3 Results:

3.1: Induction of obesity in adult female rats (F_0 generation):

3.11. Biometric parameters:

The adult female rats that received HCD for 8 weeks appeared obese compared to age matched control females (Fig. 1 A & B).

The body weight, BMI, TC and AC of females fed with HCD for 8 weeks showed a significant increase from 2nd week through 8th week, measured at weekly intervals compared to normal diet fed (ND) (controls) age matched females (Fig. 2 A, B, C & D; Table 1). The gain in body weight of HCD rats was more than 20% compared to controls.

3.12. Biochemical parameters (F_0):

The serum concentrations of fasting glucose, cholesterol and TG in HCD fed female rats after 4 and 8 weeks feeding were significantly higher than ND fed female rats (Fig. 3 A & B; Table 2).

3.13. Fertility indices:

The fertility index was 100 in ND and HCD rats. Though the parturition index lower in HCD rats compared to controls, the difference was not statistically significant whereas gestation index was significantly lower in HCD than ND group. In HCD rats the litter size and litter weight were significantly lower whereas body weight/pup was significantly higher when compared to ND group (Fig. 4 A & B;

Table 3). There was no mortality of pups in controls whereas it was 10% in HCD fed rats (Fig. 4 A & B; Table 3).

3.2. Spermatogenesis, sperm parameters and onset of puberty in male offspring (F_1) of HCD and ND fed females (F_0):

3.21. Biometric parameters:

The male offspring of HCD fed female rats appeared obese compared to lean appearance of offspring of ND rats on PND 7, 13, 17, 24, 36, 100 and day of preputial separation (Figs. 5, 6, 7 & 8). The offspring of HCD fed mother rats (OHCDM) showed a significantly higher body weight, BMI, TC and AC on PND 7, 13, 17, 24, 36, 100 and day of preputial separation when compared to those of offspring of control (ND) mother rats (OCM) (Fig. 9 A, B, C & D; Table 4). Though, the body length of OHCDM was higher than controls at all age intervals studied, it was significant only on PND 13, 17 and day of preputial separation (Table 4).

3.22. Serum biochemical parameters:

Serum concentrations of fasting glucose, cholesterol, triglyceride and LDL showed a significant increase whereas that of HDL showed a significant decrease in OHCDM compared to OCM on PND 100 and day of preputial separation (Fig. 10 A & B; Table 5).

3.23. Weight of the testes and accessory reproductive organs:

There was a significant increase in the relative weight of the testes of OHCDM on PND 7, 13, 17, 24, 36, 100 and day of preputial separation when compared to that of OCM (Fig. 11 A; Table 6). The relative weight of the epididymis of OHCDM was significantly higher on day of preputial separation and PND 100 compared to OCM whereas that of vas deference and seminal vesicle of OHCDM showed a significant increase only on PND 100 (Fig. 11 B & C; Table 7).

3.24. Histology of the testis and spermatogenesis:

The histological alterations in the testis were studied from PND 7 onwards, as on this day differentiated spermatogonia are clearly visible. The testis of both groups contained seminiferous tubules and the Leydig cells in the interstitial spaces on PND 7. Seminiferous tubules contained only primitive type of spermatogonia in both OCM and OHCDM on PND 7 (Fig. 12 A & B). On PND 13, seminiferous tubule showed the presence of leptotene spermatocytes along with spermatogonia in both the groups (Fig. 13 A & B). On PND 17, seminiferous tubule showed presence of zygotene spermatocytes along with spermatogonia and leptotene spermatocyte in both OCM and OHCDM (Fig. 14 A & B). Pachytene spermatocyes were present in the seminiferous tubule of testis on PND 24, along with spermatogonia, leptotene and zygotene spermatocytes in both groups (Fig. 15 A & B). Round spermatids were present on PND 36 in seminiferous tubules along with spermatogonia, leptotene spermatocytes, zygotene spermatocytes, pachytene spermatocytes in both the groups (Fig. 16 A & B). Round and elongated spermatids were present in the seminiferous tubules on PND of preputial separation along with all other types of germ cells in both

OCM and OHCDM (Fig. 17 A & B). On PND 100 the seminiferous tubules contained spermatozoa in addition to all these types of germ cells (Fig. 18 A & B).

Variation in number of germ cells belonging to different stages of development did not show consistent pattern up to PND 36, although there were significant variations in mean number of one or other categories of germ cells at different age intervals. On PND 17, 36, 100 and day preputial separation, OHCDM showed a significant decrease in mean number spermatogonia. There was a significant decrease in mean number of round and elongated spermatids, an increase in mean number of leptotene and zygotene speratocytes and significant increase in number of pachytene spermatocyte in OHCDM compared to OCM on the day of preputial separation. A similar pattern was found on PND 100 excepting a significant increase in pachytene spermatocytes in contrast to significant increase in leptotene and zygotene spermatocytes on day of prepeutial separation in OHCDM compared to controls (Fig. 19 A-G; Table 8).

3.25. Age of preputial separation:

The preputial separation was at a significantly lower age (days) in OHCDM compared to OCM (Fig. 20 A; Table 9).

3.26. Total epididymal sperm count:

Total cauda epididymal sperm count was significantly lower in OHCDM than OCM on PND 100 (Fig. 20 B; Table 9).

3.27. Abnormal spermatozoa:

The smear of cauda epididymal sperm preparation showed spermatozoa with different types of morphological defects viz. amorphous head, pin head, hammer head, curved tail, hook less head, double head, banana head and double tail etc. The percentage of spermatozoa with defective morphology was significantly higher in OHCDM than OCM when observed on PND 100 (Fig. 20 C; Table 9).

3.28. Serum concentrations of hormones in male offspring:

The serum concentrations of insulin and leptin were significantly higher whereas testosterone was lower in OHCDM than those of OCM on PND 13, 17, 24, 36, 100 and day of preputial separation (Fig. 21 A, B & C; Table 10).

3.3. Ovarian follicular development and onset of puberty of female offspring (F_1) born to HCD and ND fed females (F_0):

3.31. Biometric parameters:

The female offspring born to HCD fed female rats appeared obese compared to lean appearance of offspring born to control female rats on PND 1, 4, 7, 15, 21, 28 and day of vaginal opening (Figs. 22 - 25).

The body weight, BMI, TC and AC of female offspring of high calorie diet fed females on PND 1, 4, 7, 15, 21, 28 and day of vaginal opening were significantly higher than female offspring of controls (Fig. 26 A, B, C & D; Table 11).

3.32. Biochemical parameters:

The serum concentrations of fasting glucose, cholesterol, triglyceride and LDL were significantly higher whereas that of HDL was significantly lower in female offspring of OHCDM when compared to OCM on the day of vaginal opening (Fig. 27 A; Table 12).

3.33. Age (days) at vaginal opening (onset of puberty):

The vaginal opening occurred at significantly earlier age in OHCDM compared to OCM (Fig. 27 B; Table 13).

3.34. Histology of the ovary and follicular counts:

The ovary contained only naked oocytes whereas all other categories were absent in both OCM and OHCDM on PND 1 (Fig. 28 A & B). The naked oocytes and primordial follilces (type 2) were present on PND 4 ovary of both OCM and OHCDM (Fig. 28 C & D). On PND 7, ovary showed presence of primary follicle (type 3a & 3b) along with naked oocytes and primordial follicles (Fig. 29 A & B). All types of pre-antral follicles (type 4, 5a & 5b) and antral follicles (type 6) were present along with primary and primordial follicles on PND 15 in both groups (Fig. 29 C & D). The antral follicles (type 6 and 7) were present along with primordial, primary and pre-antral follilces on PND 21 and PND 28 in both groups (Fig. 30 and 31). Preovulatory follicles and corpora lutea were present on PND of vaginal opening along with all other categories of follicles on both OCM and OHCDM (Fig. 32). The different types of follicles were embedded in ovarian stroma and entire ovary was covered with surface epithelium.

The compliment of follicles belonging to different stages in the ovaries of OCM did not differ from OHCDM as same categories of follicles were found in the ovaries of both the groups on PND 1, 4, 7, 15, 21, 28 and on the day of vaginal opening. However quantitative differences were observed. The mean number of oocytes was significantly higher in OHCDM than OCM on PND 1, 4 and 7. The mean number of primordial and primary follicles were higher in all the age intervals studied in OHCDM than OCM, but significant at some age point and not significant at others. However, pre-antral, antral, pre-ovulatory and corpora lutea showed consistent pattern, i.e. their mean number in OHCDM was significantly higher than controls at all age intervals studied (Figs. 33 A- G; Table 14). There was a significant increase in the number of atretic follicles of primary, preantral, antral, preovulatory follicles at all age intervals studied in OHCDM compared to OCM (Fig. 34 A-E; Table 15).

3.35. Serum concentrations of hormones in female offspring:

The serum concentrations of insulin, leptin and estradiol of OHCDM were significantly higher than those of OCM on PND 15, 21, 28 and day of vaginal opening (Fig. 35 A, B & C; Table 16).

3.4. Reproductive performance of offspring (F_1) born to HCD and ND fed female rats (F_0):

3.41. Study of estrous cycle:

The female offspring born to ND as well as HCD mother rats showed estrous cyclicity as determined by daily observation of vaginal smear for a period of 2 months from the day of vaginal opening. However, there were some alterations in OHCDM.

The mean length of estrous cycle in OHCDM was significantly lower than OCM and mean number of cycles per 2 months period was significantly higher in OHCDM than OCM (Fig. 36; Table 17).

3.42. Biometric parameters during pregnancy of ND and HCD fed F_1 offspring:

The females (F_1) in ND♂ x HCD♀ and HCD♂x HCD♀ groups showed a significant increase in BW, BMI, TC and AC from day zero of pregnancy through 3rd week of pregnancy recorded at weekly intervals, when compared to ND♂ x ND♀ and HCD♂ x ND♀ groups. However there was no significant difference in these parameters between ND♂ x ND♀ and HCD♂ x ND♀ groups (Fig. 37 A, B, C & D; Table 18).

3.43. Fertility indices:

The fertility index of male, fertility index of female, parturition index and gestation index did not significantly differ among ND♂ x ND♀, ND♂ x HCD♀, HCD♂ x ND♀ and HCD ♂x HCD♀ (Fig. 38 A; Table 19). However, there was a drastic and significant decrease in litter size and litter weight in ND♂ x HCD♀, HCD♂ x ND♀ and HCD ♂x HCD♀ groups when compared to ND♂ x ND♀ group. The weight/pup was significantly higher in ND♂ x HCD♀ and HCD ♂x HCD♀ than ND♂ x ND♀, and that of HCD♂ x ND♀ group did not significantly differ from ND♂ x ND♀ (Fig. 38 B; Table 19). Mortality was not observed in ND♂ x ND♀ group, whereas it was 18.7%, 13.04% and 19.04% in ND♂ x HCD♀, HCD♂ x ND♀ and HCD ♂x HCD♀ groups respectively (Fig. 38 B; Table 19).