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Age Related Changes in the Ovary, Uterus and Fertility in Swiss Albino Mice

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Age related changes in the reproductive status were studied in female Swiss albino mice from 6 to 20 months of age. Signs of ageing were first apparent at 8 months when majority of the females showed prolonged estrus cycles due to extension of either estrus or diestrus phase. There was also a decline in the percentage of pregnant females and in the number of live fetuses with increasing age. Significant decrease in ovarian weight was observed at 16 months and thereafter the ratio of the number of corpora lutea and Graaffian follicles to that of atretic and primary follicles was reduced. Uterine weight also decreased suddenly after 18 months of age with a thinning of the myometrium and regression of uterine glands. Results suggest that the reduced fertility in ageing mice might be due to manifold changes in the ovary and environment of the uterus along with a decrease in the activity of hypothalamo-pituitary-ovarian axis.

Key Words: Ovary, Uterus, Fertility, Swiss albino mice, Hypothalamo-pituitary-ovarian axis

Introduction

The old age changes in the reproductive system in female rats and mice are marked by irregular estrus cycles (after 8 months age), primarily due to constant estrus or repeated pseudopregnancy and finally anestrus (Wise 1983, Felicio et al. 1984). The progressive loss of oocytes, reduced follicular recruitment (Gosden et al. 1983) and loss of preovulatory surge of LH (Belisle et al. 1991) mark transition to acycilicity. Ovulation after long and irregular intervals is probably due to impairment of estrogen regulation of LH surge or due to decreased LHRH output (Mobbs et al. 1985, Huang et al. 1990, Rubin 1992).

In female mice, the ovarian senescence is characterised by progressive loss of oocytes (Lu 1983) and development of follicular cysts in human and other animals because of disruption in hormonal balance (Coney 1984, Mobbs et al. 1984). The aging uterus too shows reduced decidual response and impaired implantation and placenta formation (Shapiro & Talbert 1974). There is also decrease in estrogen receptors whereas progesterone receptors remain unchanged although their binding capacity declines (Larson et al. 1972, Hseuh et al. 1979). Age related decline in fertility is reported to preceed the loss of regular cycles in laboratory rodents (Matt et al. 1987). Delay in fertilization, implantation, corpus luteum activity and unfavourable uterine environment (Gosden 1975) and impaired oocyte quality and alteration in oviductal environment (Day et al. 1989) result in reduced fertility.

A systematic and detailed study on age related changes in reproduction is lacking. Therefore, changes in the weight and histology of ovary and uterus (6-20 months) and

fertility (10-20 months) was studied in Swiss albino mice.

Materials and Methods

Laboratory bred Swiss albino mice were kept under standard laboratory conditions. After weighing, the animals were autopsied by decapitation at 2 months interval from 6 to 20 months of age. Ovary and uterus were weighed, and fixed in Bouins fixative and later processed and sectioned at 5 to 7μ and stained with haematoxylin and eosin for histological studies. Vaginal smear of 15 mice was observed daily from 6 to 20 months to study changes in the estrus cycles.

Female mice of 10 to 20 months age (at 2 months age difference) were divided into six groups and caged (2:1) with mature males (3 months old). Every morning, vaginal smear was checked for the presence of sperms. The day the sperms were observed, the females were isolated and sacrificed after 15 days and the number of live fetuses was counted. Some females in each age group (except 10 months age) did not become pregnant even after retaining them with males for 15 days.

The results were analysed statistically by using Students 't' test.

Results and Discussion

Body Weight

There was no significant change in body weight from 6 to 20 months (table 1).

Ovary: The ovarian weight remained unchanged till 14 months but declined significantly at 16 months continued till 20 months. After 16 months age tumour-like growth was observed in the ovaries of few animals and this incidence of tumour was high at 20 months age. Developing follicles up to Graaffian follicle stage and corpora lutea were observed up to 10 months age. After 12 months, there were more primary follicles and few corpora lutea with intense proliferation of stromal cells. At 20 months, mainly primary follicles were present. The number of atretic follicles also increased with age. Ovaries with tumour-like growth showed complete absence of follicles, but there was formation of compartments full of secretion (table 1, figure 1).

Uterus: Uterine weight increased slightly till 8 months, remained unchanged uptill 18 months, but declined significantly thereafter. From 6 to 12 months, normal histological structures were observed but later the decrease in weight was due to thinning of myomet-

Table 1 *Changes in body, ovary and uterus weight and pattern of estrous cycles in Swiss albino mice* (6-20 *months)—Live fetuses count and percentage of pregnancy in aging female mice* (10-20 *months)*]

Age (months)	Body weight (g)	Ovary weight paired (mg)	Uterus weight paired (mg)	Percentage of females with irregular cycles	Percentage of pregnancy	Number of live fetuses
6	38.0±2.5(10)	12.5±0.8	85±15	—	—	—
8	40.0±0.8(8)	11.7±0.9	139±32	66	—	—
10	45.0±2.2(9)	14.5±2.0	150±12	88	100	6.0±0.9
12	38.7±1.8(7)	12.7±0.4	150±22	100	60	5.0±1.1
14	40.7±0.8(8)	13.2±1.1[a]	136±12	"	40	4.5±1.5[e]
16	44.3±2.3(9)	6.51±0.9[b]	128±13	"	40	2.5±1.0[f]
18	44.0±1.0(7)	5.9±0.9	158±33[c]	"	20	2
20	45.5±7.3(8)	4.9±0.7	67±30[b,d]	"	20	1

No. of animals used given in parenthesis.
'P' values a vs b < 0.001; c vs d < 0.001; e vs f < 0.001

Figure 1 TS ovary in 20 months old mice showing a single Graffian follicle; a large number of atretic follicles and increased proliferation of stromal cells.

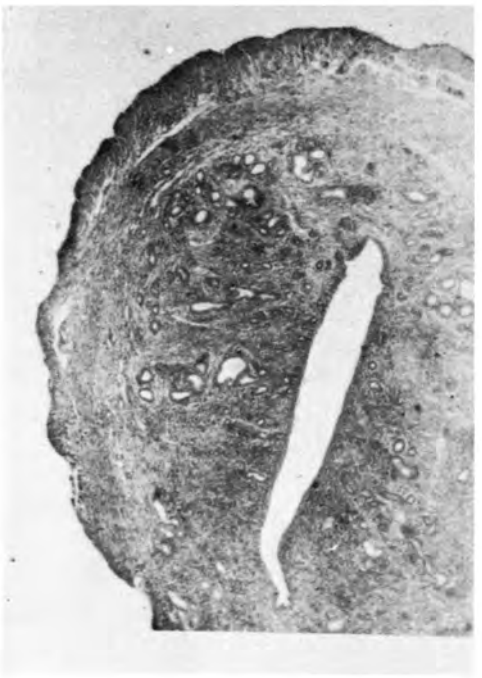

Figure 2 TS uterus in 20 months old mice showing regression of muscle layers and few regressing uterine glands

rium accompanied with regressed uterine glands. Twenty-five percent females at 20 months age showed shifting of compressed lumen to one side (table 1, figure 2).

Estrous Cycles and Fertility

Regular estrous cycles were observed till 7 months but from 8 months onwards there was progressive increase in the percentage of females showing prolonged cycles due to extended diestrus (7-11 days) or estrus phase (up to 3 days) till 20 months. There was gradual decline in percentage of pregnant mice. The number of live fetuses reduced significantly after 14 months of age and an average of one fetus was found in one 20 month-old female (table I).

Discussion

Extended estrous cycles from 10 to 12 days duration due to prolonged estrus or diestrus phase confirm the earlier reports in rat (Meites 1983) and mice (Wise 1983, Felicio et al. 1984). In the present study, ending at 20 months age, complete anestrus condition was not noted, which if at all occurs must be beyond this age. These age-dependent changes in the estrous cycles could be due to alteration in the hormones of hypothalamo-pituitary-ovarian axis (Freeman 1988).

Decrease in the weight of ovaries from 16 to 20 months is due to degenerative changes in the follicles. The number of Graaffian follicles and corpora lutea decreases and only primary follicles are observed at higher age. Similar decrease in number of oocytes and follicles is reported earlier in mice (Gosden et al. 1983). In our animals, too, higher increase in the number of atretic folicles is observed after 14 months age due to improper follicular development and large scale atresia (Nelson et al. 1987).

Senescent changes in the ovary are either due to decrease in the pituitary gonadotrophins or the responsiveness of the ovary to these hormones. On the basis of ovarian graft studies, it has been found that middle-aged ovaries support very few cycles after grafting into young hosts (Mobbs & Finch 1982). Decline in pituitary LH and hypothalamic LHRH, at higher age in these mice (Kaur & Saxena 1994), may be responsible for these senescent changes.

Histological studies of ovary, with increase of tumour-like growth in a few cases from 16 to 20 months showed decreased number of primary, developing or Graaffian follicles but only tubular structures from germinal epithelium occupy the major space. This may also be due to, changes in the pituitary and/or ovarian hormones, since FSH plays an important role in induction of ovarian cysts, and stimulation by FSH and LH like activity, induces ovarian follicular cysts in rat (Bogowich 1992).

High uterine weight, maintained up to 18 months, declined suddenly at 20 months age. In rats, with persistent diestrus, at 30 months age, showed similar reduced uterine weight (Hseuh et al. 1979). The decrease in weight at 20 months age, is probably the result of thinning of myometrium, due to loss of collagen. Regressed and non-functional, uterine glands at 20 months age, may be due to low progesterone output. The decrease in ovarian steroids, in aged mice (Parkening et al. 1980), are in general, responsible for senescent changes in the uterus. There is also decline in uterus response to steroids, as demonstrated by decrease in estrogen receptor protein in mice (Gosden 1975) and rat (Hseuh et al. 1979).

On the basis of percentage of pregnant females, and the number of live fetuses, it is evident that these gradually decreased with increase in age. This is, probably due to decrease in the pituitary and gonadal hormones, and changes in the uterine environment. The fertility, and fecundity of middle aged rats, begins to decline even prior to the loss of regular cycles due to failure of events before and/or implantation (Matt et al. 1986, 1987). In rat, abnormal pattern of embryonic development, like impaired oocyte quality and altered eviductal environment are reported to be responsible for decline in fertility (Day et al. 1989). The ratio of fetal resorption to implantation also increases gradually during aging (Matt et al. 1986). The present study, confirms that there is is decline in fertility due to regressed follicular development in the ovary, and degenerative changes in the uterine environment, on account of combined effects of the changes occurring in the hypothalamo-pituitary-gonadal axis.

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