CHAPTER-4

Histological changes in ovary, oviduct and uterus in female rats fed with different ratios of fish meal during F1 and F2 generation

The female reproductive system consists of pair of ovaries, oviduct and uterus, single cervix and vagina opening out through the vulva (Fig. 6).

4.1: Ovary

The ovaries are paired dull pinkish in colour and appear like a bunch of grapes. They are abdominal in position suspended in the dorsal body wall by ligament. Each ovary is enclosed in a sac like structure called bursa that prevents ova from escaping into the abdominal cavity. The ovary is covered externally by a transparent connective tissue capsule, tunica albuginea (Fig: 7).

The control rat ovary is divided into outer cortex and inner medulla. The cortex consists of multiple follicular cell development from primordial upto graafian follicle, stroma and corpus luteum. The medulla consists of loosely arranged connective tissue, stroma, elastic fibres, blood, lymphatic capilaries and nerves. Primordial follicle consists of single layer of flattened squamous granulosa cells. The percentage of primordial follicles was 47% and the mean diameter was $16\pm0.75\mu$. Squamous follicular cells surrounding the primordial follicles differentiate into a single layer of columnar cells forming primary follicle. The Percentage of primary follicle was 13% and the mean diameter was $115\pm5.74\mu$. Prolifereation of the mono layer of
columnar cells results in the formation of multilayered zone of granulosa cells around the oocyte, accompanied by amorphous layer of zona pellucida between oocyte and zona granulosa. It continues to grow and multiple fluid filled spaces form within the zona granulosa which is termed as vesicular follicle. Stromal cells surrounding the developing follicle become arranged into concentric layers and form theca. This layer is separated from zona granulose by a basement membrane. Percentage of secondary follicles was 18% and the mean diameter was $210 \pm 0.81 \mu$ (Table 11&13, Fig: 7) respectively.

The mature follicles consist of fully grown oocyte surrounded by zona pellucida, undifferentiated stromal cells that develop into two distinct layers the theca interna and theca externa. The theca interna have loosely arranged connective tissue with large rounded cells whereas the theca externa consist of fibres with spindle shaped cells. Both layers of thecal cells are separated from the membrane granulosa of the follicle by a basement membrane. The cells of membrane granulosa lodging the ovum and are called cumulous oophorous. The cells of cumulous oophorous are arranged radially around the zona pellucida. A large number of irregular lacunae appear among the granulosa cells which forms the antrum. The surrounding granulosa cells secrete fluid the liquor folliculi or follicular fluid filling the antrum (Fig: 8). Percentage of matured follicles was 7% and 8% and the mean diameter was $468 \pm 3.03 \mu$ and $468.1 \pm 4.02 \mu$ (Table 11 -12 & 13 - 14, Fig. 8). The corpus luteum showed polygonal cells, containing large nuclei. As the corpus luteum matures and subsequently degenerates. Numerous bloodvessels are present. Percentage of corpus luteum was 10%, 14% and the mean diameter was $764 \pm 3.18 \mu , 769 \pm 2.0 \mu $ (Table 11 -12 & 13 - 14, Fig: 8). Only a small number of primordial
follicle progress through folliculogenesis to form Graafian follicle and ovulate. The remainder undergoes follicular degeneration or atresia at various stages during follicular maturation (Fig. 8). Percentage of Atretic follicles was 5% and 4% and the mean diameter was 104±3.26μ, 109.6±0.8μ (Table 11&12 & 13 & 14) in both F1 anf F2 generation respectively.

The rats fed with 1:1 ratio of fish meal during F1 generation showed disorganised growing follicle, loosely arranged stromal and luteal cells. Matured follicles showed slight variation in organisation but not much difference was observed when compared with that of control (Fig. 9). Primordial, primary and secondary and matured follicles (46%, 12%, 15%, 5) decreased. Further increase in number of atretic follicles was noticed (14%). Corpus luteum showed decrease in percentage (8%) and decrease in diameter of primordial follicles (15± 0.51μ), primary follicle (100± 0.51μ), secondary follicles (200± 1.03μ), matured (450± 0.51μ), atretic (98± 1.22μ) and corpus luteum (750± 0.81μ) were noticed (Table 11&13).

The rats when fed with 1:2 ratios of fish meal, the ovary showed reduction in the number of primordial, primary and secondary follicles. The growing follicles were lightly stained, the stromal cells were found to be loosely arranged, and degeneration of matured follicle was noticed (Fig. 10). Increased in number of atretic follicle (25%) and reduction in corpus luteum was observed (6%). Percentage of developing primordial, primary, secondary and matured follicles cells decreased further (45%, 10%, 10%, 4%).

The hypotrophy of follicles with decreased in diameter of primordial (14.8± 0.40μ), primary (99± 0.98μ), secondary (198± 0.51μ), matured (438± 2.58μ), atretic follicles (96± 0.51μ), corpus luteum (741± 5.89μ) were noticed when compared to control (Table: 11&13) respectively. Rats fed with 1:3 ratios of fish meal
showed atrophy of growing follicles, increase in number of vacuoles, loosely arranged stroma, Hypotrophy of the matured follicles, disorganised antrum and with vacuoles (Fig. 11). Significant decrease in Percentage of developing primordial, primary, secondary and matured follicles (42%, 9%, 9%, 4%) was noticed. Further percentage of atretic follicles showed drastic increase (30%) and corpus luteum showed gradual decrease (6%) (Fig. 11). Significant hypotrophy of follicles with decreased in diameter of primordial, primary, secondary, matured, atretic follicles and corpus luteum (13± 0.40μ, 97± 0.83μ, 191± 1.03μ, 421± 1.03μ, 95± 0.51μ, 731± 3.92μ) was also noticed when compared to control (Table: 11&13) respectively. The rats when fed with only fish meal the matured follicles showed distorted ooocytes, reduction in number and atrophy of growing follicles, increase in number of atretic follicles and decrease in number of corpus luteum (Fig. 12). A significant decrease in percentage of developing primordial, primary, secondary and matured follicles (42%, 8%, 7%, 3%) was observed. Further atretic follicles showed drastic increase (35%) and corpus luteum showed a gradual decrease (5%) and significant hypotrophy of follicles with decreased in diameter of primordial, primary, secondary, matured, atretic follicles and corpus luteum (12.5± 0.54μ, 95.1± 0.40μ, 189± 1.96μ, 415.6± 0.51μ, 91.3± 1.03μ, 731± 3.92μ) when compared to control rats (16± 0.75μ, 115± 5.74μ, 210± 0.81μ, 468± 3.03μ, 764± 3.18μ) (Table 11 &13) respectively. There was no significance difference in diameter of primordial follicles between control and 1:1 (p=0.017), 1:3 and only fish meal (p=0.230), primary follicle 1:1 and 1:2 (p=0.982), secondary follicle 1:1 and 1:2 (p=0.135), 1:3 and only fish meal (p=0.596), atretic follicle, 1:1 and 1:2 (p=0.741)
During F2 generation the rats fed with 1:1 ratio of fish meal showed darkly stained blood vessels, reduction in number of growing follicles, loosely arranged luteal cells and matured follicle showed disintegration of oocytes (Fig. 13). Significant decrease in percentage of developing primordial cell (46%), primary, secondary, matured follicles and corpus luteum (11%, 14%, 5%, 12%) and further, increase in number of atretic follicles was noticed (12%) when compared to control (4%). (Table: 12 & 14). Significant decrease in diameter of primordial, primary, secondary, matured, atretic follicles and corpus luteum was (14.8±0.98μ, 97.8±0.40μ, 197.5±0.54μ, 437.5±2.73μ, 88.8±1.47μ, 729.6±0.81μ) were observed when compared to control 16.5±0.83μ, 111.5±2.34μ, 214.6±5.88μ, 468.1±4.02μ, 109.6±0.81 and 769.1±2.04μ. (Table: 12 & 14). Growing follicles were loosely arranged and showed disorganisation, increase in number of vacuoles, degeneration of stromal cells and increase number of atretic follicles when the rats were fed with 1:2 ratios of fish meal (Fig. 14). There was a drastic significant decrease in percentage of developing primordial cells (45%). Primary, secondary and matured follicles (10%, 10%, 4%) also showed much variations in number when compared with control. Further atretic follicles increased to (22%) and corpus luteum showed a significant decrease (9%). Significant hypotrophy of follicles with decreased in diameter of primordial (14.1±1.16μ), primary (96.5±0.54μ), secondary (195.5±1.76μ), matured (420.3±0.81μ), atretic follicles (82.8±0.40μ), corpus luteum (708.6±1.96μ) were noticed when compared to control (Table: 12 & 14). The rats when fed with 1:3 ratios of fish meal, growing follicles showed hypotrophy and medullary region showed more vacuolization, number of growing and matured follicles decreased with increase in number of atretic follicles (Fig. 15). There was a
drastic significant decrease in percentage of developing primordial cells decreased further (42%) primary, secondary and matured follicles (8%, 8%, 3 %). Further, atretic follicles increased to (35%) and corpus luteum showed a significant decrease (4%), Significant hypotrophy of follicles with decreased in diameter of primordial (12.6±0.51 μ), primary (95.3±0.81 μ), secondary (195.5±1.1 μ), matured (420.3±1.2 μ), atretic follicles (82.8± 0.4 μ) and corpus luteum (708.6±1.9 μ) were noticed when compared to control (Table: 12&14). Clumping and disorganised growing follicles, number of matured follicles with distorted oocyte and all the follicles undergoing atretic condition were noticed in rats fed with only fish meal during F2 generation (Fig. 16). There was a drastic significant decrease in percentage of developing primordial cells (40%), primary, secondary and matured follicles (7%, 8%, 2%). Further atretic follicles increased to (40%) and corpus luteum showed a significant decrease (3%). Significant hypotrophy of follicles with decreased in diameter of primordial (11.6±0.51 μ), primary (94.1± 0.40 μ), secondary (184.8±0.40 μ), matured (403.6±2.73 μ), atretic follicles (79.1± 0.40 μ), corpus luteum (678.5±0.54 μ) were noticed when compared to control (16.5±0.83 μ, 111.5±2.34 μ, 214.6± 5.88 μ, 468.1±4.02 μ, 109.6±0.81 and 769.1±2.04 μ) (Table: 12&14). There was no significant difference in diameter of primordial follicles 1:1 and 1:2 (p=0.653), 1:3 and only fish meal (p=0.272), primary follicle 1:1 and 1:2 (p=0.304), secondary follicle 1:2 and 1:3 (p=0.734), matured follicle 1:2 and 1:3 (p=0.734) and atretic follicle, 1:2 and 1:3 (p=0.625) respectively.

### 4.1.2: Oviduct

Oviduct is a paired coiled tubular organ extending between ovary and uterus. The anterior tip of oviduct is in the form of a ciliated funnel called infundubulam which continues
posterior in to a bulbus chamber called ampulla. The ampula opens into a short segment representing istmus which is responsible for transport of ova. (Fig. 6)

Oviduct is covered externally by a serosa with smooth muscle fibres. Beneath the serosa is muscularis layer consist of inner circular layer and outer longitudinal layer of muscle. The inner most layer lining the lumen is mucosa that consist of simple columnar epithelium (Fig. 17). Some of the epithelial cells are ciliated, the ciliary beating and the muscular contraction helps in the transport of fertilised ova. Narrow peg shaped non ciliated cells that secrete mucus and specific product into the oviducal fluid were noticed (Fig. 18).

Rats fed with 1:1 ratio of fish meal during F1 generation showed thick mucosal layer with elongated folds and ciliated cells projecting towards narrow lumen of oviduct. Serosal layer with smooth muscle fibres were observed. (Fig. 19). Rats fed with 1:2 ratio of fish meal showed large number of elongated mucosal folds with highly restricted lumen space, ciliated cells showed highly prominent dense cilia projected towards lumen with few peg shaped non ciliated secretory cells (Fig. 20). Rats fed with 1:3 ratios of fish meal showed thin muscularis, highly elongated mucosas folds with simple columnar epithelium and large number of darkly stained ciliated cells with dense cilia and few non ciliated peg shaped secretory cells (Fig. 21). Rats fed with only fish meal showed thick serosa, the degree of extension and area of involvement of mucosa increased with large number of mucosal folds, more number of dense ciliated cells and lesser non ciliated cells, (Fig. 22).

During F2 generation, rats fed with 1:1 ratio of fish meal further showed large number of mucosal folds with constricted lumen space, thin muscularis layer and highly
elongated mucosal folds with and large number of ciliated cells with dense cilia and less non ciliated secretory cells (Fig. 23). 1:2 ratios of fish meal fed rats during F2 generation showed disorganised oviduct with thick outer serosa layer and thick muscularis layer, elongated ciliated cells with dense cilia and few non ciliated cells. Extension of mucosal layer further increased with accumulation of shedded epithelial tissues into the lumen. (FIG. 24). Rats treated with 1:3 ratios of fish meal showed highly disorganised oviduct with thick serosa and, elongated mucosal folds were more prominent. Increase of ciliated cells with dense cilia and decrease in non ciliated cells projecting towards the lumen were also noticed. (Fig. 25). Rats when treated with only fish meal showed thick serosal layer and thin middle muscularis. The mucosal folds were more and significantly elongated and occupied entire area of the lumen. The branching of mucosa showed lining of columnar epithelium. Presence of lesser number of non ciliated cells was noticed (Fig. 26).

4.1.3: Uterus

The uterus is Y shaped structure with 2 horns, which are tubular anteriorly and united posteriorly (Fig. 6). The control rat uterus during F1 generation consists of outer layer of perimetrium with thickness of 18.6±2.94, middle layer of myometrium with thickness of 191.6±3.82and an inner layer of endometrium with thickness of 275.3±3.01 and number of uterine glands was 16.1±0.75. Myometrium consisted of outer layer of longitudinal muscle and inner later of circular muscle. Endometrium consists of lamina propria, uterine epithelium, uterine glands and number of blood capillaries (Table: 15& Fig. 27). Rats of F2 generation uterus showed all three layers of endometrium consisting of columnar cells n the cytoplasm and uterine glands were prominent with simple cuboidal epithelium.
Myometrium consisted of thick circular layer and thin outer smooth with blood vessels and perimetrium with evenly arranged serosa layer. The thickness of endometrium layer was 277.6±4.08, myometrial layer (191.16±2.85) and perimetrial thickness was (19.0±2.36) and the number of uterine glands was 16.33±0.81 (Table: 16 & Fig. 28).

Rats fed with 1:1 ratio of fish meal showed decrease in thickness of surface epithelium, decrease in number of uterine glands and decrease in thickness of myometrium, perimetrium 16.0±3.09 with larger spaces among cells. Uterine glands were irregularly spaced and small in number (15.5±0.83) and endometrial, myometrial, perimetrial thickness (267.6±5.16, 186.5±3.72, 16.0±3.09) also showed reduction when compared with control. (Table-16 & Fig. 29). Rats fed with 1:2 ratios of fish meal showed endometrial layer disorganized and contain many degenerating epithelial cells, irregularly arranged uterine glands, thin myometrium and perimetrium revealed spaces. Uterine glands appeared distorted with less secretory cells and significant reduction in number was seen 13.50±0.83 when compared to control, 16.1±0.75) and endometrial, myometrial, perimetrial thickness 253.3±2.58, 171.6±1.60, 12.5±0.83) also showed reduction (Table:16 & Fig. 30).

Rats fed with 1:3 ratios of fish meal showed elongated thin columnar cells of endometrium, hypotrophy with less number of shrunken uterine glands. Reduction in thickness of endometrium, myometrium and perimetrium was noticed. 252.6±0.51, 170.6±0.81, 10.1±0.40 and uterine gland number also decreased 11.6±0.51. (Table:16 & Fig. 31). Rats treated with only fish meal revealed hypotrophy of uterine glands and distortion of blood vessels and endometrium, myometrium showed lesser thickness and uterine glands was distorted remarkably (9.83± 0.57). The
cells of endometrial layer reduced, highly disorganised with inconspicuous nuclei, dense vacuolisation and extensive degeneration. The endometrium, myometrium and perimetrium also showed reduced thickness (250.83±1.72, 167.8±0.75, 10.0±0.89) when compared with that of control Values are significant at p<0.05. (Table:16 & Fig. 32). There was no significance difference in diameter of endometrial layer between 1:2 and 1:3 (p=0.995), myometrium between 1:2 and 1:3 (p=0.407), perimetrium between 1:3 and only fish meal (p=1.00).

During F2 generation rats fed with fish meal at 1:1 ratio the endometrium showed many folds appeared with decrease in epitjelial thickness, disorganised thin myometrium. Uterine glands reduced in number (14.0±0.40) when compared to control (16.33±0.81) and endometrial, myometrial, perimetrial thickness (264.5±0.83, 183.8±1.32, 14.16±1.16) also showed reduction when compared with control (277.6±4.08, 191.16±2.85, 19.0±2.36) Values are significant at p<0.05. (Table: 17 & Fig. 33). Rats fed with 1:2 ratios of fish meal showed endometrial layer disorganized and contain many degenerating epithelial cells with elongated nuclei placed irregularly in the cytoplasm, thin myometrium . Uterine glands appeared distorted, irregularly arranged and reduced in number was seen 9.5±0.54 and endometrial, myometrial, perimetrial layers (249.3±1.03, 169.3±1.86, and 12.0±3.94) also showed reduction when compared with control. (Table: 17 & Fig. 34). Rats fed with 1:3 ratios of fish meal showed elongated thin columnar cells of endometrium with many vacuolated spaces and nuclei and endometrial layer highly disorganized and distorted, irregularly arranged shrunken hypotrophy with reduction in number of uterine glands. Significant reduction in thickness of endometrium was noticed with 246.8±0.98, 167.16±1.47, 09.83.1±0.40 and
uterine gland number also decreased 8.33±0.51(Table: 17 & Fig. 35). Rats treated with only fish meal during F2 generation revealed deceased in thickness of endometrium and loosely arranged cells. The cells of endometrial layer were small, highly disorganised with inconspicuous nuclei, dense vacuolisation and extensive degeneration. Hypotrophy of the uterine glands which were distorted remarkably and showed decrease in thickness of myometrium showed reduced musculature and darkly stained, perimetrium showed loosely arranged cells with vacuolization. Glands appeared shrunken, less secretory substance and less in number (7.83±0.75) compared with control (16.33±0.81). The endometrium, myometrium and perimetrium also showed reduced thickness (242.00±1.54, 161.6±2.58, 09.33±1.96) when compared with that of control. (Table:17 & Fig. 36). There was no significance difference in diameter of myometrium between 1:3 and only fish meal (p=0.407), perimetrium between 1:3 and only fish meal (p=0.996).