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Commentary

Of Mice and (wo)Men: Purified Oogonial Stem Cells from Mouse and Human Ovaries

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When it comes to reproducing, James Brown summed up the human experience: *This is a man's world*. Women produce a limited number of viable eggs (probably considerably fewer than 400) and become reproductively senescent in the forth or fifth decade of life. In contrast, men produce millions of sperm per day, with little evidence of age-related decline, and can father offspring into their seventh decade and beyond—if they can find willing mates. Universal agreement exists that a germline stem cell population in the testis makes continuous sperm production possible. The existence of a corresponding stem germ cell population in the ovary, however, has been the subject of intense debate. The latest chapter in this debate was recently published online in *Nature Medicine* [1]. What makes this paper especially noteworthy is that it provides the first evidence that isolation techniques used to obtain putative oogonial stem cells (OSCs) from the mouse ovary can be used to isolate similar cells from human ovaries.

The findings are the result of collaborative studies between investigators at Harvard and at Saitama Medical Center in Japan. The Harvard group includes Jonathan Tilly, whose laboratory challenged the ''central dogma'' of reproductive biology—that all eggs produced by the mammalian ovary initiate oogenesis during fetal development—with a paper in 2004 reporting OSCs in the mouse ovary [2].

In males, spermatogonial stem cells (SSCs) at the basement membrane of the seminiferous tubules divide to replenish their numbers and give rise to progenitor spermatogonia that provide the steady supply of new spermatocytes required for continuous sperm production. The SSC population is thought to be small in number, making up 0.03% of the total testicular cell population in adult mice [3]. Techniques for the isolation and in vitro expansion of SSCs have been developed, and the litmus test of the putative SSC cells obtained—their capacity to regenerate continual sperm production following transplantation into a recipient testis—has been firmly established [4, 5].

Despite a flurry of research, the existence of the female germline stem cell counterpart—OSCs—has remained controversial. White et al. [1] expand our understanding of OSCs with the demonstration that a population of mitotically active cells can be isolated from both mouse and human ovarian tissue by cell sorting using an antibody that recognizes the C-terminal portion of DDX4, a germ cell-specific RNA helicase. The isolated cells express genes known to be restricted to germ cells, and like male SSCs, these appear to be a rare type (an estimated 0.014% of the ovarian cell population). Interestingly, in contrast to the male, where isolated SSCs form colonies of actively dividing cells after several days in culture [6–9], from 10 to 12 wk (mouse) and from 4 to 8 wk (human) of culture were required to obtain actively dividing OSC colonies. This smacks of in vitro transformation, raising concern about whether the population of cells that exists in the ovary are true stem cells. Further, during mitotic expansion in vitro, a proportion of OSCs appeared to spontaneously form oocytes that not only initiated but also completed meiosis. This finding contrasts sharply with the male, where SSCs can be maintained and expanded in vitro for long periods of time [6–9] but where spontaneous differentiation has not been reported.

The ultimate test is, of course, the ability of OSCs to give rise to normal eggs. White et al. [1] take a step in this direction, providing evidence that early cleavage embryos with GFP-positive blastomeres can be obtained from recipient female mice whose ovaries were injected with GFP-tagged donor mouse OSCs. Unfortunately, no evidence of normalcy is provided, because neither the developmental potential nor the genetic quality of these embryos was tested. Similarly, evidence of the formation of oocytes in vitro was based on the expression of oocyte-specific markers, cell morphology, and changes in DNA content. Importantly, the reported increase in apparent haploid cells indicates not only meiotic entry but also completion of *both* meiotic divisions. This is surprising, because oocytes never reach a haploid state in vivo. Completion of the first meiotic division and arrest of the cell at the second meiotic metaphase occur just before ovulation. Thus, the egg awaiting a sperm in the female reproductive tract is not haploid. Fertilization triggers completion of the second meiotic division, and by the time an egg becomes haploid, it is a diploid zygote. Thus, a population of haploid oocytes presents a problem.

Meiotic completion could, of course, simply be an artifact of long-term culture. Nevertheless, meiosis remains the Achilles heel of this field. Attention has focused on germ cell differentiation, with meiosis generally treated as a detail. Expression of a couple of meiotic genes (usually one component of the synaptonemal complex and one DNA repair gene) typically is provided as evidence of meiotic entry, and the production of apparently haploid cells is heralded as meiotic success. However, meiosis is a complex process, and investigators in the germline stem cell field would be well served to bear a few additional details in mind: First, under the best of circumstances, meiotic errors are common and, because

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they produce genetically abnormal individuals, cannot be tolerated. Second, although we call it meiosis in both males and females, the processes are hugely different, and we remain largely ignorant of how differences in the time of onset, sex chromosome activity, recombination levels, and meiotic duration are established. Lastly, the need to impose sex-specific epigenetic modifications and differences in the timing of these modifications adds an additional layer of complexity, as do the postmeiotic process of spermiogenesis in the male and the long process of oocyte growth in the female.

Until the germline stem cell field focuses on the complexities of meiosis and the unique sex-specific requirements of gametogenesis, we are left with the old duck adage: It looks like a duck, it quacks like a duck, but is it a duck? Female ducks prove themselves by laying eggs that hatch into ducklings. For OSCs, doubt will persist until clear evidence is provided that they give rise to genetically normal, developmentally competent eggs. In the meantime, skeptics are plagued by several nagging questions: What do these cells do in the ovary? Where do they come from? And, most importantly, if they can and do give rise to viable eggs in the adult ovary, why is female reproduction of such limited duration?

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