

Aged mouse ovaries possess rare premeiotic germ cells that can generate oocytes following transplantation into a young host environment

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Abstract: Of all the major organ systems in the body, the ovaries of females are the first to exhibit impaired function with advancing age. Until recently, traditional thinking was that female mammals are provided with a non-renewable pool of oocyte-containing follicles at birth that are depleted during postnatal life to exhaustion, driving ovarian failure. However, a growing body of evidence, including the isolation of germline stem cells (GSC) from adult mouse ovaries that produce developmentally-competent oocytes, has challenged this belief. In addition, rare germline stem-like cells capable of generating oocytes in vitro that undergo parthenogenesis to form blastocyst-like structures have recently been identified in postmenopausal human ovaries. Here we show that the germline-specific meiosis-commitment genes, *Stimulated by retinoic acid gene 8 (Stra8)* and *Deleted in azoospermia-like (Dazl)*, are highly expressed in aged mouse ovaries. However, histological and marker analyses fail to demonstrate the presence of oocytes, supporting that *Stra8* and *Dazl* are expressed in premeiotic germ cells that do not undergo further differentiation. Through the use of aged germline-specific GFP-expressing transgenic mice, we further show that these germ cells can generate GFP-positive oocytes that co-express the primordial oocyte marker NOBOX and form follicles when grafted into young adult wild-type female hosts. Thus, aged mouse ovaries possess a rare population of premeiotic germ cells that retain the capacity to form oocytes if exposed to a young host environment.

INTRODUCTION

In humans and laboratory rodent models (rats and mice), the ovaries exhibit age-related dysfunction relatively early in life, with failure noted long before aging-associated changes in other organs are manifest. In humans, this loss of ovarian function drives the menopause and its associated increased risk for development of diverse health complications, many of which are tied to disrupted ovarian hormone production [1]. Endocrine function of the ovaries is carried out primarily by structures termed follicles, which are com-

posed of a centralized germ cell arrested in meiosis (oocyte) surrounded by one or more layers of supporting somatic cells [2]. Traditional thinking has been that female mammals are provided with a non-renewable pool of oocyte-containing follicles at birth that are continuously depleted during postnatal life to the point of exhaustion [3]. However, a growing body of evidence (reviewed in [4]), including the recent purification and in-vitro propagation of premeiotic germ cells from neonatal and young adult mouse ovaries that can generate developmentally-competent oocytes in transplanted host females [5], has challenged this belief,

thus offering new avenues to consider in the context of deciphering the role that adult stem cells may play in ovarian function and aging in females [6].

For example, findings from gene mutant mice show that p16^{INK4a} and p19^{ARF}, two senescence-associated proteins that contribute to stem cell failure during aging of the hematopoietic, neural and cardiac systems [7-9], do not play a comparable role in restraining oogenesis in adult females [10]. However, another cell cycle-regulatory protein termed CABLES1 [cyclin-dependent kinase (CDK)-5 and ABL enzyme substrate 1] was identified as serving this function in the mouse female germline, uncovering a cell lineage-specificity with respect to the role that cell cycle modulators play in controlling somatic versus germline stem/progenitor cell activity [10]. Other studies have shown that post-

menopausal human ovaries devoid of oocytes possess rare stem-like cells with germline characteristics [11]. When maintained in vitro under defined conditions, these cells spontaneously generate oocytes (or oocyte-like cells) that can undergo parthenogenetic development to form preimplantation embryo-like structures [12]. Although these reports indicate that aged ovarian tissue retains at least some degree of germline cell function, it is unclear whether these cells contribute to oogenesis under physiological conditions and, if they do, why these cells would then fail to maintain the follicle reserve with advancing age. Herein we used mice as a model to further test whether changes in premeiotic germ cell function might be an important variable to at least consider in the context of understanding the mechanisms involved in ovarian aging in mammals.

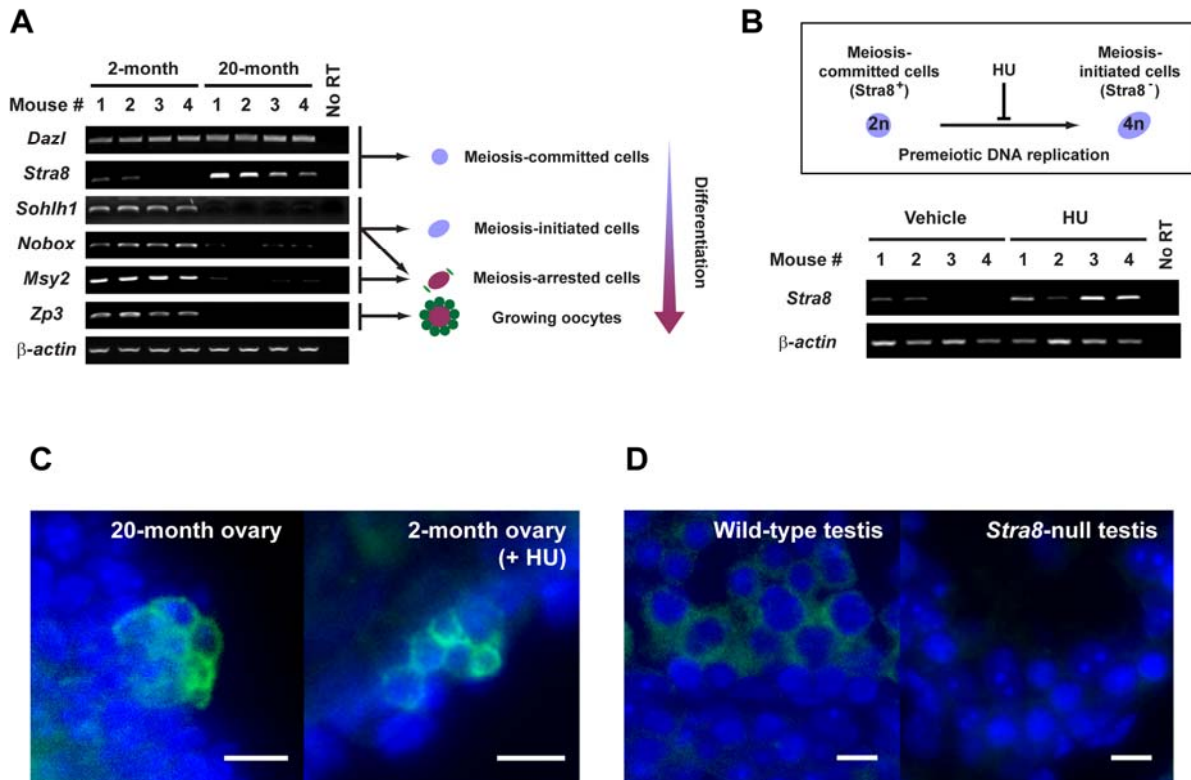


Figure 1. Premeiotic germ cells persist in aged atrophic mouse ovaries. (A) Analysis of germline marker gene expression in ovaries of young adult (2-month) and aged (20-month) female mice. Results from all 4 mice per age group are shown (β -actin, housekeeping gene used as a sample loading control). (B) In-vivo blockade of premeiotic DNA replication by HU in ovaries of young adult mice results in enhanced levels of *Stra8* expression, consistent with premeiotic germ cell accumulation. (C) Immunofluorescence analysis of STRA8 expression (green, cytoplasm) in ovaries of aged or HU-treated young adult female mice. (D) Control immunofluorescence analysis of STRA8 expression (green, cytoplasm) in testes of young adult wild-type or *Stra8*-null male mice (a representative cross-section of seminiferous tubule is shown for each.). C, D: scale bar = 10 μ m; DAPI counterstain, blue (nucleus).

