CONTINUING EDUCATION IN TOXICOLOGIC PATHOLOGY
REPRODUCTIVE SYSTEM

Third Conference

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PHYSIOLOGY OF THE RAT ESTROUS CYCLE

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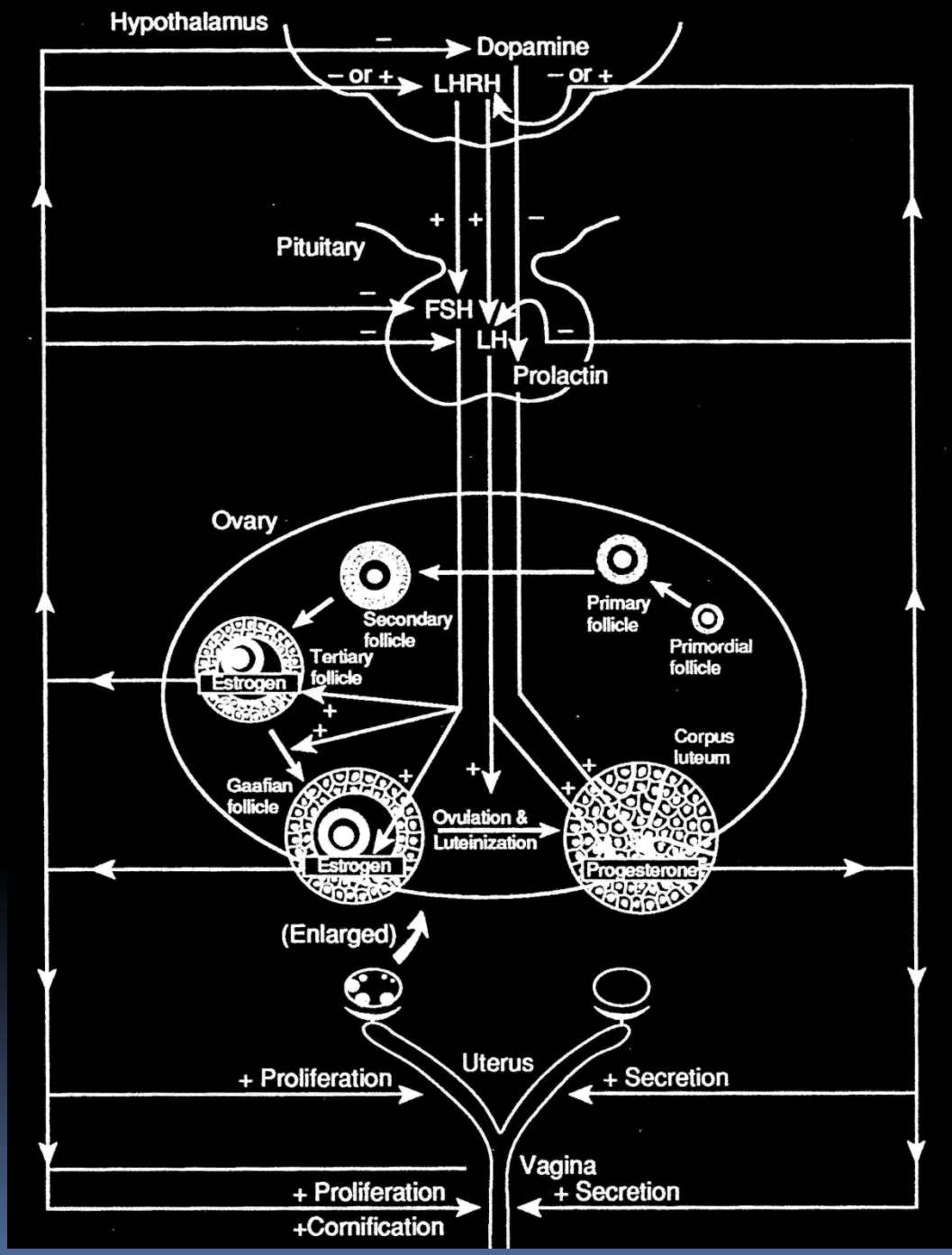
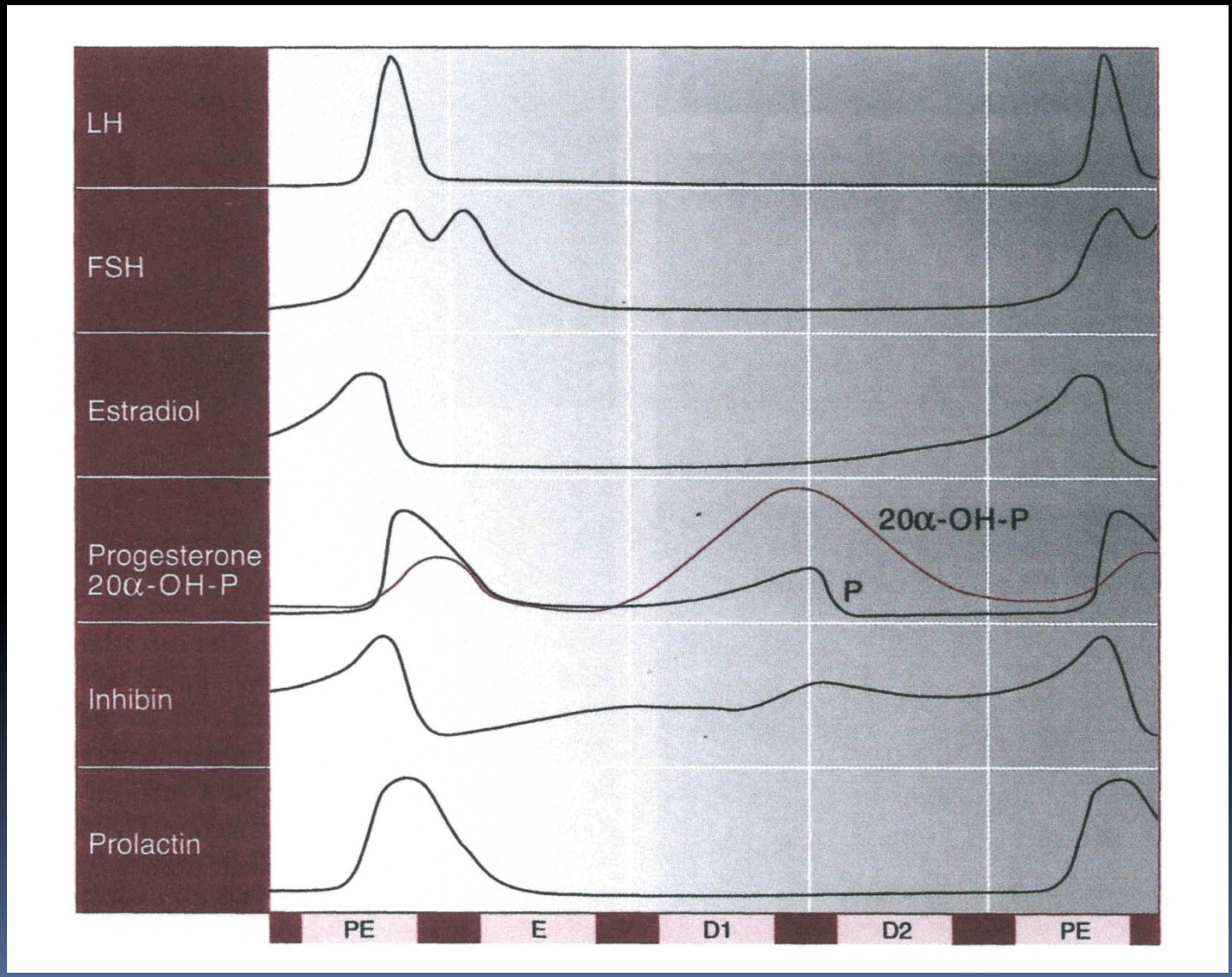
Hormonal Regulation of Reproduction



From Yuan & Foley 2002

Hormonal Profile, 4 Day Cycle



from Maeda et al 2000

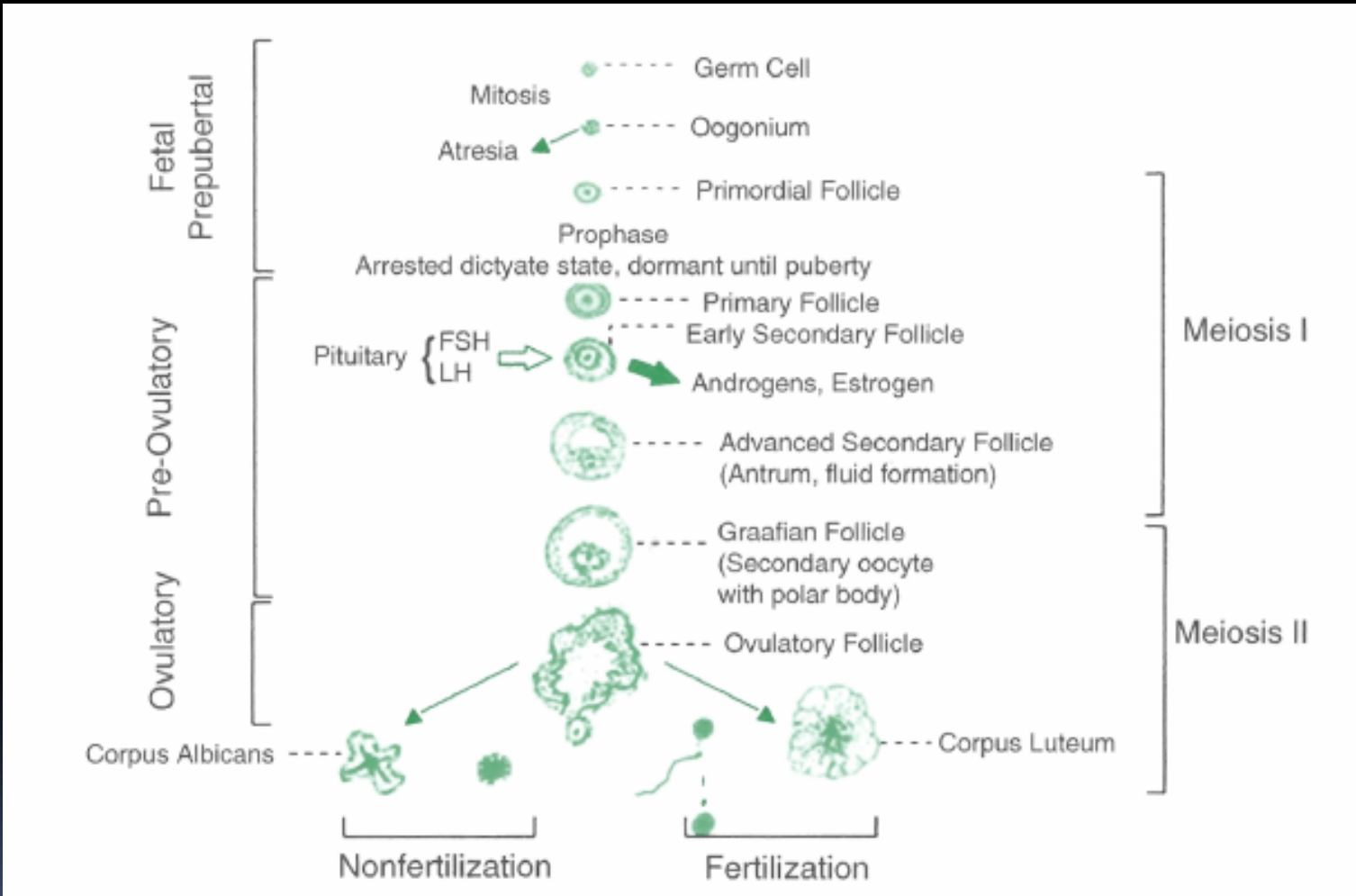
Ovarian Development

- Early embryo is sexually undifferentiated
- Bipotential gonad arises in genital ridge
- Sex determination @E12-14
  - Coelomic epithelium lining ridge gives rise to stroma and rete gives rise to granulosa cells
  - Does not require gonadotropins
- Primordial germ cells in yolk sac migrate to genital ridge E9-11.5; undergo mitosis
- In rodents, meiosis starts ~E13.5 and can continue to PND 3. Oocytes arrest in diplotene phase of meiosis I
- Major period of oocyte attrition during meiosis

Folliculogenesis

- Process by which female germ cells develop within the somatic cells of the ovary and mature into fertilizable eggs
- Early folliculogenesis is directed by signals within the ovary
- Development beyond preantral stage requires hormones from the hypothalamic/ pituitary axis

Folliculogenesis



from Foster & Gray 2008

Neonatal Follicular Development

- Progression of follicular development at birth
  - Majority of oocytes in germ cell nests at birth
  - A second period of oocyte attrition occurs
  - Formation of primordial follicles PND 1-3
  - Primary follicles observed by PND 3
  - Germ cell nests gone, primary and secondary follicles seen by PND 7

Early Folliculogenesis

- Formation of primordial follicles through preantral follicles does not require gonadotropins
- Mechanism controlling primordial to primary oocyte transition are largely unknown
  - bFGF IGF NGF AMH (-)
  - Kit/kit ligand KGF BMP_4 Nobox
- Oocyte has dominant role in directing early folliculogenesis but granulosa cell-oocyte interactions are needed

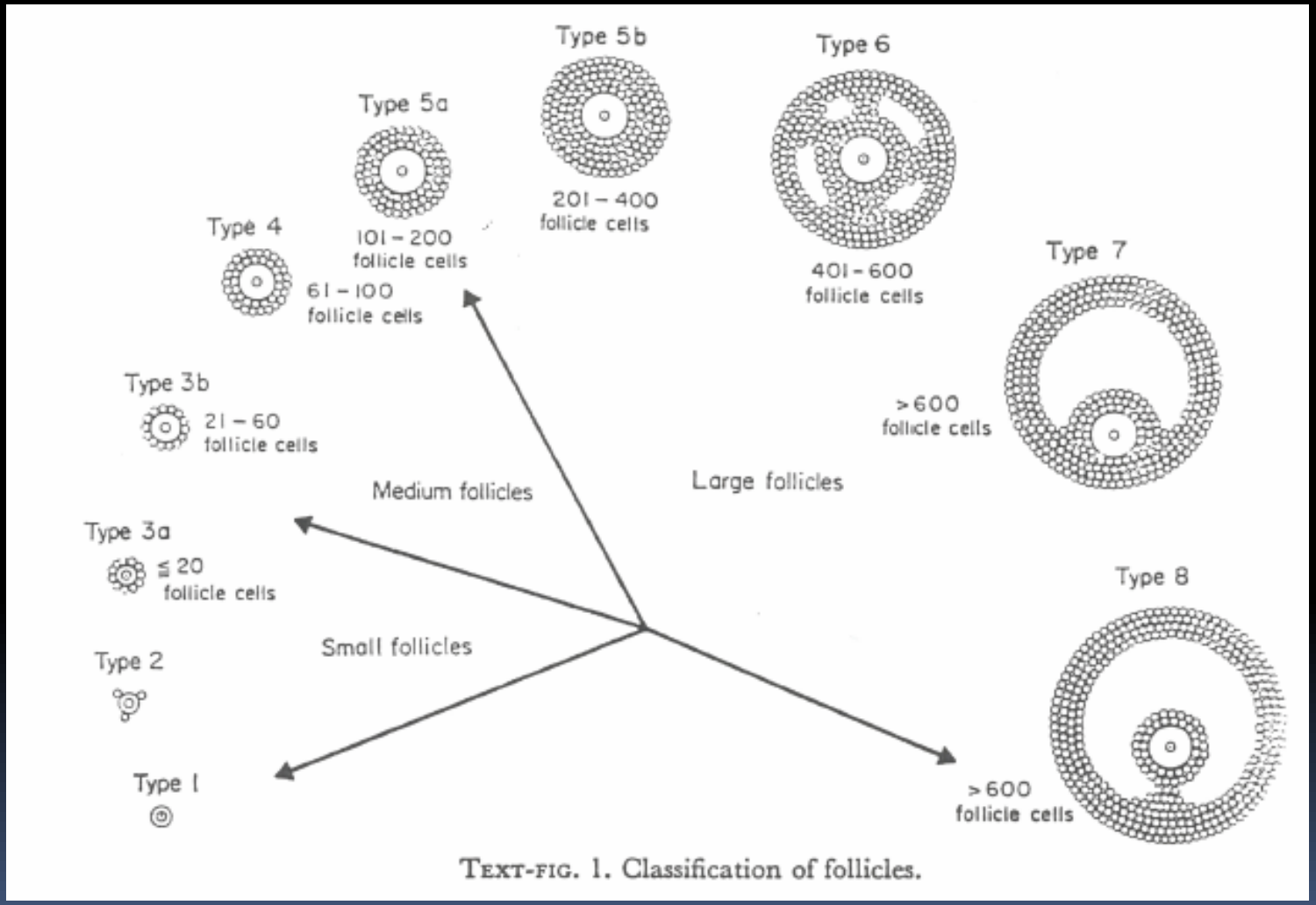
Early Folliculogenesis

- Initiation of ovarian steroidogenesis is also gonadotropin independent
  - Aromatase is present in fetal ovaries
- Folliculogenesis first becomes dependent on gonadotropin control during 2nd postnatal week of life
  - Large increased expression of FSH receptors on granulosa cells
  - Development of steroid-negative feedback on hypothalamus
- Formation of antral follicles ~PND21

Pre-Pubertal Development & Puberty

- E-positive feedback on gonadotropin develops ~PND20
- Mid-afternoon surge of PRL develops
- Rapid increase in LH receptors in follicle
  - Facilitated by increasing PRL levels
- Diurnal pattern of release of LH develops ~PND30
- First proestrus observed ~9 days later

Follicle Classification

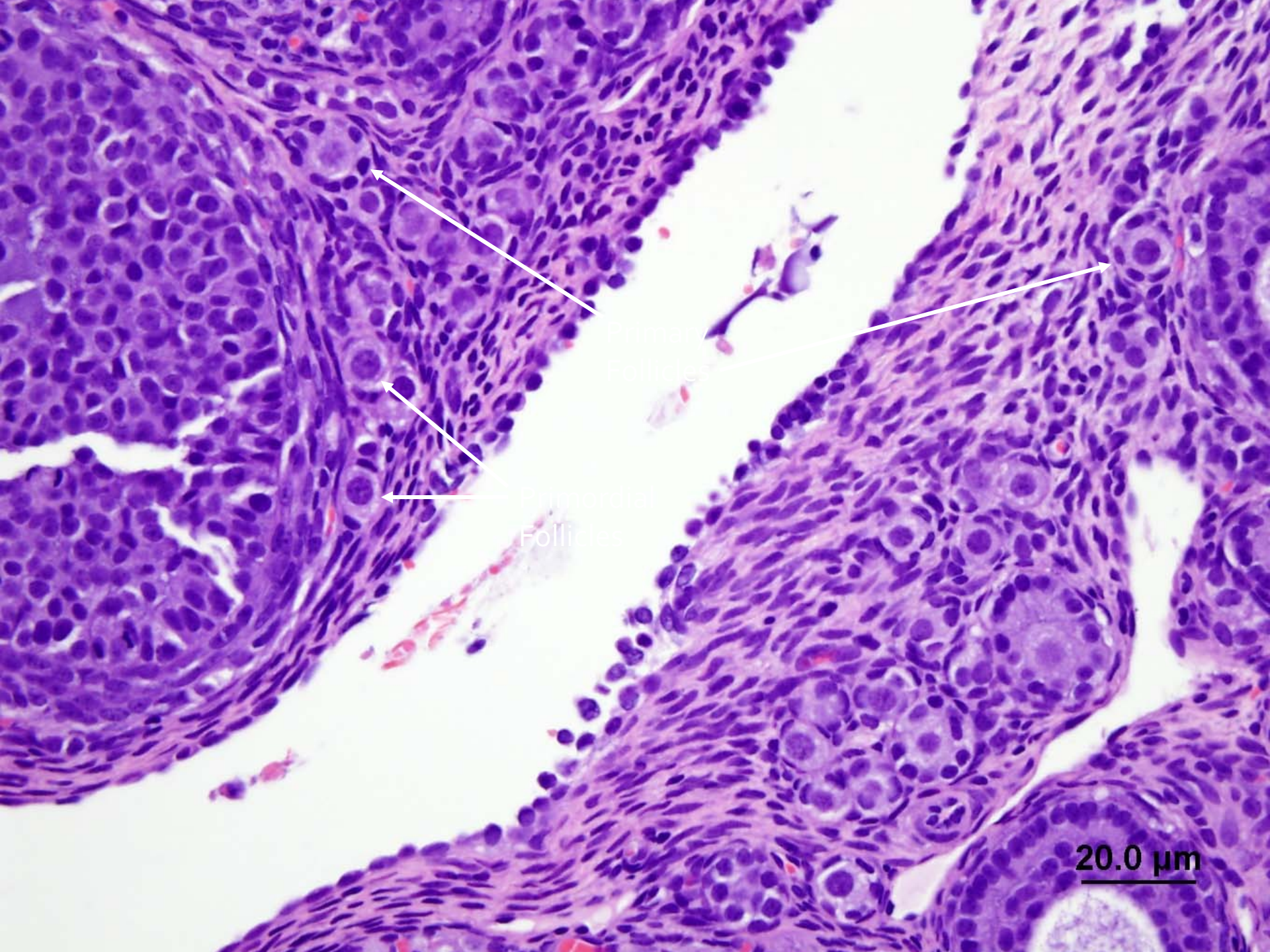


TEXT-FIG. 1. Classification of follicles.

from Pedersen & Peters, 1968

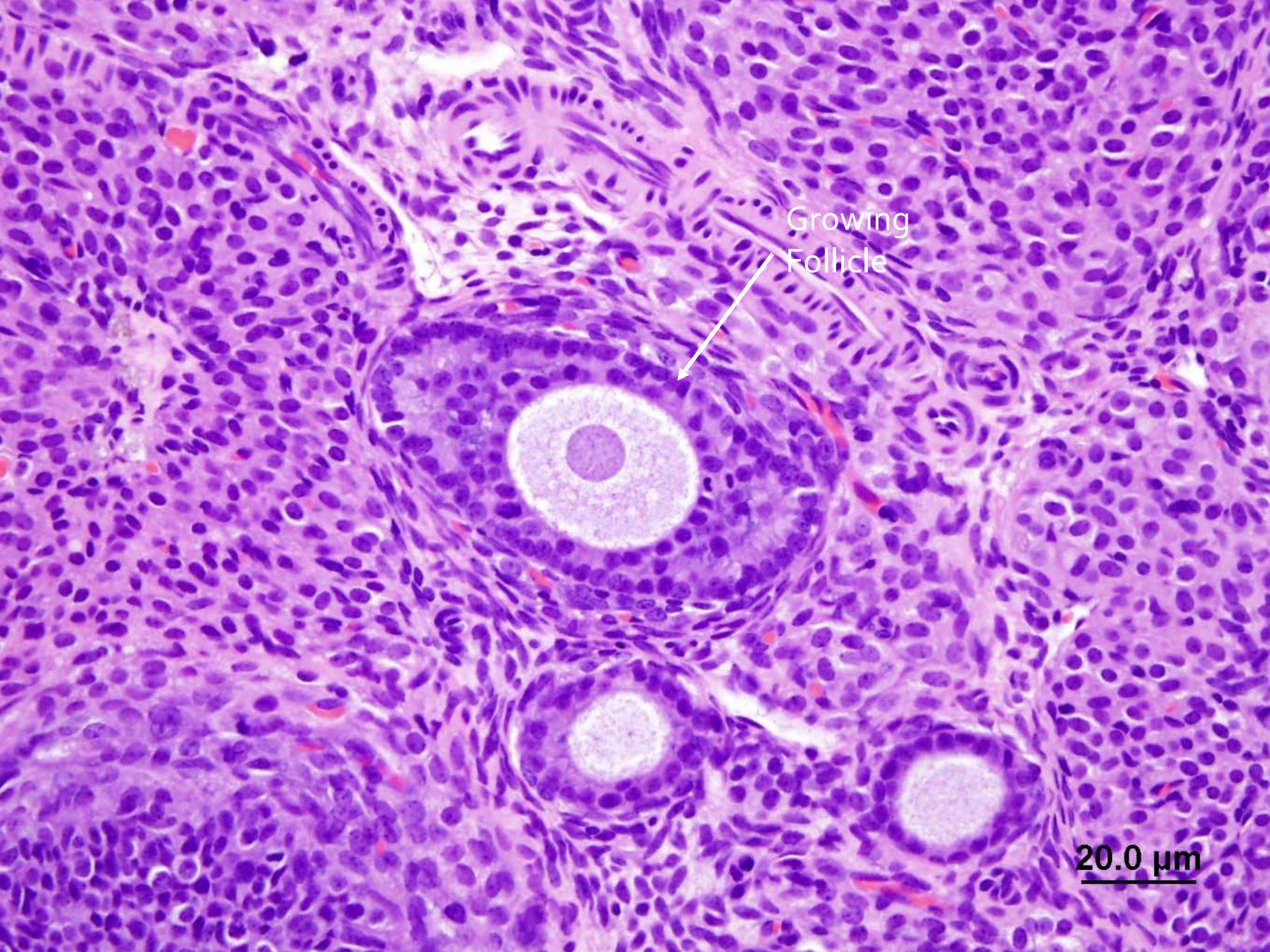
Follicular Stages

- Primordial follicles - small oocyte; nucleus surrounded by complete or incomplete single layer of flattened pregranulosa cells (P&P 1-2) – 'nongrowing', oocyte <19 µm
- Early primary follicles - small oocyte; nucleus surrounded by partial single layer of cuboidal granulosa cells (P&P 3a)


Primary
Follicle
Primordial
Follicles
20.0 µm

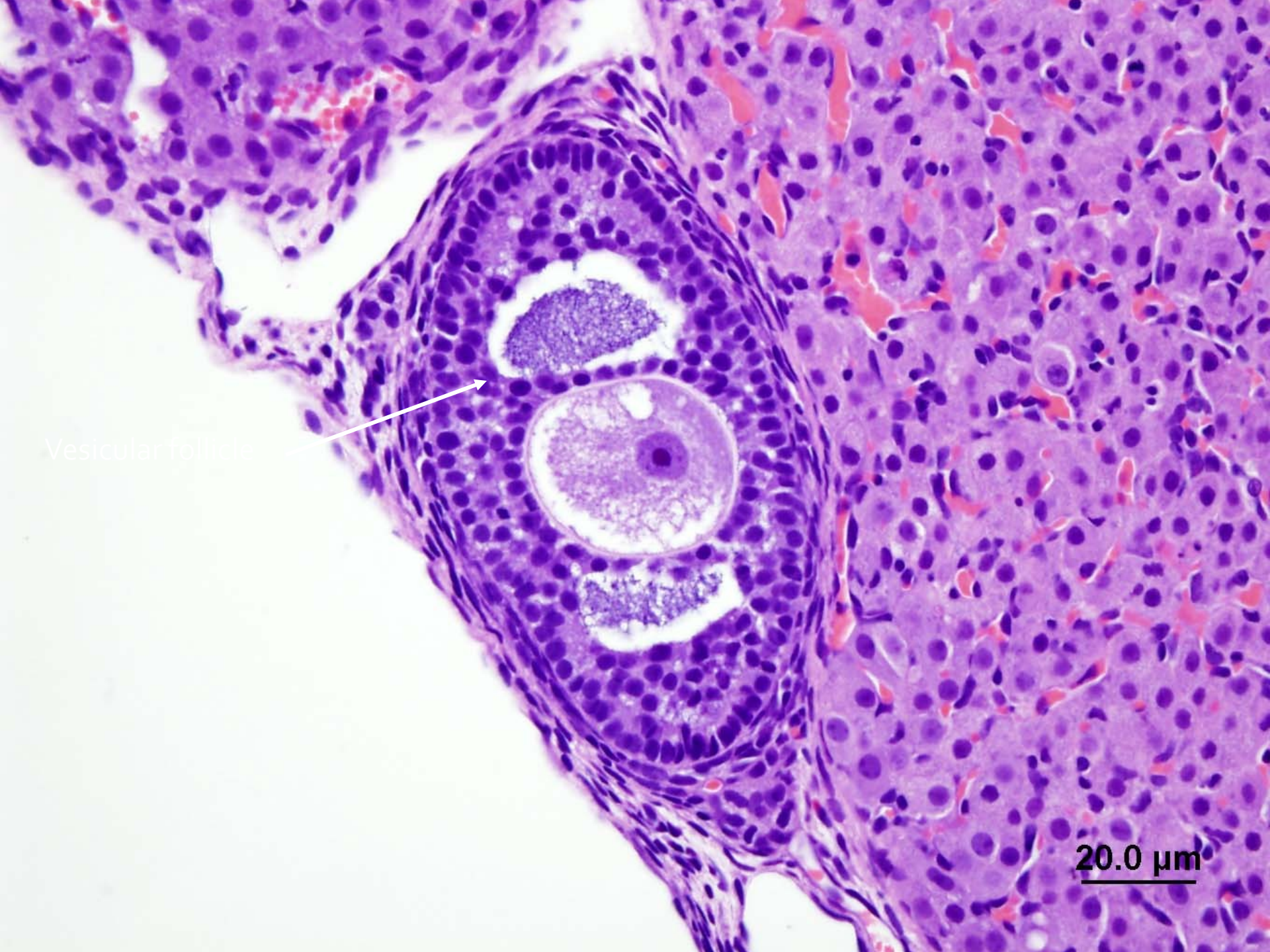
Follicular Stages

- Primary/Small growing follicles - small oocyte; nucleus surrounded by complete single layer of cuboidal granulosa cells (P&P 3b)
- Growing/Secondary follicles – Enlarging oocyte; nucleus surrounded by multilayered solid mantle of granulosa cells (P&P 4-5b); theca layer present

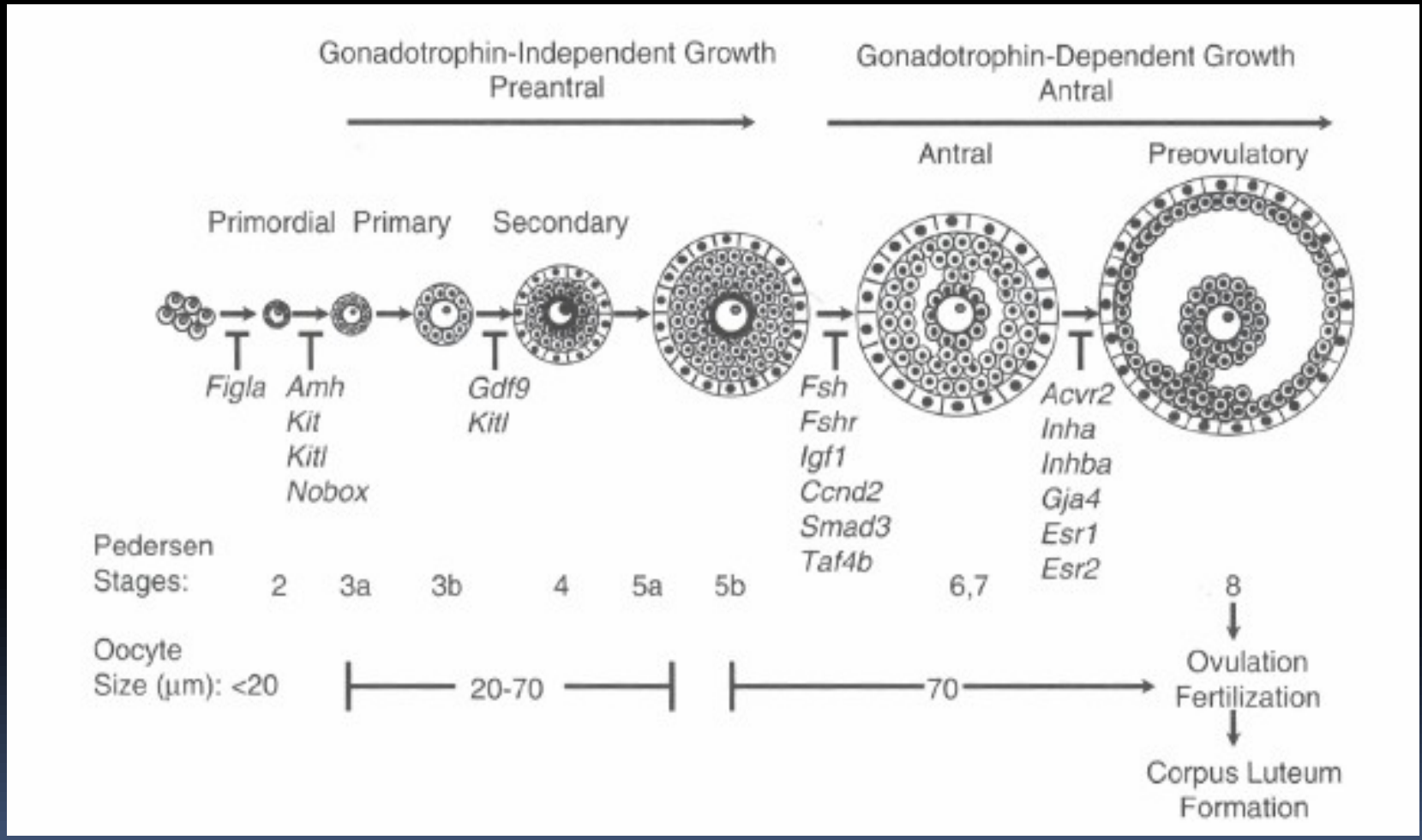

Growing
Follicle
20.0 µm

Follicular Stages

- Vesicular/Antral follicles – Growing follicles with developing fluid-filled cystic spaces (P&P 6)
- Large Antral/Preovulatory follicles – Full sized oocyte; central nucleus surrounded by fluid filled space bordered by hundreds of layered granulosa cells (P&P 7-8)


Vesicular follicle
20.0 µm

Postnatal Follicle Development



from Rajkovic et al 2006

Maturation of Antral Follicles

- Early antral follicles are responsive to Gn
  - ↑ FSH release by pituitary leads to ↑FSH-R on granulosa cells
  - Major effects of FSH binding on granulosa cells
    - proliferation
    - ↑FSH-R
    - ↑LH-R
    - ↑cyclin D2
    - ↑ PRL-R
    - ↑IGF-1
    - ↑aromatase activity (↑estrogen production)

Maturation of Antral Follicles

- Early antral follicles are responsive to Gn
  - Major effects of FSH binding on granulosa cells (cont')
    - ↑P production (via induction of cholesterol side chain cleavage enzyme)
    - Inhibition of apoptosis
    - ↑α inhibin
  - Estrogen generally potentiates effects of FSH via ERβ

Maturation of Antral Follicles

- Early antral follicles are responsive to Gn
  - Basal LH release by pituitary binds to LH-R on thecal cells
  - Major effects of LH binding to thecal cells:
    - ↑lipoprotein receptors
    - ↑androgen (androstenedione) production from cholesterol via induction of cyp17

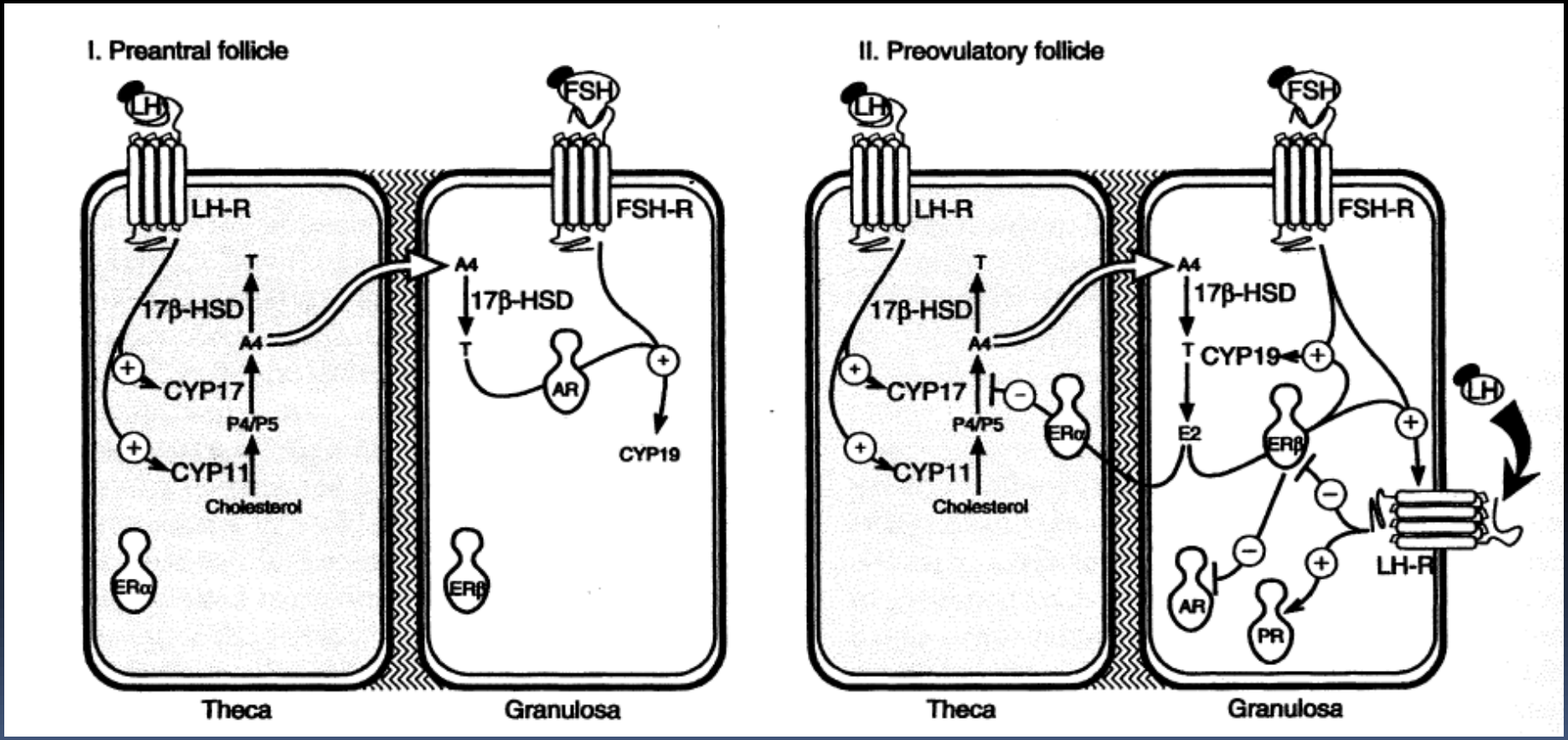
2 Cell, 2 Gonadotropin Theory

- Describes complex paracrine-endocrine interactions between the cells of different compartments within the ovary and the pituitary

2 Cell, 2 Gonadotropin Theory

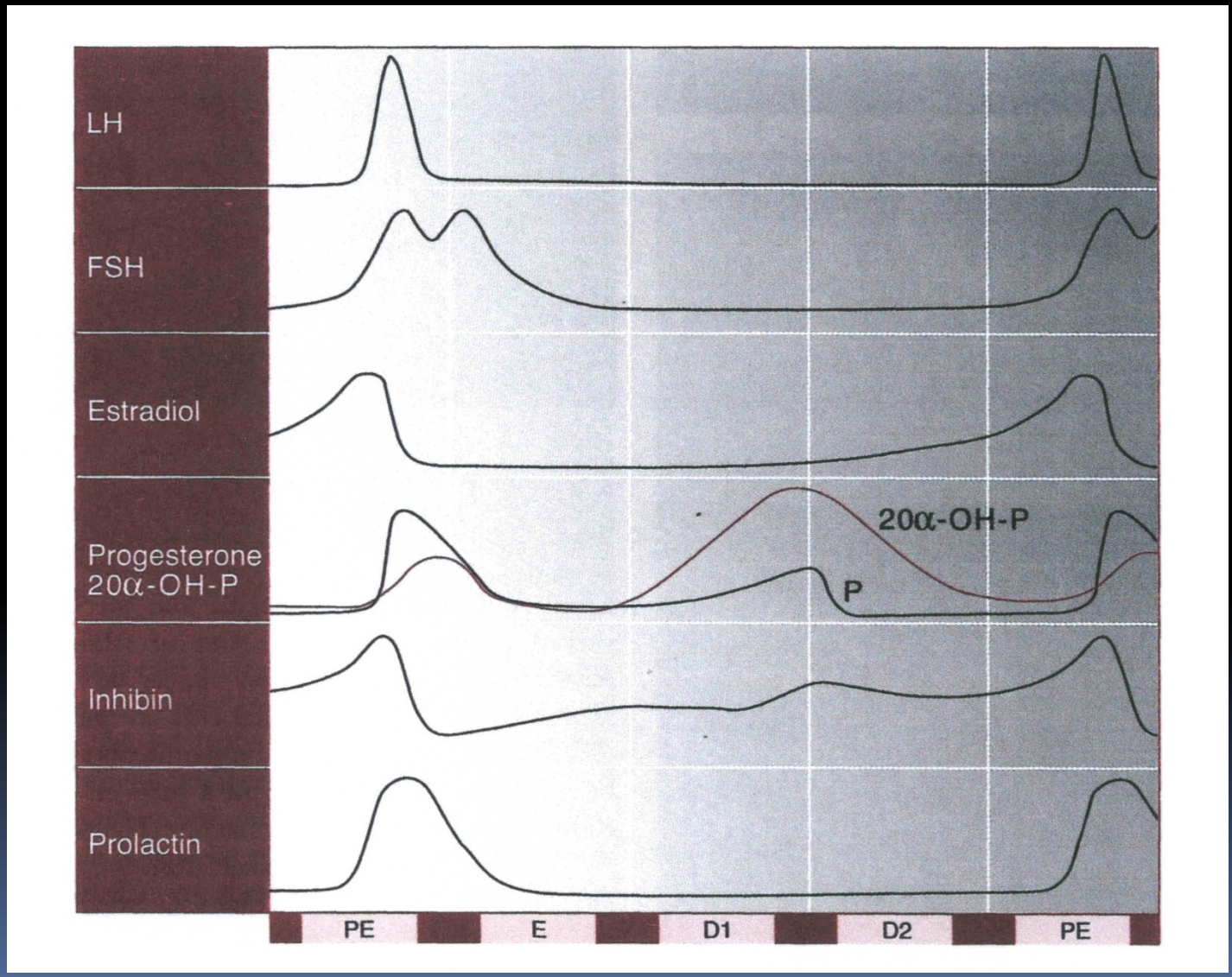
- LH/thecal cell
  - LH stimulates thecal cells to ↑ androstenedione
  - Androstenedione diffuses into granulosa cells
    - Further induces aromatase
    - Is substrate of aromatase to synthesize E
- FSH/granulosa cell
  - FSH stimulates granulosa cells to ↑E and P
    - ↑aromatase activity (↑ E)
    - ↑cholesterol SCC enzyme (↑P)
  - P diffuses into theca cells
    - Is used by the theca cell to make androstenedione

2 Cell, 2 Gonadotropin Theory



from Couse et al 2006

Hormonal Profile, 4 Day Cycle

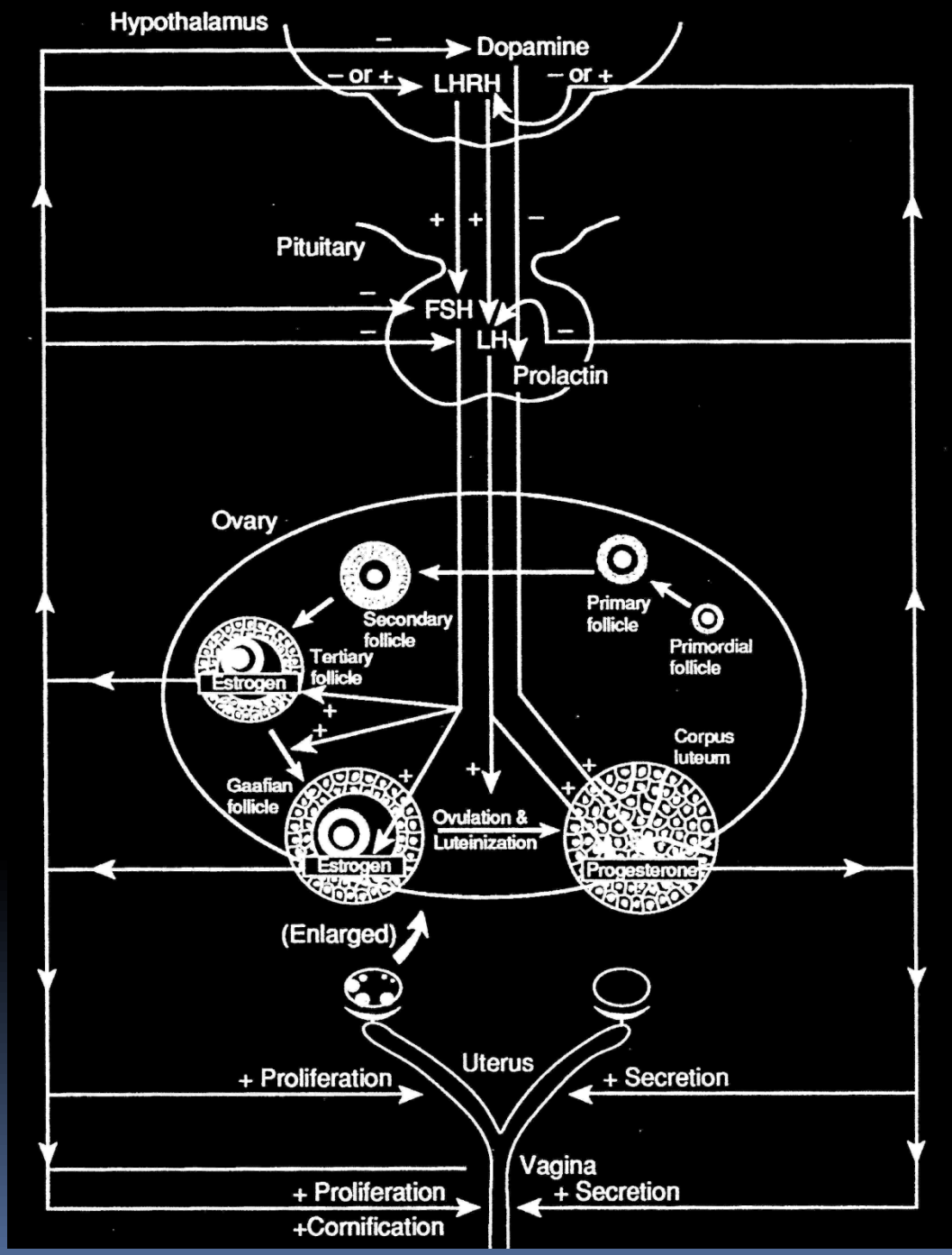


from Maeda et al 2000

Effects of Elevated Estrogen

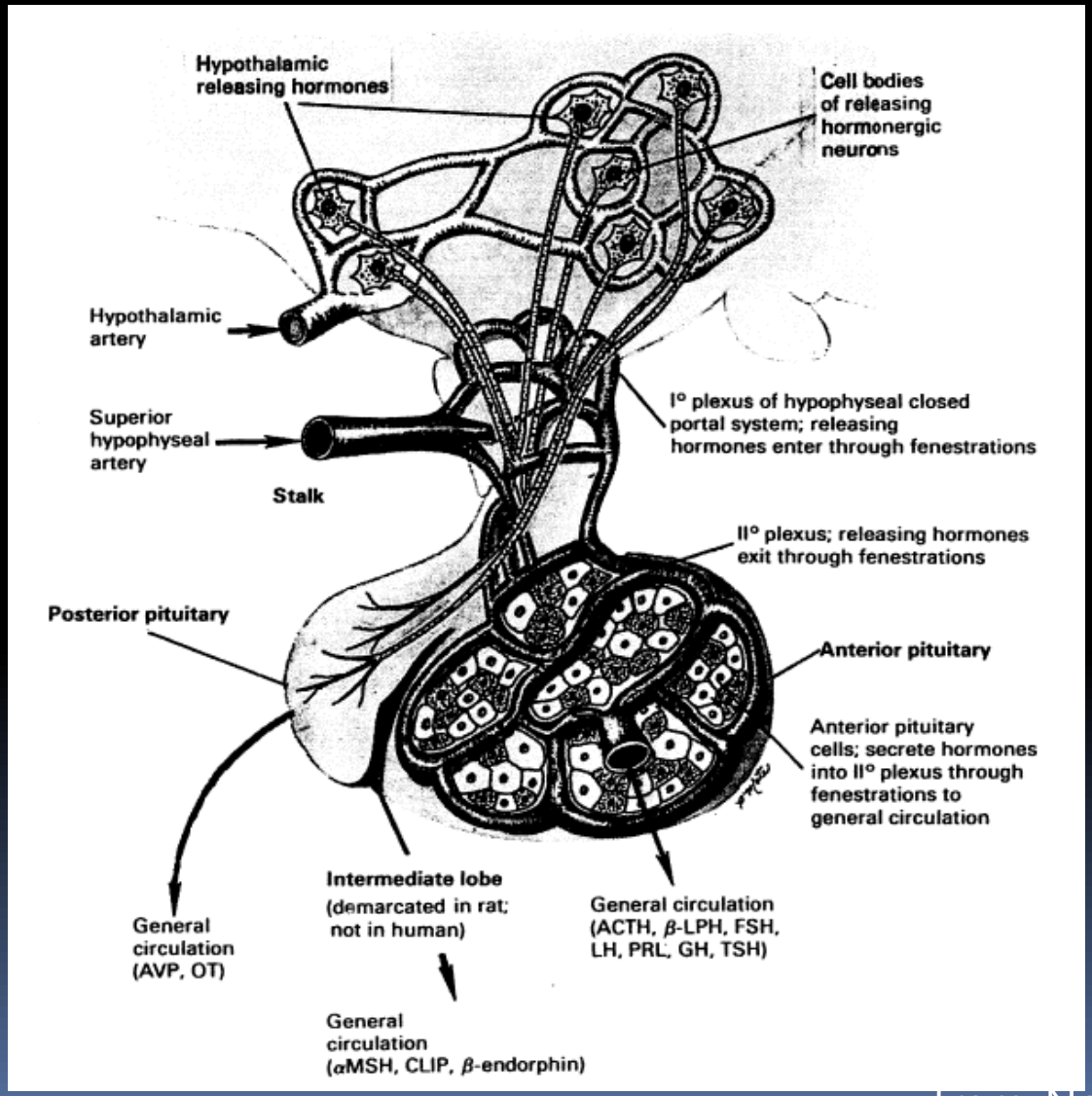
- Preovulatory PRL surge (prior to LH surge)
  - Primarily via positive feedback loop to anterior pituitary lactotrophs
- Preovulatory LH and FSH surges
  - Via a positive feedback loop involving kisspeptin neurons of the AVPV , hypothalamic GnRH neurons , and anterior pituitary gonadotrophs
- Preovulatory ↑follistatin in pituitary
  - Via ↑FSH

Hormonal Regulation of Reproduction



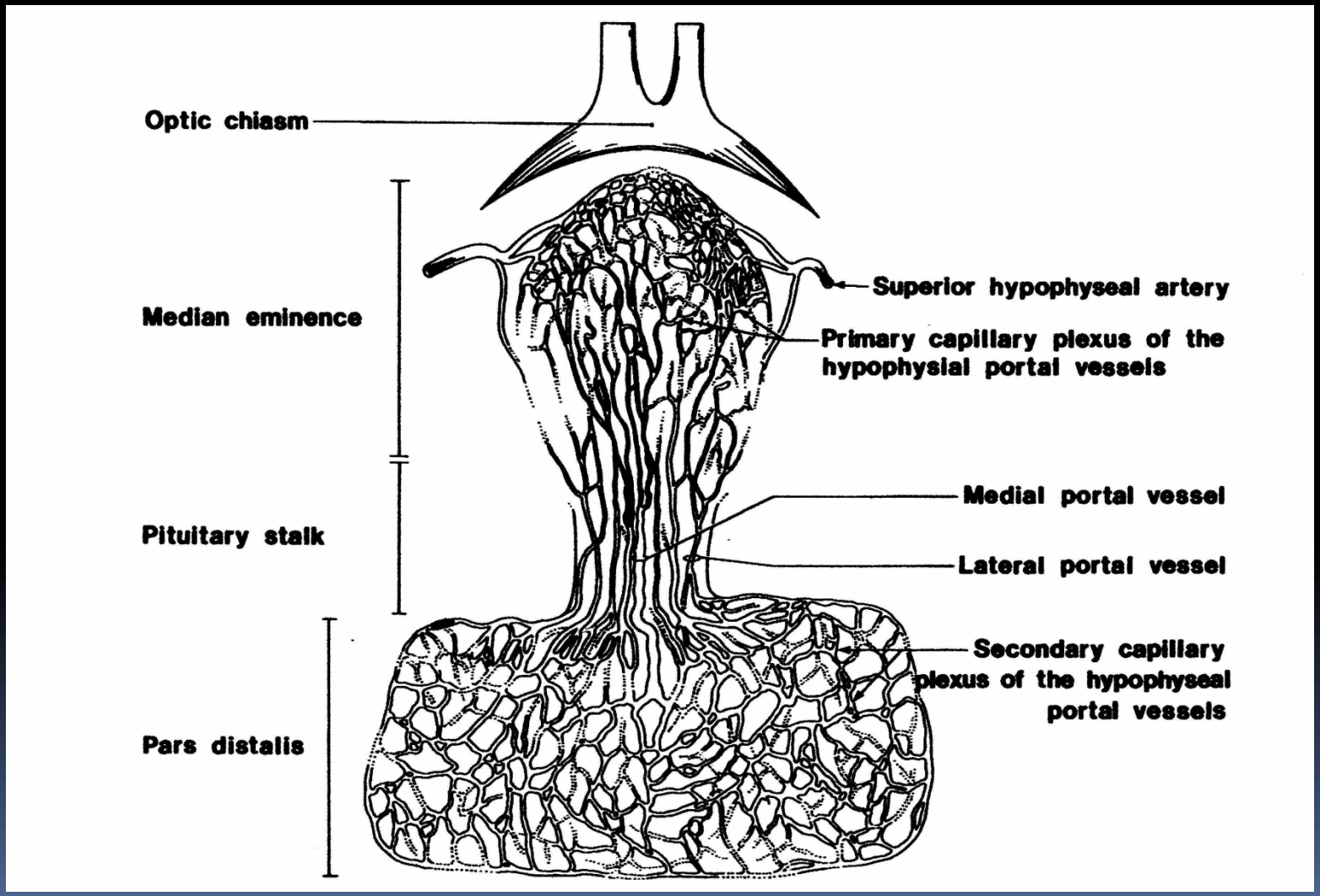
From Yuan & Foley 2002

Neuroendocrine Control of Estrus



from Norman et al 1997

Portal Circulation



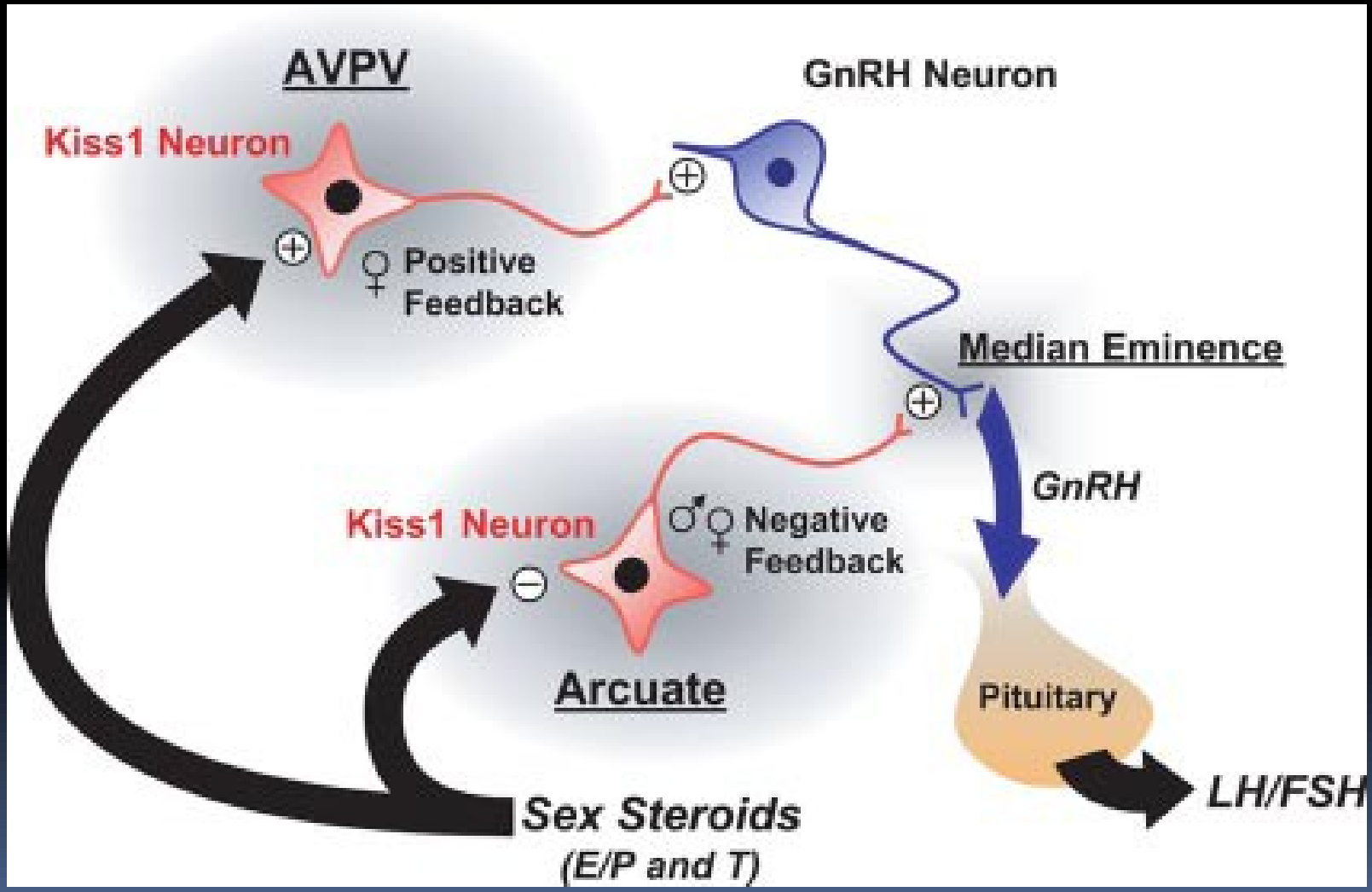
Neuroendocrine Control of Estrus

- GnRH
  - Central control of estrous cycle
  - Secreted by hypothalamic neurons into portal circulation
  - Induces release of LH and FSH from the anterior pituitary
  - Release is under negative and positive feedback control by E/P and T

Neuroendocrine Control of Estrus

- GnRH
  - Release is under negative and positive feedback control by E and T via kisspeptin neurons
    - GnRH neurons do not have ER or AR receptors
    - Kisspeptin neurons have ER and AR receptors
    - Kisspeptin neurons in arcuate nucleus regulate negative feedback control
    - Kisspeptin neurons in AVPV regulate positive feedback control

Kiss1 Signaling in the Forebrain

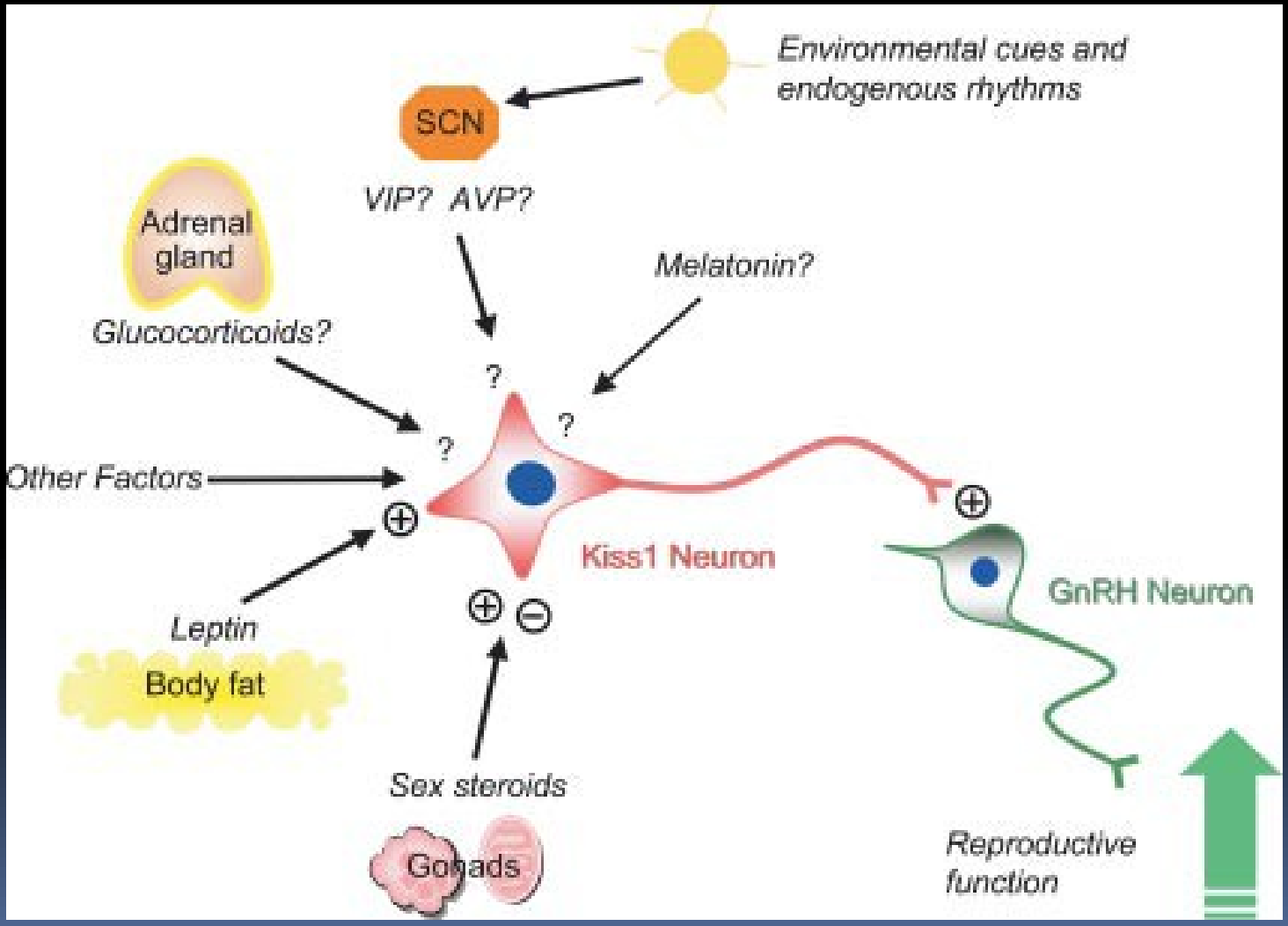


from Oakley et al 2009

Neuroendocrine Control of Estrus

- GnRH
  - Release further refined by inputs from:
    - Environment (light and other circadian rhythms)
    - Glucocorticoids (stress)
    - Nutrition (leptin)
    - Others defined and yet undefined
  - Main effects proposed to occur on kisspeptin neurons with modulatory control on GnRH neurons in hypothalamus

Potential Roles of Kisspeptin Neurons



from Oakley et al 2009

Meanwhile, back at the ovary…

Preovulatory LH Surge

- ↑↑E from ovary induces GnRH release and ultimately LH and FSH release from the pituitary
- Within hours the ovary produces ↑↑P
  - Occurs in granulosa cells and theca interna cells
  - ↑ regulatory proteins to transport cholesterol into the mitochondria
- ↑PR on granulosa cells
- Essentially, luteinization has begun
  - Alternations in timing of luteinization events result in luteinization without ovulation

Preovulatory LH Surge

- Resumption of meiosis I
  - Starts 2-3 hours after LH surge
  - Reduction division (metaphase I) and extrusion of first polar body occurs within follicle
- Induction of COX2 in cumulus cells
  - Results in ↑prostaglandins
  - Prostaglandins are critical for cumulus expansion and ovulation
  - Ovulation occurs 10 hours after COX-2 expression

Preovulatory LH Surge

- Decreased proliferation of granulosa and thecal cells
  - ↓cyclins, ↑cyclin inhibitors
- ↓FSH-R on granulosa cells
- ↓LH-R on granulosa cells
- ↑PRL-R on granulosa cells

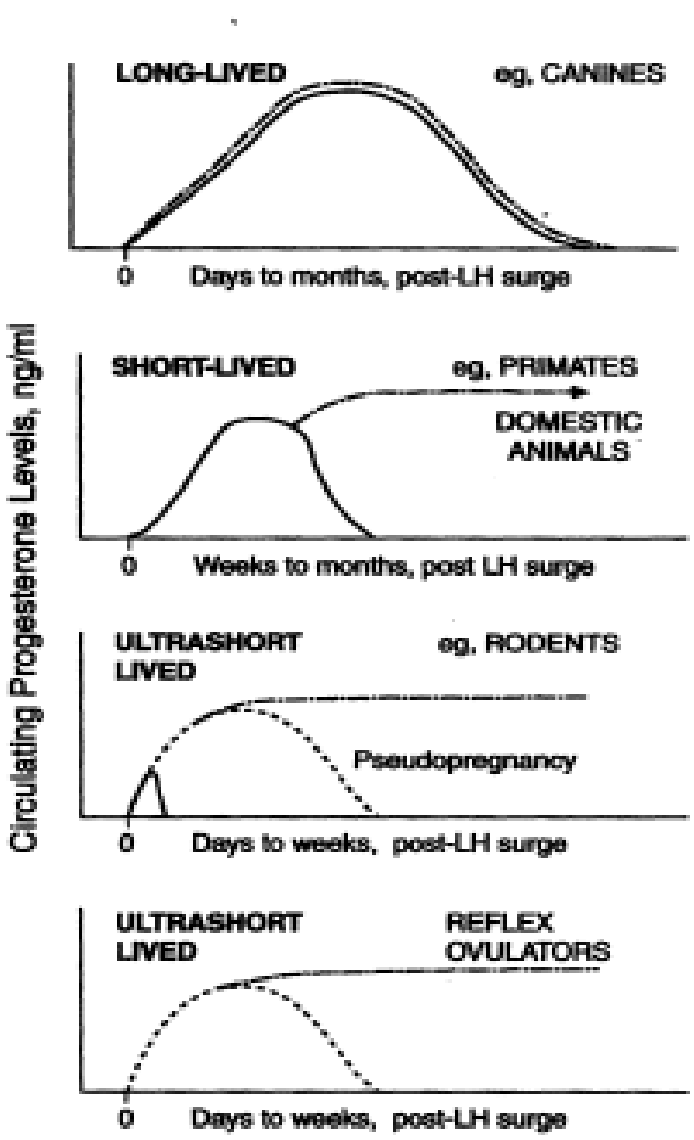
Ovulation

- Is an acute inflammatory reaction
  - Occurs throughout the follicular wall
  - Starts with hyperemia
  - Inflammatory process generates protease activity in granulosa and thecal layers
  - Degradation extracellular matrix (ECM) of wall
- Stigma formation, rupture of wall and extrusion of cumulus + oocyte within minutes
- Ovulated oocyte is in metaphase II of 2nd meiotic division (haploid)

Corpus Luteum

- Life span
  - Short lived (e.g. primate)
    - CL fully functional for finite time
    - Life span extended if pregnancy occurs
  - Ultra short lived (e.g. rat)
    - CL of non-fecund cycle does not secrete sufficient P to support pregnancy
    - Cervical stimulation results in functional CL that secretes P for 12-14 days (pseudopregnancy)
  - Long lived (e.g. dog)
    - CL fully functional in fecund and non-fecund cycles
    - CL lasts same amount of time in pregnant and non-pregnant animals

Differences in Functional Life Span of Corpora Lutea



from Stouffer 2006

Luteinization

- 2 major processes of luteinization
  - Terminated proliferation but rapid hypertrophy and differentiation of steroidogenic cells
  - Angiogenesis
- In the dog, these processes start before ovulation
- In most species both granulosa cells and thecal cells are incorporated within the CL
  - Fate of theca externa is not clear
- In some primates, granulosa and thecal cells remain compartmentalized within the CL

Luteinization

- Hallmark of mature luteinization is the switch of principal steroid product from E to P
  - Caused by increased ability to sequester cholesterol from blood and induction of enzymatic pathways for P synthesis
- Structural and functional involution of CL
  - Occurs at end of non-fecund cycle, end of gestation, or when its progestagenic output is taken over by other sources during a pregnancy
  - Primate: 3-4 days before start of menstruation
  - Dog: cycle day 65-66
  - Rat: at proestrus of following cycle

Luteal Regression

- Structural and functional involution of CL
  - Occurs at end of non-fecund cycle, end of gestation, or when its progestagenic output is taken over by other sources during a pregnancy
  - Primate: 3-4 days before start of menstruation
  - Dog: cycle day 65-66
  - Rat: at proestrus of following cycle

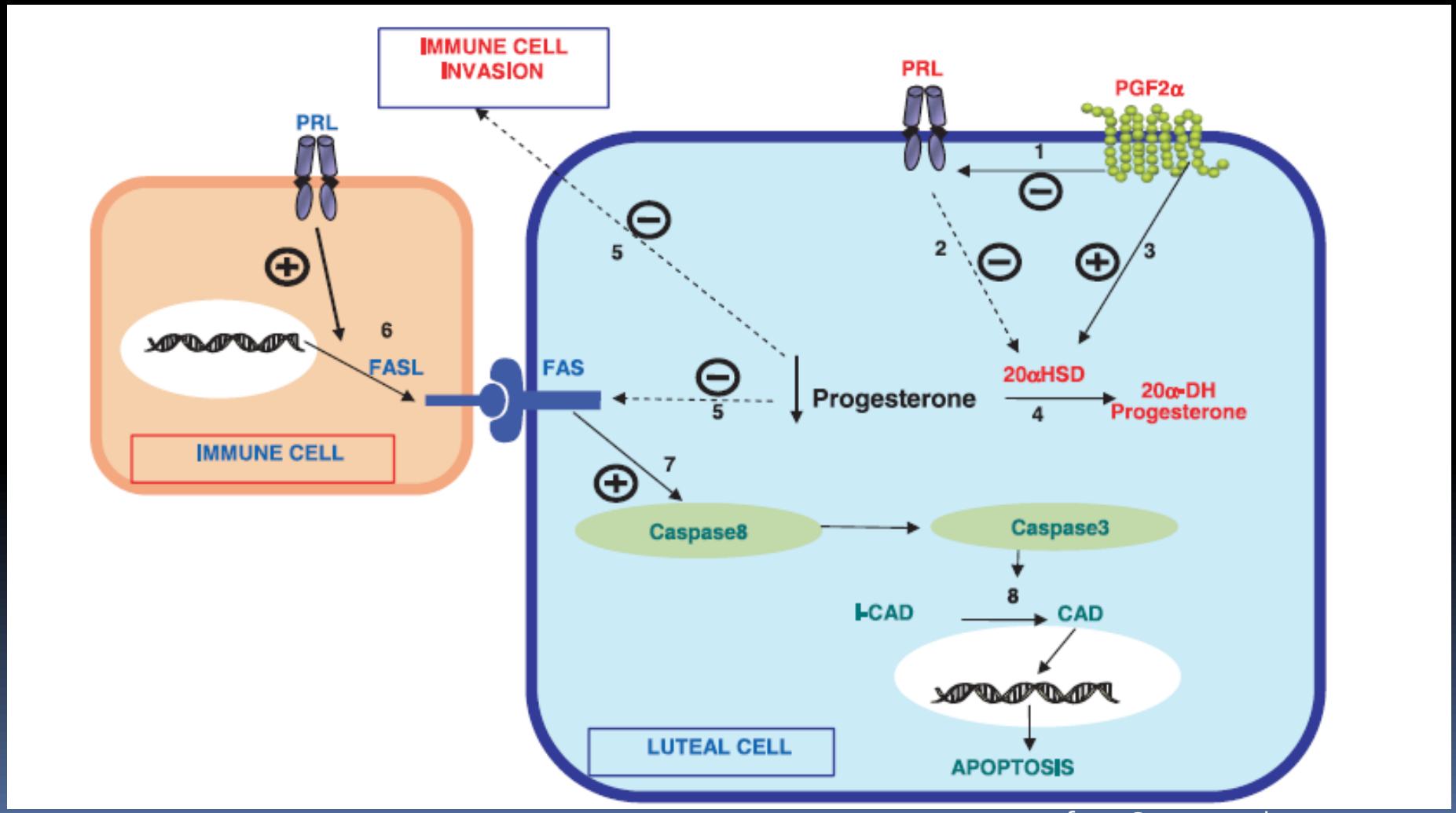
Luteal Regression

- The functional life span of the CL after the LH surge depends on the balance of luteotropins and luteolysins
- Hallmark of luteal regression is declining P
- In cycling rats, regression begins with the proestrous preovulatory surge of PRL in the following cycle
  - PRL is likely an indirect pro-apoptotic signal
  - PRL is associated with influx of inflammatory cells (monocytes/macrophages) into the CL

Luteal Regression

- Proposed PRL effects on luteal regression:
  - PRL induces ICAM expression on luteal endothelial cells (enhances inflammatory cell migration)
  - ↓P levels during regression upregulates FasL in luteal cells
  - Influx of lymphocytes with PRLR into the CL express Fas and induce apoptosis when bind with FasL on luteal cells

Proposed Immune Cell Infiltration in Corpora Lutea

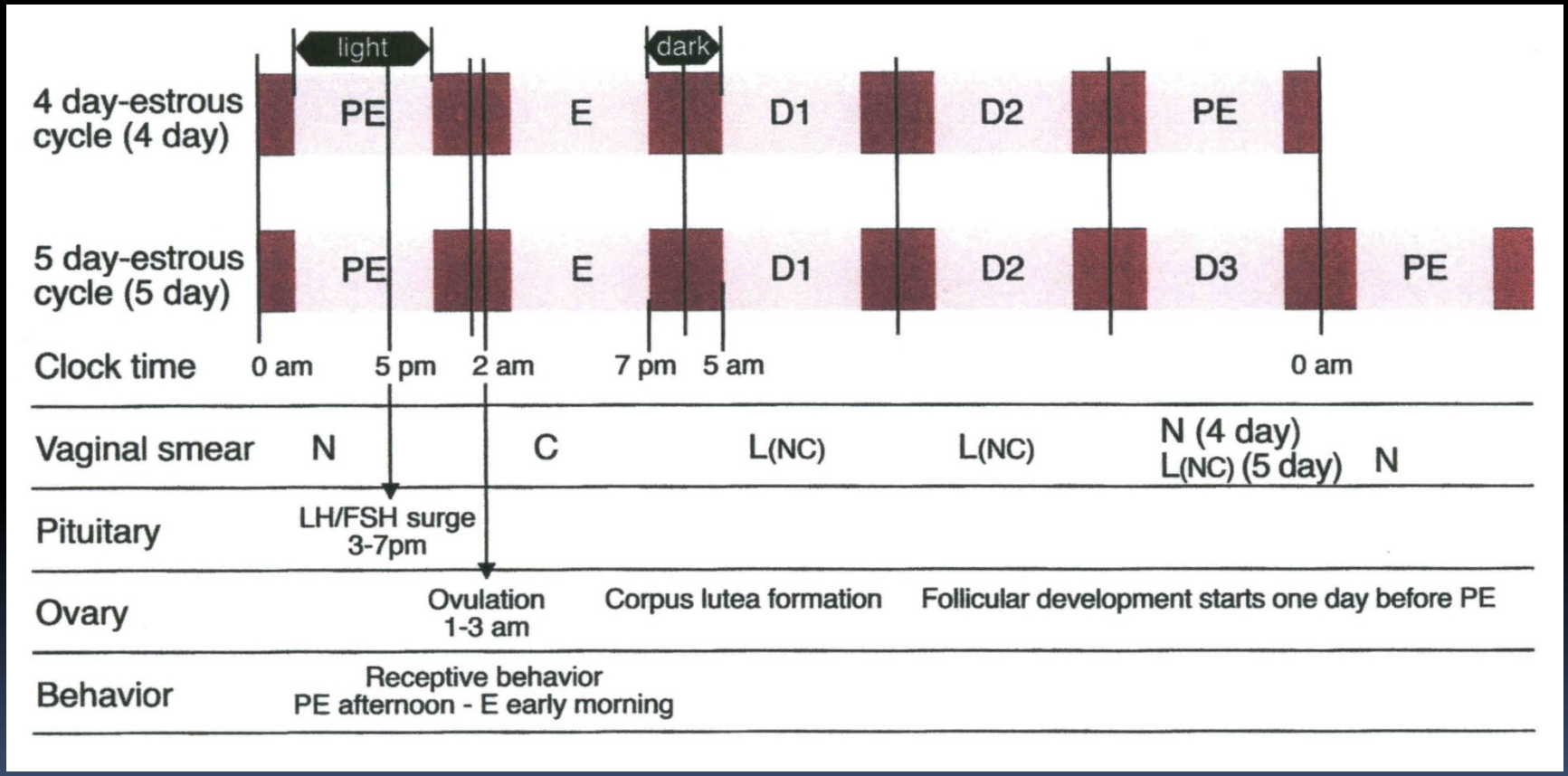


from Stocco et al 2007

Initiation of Next Cycle

- Second FSH peak during estrus
  - Due to decreased inhibin from preovulatory FSH peak
- Preovulatory follicles initiate events for ovulation at next LH surge

Timing of Ovulation in 4 & 5 Day Cycles



from Maeda et al 2000

We've gone full cycle