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Aging and Ovarian Δ^5-3β-Hydroxysteroid Dehyrogenase in Rats[1] (38633)

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A decline in reproductive capacity associated with increasing maternal age is well known (1). This change has been related to functional modifications in the brain (2–4), pituitary (3–7), uterus (8–11) and ovary (12). In view of the continuous loss of oocytes and the absolute reduction in estrogen production by the human ovary (13) despite elevated serum gonadotrophins, further study of this organ during aging was suggested. In rodents, the metabolic and steroidogenic competence of the aging ovary has had little consideration. Talbert (14) has postulated a corpus luteum deficiency in older mice and modest differences in progesterone synthesis have been noted in aging rabbits (15). Furthermore, modest changes in ovarian Δ^5-3β-hydroxysteroid dehydrogenase have been observed in hamster (16) and human (17) when examined histochemically. The essentiality of this enzyme in ovarian steroidogenesis prompted an examination of the biochemical activity in the ovaries of rats of 1–24 mo of age.

Materials and Methods. Long–Evans strain rats, born and raised in our laboratory were used. All animals were of known birth date and were fed Purina laboratory chow *ad lib.* supplemented weekly with cod liver oil on bread. The rats were housed in groups of three to five in a temperature (78°F) regulated room with a light–dark cycle of 12:12 hr. Untreated virgin female rats of 1–24 mo of age were studied. The rats were killed by decapitation between 8 and 10 AM. Ovaries were quickly removed, weighed and frozen on dry ice until assayed within 24 hr. Ovarian Δ^5-3β-hydroxysteroid dehydrogenase (3β-OHSD) was estimated biochemically (18) using dehydroepiandrosterone as the substrate. Activity was reported as micrograms of androstenedione formed per minute or per milligram of protein (19). Total activity is presented as the androstenedione formed in micrograms per minute per ovary. Significant differences were estimated by the Student's t test and a P value of 0.01 was considered as significant.

Results. Actual ovarian weight (one ovary) increased throughout the first 4 mo of age and then remained essentially unchanged to 18 mo. Although ovarian weight did not differ significantly between 18 and 24 mo the ovarian weight at 24 mo was significantly less than that recorded at 4 mo. A similar decline was noted when ovarian weight was related to body weight.

Ovarian protein concentration increased between 1 and 2 mo of age but did not change significantly thereafter. Total ovarian protein increased between 1 and 2 mo of age and between 2 and 4 mo of age and then remained unchanged to 18 mo. Ovarian protein at 24 mo was significantly less than at 4 but not at 18 mo of age.

A significant increase in ovarian 3β-OHSD activity/mg protein occurred between 1 and 2 mo of age but after 4 mo the specific activity declined. Between 2 and 12 mo of age, the ovarian enzyme activity decreased 40% with significant changes recorded between 4 and 6 mo and between 6 and 12 mo. Furthermore, 3β-OHSD activity continued to decline after 1 yr of age so that at 24 mo the 3β-OHSD specific activity was approximately half that of the prepubertal ovary. Total ovarian 3β-OHSD activity was greatest at 4 mo of age and declined thereafter.

Discussion. The increase in Δ^5-3β-hydroxysteroid dehydrogenase activity between 1 and 2 mo of age in the rat was noted previously (18) and appeared to be due primarily to the appearance of corpora lutea. Thus the decline in 3β-OHSD activity in 18- and 24-mo old rats could relate to the decline or absence of corpora lutea noted in some senescent ovaries (20). However at 6

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TABLE I. EFFECT OF AGING ON OVARIAN Δ^5-3β-HYDROXYSTEROID DEHYDROGENASE ACTIVITY IN THE RAT.*

Age (mo)	No. of rats	Body wt (g)	Ovary (one) wt (g)	Δ^4 Androstenedione (μg/min)			Protein	
				mg	mg/protein	Total (per organ)	mg%	Total
1	10	67	9.4 ± 0.6	0.11 ± 0.01	1.06 ± 0.13	0.98 ± 0.11	9.8 ± 0.13	0.9 ± 0.1
2	7	165	24.1 ± 1.6[a]	0.16 ± 0.01[a]	1.43 ± 0.03[a]	3.92 ± 0.30[a]	11.4 ± 0.13*	2.7 ± 0.2*
4	7	246	33.0 ± 1.0[a]	0.15 ± 0.01	1.32 ± 0.06	4.96 ± 0.16[a]	11.5 ± 0.13	3.8 ± 0.1*
6	7	249	31.6 ± 1.7	0.13 ± 0.01[a]	1.15 ± 0.04[a]	4.13 ± 0.31[a]	11.4 ± 0.32	3.6 ± 0.2
12	8	285	29.8 ± 3.7	0.10 ± 0.01[a]	0.87 ± 0.05[a]	3.04 ± 0.58	10.9 ± 0.44	3.4 ±.0.5
18	7	308	29.5 ± 4.0	0.09 ± 0.01	0.79 ± 0.11	2.67 ± 0.68	10.8 ± 0.31	3.2 ± 0.4
24	8	307	25.6 ± 3.4	0.06 ± 0.01	0.59 ± 0.08	1.83 ± 0.52	10.6 ± 0.32	2.8 ×.0.5

* Values expressed as means ± SEM.
* Significant difference from rats of the prior age group: $P = 0.01$.

mo of age the ovarian enzyme activity had declined to that of a prepubertal animal despite the known fertility of the Long–Evans rat at this age.

An indication of ovarian functional change with advancing age is the decline in oocyte number, the increase in ovulation failures and the modified estrous cycles (1, 12). The ability of LH administration and hypothalamic stimulation to induce ovulation and to restore estrous cycles in old constant estrous rats has indicated a deficiency in secretion of pituitary gonadotrophins with ovarian senescence (3, 21). However, the ovarian response to exogenous (12) and endogenous (5) gonadotrophin is subnormal. Then too, FSH, LH and prolactin administration had no effect on 3β-OHSD activity in the rat ovary (22) although a single injection of chorionic gonadotrophin did increase total ovarian 3β-OHSD (23). The relative independence of ovarian 3β-OHSD from gonadotrophin regulation suggests that the decline in reproductive potential with advancing age may be due, in part, to inherent changes in the ovary that are not necessarily influenced by other endocrine glands.

Summary. Ovarian Δ^5-3β-hydroxysteroid dehydrogenase activity and protein content were determined in Long–Evans rats 1–24 mo of age. Activity of the enzyme per milligram of protein was maximal between 2 and 4 mo of age and declined thereafter. Ovarian enzyme activity of the 24-mo old rat was significantly less than that of the prepubertal animal. Total ovarian enzyme activity was maximal in 4-mo old rats. A decline in ovarian protein was noted between 4 and 24 mo of age.

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