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On Regenerating the Ovary and Generating Controversy

For more than a half a century, biologists have upheld the theory that in most mammalian species, oocytes are formed before or shortly after birth, but never in adulthood. This foundation of reproductive science has survived the rapid growth of new technology and knowledge and has remained virtually unchallenged until two recent papers were published by the group headed by Jonathan Tilly. The first paper claims that mouse germline stem cells (GSCs) replace ovarian follicles that have been rapidly lost through follicle death (Johnson et al., 2004). The second paper, recently published in *Cell*, proposes continuous immigration into mouse ovaries of GSCs derived from bone marrow (Johnson et al., 2005). How could so many investigators have overlooked these basic mechanisms for so long, or have they?

One of the major conclusions of their first paper—that GSCs reside in the surface epithelium of mouse ovaries and are active throughout life (Johnson et al., 2004)—has now been modified by the authors themselves in their second paper (Johnson et al., 2005) in response to several critical comments (Gosden, 2004; Albertini, 2004; Greenfeld and Flaws, 2004; Telfer, 2004). Indeed many of the experiments described in the first paper are open to alternate explanations, and independent corroboration of their conclusions has still to be obtained. Here we express concerns about their second paper regarding the putative role of bone marrow cells in reproduction and the experimental rigor needed to verify this revolutionary hypothesis.

Johnson et al. (2005) claim that "adult mouse ovaries can produce hundreds of new oocytes within 24 hours" after follicle destruction by doxorubicin. Oogenesis—the process by which mitotic germ stem cells undergo meiosis to the diplotene stage followed by the formation of follicles—normally requires at least one week in the developing mouse ovary. It is astonishing if this process can be completed within 24 hr as Johnson et al. (2005) propose. To reconcile these observations, it is important to test whether pre-existing follicles can recover after doxorubicin treatment or whether new germ cells undergoing accelerated oogenesis can be identified and, in either case, whether the purported oocytes are capable of supporting fertilization and subsequent embryo development. Indeed it is central to their hypothesis that the rates of meiosis and of follicle formation are dramatically different and, therefore, it falls upon these authors to show this experimentally.

The authors have also shown that molecular markers normally associated with germ cells (Oct4, Mvh, Dazl, Stella, and Fragilis) are expressed in blood and bone marrow and, even more surprising, that expression levels of these proteins vary during the mouse estrous cycle. All of these proteins have been shown to be expressed in other cells and organs including brain and bone, so they cannot be considered germ cell markers; rather, they should be considered as stem cell markers. Such data based purely on RT-PCR are interesting but do not prove that a reservoir of GSCs exists in extraovarian sites. Besides, markers expressed by peripheral blood cells are likely to be detected in other organs, thus the case for the exclusivity of these markers in bone marrow is untenable. In future experiments, it will be crucial to isolate candidate GSCs from various organs for a detailed characterization of their phenotype and developmental potential.

Studies with cell markers in crude tissue extracts will always be open to alternative interpretations, but transplantation studies can produce more decisive results. Johnson et al. (2005) have shown that transplantation of bone marrow from normal donor mice appears to regenerate follicles in the ovaries of young mice sterilized by combination chemotherapy and in those of the congenitally sterile *Atm* mutant mouse. Some of these new follicles were still present even as late as 11 months of age despite the small number shown to have been formed initially.

Furthermore, by using transgenic animals overexpressing Oct4 linked to green fluorescent protein (GFP), GFP-labeled oocytes were recognized in ovaries as early as 28 to 30 hr after intravenous injection of nucleated peripheral blood cells from the transgenic mice into infertile female recipients. These results are certainly intriguing, but we are concerned that the authors have not properly considered other explanations or thoroughly examined the new putative germ cells. First, there is a possibility that GFP could have been taken up from the blood. This makes it essential to carry out the reciprocal experiment to determine whether non-GFP oocytes appear in *Atm*-Oct4/GFP mutant mouse ovaries after injection of nucleated peripheral blood cells from wild-type animals. Further studies of the lineages of cell types migrating into the ovaries are also needed, and these should use a nuclear rather than a cytoplasmic label to track the cells. We emphasize again the importance of verifying the fertility and origin of "new" germ cells: the results published to date do not even show that these new "oocytes" have entered meiosis, far less that they can complete it and undergo fertilization. Indeed, the Johnson et al. (2005) paper raises two separate issues. The first is whether pluripotent stem cells, such as those from bone marrow, are able to develop into germ cell-like cells, and the second is whether this mechanism of oocyte development exists in the normal functional ovary? It has already been shown that the answer to the first is yes (Hubner et al., 2003), but no evidence is presented to support the second.

Although the authors are careful in the published paper to restrict speculation beyond the data, in media interviews following publication Jonathan Tilly presented an overly optimistic and enthusiastic vision with respect to clinical applications and has suggested that blood transfusion alone could solve infertility. "They're your own cells; you don't need anybody's approval.

They go right into your blood supply and go right to your ovaries, where they mature into eggs" (J. Tilly in Goldberg, 2005). Although this speculation may be exciting, it contradicts the experience to date of young women undergoing medical treatments (comparable to those given to mice in the Johnson et al. study) that may render them infertile. Young cancer patients undergoing simple chemotherapy often exhibit a certain amount of oocyte recovery, albeit with an earlier menopause and a lower ovarian reserve. In contrast, the stronger chemotherapy regimens that precede bone marrow transplantation almost never result in oocyte recovery. This clinical experiment has been ongoing for the last two decades in humans undergoing bone marrow transplantation to treat cancer. If the bone marrow contained putative oogonial stem cells, why do these patients not usually recover ovarian function?

At a time when radical and far-reaching discoveries are commonplace and old assumptions are sometimes overturned, we should be prepared for unexpected results. Whether the hypothesis of Johnson et al. (2005) proves to be a blind alley of science or to have dramatic implications for medicine and animal reproduction will depend on whether their findings can be extended as outlined above and independently corroborated. On the basis of the evidence so far, we find their conclusions from their mouse model not proven and extrapolation of the data to humans premature. Moreover, we are concerned that their hypothesis is less reconcilable with pure reason and human biology than conventional theory. Stem cells are characterized by an indefinite life span and proliferative potential, but, according to their hypothesis, the phenomena of menopause and oocyte aging may be attributable to bone marrow senescence. Although modern biology has made us more accustomed to the variety and plasticity of living systems, a finite stock of oocytes formed early in life still seems to better account for our current understanding of ovarian physiology.

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