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Fate of the initial follicle pool: Empirical and mathematical evidence supporting its sufficiency for adult fertility

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Abstract

The importance of the initial follicle pool in fertility in female adult mammals has recently been debated. Utilizing a mathematical model of the dynamics of follicle progression (primordial to primary to secondary), we examined whether the initial follicle pool is sufficient for adult fertility through reproductive senescence in CD1 mice. Follicles in each stage were counted from postnatal day 6 through 12 months and data were fit to a series of first-order differential equations representing two mechanisms: an initial pool of primordial follicles as the only follicle source (fixed pool model), or an initial primordial follicle pool supplemented by germline stem cells (stem cell model). The fixed pool model fit the experimental data, accurately representing the maximum observed primary follicle number reached by 4–6 months of age. Although no germline stem cells could be identified by SSEA-1 immunostaining, the stem cell model was tested using a range of *de novo* primordial follicle production rates. The stem cell model failed to describe the observed decreases in follicles over time and did not parallel the accumulation and subsequent reduction in primary follicles during the early fertile lifespan of the mouse. Our results agree with established dogma that the initial endowment of ovarian follicles is not supplemented by an appreciable number of stem cells; rather, it is sufficient to ensure the fertility needs of the adult mouse.

Keywords: Primordial follicles; Germ cells; Germline stem cells; Follicle counts; Oocyte; SSEA-1

Introduction

A central theory in mammalian ovarian biology is that the number of follicles continually decreases with age due to both atresia and ovulation, ultimately resulting in menopause or reproductive senescence. For over 50 years, it has been widely accepted that germline stem cells in the ovary, unlike the testis, stop proliferating around the time of birth and establish an initial quota of follicles that is the only source of adult oocytes in rodents (Peters et al., 1962; Zuckerman, 1951). Hence, the processes of oogenesis and neo-folliculogenesis occur in the embryonic and neonatal animal, but not in the adult. Results reported in the companion paper indicate that follicle assembly can be regulated by activin A in the neonatal mouse, leading to an increase in primordial follicle number (Bristol-Gould, et al., this issue). Follicle loss was higher in activin-treated vs. vehicle-treated animals resulting in similar populations of follicles by the time the animals reach puberty. The follicles that are eliminated are of poor quality, leading to a quorum sensing mechanism that relies on the health of the oocyte in shaping the adult follicle pool.

To directly examine if the initial follicle pool contains a sufficient supply of oocytes for adult fertility, follicle counts from our previous study were extended through 1 year and mathematical modeling was employed to analyze the dynamics

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Table 1
Normalization factors and follicle counts

Age	n=ovaries	Normalization		Number of follicles per animal[a]		
		PF/1°	2°	PF	1°	2°
6 days	11	37.5	75	10,265±489	414±29	447±49
10 days	8	50.0	100	8662±660	567±82	984±85
19 days	6	62.5	125	5127±488	294±21	656±74
45 days	4	92.5	185	2706±387	480±54	596±90
4.5 months	4	136.0	272	1583±81	595±21	531±49
6 months	4	122.5	245	1487±109	595±23	519±55
12 months	4	115.0	230	477±145	281±67	166±41

[a]Data given as average±SEM. Abbreviations used: PF=primordial follicle, 1°=primary follicle, 2°=secondary follicle.

of follicle progression throughout the adult lifespan. This approach permitted an examination of the adequacy of the initial follicle pool to support adult fertility and the potential role of a germline stem cell population in the mouse. Both empirical and mathematical approaches were used to determine whether the initial follicle pool provides a sufficient population of germ cells necessary for fertility throughout the reproductive lifetime of the mouse.

Materials and methods

Tissue processing, follicle counting and immunohistochemistry

Follicle counts were performed on ovaries collected on postnatal days 6, 10, 19 and 45; and at 4.5 months, 6 months and 12 months using the protocols and follicle classification schemes described in the companion paper. The correction factors for determining total follicles per ovary from the average follicles per section are given in Table 1. Immunohistochemical staining for stage-specific embryonic antigen-1 (SSEA-1) was performed as described using 4- and 6-month-old mouse ovaries (Bristol-Gould, et al., this issue).

Mathematical modeling

The persistence of primordial (F_0), primary (F_1) and secondary follicles (F_2) was investigated using a mathematical model that describes primordial follicle loss and the transitions between follicle stages (depicted in Fig. 1A). These processes were modeled as first-order and equations were solved numerically using an ordinary differential equation solver in Matlab (The Mathworks, Inc, Natick, MA). In the model, k_{T0} describes the transition from the primordial to primary follicle stage, k_{L0} describes the loss of primordial follicles, k_{T1} describes the transition from the primary to secondary follicle stage, k_{T2} describes the transition from secondary to more advanced follicle stages and k_{SC}

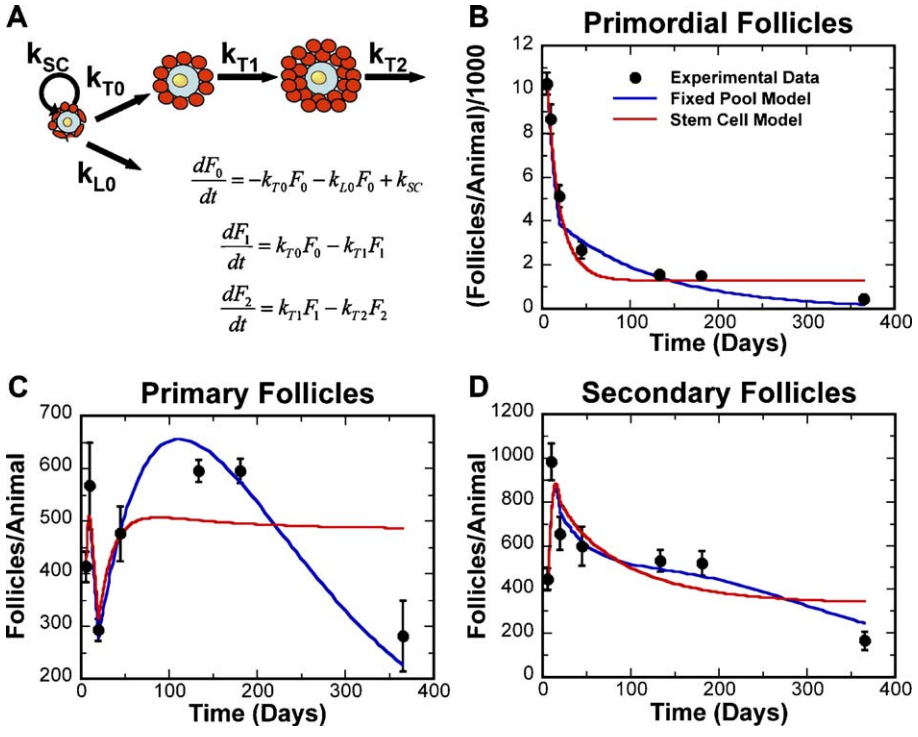

Fig. 1. Persistence of initial ovarian follicle pool. (A) Follicle dynamics were described by a series of first-order processes for loss (k_{L0}) and transition from primordial to primary (k_{T0}), primary to secondary (k_{T1}) and secondary to more mature stages (k_{T2}). In addition, the initial follicle pool was modeled as either static (fixed pool model) or as supplemented by germline stem cells (stem cell model) in a zero-order process (k_{SC}). (B–D) Follicle counts were performed on CD-1 mice from postnatal day 6 to 12 months of age and used to fit the kinetic parameters. Both models were able to capture the behavior of primordial and secondary follicles through approximately 200 days. However, the stem cell model was unable to mimic the peak in primary follicles observed at 100 days and the ultimate decline in all follicle populations (primordial, primary and secondary) beyond 200 days.