Clinical Reproductive Endocrinology

Jacky Chan
Clinical Reproductive Endocrinology

- Reproductive hormones
- Assay methods
- Physiology of reproductive hormones in the female
- Clinical aspects of reproductive endocrinology
- General comments
Reproductive hormones

- Definition of hormones
- Chemical classes of reproductive hormones
- Hormone receptors
- Interconversion of steroids in target tissues
- Synthesis and clearance of hormones
Definition of hormones

• synthesized and secreted by ductless endocrine glands
• minute quantities
• directly into the blood vascular system
• Classic endocrine glands:
  – pituitary, thyroid, parathyroid, adrenal, pancreas, ovary, testis, placenta, pineal gland
Definition of hormones

• Reproduction
  – pituitary, pineal glands, gonads, placenta
  – adrenal and thyroid glands
  – uterus, hypothalamus
Reproductive endocrine

1. Gonadotropin-releasing hormone
2. Gonadotropic hormone
3. Sex steroid hormones
Chemical classes of reproductive hormones

• Peptide and protein hormones
  – Releasing hormones, Inhibitory hormones
  – Hypophyseal hormones
  – Neurohypophyseal hormones
  – Placental gonadotropins
  – Subunits

• Steroid hormones

• Prostaglandins
The hormones secreted from the anterior pituitary include:

- **Oxytocin**
  - Uterine muscles
  - Mammary glands

- **ADH**
  - Kidney tubules

Other hormones released from the hypothalamus include:

- **TSH**: Thyroid
- **ACTH**: Adrenal cortex
- **FSH and LH**: Testes or ovaries
- **Growth hormone (GH)**: Entire body
- **Prolactin (PRL)**: Mammary glands (in mammals)
- **Endorphins**: Pain receptors in the brain
Peptide and protein hormones

  - Peptide hormones
  - produced within the hypothalamus
  - Adenohypophysis

- **Inhibitory hormones**
  - Growth hormone (GIH)
  - Prolactin (PIH)

\[ \text{TSH} \uparrow, \text{Insulin} \downarrow, \text{glucagon} \downarrow \]
Releasing hormones

- Gonadotropin-releasing hormone (GnRH)
  - pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH2 (MW of GnRH \( \approx 1,182 \) )
  - regulate the release of FSH and LH
hypothalamic-pituitary-ovarian axis
LH and FSH are released into the systemic circulation, traveling to the target reproductive organs (testes and ovary) where sex steroid hormones are controlled.

LH sends the same message and results in increased testosterone production by the testis due to LH effect on the Leydig cells of the testis.
Releasing hormones

- Thyrotropin-releasing hormone (TRH)
  - pGlu-His-Pro-NH2 (MW of TRH $\cong 362$)
  - regulate the release of TSH, GH, and prolactin

Pathways of thyroid hormone metabolism

[pic]

(pyro)Glu-His-Pro-(NH₂)
Releasing hormones

• Corticotropin-releasing hormone (CRH)
  – CRH is a 41-amino acid peptide
  
  regulate the release of ACTH
Peptide and protein hormones

• Hypophyseal hormones
  – Glycoprotein, FSH and LH, prolactin
  – MW of LH ≈ 30,000 (bovine, ovine, procine, equine)
  – MW of FSH ≈ 32,000 (ovine, equine)
  – MW of prolactin ≈ 23,000 (bovine, ovine, procine)

• FSH, LH, TSH, ACTH – produced in basophilic cells

• Prolactin– localized in acidophilic cells
• Prolactin
  – Luteotrophic hormone (LTH): a corpus luteum-stimulating effect in mouse, rat, dog and cat
  – 198 a.a, MW 23,300 and a 15-minute half-life in sheep
  – increasing in lactation (mamotropin)
  – stimulating the pigeon crop gland to secret “crop milk”
Peptide and protein hormones

• Neurohypophyseal hormones

Oxytocin, Vasopressin (synthesized in hypothalamus)

Axoplasmic fluid

Posterior pituitary

Stimulation of
the N cell

release
Peptide and protein hormones

• Placental gonadotropins
  – **PMSG (eCG, MW \( \geq 70,000 \))**, a protein hormone in serum, produced by fetal trophoblastic cells of pregnant mares
    • The function of endometrial cups begins on day 36 until 120 days of pregnancy.
  – **hCG (MW \( \geq 36,000 \))**, a protein hormone, extracted from the urine of pregnant women from 30-40 days of pregnancy
  – *glucocorticoid, estrogen, progestin, relaxin*...
Endometrial cups develop from cells of the chorionic girdle, circumferentially around the conceptus at a point where the membranes of the allantois and yolk sac meet.
1. The relationship of eCG and CL during equine pregnancy
2. eCG has both FSH-like and LH-like activity, it provides the stimulus for the formation of accessory CLs and maintain a plateau of plasma progesterone from about 50-140 days.
Clinical use of hCG

1. One Step hCG Urine Pregnancy Test
2. hCG is also used to treat women with certain ovarian disorders.
Clinical use of hCG

- Athletes use hCG to increase the body natural production of testosterone which is often depressed by long term steroid use.

- HCG therapy has been found to be very effective in the prevention of testicular atrophy and to use the body own biochemical stimulating mechanisms to increase plasma testosterone level during training.
Peptide and protein hormones

hCG - subunit $\alpha$: 96 a.a
subunit $\beta$: N-terminal specific for hCG

LH - subunit $\alpha$: 96 a.a
subunit $\beta$: 119 a.a specific for LH

FSH - subunit $\alpha$: 96 a.a
subunit $\beta$: specific for FSH

TSH - subunit $\alpha$: 89 a.a in human
subunit $\beta$: 112 a.a in human
Peptide and protein hormones

- **Subunits, $\alpha, \beta$**
  - $\beta$-subunit, unique for each hormone within a species, determines the biological activity.
Chemical classes of reproductive hormones

- Steroid hormones
  - derived from the precursor, cholesterol
  - MW < 500
    - Estrogens (estradiol)
    - Androgens (testosterone)
    - Progesterones (progesterone)
Major Pathways in Steroid Biosynthesis

- Cholesterol
  - Methyl group
  - Methyl group

- Pregnenolone
  - CYP17
  - 3βHSD

- Progesterone
  - CYP21A2
  - 17-hydroxy progesterone
  - CYP21A2

- Deoxycorticosterone
  - CYP11B1
  - 11-deoxycorticisol

- Corticosterone
  - CYP11B2
  - Cortisol

- Aldosterone
  - CYP11B1

- 17-hydroxy pregnenolone
  - CYP17
  - 3βHSD

- Androstenedione
  - CYP19
  - 17βHSD

- Estrone
  - Testosterone
  - Estradiol

Legend:
- Major progestagen
- Major mineralocorticoid
- Major glucocorticoid (species variation)
- Major gonadal estrogens
- Major gonadal androgen
Chemical classes of reproductive hormones

• Prostaglandins
  – a group of 20-C unsaturated fatty acids
  – MW: 300-400
  – local hormones, short biological half-life with a local action
  – found in mammalian tissues, uterus and prostata
Biosynthesis of PG from arachidonic acid
Synthesis of prostaglandin affected by estrogen

- Estrogen ↑ oxytocin receptors ↑
- Inactivated Phospholipase A2
- Activated Phospholipase A2
- Progesterone ↓
- Prostaglandin H2 synthetase
- Phospholipids
- Arachidonic acid
- Prostaglandin H2
- PGE
- PGF
- PGD

Estrogen ↑ Progesterone ↓:
1. proestrus → estrus
2. prior to parturition
Function of prostaglandins

- **PGE<sub>2</sub>** stimulates:
  - vascular dilation
  - bronchiodilation
  - gastrointestinal and uterine contraction
  - inflammatory response

- **PGF<sub>2</sub>** stimulates:
  - vascular constriction
  - bronchioconstriction
  - smooth muscle contraction, e.g. intestinal tract, uterus

- **PGI<sub>2</sub>** inhibits:
  - platelet aggregation (increases cAMP)
  - vascular constriction
  - bronchioconstriction
  - gastrointestinal and uterine contraction

- **PGI<sub>2</sub>** mimics hormones that act through cyclic AMP

- **PGF<sub>2</sub>** inhibits platelet aggregation
vascular uteroovarian pathways for PGF$_{2\alpha}$
vascular uteroovarian pathways for PGF$_2\alpha$

- **Systemic uteroovarian pathway:** horse, rabbit
- **Local uteroovarian pathway:** sheep, cattle, (guinea pig, hamsters, rats)?
- **Combined systemic and local uteroovarian pathway:** swine
Clinical use of PGF$_2$\textalpha

endometritis/pyometra
Clinical use of PGF$_{2\alpha}$

**Option 1 – Two Prostaglandin Injections**

- **Infect Prostaglandin**
- **Day of Schedule**: 1 2 3 4 5 6 7 8 9 10 11 12
- **Estrous Detection and Inseminate**: 13 14 15 16 17
- **Most Repeat Estrus**: 32 33 34 35 36 37 38

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**Lutalyse**

- 1 injection + palpation
- Palpation to detect CL
- Inject Lutalyse to those not getting the 1st injection
- Heat Check & Breed

induction of estrus
Onset of estrus

Ovulation

Corpus luteum/
progesterone

PGF$_{2\alpha}$ injection for
induction of estrus

Onset of estrus

Ovulation

Shorten
estrus cycle

with PGF$_{2\alpha}$

Onset of estrus

Ovulation

Normal estrus cycle
Clinical use of PGF$_{2\alpha}$

induction of abortion/parturition
induction of abortion/parturition
Clinical use of PGF$_2$\textsubscript{α}
Clinical use of PGF$_{2\alpha}$

*induction of abortion*

*endometritis/pyometra*

*misalliance*

only in the case of open pyometra!!
Hormone receptors

• Steroid hormones
  – fat soluble, able to enter all cells
  – low concentration (estrogen: 10-150 pmol/L in non-pregnant female)
    • Receptors enable the target cells
      -to concentrate the hormone within the cell
      -to elicit particular cellular responses
      -to create a biological response on specific binding
The steroid hormone receptor is made up of three domains:
1. a modulating N-terminal domain (transcription-activation domain),
2. a DNA-binding domain,
3. a C-terminal steroid-binding domain.
Hormone receptors

- Protein-bound steroid hormone
- Non-protein-bound hormone

Steroid-receptor complex

- Translocation of complex into nucleus
- RNA synthesis \(\uparrow\)
- Protein synthesis \(\uparrow\)
- Target cells

E2: mammary gland
P4: endometrium
T: sertoli cell
Hormone receptors

Protein hormone

protein-receptor complex

G protein

cyclic AMP

protein kinase

Target cells

actions of FSH and LH in gonads

actions of cholesterol
Activation / Deactivation Cycles for G Proteins and G Protein-Coupled Receptors

1. Outside of cell
   - Hormone
   - Receptor molecule
   - Plasma membrane
   - Inactive G-protein

2. Inside of cell
   - Receptor binds hormone
   - Hormone–receptor complex activates G-protein
   - Inactive adenylate cyclase

3. Active G-protein activates adenylate cyclase
   - ATP
   - cAMP
   - Other reactions

A. Basal state (Resting state)
B. Receptor activation

Activation / Deactivation Cycles for G Proteins and G Protein-Coupled Receptors

B. Receptor activation

C. Subunit dissociation to α and βγ
Tissues responsive to androgens

The production of testosterone by the testis is controlled by the pituitary gland.

Testosterone causes beard and body hair growth, promotes the growth of the prostate gland, contributes to male sexuality and causes bone and muscle growth.

Testosterone stimulates cells in the testis to produce sperm.
Interconversion of steroids in target tissues

Testosterone $\xrightarrow{5\alpha\text{-reductase}}$ 5α-dihydrotestosterone (5α-DHT)

Target cells
Progestosterone conversion in the placenta

Cholesterol → Pregnenolone → Progesterone

17α-Hydroxylase

Cortisol

17α-Hydroxyprogesterone → Androstenedione

17α-Hydroxylase

Testosterone

PGF2α ↑

Estradiol 17β
Synthesis and clearance of hormones

- Synthesis and clearance are main the factors, which influence the hormone concentrations.

  - The concentration of a hormone reflects its rate of synthesis or secretion.
  - metabolized by liver
  - detection of the body fluids (plasma, urine, saliva, feces...)
  - reproductive condition, ex wild animals
Synthesis and clearance of hormones

• under the control of gonadotropins in pulsatile mode

♂ – The secretion of testosterone changes in pulsatile rate.
  – varying from 3.5 – 20 nmol/L within hours

♀ – The pulse rate of \( E_2 \) and \( P_4 \) remains stable.
Synthesis and clearance of hormones

• Placenta gonadotropins (hCG, eCG)
  – produced in high concentration
  – longer half-life than the pituitary gonadotropins
    • FSH, LH ≈ 10-30 minutes
    • hCG ≈ 1.5 days
    • eCG ≈ 6 days
    • equine LH > other species
Synthesis and clearance of hormones

- PGF$_2\alpha$ $\rightarrow$ 15-keto-13,14-dihydro- PGF$_2\alpha$
  - Half life
    - primary PGF$_2\alpha$ $<$ 20 seconds
    - 15-keto-13,14-dihydro-PGF$_2\alpha$ $\approx$ 8 minutes
  - 25 mg PGF$_2\alpha$, IM in cows
    - $>$ 90% excreted in the urine and feces (2:1) over 48 hours
Assay Methods

• Quantification of specific hormones is necessary for the endocrinological events.
  – Weight-gain change
  – Chemical determinations for steroid hormones
  – Urine analysis
Immunoassay technique
Assay Methods

- **Immunoassay**
  - Radio-immunoassay (*Berson et al.*, 1959), Sen: $10^{-9}$
  - Enzyme multiplied immunoassay technique, Sen: $10^{-6}$
  - Fluoescine immunoassay, Sen: $10^{-9}$
  - EIA (*Engvall et al.*, 1970s), Sen: $10^{-12}$
  - Chemiluminescence immunoassay, Sen $10^{-15}$
Because of the ease with which iodine atoms can be introduced into tyrosine residues in a protein, the radioactive isotopes $^{125}$I or $^{131}$I are often used as the radioactive antigen.
Sandwich EIA

1st incubation
Antigen
Immobilized antibody

2nd incubation
Labeled antibody
Substrate
Color formation
Sandwich complex
**Competitive EIA**

**Step 1**
Specific antigen is attached to a solid-phase surface.

**Step 2**
Test specimen, which may or may not contain the antibody, and an enzyme-labeled antibody (conjugate) are added together.

**Step 3**
Chromogenic substrate is added, which in the presence of the enzyme, changes color. The amount of color that develops is inversely proportional to the amount of antibody in the test specimen.
semi quantitative ELISA test
The chemical luminol is oxidised by \( \text{H}_2\text{O}_2 \), to produce a molecule in an excited state. When the excited electrons in the molecules fall back to their ground states, light is given out.
Assay Methods

- **Immunoassay**
  - Radio-immunoassay (Berson *et al*., 1959), Sen: $10^{-9}$
    - Competitive protein binding
  - EIA (Engvall *et al*., 1970s), Sen: $10^{-12}$
  - Chemiluminescence immunoassay, Sen $10^{-15}$

**Diagnostic aids**

**Therapy-monitoring**
Monitoring the seasonal estrus activity of mare

Northern semi-sphere

Subtropical Taiwan
Physiology of reproductive hormones in the female

- Estrous cycle
- Control of the corpus luteum
- Early pregnancy
- Pregnancy and parturition
Endocrine control of cyclical reproductive activity
The duration of estrus, time of ovulation and duration of CL function in domestic animals

<table>
<thead>
<tr>
<th>Animal</th>
<th>CL life span</th>
<th>Estrus</th>
<th>Ovulation</th>
<th>Day of cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cow Doe</td>
<td></td>
<td></td>
<td></td>
<td>1 3 5 7 9 11 13 15 17 19 21 1 3 5</td>
</tr>
<tr>
<td>Ewe</td>
<td></td>
<td></td>
<td></td>
<td>1 3 5 7 9 11 13 15 17 1 3 5</td>
</tr>
<tr>
<td>Sow</td>
<td></td>
<td></td>
<td></td>
<td>1 3 5 7 9 11 13 15 17 19 21 1 3 5</td>
</tr>
<tr>
<td>Mare</td>
<td></td>
<td></td>
<td></td>
<td>1 3 5 7 9 11 13 15 17 19 21 1 3 5</td>
</tr>
<tr>
<td>Queen</td>
<td></td>
<td></td>
<td></td>
<td>1 3 5 7 9 11 21 1 3 5 7 9 15 17 19 21 1 3 5 35 37 39 41 1 3 5 7 50 - 80</td>
</tr>
<tr>
<td>Bitch</td>
<td></td>
<td></td>
<td></td>
<td>1 3 5 7 9 11 50 - 80</td>
</tr>
</tbody>
</table>
Influence of the length of day light on the ovary function in mares
estrous behavior of mares
Large amounts of equine LH are released during an 8- to 9-day period with ovulation occurring on the 3rd to 5th day.

**Control of the corpus luteum**
The Ovum

Graafian follicle

Azan 75

- FA = follicular antrum
- CR = corona radiata
- O2 = secondary oocyte
- ZG = zona granulosa
- TI = theca interna

- The oocyte is released along with the corona radiata.
- Notice the clear ring just around the ovum (arrow tip): this is the zona pellucida.

From Wheater’s Functional Histology, 4th ed., 2003
1. theca interna has LH receptor and produces testosterone.

2. granulosa cell has FSH receptor which causes cell to take testosterone from theca and convert it to estradiol.

3. dominant follicle has LH receptors on granulosawhich can respond to LH to drive progesterone production.

Each granulosa cell has approximately 1500 FSH receptors.
Ovarian steroidogenesis

FSH causes increased aromatase activity, which converts testosterone to estradiol

### Production Rate of Sex Steroids in Women at Different Stages of the Menstrual Cycle

<table>
<thead>
<tr>
<th>SEX STEROIDS*</th>
<th>Early Follicular</th>
<th>Preovulatory</th>
<th>Midluteal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Progesterone (mg)</td>
<td>1</td>
<td>4</td>
<td>25</td>
</tr>
<tr>
<td>17Hydroxyprogesterone (mg)</td>
<td>0.5</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Dehydroepiandrosterone (mg)</td>
<td>7</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Androstenedione (mg)</td>
<td>2.6</td>
<td>4.7</td>
<td>3.4</td>
</tr>
<tr>
<td>Testosterone (mg)</td>
<td>144</td>
<td>171</td>
<td>126</td>
</tr>
<tr>
<td>Estrone (mg)</td>
<td>50</td>
<td>350</td>
<td>250</td>
</tr>
<tr>
<td>Estradiol (mg)</td>
<td>36</td>
<td>380</td>
<td>250</td>
</tr>
</tbody>
</table>
ovulation

- cumulus cells produce hyaluronic acid and proteins to cause **cumulus expansion**
- increased blood flow to follicle (vascular permeability ↑)
ovulation

- Triggers increase protein and steroid synthesis, and cellular differentiation within follicle
- Plasminogen is converted to plasmin by plasminogen activator (active collagenase $\uparrow$).
- Prostaglandin stimulates smooth muscle to contract and force the ruptured follicle to empty.
Control of the corpus luteum

- **CL** is responsible for the estrous cyclicity.
- **Uterus** controls the lifespan of the CL in the cow, ewe, sow and mare.
  - Estrogens initiate uterus to synthesizes PGF$_2\alpha$.
  - Regression of CL is accomplished within 48 hrs.
Control of the corpus luteum

- **Estrogens** → endometrial oxytocin receptors initiate the release of PGF$_2\alpha$, which causes the reaction of luteal regression.
- In ruminants, releasing of PGF$_2\alpha$ is induced by oxytocin secreted by the CL.
- In dog and cat, PGF$_2\alpha$ → CL?
Control of the corpus luteum

LH stimulates the secretion of progesterone from small luteal cells while PGF$_2$α inhibits secretion of progesterone from large luteal cells and causes degeneration of these cells.
Early pregnancy

• Maternal recognition
  – modification of PGF$_{2\alpha}$ by the luteotropic products from the conceptus and uterus
    • Trophoblast proteins (cow, ewe, doe)
    • Estrogens (sow, mare)
    • intrauterine mobility of the embryo (mare)
    • ? (bitch, queen)

  • antiluteolytic action or luteotrophistic effect
  • to extend the luteal activity
Early pregnancy

• **Trophoblast proteins** (*cow, ewe, doe*)
  – oTP-1 secreted between 10 and 21-24 days
  – bTp-1 secreted between 16-19 days
  – cTP-1 secreted on Day 17
    • to bind uterine oxytocin receptors
    • to block the uterine PGF\(_2\alpha\) secretion
Trophoblast proteins

• **Ovine trophoblast protein (oTP-1, IFN- τ )**
  – MW 18,000, produced on Day 10
  – inhibiting the uterine oxytocin receptors
  – inhibiting the synthesis of PGF$_2$α

\[ \text{PGF2} \downarrow, \text{PGE2} \uparrow \]
Early pregnancy

• **Conceptus-derived estrogens (sow, mare)**
  - estrone and estradiol 17-β converted from progesterone on Day 11, Day 14-18
  - rapid elongation of the blastocyst
  - releasing of calcium, specific proteins
    - altering an endocrine of PG to an exocrine PG
    - metabolized to inactive PGFM
    - PGF2 → PGE2
The secretion of estrogens by the blastocyst stimulates the release of calcium, specific polypeptides and proteins.

1. altering the PGF2 from the endocrine to an exocrine
2. intrauterine PGF2 metabolised to inactive PGFM by fetal membranes

Diagram:
- Conceptus
- Estrogen
- Exocrine
- Endocrine
- PGF2α
- PGE2
- Endometrium
- LHR
- Corpus Luteum
- Counter Current System in Mesometrium
• There is extensive mobility of the equine conceptus within the horns and uterine body before fixation occurs between days 16 and 18.

• ?? Endometrial Prostaglandin Synthesis Inhibitor, (EPSI) from endometrium
<table>
<thead>
<tr>
<th>Animal</th>
<th>Day of recognition</th>
<th>Day of definite attachment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mare</td>
<td>14-16</td>
<td>36-38</td>
</tr>
<tr>
<td>Cow</td>
<td>16-17</td>
<td>18-22</td>
</tr>
<tr>
<td>Sow</td>
<td>12</td>
<td>18</td>
</tr>
<tr>
<td>Ewe</td>
<td>12-13</td>
<td>16</td>
</tr>
<tr>
<td>Goat</td>
<td>17</td>
<td></td>
</tr>
</tbody>
</table>
Corpus luteum & Pregnancy

- \( P_4 \) from CL: cow, sow, goat, dog and cat
- \( P_4 \) from CL + fetoplacental unit: mare, sheep
Pregnancy

1. The sheep placenta produces enough progesterone by day 60-70.

2. Luteal progesterone is required throughout gestation in cattle and goats because their placenta secrete much smaller quantities of progesterone.

Relative concentrations of progesterone (■) and estrogens (●) in maternal serum

Circulating Hormone Concentrations in Mares During Pregnancy

- Chorionic gonadotropin
- Estrogens - from ovaries
- Estrogens - from placenta
- Progesterone - from ovaries
- Progestins - from placenta
- Relaxin
Fetal-Placental Unit

Dihydroepiandrosterone

Cholesterol

Fetal blood

Maternal blood

Adrenal

Compartments

Fetal

Liver

16-OH Dihydroepiandrosterone
Parturition within 12 hours

Relative Changes in Hormone Levels

Days from Parturition

Progesterone ↓

rectal temperature (°C)

Parturition within 12 hours

weeks after mating
Parturition

Parturition

Infusion induces parturition

Fetal Adrenal

Adrenergic corticotrophic hormone (ACTH)

Corticotrophin Releasing Hormone

Hypothalamus

Removal blocks parturition

Corticosterone

Estrogen (increase)

Glucocorticoid

Progesterone (decrease)

Prostaglandin F2α (increase)

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Parturition

- Fetal hypothalamus
  - ACTH
  - Fetal pituitary
    - Adrenal corticosteroids
      - Fetal adrenal
      - Cotyledons/myometrium
      - Release of PGF2α
        - Myometrial contractions
          - Oxytocin
            - Posterior pituitary
            - Oxytocin
              - Myometrial contractions
                - Relaxation and softening of cervix, pelvic ligaments, perineum
                - Relaxin
                  - Expulsion of fetus
                    - Stimulates vagina and cervix

- Cortisol
  - Fetal maturation
    - Decrease in placental P4
      - Luteolysis of the CL of pregnancy
        - Decrease in serum P4
          - Increased myometrial excitability

- Adrenaline
  - Relaxation and softening of cervix, pelvic ligaments, perineum
Parturition

• Prostaglandins are soluble in fat and water, Prostaglandins → pass from cell to cell

• Actions:
  – smooth muscle contraction
  – luteolysis
  – softening of cervical collagen
  – developing gap junctions
  – movement of Ca^{2+} between the myofibrils
    • PGF$_2\alpha$, PGE $\uparrow$, PGI$_2$ $\downarrow$
Smooth muscle cells

- Intermediate filament
- Thick filament
- Thin filament
- Membrane dense area
- Dense body
- Mechanical junction coupling cells
- Gap junction for electrical and chemical communication

(a) Cell 1
Gap junction
Cell 2
Cell 1 cytoplasm

(b) Channel formed by pores in each membrane
Extracellular space
Open
Closed
6 connexins = 1 connexon
Extracellular side
Cytoplasmic side

Bob Crimi
molecules (< 1000 daltons) that can pass include calcium ions, cAMP, ATP, glutathione, and large molecules like amino acids and sugars etc.
Smooth muscle contraction

- **Muscle protein: myosin and actin**
  - Calmodulin activates MLC kinase

- **Calcium-Calmodulin**
  - MLC kinase
  - Inactive myosin light chain
    - $\text{(M}^*-\text{ATP}) + A \rightleftharpoons \text{(M}^*-\text{ADP-A}) + \text{Pi}$
    - $\text{(M}^*-\text{ADP-A}) \rightleftharpoons \text{(M-A)} + \text{ADP}$

- **Actomyosin complex**
  - Contraction
Smooth muscle contraction

1. Calcium ions come from outside of the cell.
2. Calcium ions bind to calmodulin-MLCK complex on myosin.
3. The enzyme complex breaks up ATP into ADP and transfers the Pi directly to myosin.
4. This Pi transfer activates myosin.
5. Myosin forms cross-bridges with actin.
6. When calcium is pumped out of the cell, the Pi gets removed from myosin by phosphatase.
7. The myosin becomes inactive, and the muscle relaxes.
The top view shows a relaxed smooth muscle cell. Note the focal densities and the network of actin and myosin filaments. When contracted, the filaments slide together and pull the cell to a more rounded appearance.
Calmodulin is one of the primary receptors of intracellular calcium. 4 $\text{Ca}^{2+}$ bind to calmodulin. At each binding site, $\text{Ca}$ interacts with oxygen atoms, mainly glutamate and aspartate side-chain carboxyl group.

Calmodulin mediates processes such as inflammation, metabolism, apoptosis, muscle contraction, nerve growth, immune response...
Parturition

- **Actions of estrogens**
  - contractile protein synthesis $\uparrow$
  - receptors of oxytocin and PGF$_2\alpha$ $\uparrow$
  - calmodulin synthesis $\uparrow$
  - MLC kinase activity $\uparrow$
  - gap junctions $\uparrow$
Parturition

• **Actions of progesterone**
  - gap junctions ↓
  - receptors of oxytocin and PGF$_2\alpha$ ↓
  - synthesis of oxytocin and PGF$_2\alpha$ ↓
  - calmodulin binding ↑
Parturition

- **Actions of oxytocin**
  - dependent on progesterone ↓, estrogens ↑
  - stimulation of receptors in the anterior vagina and cervix → oxytocin release
    - increasing PG release
    - increasing Ca^{2+} release
Clinical aspects of reproductive endocrinology

- Steroid hormone concentrations are lower in animals than in human. (estrogens < 1/10)
- Progesterone analysis give the most useful information on the reproductive status.
- Blood, milk, urine, saliva and feces are sources for endocrine information.
Clinical aspects of reproductive endocrinology

- **Cow:**
  - **P4 for early diagnosis of pregnancy**
    - Negative forecast (98%) is better than positive forecast (67-90%).
  - **P4 for postpartum ovarian activity**
  - **Estrone sulfate for diagnosis of pregnancy**
    - from Day 120, 96% accuracy rate
  - **Bovine pregnancy specific protein B for early diagnosis of pregnancy on Day 30**
P₄ for postpartum ovarian activity or early diagnosis of pregnancy
Follicular cyst
Ovarian cyst

$P_4$ for postpartum ovarian activity

Ovarian cyst
$P_4$ for postpartum ovarian activity

Luteal cyst
Diagnosis of the ovarian diseases in cattle

Days of estrus cycle

Progestosterone concentration (ng/ml)

Persistent CL or luteal cyst

Normal cycle

Ovarian atrophy or follicular cyst

Sampling time
Treatment of bovine follicular cyst

GnRH

Hypothalamus
Hypophyse

Regulation of estrus cycle

LH

Progesterone

ovulation stimulation or **cyst luteinization** or ovulation of cyst
Monitoring the treatment of bovine follicular cyst

Progestosterone concentration (ng/ml) vs. Days after application of GnRH

- GnRH inj.
- PGF2α inj.
- AI
- Sampling times

Days after application of GnRH range from -10 to 24.
Clinical aspects of reproductive endocrinology

• Sheep:
  – P₄ for early diagnosis of pregnancy
    • P₄ in estrus: 0.15-0.25 ng/ml, in CL phase: 2-4 ng/ml,
      in pregnancy from 60 days: 12-20 ng/ml
  – Estrone sulfate (fetoplacenta) for diagnosis of pregnancy from 70 days (≒ 88%)
  – Pregnancy specific protein, ovine placental lactogen for diagnosis of pregnancy
Clinical aspects of reproductive endocrinology

• **Sow:**
  – $P_4$ for early diagnosis of pregnancy
  • $P_4$ in pregnancy: 20-50 ng/ml
  – Estrone sulfate for early diagnosis of pregnancy from Day 20-26, 98% accuracy rate
Clinical aspects of reproductive endocrinology

• Horse:
  – eCG for diagnosis of pregnancy
  – P₄ for ovarian activity
    • Persistent CL exhibits low P₄ value
    • Presence of cyclic ovarian activity
    • Ovulation prediction (88%)
  – Estrone sulfate for diagnosis of pregnancy
  – Testosterone for diagnosis of granulosa cell tumor, cryptorchidism
• **Dog:**
  - P₄ for preovulatory breeding
  - P₄ for diagnosis of remaining ovarian remnant
  - Testosterone for checking the secretory status of the Ledig cells
  - Confirmation of sertoli cell tumors: LH ↓, T ↓ (Cryptorchidism)
  - Relaxin for confirmation of pregnancy status
- **Cat:**
  - $P_4$ for confirmation of ovulatory failure, $P_4 \downarrow$ 10 days postbreeding ($P_4 < 0.15 \text{ ng/ml}$)
  - Estrogen analysis (from feces) for ovarian follicular activity, completeness of an OHE
  - Testosterone for evaluation of Ledig cell function in the testis
  - Relaxin for confirmation of pregnancy status
General comments

• Reference values developed by laboratories are depend on the type of assay used.
  – 1 ng/ml as the basal value of $P_4$

• Harvesting serum soon after collection
  – RBC can metabolize $P_4$ to other steroids
  – Temperature ↓, $P_4$ decline slowly
  – Skim milk for the immunoassay of $P_4$
contraception