The role of peripheral gonadotropin-releasing hormone receptors in female reproduction

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Objective: To review the physiologic functions and clinical significance of peripheral GnRH receptors.

Design: Literature review. All peer-reviewed journal articles published before 2010 on peripheral GnRH receptors were searched for in the Pubmed database, and relevant findings were summarized.

Results: Peripheral GnRH/GnRH receptor systems may serve as regulators of hCG synthesis and implantation, and play crucial roles in antiproliferation and apoptosis. Currently, GnRH agonists have been used in cancer treatment and ovary protection during chemotherapy, taking advantage of the local direct effect mediated by peripheral GnRH receptors.

Conclusions: The ubiquitous GnRH/GnRH receptor system in human tissues has been shown to have some important physiologic functions. Further research to clarify functions of these peripheral GnRH receptors may lead to discovery of new therapeutic options. (Fertil Steril 2011;95:465–73. ©2011 by American Society for Reproductive Medicine.)

Key Words: GnRH, peripheral GnRH receptor, central GnRH receptor, GnRH agonist, chemotherapy, apoptosis, ovarian damage, implantation, placenta

Gonadotropin-releasing hormone (GnRH) is a ten–amino acid peptide that is secreted from the hypothalamus and acts as a key regulator of reproductive functions. By binding to GnRH receptors on pituitary gonadotropes, GnRH stimulates the release of LH and FSH and subsequently the secretion of steroid hormones from the gonads. The gene for the pituitary GnRH receptor is located on chromosome 4; it is organized into three exons and two introns and codes for a 328–amino acid protein. The GnRH receptor is a seven–transmembrane-domain G protein–coupled receptor (GPCR). In contrast to most members of the GPCR superfamily, mammalian GnRH receptor lacks the characteristic intracellular carboxyl-terminal domain. Furthermore, the GnRH receptor has a very short extracellular amino terminus of only 35 residues. Because of these two characteristics, this is one of the smallest GPCRs. A growing body of evidence indicates that GnRH receptors are expressed not only in the pituitary, but also in normal and tumor peripheral tissues.

The present review focuses on physiologic functions and possible clinical significances of peripheral GnRH receptors in female reproductive tissues.

DISTRIBUTION OF PERIPHERAL GnRH RECEPTORS

In 1981, the presence of GnRH receptors was reported in the human placenta using radioreceptor assays (1). This was the first demonstration of extrapituitary GnRH receptors in humans. Since then, GnRH receptors have been identified in a wide variety of human tissues using different techniques. For example, GnRH receptor mRNA and complementary DNA (cDNA) have been detected in several types of human placental cells. Using in situ hybridization, GnRH receptor mRNA was detected in the human placenta and localized to both cytotrophoblast and syncytiotrophoblast cell layers (2). In addition, a full-length GnRH receptor cDNA was isolated from various human placental cells, including a choriocarcinoma cell line (JEG-3), immortalized extravillous trophoblasts, and first-trimester cytotrophoblast cells.

Because the ovary is the target organ of the hypothalamic-pituitary-gonadal axis, which is centrally regulated by GnRH, significant research effort has been focused on identifying the presence and function of GnRH receptors in the ovary. GnRH receptor mRNA has been found to be expressed in multiple ovarian cell types, including preovulatory granulosa cells, luteinized cells, and ovarian compartments other than follicular or luteal structures, across different functional stages (3).

Other human reproductive tissues, such as breast and endometrium (but not adenomyosis or myometrium), have been proven to express GnRH receptor mRNA on the cell surface. GnRH receptors have also been identified on the surface of various tumor cells, including endometrial carcinomas, leiomyomas, leiomyosarcomas, breast cancer, choriocarcinoma, epithelial ovarian cancer, and...
from a number of fishes, amphibians, and primates raised the proba-
tein sequence obtained from the pituitary encoding a 328–amino acid
sequences for the two GnRH receptor gene transcripts are confirmed to
be identical, the encoded proteins could perhaps differ in their post-
translational processing, yielding proteins that differ in their phos-
horylation or glycosylation states. Generation of these multiple
isoforms could have physiologic significance and could be another
level of control of GnRH receptor gene expression (15).

It is therefore unclear whether central and peripheral GnRH
receptors are actually identical in protein structures. Even if the se-
quences for the two GnRH receptor gene transcripts are confirmed to
be identical, the encoded proteins could perhaps differ in their post-
translational processing, yielding proteins that differ in their phos-
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isoforms could have physiologic significance and could be another
level of control of GnRH receptor gene expression (15).

GnRH receptors in peripheral tissues differ from those in pituitary
gonadotrophs regarding the transmembrane signaling cascade. Both
pituitary and peripheral GnRH receptors are G protein–coupled
receptors. Activation of the pituitary GnRH receptor leads to the
stimulation of the Gq/11 protein, which activates phospholipase C.
The generated second messengers inositol 1,4,5-trisphosphate and
diacylglycerol lead to mobilization of Ca\(^{2+}\) from intracellular stores
and activation of various protein kinase C subspecies. This intracel-
lunar cascade of events mediates the biologic activity of GnRH,
which stimulates the synthesis and secretion of gonadotropins.
On the other hand, the peripheral GnRH receptor is coupled to the
Gi protein in uterine leiomyosarcoma, ovarian carcinoma, and endo-
metrial carcinoma (17, 18). Activation of the peripheral GnRH

### TABLE 1

Comparison of central and peripheral GnRH receptors.

<table>
<thead>
<tr>
<th></th>
<th>Central GnRH receptor</th>
<th>Peripheral GnRH receptor</th>
</tr>
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<tbody>
<tr>
<td>Complementary DNA sequence</td>
<td>Same</td>
<td>Normal and tumoral reproducive tissues and nonreproductive tissues</td>
</tr>
<tr>
<td>Affinity for native GnRH</td>
<td>Same</td>
<td>Same</td>
</tr>
<tr>
<td>Affinity for agonist buserelin</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Action of cetrorelix</td>
<td>Competitive antagonist</td>
<td>Inhibitory—decreases cell proliferation in tumors</td>
</tr>
<tr>
<td>Intracellular activity caused by activation of receptor</td>
<td>Stimulatory—increases gonadotropin synthesis and secretion</td>
<td>Gi protein activates cAMP and PTP which reduce activity of MAPK</td>
</tr>
<tr>
<td>Signaling pathway</td>
<td>Gq/11 protein stimulates PLC which stimulates PKC</td>
<td>Dynamic in ovary, depends on degree of follicular development and stage of estrous cycle</td>
</tr>
<tr>
<td>Receptor expression on cell surface</td>
<td>Dynamic, highest level before LH surge</td>
<td></td>
</tr>
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</table>

Note: cAMP = cyclic adenosine monophosphate; MAPK = mitogen-activated protein kinase; PKC = protein kinase C; PLC = phospholipase C; PTP = phosphotyrosine phosphatase.


stromal tumors of the ovary (4). GnRH receptor cDNA has also been
obtained from normal prostate tissue, prostate cancer cells, and non-
reproductive peripheral tissues, including liver, heart, skeletal muscle,
kidney, and melanoma (5, 6).

Recent discovery of GnRH-II in humans and cloning of its receptor from a number of fishes, amphibians, and primates raised the probable
existence of this second type of GnRH receptor in humans (7). GnRH-II is a decapeptide that has three amino acid differences
from GnRH. The GnRH-II peptide is widely distributed in the central and peripheral nervous system and peripheral tissues. Inquiry on the
human genome database revealed that a putative GnRH-II receptor
gene was located on chromosome 1. Nevertheless, to date, direct
evidence to demonstrate the existence of full-length functional
GnRH-II receptor RNA transcript in human tissues is lacking. Therefore, the present review does not cover GnRH-II receptors.

### CENTRAL VERSUS PERIPHERAL GnRH RECEPTOR

Peripheral GnRH receptors resemble central or pituitary GnRH re-
ceptors in terms of cDNA nucleotide sequence and protein molecular
weight, but they do not share the same pharmacologic profile in
terms of binding affinity for different synthetic GnRHaalogues. Genomic Southern blot analysis indicated that the GnRH receptor
gene exists as a single-copy gene in the human genome. Nucleotide
sequence analysis of GnRH receptor cDNAs from normal ovarian
and placental tissue as well as from a breast tumor cell line
(MCF-7) and an ovarian tumor revealed 100% homology with the
sequences obtained from the pituitary encoding a 328–amino acid protein (5, 8–10). Native GnRH was found to have the same
affinity for the pituitary GnRH receptor as it had for GnRH receptors in placenta cells, rat luteal cells, and rat Leydig cells. However, GnRH agonists bound to the pituitary with a 100-fold higher affinity than to the placenta (11). Cetrorelix, which is a
GnRH antagonist at the pituitary level, has been consistently re-
ported to act as a GnRH agonist peripherally and exerts antiprolifer-
avative activity on tumor cells from the reproductive tract (12–14). The reason cetrorelix behaves as an antagonist on pituitary gonadotropes and as an agonist on cancer cells is still unclear. However, this could indicate that differences at the molecular level may exist between
central GnRH receptors in the pituitary and peripheral receptors present in tumors of the reproductive system (Table 1).

The hypothalamic GnRH gene was found to have a 61–base pair
first exon, and its transcriptional start site was determined. The hu-
man hypothalamic GnRH cDNAs isolated to date have all contained
a short 5' untranslated region which would correspond to this start
site. However, all human placental GnRH cDNAs reported to date
have a long 5' untranslated region, which extends more than 140
base pairs 5' to this start site in the hypothalamus, suggesting the uti-
ilization of an alternative promoter in the placenta. In addition, the
human GnRH gene undergoes differential splicing in these tissues.
The first intron is removed from the hypothalamic, but retained in the
placental, GnRH mRNA. Thus, the GnRH gene has a very long first exon in the placenta, whereas in the hypothalamus it has a comparatively short first exon, followed by a long first intron. This characterization of the human GnRH gene will now allow hormonal regulatory studies to be performed using gene transfer techniques (15).
receptor leads to a decrease of intracellular cyclic adenosine monophosphate (cAMP) level, which causes activation of protein tyrosine phosphatase. Tyrosine phosphatase interferes with growth factor–induced tyrosine phosphorylation and with mitogen-activated protein kinase (MAPK) cascades. Decreased cytoplasmic levels of cAMP and reduced activities of MAPK pathways ultimately down-regulate gene transcription in the cell nucleus. These intracellular molecular events mediate antiproliferative activity of GnRH and its receptors in tumor cells (19) (Fig. 1).

Under physiologic conditions, the central GnRH receptor number varies and is usually directly correlated with the gonadotropin secretory capacity of pituitary gonadotrophs. For example, across the rat estrous cycle, a rise in GnRH receptors is seen just before the surge of gonadotropins that occurs on the afternoon of proestrus. GnRH receptor message levels are regulated by a variety of hormones and second messengers, including steroid hormones (E2 can both suppress and stimulate, and P suppresses), gonadotropins (which suppress), and calcium and protein kinase C (which stimulate). When there is a decline in GnRH stimulation to the pituitary, as occurs in a variety of physiologic conditions including states of lactation, undernutrition, or seasonal periods of reproductive quiescence, the number of GnRH receptors on pituitary gonadotropes declines dramatically. Subsequent exposure of the pituitary to pulses of GnRH restores receptor number by a Ca2+-dependent mechanism that requires protein synthesis. In contrast to up-regulation of GnRH receptors by pulsatile regimens of GnRH, continuous exposure to GnRH leads to down-regulation of GnRH receptors and an accompanying decrease in LH and FSH synthesis and secretion.

Similarly to pituitary GnRH receptors, the expression of GnRH receptors is dynamic in ovarian tissue. In the rat ovary, in situ hybridization analysis reveals that GnRH receptor gene expression is dependent on the degree of follicular development and the stage of the estrous cycle. The GnRH receptor expression was greatest in the granulosa cells from graafian and atretic follicles, with lower levels of expression present in preantral, small antral follicles, and the corpus luteum (20). GnRH receptor mRNA levels in atretic follicles increased up to threefold on the day of proestrus, coincident with the preovulatory gonadotropin surge, whereas the level of GnRH receptor gene expression in the corpus luteum significantly increased between the morning of metestrus and the afternoon of proestrus (19). Interestingly, GnRH receptor expression in the rat ovary is correlated with the expression of pituitary GnRH receptors. The highest level of receptor expression was observed in proestrus, just before the gonadotropin surge, and that level were maintained throughout the gonadotropin surge, followed by a decline in metestrus. In preovulatory rat granulosa cells, GnRH induced an increase in the receptor levels in a dose-dependent manner, whereas LH decreased GnRH receptor mRNA levels (19). In the rat, treatment with E2 enhanced GnRH receptor gene expression in granulosa cells from both actively growing and atretic follicles (21).

Although GnRH receptor gene expression is well characterized in the rat ovary, little is known about the primate ovary. In cultured human granulosa cells (10) and ovarian surface epithelial cells (22), treatment with GnRH induced a biphasic effect (up- and down-regulation) on GnRH and GnRH receptor mRNA expression in these cells. GnRH and GnRH receptors were not immunostained in the follicles from the primordial to the early antral stage. In preovulatory follicles, GnRH and its receptor were localized predominantly to the granulosa cell layer, whereas the theca interna layer was weakly positive. In the corpus luteum, significant levels of GnRH and GnRH receptors were observed in granulosa luteal cells, but not in theca luteal cells.10 GnRH and GnRH receptors were localized also to the ovarian surface epithelium from which >85% of ovarian

![Figure 1](image-url)
cancers are thought to be derived (22). The expression of GnRH and GnRH receptor protein in the human ovary is temporally and spatially specific and further supports the physiologic role of an autocrine regulatory system involving GnRH and GnRH receptor in follicular development and corpus luteal function.

**PHYSIOLOGICAL FUNCTIONS AND MECHANISM OF ACTIONS**

With the discovery of GnRH receptors in a wide variety of human tissues, there has been a growing interest in characterizing the physiologic functions of these peripheral receptors. Research suggests that peripheral GnRH receptors may be involved in regulating hCG secretion and implantation, as well as in decreasing cell proliferation and mediating apoptosis in tumor cells. Continuing research in this area will further elucidate these and perhaps additional physiologic functions of the GnRH receptor.

**Regulation of hCG Secretion**

The hypothesis that placental GnRH may be involved in the autocrine/paracrine regulation of the biosynthesis of hCG is supported by two observations: First, the highest GnRH concentrations in the placenta are present during the first trimester of pregnancy, coinciding with the temporal distribution of hCG synthesis (23); second, the secretion of hCG from placental cells has been found to be suppressed by treatment with a GnRH antagonist (24).

The role of GnRH in controlling placental hCG production and secretion has been fully demonstrated both in vitro and in vivo. Studies using placental cells from anencephalic fetuses demonstrated a decreased binding capacity for GnRH and its agonists, as well as a reduced capacity to produce hCG, compared with normal trophoblastic cells. This indicates that GnRH secreted from the pituitary up-regulates peripheral GnRH receptors and stimulates hCG production in the placenta. This stimulatory effect appears to be receptor dependent, because GnRH antagonists block both GnRH- and GnRH agonist–induced effects. Furthermore, GnRH antagonists reduce the amplitude of spontaneous placental hCG pulses, suggesting a direct blockade of endogenous placental GnRH (25).

Using immunohistochemistry, the most intense staining for GnRH in the placenta was found during the eighth week of gestation, with low staining during the remainder of the gestation period (26). Using solution hybridization protection assay and in situ hybridization assay, the level of GnRH mRNA was shown to remain constant throughout gestation (27). In contrast, GnRH receptor mRNA is expressed in both cytotrophoblasts and syncytiotrophoblasts and exhibits changes paralleling the time course of hCG secretion during pregnancy. These data provide a mechanistic understanding that paracrine/autocrine regulation of hCG secretion by placental GnRH is mediated through an increase followed by a decline in GnRH receptor gene expression from the first-trimester to term placenta (2). Taken together, these findings implicate an important role for the GnRH receptor in regulating hCG secretion during pregnancy.

With the demonstration that GnRH receptor and GnRH are synthesized within the same cell, Wolfarth et al. (28) proposed that an ultrashort feedback mechanism regulating the neuroendocrine network in placenta may exist.

**Regulation of Implantation**

Human implantation is a complex series of steps that under normal circumstances begins even before the blastocyst reaches the uterine cavity and attaches to the endometrial epithelium after the loss of zona pellucida. To complete this series of events and to accomplish successful implantation and placentation, the embryo and the uterine endometrium must be synchronized during this limited period of uterine receptivity known as the “window of implantation.” This complex process is the result of an embryonic-maternal dialogue, in which the embryo and the endometrium induce changes in each other to promote receptivity. Growth factors and cytokines are secreted by the developing blastocyst to enhance uterine receptivity, directly by influencing the endometrial epithelial cells to undergo cellular changes such as down-regulation of cell polarity, or indirectly by stimulation of ovarian steroidogenesis through hCG, until placental P production is sufficient to maintain the continuing pregnancy. After adhesion of the embryonic pole of the human blastocyst to the endometrial epithelial surface, trophoblastic differentiation into cytotrophoblast and syncytiotrophoblast occurs and the trophoblast invades the stromal endometrium.

Seshagiri et al. (29) showed that immunoreactive GnRH and chorionic gonadotropin (CG) were produced in vitro by cultured rhesus monkey embryos during the entire periattachment period, from morula to attached blastocyst stage, and that the GnRH secretion commenced before that of CG. Moreover, embryos that failed to hatch and attach secreted lower amounts of GnRH into the medium compared with those that did reach later stages of development; at the same time, CG was very low or absent in the medium of these embryos. GnRH and GnRH receptors have also been shown to be present in preimplantation human embryos and the fallopian tubes in the luteal phase at both mRNA and protein levels (30, 31). After embryo-endometrial adhesion, as the embryo invades the uterine endometrial stroma, trophoblastic differentiation into cytotrophoblast and syncytiotrophoblast layers occurs, and both are known to produce GnRH as well as to express the GnRH receptor in the first-trimester human placenta (32).

The presence of a dynamic pattern of expression of GnRH and its receptor in both the epithelium and the stroma of the human endometrium provides evidence that this hormone plays a substantial role as a molecular autocrine-paracrine regulator in embryonic-endometrial interactions during early implantation (32). Both GnRH and its receptor were shown to be expressed at the mRNA level in vivo by the human endometrium throughout the entire menstrual cycle of fertile patients, with a significant increase in the secretory phase compared with the proliferative phase. Furthermore, isolated endometrial stromal and epithelial cells expressed both GnRH and its receptor, with higher mRNA levels in the luteal phase in both compartments. GnRH immunostaining was localized in all major compartments, with the most intense staining during the luteal phase. On the basis of these data, Raga et al. (32) suggested that during reproductive life, endometrial GnRH may play a paracrine-autocrine role in the early stages of implantation by modulating embryonic trophoblastic secretion of hCG. GnRH mRNA and protein expression are increased in the hatching blastocyst stage compared with the morula stage, and GnRH receptor was found to maintain a constant level during all developmental embryonic stages, reinforcing the hypothesis that the embryo communicates with the maternal tubal epithelium and endometrium through the GnRH system to promote embryonic development and endometrial receptivity (31, 33).

A Cochrane review of 27 randomized control trials comparing the GnRH antagonist to the long protocol of GnRH agonist in assisted reproductive technology (ART) cycles revealed significantly lower clinical pregnancy, ongoing pregnancy, and live birth rates in the antagonist group, despite the transfer of an equivalent number of good quality embryos in both groups (34). The persistent lower pregnancy...
rate with antagonist may raise questions about the impact of GnRH antagonist on the endometrium and subsequently on implantation. A recent study using endometrial stromal cells derived from fertile women during implantation window demonstrated that GnRH agonist or antagonist had no detrimental effect on trophoblast invasion and stromal cell decidualization in vitro. However, the elevated levels of GnRH receptors in decidualized stromal cells suggested a function for the GnRH–GnRH receptor pathway in these early implantation events (35).

Increased implantation and pregnancy rates in ART cycles that used GnRH agonist for additional luteal phase support also imply potential local effect from endometrial GnRH receptors (36). In a randomized control trial, 164 patients in GnRH agonist protocol who underwent intracytoplasmic sperm injection (ICSI) after ovarian stimulation were randomized to either P alone or GnRH agonist in addition to P for luteal phase support. Although the numbers and grades of embryos transferred were similar in the two groups, the patients with additional GnRH agonist supplementation had significantly higher implantation, clinical pregnancy, and live birth rates (36).

An antibody against GnRH has been described in the maternal circulation of pregnant women with previous miscarriages and low levels of hCG (37), reinforcing the important role of GnRH in human implantation and placentation.

**Cell Proliferation and Apoptosis**

The demonstration of presence of both GnRH and GnRH receptors in tumors of the reproductive tract prompted investigation into the possible role played by the peripherally expressed GnRH system in controlling tumor growth. GnRH agonist in vitro suppresses the growth of endometrial cancer, ovarian cancer, and estrogen-dependent and estrogen-independent breast cancer. Cell growth arrest in response to GnRH can be observed not only in malignant diseases but also in benign tumor leiomyoma (13, 38). GnRH analogues in nanomolar-order concentrations reduced the number of GnRH-responsive carcinoma cells and their cell lines to 50%–80% compared with control cells (39). Antiserum to GnRH greatly increases the growth of ovarian cancer cells (40). These findings strongly suggest that the antitumor effect of GnRH agonists is specific and directed in part through GnRH receptors present on membranes of tumor cells (4).

Although the mechanism by which GnRH agonist inhibits tumor cell proliferation is not known, the GnRH-mediated antiproliferation of endometrial cancer and ovarian cancer may result from apoptotic cell death mediated by Fas and Fas ligand. Fas and Fas ligand are transmembrane proteins of the tumor necrosis factor family of receptors and ligands. Engagement of Fas by Fas ligand triggers a cascade of subcellular events that results in programmed cell death, or apoptosis. Fas has been detected in a variety of normal and neoplastic cells, whereas Fas ligand was initially thought to be expressed only in activated T cells. Recently, it has been reported that Fas ligand is also expressed in some kinds of tumors, such as resected human colorectal cancers, melanomas, hepatocellular cancers, astrocytomas, and lung cancers (41), and that GnRH agonist induces Fas ligand expression in GnRH receptor–bearing tumors (42). It was found that GnRH agonist induced apoptosis in the cultured leiomyoma cells associated with increased expression of Fas and induction of Fas ligand, suggesting that the Fas–Fas ligand system may participate in GnRH agonist–induced apoptosis in leiomyoma cells in vitro. It is likely that GnRH agonist not only increases the expression of Fas but also has a similar effect on p53, which may transport Fas from the Golgi complex to the membrane surface and then play a role in the induction of apoptosis by combining Fas with Fas ligand (43).

Other studies have demonstrated that treatment with GnRH analogues such as triptorelin and leuprolide induce a reversible reduction of cell proliferation through an increase in the portion of cells in resting phase G0/G1, with a corresponding decrease of cells in DNA synthesis and cell proliferation phases (44, 45). It was recently reported that JunD, which is the most widely distributed member of a family of transcription factors, may function as a negative regulator of cell proliferation, because overexpression of JunD slowed cell growth and resulted in an increase in the percentage of cells in G0/G1 phase of the cell cycle. Therefore, Gunthert et al. (45) propose that JunD activation by GnRH plays an important role as a modulator of cell proliferation and cooperates with the antiapoptotic and antimitogenic actions of GnRH.

It has been suggested that GnRH may be involved in the process of luteinization and luteolysis. GnRH induces remodeling of the extracellular matrix by stimulating matrix metalloproteinase, which degrades collagens (46). GnRH-induced apoptosis also contributes to the acceleration of the ongoing luteolytic and endometriolytic processes (47). There is increasing evidence for a role of GnRH in the regulation of follicular atresia. During the follicular phase, GnRH receptor expression is high in atretic rat follicles. In vitro, GnRH inhibits DNA synthesis and induces apoptosis in rat granulosa cells. During the periovulatory period, GnRH induces transcription of several genes that are involved in follicular rupture and oocyte maturation. GnRH induces an increase in the number of apoptotic human granulosa cells obtained during oocyte retrieval for in vitro fertilization (IVF) (47).

**CLINICAL APPLICATION**

**Cancer Therapy**

GnRH analogues have been used in the treatment of many endocrine-dependent cancers, including breast cancer, endometrial cancer, ovarian stromal tumor, and ovarian epithelial cancer. The antitumor action of GnRH analogues was presumed to result from desensitization of or decrease in GnRH receptors in the pituitary, with the consequent decline in gonadotropin secretion and gonadal hormone production. However, there are indications that a GnRH analogue directly suppresses the growth of endometrial, ovarian, breast cancer, and leiomyoma cells in vitro (48).

The observation that GnRH agonists inhibit the proliferation of cancer cells from tumors of the reproductive tract (either hormone-dependent or hormone-resistant) as well as from tumors not classically related to the reproductive system, such as melanoma, supports the following speculations: 1) GnRH agonists might exert a more direct antiproliferative action at the level of the tumor in addition to the suppression of pituitary-gonadal axis; and 2) the clinical use of GnRH analogues might be extended to nonreproductive tissue tumors that express GnRH receptors (49).

The presence of GnRH receptors in tumor cells has recently opened a new field of research based on the possibility of using GnRH analogues to carry cytotoxic agents directly to cancer cells expressing GnRH receptors (50). Conjugated compounds have been made by covalently linking GnRH agonists to cytotoxic radicals; for example, AN–152 conjugate is made from doxorubicin being linked to [D-Lys6]GnRH agonist (51). Once bound to cancer cells that express GnRH receptors, the complex is supposed to be
internalized so that the cytotoxic agent can selectively destroy the cancer cells. AN-152 has been shown to reduce the proliferation of breast, ovarian, and endometrial cancer cells, either in vitro or in vivo when xenografted into nude mice (51, 52). Rahimipour et al. (53) have synthesized conjugated compound composed of either a GnRH agonist or a GnRH antagonist linked to a photosensitizer agent (protoporphyrin IX). When tested on human breast cancer cells (MCF-7) transfected with GnRH receptors, the conjugates decrease cell proliferation. Clinical trials are needed to verify the effectiveness of GnRH analogue–targeted anticancer treatments. One of the potential limitations of the efficacy of these conjugates is the low number of GnRH receptors on tumor cells and the poor internalization of the GnRH receptors (49).

Prevention of Chemotherapy-Induced Ovarian Damage
Owing to improvements in cancer therapy, cure rates of both adult and childhood cancers have increased significantly over the past 3 decades. However, long-term consequences of cancer therapy and impact on quality of life are now being recognized. One of the major sequelae of cytotoxic chemotherapy is gonadal failure. Cytotoxic chemotherapy and/or radiotherapy are used to treat not only malignant diseases but also nonmalignant systemic conditions. Candidates who can benefit from ovarian preservation include those who are treated for childhood cancers, breast cancer, cervical cancer, benign ovarian diseases, systemic lupus erythematosus, and other autoimmune diseases, as well as patients receiving pelvic radiation, prophylactic oophorectomy, or hematopoietic stem cell transplantation. Currently the only unequivocal and clinically available option is cryopreservation of fertilized ova or embryos after IVF and before chemotherapy; all other options, such as mature and immature oocyte cryopreservation, ovarian tissue cryopreservation, in vitro oocyte maturation, and human ovarian transplantation, are still experimental and await clinical experience (54).

It has been hypothesized that ovarian suppression can be protective. Some animal studies demonstrated a protective role of GnRH analogue treatment against chemotherapy-induced gonadal damage (55, 56). Ataya et al. (55) have found that GnRH analogue protected the ovary against cyclophosphamide-induced damage in Rhesus monkeys by significantly decreasing the total amount of follicle loss during the chemotherapeutic insult and by decreasing the daily rate of follicular decline.

A few nonrandomized studies suggested a protective role for GnRH analogue treatment against ovarian damage during chemotherapy (57–60). In the most recent study by Blumenfeld et al. (61), 111 patients with Hodgkin lymphomas treated with chemotherapy were followed for 2–15 years. In the group who received a monthly injection of GnRH agonist, administered before starting chemotherapy until its conclusion, up to a maximum of 6 months, 63 out of 65 patients resumed ovulation and regular menses (96.9%), compared with 63% of the 46 control subjects.

There have been four phase II studies in premenopausal breast cancer patients which have demonstrated that GnRH agonist treatment enables the resumption of ovarian function in a high percentage of treated patients, in the range of 83%–96% (62–67). All 13 patients in one study (62), aged 26–39 years, resumed normal ovarian function after a mean of 4.9 months after chemotherapy. In another study (63), 86% of 64 patients, aged 27–50 years, resumed regular menstruation, despite a relatively advanced median age of 42 years.

The protective role of GnRH agonist has been demonstrated also in patients undergoing chemotherapy for diseases other than cancer. In a study of 40 young women with severe systemic lupus erythematosus (SLE) treated with cyclophosphamide, Somers et al. (68) found that premature ovarian failure developed in 1 of 20 women treated with GnRH agonist (5%), compared with 6 of 20 in the control group (30%) after a minimum of 3 years’ follow-up. That was the first study to analyze a large group of lupus patients with control subjects individually matched for age and cumulative dose of chemotherapy agent. That study also included add-back E2 therapy, demonstrating that the protective effect of GnRH analogue was not merely the result of a hypoestrogenic environment.

A meta-analysis that included 9 studies and 366 women concluded that GnRH agonists appear to improve ovarian function and the ability to achieve pregnancy after chemotherapy (69). The use of a GnRH agonist during chemotherapy was associated with a 68% increase in the rate of preserved ovarian function compared with women not receiving a GnRH agonist (summary risk ratio [RR] 1.68, 95% confidence interval [CI] 1.34–2.1). Among the GnRH agonist–treated women, 22% achieved pregnancy after treatment compared with 14% of women without GnRH agonist therapy (summary RR 1.65, 95% CI 1.03–2.6) (69).

Two recent randomized controlled studies also support the protective role of GnRH agonists during chemotherapy, although each study had limitations in design and data collection. Badawy et al. (70) randomized 80 patients between ages 18 and 40 years with early-stage breast cancer after surgical treatments to two groups: chemotherapy alone, and combined goserelin and chemotherapy. After up to 8 months of follow-up, 89.6% women in the study group and 33.3% in the control group resumed menses. Premature ovarian failure (POF), which was not defined in the study, occurred in 11.4% of patients in the study group and in 66.6% of the control group (70). However, several concerns were raised about the study (71, 72). The incidences of amenorrhea and POF in the control group were significantly higher than data from earlier literature. The baseline E2 and FSH levels were statistically different between the two groups, and estrogen receptor status and tamoxifen usage were not controlled. The length of follow-up was not clarified to be from the initiation or the completion of chemotherapy (71, 72).

In the randomized control study by Sverrisdottir et al. (73), ~36 months of follow-up was available for 94 of 123 premenopausal breast cancer patients treated with chemotherapy who were randomized, in a 2 × 2 factorial design, to goserelin or no goserelin and to tamoxifen or no tamoxifen, each for 2 years. One year after completed therapy, 20% of goserelin-treated patients and 12% of patients not receiving goserelin resumed menstrual cycles (74). The study was underpowered to firmly conclude the gonadal protective effect of goserelin. The study protocol also called for administration of the first goserelin dose on the same day as the first chemotherapy dose, which may have attenuated the protective benefit of goserelin (74).

Only one small prospective randomized study demonstrated that GnRH analogue treatment was not effective in preserving fertility in patients receiving chemotherapy for Hodgkin disease (75). In that study, 30 men and 18 women were randomly allocated to receive GnRH analogue before and for the duration of cytotoxic chemotherapy. Twenty men and eight women received buserelin. After 3 years of follow-up, all of the men in both the study and the control groups became oligo/azoospermic. Among the women, four of eight in the treatment and six of nine control subjects became amenorrhoeic.

A few ongoing phase III randomized controlled trials (76–78) will probably be able to unequivocally answer the question regarding the role of GnRH agonists as a possible effective adjunct to minimize chemotherapy-associated gonadotoxicity in
young premenopausal patients. Ovarian protection may enable the preservation of future fertility in survivors and, in addition, prevent the bone demineralization and osteoporosis associated with hypoestrogenism and ovarian failure.

The mechanism of the possible protective effect of GnRH agonist is unclear. Because murine ovaries contain GnRH receptor sites and luteinized immature granulosa cell lines express GnRH receptors (79), the protective ovarian effects of GnRH agonists have been suggested to be mediated by the direct effects of the GnRH agonist on the ovarian follicles. However, presence of GnRH receptors on human primordial follicles or oocytes has yet to be demonstrated. Fister et al. (80) showed that after knock-down of GnRH receptor expression, doxorubicin-induced apoptosis in human endometrial and ovarian cancers and in the human breast cancer cell line MCF-7 was increased. These data demonstrated that peripheral GnRH receptor activation suppressed chemotherapeutic drug-induced apoptosis in these cancers. Imai et al. (81) studied the E2 productivity of GnRH receptor–positive granulosa cells during simultaneous exposure to doxorubicin and GnRH agonist in culture, and demonstrated that a GnRH agonist may retard doxorubicin-induced granulosa cell damage, suggesting that the ability of GnRH agonist to protect the gonads during chemotherapy may be through local GnRH receptor–mediated mechanism(s).

Several other possible mechanisms have been suggested. GnRH agonists may preferentially steer cells into cell cycle arrest, i.e., resting G0/G1 phase, which decreases the number of the most vulnerable primordial follicles (82). The hypogonadotropic state induced by GnRH agonist to protect the gonads during chemotherapy may be through local GnRH–GnRH receptors may serve as regulators of hCG secretion. Although the reasons for the discrepancies are unknown, it is possible that they are due to differences in species and/or dosing regimens (85).

**SUMMARY**

GnRH receptors have been found in a wide variety of normal and human reproductive tissues. It has been indicated that GnRH in peripheral tissues functions as an autocrine-paracrine regulator by activating peripheral GnRH receptor and involving special signaling pathway. The ubiquity of GnRH–GnRH receptor system in human tissues implies that this system may have some important physiologic functions. Studies have indicated that peripheral GnRH–GnRH receptors may serve as regulators of hCG synthesis and implantation and play crucial roles in antiproliferation and apoptosis. Currently, GnRH agonists have been used in cancer treatment and ovary protection during chemotherapy, taking advantage of the local direct effect mediated by peripheral GnRH receptors. Further research to clarify functions of these peripheral GnRH receptors may lead to discovery of new therapeutic options.

**REFERENCES**


77. National Cancer Institute. Triptorelin in preventing early menopause in premenopausal women who are receiving chemotherapy for stage I, stage II, or stage III breast cancer that has been removed by surgery. Available at: http://www.clinicaltrials.gov/ct/show/NCT00311636.


