Embryonic Estrogen Receptors: Do They Have a Physiological Function?

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In normal estrogen target tissues, estrogen action is mediated through a specific nuclear transcription factor, the estrogen receptor (ER). The site of estrogen action in the developing organism is therefore determined by cells that contain ER and other necessary tissue and gene-specific components for estrogen-mediated transcription. Immunocytochemical methods were used to determine the cellular localization and tissue distribution of ERs in reproductive tracts of mouse fetuses. Nuclear staining for ER was observed in reproductive tracts at fetal days 13 to 15. ERs were present in the precursors of both male and female reproductive tracts at these early developmental stages, which may be attributable to their similar embryonic origins. However, as the tissues undergo sexual differentiation at later fetal and early neonatal ages, ER increases in the female reproductive tracts as compared with the male. ER was detected by immunoblotting on fetal day 10 (before sexual differentiation) in extracts of whole mouse embryos. To determine whether ER and progesterone receptor genes are expressed in mouse preimplantation embryos, we examined DNA from preimplantation mouse embryos using reverse transcriptase–polymerase chain reaction techniques. ER mRNA was found in oocytes and fertilized eggs. Message concentration declined at the 2-cell stage and reached its lowest level at the 5- to 8-cell stage. ER mRNA was not detectable at the morula stage but reappeared at the blastocyst stage. Progesterone receptor mRNA was not detectable until the blastocyst stage. The embryonic expression of ER and progesterone receptor genes in the blastocyst suggests a possible functional requirement for estrogen and progesterone receptors in preimplantation embryos. — Environ Health Perspect 103(Suppl 7):69–72 (1995)

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Introduction

A combination of recent and older observations has raised intriguing questions about the role of estrogenic hormones in the early embryo. The new data, which are based on two different approaches, paradoxically appear to be contradictory. The results of the first approach are summarized in Figure 1 (1) and show that early mouse embryos from the unfertilized state to the 3- to 4-cell stage contain what appears to be maternal messages for the estrogen receptor (ER) (1,2). Furthermore, the ER mRNA reappears at the blastocyst stage, indicating that the embryonic genome expresses the gene (Figure 2) (1).

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Abbreviations used: DES, diethylstilbestrol; ER, estrogen receptors; PCR, polymerase chain reaction.

Immunocytochemical studies show that all the cells of the blastocyst appear to have ER present (3). Additional data indicate that the progesterone receptor mRNA, which is usually considered to be responsive to estrogen, also appears for the first time at the blastocyst stage. It is still not possible to say conclusively whether these receptors are physiologically active, but there are few examples of nonmutated ER receptors in target cells that are inactive. These studies coupled with older reports in the literature support the notion that ER may have a novel role in the development of the mammalian embryo. The presence of ER in all cells of the blastocyst increases the possibility of estrogenic effects that lead to changes in tissues and in cells that are not ordinarily considered to be estrogen targets.

In marked contrast to the above observations, the elegant studies from Korach’s group (4) describe “gene knockout” strategies to produce a mouse with a defective ER gene. This defective gene has an insert that should prevent the production of normal mRNA and limit the production of a normally active ER protein. These mice showed grossly abnormal reproductive tract development as predicted. It was also noted that the males were infertile.

A puzzling aspect of these observations is that the presence of the receptor in the early embryo implies an essential role for
the ER in early embryonic development such as implantation or some other critical function. Furthermore, the widespread distribution of embryonic ER suggests a more generalized requirement for ER than solely reproduction. Yet, the ER-deficient mice appear to develop normally except for their reproductive organs. In several other cases, important genes that were "knocked out" also showed only limited effects on development (5–7). It has been suggested that in such cases the organism has developed more than one gene to regulate such a critical mechanism. Whether or not such a second mechanism exists for ER-dependent developmental regulation is not yet clear.

Results from older literature lead us to ask other questions about the role of ER in the embryo. It has been known since the 1960s that administration of high doses of the synthetic estrogen diethylstilbestrol (DES) to pregnant women caused dramatic and widespread deleterious effects on the offspring from those pregnancies (8). The resulting clear-cell carcinomas of the vagina of adolescent daughters and widespread abnormalities in sons emphasized the powerful effects of estrogens on this developmental stage and revealed the long time interval between embryonic exposure and resulting pathology in the adult. These observations also point out that the treated mothers did not develop any of the symptoms observed in the fetuses. This suggests that embryonic exposure to estrogen represents a new and different environment in which estrogen and its receptor functions, as compared with the adult.

More carefully controlled experiments have been carried out in which DES was injected into pregnant mice on days 9 to 16 of gestation (9–12). These studies also resulted in definite developmental abnormalities in both male and female offspring from the DES-treated pregnancies. As in the human studies, the DES effects were only seen in the fetal animals exposed to estrogen and not in the maternal hosts. Thus, the cells of the midgestational mouse fetus, which can be assumed to be in an early state of development, respond differently than cells from the same tissues of the adult, which are in a later stages of development.

Another widely used experimental system is the estrogen-injected neonatal mouse or rat (8,13–15). These treated mice also respond differently to estrogen than adults do. The estrogen-treated neonates exhibit a high incidence of epithelial cancer after they reach sexual maturity compared to controls.

![Figure 2](image-url)

**Figure 2.** Expression of the ER gene. (A) Agarose gel showing the polymerase chain reaction (PCR) product amplified with primers mERa and mERp. RNA from 1.8 embryos was included in each sample. Uterine RNA (50 µg) was used as a positive control. The annealing temperature for PCR was 65°C, and 17 plus 33 cycles were run. (B) Autoradiogram of Southern blot for the gel shown in A. (C) Southern blot analysis of the PCR product amplified with primers mERa and mERp. RNA from 3.8 embryos was included in each sample. Uterine RNA (100 µg) was used as a positive control. The annealing temperature for PCR was 62°C, and 20 plus 35 cycles were run. Lane M, molecular size markers (lengths at left in nucleotides). Reproduced with permission from Hou and Gorski (1).
Thus, from day 9 of gestation to approximately 5 days after birth (9–25 days postconception), estrogen target cells respond to estrogen in a manner that leads to an abnormal or cancerous response from subsequent exposure to estrogen when the animals reach sexual maturity. Since differentiation of the reproductive tracts starts about days 12 to 13 in these species, the ER is no longer distributed randomly, which appears to be the case in the very early embryo at the blastocyst stage (gestation days 4 to 5). This raises the question as to whether exposure to estrogen at the blastocyst stage might lead to an even broader array of effects in the adult. Perhaps such effects are limited to cells that contain ER in the adult, but it is possible that changes in the cellular environment induced by embryonic exposure to estrogen might not be dependent on later exposure to estrogen.

These observations also recall reports in the literature about breast cancer in which early menarche is associated with increased risk of breast cancer, whereas early pregnancy is associated with lower risk of breast cancer (8). In this case it is believed that the developmental state of the mammary cells may explain this phenomenon. The early development of mammary cells under the influence of the hormones associated with pregnancy may result in a cellular environment that is comparable to the adult mice or humans in the experiments described above. Fetal cells, in contrast, have not yet differentiated and are susceptible to abnormal differentiation in response to estrogen exposure at these early stages of development. Early menarche may principally involve exposure to estrogen whereas pregnancy exposes the target cells to a complex array of hormones that includes estrogens, progestins, placental gonadotropins, placental lactogens, and probably others. Thus, the estrogens and progestins produce different effects in cells that are in varying states of differentiation; at the same time these hormones are causing changes in the differentiation of these same target cells.

The development of the reproductive tracts of both males and females is controlled by sex steroid hormones. In previous studies from this laboratory, Greco et al. (16–18) showed that ER is present in both male and female mice from gestational day 10 and later, with definite evidence of ER being present in reproductive tissues at gestational day 1 or later. This includes the gonads (Figure 3) (18) as well as the reproductive tracts (Figure 4) (16–18).

**Figure 3.** Immunocytochemistry for ER on frozen sections from fetal mouse gonads taken on 13, 15, 17, and 19 days postconception. An asterisk (*) indicates that on day 13 gender was not apparent and the fetus could be either female or male before sexual differentiation. Female: nuclear staining was only observed on day 15, and the intensity of staining varied between experiments. Male: nuclear staining was observed on days 13 and 15; Leydig cells stained on fetal day 15. On days 17 and 18, peritubular cells of the testes stained for ER. Bar = 50 µm. Reproduced with permission from Greco et al. (18).

**Figure 4.** Immunoblots of extracts prepared from female and male reproductive tracts collected 15, 17, and 19 days after fertilization; 19 days after fertilization is date of birth (DOB). For males, the testes were separated from the associated ducts of the reproductive tract and prepared separately for the immunoblots. ND, not determined. Protein concentration (µg) and approximate number of fetuses pooled are listed below the photograph. Reproduced with permission from Greco et al. (16).
as the primitive reproductive tracts (Figure 4) (18) of both fetal males and females. These observations likely explain why both sexes show abnormalities after exposure to DES in utero.

None of the above observations provides an explanation for the apparent contradiction presented by the presence of ER in the embryo and, with the exception of the reproductive organs, the apparent normal development of the ER-negative mice. This paradox may have little relevance to the problem of cancer induced by embryonic exposure to estrogen. If embryonic ERs are physiologically active, then cellular responses in the embryonic cells will occur in a cellular environment that is unique and different from the cellular environment normally present in cells from estrogen-regulated adult tissues. This could be one step in the multistep sequence that leads to the transformed state of cancer cells found in the adult. The embryo may normally be well protected from estrogen exposure due to a variety of mechanisms that limit the concentration of 17β-estradiol, the estrogen that is normally found in most species. However, such mechanisms may not protect against estrogens such as DES, which are not affected by the mechanisms that limit 17β-estradiol. For example, DES is not bound by α-feto protein, which sequesters most of the estradiol in the blood of fetal and neonatal rats. Also, DES is not metabolized in the same manner as estradiol and therefore is cleared from the animal at a much slower rate. That mechanisms may be involved at very early embryonic stages, such as the blastocyst, is not clear but could have great importance in relation to normal development.

Currently there is a great deal of interest in environmental estrogens that originate from sources such as chemical processing or foods that contain mycotoxins or phytoestrogens. Whether such estrogens have effects on the developing embryo is not clear at this time, but one critical component of such a response is the ER, appears to be present in all cells of the early embryo. Therefore, such a response is a possibility that must be considered.

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