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To β or Not To β: Estrogen Receptors and Ovarian Function

Much of what we know about hormone action in the vertebrate endocrine system comes from studies of estrogen and its signaling pathways, and, in turn, a good deal of this information derives from studies of the ovary. The identification of an estrogenic activity produced in the ovary dates to the studies of Allen and Doisy in 1923 (1) and eventually led to the crystallization of estrone in 1929 (2). The ability to prepare labeled [^{3}H]estradiol allowed the identification of specific target tissues, solidified the receptor concept, and was crucial to the identification of the estrogen receptor, the first hormone receptor (3, 4). Even at these early stages, it was recognized not only that estrogens are made in the ovary, but that the ovary is itself a target for estrogen action (5, 6). With the identification of a second estrogen receptor, ERβ, in 1996 (7) and the finding that this form predominates over ERα in the ovary (8, 9), new interest emerged in the intraovarian roles of estrogen and the estrogen receptors that mediate these effects. Definitive evidence regarding the importance and relative contributions of the two estrogen receptors in the ovary began to accumulate after the targeted disruption of estrogen receptors α (10), β (11, 12), or the combination (12, 13) in mice. The story of estrogen, its receptors, and the ovary continues to be written with the study by Couse *et al.* (14), which provides new insights into the ovarian molecular phenotypes of mice lacking each estrogen receptor, in this issue of *Endocrinology*.

In many respects, the ERα knockout mice (αERKO) have the most severe ovarian phenotype, in which follicles fail to mature or ovulate and form hemorrhagic cysts, leading to infertility (10, 12). However, subsequent studies revealed that much of the αERKO ovarian phenotype could be explained by the lack of estrogen-mediated negative feedback on pituitary LH secretion, resulting in chronically elevated LH levels and enhanced ovarian steroidogenesis (15, 16). Indeed, chronic treatment with a GnRH antagonist to suppress LH reverses the cystic ovarian phenotype, and immature αERKO mice can be successfully ovulated with exogenous gonadotropins before the onset of overt LH hypersection (17), although in the ERα knockout model generated by Dupont *et al.* (12) no ovulations in response to exogenous gonadotropins were observed. In contrast to the αERKO phenotype, gonadotropins and steroids are largely normal in βERKO mice (16), implicating ERα as mediating most of the negative feedback effects of estrogens on pituitary gonadotropin secretion.

The βERKO mouse ovaries appear grossly normal, with follicles at all stages of development but fewer corpora lutea. In agreement with this finding, these female mice exhibit reduced fertility, although there appears to be a range that extends from mild subfertility to complete infertility (11, 12). The βERKO mice also fail to respond to exogenous gonadotropins and exhibit mature follicles containing "trapped" oocytes, suggesting a deficiency in the response to the LH/human chorionic gonadotropin (hCG) ovulatory stimulus. Not surprisingly, the combined αβERKO mice are also infertile and have the attenuated folliculogenesis and anovulatory phenotype characteristic of the αERKO mice (12, 13). However, an unexpected finding was the apparent transdifferentiation of granulosa cells toward a male Sertoli cell phenotype in the αβERKO mice, a phenotype that is also seen in mice with a targeted disruption of the cyp19 gene encoding aromatase, the critical enzyme in estrogen biosynthesis, generating a complete estrogen-deficient state (18–20).

All of this work is suggestive of a primary role for ERβ in regulating follicle development and ovulation but does not provide for a complete mechanistic understanding of the roles or targets of ERβ in the ovary. The studies by Couse *et al.* (14) in this issue of *Endocrinology* begin to address these issues with a detailed analysis of the molecular phenotypes of the αERKO and βERKO mice in the pre- and periovulatory period. The overall picture that emerges is that the predominant ERβ-expressing cells of the ovary, the granulosa cells, exhibit an attenuated response to FSH (or pregnant mare serum gonadotropin, PMSG) in the βERKO ovary and, as a consequence, are not able to respond appropriately to the LH (hCG) stimulus to initiate cumulus expansion, follicle rupture, and ovulation. Supporting these conclusions regarding aberrant PMSG-induced granulosa cell differentiation are the findings that two classical markers of granulosa cell differentiation, the LH receptor and aromatase, show attenuated or delayed expression in the βERKO ovary. Although overall ovarian LH receptor mRNA levels are more or less normal, there is a selective failure of PMSG-induced LH receptor expression in the granulosa cells, and aromatase induction is delayed and does not occur until after the hCG stimulus, resulting in reduced estradiol secretion in the preovulatory period. Thus, the follicle is delayed in its maturation and is not poised to respond to the ovulatory stimulus. As a consequence, several key genes known to be critical for ovulation, including prostaglandin synthase 2 (21) and the progesterone receptor (22), fail to be appropriately induced by LH-hCG in the βERKO mouse ovaries. Morphological examination of the βERKO ovaries points to a failure of LH to induce expansion of the cumulus-oocyte complex in a subset of the preovulatory follicles, consistent with attenuated LH action and a periovulatory defect in the follicle. It is curious that only some follicles exhibit the reduced cumulus-oocyte complex expansion, and it seems reasonable to spec-

Abbreviations: ER, Estrogen receptor; ERKO, ER knockout; hCG, human chorionic gonadotropin; PMSG, pregnant mare serum gonadotropin.

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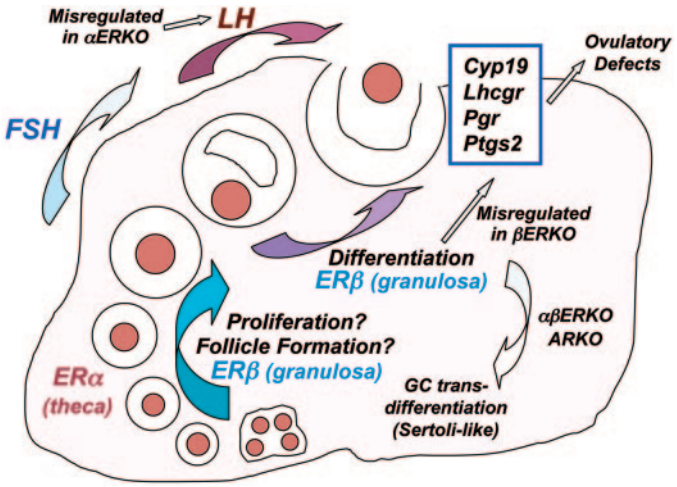

FIG. 1. Model for estrogen and estrogen receptor actions in the ovary. A pathway of follicle formation and maturation is shown, with later stages of follicular development regulated by FSH and ovulation and luteinization triggered by LH. ERα is expressed in thecal/interstitial cells but appears to have few discernable intraovarian effects; rather, LH hypersection in the αERKO mouse is responsible for most of the observed ovarian phenotypes. ERβ is expressed in the granulosa cell, and emerging evidence implicates it and estrogens in the formation or activation of early follicles. The study by Couse *et al.* (14) in this issue demonstrates a requirement for ERβ in FSH-induced differentiation of the granulosa cells of antral follicles. In the absence of this key signal, a variety of genes critical to follicular maturation and ovulation are misregulated (indicated in the *box*), leading to impaired fertility of βERKO mice.

ulate that those that appear morphologically normal are capable of subsequent ovulation, consistent with the wide variability in ovulation rate by the βERKO female mice observed in this study.

Recent related work from the Korach laboratory (23) that investigated the growth and maturation of follicles from the αERKO and βERKO ovaries *in vitro* further supports the concept that ERβ plays a predominant role in follicular maturation. These data demonstrate that preantral follicles from βERKO mice maintained in culture exhibit slower growth, decreased estradiol secretion, and reduced ovulatory capacity compared with follicles from wild-type or αERKO mice. Importantly, follicles cultured from αERKO mice, removed from the environment of elevated LH and androgen that exists in the αERKO mouse, behave normally with respect to the parameters tested, consistent with the view that ERα does not have a significant role in mediating the intraovarian responses to estrogens. Furthermore, this paper reports detailed *in vivo* follicle counts from βERKO mice, confirming that the pool of larger antral follicles is reduced, an observation also made by Dupont *et al.* (12). These effects on later-stage follicles would appear to impact survival or differentiation of the follicle, as proliferation of the granulosa cells appears to be relatively normal.

Interestingly, the adult βERKO ovary had a significantly increased pool of primordial follicles with correspondingly fewer primary follicles (23), hinting at an effect of ERβ on much earlier stages of follicle formation than heretofore appreciated. Two related observations are consistent with a role for estrogens and ERβ in early folliculogenesis. First, aromatase (and thus estrogen)-deficient (ArKO) mice have reduced numbers of primordial and primary follicles at 10 wk of age compared with wild type (24). This phenotype was not impacted by estradiol replacement from wk 7–10, arguing that the impact of estrogen deficiency may be manifest at the time of early follicle formation from the germ cell syncytium or may represent earlier or enhanced activation of the ordinarily static primordial follicle pool. Second, studies to treat hypophysectomized rats or GnRH antagonist-treated mice with ERα or ERβ selective agonists (25) demonstrate that the ERβ agonist 8-vinylestra-1,3,5 (10)-triene-3,17β-diol caused a robust increase in the numbers of primary stage 3a/3b follicles as well as later-stage preantral and antral follicles compared with the vehicle control, an effect not seen with the ERα selective agonist. Together, these observations suggest an estrogen effect mediated by ERβ on the earliest stages of follicle formation and fate.

The new studies by Couse *et al.* (14) reported in this issue, together with other recent work in related models, go a long way toward demonstrating that it is indeed ERβ that mediates the critical actions of estrogens in the ovary and that, in the absence of this signaling pathway, FSH-stimulated granulosa cell differentiation is attenuated, resulting in a follicle that cannot fully respond to the LH ovulatory stimulus (Fig. 1). Although changes in several key receptors and enzyme activities have been identified, there is still likely much to be learned about interactions between FSH and estrogen signaling and about the proximal targets for ERβ regulation in the granulosa cell. Also new on the horizon are the ligand-independent activities of the estrogen receptors and the nonclassical roles such receptors might play in ovarian function (26). Equally exciting is the growing body of evidence suggesting early actions of estrogens in follicle formation, maturation or atresia, and, indeed, in regulating the sexual differentiation of ovarian granulosa cells. The evidence thus far suggests that these actions are also likely to be mediated by ERβ. In much the same way as ERβ appears to enhance FSH signaling in regulating later stages of follicle maturation (14), it seems likely that ERβ will have a modifying influence on signaling pathways for hormones such as activin or other TGFβ family proteins in regulating earlier stages of folliculogenesis (27). We know surprisingly little about the regulatory events controlling follicle formation, activation, and maturation before the onset of gonadotropin dependence, and it looks as though this is yet another research area in which estrogens might help lead the way.

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