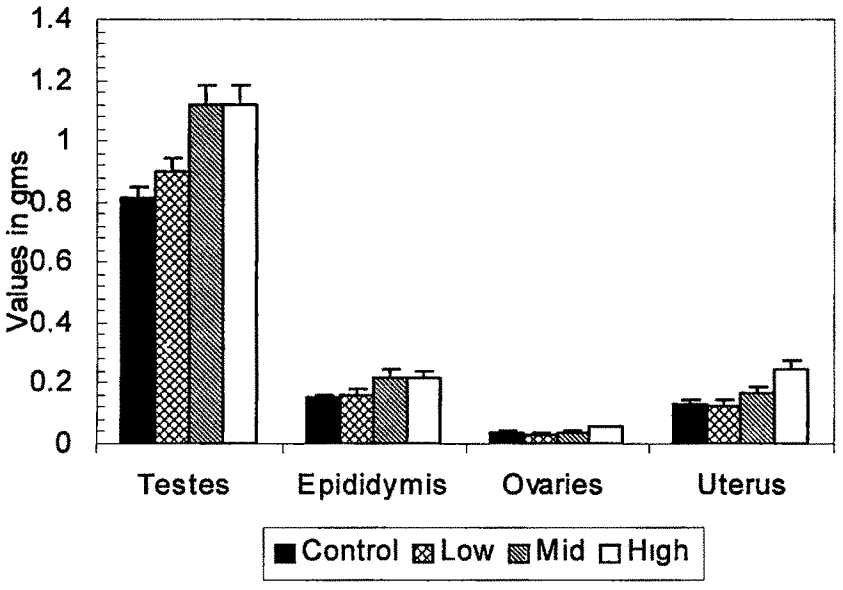


Figure 3 10 Litter Size

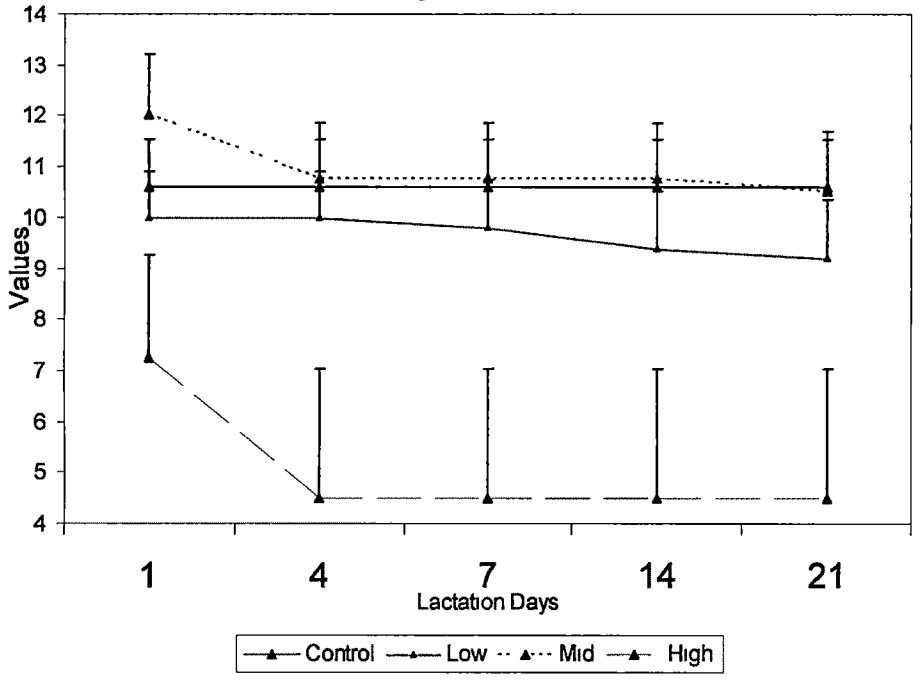



Figure 3.11: Pups on Day 21 of Lactation

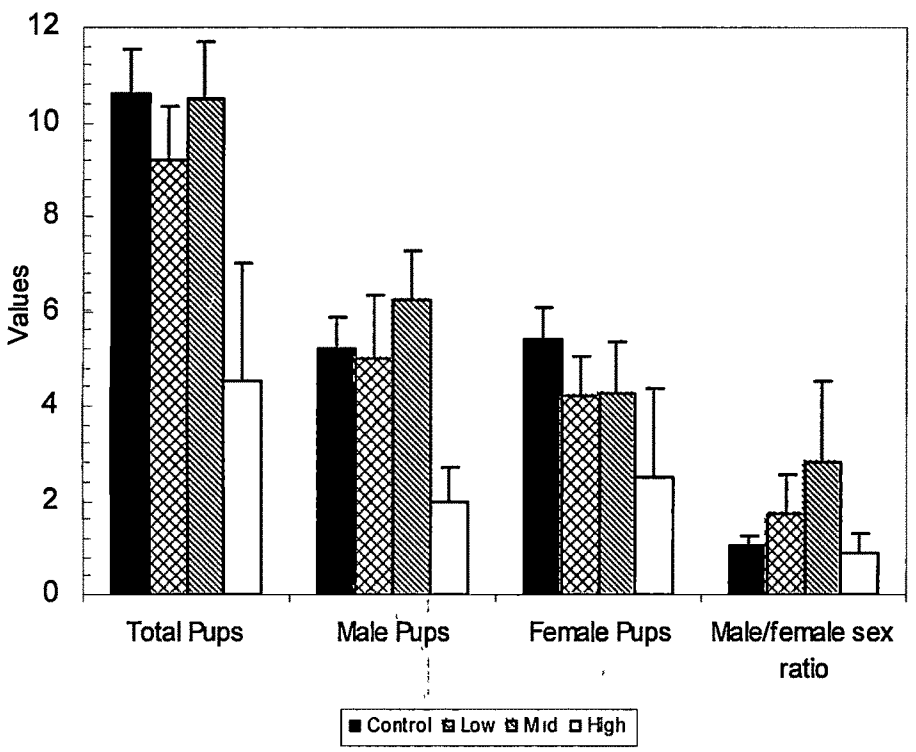


Figure 3.12. Pups Weight

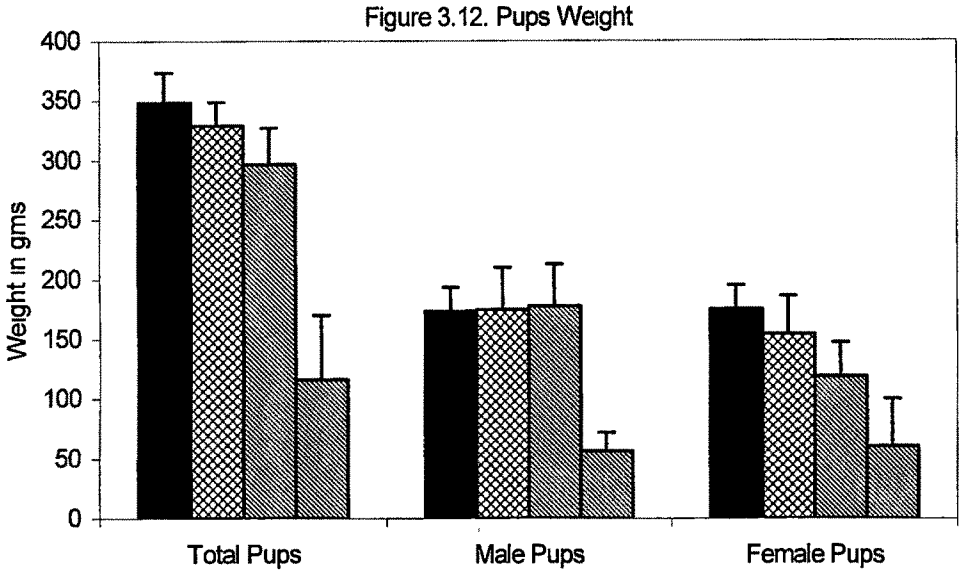
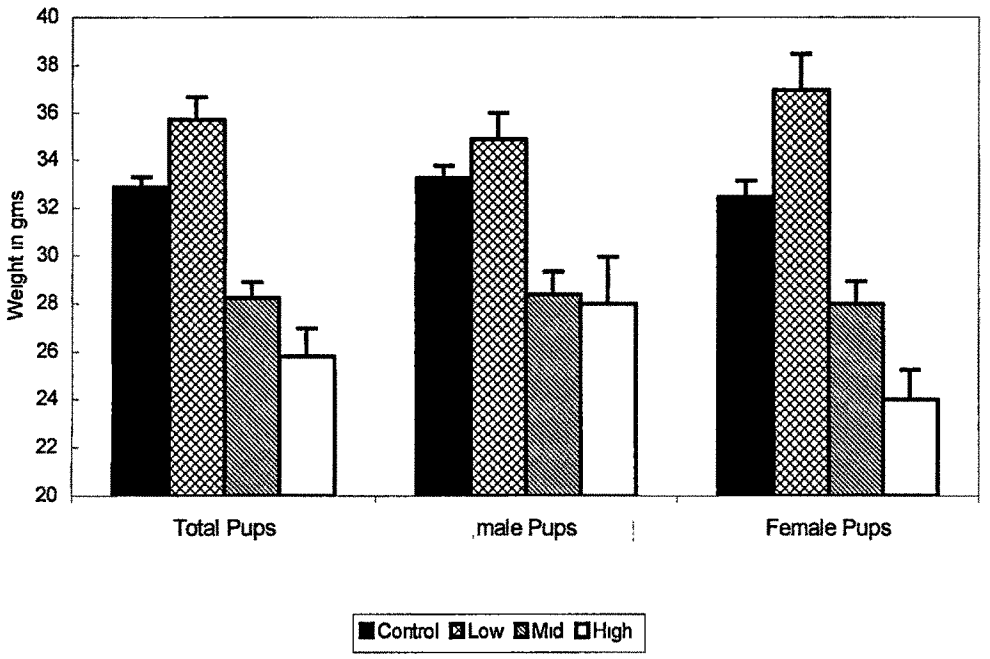



Figure 3 13 Average Pups Weight



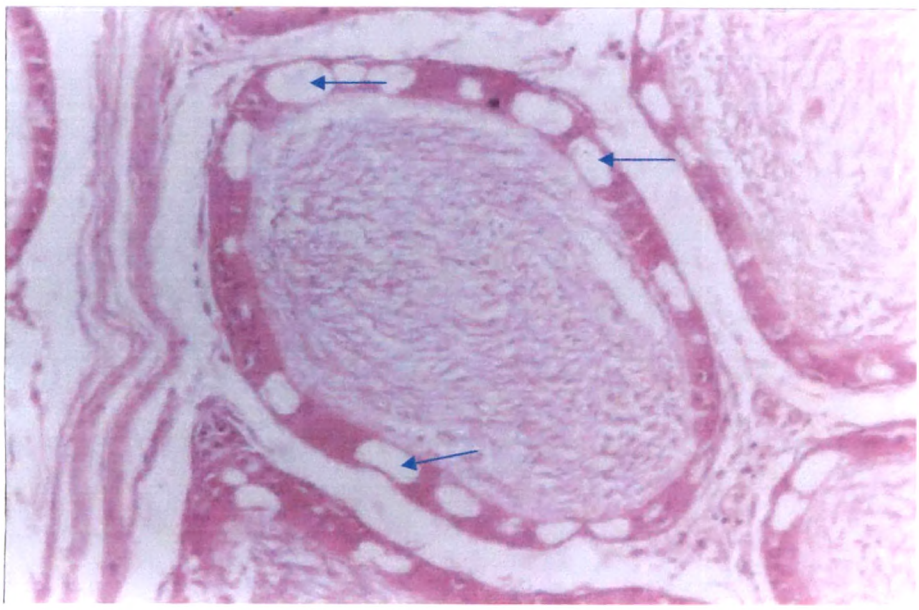


FIGURE 3.14 Epididymis showing epithelial vacuolation (⟵ )

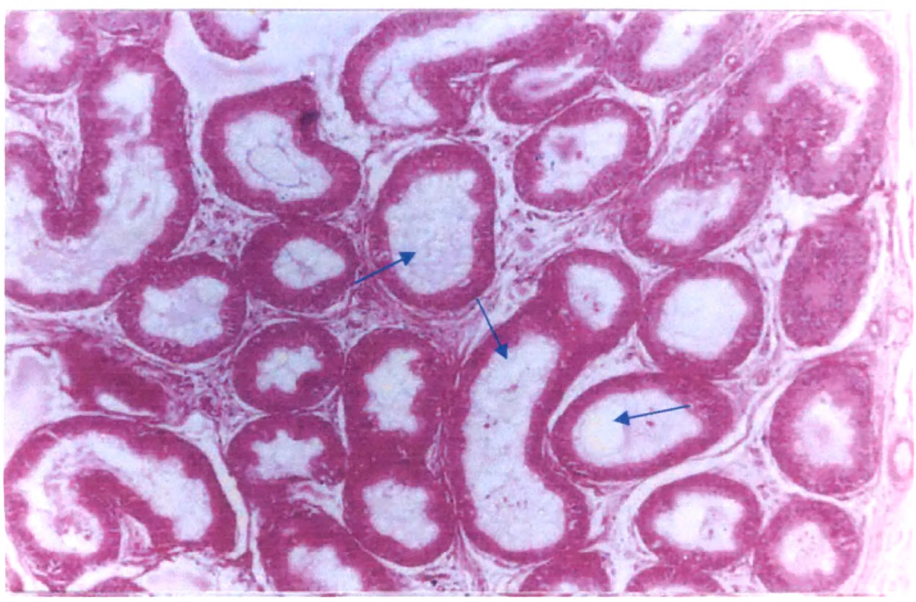


FIGURE 3.15 Epididymis showing complete absence of spermatozoa in lumina of ductus epididymidis X 4 (⟵ )

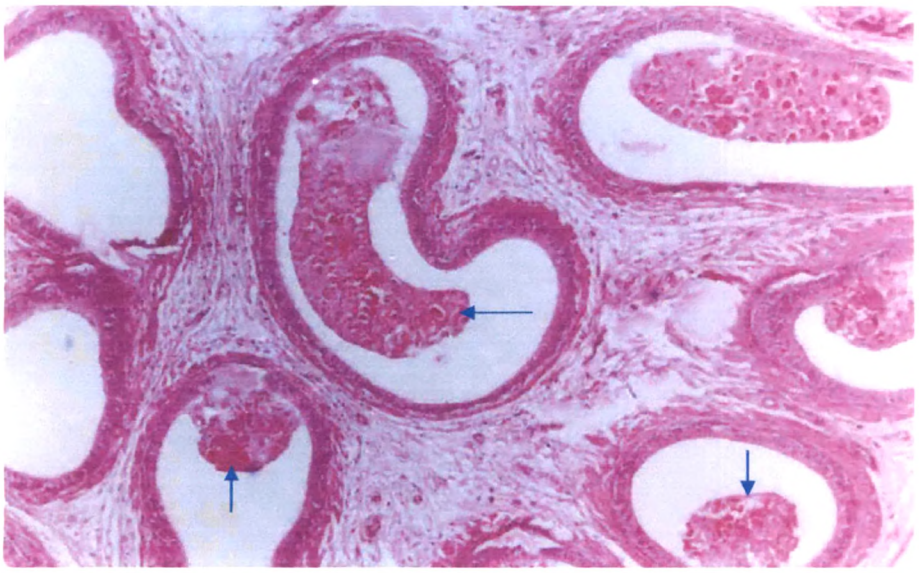


FIGURE 3.16 Epididymis: Lumina devoid of spermatozoa but containing tissue detrifitai X 4 (← )

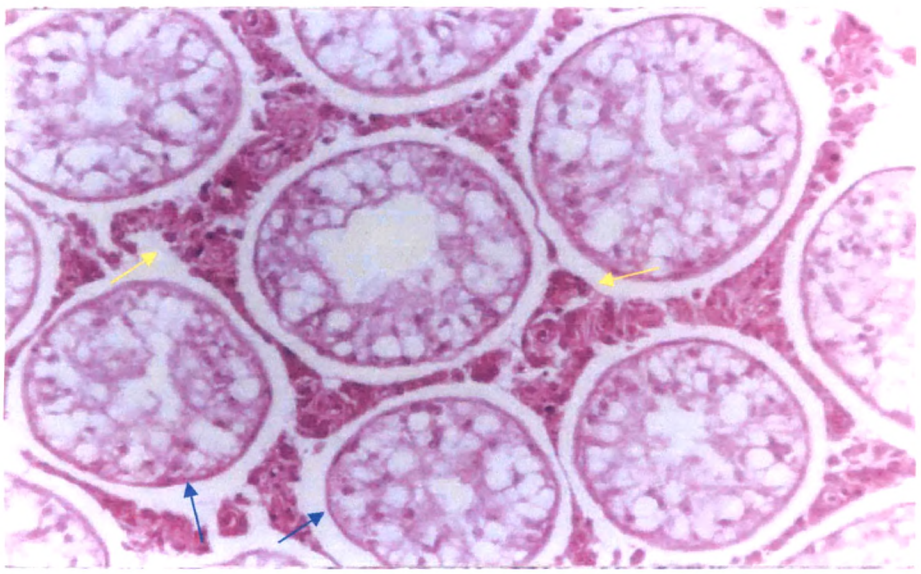


FIGURE 3.17. Testis: Diffuse (100%) degeneration changes ( ← )in seminiferous tubules with interstitial cell hyperplasia X 10 ( ← )

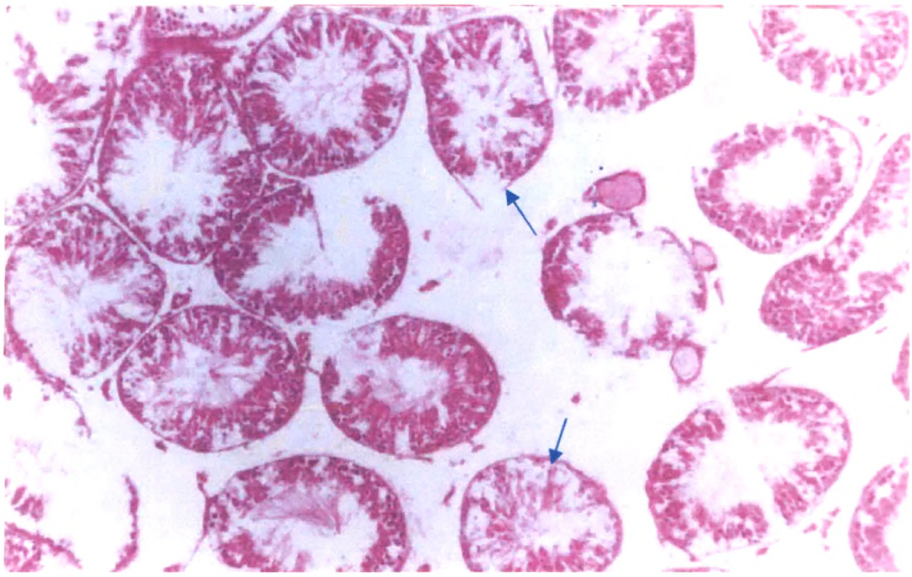


FIGURE 3.18 Testis Showing Focal Degenerative changes in seminiferous tubules ( ← )

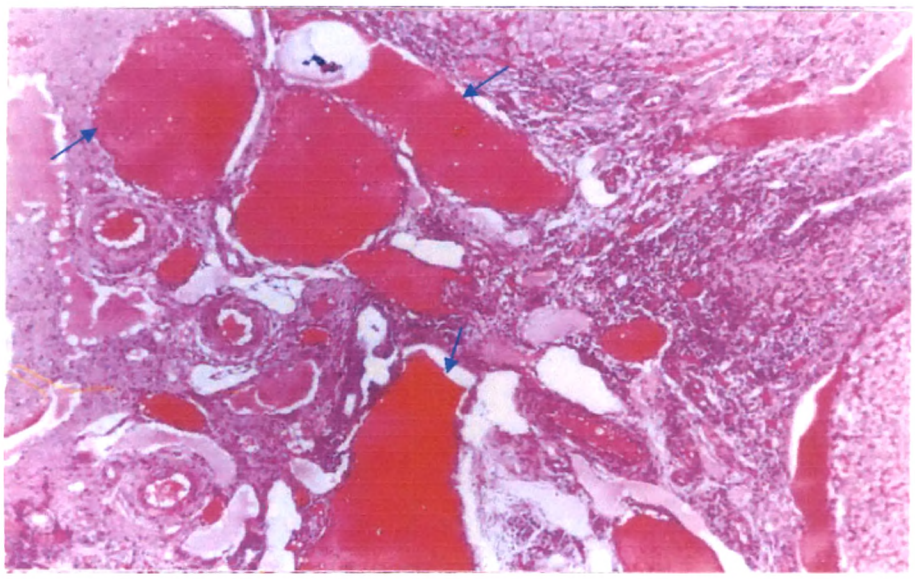


FIGURE 3.19 Ovary : Angiectasis X 4 ( ← )

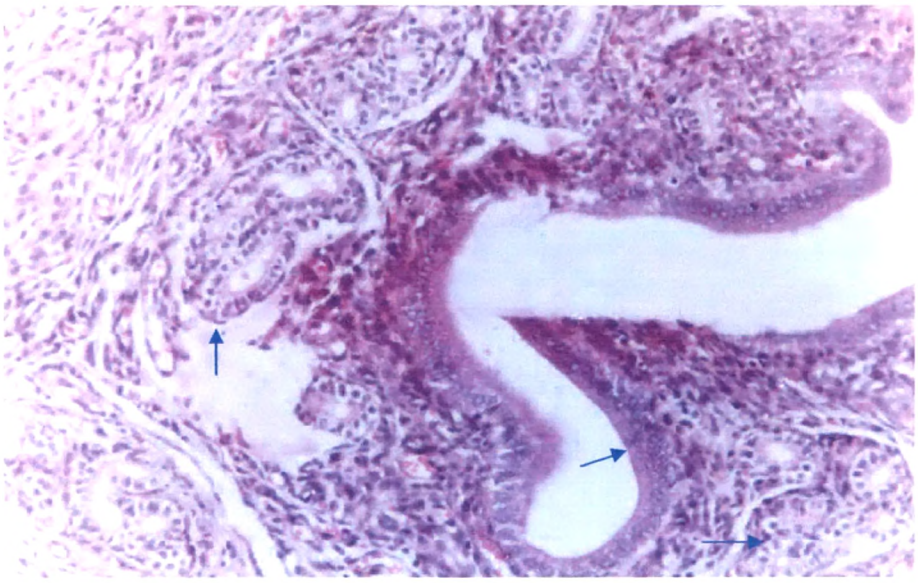


FIGURE 3. 20 Uterus showing endometrial glandular hyperplasia X 10 (← )

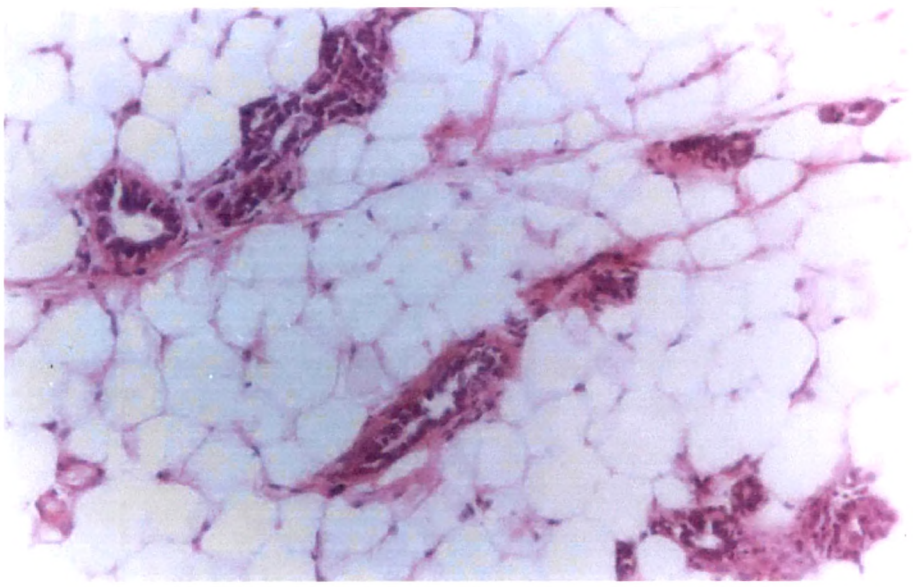


FIGURE 3.21 Mammary gland: undeveloped X 10