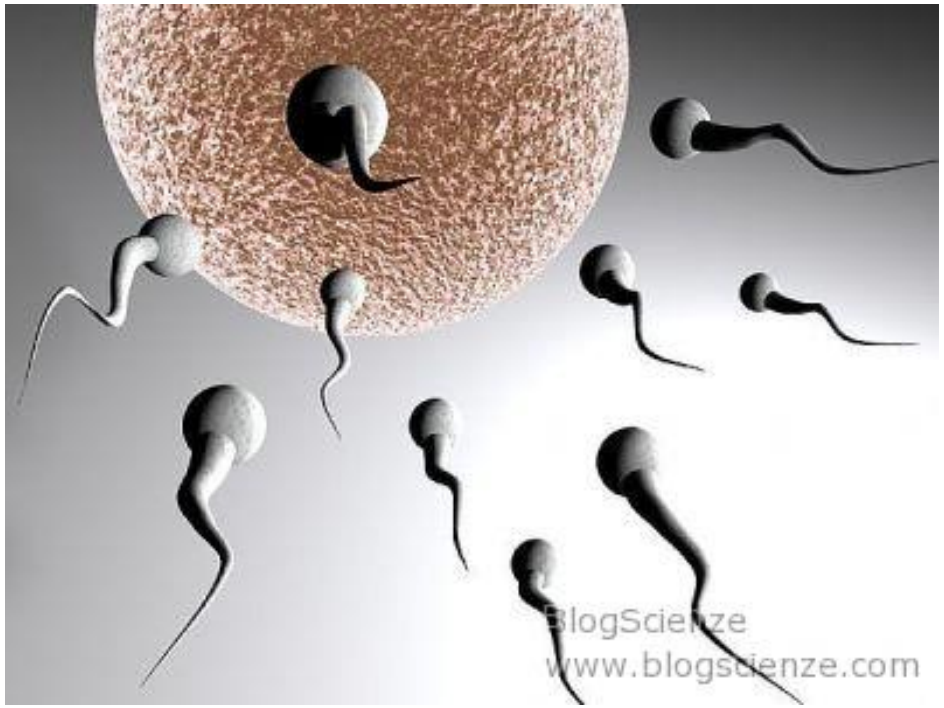
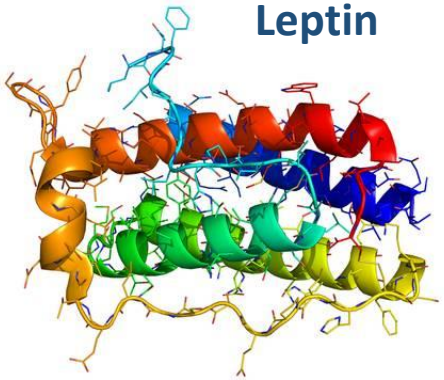
Costanzo Moretti
Reproductive Endocrinology Fatebenefratelli Hospital
San Giovanni Calibita, Tiber Island Rome

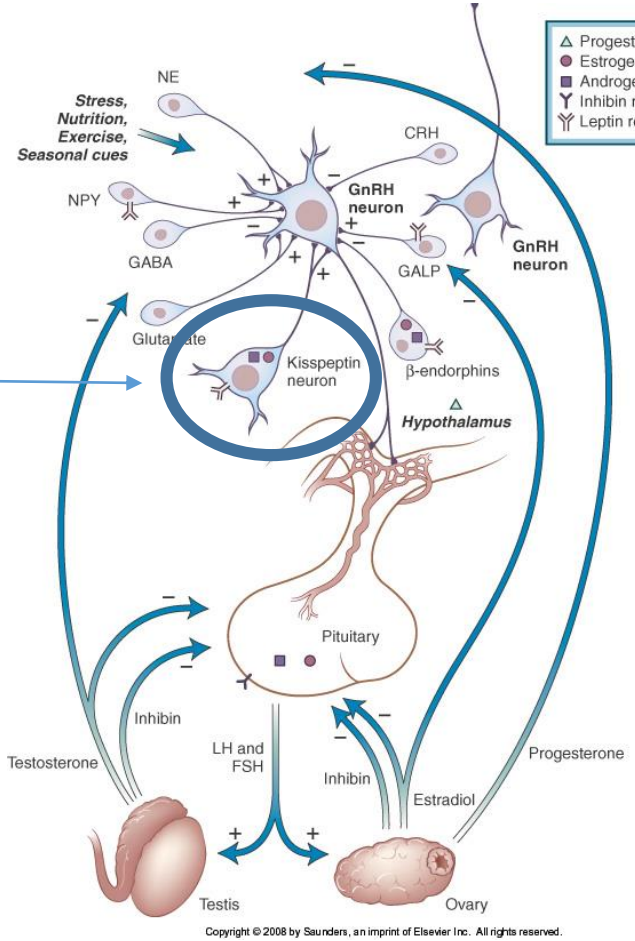
Some intriguing aspects of the female reproductive system



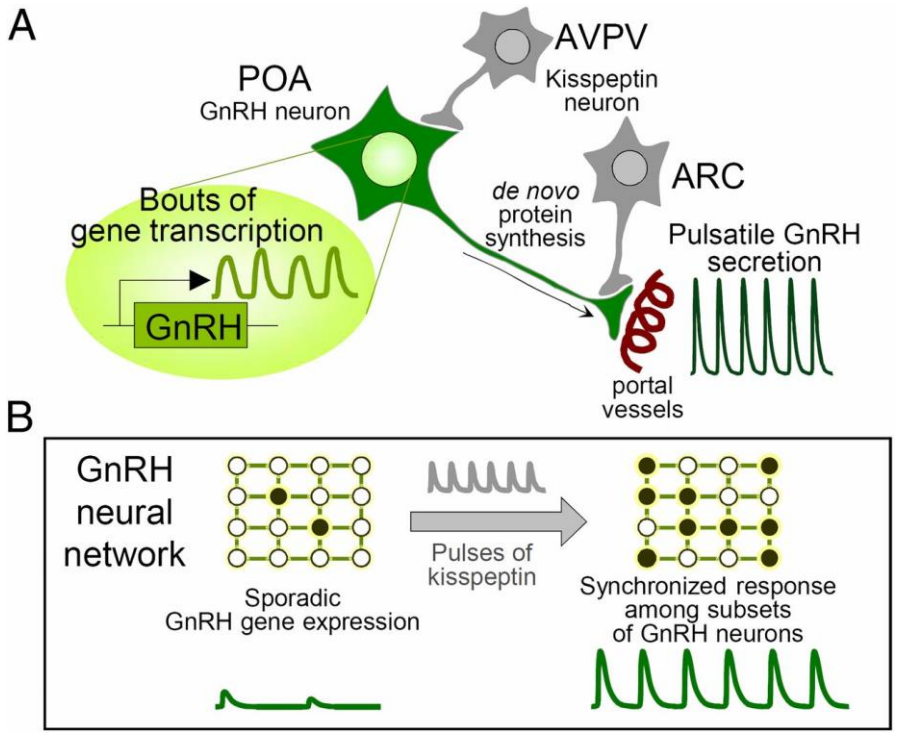
Successful reproduction is essential to the survival of a species. The reproductive system represents a highly-complex functional organization of diverse tissues and signaling pathways that, when properly functioning, ensures a number of key endpoints, the most important of which are the adequate production of gametes (ova and sperm); successful delivery of gametes for fertilization; and, in women, physiological preparation for possible pregnancy.

Leptin





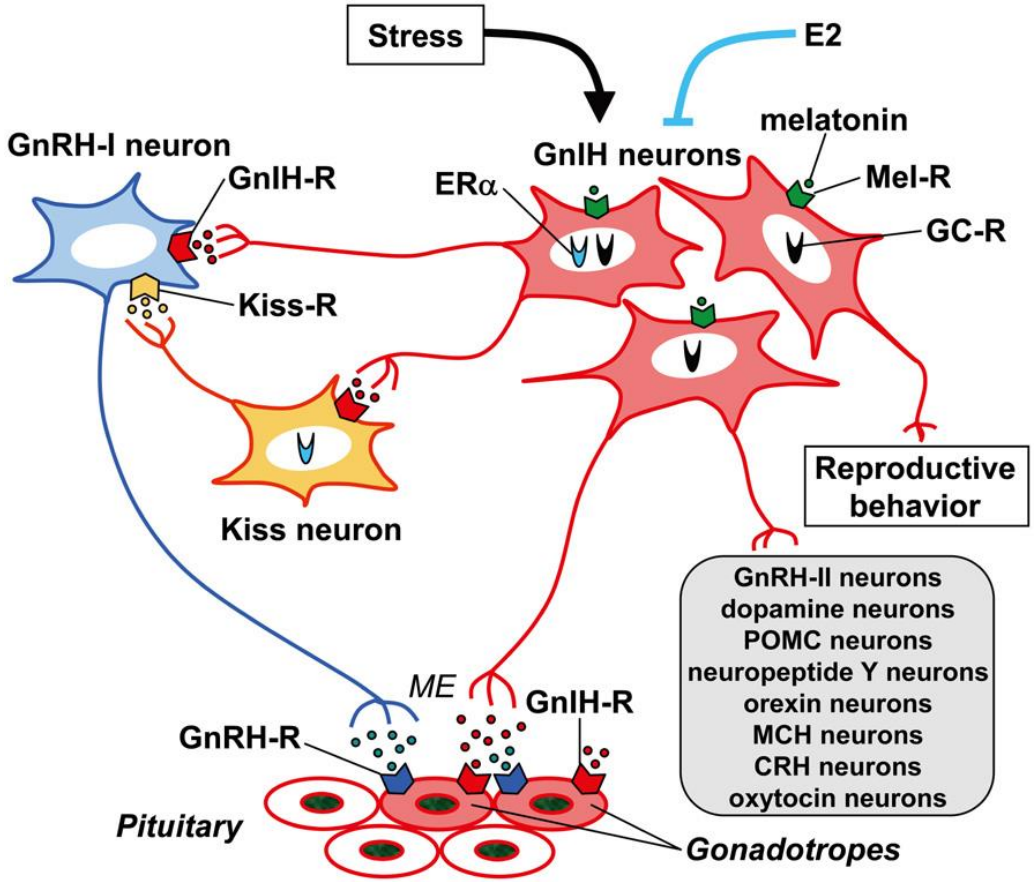
Pulsatile mode of GnRH secretion



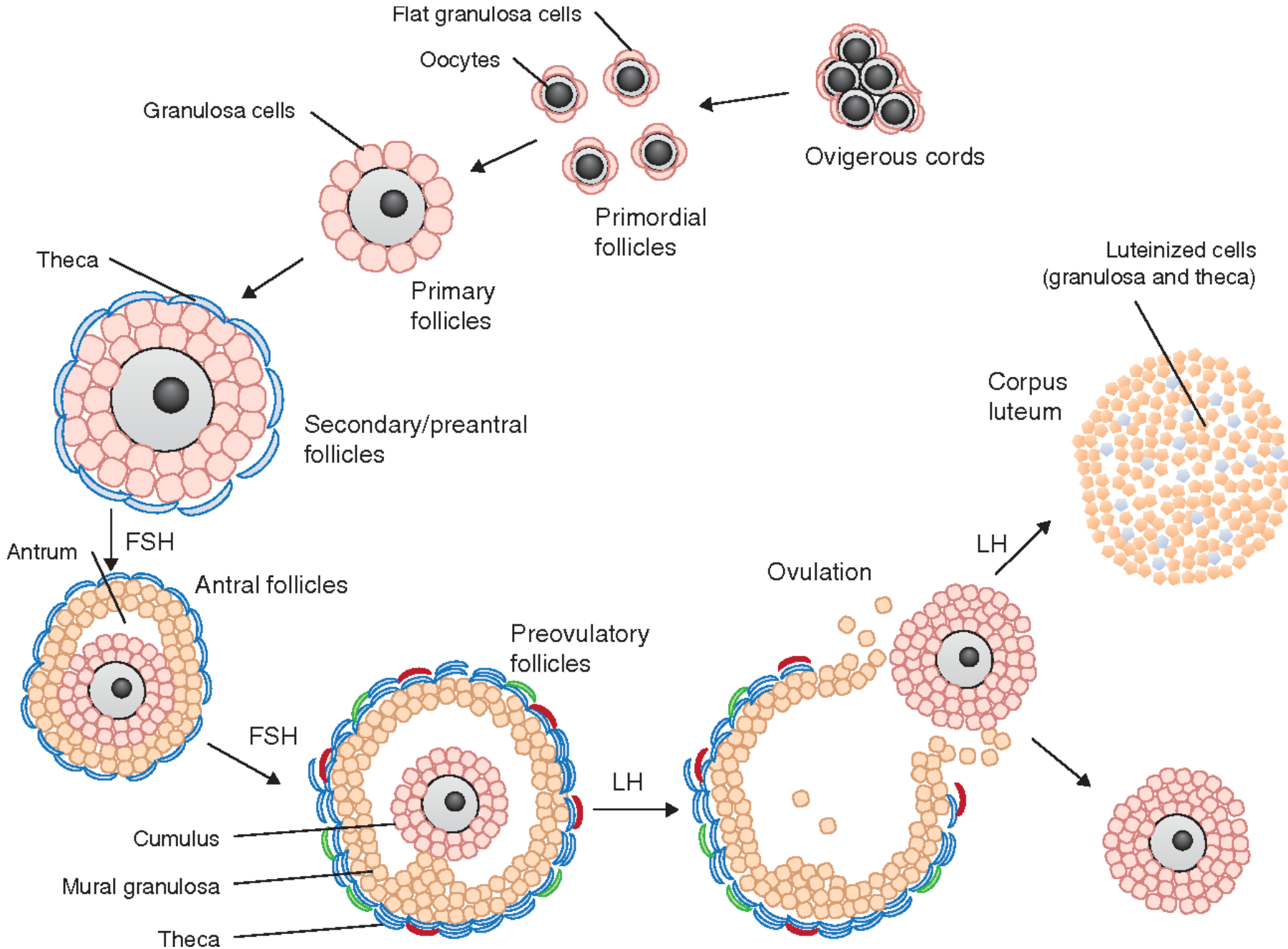
Neuroendocrine systems are the principal drivers of reproductive function in both men and women. In particular, hypothalamic gonadotropin-releasing hormone (GnRH) is the primary, if not exclusive, feedforward signal to gonadotrope cells of the anterior pituitary, stimulating the synthesis and secretion of both luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Together, these two gonadotropins direct the primary functions of the reproductive axis: gametogenesis and gonadal sex steroid synthesis.

Intermittent GnRH receptor stimulation is an absolute requirement for physiological maintenance of gonadotropin secretion

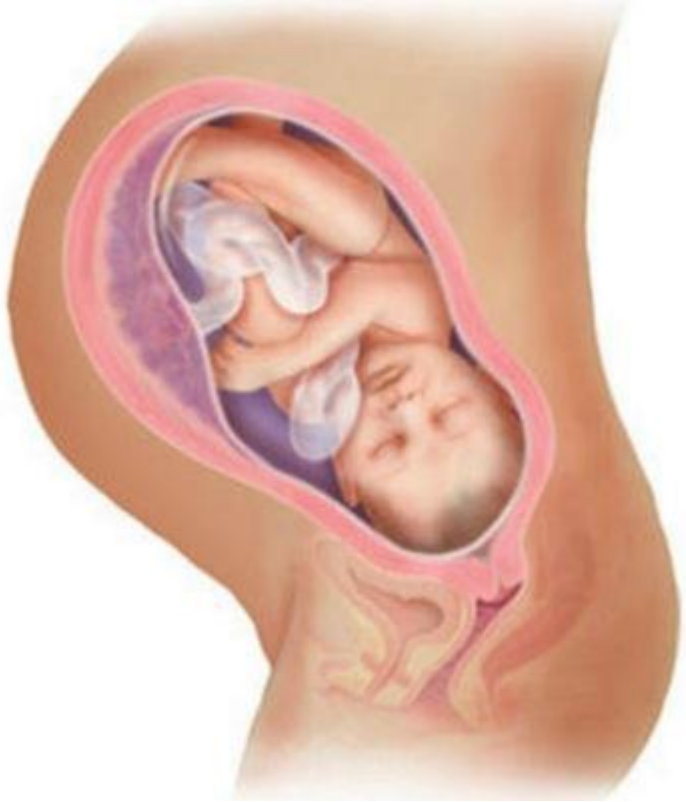
Human mediobasal hypothalamus secretes GnRH pulses every 60 to 100 minutes

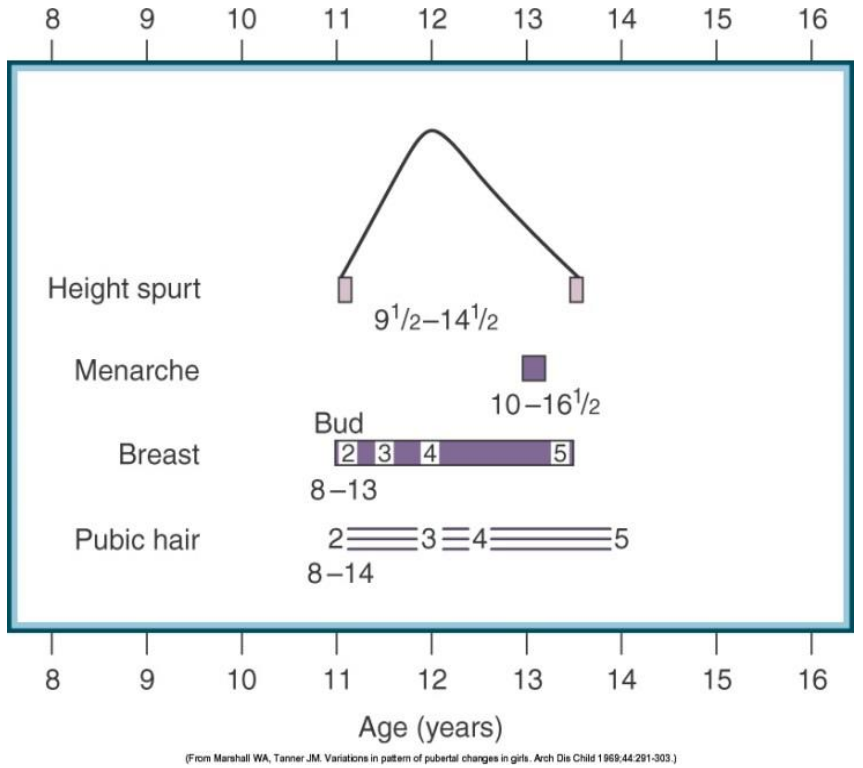


LH pulse amplitude changes across the menstrual cycle. LH pulse amplitude decreases slightly across much of the follicular phase, but it is greatly amplified at the mid-cycle LH surge. During the luteal phase, LH pulse amplitude is variable, but in general approximately twofold higher than that of the follicular phase

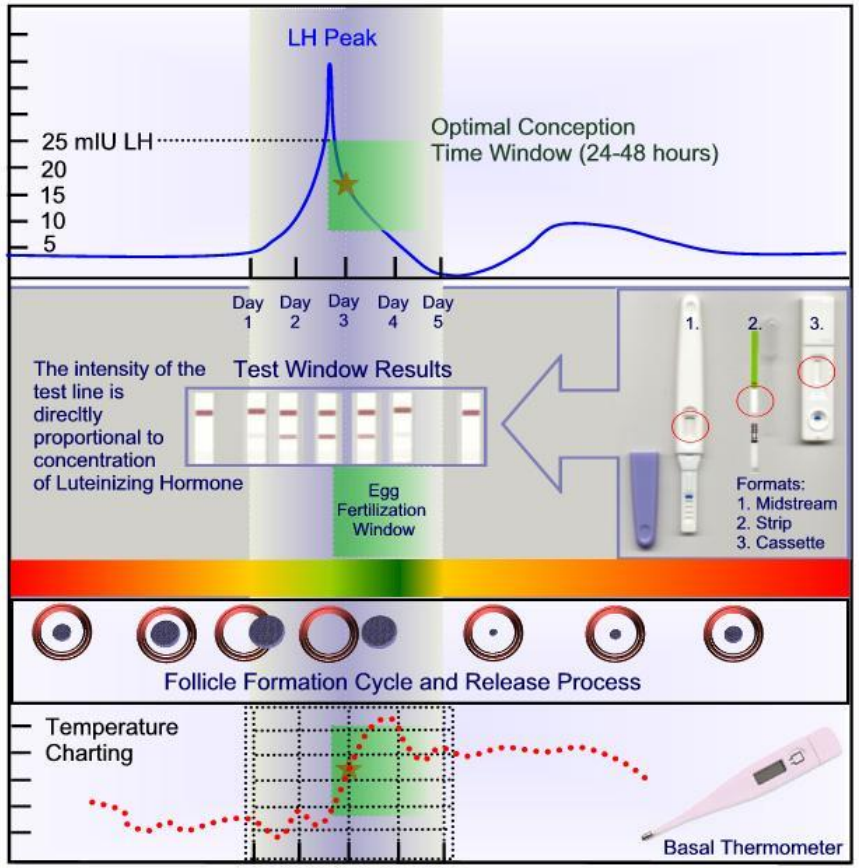

Flat granulosa cells
Oocytes
Granulosa cells
Ovigerous cords
Primordial follicles
Primary follicles
Theca
Secondary/preantral follicles
Antrum
FSH
Antral follicles
Luteinized cells (granulosa and theca)
Corpus luteum
LH
Ovulation
Preovulatory follicles
FSH
LH
Cumulus
Mural granulosa
Theca

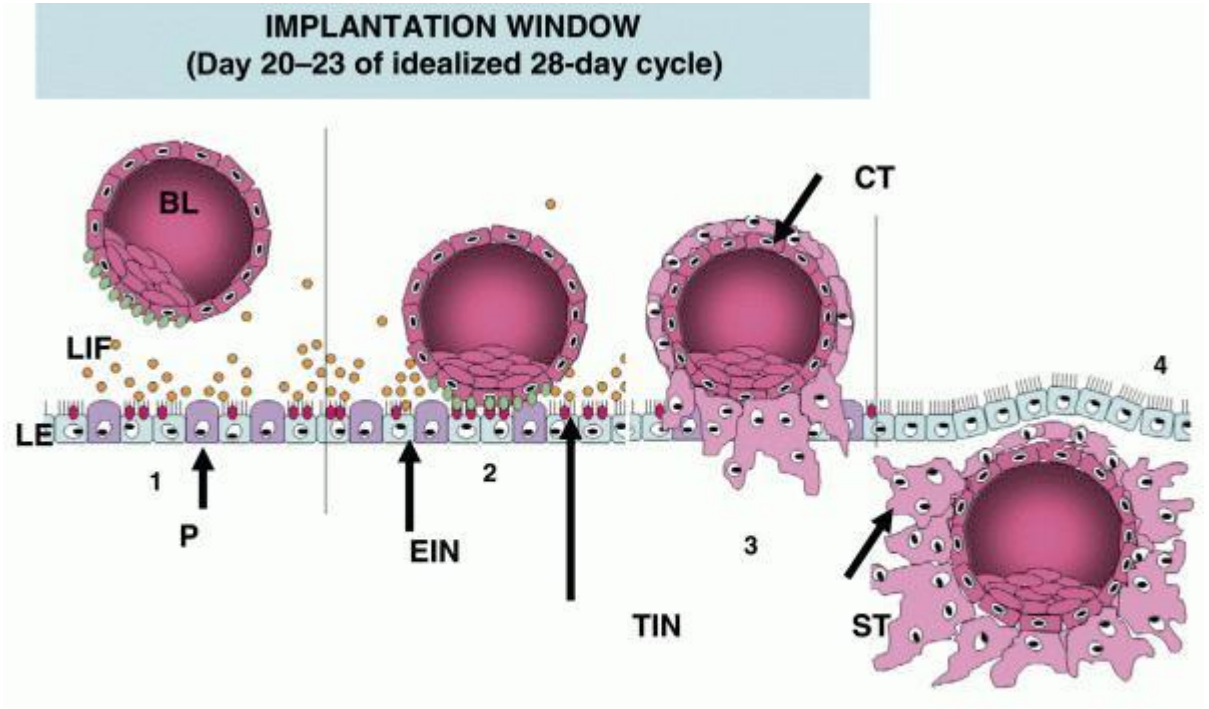
In women, dynamic changes of gonadotropin secretion are required to achieve follicular development, ovulation, and preparation for possible pregnancy.





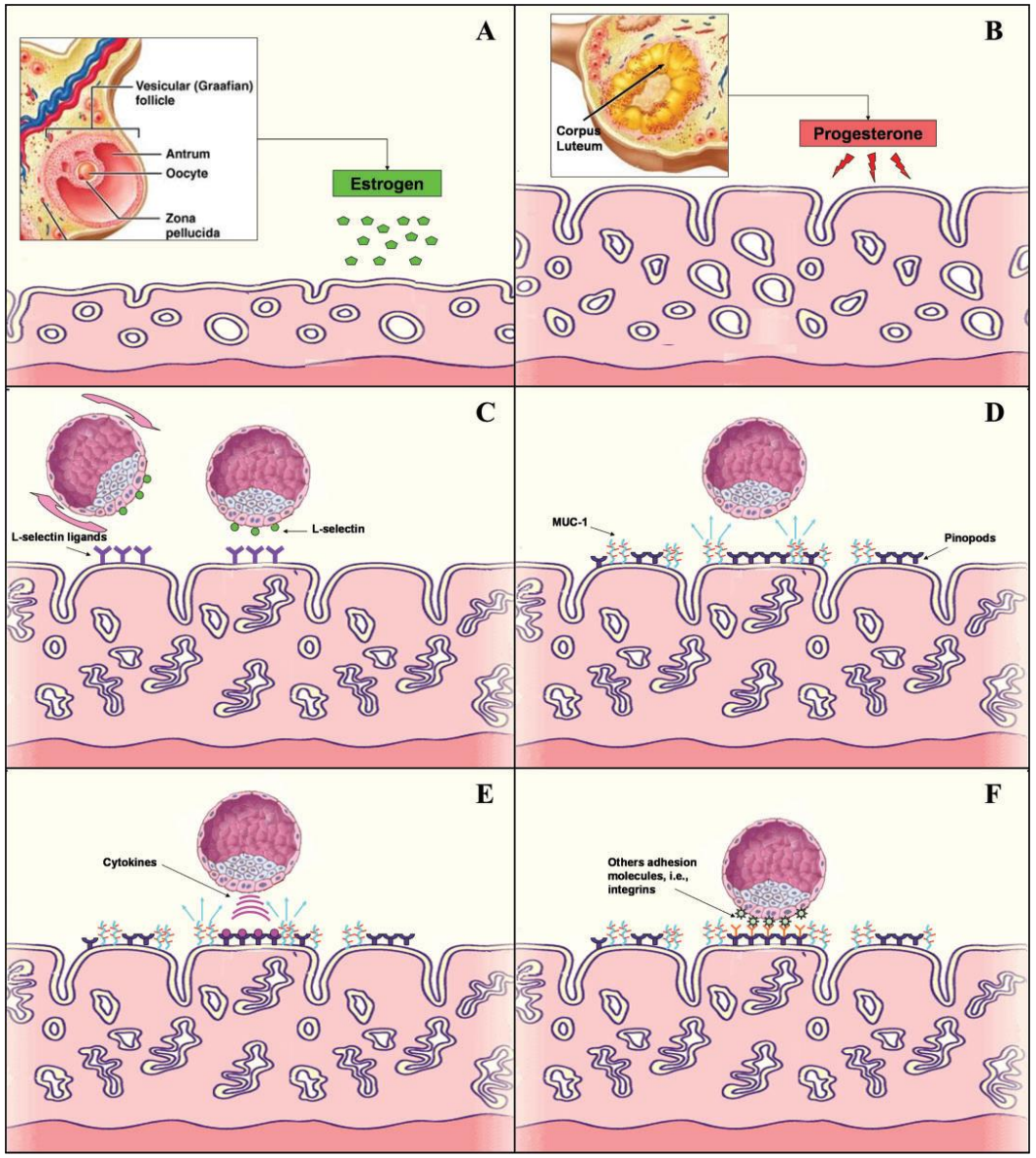
The gestational increase of sex steroid (e.g., estradiol) production from the fetoplacental unit provides negative feedback to limit fetal GnRH and gonadotropin secretion. Birth is followed by a marked but transient (3 to 9 month) increase of GnRH and gonadotropin secretion (the "mini-puberty of infancy"), perhaps related to the withdrawal of fetoplacental sex steroids. A marked sex difference of gonadotropin release is evident at this time, with LH concentrations being higher in males and FSH levels higher in females.





Markert U et al. Journal für Reproduktionsmedizin und Endokrinologie - Journal of Reproductive Medicine and Endocrinology 2007; 4 (6): 322-330 ©

High estradiol concentrations from the dominant ovarian follicle can produce a marked increase in gonadotropin release—a midcycle (or preovulatory) gonadotropin surge. In effect, estradiol from the preovulatory follicle signals to the hypothalamo-pituitary unit that follicular development is adequate for ovulation. Estradiol positive feedback appears to be related to both achieved estradiol concentrations and the duration of estradiol elevation

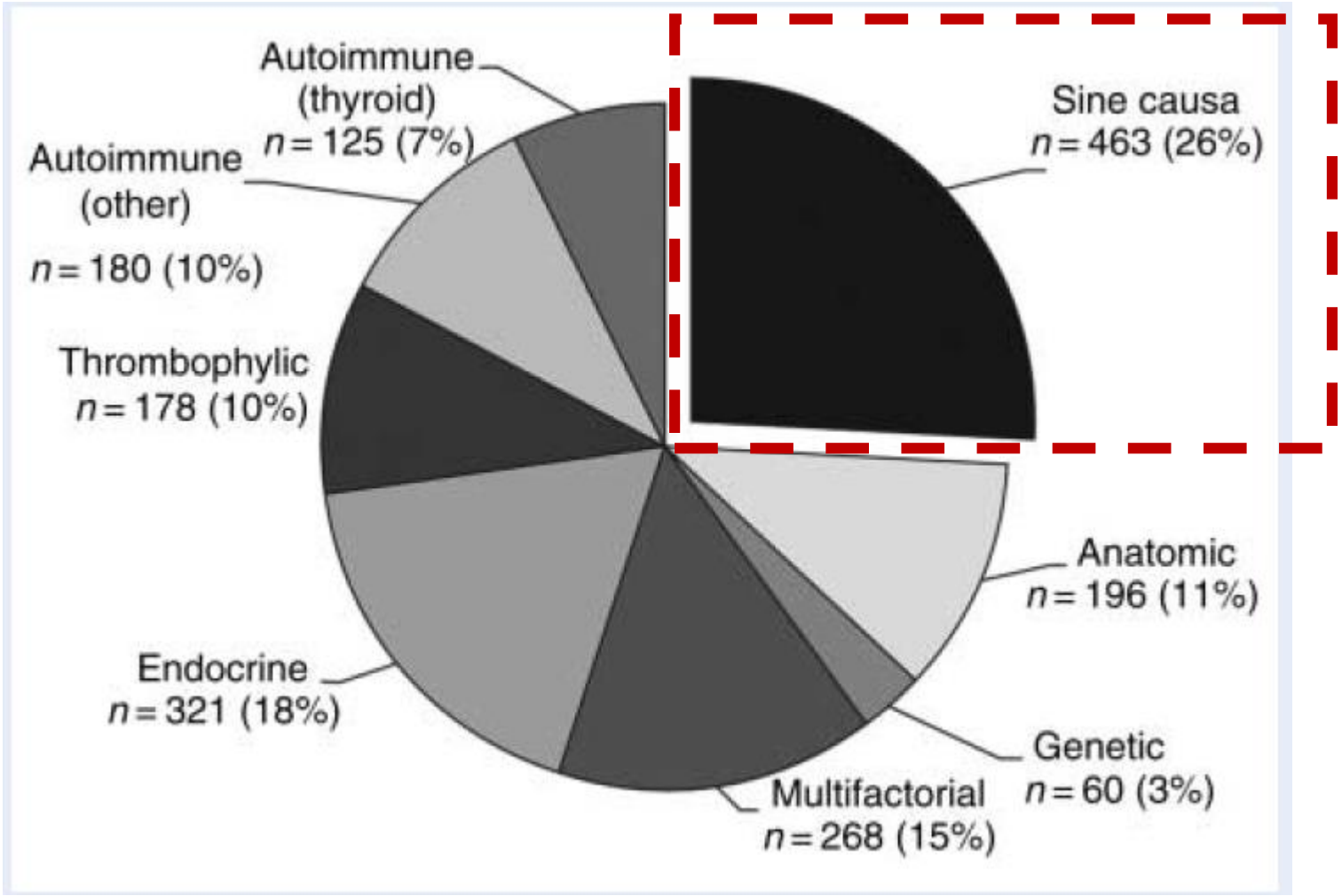


HyperModules algorithm for embryo-endometrium interaction network during pre- and post implantation

- **PREPARATION**
  Proliferation
  Differentiation
- **ROLLING**
- **REPELLING**
- **ATTRACTION**
- **ATTACH**

Achache H e Revel A *Human Reproduction Update* 12(6):731-746,2006

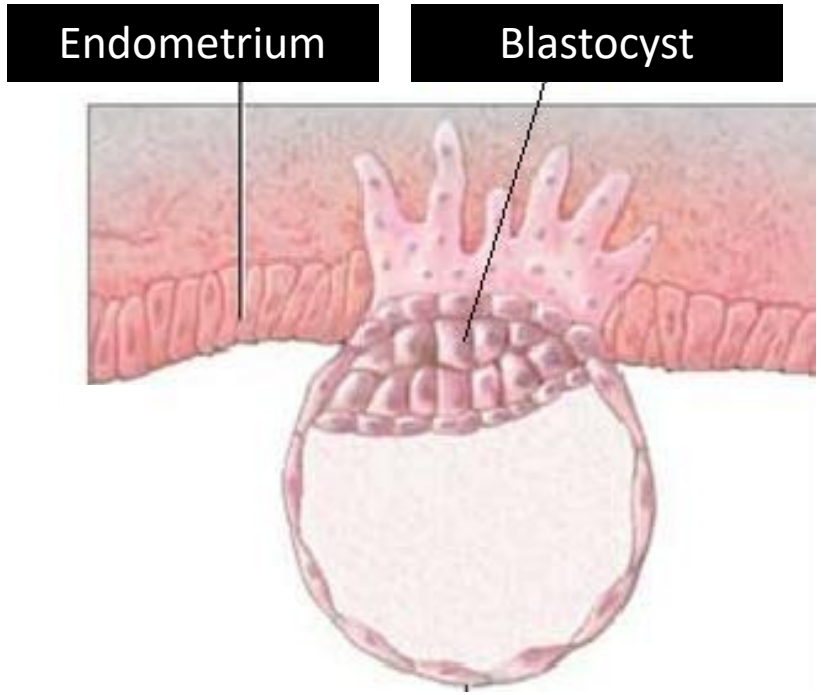
Frequency of the most important etiological factors involved in the early recurrent miscarriage

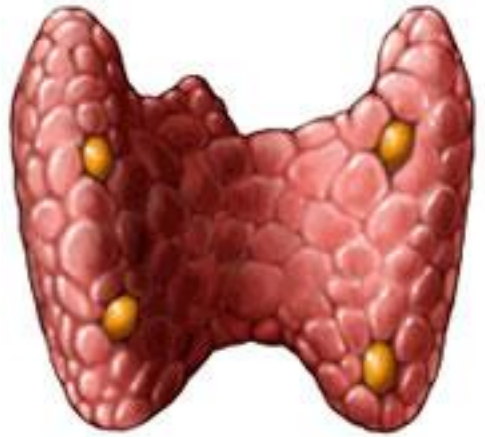


Dal Lago A, Moretti C et al *Human Reproduction* 2011

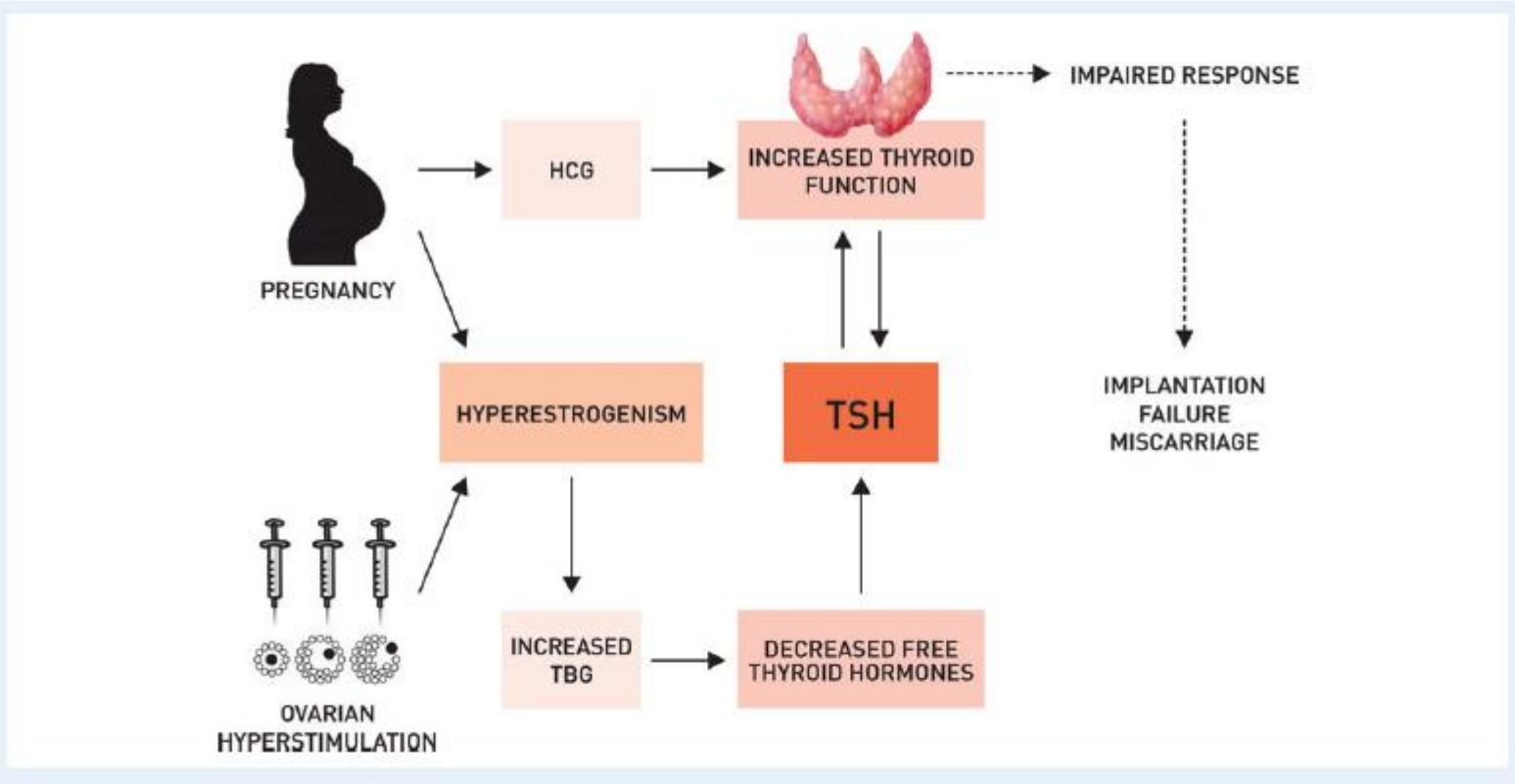
Can thyroid function influence the implantation and the early progression of pregnancy?

Contact between maternal tissue and fetal interface before invasion of extravillous trophoblast in maternal spiral arteries





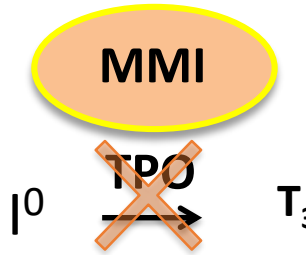
Correlations between pregnancy, ovarian hyperstimulation and thyroid function



Colicchia M et al. Human Reproduction Update 20 (6):884, 2014

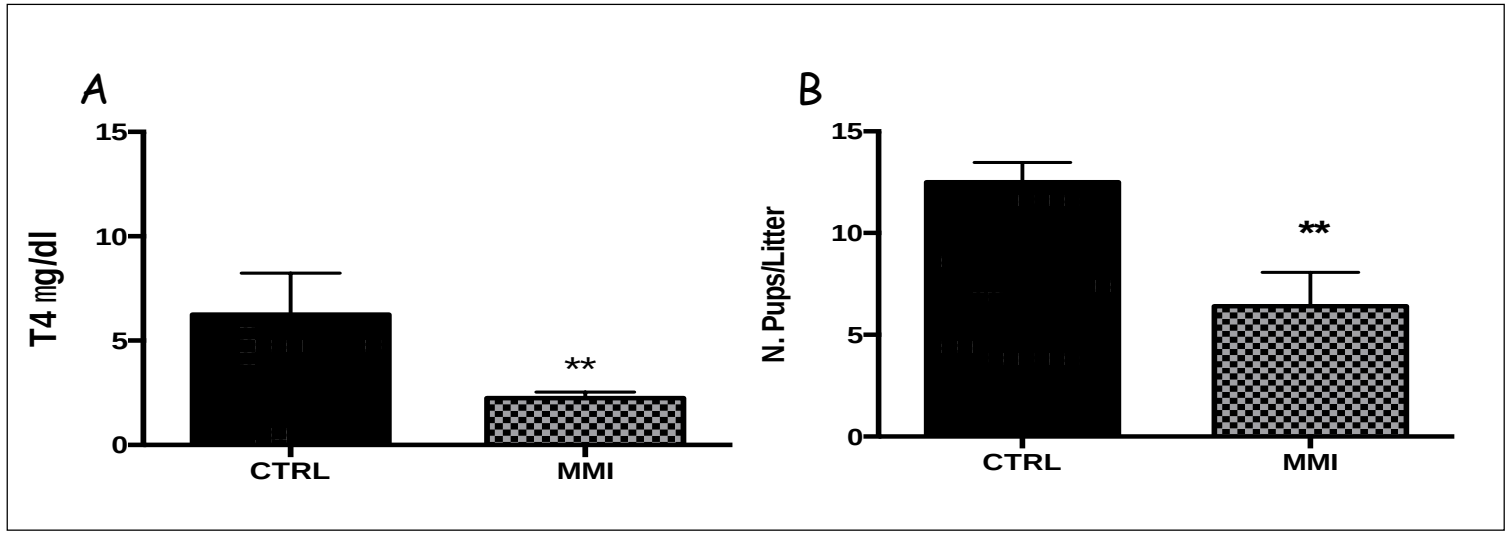
In vivo studies in the mouse model:

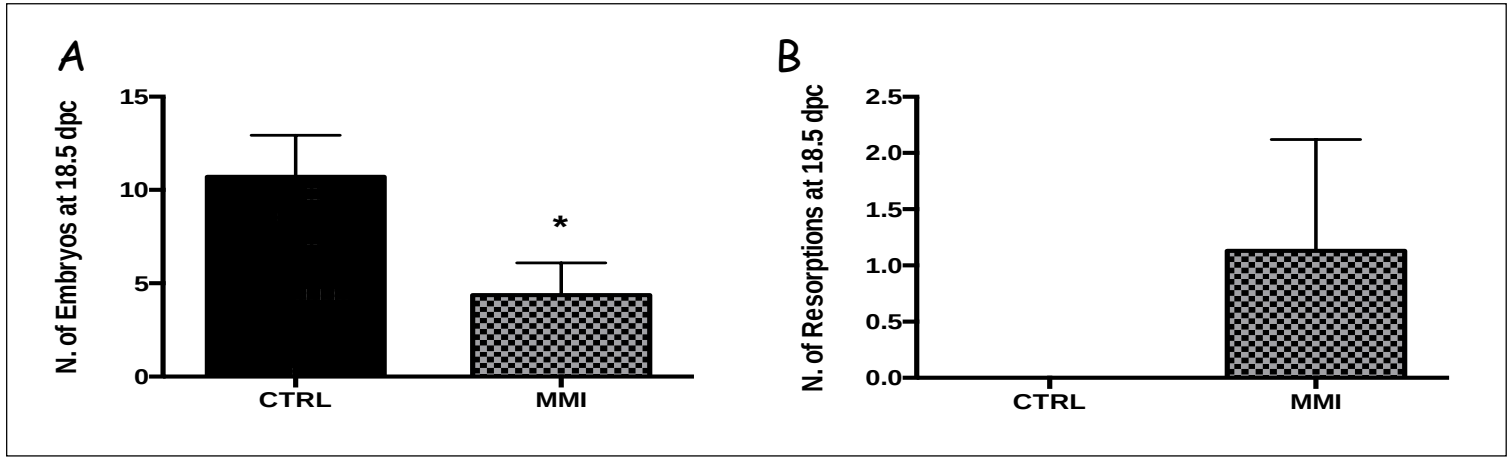
Hypothyroidism Induction with Methimazole (MMI) Treatment 0.02%



CTRL	MMI
Water	8 weeks treatment 0.2% MMI

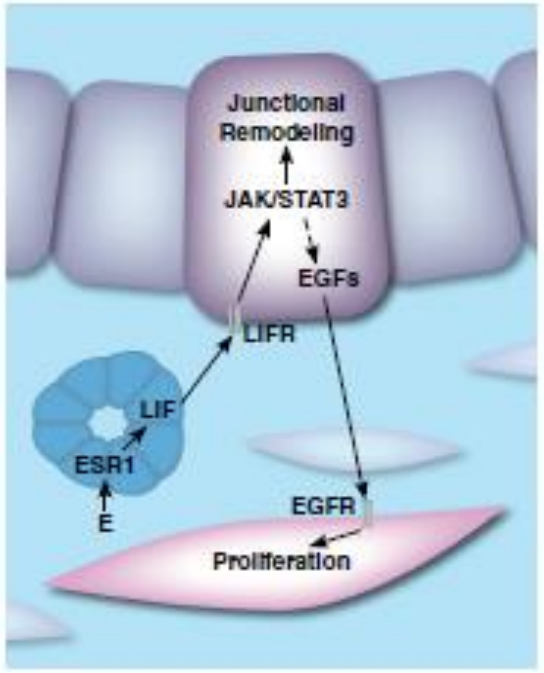
Picirilli D, Campagnolo L, Moretti C 2016


A
T4 mg/dl
15
10
5
0
**
CTRL
MMI
B
N. Pups/Litter
15
10
5
0
**
CTRL
MMI

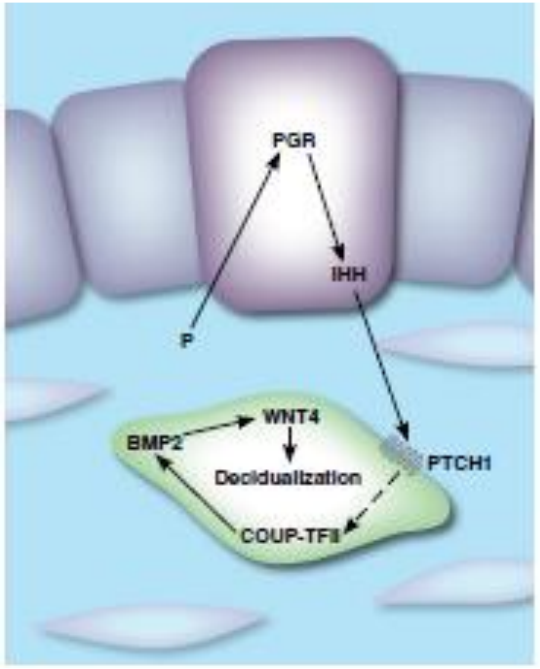

A
N. of Embryos at 18.5 dpc
15
10
5
0
*
CTRL
MMI
B
N. of Resorptions at 18.5 dpc
2.5
2.0
1.5
1.0
0.5
0.0
CTRL
MMI

Implantation involves a huge series of endocrine modulators, paracrine and iuxotacrine, depending on cellular and molecular cross talk between the blastocyst and the selected endometrium

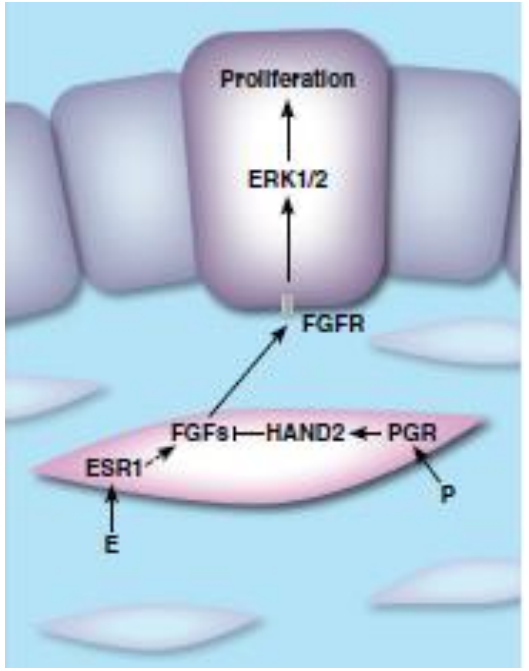
Steroid induced cross talk epithilium-stroma



Steroid-induced paracrine signals

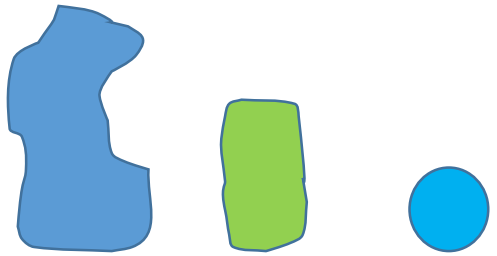


Rule of the stroma

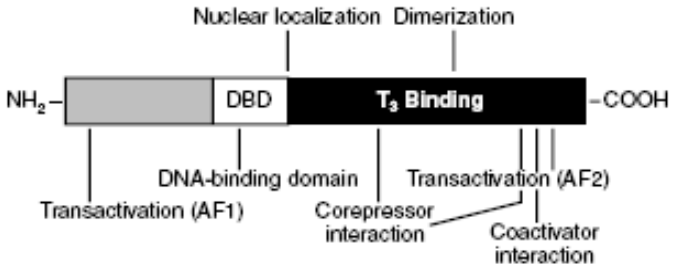


Pawar et al. Epithelial-Stromal Dialogue in Implantation Mol Endocrinol, 2014, 28(9):1408–1422

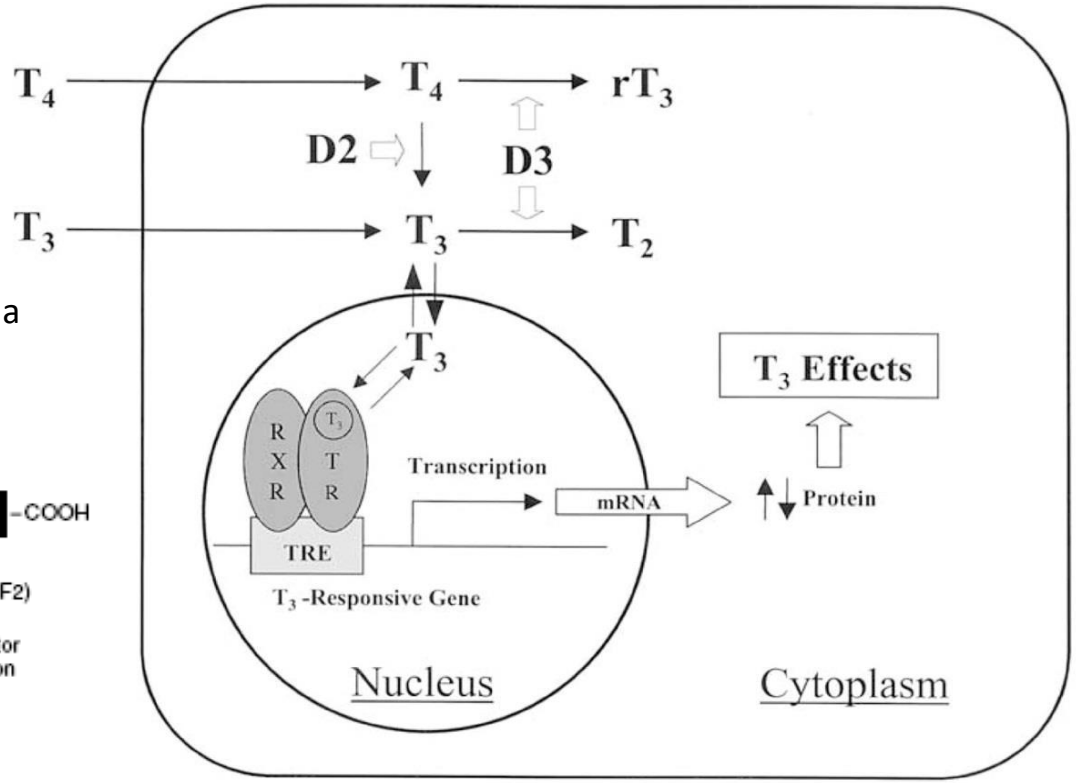
Plasma carriers of thyroid hormones and their action at the level of target cells



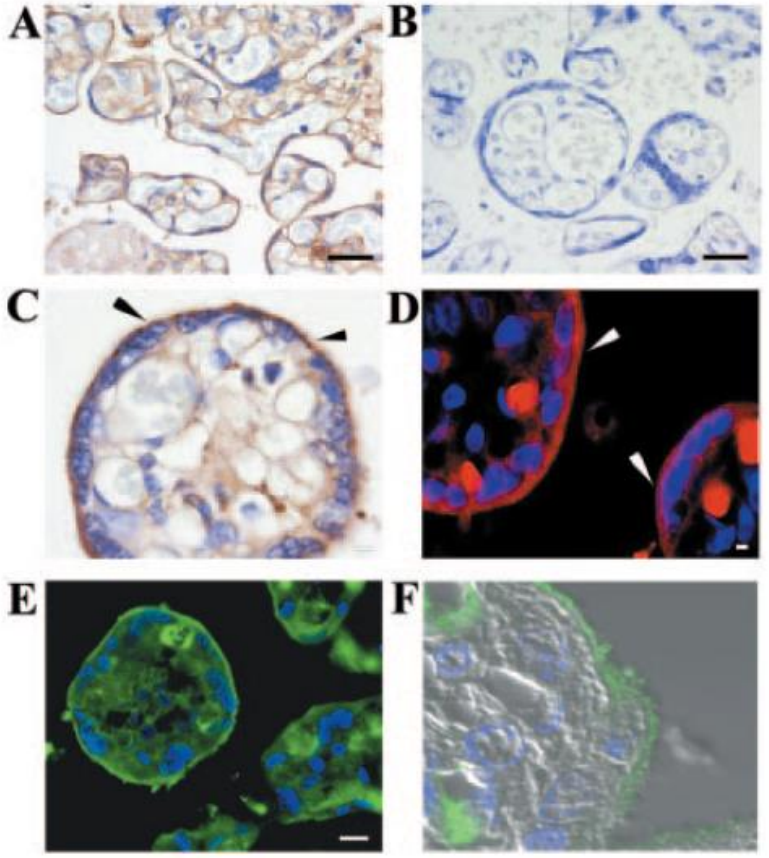
TBG Transtiretina Albumina



Thyroid receptor

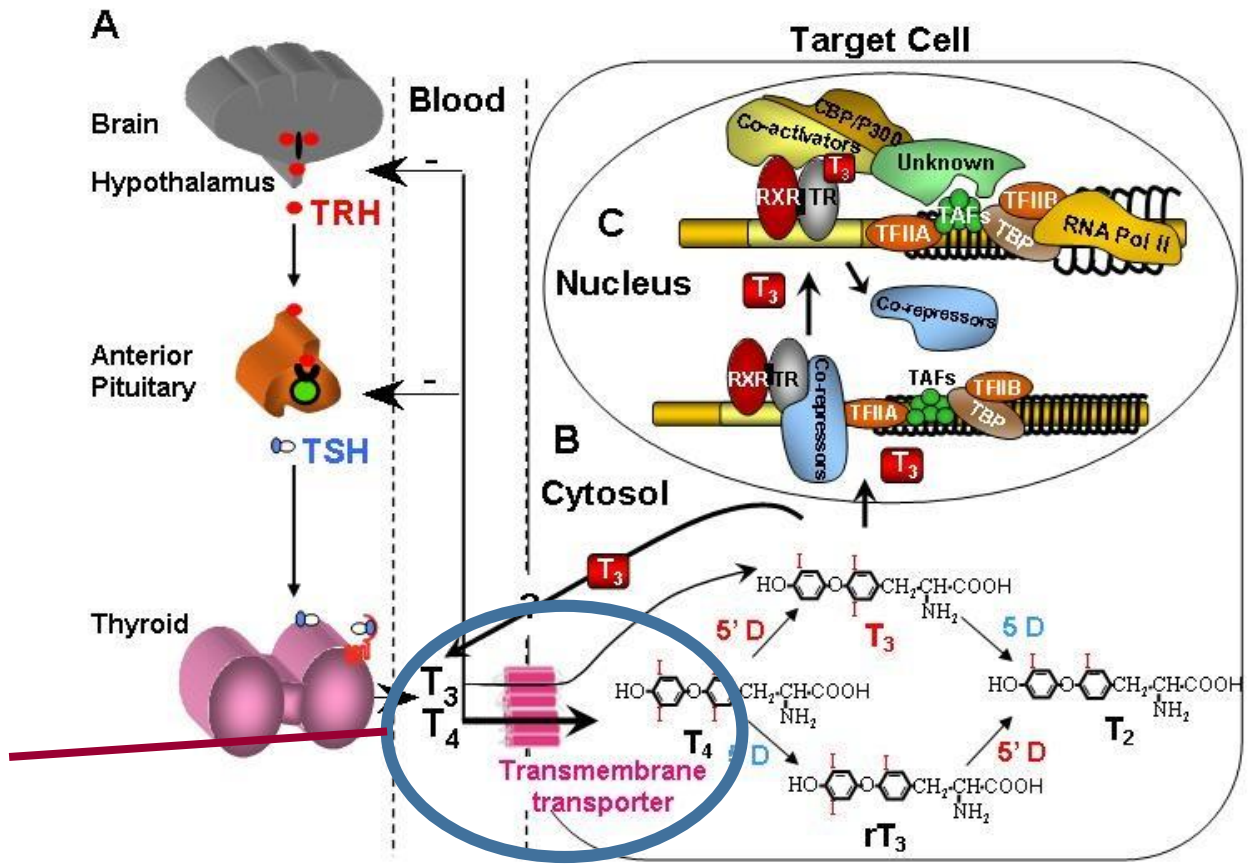


Immunohistochemistry and immunofluorescence of transthyretin and albumin in human placenta



McKinnonet al. JCE M 2005

Thyroid hormones transmembrane transporters



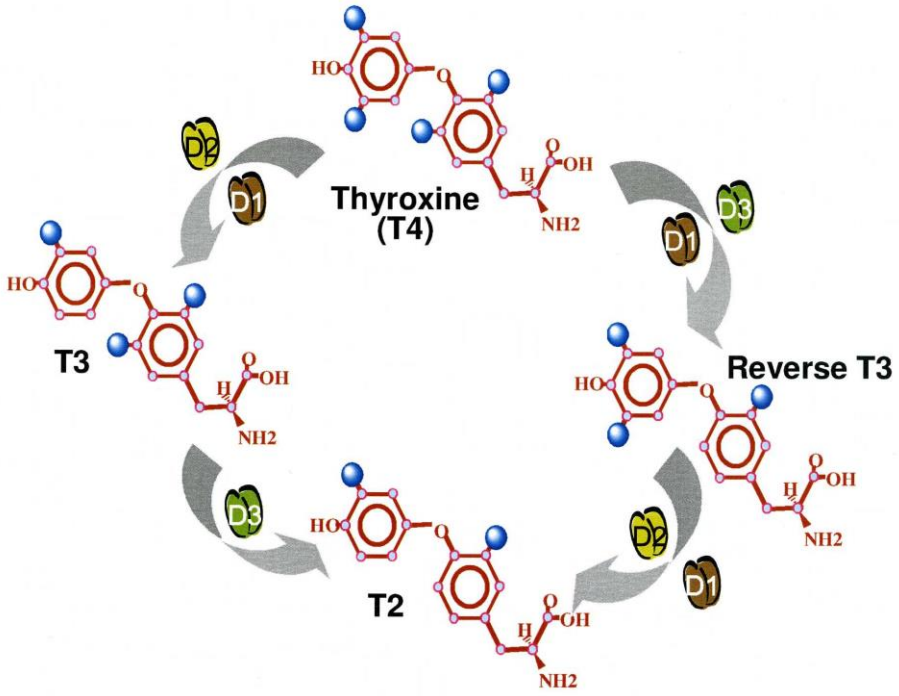
MCT
LAT
Oatp

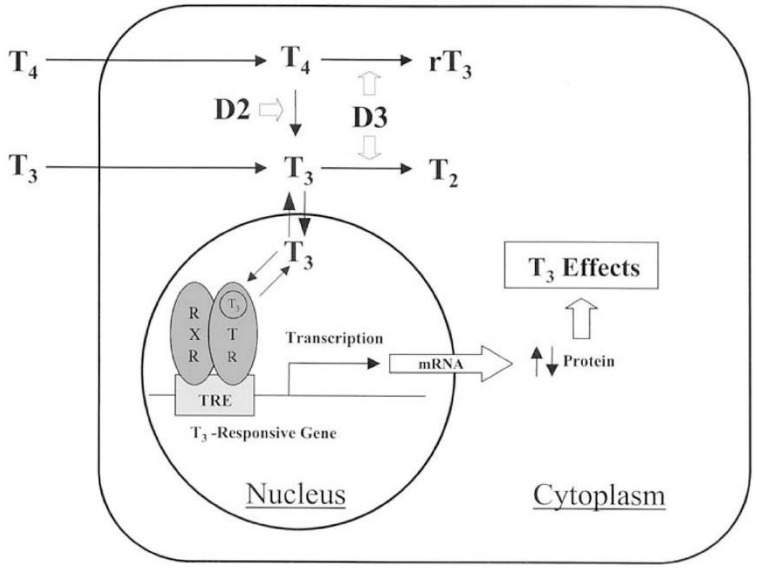
TH trasporters expressed at the placental level

MCT8 : monocarboxylated
MCT10
LAT1 : heterodimeric L-type amino acid
LAT2
Oatp1 : Organic anion transporting polypeptides
Oatp4

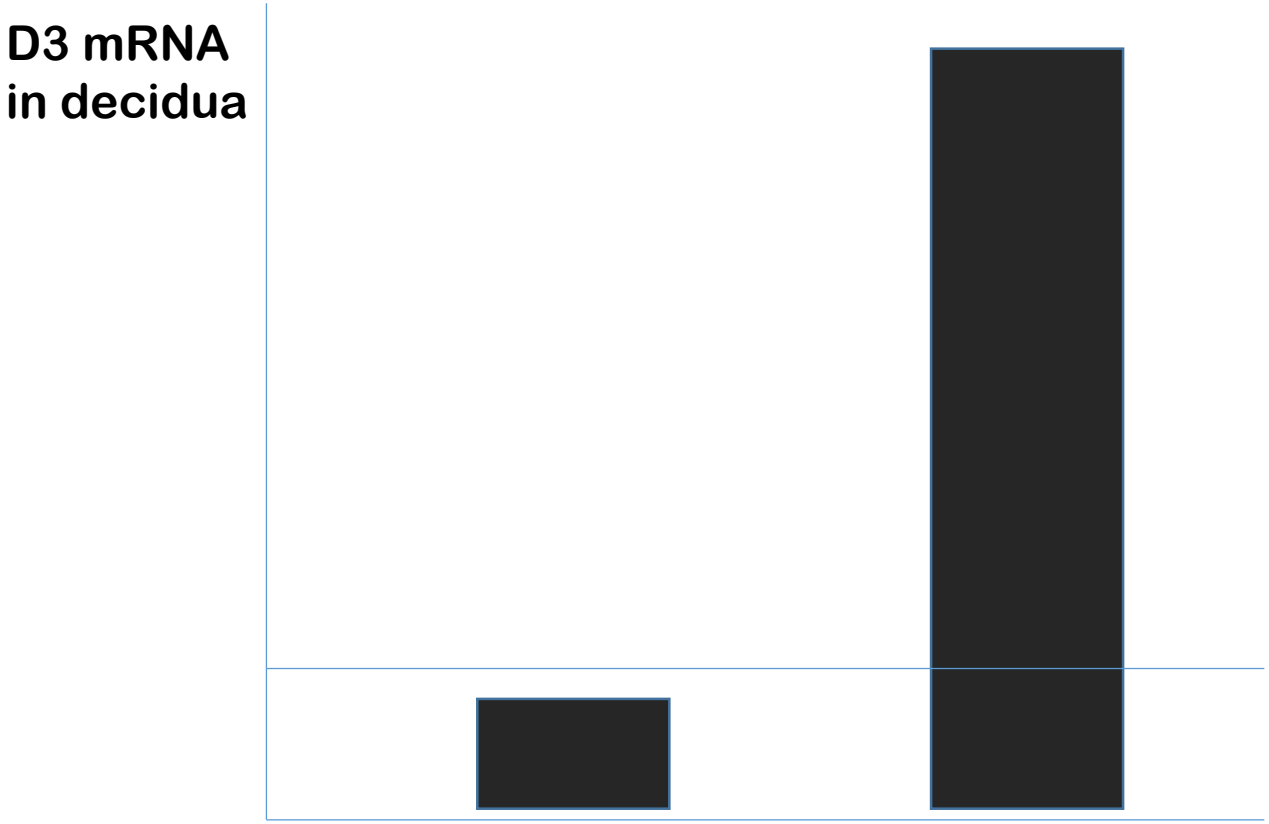
Van Herk SL et al. Gen Comp Endocrinol 2015 Apr 1;214:30-9

DEIODINASES



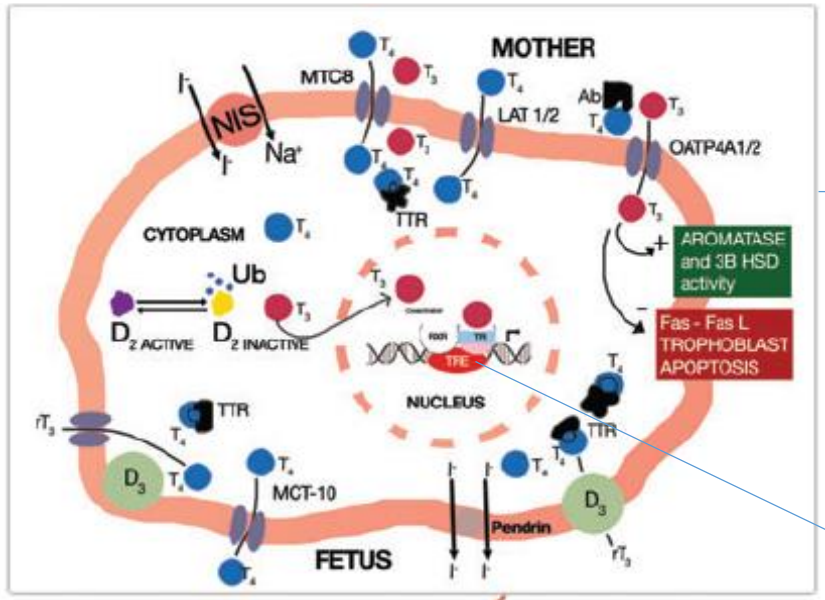


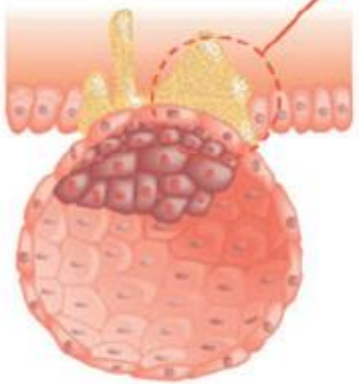
Bianco e Kim The Journal of Clinical Investigation 2006

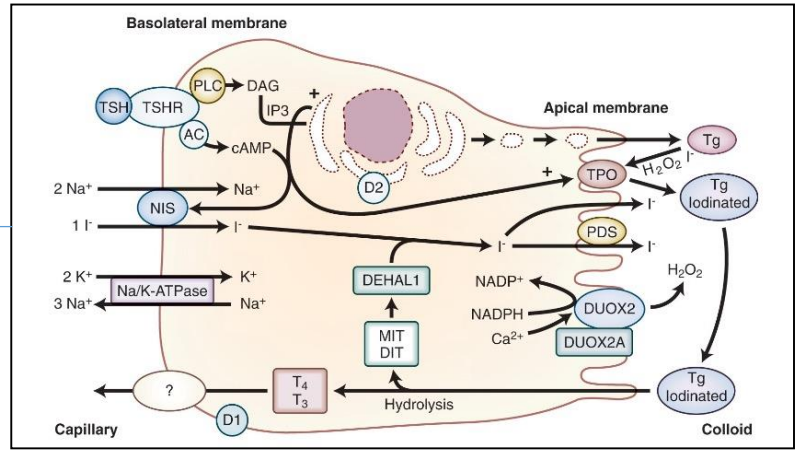


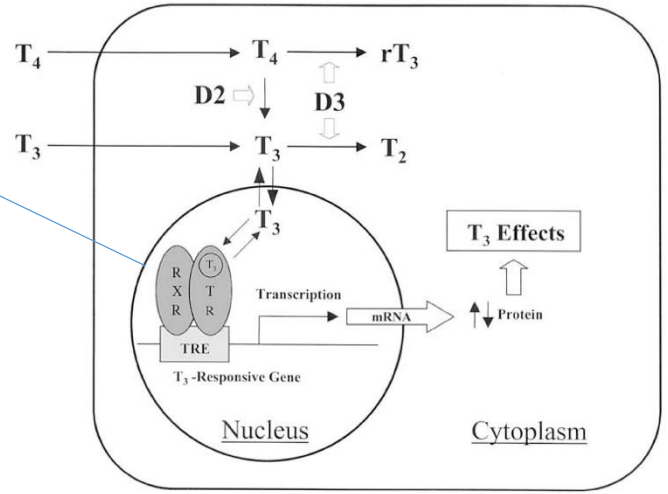
Huang SA Thyroid 2005

TH expression beyond the window of implantation





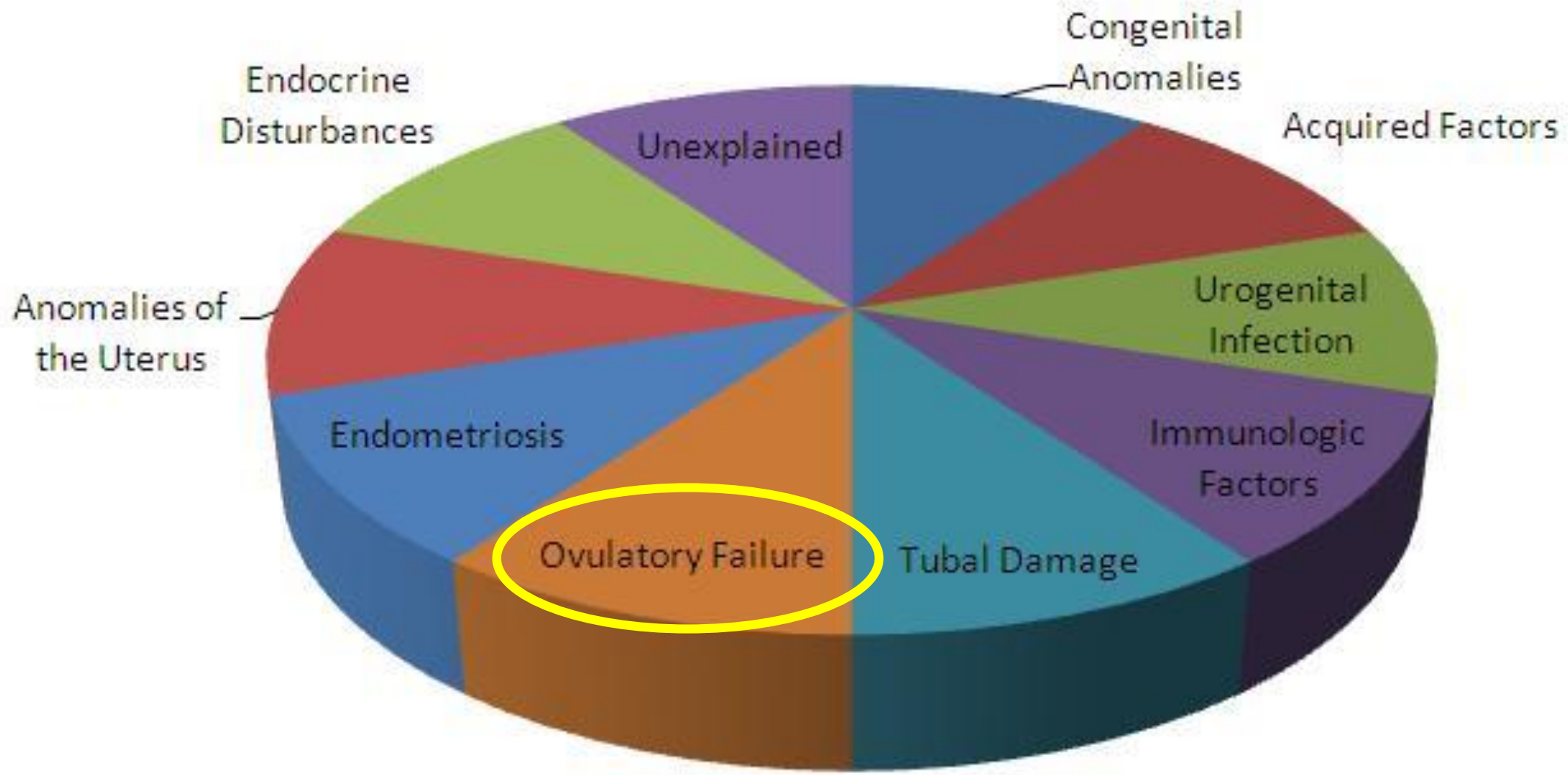




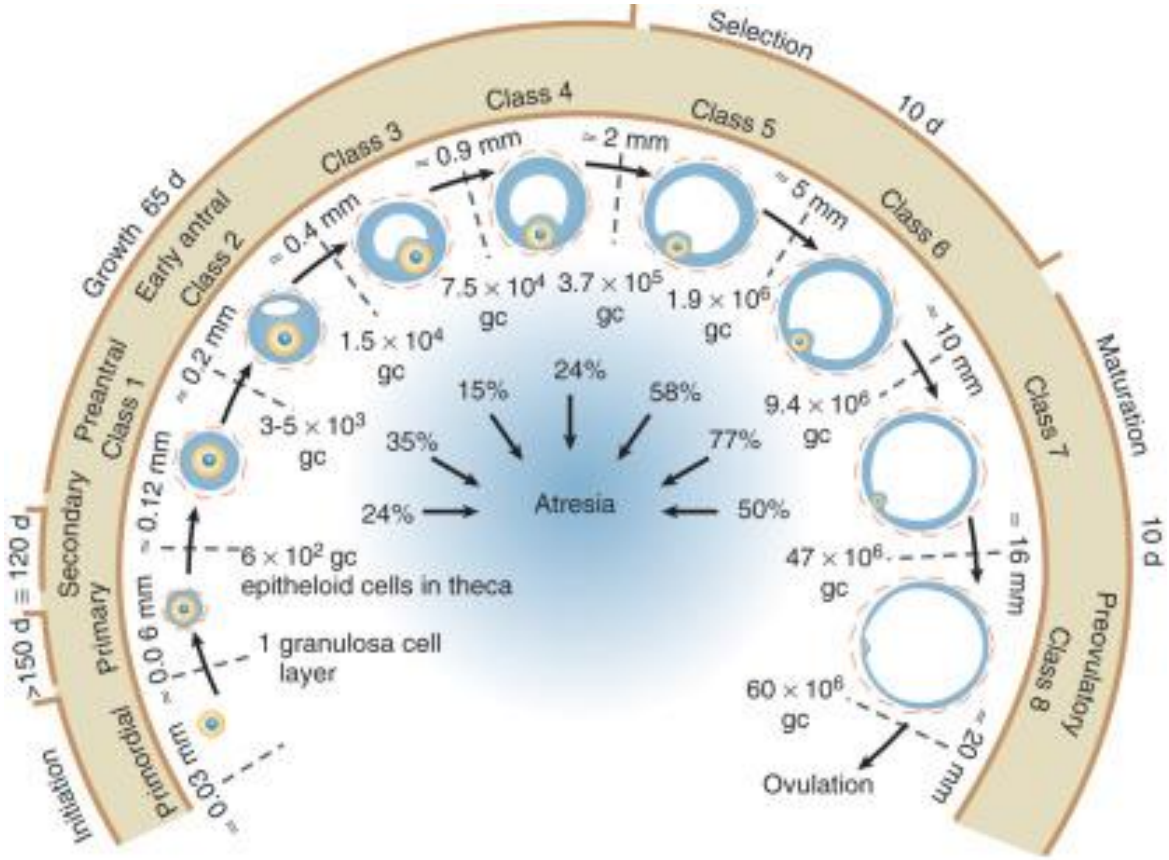
Colicchia et al. HRU 2014

the uncomfortable delay in conception

CAUSES OF FEMALE INFERTILITY

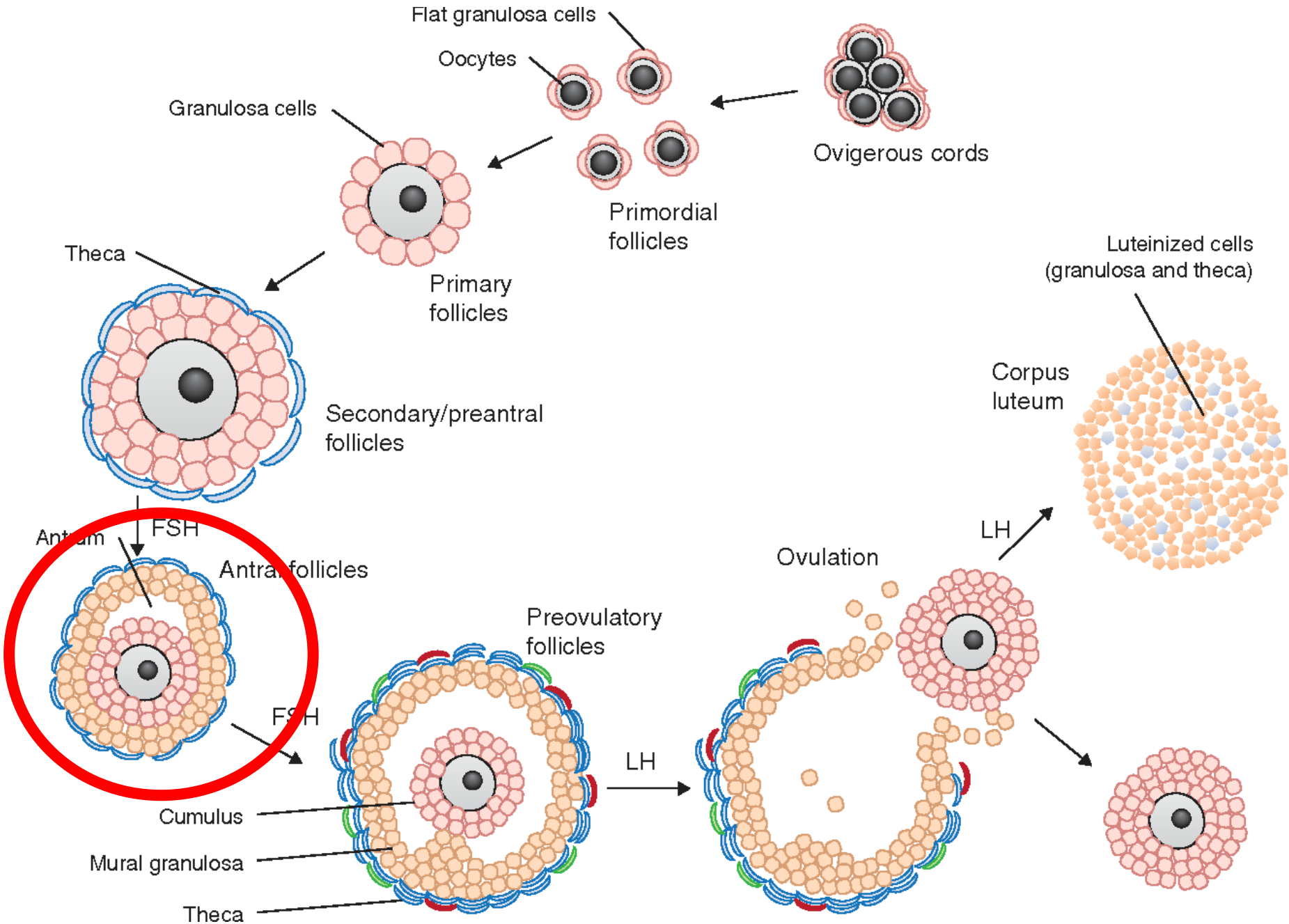


The various follicular classes, defined largely by size and number of granulosa cells, represent sequential stages of development on the way to maturity



Approximately 1 year may elapse in the maturation of a primordial follicle to a dominant follicle. During much of this remarkably long period (approximately 300 days), follicles are believed to grow in a gonadotropin-independent manner. Gonadotropins influence the last 50 days of the maturation process.

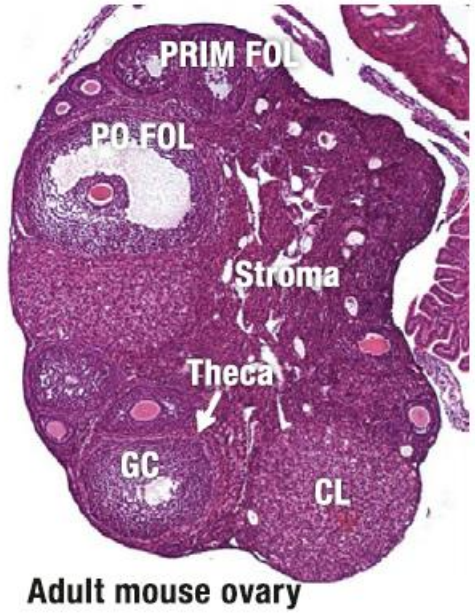
Intraovarian factors are believed to play key roles in regulating the early phases of follicular growth either as activators or inhibitors. Activators of follicular growth and development include endometrial leukemia inhibitory factor (LIF), basic fibroblast growth factor (FGF), and Kit ligand. Kit ligand, which is produced by granulosa cells, and acts on Kit, a receptor on the oocyte and theca cells, is required for initiation of follicular growth and growth of the oocyte.

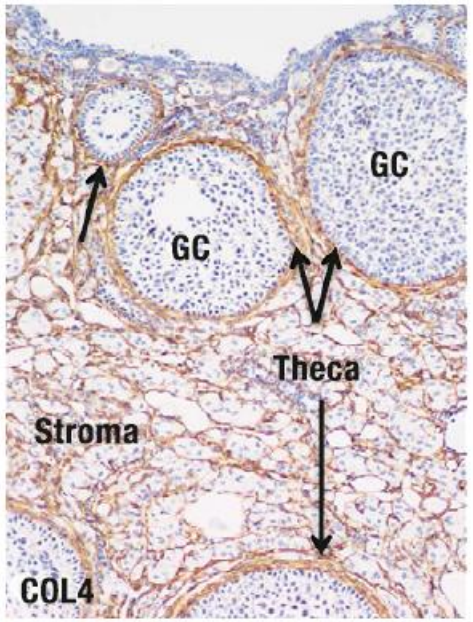

Flat granulosa cells
Oocytes
Granulosa cells
Ovigerous cords
Primordial follicles
Primary follicles
Theca
Secondary/preantral follicles
Luteinized cells (granulosa and theca)
Corpus luteum
FSH
Antrum
Antral follicles
LH
Ovulation
Preovulatory follicles
FSH
LH
Cumulus
Mural granulosa
Theca

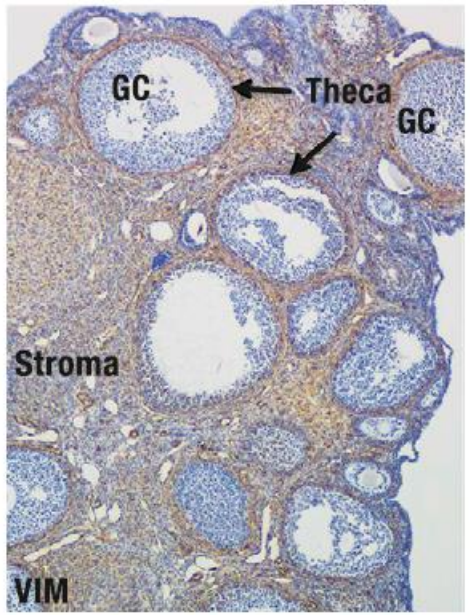
The follicular theca cell recruitment and differentiation may impact on fertility: the lesson of Polycystic Ovarian Syndrome

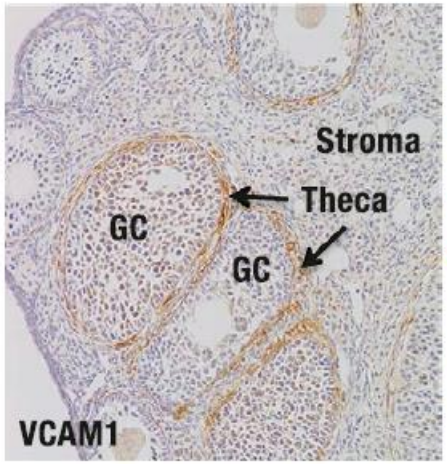
The histology of an adult mouse ovary illustrates the presence of primary follicles (PRIM FOL), preovulatory follicles (PO FOL), granulosa cells (GC), theca cells, corpora lutea (CL), and stroma.

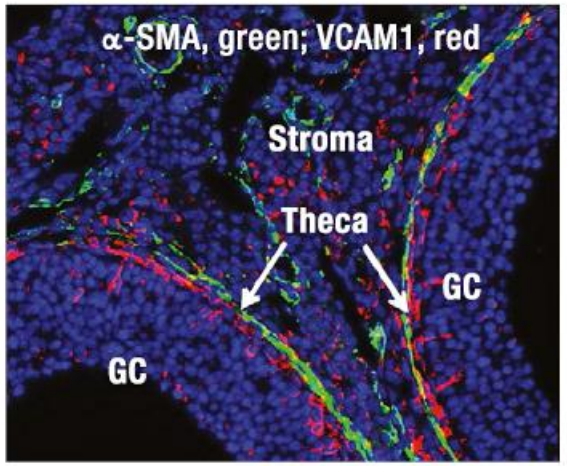
Markers of the theca layer during follicle development, stroma, and immune cells are illustrated by immunostaining for collagen 4 (COL4), vimentin (VIM), vascular cell adhesion molecule (VCAM)1, a-SMA/ACTA2, and epidermal growth factor-like module-containing Mucinlike hormone receptorlike 1 (EMR1; also known as F4/80, encoded by ADGRE1), a macrophage marker in mice

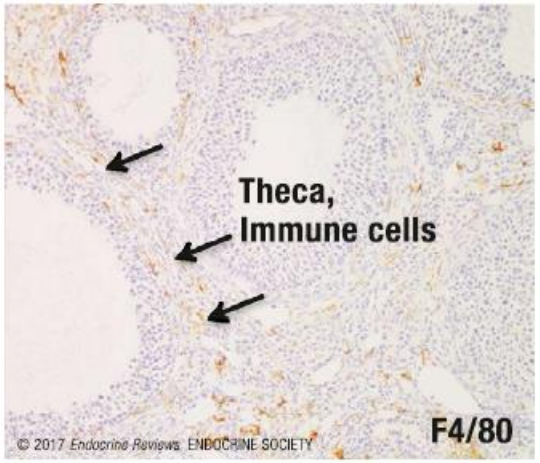


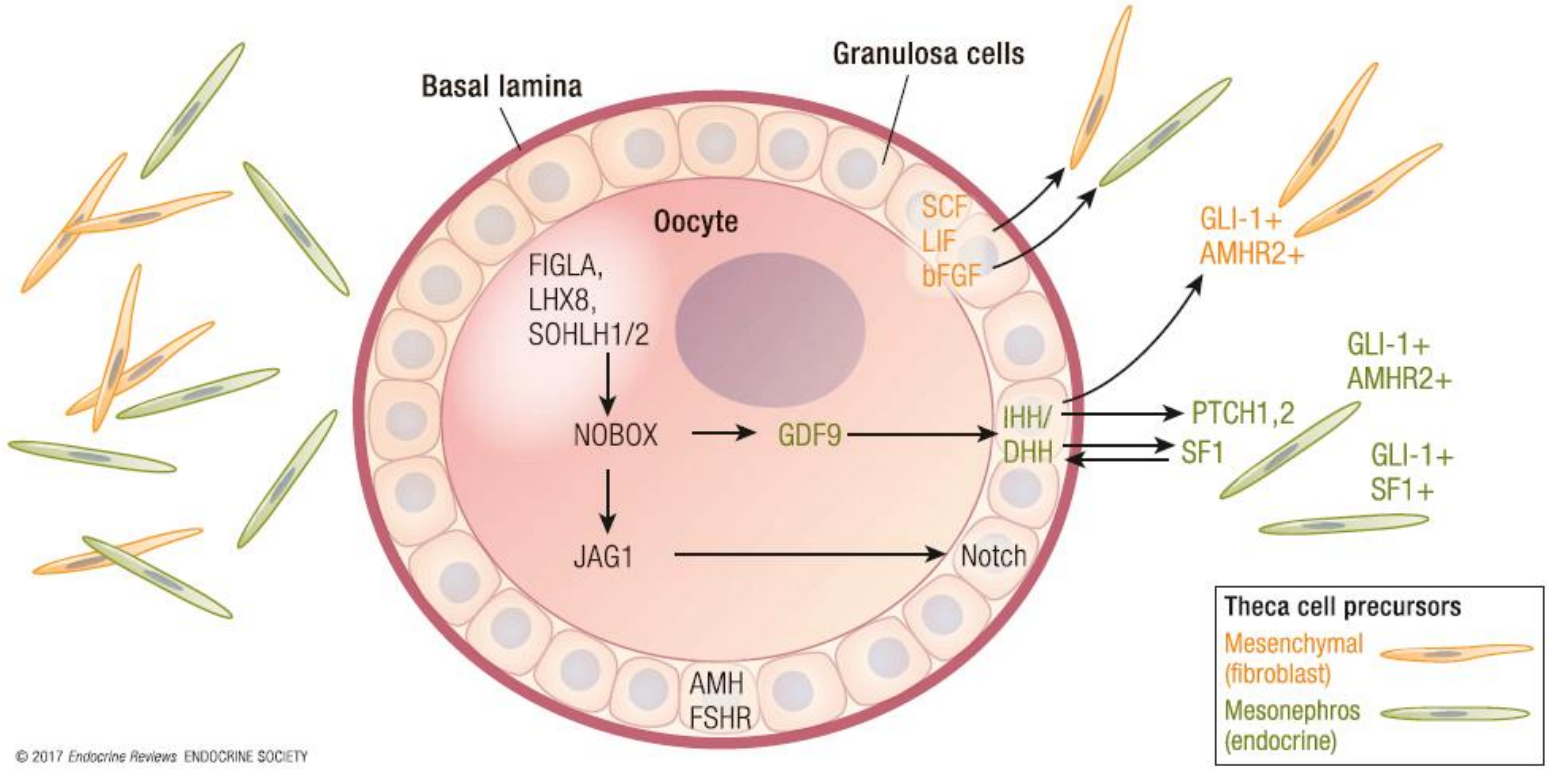




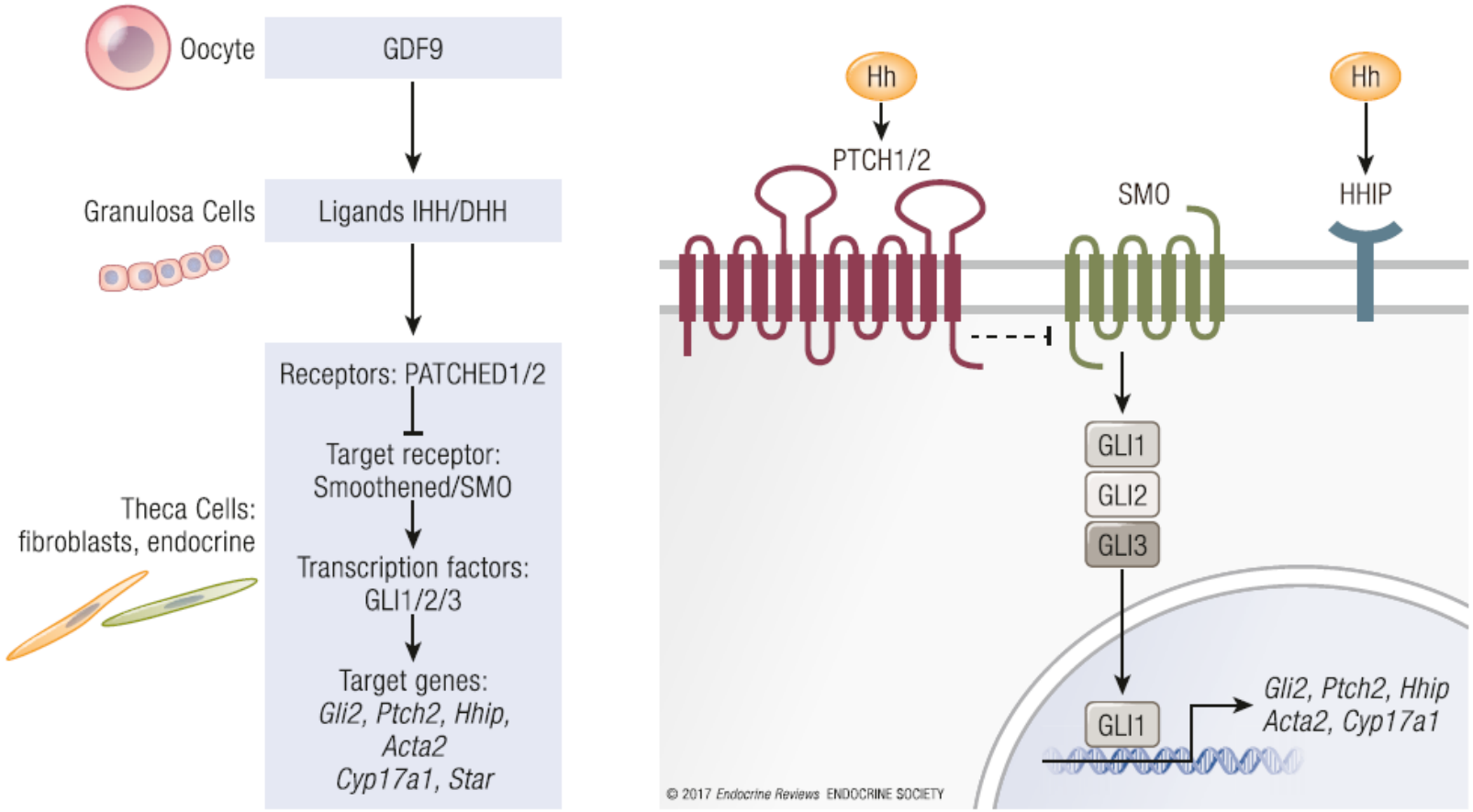






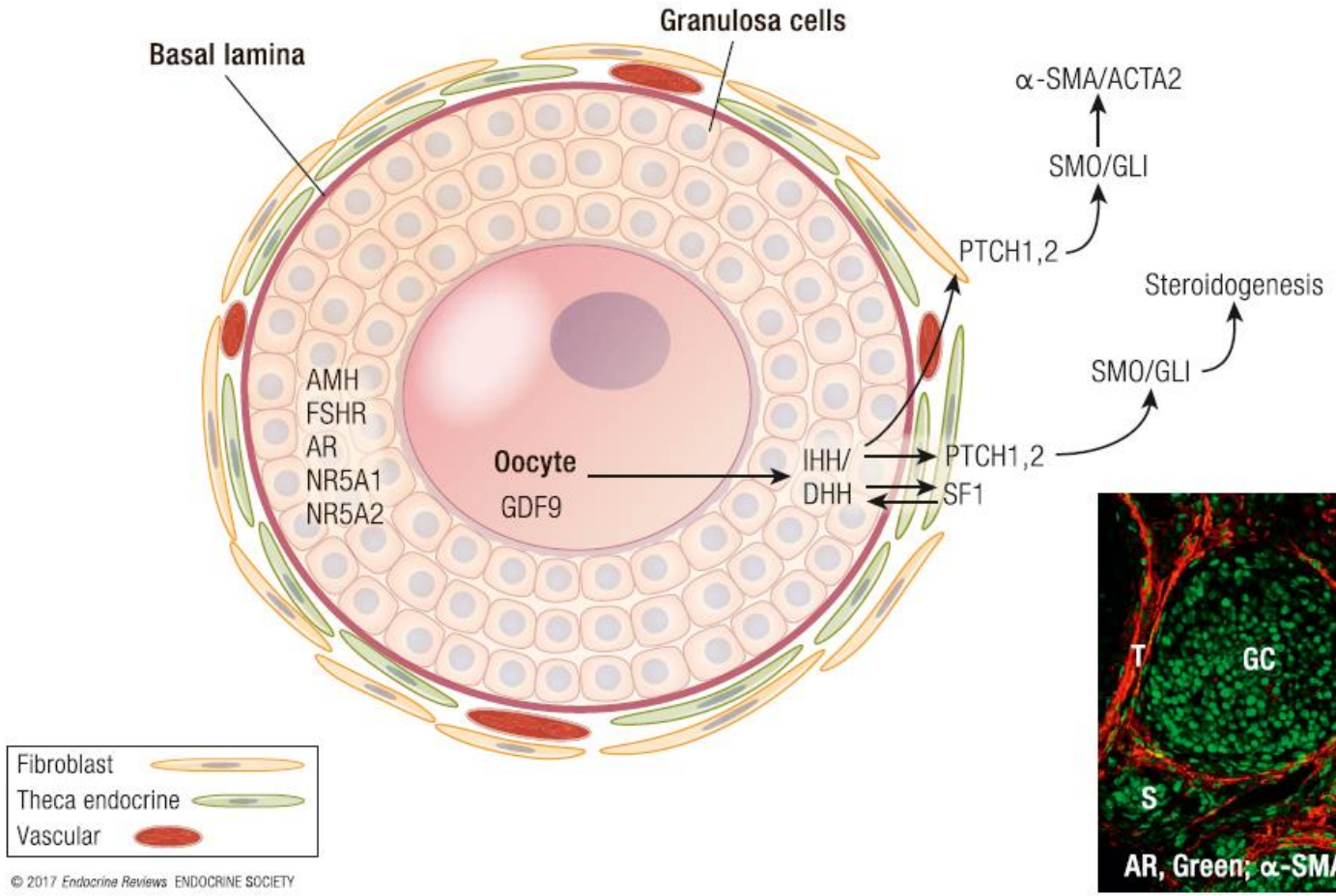


Specific oocyte factors regulate follicle formation. JAG1 activates notch signaling in pregranulosa cells that then become associated with the oocytes and promote oocyte–cyst breakdown and primordial follicle formation. Other factors (FIGLA) regulate the formation of the zona pellucida proteins. Additional factors LHX8, SOHLH1/2 control the expression of NOBOX that regulates early follicle formation and the expression of the oocyte-derived, TGF-b–related factor, GDF9. GDF9 from the oocyte induces expression of IHH and DHH in granulosa cells. These ligands then activate PTCH1/2 in theca cells. The theca precursor cells that become the theca endocrine cells are derived from SF1+ cells that migrate to the ovary from the mesonephros (denoted in green); the theca precursors that become fibroblast or express a-SMA are derived from the mesenchymal cells of the ovarian medullary region that express AMHR2

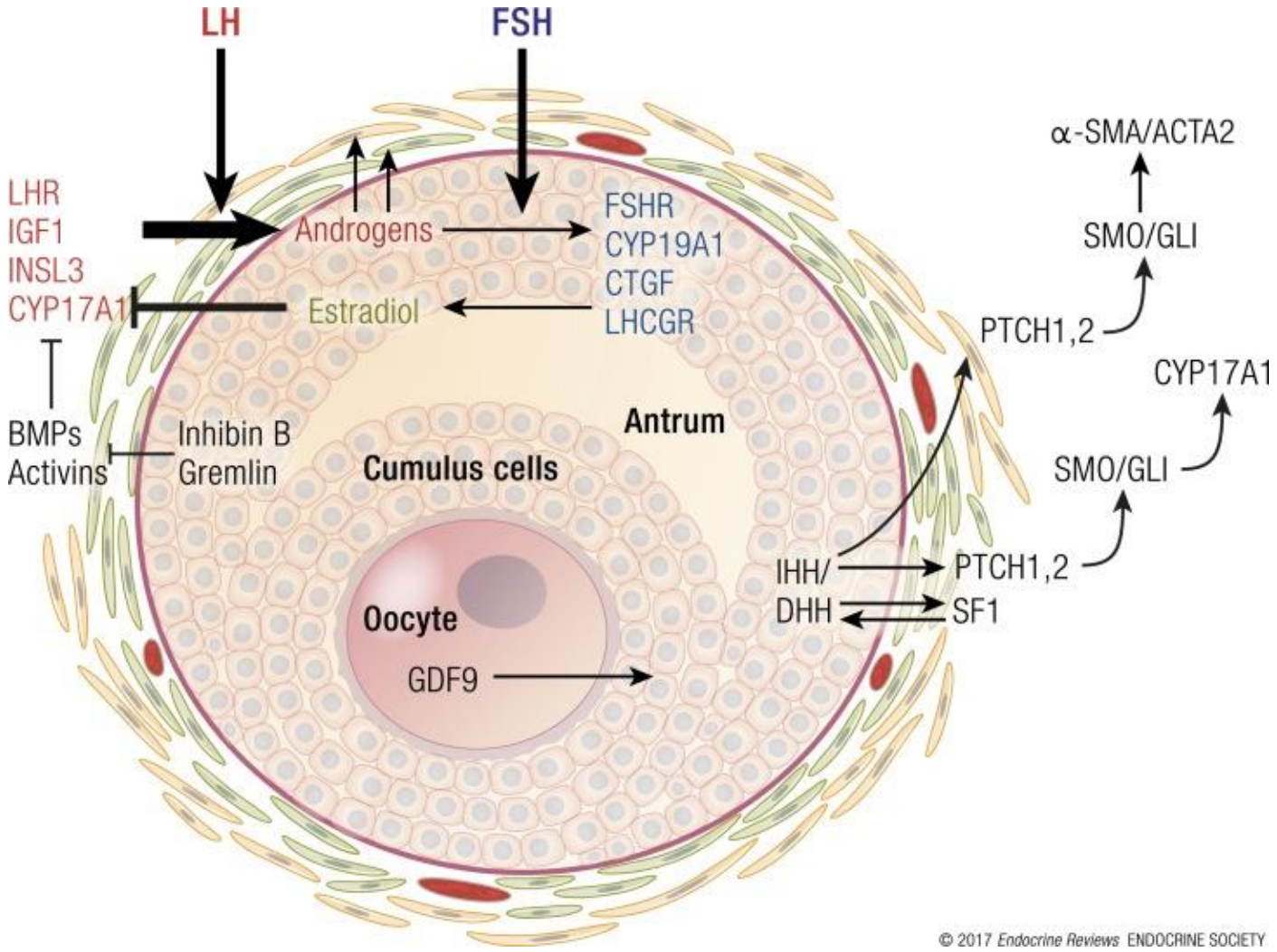


Schematic of hedgehog signaling pathway components in the ovary. GDF9 made by oocytes induces the expression of the ligands IHH and DHH in granulosa cells. These ligands bind to and activate cell surface receptors PTCH1/2 in theca cells. This activation blocks PTCH inhibition of the target receptor SMO, leading to the activation of the transcription factors GLI1, 2, or 3. These transcription factors regulate the expression of selected genes, including inhibitory feedback molecules (Hhip and Ptch2) and enzymes in the steroidogenic pathway, including Cyp17a1

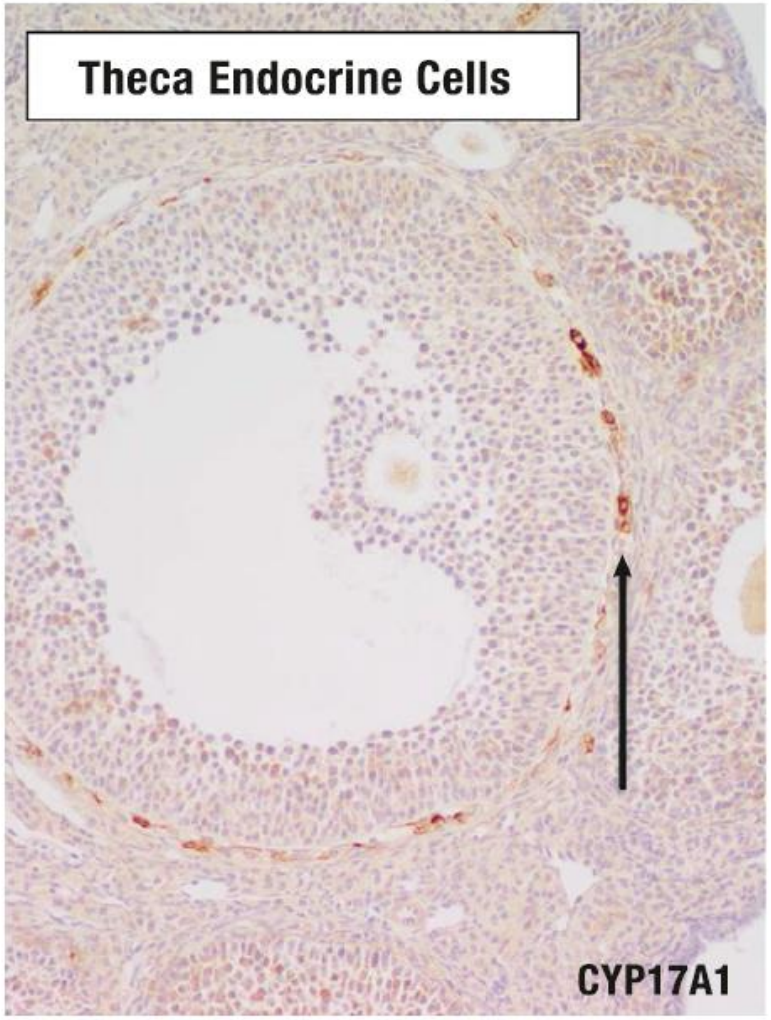
The theca layer of growing follicles becomes functional and contains theca endocrine cells that produce steroids (androgens) and theca externa cells that express α-SMA/ACTA2

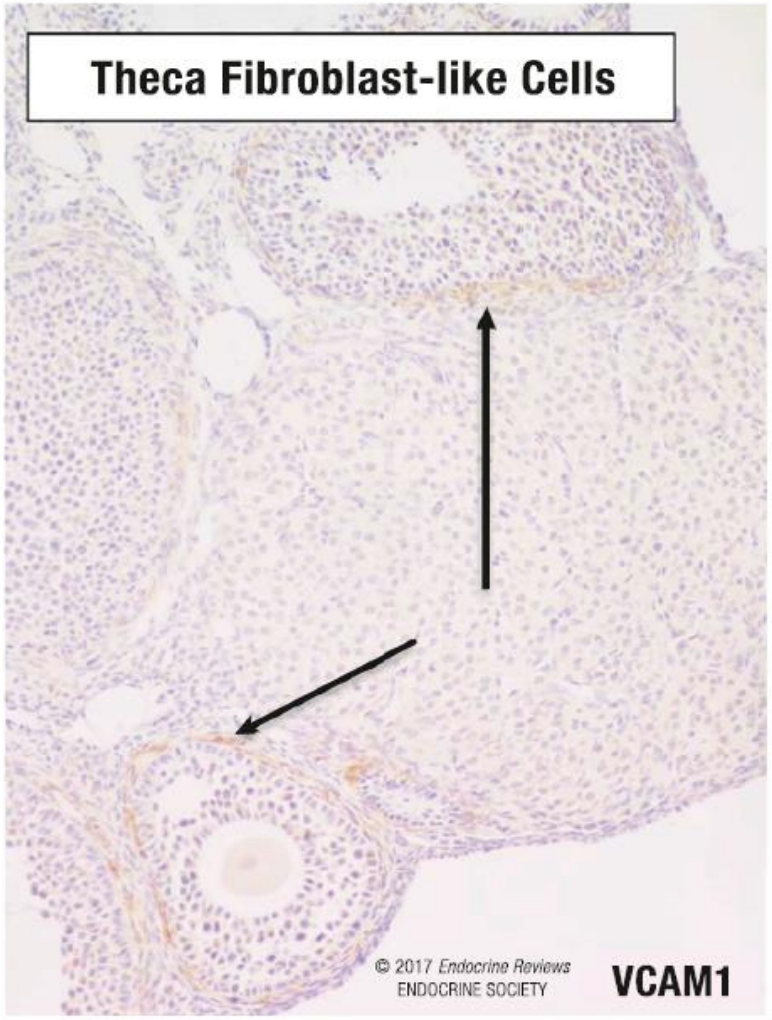


LH and FSH act to stimulate theca cell and granulosa cell differentiation, respectively, in growing antral follicles



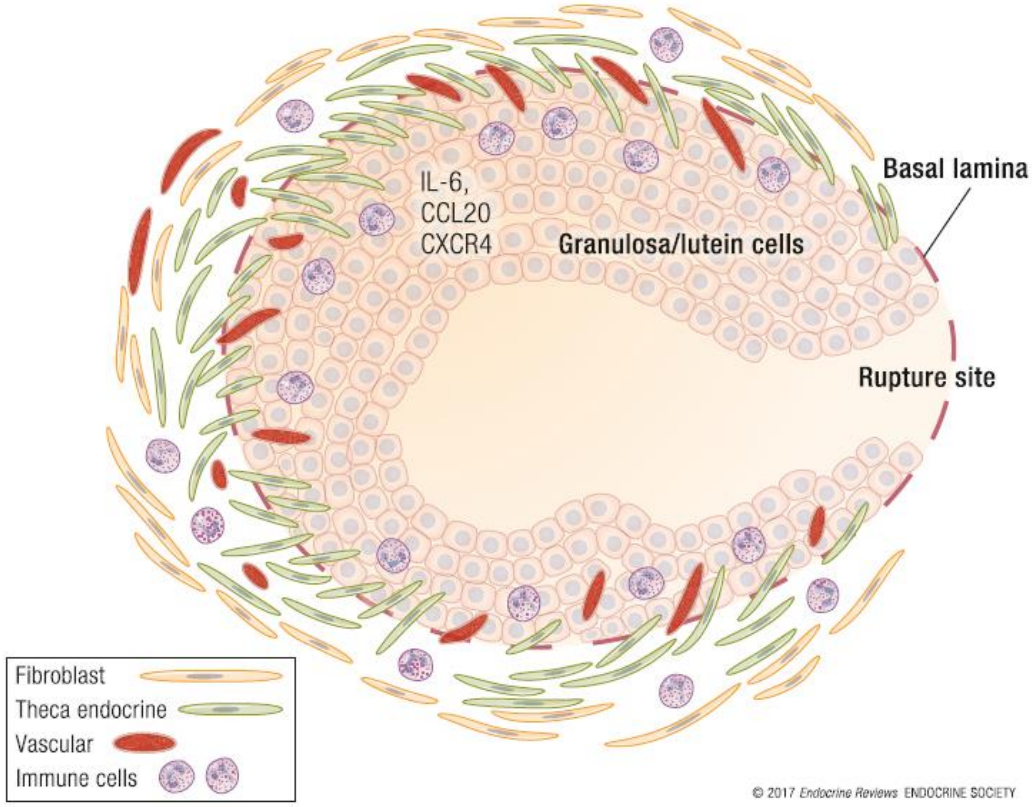
Young JM et al. **Theca the forgotten cell of the ovarian follicle**. *Reproduction* 2010





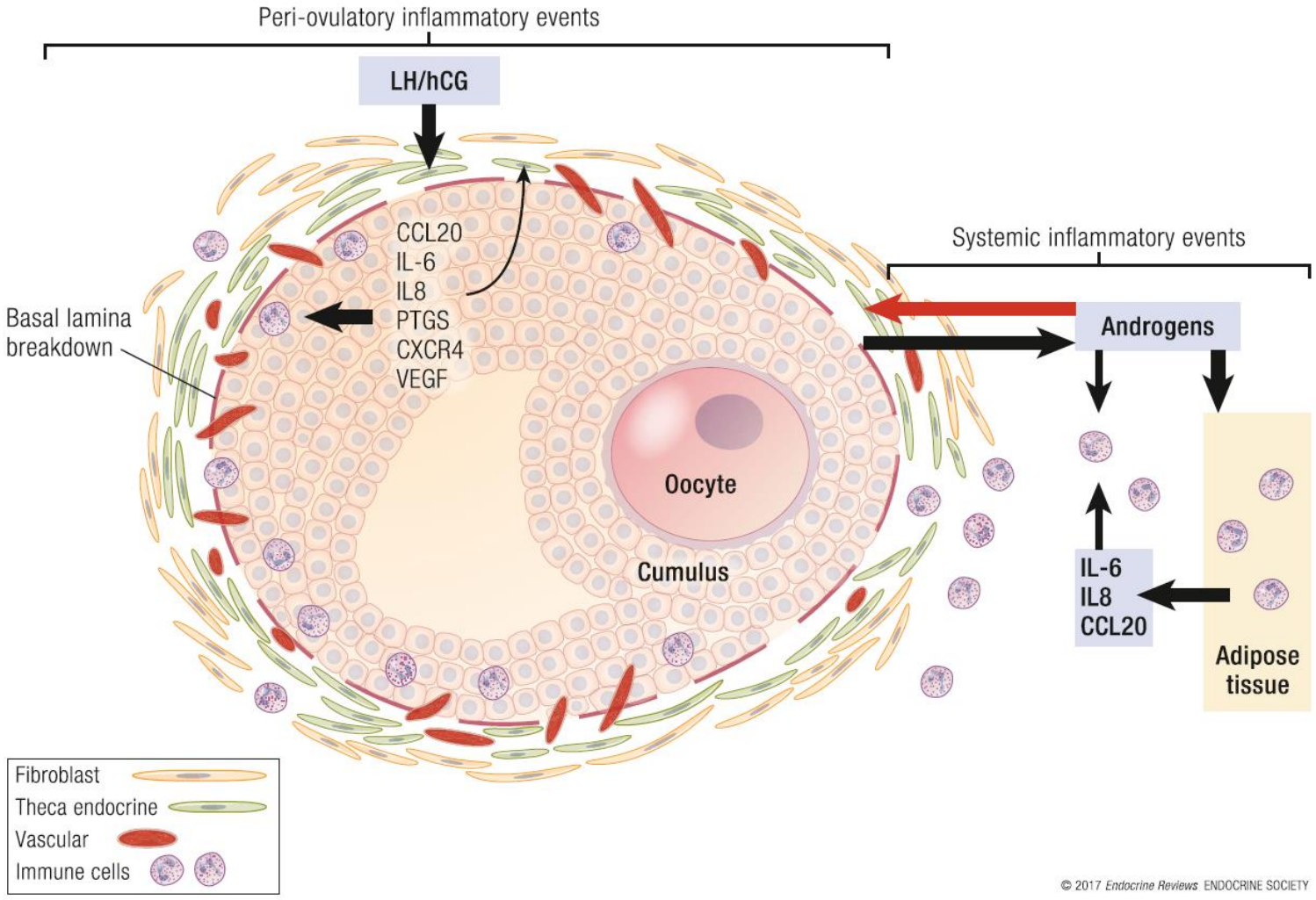
Immunostaining of CYP17A1 illustrates that it is a marker of theca endocrine cells

The breakdown of the basal lamina and invasion of vascular cells



Many theca-related events occur following ovulation. The most dramatic changes in the ovulated follicle include the breakdown of basal lamina (denoted by dashed line), the invasion of theca endocrine and vascular cells into the granulosa cell layer, the increased homing of immune cells associated with the vascular elements, and the increased expression of cytokines by granulosa cells (IL-6, CCL20, CXCR4) and immune cells (IL-6). These changes lead to the formation of the corpus luteum.

PCOS is associated with a systemic proinflammatory state and a hyperinflammatory response in ovulating follicles

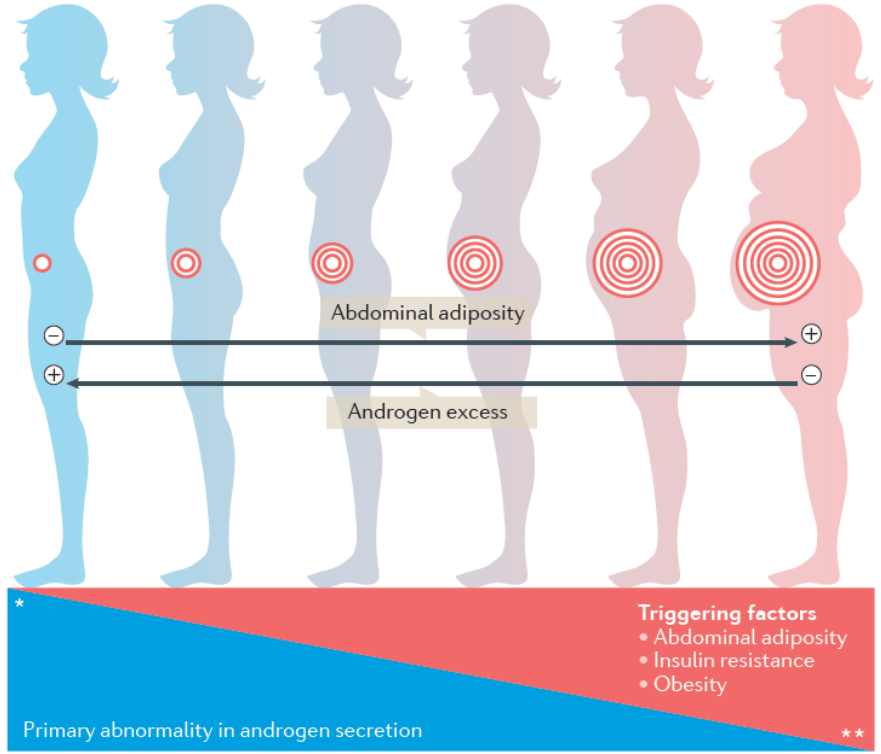


Elevated androgens produced by theca cells in patients who have PCOS impact adipose tissue, leading to a proinflammatory state in the peripheral tissues, even prior to ovulation. The LH surge further increases androgen production by theca cells and induces prostaglandin and cytokine expression by granulosa cells (CCL20). Androgens can act on theca, vascular, and immune cells that are recruited to the periovulatory follicle by cytokines. These recruited immune cells then release additional cytokines, leading to a proinflammatory perifollicular milieu, most evident in patients who have PCOS that are obese

KEY POINTS

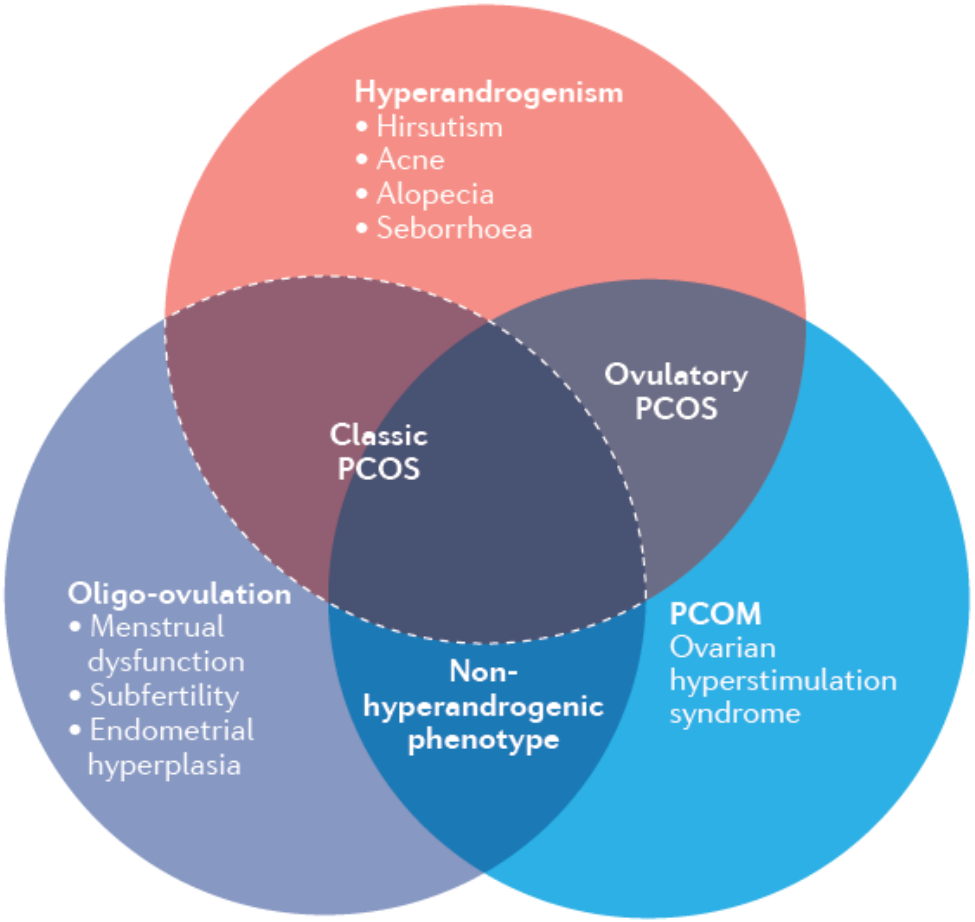
- Theca cells within the theca layer of growing follicles are derived from two different sources in the embryonic gonad; mesenchymal cells migrating into the ovary from the mesonephros region become the steroidogenic cells, and WT1+ stromal cells indigenous to the embryonic ovarian medullary region become fibroblasts, perivascular smooth muscle cells, and interstitial ovarian tissue, respectively, in the adult ovary

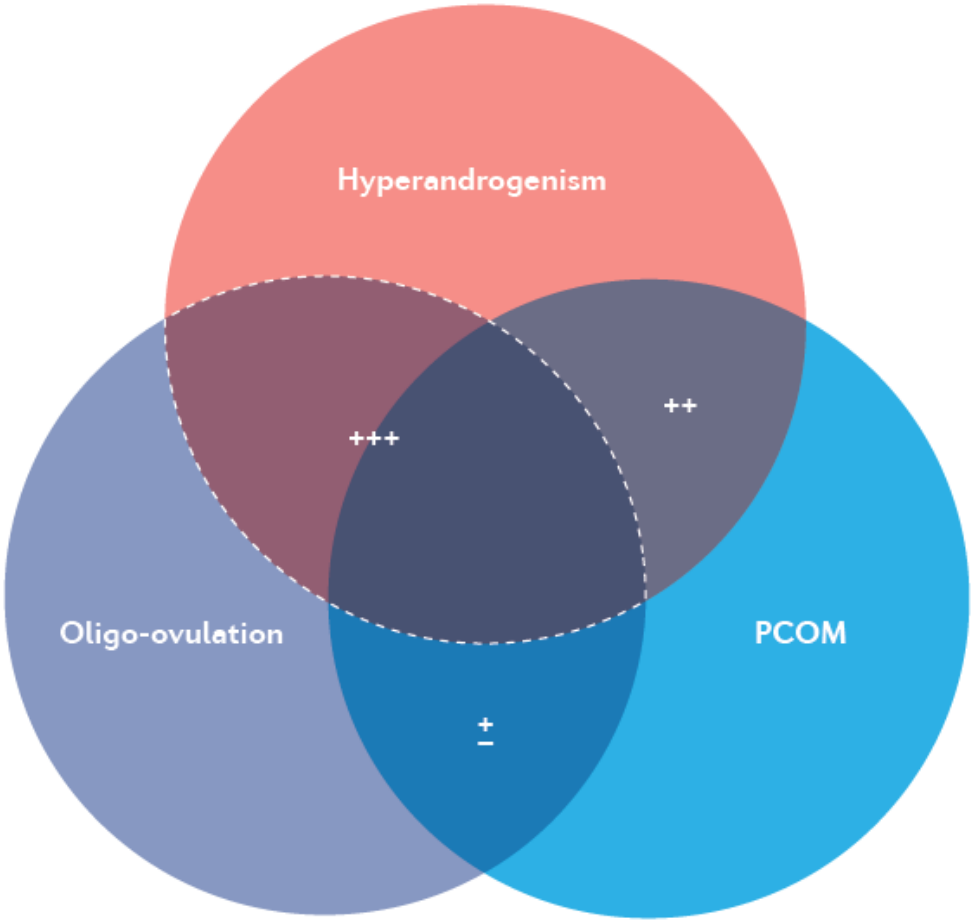
- Theca cell functions are altered in polycystic ovarian syndrome and at least in some cases of premature ovarian failure where mutations in GDF9 and NOBOX have been observed



Pathophysiological heterogeneity in patients with PCOS. Polycystic ovary syndrome (PCOS) is the result of the interaction of a primary abnormality in androgen synthesis (manifesting as androgen excess) with other factors, such as abdominal adiposity (red and white targets), obesity and insulin resistance. At one extreme (*), the disorder in some patients is severe enough to result in PCOS even in the absence of triggering factors (light-blue-shaded woman on the left). At the other extreme (**), a very mild defect in androgen secretion is amplified by the coexistence of abdominal adiposity, obesity and/or insulin resistance (pink-shaded woman on the right). Between the two extremes, there is a spectrum in the severity (range of blue-shaded to pink-shaded women in the middle) of the primary defect in androgen secretion, providing an explanation for the heterogeneity of patients with PCOS with regards to the presence of obesity and metabolic comorbidities. However, all patients share a primary defect in androgen secretion

The heterogeneous nature of PCOS





PCOS

- Polycystic ovary syndrome (PCOS) is defined by a combination of signs and symptoms of androgen excess and ovarian dysfunction in the absence of other specific diagnoses.
- Heterogeneity, from aetiology to clinical presentation and long-term prognosis, is intrinsic to PCOS.
- Mounting evidence suggests that PCOS might be a complex multigenic disorder with strong epigenetic and environmental influences, including diet and other lifestyle issues.
- The diagnosis of PCOS is uncomplicated, requiring only the careful application of a few well-standardized diagnostic methods.
- Treatment should be symptom-oriented, long term and dynamic and adapted to the changing circumstances, personal needs and expectations of the individual patient.
- Therapeutic approaches should target hyperandrogenism, the consequences of ovarian dysfunction and/or the associated metabolic disorders.