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Original Research Article

Populations of follicles in F344/N rats during aging

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ABSTRACT

Follicular populations were investigated in female F344/N rats to better understand the aging process of the rat ovary. Ovaries dissected at various ages (spanning 1–36 months old) were submitted for histological examination. The total number of primordial, growing (primary and secondary), tertiary, and atretic follicles as well as corpora lutea (CL) were counted in hematoxylin–eosin- and azocarmine–aniline-blue-stained ovarian sections. The number of healthy follicles including primordial, growing and tertiary follicles decreased rapidly between the first and third months and gradually thereafter. CL were found in 3-month-old rats, and their number remained unchanged until 18 months of age, at which point it decreased. The number of atretic follicles started to increase in rats older than 18 months, which corresponded to the cessation of estrous cyclicity. Several healthy follicles and CL were observed even in 36-month-old rats.

1. Introduction

Development and aging of the reproductive system are important factors determining reproductive performance. In particular, physiological conditions in female animals are more profoundly affected by reproductive status. Moreover, menopausal symptoms, a major complaint of middle-aged women, often lead to osteoporosis and cardiovascular disorders and are of clinical concern [1,2]. Therefore, the elucidation of the mechanisms underlying the aging of the reproductive system is of interest not only for scientists but also for public health. The characteristics of aging and associated changes vary in animals depending on species, strain and sex [3,4]. The distinction and assessment of these differences are crucial for streamlined and waste-free animal experiments. The National Institute for Longevity Sciences (NILS) of Japan has established the NILS Aging Farm, and successfully produced F344/N rats that can survive up to 30 months [5]. Aging and/or aged rats have been extensively

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studied to reveal the relationship between aging and morphological changes [6–9]. Sone et al. [10] monitored the estrous cycle of aging F344/N rats by vaginal smear cytology, and found that the cornified cell phase was no longer displayed in 16.4-month-old rats, indicating that the cessation of the estrous cycle in F344/N rats occurs approximately halfway through their lifespan. However, other reproduction-driven changes in aging female F344/N rats remain to be elucidated. In the current study, changes in the number of different populations of follicles and corpora lutea (CL) were examined in the ovaries of F344/N rats to better understand reproductive aging.

2. Materials and methods

The experimental protocol was conducted with the permission of the Committee for Animal Ethics of NILS according to the Guidelines of NILS Animal Experimentation with due care for animal rights and welfare. Aging and aged female F344/N rats introduced from Japan SLC, Inc. (SLC, Hamamatsu, Japan) had been maintained at the NILS Aging Farm, while younger animals were purchased from the SLC. The animals were housed at the NILS Aging Farm under a 12-h light (08:00–20:00) and 12-h dark cycle, at 22–23 °C, and had ad libitum access to food (Labo MR Stock, Nihon Nousan Co., Yokohama, Japan) and tap water.

The animals were divided into twelve groups by age and each group was sacrificed at the indicated age (3 animals/group; 1, 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, and 36 month-old females) by exposure to lethal concentrations of ether vapor in a closed chamber. The dissected ovaries were weighed (at 1, 3, 6, 12, 18, 21, 24, 30 and 36 month-old) and immersed in 10% formaldehyde and processed using a standard procedure: hydration in a graded series of ethanol, clearance by xylene and embedding in paraffin. Serial sections (2 μm thick) were obtained and deparaffinized. One of the serial sections was stained with hematoxylin and eosin (HE), and its neighboring section with azocarmine–aniline blue stain. These preparations were then dehydrated and mounted onto glass slides.

The follicles were classified by morphology [11], by the number of primordial, growing (primary and secondary), and tertiary follicles, and by the number of atretic immature and mature follicles counted from two sets of two neighboring sections from the cross-section of maximum diameter of a unilateral ovary (i.e., counts were obtained for four different sections per animal). The section sets were spaced at a distance greater than 200 μm. Primary and secondary follicles were counted as growing follicles. The number of corpora lutea (CL) was counted in a similar manner. The number of healthy (primordial, growing and tertiary) and atretic (immature and mature) follicles as well as CL were scatter-plotted against age. The number of atretic follicles originating from immature (primordial or growing) follicles per section was approximated by means of a 4th order polynomial (Excel 2007, Microsoft, Redmond, WA, USA). One-way ANOVAs followed by Bonferroni correction were used to compare the ovarian weight and the number of follicles or CL among groups (12 sections per group; PASW Statistics 18, IBM Japan, Tokyo). Additionally, Kendall's rank correlation test was conducted to analyze the linear relationships between age and the number of follicles (or CL).

3. Results

The total weight of ovaries increased dramatically ($p < 0.01$) between the first and third month, and then eventually peaked in the 21st month after birth in F344/N rats (Fig. 1). Follicles at various maturational stages were observed in the ovarian cortex (Fig. 2). Primordial follicles contained an oocyte enclosed by a single layer of epithelial cells (Fig. 2). Epithelial cells of the primary follicle elongated to take either a cuboidal or columnar form. Oocytes of the secondary follicles were surrounded by a stratum granulosum with more than two layers of epithelial cells (Fig. 2). The follicular antrum increased in size as the follicle grew larger. Atretic follicles originating both from mature and immature follicles were observed in the ovarian sections (Fig. 2).

Ovaries of 1-month-old rats contained many follicles of different types, but they did not contain CL. The distribution of follicles in 1-month-old rats differed from that in older rats (Table 1 and Fig. 3). The total number of healthy follicles as well as the number of primordial and tertiary follicles decreased rapidly in 3-month-old rats (Table 1, $p < 0.01$) and gradually thereafter (Fig. 3, Kendall's tau = −0.787, $n = 36$, $p < 0.001$). In contrast, the number of atretic follicles initially decreased but then gradually increased, and in rats older than 18 months this count was higher than the number of healthy follicles (Fig. 3). The number of atretic mature follicles decreased (Fig. 4, Kendall's tau = −0.596, $n = 39$, $p < 0.001$) and these follicles were difficult to find in rats older than 30 months (Fig. 4). The number of atretic immature follicles in rats older than 18 months was higher than in younger rats (Fig. 4). CL were found earliest in 3-month-old rats, with their number remaining unchanged until 18 months of age before beginning to decline (Kendall's tau = −0.552, $n = 21$, $p < 0.001$), and becoming very low in rats aged 27 months and older (Fig. 3, $p < 0.01$).

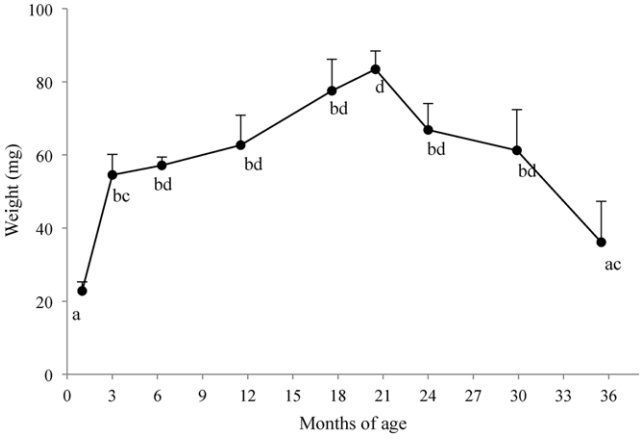

Fig. 1 – Ovarian weight (mean ± SD) in F344/N rats ($n = 3$ rats per time point). Different superscripts depict significant ($p < 0.05$) differences.