

## Eggs in the Nest

The last several years have produced a great deal of evidence supporting the once revolutionary notion that the formation of the ovary is a directed rather than passive process. For example, factors such as follistatin, respondin-1, and Wnt-4 are differentially expressed in the XX gonad and appear to play a pivotal role in differentiation of the bipotential ovary (1). Primordial germ cells are also actively directed along different pathways in the testis and ovary (2). The hormone retinoic acid diffuses from the mesonephros into the gonad to initiate meiosis in germ cell nuclei in the ovary. In contrast, a testis-specific enzyme, Cyp26b1, actively degrades retinoic acid and prevents germ cell entry into meiosis in the testis. These early steps of somatic cell differentiation and the initiation of germ cell fate are soon followed by oogenesis and follicle formation. Pepling and colleagues (3–5) have done much to advance our understanding of this step of the process, with their work resulting in a new paper exploring the direct contribution of maternal steroids to primordial follicle assembly (6). With their contribution, the molecular, cellular, and endocrine basis of ovarian follicle formation is being solidified, and the field is poised to address essential mechanisms underlying this process and its impact on both fertility and infertility.

It has been known for some time that germ cell nuclei maintain a shared local environment by undergoing incomplete cytokinesis during their early mitotic proliferation, thus creating clusters variously called germ cell nests, germ cell cysts, or germ cell syncytia (5, 7). The utility of a syncytium is not completely clear, but it has the advantages of shared resources and the potential to respond to the changing maternal/fetal environment in a coordinated manner. In this regard, it has been recognized for some time that similar cytoplasmic sharing occurs during male germ cell maturation (8). Germ cell nests persist until a few days after birth in the mouse, when the syncytium breaks down into individual oocytes that become encased in somatic pregranulosa cells to form primordial follicles. At the same time, the number of oocytes within the ovary is drastically reduced by cell death. Two critical questions in ovarian biology are: 1) what regulates germ cell nest breakdown and 2) how does germ cell apoptosis contribute to, or result from, this process? In this issue, Chen *et al.* (6) contributed key insights to this process that bring us one step closer to the answers.

A number of observations have contributed to the hypothesis that maternal hormones are necessary for maintaining germ cell nests in the fetus and that the abrupt loss of exposure to these hormones at birth triggers nest breakdown and primordial follicle formation. Ovaries from mice exposed to estrogen or estrogenic compounds prenatally or as

neonates contain a larger proportion of multiocyte follicles (MOFs) (9–12), suggesting that continued exposure to estrogen preserves germ cell nests, which leads to the assembly of pregranulosa cells around multiple oocytes. Aromatase-deficient mice that lack estrogen production exhibit reduced numbers of primordial follicles in the neonatal ovary, although germ cell nests have not been directly examined (13). Studying the cultured rat ovary, Kezele and Skinner (7) found that both estrogen and progesterone delay nest breakdown and slow the transition from primordial to primary follicles, which is otherwise accelerated in culture. Similarly, using both *in vivo* systems and an *in vitro* culture mouse ovary system to mimic the loss of maternal steroids at birth, Chen *et al.* (6) demonstrated that estrogen (or progesterone) is necessary for germ cell nest breakdown and that breakdown can be arrested if the ovary is reexposed to the steroid. It is useful to note that many normal follicles do form in the presence of these steroids, suggesting that even at the earliest stage of follicle formation, there may be responsive and non-responsive pools of germ cells or follicles. This phenomenon may be an early indication of the adult restraint on all but a few follicles within the ovarian reserve, leading to a limited number of ovulations per month. How small numbers of follicles are parsed out over time remains an unsolved problem in ovarian biology.

The Pepling model, that falling maternal steroids control germ cell nest breakdown, is somewhat challenged by the timing of human follicle formation. Human follicles emerge from the nest during the second trimester of pregnancy, a time of relatively high steroid support (14). One plausible explanation is that circulating steroid binding proteins, such as  $\alpha$ -fetoprotein, may increase during this time, resulting in reduced steroid exposure. A study published by Pepe and colleagues (15) directly tested this prediction by delivering an aromatase inhibitor to baboons during the time of follicle formation, which resulted in fewer primordial follicles and more follicles retained in a nest. The Pepling model predicts that an acute drop in estradiol initiates follicle release from the nest, but the model does not address what requirements the follicles have after formation. The Pepe model is one of chronic loss of estrogen and there may be a requirement for estrogen once follicles form that would confound the evaluation of these ovaries relative to the process of cyst breakdown. Moreover, the few cases of reported aromatase deficiency in human females reveals a range of ovarian pathology (from ovaries with primary follicles to cystic follicles) but provide no information on the absolute number of follicles nor on the presence of unassembled follicles (16). Not surprisingly, no cases of maternal estrogen loss have been reported. Clearly, precise models testing the role of estrogen in nest breakdown in humans or nonhuman primates are difficult. Even so, it may be possible to use the *in vitro* system to further evaluate primary and secondary effects of estrogen depletion on follicle formation and persistence in rodent and

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Abbreviations: ER, Estrogen receptor; MOF, multiocyte follicle.

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nonhuman primate models and to further clarify the similarities and differences in various mammals.

Jefferson *et al.* (11) addressed the receptor type by which estrogen exerts its influence over germ cell nest maintenance in past work. Estrogen receptor (ER)- $\beta$  knockout mice exposed to the estrogenic compound genistein do not form MOFs, whereas ER $\alpha$  knockout and wild-type mice do (11), suggesting that estrogens maintain germ cell nests via ER $\beta$ . It remains unclear whether these actions of estrogen occur through classical or nonclassical signaling, and mouse models, such as the nonclassical ER knock-in mouse, provide a useful approach to the problem (17).

From its earliest days, the ovary is exposed to a host of factors that influence and direct oocyte differentiation and follicle formation. The transcription factor Fig $\alpha$  is genetically required for the normal formation of primordial follicles, and in its absence germ cells are rapidly lost (18). The metalloprotease Fxna is expressed in granulosa cells at time of ovarian follicle formation, and loss of this factor leads to disorganized ovarian structure and loss of follicles (19). Inhibition of synaptonemal complex protein-1 expression prompts oocytes to undergo meiosis early and causes acceleration of primordial follicle assembly, suggesting a link between cell cycle stage and primordial follicle development (20). Several studies have suggested that germ cell nest breakdown is also influenced by locally acting factors. The TGF $\beta$  superfamily members growth differentiation factor-9, bone morphogenetic protein-15, and activin are required for early oocyte differentiation (21, 22), and knockout mice or mice with attenuated synthesis or signaling by these factors exhibit a greater proportion of MOFs (23–25), supporting a direct role for TGF $\beta$  family proteins in nest breakdown as well as subsequent follicle development. Furthermore, it has been suggested that estrogen regulates the activin to inhibin ratio within the ovary to favor follicle development (23, 25), and a recent study demonstrated that exposure of neonatal mice to estradiol, diethylstilbestrol, or genistein leads to reduced ovarian activin expression (12). Taken together, the effects of maternal estrogen and progesterone and those of various locally acting factors likely converge to regulate timing of the very first stages of follicle development: initiation of meiosis, germ cell nest breakdown, and primordial follicle formation.

Turning to the question of the role of apoptosis in germ cell nest breakdown, a previous model proposed that successive rounds of germ cell apoptosis lead directly to germ cell nest breakdown, producing progressively smaller and smaller nests that eventually become individual oocytes around which somatic cells assemble (26). Mice null for the cell death regulator, Bax, have a greater number of oocytes and exhibit a delay in nest breakdown (27), supporting a direct role for apoptosis in this process. Previous studies demonstrated that exposure to neonatal genistein increased oocyte survival *in vivo* and reduced apoptosis (3), suggesting a direct linkage between nest breakdown and germ cell death or survival. Pepling and coworkers (6) have now tested this hypothesis directly using an *in vitro* ovary culture system and found that although genistein, estradiol, and progesterone treatments inhibited nest breakdown, these hormones had no effect on oocyte survival or subsequent follicle development. Thus,

the link between apoptosis and germ cell nest breakdown remains uncertain.

Finally, the developmental programming hypothesis (Barker hypothesis) proposes that exposure of embryonic tissues to an adverse stimulus or insult at critical times during development can permanently reprogram normal physiological responses and so give rise to metabolic and hormonal disorders later in life. The female reproductive tract is a particularly susceptible tissue, and the work by Chen *et al.* (6) illuminates the critical time window when alterations to the steroid-peptide hormone ratio have the potential to substantially alter follicle quantity or quality. In the wild, environmental estrogens cause retention of unassembled follicles and altered sex ratios in alligators, which could provide significant stress on the persistence of the indigenous American alligator (28). Implications of an altered maternal steroid milieu during follicle formation have been addressed by Jefferson and coworkers (11) in previous papers, which asked whether ingesting soy products during pregnancy might impact follicle formation or function in the ovary of the offspring. The impact of nutritional supplements or environmental disruptors have not been addressed adequately in the human, but falling levels of testosterone in American men may be the harbinger of changes that might have their antecedent during gonadal development (29).

Many questions remain regarding the earliest events of follicle formation. In particular, the signals that direct somatic cell invasion of the nest and the process by which somatic cells establish direct connections with the newly autonomous oocyte are not known. And, as discussed, the relationships between nest breakdown and germ cell death remain unresolved. Nevertheless, the work presented by Pepling and coworkers (3–5) adds to our growing understanding of ovarian development and the processes responsible for the establishment of the initial follicle pool at birth. It is likely that the findings of this and other studies will have profound implications for human health and for wildlife management. The transition from maternal to neonatal control is never easy, but eventually all eggs must leave the nest. The Pepling model identifies estrogen and progesterone as important keys to this process.

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