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Ovarian follicle burnout
A universal phenomenon?

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The ovarian follicle reserve is maintained in a delicate state of homeostasis aimed at preserving the majority of follicles in a dormant state. Key regulatory factors in follicle activation and quiescence include the PI3K/PTEN/Akt signaling pathway in the oocyte, as well as external paracrine inhibitory factors such as anti-Mullerian hormone (AMH) (**Fig. 1A**). Mice with oocyte-specific deletion of one or more elements in the PI3K/PTEN/Akt pathway, or AMH-knockout mice, exhibit premature activation and rapid depletion of ovarian follicle reserve.[1]

Disturbance of ovarian homeostasis has been shown to be a mechanism of follicle loss in the case of iatrogenic ovotoxicity. We recently showed that ovotoxic chemotherapy agent cyclophosphamide (Cy) triggers upregulation of the PI3K pathway, initiating a wave of follicle recruitment and growth and, ultimately, burnout of the ovarian follicle reserve.[2] In addition, Cy also induced apoptosis in growing follicles within 24 h of exposure. Co-treatment with immunomodulator, AS101, reduced both apoptosis of growing follicles and activation of the PI3K/PTEN/Akt pathway, thereby reducing follicle activation and loss.

We further demonstrated that AMH expression levels (as measured by qRT-PCR) dropped to below control levels as early as 12 h post-Cy treatment, reflecting the loss of growing follicles at this time point. AMH levels in Cy-treated ovaries remained lower than control levels up to 3 d post-Cy; however, between 3 and 7 d post-treatment, AMH expression in Cy-treated ovaries increased not just to equivalent levels to PBS treated mice, but to twice the relative expression and maintained for at least 14 d post-treatment.[3] This increase in AMH levels reflects the increase in early growing follicles as a result of Cy-induced follicle activation. The reduction in AMH expression together with the activation of the PI3K pathway, which occur within 24 h of Cy treatment, combine to create a "window of unregulated growth" of the dormant primordial follicle population.

This is a new understanding of the mechanisms of ovotoxicity that carries significant implications, reaching further than cancer treatments. This observation is supported by additional studies that have suggested that primordial follicle activation via the PI3K/PTEN/Akt pathway is also the mechanism behind ovarian follicle loss seen after exposure to a number of other environmental carcinogens and ovotoxins.

Of particular interest is the implication of the "burnout" phenomenon for ovarian tissue transplantation. Ovarian tissue cryopreservation is a widely used strategy for fertility preservation in young patients with a high risk of ovarian failure after cancer treatment.[4] However, the duration of graft survival following ovarian tissue transplantation is extremely variable, in some cases as short as a few months, largely due to massive loss of primordial follicles that occurs following grafting.[5] In addition the vast majority of large follicles in the graft are lost during processing, freezing, and thawing, leaving the ovarian reserve unregulated. Our recent observations on follicle dynamics post-ovarian tissue transplantation show that recovered grafts have higher ratios of growing to total follicles and higher levels of proliferation staining than non-transplanted control tissue, and that this is seen to a greater degree in thinner grafts, which have fewer large follicles.[6] This data strongly suggests that ovarian tissue grafts undergo a similar process of follicle activation and "burnout" to that seen following Cy treatment (**Fig. 1B**). It is likely that the removal of follicles from their normal physiological environment disturbs the ovarian homeostasis. The absence of larger follicles in the ovarian cortical strips in particular results in a decrease in the secretion of inhibitory signaling factor AMH,[7] leading to follicle activation.

The implications of imbalances in ovarian homeostasis may also extend to the normal physiological state. Age-related changes to the ovarian environmental milieu may have important effects on the regulation of follicle activation. With increasing age, the numbers of all follicles in the ovary decrease, and the decrease in the number of growing follicles results in an age-related decline in AMH levels.[8] AMH levels do not only reflect the decline in ovarian follicle reserve, they also indicate a decrease in inhibition exerted on the same population of follicles. This may explain the age-related dynamics of ovarian reserve, which show a fairly constant rate of decline in follicle number until age 35–37, when there is an acceleration of follicle loss. We hypothesize that with age-related reduction in follicle inhibition, follicle activation is accelerated, resulting in increased ovarian follicle loss.

In our study, we showed that co-administration of AS101 attenuated follicle burnout via its effect on the PI3k pathway and by reducing apoptosis in growing follicles. This raises the possibility that other agents that act on this crucial activation

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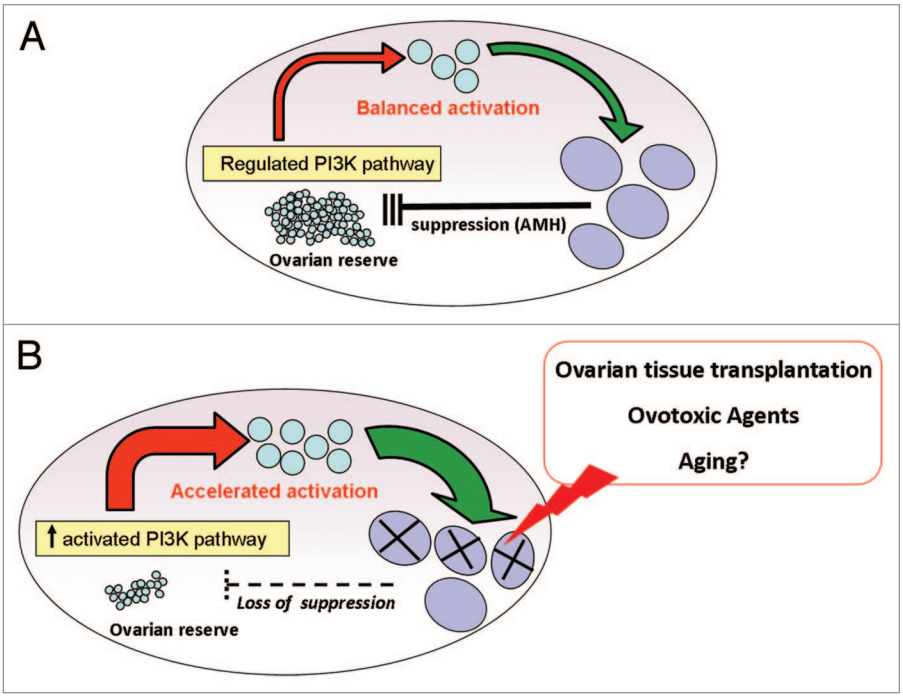

Figure 1. (**A**) During normal follicular development, the ovary is in a state of equilibrium. The primordial follicles (PMFs) are under balanced regulation by the PI3K/PTEN/Akt pathway and suppressive factors produced by growing follicles ensure that the vast majority of PMFs are maintained in a state of dormancy and very few are activated into growth. (**B**) Exposure to ovotoxic agents, as well as processing, freezing, and thawing of ovarian tissue grafts (and aging?), disturb this balance by destroying or removing growing follicles and activating the PI3K/PTEN/Akt follicle activation pathway. This reduces the negative suppression of the dormant PMF population, triggering increased recruitment of PMFs causing the reservoir to "burnout".

pathway may have the potential to reduce follicle burnout and preserve ovarian follicle reserve in the face of ovotoxic treatments or ovarian tissue transplantation.

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