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**PATHOLOGY OF THE REPRODUCTIVE SYSTEM
OF JAPANESE QUAILS (*Coturnix coturnix japonica*)**

**By
SIVAKUMAR. V.**

**Thesis submitted in partial fulfilment of the
requirement for the degree of**

Master of Veterinary Science

**Faculty of Veterinary and Animal Sciences
Kerala Agricultural University**

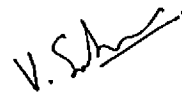
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**Centre of Excellence in Pathology
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
MANNUTHY, THRISSUR - 680651
KERALA, INDIA**

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I hereby declare that this thesis entitled "**PATHOLOGY OF THE REPRODUCTIVE SYSTEM OF JAPANESE QUAILS (*Coturnix coturnix japonica*)**" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

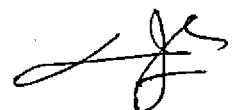
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SIVAKUMAR, V.

CERTIFICATE

Certified that the thesis entitled "**PATHOLOGY OF THE REPRODUCTIVE SYSTEM OF JAPANESE QUAILS (*Coturnix coturnix japonica*)**" is a record of research work done independently by **Shri. Sivakumar, V.**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.



Dr. Mammen J. Abraham

(Chairman, Advisory Committee)

Associate Professor

Centre of Excellence in Pathology

College of Veterinary and

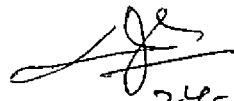
Animal Sciences, Mannuthy

Mannuthy

23.09.2003

CERTIFICATE

We, the undersigned members of the Advisory Committee of **Shri. Sivakumar, V.**, a candidate for the degree of Master of Veterinary Science in Pathology, agree that the thesis entitled "**PATHOLOGY OF THE REPRODUCTIVE SYSTEM OF JAPANESE QUAILS (*Coturnix coturnix japonica*)**" may be submitted by **Shri. Sivakumar, V.**, in partial fulfilment of the requirement for the degree.



24-1-2004

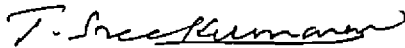
Dr. Mammen J. Abraham

Associate Professor

(Chairman, Advisory Committee)

Centre of Excellence in Pathology

College of Veterinary and Animal Sciences, Mannuthy




Dr. T. Sreekumaran

Director i/c and Head

Centre of Excellence in Pathology

(Member)



Dr. N. Divakaran Nair

Assistant Professor (Sel.Gr.)

Centre of Excellence in Pathology

(Member)



Dr. M. Mini

Assistant Professor

Department of Microbiology

(Member)



24.1.04

EXTERNAL EXAMINER

(B. MURALI MANOHAR)

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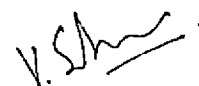
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SIVAKUMAR, V.

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Introduction

1. INTRODUCTION

Rearing of Japanese quails (*Coturnix coturnix japonica*, ITIS No. 176013) as a large scale industry has now been taken up by the farmers and it is gaining momentum by the years. Ever since the introduction of Japanese quails in India during the seventies, the industry has developed into a major alternative for diversification.

The quails are hardy birds as compared with chicken and ducks and hence are not prone to develop a large number of diseases. However, managerial errors like adopting lighting pattern formulated primarily for domestic fowls and non - practice of debeaking have predisposed the birds to certain ailments. This makes them vulnerable to pick up vices like vent pecking and cannibalism. Recently, the farmers have started realizing the importance of adopting managerial practices designed specifically for quails.

The loss by way of pullet mortality and loss of production due to non-specific diseases are of paramount importance while considering the obstacles for profitable quail farming, apart from the losses due to infectious diseases. The quails are reared mainly for egg production and this is better revealed by their production profile. On an average, a quail layer produces about 250 - 300 eggs in a year. The egg weight is nearly eight per cent of the body weight as against three per cent in chicken and one per cent in turkey. Apart from the fact that the eggs obtained from quails are delicacy items, they are of medicinal value especially in curing asthmatic conditions. Any disorder that affects the reproductive system will have a great impact on the production potential of the quail. A sound knowledge of the pathological conditions affecting the reproductive tract of Japanese quails is important to ensure persistent and better production in the flock.

Acute and generalized infections and epidemics which cause damage to the reproductive system can be recognised by the clinical manifestations and by the bacteriological examinations. But conditions which are slow and insidious in

their onset and without pronounced clinical manifestations pass unnoticed and they almost remain as the permanent cause of production loss and maintain their presence through cryptic spread. This group of diseases remains to be a poorly attended field of investigation and research in India and abroad. Though reproductive diseases form an important cause of production loss and mortality, their pathogenesis remains unexplored.

Though systematic investigations have been carried out on the pathology of the reproductive organs in the chicken and ducks, detailed knowledge on the reproductive pathology of the Japanese quail is lacking. Though bacterial organisms are considered to be the main etiological agents in reproductive diseases of domestic fowl and ducks, their involvement in reproductive diseases of quail has not been ascertained. The present work was undertaken to study the prevalence, pathogenesis, gross and histopathology of spontaneously occurring reproductive disorders and to characterise etiological agents, especially bacterial organisms, in Japanese quails. It is expected that this study will form a basis for understanding the pathological conditions affecting the reproductive tract of Japanese quails.

Review of Literature

2. REVIEW OF LITERATURE

2.1 PREVALENCE AND PATHOLOGY OF THE REPRODUCTIVE SYSTEM IN THE CHICKEN

The reproductive diseases have always been a significant entity among the various diseases affecting adult bird population. Valsala (1968) studied the pathology of reproductive system of poultry. Keymer (1980) noticed that obstruction of the oviduct and ectopic ovulation was the most prevalent reproductive disorder among birds which accounted for 28.6 per cent of the reproductive disorders. Mohiuddin (1982) found that egg peritonitis (7.82 per cent) and egg bound conditions (2.72 per cent) were the major causes of death in adult chicken. Bhowmik (1983) observed that the highest mortality in the adult duck was due to egg peritonitis (11.53 per cent), followed by non-specific enteritis (9.88 per cent), post-vaccinal paralysis (7.54 per cent) and impaction of oviduct (2.50 per cent). Christensen (1986) reported that egg peritonitis/impacted oviduct was the cause of mortality in 25 out of 37 birds autopsied.

Jayakumar (1986) studied the pathology of reproductive system of ducks. Yadgirkar *et al.* (1992) studied the mortality pattern in White Leghorn pure line stocks under continuous selection for egg production and concluded that the major causes of mortality were Marek's disease, lymphoid leukosis, fatty liver syndrome, coccidiosis, egg peritonitis and chronic respiratory disease. Reddy *et al.* (1994a) studied the prevalence of reproductive disorders in poultry. Randall and Reece (1996) described the histopathological details of various avian reproductive tract ailments like oophoritis, ovarian adenocarcinoma, granulosa cell tumour, arrhenoblastoma, salpingitis, sertoli cell tumour and seminoma.

2.1.1 Pathology of the Female Reproductive System in Chicken

2.1.1.1 *Developmental Defects*

Valsala and Sivadas (1970) encountered atrophy and hypoplasia of the left ovary and left oviduct in 7.5 per cent of the cases. The occurrence was more (88 per cent) in birds aged between six months and one year and the rest appeared in the older birds. They also reported "varicose follicles" also called haemorrhagic cysts of the ovary in hens.

Tudor (1979) reported a case of separate rectal and oviduct openings in a hen. Simultaneous presence of functional ovaries and oviducts both on the left and right sides was also reported.

Persistence of both non-functional ovary and oviduct on the right side as well as right oviduct only has also been reported (Jayakumar, 1986).

2.1.1.2 *Defects in the Process of Egg Laying and Oviduct*

Impaction of the oviduct and egg bound were considered to be the most common clinical conditions seen in chicken. Several authors (Valsala and Sivadas, 1970; Peckham, 1978) have described these conditions in chicken. Clinically the affected birds were observed to adopt the egg laying posture and strained to discharge the egg into the oviduct. At autopsy, one or more eggs in various stages of formation were seen in the oviduct.

Internal laying, also called as "Abdominal laying" was reported (Valsala and Sivadas, 1970). They reported the internal laying in hens at an incidence rate of less than one percent. The abdominal cavity of one bird was found to have ten eggs of varying shape and size. Three of the eggs were fully formed and had hard shells while others were soft shelled.

Cystic conditions of the right oviduct were reported by Valsala and Sivadas (1970). Forty-four of the forty-seven instances of the developmental abnormalities of the right oviduct were in the nature of cysts. The cysts borne on

narrow stalks which differed in size and shape, were thin walled and usually distended with clear watery fluid, which in some instances appeared cloudy. Most of them appeared as a single cyst of globular or cylindrical shape. Multiple discontinuous cysts were also observed which were in the form of blind saccular structures connected by intervening greyish white bands. They did not manifest any luminal communication with one another. They also encountered bulbous "parovarian cysts" containing clear fluid. The cysts were solitary and occurred in the mesosalpinx of the left oviduct towards the region of the infundibulum. In one case, the cyst appeared to be attached to the wall of the oviduct, while in the other three cases reported, such a relationship was distinctly absent. Histologically the cyst wall was composed of a layer of smooth muscle fibres and fibrous connective tissue enveloped by a serous coat. The cavity of the cyst was lined by a single layer of ciliated columnar or cuboidal cells, which sometimes formed minute papillary folds. The contents stained homogeneously pink.

According to Riddell (1991), internal laying was the condition in which soft-shelled eggs or fully formed eggs were found in the peritoneal cavity. This indicated that the yolk progressed normally through the oviduct to a certain point and then by reverse peristalsis the egg was discharged into the body cavity. The live bird assumed a penguin like posture. A condition called "False layer" was described by Riddell (1991). The affected bird was in production and visited the nest regularly but did not lay eggs. The bird had a normal-appearing ovary and oviduct, but the infundibulum failed to engulf the ovum after it was ovulated. At necropsy these birds showed excessive amounts of orange-colored fat and had liquid yolk in the body cavity.

According to Riddell (1991), "Egg bound" or "Egg binding" is the term used to describe a condition in which an egg is lodged in the cloaca but cannot be laid. It may result from inflammation of the oviduct, partial paralysis of the muscles of the oviduct, or production of an egg so large that it is physically impossible for it to be laid.

A case of ectopic egg yolk was noticed by Gorham *et al.* (1992) in a 6 year-old intact Cockatiel. The presence of an intact egg yolk and the absence of both egg white and uterine rupture suggested that the egg yolk entered the abdominal cavity when extruded from the ova and not after entering the oviduct. The small size of the abdominal cavity and relatively large size of their eggs could predispose them to develop dyspnoea and die from acute respiratory distress. Jordan and Pattison (1996) also described the same condition, however they did not mention the exact etiology of this condition.

Prolapse of the oviduct was seen in layers and initially it was the result of oviposition. The everted oviduct protruded through the vent. It tended to recur (Jordan and Pattison, 1996).

A case of oviduct impaction was reported in a duck (Sreramam *et al.*, 2003). The oviduct contained one large creamy white colored mass along with four small masses. These were arranged in a single row, occluding the oviduct. On cross section concentric layers of coagulated albumen, yolk and shell membranes were noticed.

2.1.1.3 Bacterial Diseases

Among the specific bacterial diseases of poultry, involvement of the reproductive system was reported to be common in salmonellosis. Dixit (1952) and Singh (1967) observed that the ovary and the oviduct manifested pronounced changes in chronic pullorum disease. According to them, discoloration and changes in the shape and consistency of the ova were very perceptible in the carrier hen. Besides being misshapen, discoloured or cystic, the ova also developed long stalks instead of the normal short attachments.

Valsala and Sivadas (1971) isolated *Escherichia intermedia* and *Alkaligenes faecalis* from the oviduct of five birds with salpingitis. They observed severe diffuse congestion of the ovary and turbid, blood tinged exudate in the abdominal cavity. They experimentally induced salpingitis in progesterone treated birds on subsequent infection with culture of *Escherichia coli*. The gross

lesions were swollen dark red ovaries, misshapen and congested ova and inflammatory changes in the oviduct. Histopathological lesions were congestion, mild haemorrhage, fibrinous exudation and foci of lymphoid and plasma cells.

Pradhan *et al.* (1973) studied the pathogenicity of *Escherichia coli* organisms isolated from egg peritonitis.

In a study of mycoplasmal salpingitis, a total of 72 isolates of *Mycoplasma* were isolated from 1001 specimens showing gross lesions of salpingitis (Rathore, 1976).

In acute cases of fowl cholera in laying hens, Heddleston and Rhoades (1978) observed flaccid follicles with less evident thecal blood vessels, hyperemia of the follicles and ovarian stroma, ruptured yolk sac and accumulation of cheesy material with resulting peritonitis.

Sharma *et al.* (1980a) isolated *Escherichia coli*, *Pseudomonas*, *Klebsiella*, *Proteus*, *Micrococcus* and *Salmonella* from cases of oophoritis, salpingitis, egg peritonitis and other conditions.

Sharma *et al.* (1980b) studied the drug sensitivity pattern of bacteria related with reproductive disorders of poultry.

Batra *et al.* (1982) isolated *Escherichia coli* from domestic fowl showing gross lesions of egg bound and oviduct impaction.

Sharma and Joshi (1985) studied the pathology of reproductive disorders of poultry and concluded that *Escherichia coli* was the main organism associated with reproductive disorders of poultry leading to low egg production, morbidity and mortality.

Choudhary *et al.* (1986) studied the bacterial etiology of oophoritis in chicken. They isolated *Escherichia coli*, *Proteus* and *Salmonella* from natural cases of oophoritis in layers.

Escherichia coli, *Proteus*, *Klebsiella* and *Pseudomonas* were isolated from various reproductive disorders in poultry (Reddy, 1990).

Das and Biswas (1998) recorded oophoritis with cystic salpingitis in Khaki Campbell ducks caused by *Escherichia coli*.

Mukhopadhyay *et al.* (1999a) observed changes similar to those described by Valsala and Sivadas in naturally occurring *Escherichia coli* infections involving the reproductive tract. The former authors also recorded distension of the oviduct due to the presence of cheesy fibrinous exudate.

2.1.1.4 Viral Diseases

Few viral diseases directly affect the reproductive system. Newcastle disease produced pathological changes in the ovary and oviduct. Biswal and Morril (1954) observed a decrease in the shell weight and shell thickness of the egg in the Newcastle disease virus infection. The magnum showed a variable picture. Cellular infiltration, edema and focal necrosis were observed in a majority of the birds in the earlier stages of infection while in later stages, lymphocytic aggregates in contact with the corial vessels showed mild hyperplasia. The most marked and characteristic change involved the ovarian follicles. Degeneration of majority of follicles was observed. This process affected both the nearly mature and immature follicles, and was most extensive in a majority of the ovaries.

Easterday and Tumova (1978) reported involvement of the ovary and oviduct in fowl plague. These were in the nature of severe hyperemia of the larger follicles and greyish exudation into the edematous oviduct.

Infectious bronchitis, a viral respiratory disease, affected chicken of all ages. The virus was known to cause permanent damage to the oviduct, which subsequently led to conditions like soft-shelled eggs or to a decrease in egg production (Hofstad, 1978).

Maiti *et al.* (1985) reported the isolation of infectious bronchitis virus from the ovaries and oviduct of hens with a history of a 25 to 40 per cent drop in egg production.

Reddy (1990) isolated CELO (Chick Embryo Lethal Orphan) virus from two cases of atrophied ovary and oviduct.

Mukhopadhyay *et al.* (1999b) isolated infectious bronchitis virus from birds sacrificed in various poultry farms in and around Namakkal.

2.1.1.5 Parasitic Infections

As compared with the parasitic conditions of the gastro-intestinal tract, parasites of the oviduct are rare in birds. Valsala (1968) reported the incidence of *Prosthogonimus* spp. belonging to the order Plagiorchiidae, in the oviduct of chicken.

Kingston (1978) also observed the presence of trematode *Prosthogonimus* in the oviduct and bursa of Fabricius of chicken, ducks and other avian species. Affected birds went off production, laid thin-shelled eggs or eggs without shell and became emaciated and anaemic. Fibrinous peritonitis with adhesion and fibrino-purulent salpingitis with rupture of the oviduct and discharge of its contents into the abdominal cavity were also seen.

2.1.1.6 Protozoan Infections

Specific protozoan diseases affecting the reproductive organs were not reported in birds. The diseases causing generalized infection, leading to pathological changes in most of the visceral organs including the reproductive organs were reported. Bangkok Haemorrhagic disease caused by *Akiba caulleuryi*, a leucocytozoan, was reported in India by various authors (Sivadas *et al.*, 1965; Valsala, 1968). They observed minute whitish glistening translucent globular cysts of uniform size in almost all organs. The mucosa of the oviduct was practically studded with cysts, many of which appeared haemorrhagic. Pronounced lesions were seen in the lower portions of the oviduct. The cysts were seen in the ovary also.

2.1.1.7 *Neoplastic Conditions*

Ovarian teratoma in chicken was reported by Narayana *et al.* (1966). The growth was deep brownish to pearly white in colour and rounded in shape. Microscopically, the growth was made up of mature tissues and the following structures were identified: (1) masses of squamous epithelium with keratinized centres (2) tubular, glandular or minute cystic structure lined by a high columnar to cuboidal epithelium (3) numerous cystic structures lined by cuboidal epithelium (4) well-formed bony spicules, (5) mesenchymal tissue differentiating into spindle cells and hyaline cartilage and (6) a loose fibrous tissue stromal elements.

Valsala (1968) recorded leiomyoma, adenocarcinoma, cystadenoma and haemangioma in the reproductive organs of chicken.

Mayor (1968) reported that the most frequent cause of death among the adult hen was neoplasms of the reproductive tract and majority of the carcinomas occurring in the fowl arose from the ovary. According to him, firm, pink, cauliflower like growth with secondary implantations often accompanied by ascites were the changes frequently encountered.

Gill and Iyer (1973) recorded the occurrence, types and histology of neoplasms in various organs in chicken.

Sreraman *et al.* (1981) made a comparative study on the occurrence of tumours in ducks and chicken. According to them, incidence of ovarian adenocarcinoma was significantly more in chicken than in ducks.

Goswami *et al.* (1988) and Sharma (1990) described the histological details of neoplastic conditions affecting the female reproductive tract in chicken.

2.1.1.8 *Salpingoperitonitis*

Valsala (1968) observed that salpingoperitonitis was common especially in birds reared under intensive system. High level of estrogen was

attributed to be a factor influencing the pathogenesis. It was presumed to be a complication of an initial salpingitis or oophoritis.

2.1.1.9 Oophoritis and Salpingitis

Yamagiwa *et al.* (1972) described the histopathological observations of necrotizing follicular oophoritis in chicken.

In a study conducted by Batra and Singh (1978), out of 2180 adult hens examined, 375 birds were found to be having reproductive lesions like oophoritis, salpingitis, impaction of the oviduct, cystic oviduct and egg bound.

2.2.1.10 Vent Gleet

Vent Gleet also called "Cloacitis" is an inflammatory condition of the vent affecting the laying females. It commences as a red area around the vent, which becomes swollen and covered with a yellow, moist, necrotic membrane. The lesion is associated with a distinct, pungent and characteristic odour (Jordan and Pattison, 1996).

2.2.1.11 Effect of Toxins on the Reproductive Organs

Davison (1978) studied the ^{45}Ca uptake by shell gland, oviduct, plasma and egg shell of 1,1,1 - trichloro - bis (P - Chlorophenyl) ethane (DDT) - dosed ducks and chicken. He concluded that species differences in the distribution of ^{45}Ca were present between control ducks and control chicken and treatment with DDT or DDE caused egg shell thinning in ducks but not in chicken.

Gallinarum cytotoxin I (GCTI) purified from a strain of *Salmonella gallinarum* isolated from an outbreak, caused lethality in White Leghorn birds in doses as low as 0.1 mg/bird with organs like ovaries and testes exhibiting petechial haemorrhages (Singh *et al.*, 1998).

2.2.1.12 Other Conditions

Reddy *et al.* (1994b) observed a case of ectopic ovarian follicles in a Kadaknath hen. The wall of the oviduct along with the mesosalpinx was thickened and edematous. Microscopically, aberrant follicles were noticed in the

submucosa, tunica muscularis and serosa of the infundibulum and magnum of the oviduct and in the mesosalpinx.

Premkumar *et al.* (1999) observed histological changes in the gonads due to manganese deficiency in both sexes of chicken.

2.1.2 Pathology of the Male Reproductive System in Chicken

Sinha *et al.* (1981) observed that the White Leghorn cocks, exposed to sumithion in doses of 12 and 36 milligrams/bird/day, showed reduction in the semen volume, sperm motility and percentage of live sperm along with an increase in the seminal plasma cholesterol. The author attributed these findings to the degenerative effect of sumithion on the testicular tissue and accessory sexual structures.

Reddy *et al.* (1994c) studied the prevalence and pathology of the male reproductive system in chicken. They described different conditions like hypoplasia of the testes, pressure atrophy due to nephroblastoma, sertoli cell tumor and Marek's disease.

George and Sundararaj (1995) studied the effect of 1,1,1 - trichloro - bis (P - Chlorophenyl) ethane (DDT) on the reproductive system of White Leghorn cockerels. They described the clinical and histological changes.

Kaur *et al.* (1998) observed that excess sodium chloride level in young chicks caused gigantic enlargement of the testicles leading to cystic tubules. However, in the adults excess feeding of sodium chloride resulted in cellular casts within the lumen of the seminiferous tubules and reduction in the size of the tubules.

2.2 PREVALENCE AND PATHOLOGY OF THE REPRODUCTIVE SYSTEM IN JAPANESE QUAIL

Srinivasan *et al.* (1980) observed that the major cause of mortality in female adult Japanese quails was egg bound/peritonitis.

In a study conducted at the Centre of Excellence in Pathology, College of Veterinary and Animal Sciences, Mannuthy, during the period between 1984 and 1988, oophoritis ranked sixth among the diseases causing mortality in adult Japanese quail stock (Gangadharan *et al.*, 1989).

2.2.1 Pathology of the Female Reproductive System in Japanese Quail

2.2.1.1 *Defects in the Process of Egg Laying and Oviduct*

A case of egg in egg (ovum in ovo) was reported in a quail (Szabo and Bardos, 1996). This was attributed to the reverse peristalsis of a developing egg and its encapsulation by ensuing egg.

2.2.1.2 *Bacterial Diseases*

Silva *et al.* (1989) reported coligranulomatosis in a Brazilian flock of 8 to 12 month-old Coturnix quail (*Coturnix coturnix coturnix*). Whitish-grey nodules were present on the serosal surface of visceral organs including the ovary and oviduct.

Das and Som (1992) reported colisepticemia without any reproductive tract lesions in adult quails.

An outbreak of *Escherichia coli* infection was observed in a farm maintaining 5000 Japanese quails. The birds showed low morbidity, high mortality and low levels of egg production (Franchesi *et al.*, 1995).

A total of 298 isolates of *Salmonella gallinarum* were isolated from both ailing and dead birds. There were gross lesions suggestive of general septicemia, however no gross lesions involving the reproductive tract were seen (Batabyal *et al.*, 2003).

2.2.1.3 *Viral Diseases*

The occurrence of Marek's disease was reported in quails. Lesions, which were present in the liver, spleen, kidney, and small intestine, were characterized by the presence of heterogenous lymphoid cells and occasional plasma cells. The affected ovary, although rarely enlarged, appeared as a fleshy pink mass 3 to 4 times the normal size (Dutton *et al.*, 1972).

Eastern Equine Encephalomyelitis (EEE) outbreak was reported in a Coturnix quail (*Coturnix coturnix coturnix*) farm in South Carolina (Eleazer *et al.*, 1978). Many soft-shelled eggs were noticed among a few eggs laid during the disease outbreak.

May (1979) reported an outbreak of Quail Bronchitis in two flocks from Singapore. The symptoms observed were rales, 10 to 15 per cent reduction in egg production and the eggs were soft-shelled and white, with no pigment on their shells. Purulent egg peritonitis was seen in 3 birds.

Granular patches or tumorous growths were seen in the ovaries of some Japanese quails affected by Marek's disease (Pradhan *et al.*, 1985).

Nair *et al.* (1986) recorded the incidence of Marek's disease in the Japanese quail flock.

Newcastle disease virus was isolated from the Japanese quails showing clinical symptoms of off-feed, malaise, diarrhoea and nervous symptoms. No reproductive tract lesions were detected. The virus isolate was pathogenic to day-old and six-week-old chicken, which indicated its velogenic nature (Sulochana, 1991).

Inclusion body hepatitis was reported in Japanese quails, though it did not cause any significant lesion in the reproductive organs (Grewal *et al.*, 1994).

Shaw *et al.* (1994) reported a mixed infection of Newcastle disease virus and adeno virus in Japanese quails.

2.2.1.4 Fungal Diseases

Singh *et al.* (1994) reported a case of mycotic salpingitis in an adult Japanese quail showing grayish white nodules on the serosal surface of the oviduct. They isolated *Aspergillus flavus* from the nodules.

2.2.1.5 Parasitic Infections

Prosthogonimus ovatus was seen in the abdominal cavity, mesentery, oviduct, uterus and cloaca of coturnix quails (*Coturnix coturnix coturnix*) on experimental infection with metacercarial cysts (Nath, 1971).

2.2.1.6 Neoplastic Conditions

Ramadevi *et al.* (2003) reported adenocarcinoma in two Japanese quails. Massive nodular growths were seen involving various visceral organs including the ovary and oviduct.

2.2.1.7 Effect of Toxins on the Reproductive Organs

Solecki *et al.* (1996) investigated the effects of methyl parathion on reproduction in the Japanese quails. The results suggested that at the highest concentration of 48 ppm methyl parathion decreased the laying performance of hens and therefore the reproductive outcome.

Furazolidone caused reversible changes in the reproductive tract of female Japanese quails when fed at the concentration of 400 or 600 ppm (Ullah *et al.*, 1998). Ovaries of the furazolidone-fed quail showed reduction in weight and size and were studded with small follicles. Magnum, isthmus and uterus had decreased area, height and number of mucosal folds compared with the control group.

2.2.1.8 Nutritional Disorders

Kling and Soares (1980) reported that vitamin E deficiency resulted in decreased percent ^{of} fertile egg production, hatchability and plasma free tocopherol.

The role of vitamin A in reproduction is well documented. Kerti *et al.* (2002) studied the content of retinol and retinyl esters in the blood plasma, liver,

kidney and reproductive organs of Japanese quails. They concluded that the predominant function of the uterus and isthmus might be storage of vitamin A and in the infundibulum and magnum, the vitamin A acted as a precursor for the modulation of the cellular metabolism.

2.2.1.9 *Other Causes*

Sturkie and Mueller (1976) described the mechanism of ovulation and oviposition in female birds, hormonal interactions and the effect of light on ovary and ovulation.

Camper and Burke (1977) studied the effect of prolactin on the reproductive function in the female Japanese quail. They concluded that treatment of mature regularly laying quail with 16 I.U. of ovine prolactin per day for 7 or 14 days caused a significant decrease in the ovarian and oviductal weight and a reduction in egg production. The most frequent response to prolactin was a complete cessation of ovulation.

Rai and Mahapatro (1979) observed that estrogen treatment caused an increase in the tissue glycogen and RNA in the oviduct while progesterone acted synergistically for the change in the glycogen content but not for RNA.

Prabakaran *et al.* (1991) observed that lesser duration of light viz., 10 h during growing delayed age at sexual maturity among quails and the best light regime for egg production among quails was either 14 h continuous or 14 h at fourth week which could be gradually increased by weekly increments of 30 minutes to reach a constant 16 h light regime.

Shukla *et al.* (1994) reported that the Japanese quails receiving layer ration from the 6th week onwards had the maximum production potential. In birds receiving feed comprising 50 per cent grower ration and 50 per cent layer ration, percent ^{of} shell weight and shell thickness were significantly affected.

Lucy and Harshan (1998) conducted histochemical studies on the oviduct of Japanese quails. They found glycogen granules at the apex of the

pseudostratified ciliated columnar epithelial cells of the isthmus. Glycogen granules might contribute to the carbohydrate portion of the shell membrane.

2.2.2 Pathology of the Male Reproductive System in Japanese Quail

Ottinger and Doerr (1980) concluded that exposure to aflatoxin at an early age resulted in a delay in the physiological and behavioural sexual maturation.

Gorham and Ottinger (1985) reported occurrence of Sertoli cell tumor in 3 out of 33 Japanese quails studied. The birds were aged between 3 and 5 years.

Ruff *et al.* (1988) observed that quails infected with high doses of coccidia (5×10^5 sporulated oocysts/quail) had reduced testes weight and lowered circulating concentrations of androgen as compared with the control males. Low doses of coccidia (5×10^3 sporulated oocysts/quail) did not affect the testes weight or plasma androgen levels. They concluded that exposure of quails to coccidiosis before sexual maturation might result in long-term effects on their later reproductive capability.

A new inherited muscular disorder resembling myotonic dystrophy in man was observed in a mutant strain of Japanese quails. Along with the muscular lesions, testicular degeneration, atrophy and bilateral lenticular cataract were noticed (Braga III *et al.*, 1995).

Fu *et al.* (1997) studied the role of vitamin A in testicular maturation in male Japanese quails. They noticed that the testes and the cloacal gland grew more quickly in quails fed retinoic acid than in those fed retinyl acetate, the precursor form of vitamin A.

Wechsler and Schmid (1998) concluded from an elaborate experiment that head injuries caused by aggressive pecking were an important welfare problem in quail farming. Factors such as visual barrier, age of introduction of male into the pens, number of hens per 5 males, number of males per 14 hens and light intensity had no significant effect on the rate of aggressive pecking interactions between males.

Materials and Methods

3. MATERIALS AND METHODS

3.1 RETROSPECTIVE STUDY

A retrospective study on the various disease conditions prevalent in Japanese quails was undertaken based on the records maintained at the Centre of Excellence in Pathology, Mannuthy. The data covering a five-year period from 1997-2002 at the Centre of Excellence in Pathology, Mannuthy were collected and analysed. The diseases encountered were classified and the percentage of mortality due to various diseases was estimated. The variations in the mortality pattern due to parameters like age, sex and season were ascertained. Analysis was carried out to reveal the causes of mortality, yearly incidence of diseases and nature of diseases prevalent. Based on the analysis of the data documented an assessment of the prevalence of various diseases was made.

3.2 STUDIES ON SPONTANEOUS DISORDERS OF THE REPRODUCTIVE SYSTEM

3.2.1 Source of Material

Japanese quails brought for post mortem examination at the Centre of Excellence in Pathology, College of Veterinary and Animal Sciences, Mannuthy, from various parts of the state and from the University Poultry Farm, Mannuthy formed the materials for the study.

3.2.2 Method of Examination

The live birds were sacrificed by decapitation and exsanguination. Autopsy was performed as per the protocol prescribed. The gross lesions encountered were recorded. The reproductive organs were dissected out for further examination.

3.2.3 Collection of Materials

The ovary was collected separately and the oviduct and uterus were collected together. They were weighed. The organs were examined in detail exposing the oviduct and uterus and gross lesions, if any, were recorded. Portions of the oviduct were tied at the ends and the lumen was infused with ten per cent

formalin. Representative samples of tissues from the ovary and different parts of the oviduct (infundibulum, isthmus, magnum and uterus) were preserved in ten per cent formalin for histopathological examination. Samples for isolation of bacterial organisms were collected from appropriate cases.

3.2.4 Isolation and Identification of Microorganisms

Primary isolation was done using nutrient agar and MacConkey's agar from samples collected at autopsy. Using nutrient agar slant, routine subculturing was done. Identification was performed on the basis of gram's staining, growth pattern on MacConkey's agar and biochemical tests using KB01 kit purchased from HiMedia Laboratories Private Limited, Chennai. The results were recorded and interpreted (Barrow and Feltham, 1993).

3.2.5 Histopathological Studies

Tissues collected as mentioned above were processed by routine paraffin embedding techniques. Paraffin sections cut at four-micron thickness were stained with Haematoxylin and Eosin method of Harris (Luna, 1968). Sections were also stained with Gomori's one-step trichrome, Phosphotungstic acid Haematoxylin (PTAH) and McManus Periodic acid - Schiff stain for glycogen wherever required (Sheehan and Hrapchack, 1980).

Results

4. RESULTS

4.1 RETROSPECTIVE STUDY

A detailed retrospective study on the reproductive diseases of Japanese Quails was conducted, based on the post mortem data available at the Centre of Excellence in Pathology, College of Veterinary and Animal Sciences, Mannuthy. During the period from April 1997 to March 2002, 4304 Japanese quails were autopsied. The reproductive diseases were encountered at the prevalence rate of 14.15 per cent among various diseases. Egg bound, oophoritis, salpingitis, orchitis and oviduct impaction constituted the reproductive diseases. The yearly incidence of the reproductive diseases encountered has been graphically represented (Fig.1). The incidence rate of various reproductive diseases is shown in Table 1.

4.2 DISORDERS OF THE REPRODUCTIVE SYSTEM OF JAPANESE QUAILS

The occurrence of various reproductive diseases in Japanese quails is shown in Table 2 based on the systematic autopsy conducted at the Centre of Excellence in Pathology, Mannuthy for a period from December 2001 to March 2003.

4.2.1 Disorders of the Female Reproductive System

4.2.1.1 *Egg Bound*

Egg bound was one of the most prevalent conditions among the quail reproductive disorders. The egg binding was observed in the caudal portions of the oviduct, uterus or isthmus-uterus junction. In ten per cent of the cases, egg was bound in the vagina or cloaca. In 80 per cent of the cases, egg was medium to large sized. Egg recovered from 40 per cent of the cases was white in colour without pigment formation or moderately pigmented. Egg with albuminous tail or masses of albumen were also seen (Fig. 2). In one case, malformed egg was

found in the lumen of the oviduct without proper shell formation (Fig. 3). In 15 per cent of egg bound cases, two eggs were bound one after the other (Fig. 4). The blood vessels of the reproductive tract, especially in those regions containing egg, were engorged (Fig. 5) when compared to that of the normal birds (Fig. 6). Hence, the area was reddish-black in colour. Ovary was normal and was bearing follicles of varying sizes in most of the cases.

The microscopic examination of tissue sections obtained from the isthmus of egg bound cases revealed cystic dilatation of the glands in the submucosa along with necrosis of the submucosal glandular epithelium. Mild heterophilic infiltration of the submucosa was also noticed (Fig. 7). Deciliation, degeneration and desquamation of the lining columnar epithelial cells were pronounced in the uterus. Homogenous pink staining exudate composed of heterophils, bacterial clumps and necrotic cells, was present in the lumen. Hyperemia of the blood vessels was a consistent finding. In some places, separation of muscular layer due to the edema and accumulation of exudate was seen. Isthmus revealed congestion, degeneration and desquamation of lining epithelium (Fig. 8). Glycogen could not be demonstrated in the isthmus using McManus method of Periodic acid - Schiff procedure (Fig. 9).

4.2.1.2 Oophoritis

Oophoritis constituted the second most common disease entity affecting the reproductive system of Japanese quails. Acute, subacute and chronic oophoritis were recorded. Haemorrhagic oophoritis was seen in two instances (Fig. 10). In this condition, the ovary was diffusely congested, haemorrhagic with engorgement of the blood vessels in the follicle. Mostly, the ovary was active and was studded with follicles in varying stages of development.

Chronic oophoritis was observed most frequently. The ovary was shrunken and greyish white in colour. Follicles were very few and smaller in size. The follicles were covered with greyish white sheets of necrotic material, which in certain instances caused the ovarian follicles to be matted together. Discoloration of the follicles, varying from opaque white to grey, was noticed. The follicles in such cases were filled with a small quantity of viscid, opaque fluid varying in colour between golden yellow to brownish yellow.

In acute oophoritis, congestion of the blood vessels (Fig. 11) and focal areas of haemorrhage were observed (Fig. 12) on microscopic examination. Focal degeneration and desquamation of the ovarian germinal epithelium was noticed. Infiltration of heterophils and mononuclear cells was a consistent lesion. Haemorrhagic cysts were found in two cases. They were seen in the ovary as cysts containing masses of blood clot or large blood clots (Fig. 13). The wall of the cyst was composed of collagenous tissue, which was rich in capillary spaces filled with blood.

In chronic oophoritis, the stromal fibroplasia was the predominant lesion observed. It was seen in varying degrees. Mild and focal proliferation of the fibroblasts, which stained green with Gomori's one - step trichrome procedure, could be seen in most cases (Fig. 14). In some cases, extensive proliferation of the fibrous tissue staining varying shades of reddish brown with Phosphotungstic acid - haemtoxylin procedure, was observed in the ovarian stroma (Fig. 15). Cellular infiltration was observed in varying degrees ranging from mild to massive. It was also seen in varying distribution pattern ranging from focal to diffuse. In some cases, active proliferation of the fibroblasts and blood vessels were noticed (Fig. 16). In one case, the granulosa cell layer of a disintegrated follicle showed focal hyperplasia (Fig. 17).

4.2.1.3 Salpingitis

The most consistent finding in almost all the cases was congestion of the blood vessels on the serosal surface of the oviduct and mesosalpinx. The lumen was congested and swollen. Caseous, catarrhal and fibrinous exudates were noted. The exudate was present in the magnum, isthmus and uterine portions of the oviduct. The mucosal folds of the lumen were edematous. There were focal necrotic spots both on the mucosal folds and crypts in some cases. Mostly the salpingitis occurred in conjunction with egg bound.

Histologically, extensive degeneration, necrosis and desquamation of the lining epithelial cells were seen. Severe infiltration of the submucosa with heterophils and mononuclear cells was noticed. The blood vessels in the tunica muscularis showed severe congestion. These features were consistently seen in

almost all portions of the oviduct in different cases. Desquamation of the lining epithelium, vascular congestion and the presence of exudate containing necrotic tissue and leucocytes were observed in the infundibulum (Fig. 18) and magnum. Haemorrhage was seen in the submucosa of the magnum (Fig. 19). Loss of the lining epithelium was seen in the magnum (Fig. 20) and uterus (Fig. 21).

Two out of 55 cases showed focal fibrous tissue proliferation with numerous capillaries in the serosal layer. The lumen of a large blood vessel was found to be occluded by a thrombus in one case. In one case, focal hyperplasia of smooth muscle fibres were seen in the tunica muscularis of the magnum. However, leiomyoma was ruled out due to the absence of neoplastic changes (Fig. 22).

4.2.1.4 *Impaction of the oviduct*

The location and the degree of impaction varied in different cases. The impaction of the oviduct was seen mostly in the uterine portion and occasionally in the magnum - isthmus region. Inspissated, caseous, cheesy or moderately hard onion shaped yellowish white material was seen in the lumen. The wall of the oviduct was thin and the vessels of the oviduct were diffusely engorged varying from moderate to severe degree (Fig. 23). The ovary was active and contained three to four mature follicles and numerous developing follicles.

The sections of the oviduct derived from cases of egg impaction, revealed areas of degeneration, necrosis and desquamation of the lining epithelial cells (Fig. 24). Moderate to severe infiltration of the submucosa with heterophils and mononuclear cells was noticed. Focal glandular hyperplasia was also noticed. The tunica muscularis showed degeneration and separation of smooth muscle fibres (Fig. 25).

4.2.1.5 *Fibroma of the left ovary*

A case of fibroma of the left ovary was encountered during routine post mortem examination. The ovary was slightly enlarged, pinkish white in colour. The ovary was studded with a few mature follicles. The number of developing follicles were seen in much lesser number than normal.

Microscopically, abundant fibrous tissue proliferation in a haphazard manner was observed. The follicles were encircled by fibrous tissue. Few haemorrhagic cysts were also seen. Interlacing bundles of fibrous connective tissue ran in all directions. Collagen fibres stained green with Gomori's one-step trichrome procedure, were seen in varying stages of maturation around the fibroblasts (Fig. 26). Neovascularisation was noticed in and around the proliferating fibroblasts (Fig. 27). Infiltrating inflammatory cells were also present. Nucleus of the fibroblasts showed pleomorphism (Fig. 28).

4.2.2 Disorders of the Male Reproductive System

4.2.2.1 *Hypoplasia of the Testicles*

A single case of hypoplasia of the testicles was observed in a 60 days old male Japanese quail. Both the testicles were much smaller in size (Fig. 29) than that of the normal adult (Fig. 30). The testicles had the size only as that of a quail chick.

On microscopic examination, seminiferous tubules appeared narrow and were lined by flattened cells and were arranged as single layers or multiple layers. Spermatozoa were very few. In some areas, they were absent whereas the tubules of normal testicles were lined by multilayered spermatogonial cells with spermatozoa in the lumen. Marked intertubular fibrous tissue proliferation was observed (Fig. 31) when compared to sections from normal testicles (Fig. 32).

4.2.2.2 *Orchitis*

A single case of orchitis was observed. Both the testicles showed enlargement and slight edema. They were soft to touch and the blood vessels on the surface were engorged. On section, the cut surfaces bulged.

Histologically, degenerative changes were seen in the spermatogonial cells along with infiltration of the heterophils. The lumen of the tubules showed clumps of leucocytes and necrotic tissue. Focal accumulation of fibrinous exudate was present in the interstitial tissue. Blood vessels were congested (Fig. 33).

4.3 ISOLATION AND IDENTIFICATION OF BACTERIAL ORGANISMS

The details about the bacterial organisms isolated and identified are presented in Table 3.

Table 1. Prevalence of reproductive disorders encountered in japanese quails from april 1997 to march 2002

Disorders encountered	Number of cases	Prevalence rate in percentage	Percentage of the reproductive disorders
Egg bound	132	3.07	21.67
Oophoritis	407	9.46	66.83
Salpingitis	65	1.51	10.67
Orchitis	1	0.02	0.16
Oviduct impaction	4	0.09	0.66

Total number of birds examined: 4304

Table 2. Prevalence of reproductive diseases in japanese quails from december 2001 to march 2003

Disorders encountered	Number of cases	Prevalence rate in percentage
Oophoritis	144	12.64
Egg bound	134	11.75
Salpingitis	55	4.83
Orchitis	1	0.09
Oviduct impaction	4	0.35
Fibroma of left ovary	1	0.09
Hypoplasia of testicles	1	0.09
Orchitis	1	0.09

Total number of birds examined: 1139

Table 3. Bacterial organisms isolated and identified from various reproductive diseases of japanese quails

Condition	<i>Escherichia coli</i>	<i>Klebsiella</i> spp.	<i>Proteus</i> spp.	Total number of isolates
Oophoritis	4	0	2	6
Egg bound	5	2	1	8
Egg impaction	1	0	1	2
Orchitis	1	0	0	1
Salpingitis	1	0	0	1
	12	2	4	18

Total number of isolates obtained: 18

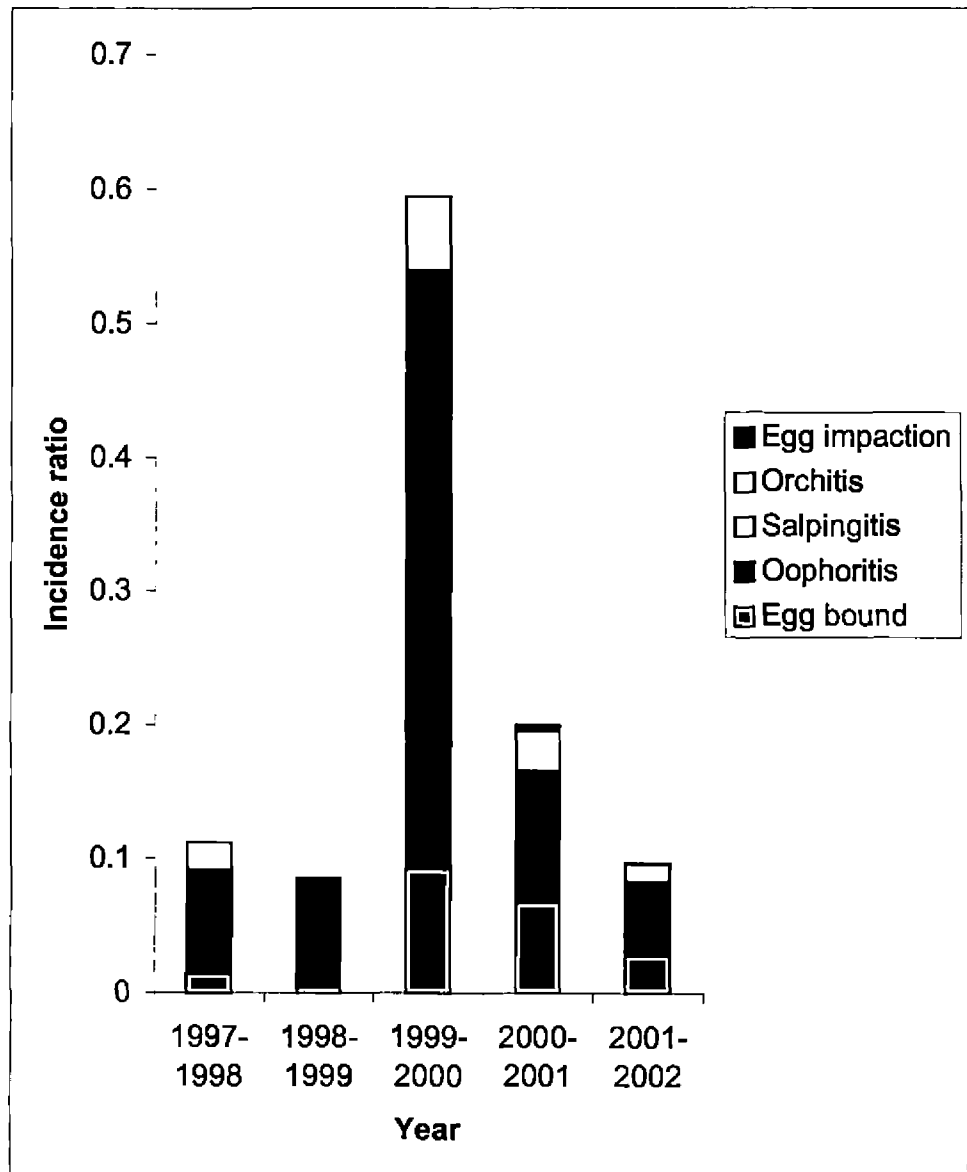


Fig. 1. The yearly incidence of reproductive diseases in Japanese quails (1997 - 2002)

Fig. 2. Egg with albuminous tail in the oviduct

Fig. 3. Malformed egg with improper shell

Fig. 4. Double egg binding

Fig. 5. Egg bound

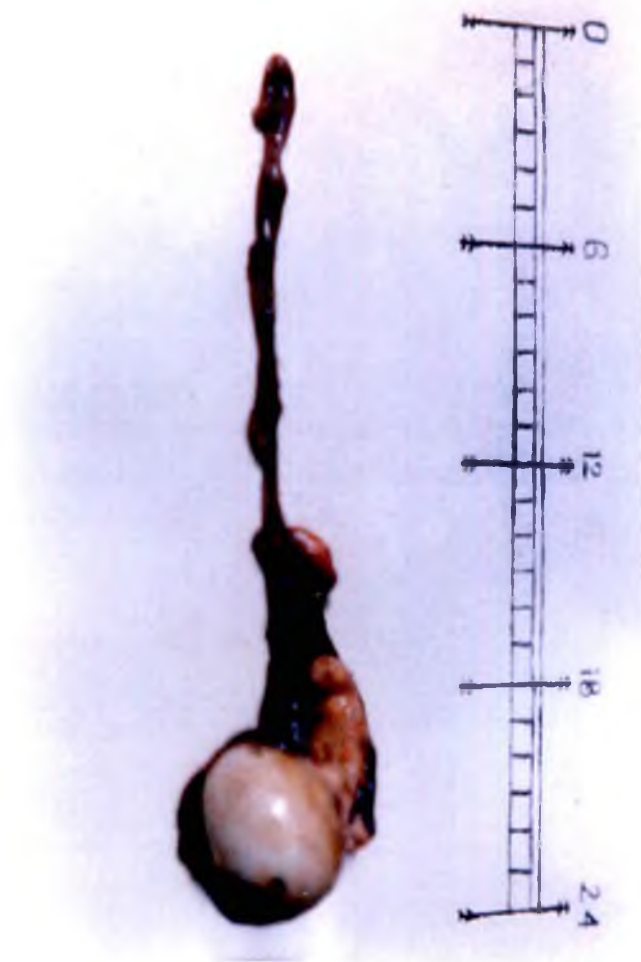


Fig - 2



Fig - 3



Fig - 4



Fig - 5

Fig. 6. Ovary and oviduct in a normal bird

Fig. 7. Uterus - necrosis of the submucosal glandular epithelium with heterophilic infiltration. H&E x 160

Fig. 8. Isthmus - degeneration and desquamation of the mucosal epithelium with congestion. H&E x 63

Fig. 9. Isthmus - absence of glycogen particles in the isthmus. McManus Periodic acid - Schiff stain x 63



Fig - 6

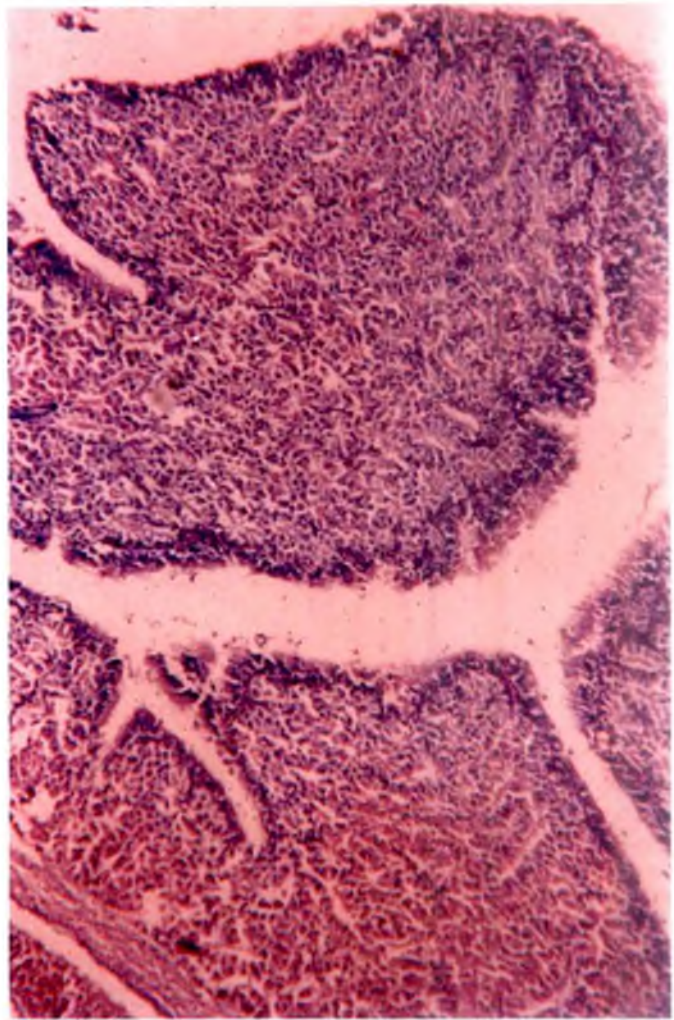


Fig - 7

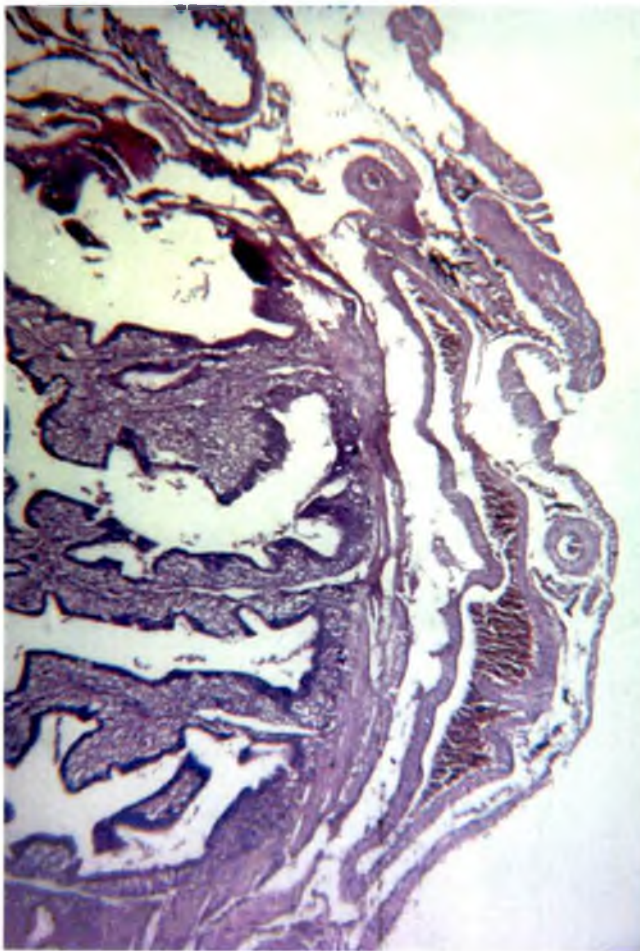


Fig - 8

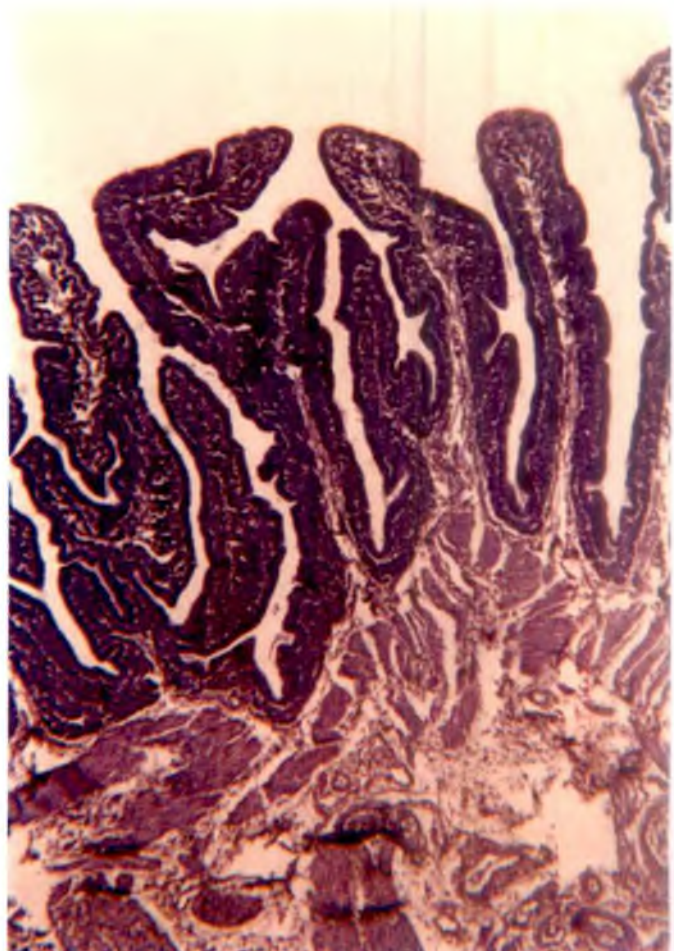


Fig - 9

Fig. 10. Haemorrhagic oophoritis with egg bound

Fig. 11. Ovary - congestion of the blood vessels in acute oophoritis. Phosphotungstic acid - haematoxylin x 250

Fig. 12. Ovarian cortex - haemorrhage and congestion. H&E x 250

Fig. 13. Ovary - haemorrhagic cysts in the ovary. Phosphotungstic acid - haematoxylin x 250



Fig - 10

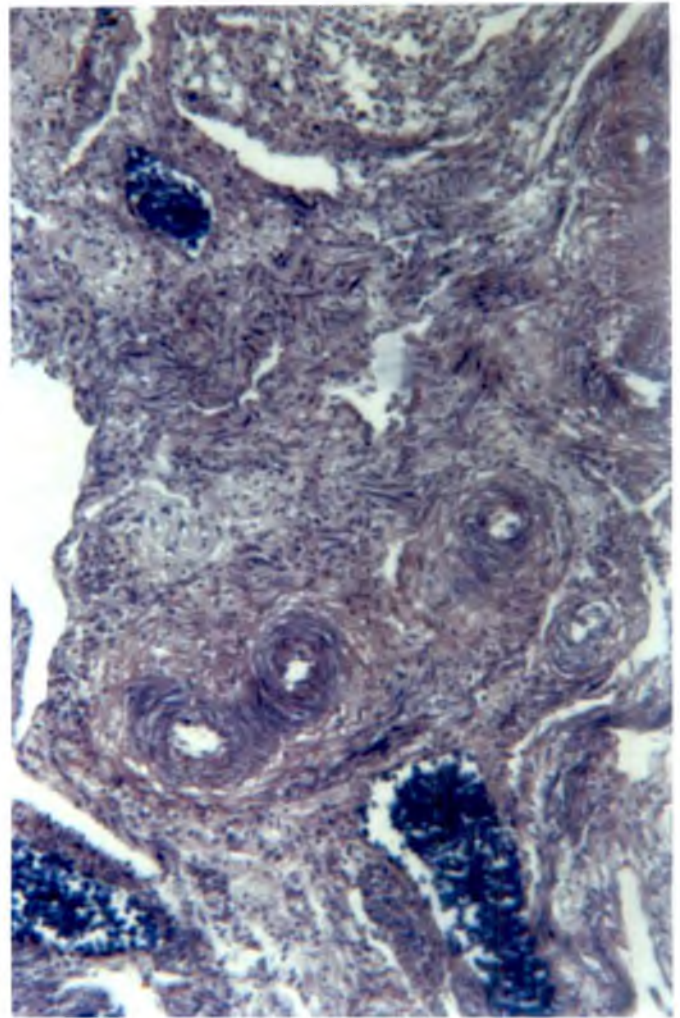


Fig - 11

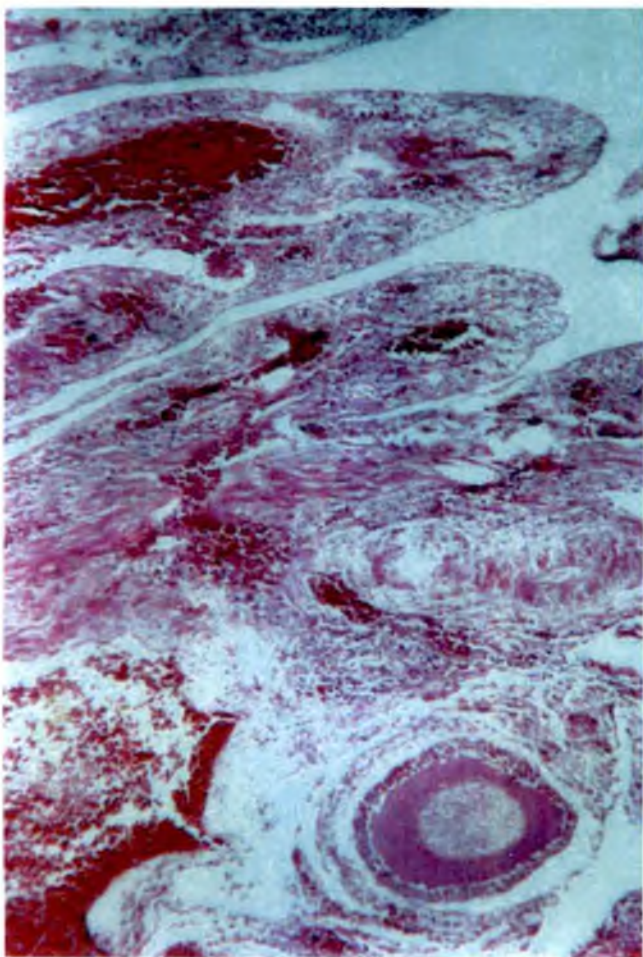


Fig - 12

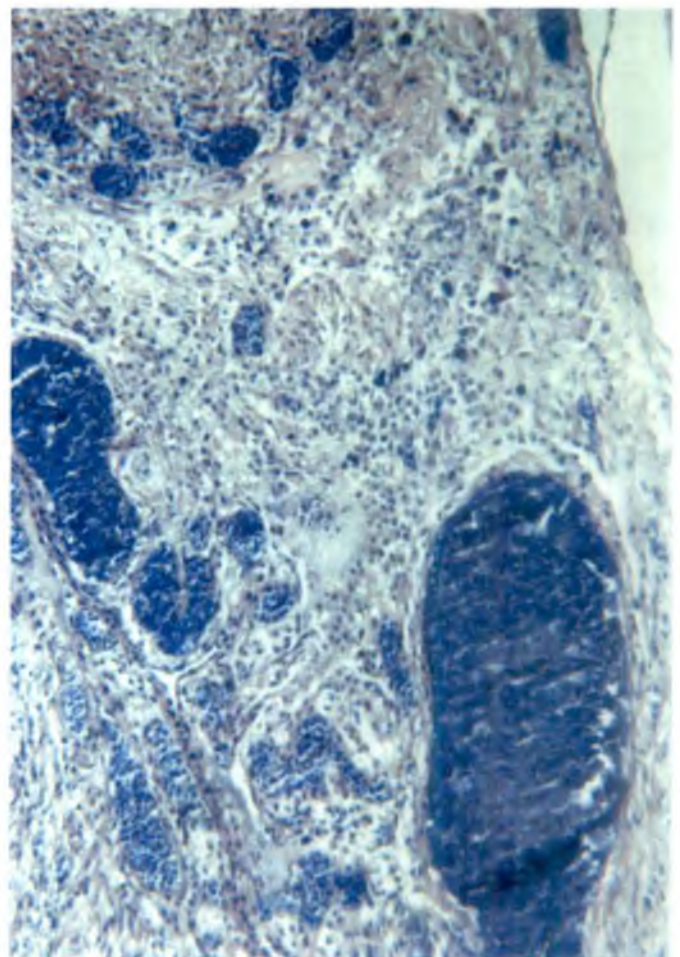


Fig - 13

Fig. 14. Ovary - mild fibroplasia. Gomori's one-step trichrome
x 250

Fig. 15. Ovary - massive fibroplasia. Phosphotungstic acid -
haematoxylin x 250

Fig. 16. Ovary - proliferation of fibrous tissue and blood
vessels. H&E x 250

Fig. 17. Ovary - focal hyperplasia of the granulosa cell layer in
a disintegrated follicle. Phosphotungstic acid -
haematoxylin x 250

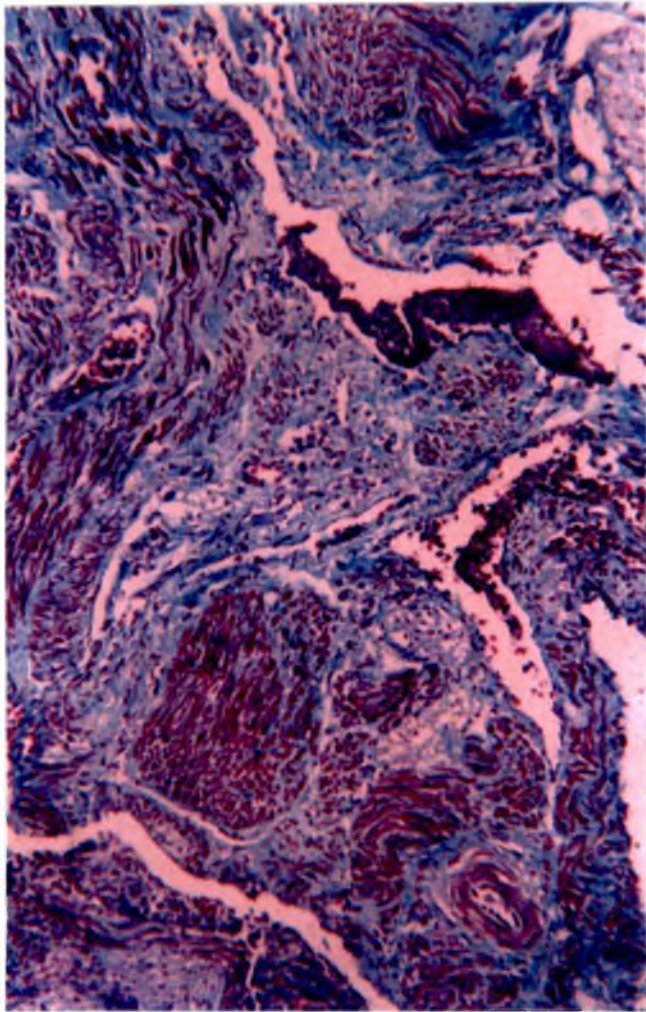


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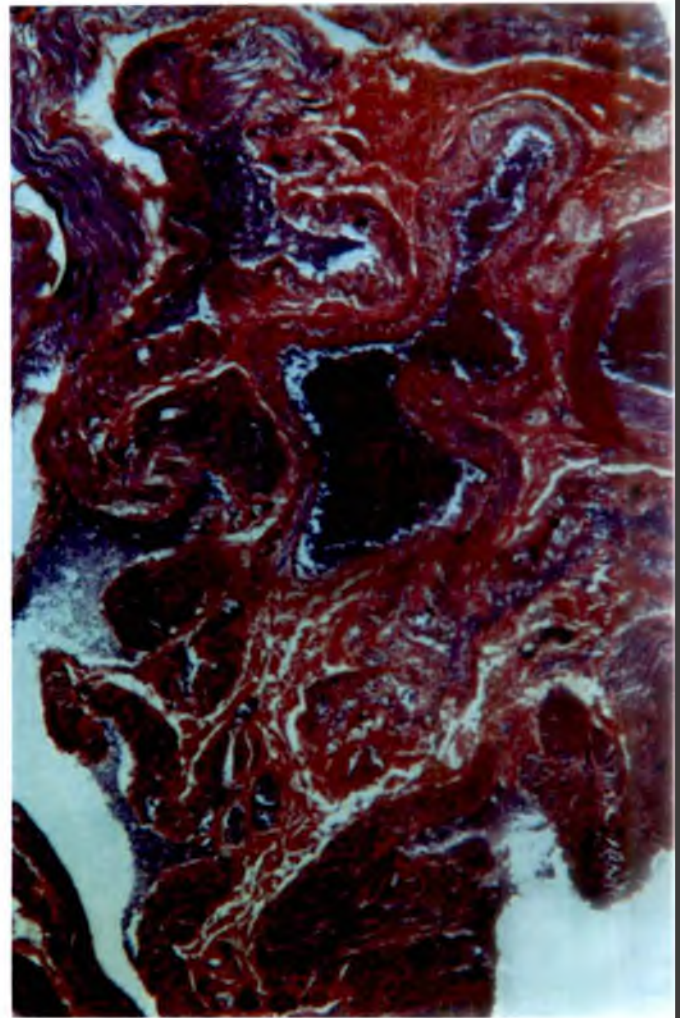


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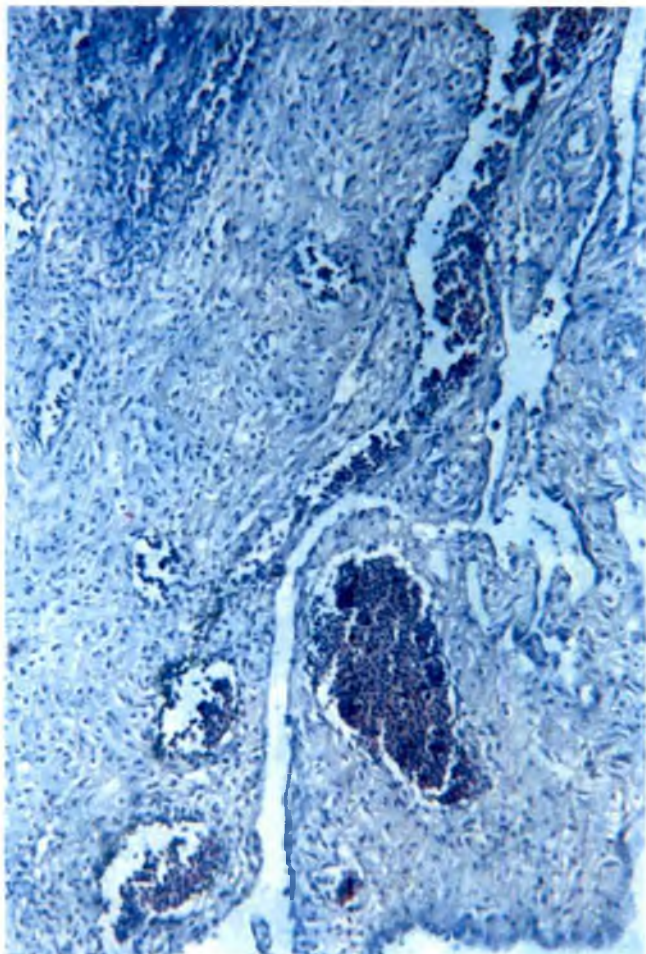


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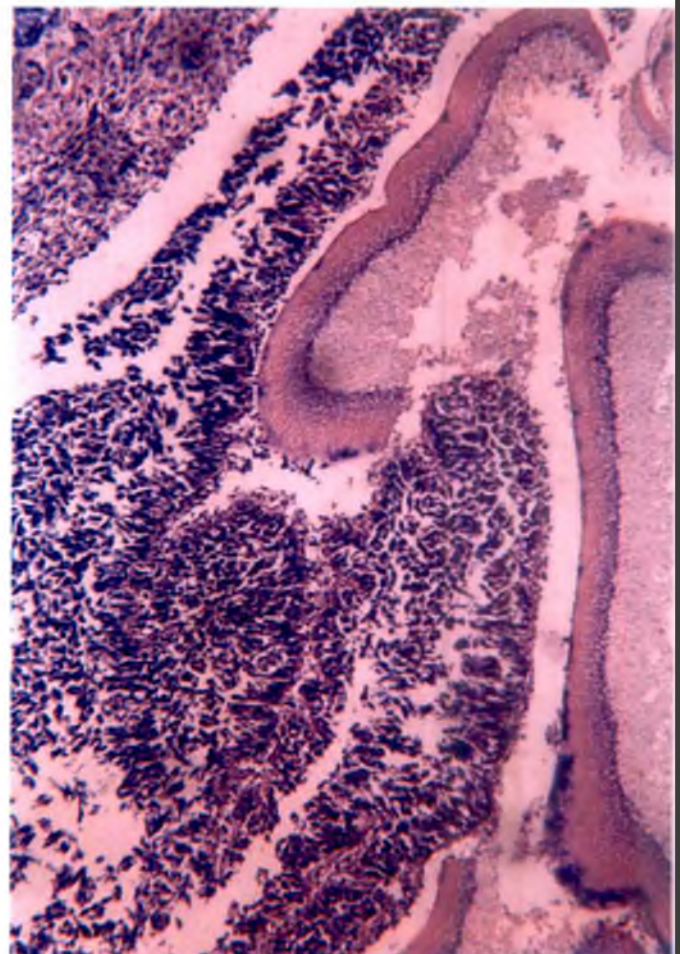


Fig - 17

Fig. 18. Infundibulum - degeneration and desquamation of the mucosal epithelium with leucocytic infiltration. H&E x 250

Fig. 19. Magnum - haemorrhage in the submucosa. H&E x 250

Fig. 20. Magnum - mild degeneration and desquamation of the lining epithelium. H&E x 250

Fig. 21. Uterus - mild degeneration and desquamation of the lining epithelium. H&E x 250

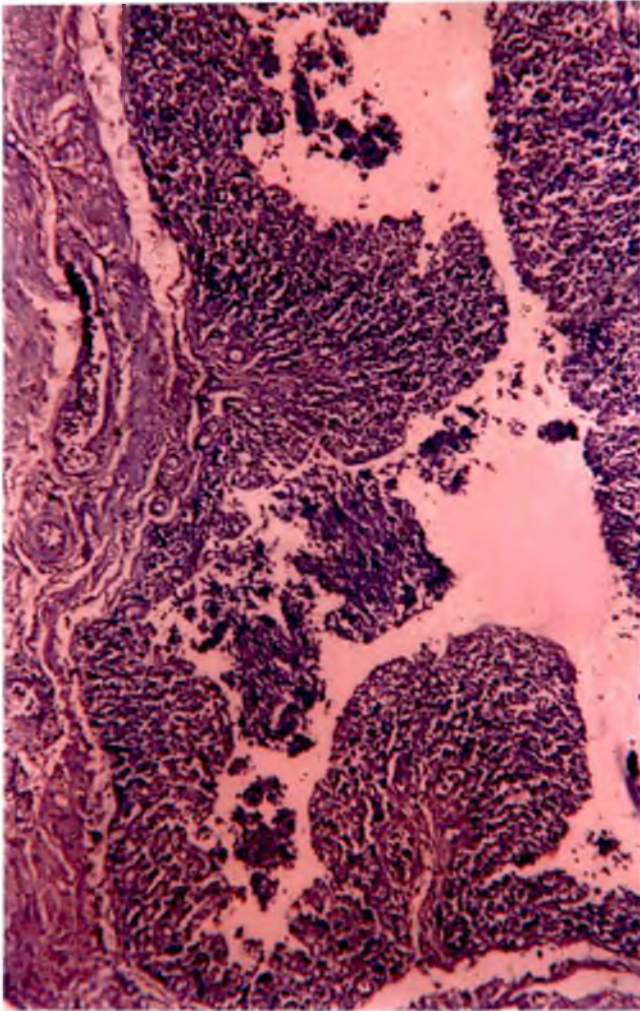


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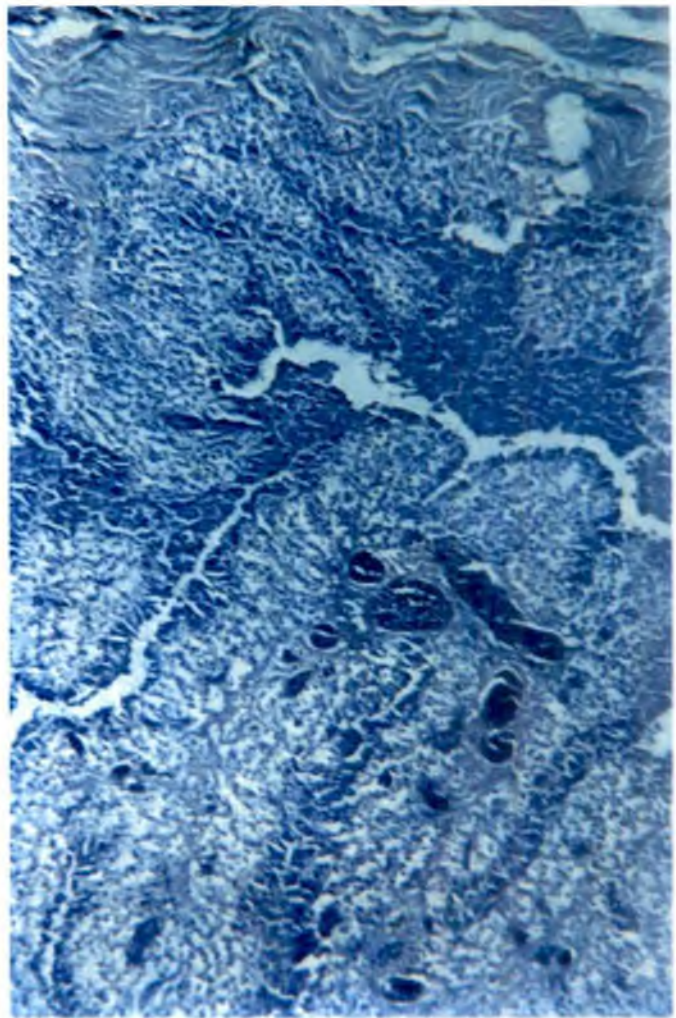
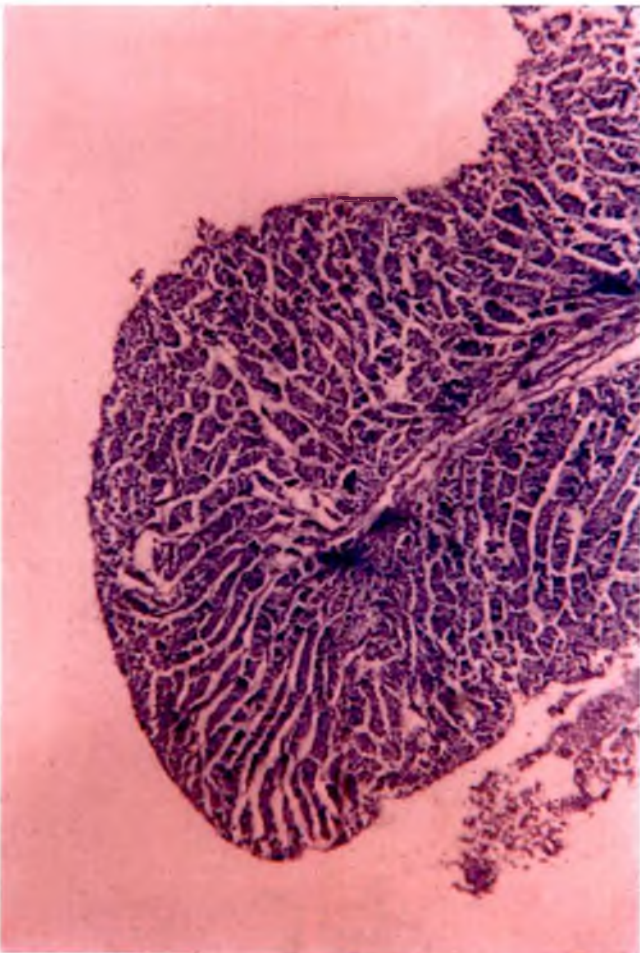


Fig - 19



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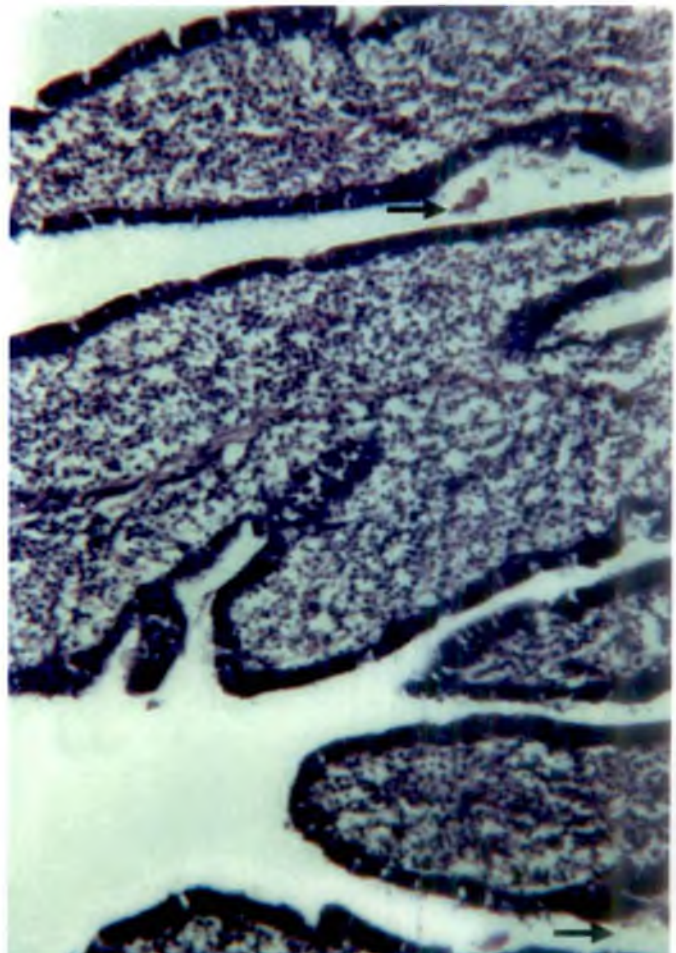


Fig - 21

Fig. 22. Magnum - focal hyperplasia of smooth muscle fibres in the tunica muscularis. Phosphotungstic acid - haematoxylin x 250

Fig. 23. Impaction of the oviduct

Fig. 24. Uterus - degeneration and desquamation of the lining epithelium with leucocytic infiltration. H&E x 250

Fig. 25. Uterus - leucocytic infiltration in the submucosa with degeneration of smooth muscle fibres. H&E x 250

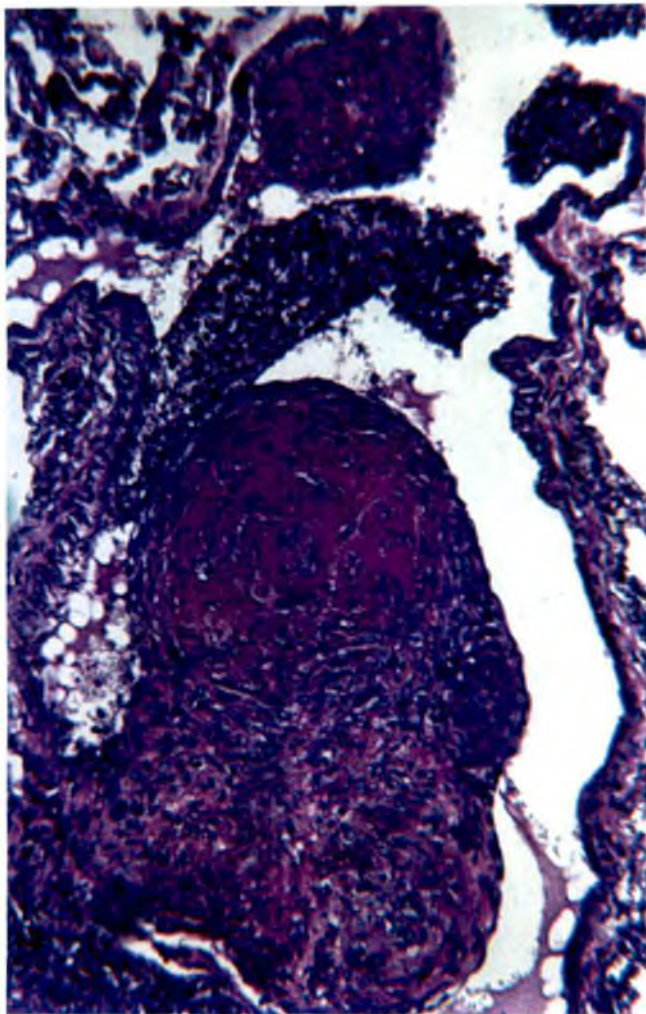


Fig - 22



Fig - 23

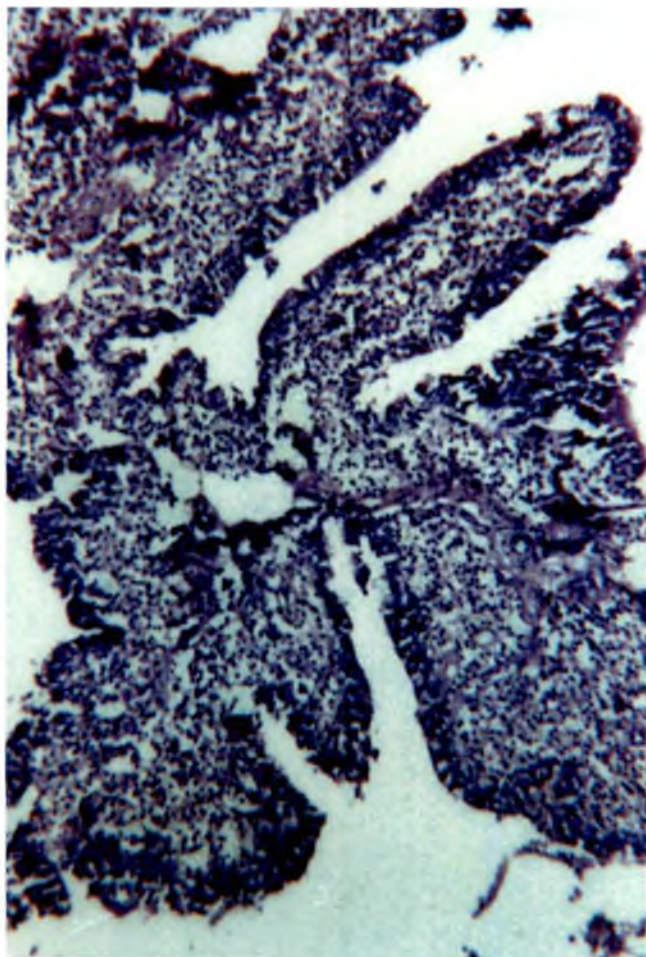


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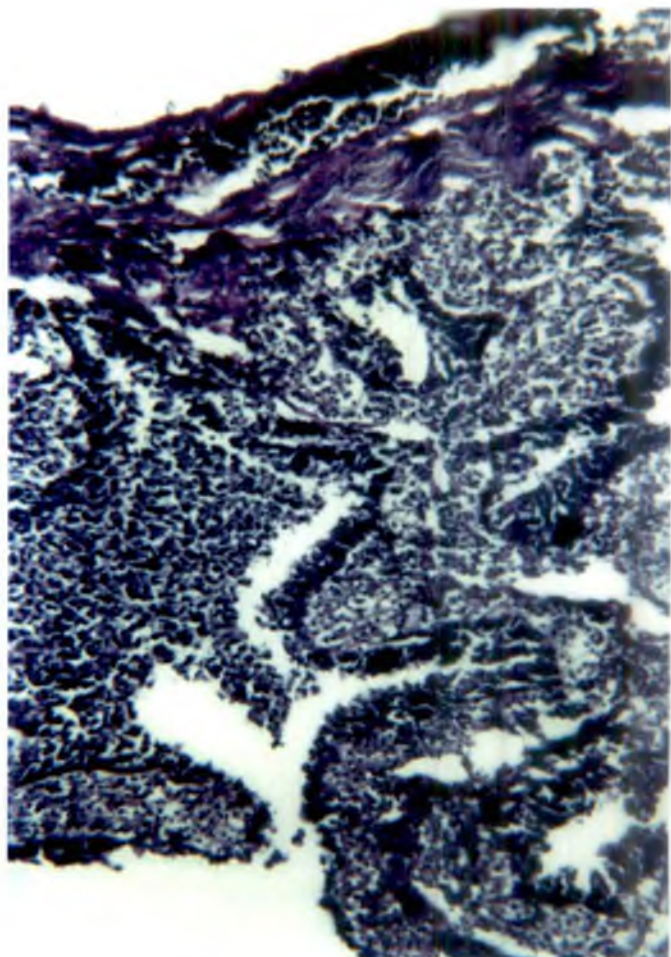


Fig - 25

Fig. 26. Actively proliferating fibroblasts - ovarian fibroma. Gomori's one-step trichrome stain x 250

Fig. 27. Neovascularisation with proliferating fibroblasts - ovarian fibroma. Gomori's one-step trichrome stain x 160

Fig. 28. Nuclear pleomorphism - ovarian fibroma. Gomori's one-step trichrome stain x 1000

Fig. 29. Testicular hypoplasia

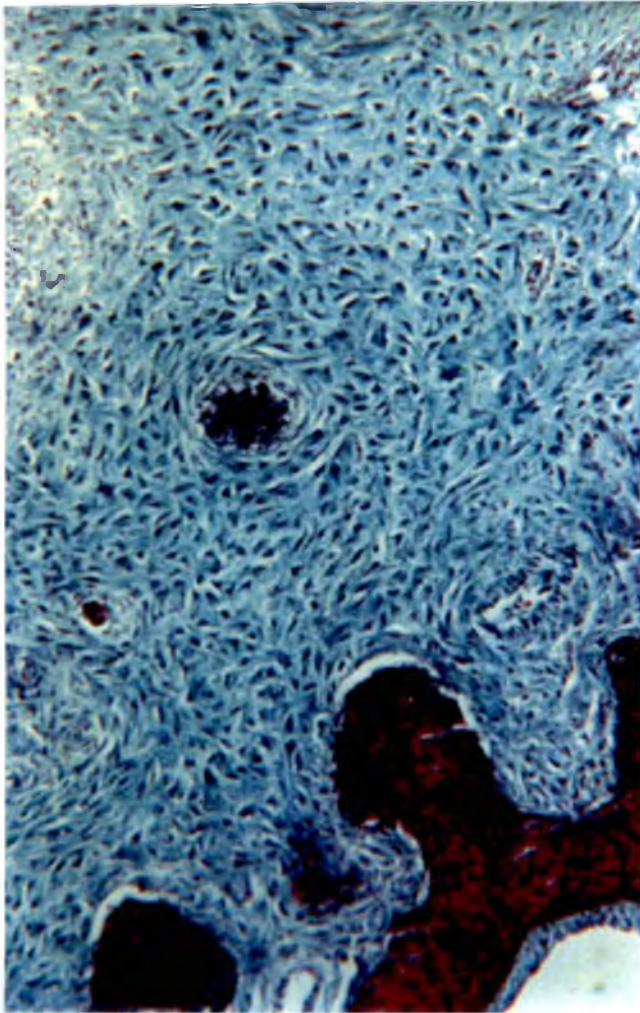


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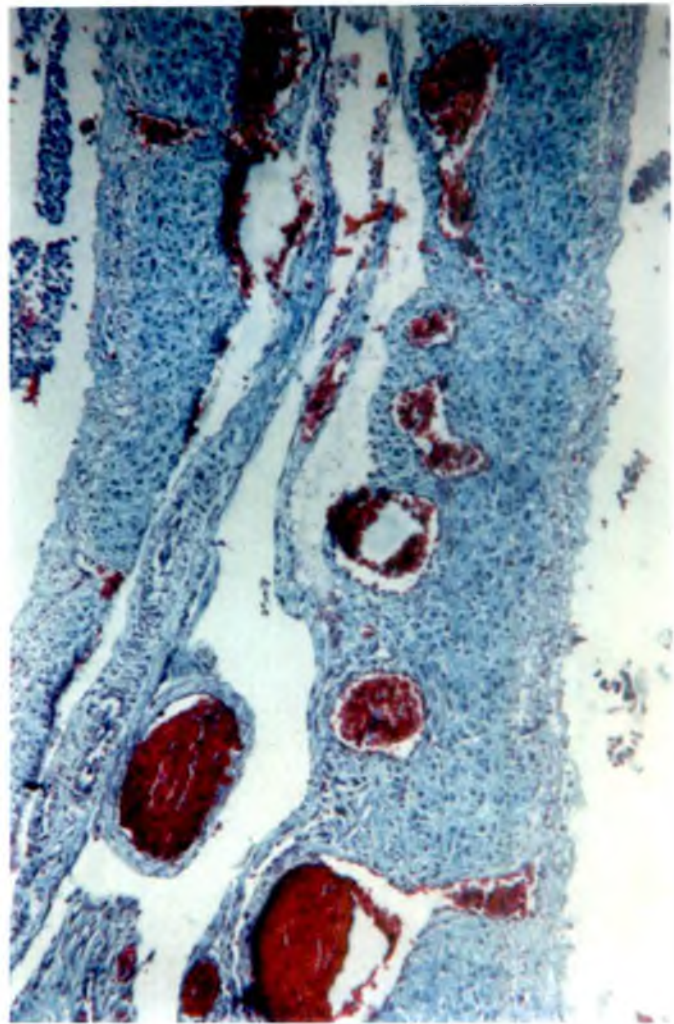


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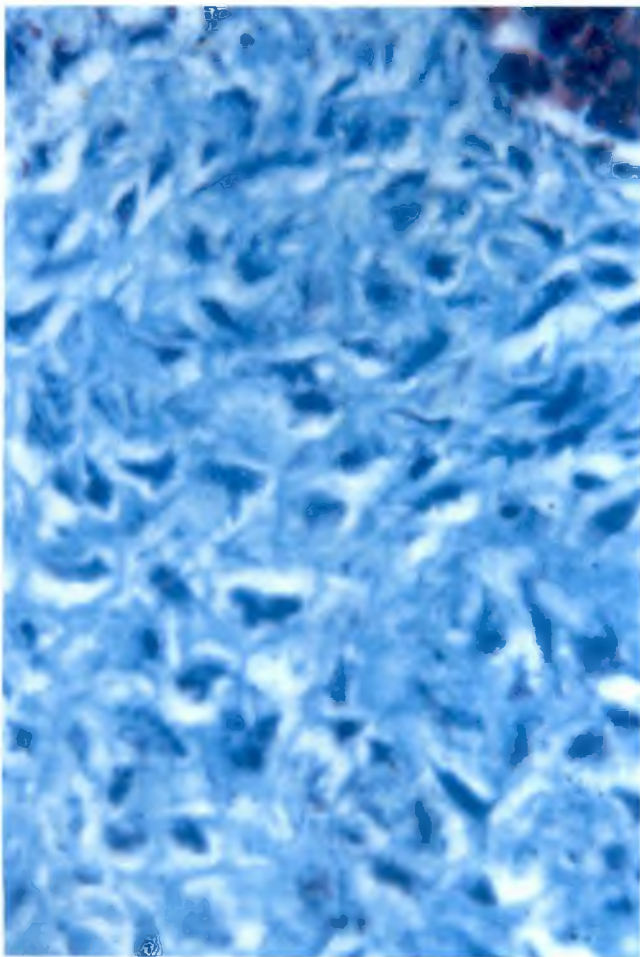


Fig - 28



Fig - 29

Fig. 30. Testicles of a normal adult male

Fig. 31. Testis - narrow seminiferous tubules with marked intertubular fibrous tissue proliferation. H&E x 160

Fig. 32. Testis - seminiferous tubules and interstitial tissue in a normal adult male. H&E x 63

Fig. 33. Testis - degeneration of spermatogonial cells with leucocytic infiltration. H&E x 250



Fig - 30

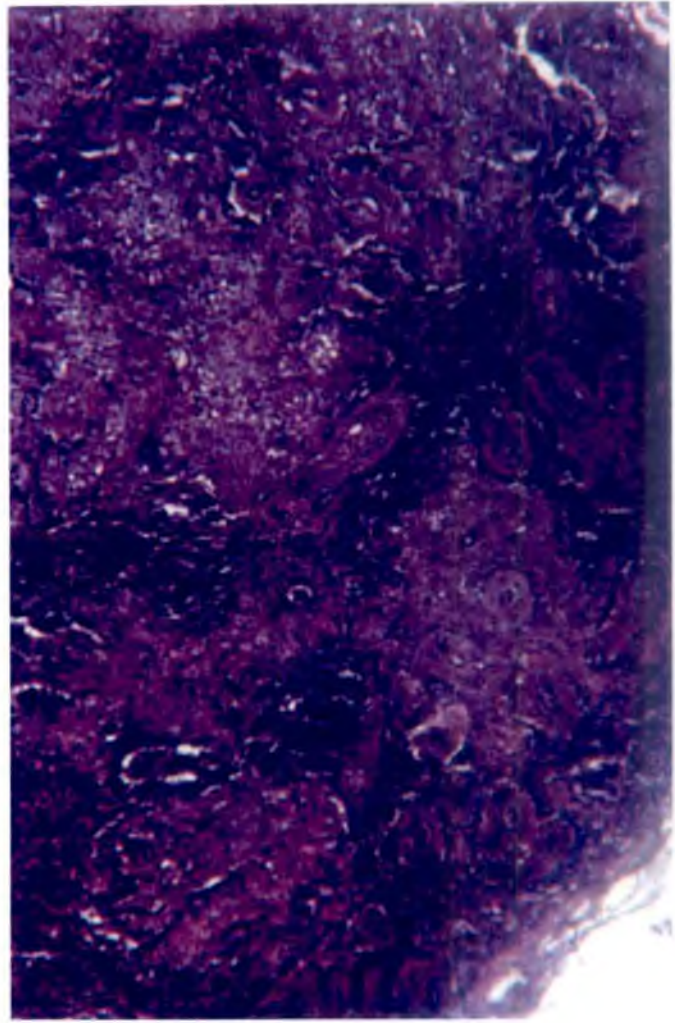


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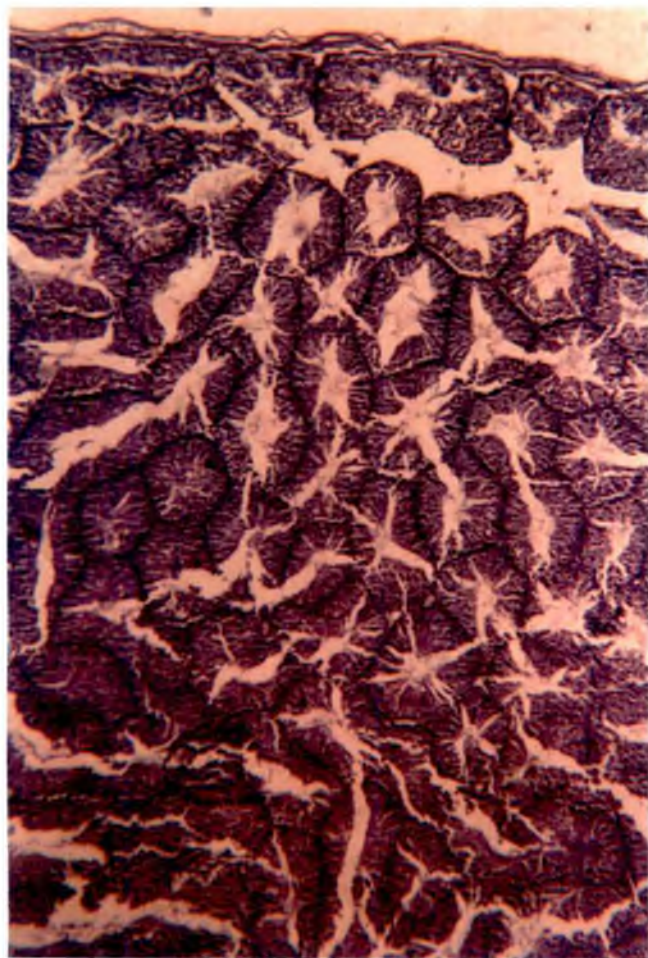


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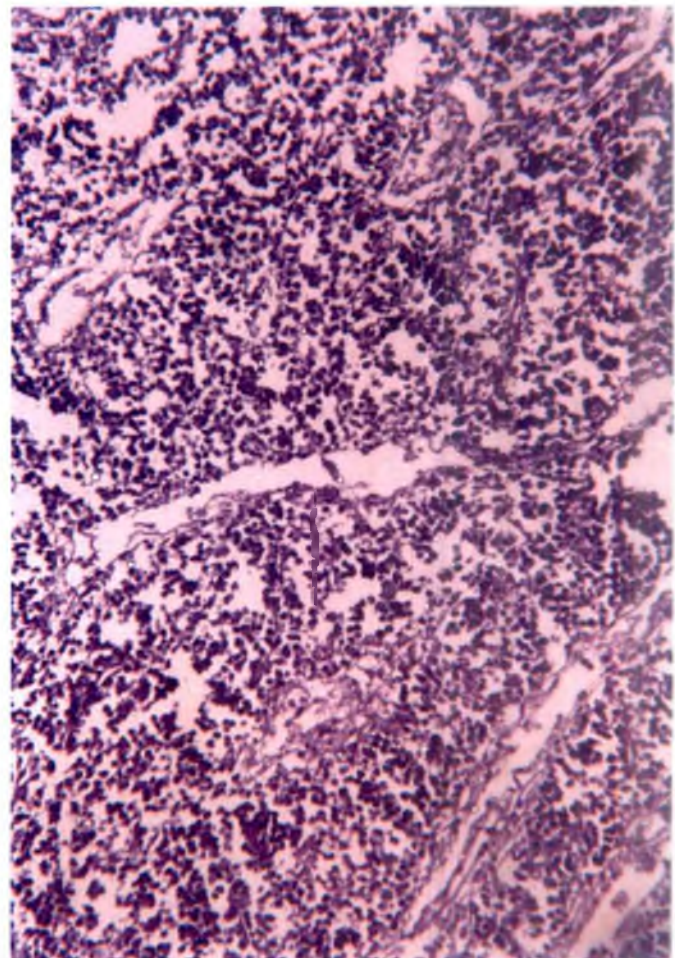


Fig - 33

Discussion

5. DISCUSSION

The present study was undertaken to investigate the prevalence and pathology of the reproductive tract disorders of Japanese quails (*Coturnix coturnix japonica*). The data collected from the autopsy records maintained at the Centre of Excellence in Pathology and gross and histopathological observations of the samples collected from necropsy done during study period formed the materials for the study.

The occurrence of reproductive disorders is steadily rising. This is evident from the fact that incidence rate of reproductive disorders are increasing from 1997-98 to 1999-2000 and this view reconciles with that of Srinivasan *et al.* (1980). They recorded that the major cause of mortality in the adult quails was egg bound/peritonitis and the cause-specific mortality rate of egg bound as 2.40 during 1976 and 6.60 during 1977 underlining the upward trend of the disease. One obvious reason was the increase in flock strength.

Egg bound was observed in 134 out of 1139 adult quails at an incidence rate of 11.75 per cent and this observation is in agreement with that of Valsala and Sivadas (1970) in chicken (12.7 per cent), Mohiuddin (1982) in chicken (10.54 per cent) and Bhowmik (1983) in ducks (11.53 per cent). Various other authors (Keymer, 1980; Christensen, 1986; Yadgirkar *et al.*, 1992) shared the common view that egg bound/egg peritonitis was the predominant non-specific cause for mortality in adult female birds and the results of the current investigation reconciles with their view.

Necrosis of the mucosal epithelium, focal glandular hyperplasia, heterophilic infiltration, hyperemia of the vessels and focal haemorrhage were the observations noted in the oviduct collected from egg bound cases, which are in agreement with the findings of Valsala (1968) in chicken. As the egg bound is an acute or sometimes peracute condition, the histology revealed mostly infiltration of heterophils, necrosis of the mucosal epithelium and hyperemia.

In the present study, *Escherichia coli*, *Klebsiella* and *Proteus* were isolated from 5, 2 and 1 cases of egg bound respectively as Sharma *et al.* (1980a) isolated *Escherichia coli*, *Proteus mirabilis* and *Klebsiella* from egg peritonitis and Sharma and Joshi (1985) isolated *Escherichia coli* from a case of egg bound.

In this study it was noted that the prevalence of egg bound was higher in birds exposed to higher photoperiod (18L - 6D or more light hours). This correlates with the observations of Sturkie and Mueller (1976) who reported that light stimulates the pituitary to secrete gonadotropins, particularly FSH. The light hour pattern suitable for Japanese quails was studied by Prabakaran *et al.* (1991) who concluded that the best light regime for egg production was either 14 h continuous or 14 h at the fourth week, which could be gradually increased by weekly increments of 30 minutes to reach a constant 16 h light regime.

Sixty per cent of the egg bound birds were presented with poor conditions of the body and breast muscles, which might have caused weaker muscular contractions and inadequate abdominal press subsequently leading to impaired oviposition. Sturkie and Mueller (1976) stated that the impaired abdominal press delayed oviposition slightly. This is consistent with the present observation.

About 20 per cent of the eggs recovered from egg bound cases were thin shelled or leathery indicating a deficiency of calcium as the quails were fed with broiler ration, which contained inadequate calcium and this finding fell in line with Davison (1978) who attributed egg shell thinning to hypocalcaemia in ducks and chicken. Shukla *et al.* (1994) reported that the Japanese quails receiving layer ration from the 6th week onwards had the maximum production potential. In birds receiving feed comprising 50 per cent grower ration and 50 per cent layer ration, percent shell weight and shell thickness were significantly affected. This finding supports the present observations. The role of calcium in causing egg bound in Japanese quails needs to be studied.

In the present study, two eggs were bound one after the other and this was seen in 15 per cent of the egg bound cases. This is in contrast to the observations made by Valsala and Sivadas (1970) that usually a single egg was lodged in the

caudal portion of the oviduct in chicken. This variation could be attributed to the difference in laying pattern between Japanese quails and chicken.

In the current study, the egg bound was seen in both pullets and old birds in equal proportion and this is in contrast to the reports of Riddell (1991) that young pullets laying an unusually large egg were prone to egg bound. This could be due to the presence of a large number of aged breeder quails in the farm, which formed the main source of necropsy specimens.

Salpingitis and egg bound occurred simultaneously in five cases, ^{and} larger eggs were found in the lumen of the oviduct in 80 per cent of the egg bound cases and these observations are in agreement with that of Riddell (1991). He reported that the egg bound might result from salpingitis, paresis of muscles of the oviduct, or production of an egg so large that it is physically impossible for it to be laid. Gorham *et al.* (1992) suggested that ectopic egg or egg binding might result due to the small size of the abdominal cavity and relatively larger size of the eggs in pet birds. Lucy (1994) reported that quails laid eggs, which weighed about eight per cent of their body weight and this was much higher than that of chicken (three per cent) and turkey (one per cent). Relatively larger eggs could be one of the predisposing factors for the prevalence of egg bound in quails when compared to chicken and ducks. Therefore, the present finding is in accordance with the observations of Riddell (1991), Gorham *et al* (1992) and Lucy (1994).

In the current investigation, glycogen was demonstrated neither in the isthmus epithelium nor in the smooth muscles of the oviduct from egg bound cases using the McManus method of Periodic acid - Schiff stain. Lucy and Harshan (1998) were able to demonstrate glycogen granules in the apex of the ciliated columnar cells of the pseudostratified epithelium of the isthmus in Japanese quails, which might contribute to the carbohydrate portion of the shell membrane. However, they could not demonstrate the presence of glycogen in the smooth muscles of the oviduct. The role of derangement of energy metabolism in the pathogenesis of egg binding needs further exploration.

Presence of pecking lesions near the vent and the history indicated that pecking was prevalent in the farm. This finding gains importance due to the fact that pecking predisposes the birds to egg bound condition. Wechsler and Schmid (1998) concluded from an elaborate experiment that injuries caused by aggressive pecking are an important welfare problem in quail farming and recommended that the optimum sex ratio of 1:8 or 1:12 in the flock could help to reduce the prevalence of pecking.

Oophoritis was observed in 144 cases encountered with a prevalence rate of 12.64 per cent and this was mostly seen in association with egg peritonitis or salpingitis, which indicated the extension of infection to the peritoneum or descending to the salpinx respectively. *Escherichia coli* and *Proteus* were isolated from 4 and 2 cases of oophoritis respectively and this is in accordance to the observations of other authors. Valsala (1968) reported that *Escherichia intermedia* was mainly involved in the pathogenesis of salpingitis and oophoritis in poultry. *Escherichia coli*, *Salmonella* and *Proteus* were isolated by Choudhary *et al.* (1986) in chicken.

Haemorrhagic cysts were seen in the ovary as cysts containing masses of blood clot or large blood clots and the wall of the cyst was formed by collagenous tissue, which was rich in capillary spaces filled with blood as observed by Valsala and Sivadas (1971).

Fibroplasia was the most common microscopic feature observed in the reproductive organs of Japanese quail in chronic oophoritis conditions and this view is similar to that of Mukhopadhyay *et al.* (1999a) who studied the chicken reproductive organs infected with *Escherichia coli*. They also observed mononuclear cell infiltration in the wall of the oviduct and diffuse area of edema in the magnum in a few cases. The histopathological details were consistent with the studies on the reproductive organs of chicken by Valsala (1968) and Randall and Reece (1996) and in ducks by Jayakumar (1986).

Focal hyperplasia of granulosa cell layer with disintegrating follicle was seen, which needed differentiation from granulosa cell tumour. Randall and

Reece (1996) recorded the histological feature of ovarian granulosa cell tumour as composed of polyhedral neoplastic cells with eosinophilic cytoplasm arranged in the form of tubules. No such tubular pattern and neoplastic changes were found in this case, therefore, the granulosa cell tumour was ruled out.

Salpingitis was seen in 55 instances out of 1139 autopsies performed. Mostly the salpingitis occurred in conjunction with egg bound. The most consistent finding in almost all the cases was congestion of the blood vessels on the submucosal and serosal layer of the oviduct, which is in agreement with that of Jayakumar (1986). Extensive degeneration, necrosis and desquamation of the epithelial lining cells and severe infiltration of the submucosa with heterophils and mononuclear cells were also noticed. These observations are consistent with that of Valsala and Sivadas (1971) in chicken and Jayakumar (1986) in ducks. Bacterial clumps were visible in the core of the fibrinous exudate in the lumen of the oviduct as observed by Randall and Reece (1996) in chicken. The lesions were suggestive of bacterial etiology. One isolate of *Escherichia coli* was obtained from a single case, which is in accordance with the findings of Valsala (1968) and Batra *et al.* (1982) in chicken.

Impaction of the oviduct was observed in four out of 1139 cases examined at an incidence rate of 0.66 per cent. The occurrence of oviduct impaction was minimal when compared to other avian species as observed by Valsala (1968) in hens (2.81 per cent) and Jayakumar (1986) in ducks (1.55 per cent). Keymer (1980) noted that the Galliformes, to which the domestic fowl and quails belong, appeared to be the most susceptible to oviduct impaction than other birds.

The accumulation of a cheesy mass, concentrically laminated and arranged in onion skin formation in quail oviduct was observed and this is in accordance with the findings of Valsala (1968), Jayakumar (1986) and Sreraman *et al.* (2003).

The number and size of small masses was variable as reported by Sreraman *et al.* (2003). In all the instances, the impaction occurred in the magnum and uterine portion of the oviduct, which is consistent with that of

Jayakumar (1986). He also recorded the impaction of oviduct in the infundibulum in one case.

The histological features are in accordance with the observations of Valsala (1968) in chicken and Jayakumar (1986) in ducks. Severe infiltration of the submucosa with heterophils and lymphocytes was seen in the submucosal layers of the oviduct as reported by Valsala (1968). Focal degeneration, necrosis and desquamation of the lining epithelium were observed as recorded by Jayakumar (1986).

Escherichia coli and *Proteus* were isolated in one case of the oviduct impaction and this finding reconciles with that of Batra *et al.* (1982) who isolated *Escherichia coli* from the impacted oviduct of chicken. Since *Escherichia coli* are the most common enteropathogenic bacteria, they could infect the impacted oviduct through cloaca.

The only neoplastic condition encountered in this study was a single case of fibroma of the left ovary. Ovarian fibroma was seen as a firm mass attached to the ovary. Histological examination revealed haphazardly arranged masses of connective tissue, which was in accordance with that of Goswami *et al.* (1988). They recorded two cases of fibroma in the mesosalpinx of chicken, which were hard, round to oval in shape and about an inch in diameter.

Hypoplasia of the testicles was observed in a 60 days old male Japanese quail characterised by narrow seminiferous tubules lined by single layer of flattened cells along with interstitial fibrous tissue proliferation, which was consistent with the findings of Jayakumar (1986) in a duck.

A single case of orchitis was observed. The histologic picture of degeneration of the spermatogonial cells, infiltration of heterophils and fibrinous exudate was similar to that of Randall and Reece (1996). *Escherichia coli* was isolated.

The present investigation was primarily aimed at studying the gross and histological changes in the quail reproductive organs along with bacterial etiology involved. From the present study, it may be concluded that a multitude

of factors like excessive photoperiod, improper ration, improper sex ratio in the flock, specific and non-specific bacterial infections are involved in causing reproductive diseases. Proper managerial practices like feeding recommended level of quail layer ration, photoperiod, sex ratio and practices like debeaking coupled with antibiotic regime could be helpful in reducing the incidence of reproductive diseases and in achieving the farmer's goal of profitable quail farming. .

Summary

6. SUMMARY

The present investigation was primarily aimed at studying the prevalence, gross and histological changes in the quail reproductive organs along with the bacterial causes involved. The incidence and pathological features of reproductive diseases in quail were investigated based on the records available and also on the gross and histopathological lesions encountered. The retrospective survey revealed that the incidence of reproductive diseases were oophoritis (3.07 per cent), egg bound (9.46 per cent), salpingitis (1.51 per cent), orchitis (0.02 per cent) and oviduct impaction (0.09 per cent).

Samples collected from 102 Japanese quails were subjected to detail gross and histopathological examination. The conditions encountered were clarified and pathological features were recorded. Samples for isolation of bacterial organisms were also collected. Isolation and identification of bacterial organisms were performed. Egg bound was observed in 134 out of 1139 adult quails autopsied and it had a prevalence rate of 11.75 per cent. From the present study, it was noted that the prevalence of egg bound was higher in birds exposed to higher photoperiod (18L - 6D or more light hours). It was advised to decrease the light hours along with suitable antibiotic regime. When the photoperiod was reduced to 14L - 10D or 16L - 8D, there was a drop in the incidence of egg bound.

Escherichia coli, *Klebsiella* and *Proteus* were isolated from 5, 2 and 1 cases of egg bound respectively. Histopathologically, necrosis of the mucosal epithelium, focal glandular hyperplasia, heterophilic infiltration and hyperemia of vessels were observed.

Oophoritis was observed in 144 cases out of 1139 cases encountered with a prevalence rate of 12.64 per cent and this was mostly associated with salpingitis or egg peritonitis. *Escherichia coli* and *Proteus* were isolated from 4 and 2 cases of oophoritis respectively. The non - specific stress associated with high

production could have predisposed the birds to *Escherichia coli* infection. Fibroplasia was the most common microscopic feature observed.

Salpingitis was seen in 55 out of 1139 autopsies performed. Mostly the salpingitis occurred in conjunction with or as sequelae to egg bound. One isolate of *Escherichia coli* was obtained from a single case. Impaction of the oviduct was observed in four cases. *Escherichia coli* and *Proteus* were isolated.

A case of fibroma of the left ovary was encountered in the present study. Histological examination revealed haphazardly arranged masses of collagen. Few haemorrhagic cysts were also seen.

A single case of hypoplasia of the testes was observed in a 60 days old male Japanese quail. Orchitis was observed in one case from which *Escherichia coli* was isolated. Infiltration of heterophils, congestion of blood vessels and degenerative changes in gonadal cells were noticed.

From the present study, it was concluded that proper managerial practices coupled with antibiotic regime could be helpful to reduce the incidence of reproductive disorders and to achieve the farmer's goal of profitable quail farming.

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**PATHOLOGY OF THE REPRODUCTIVE SYSTEM
OF JAPANESE QUAILS (*Coturnix coturnix japonica*)**

By
SIVAKUMAR. V.

ABSTRACT OF THE THESIS

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Centre of Excellence in Pathology
COLLEGE OF VETERINARY AND ANIMAL SCIENCES
MANNUTHY, THRISSUR - 680651
KERALA, INDIA

ABSTRACT

The present study was undertaken to assess the prevalence and pathology of various diseases affecting the reproductive system of Japanese quails. The data collected from the autopsy records maintained at the Centre of Excellence in Pathology, Mannuthy and one hundred and two samples of reproductive organs obtained from the necropsy cases formed the basis of the study. Retrospective survey revealed the increasing incidence of the reproductive diseases. Out of the 1139 cases encountered, various reproductive diseases were seen in quails at an incidence rate of 25.11 per cent. Oophoritis was the most prevalent among them at an incidence rate of 9.46 per cent. The higher incidence of reproductive diseases could be attributed to the maintenance of large number of breeder stock in the quail farm, which formed the major source for the necropsy samples. The gross and histopathological examination revealed that among the reproductive diseases the highest mortality was due to oophoritis (12.64 per cent) followed by egg bound (11.75 per cent). Other cases were salpingitis, egg impaction, ovarian fibroma, testicular hypoplasia and orchitis. *Escherichia coli*, *Klebsiella* and *Proteus* were isolated from various cases. The incidence of pathological disorders encountered was relatively high. The need and scope for investigation into the diseases affecting quail reproductive system were highlighted.