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# Invasion of Foreign White Blood Cells into Vaginal Epithelium

Brent Ibata
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## Introduction

Lymphocytes and macrophages, the tiny warriors of the immune system, constantly patrol the mucosal borders of the body to fend off possible intruders. But can the Common Mucosal Immune System (CMIS) fall prey to a Trojan Horse? HIV infected cells have been theorized to be the Trojan Horse that caries the virus' genetic code to the mucosal barriers of a potential victim. The question is where, in the reproductive tract does the infection initially take root and by which vector? One suggestion is that lymphocytes may transmit HIV to CD4-negative epithelial cells.(Phillips, 1994) Another suggestion is that HIV initially infects host macrophages in the cervical transformational zone.(Nuovo, 1994) It hypothesized here, in this paper, that foreign leukocytes can invade the female reproductive mucosal epithelium and enter into the lymphatic system. This hypothesis is partially supported by the unpublished observations (Quayle, et al 1995) of mononuclear cell adherence and penetration into endocervical epithelium, in-vitro.

In our experiment, a DNA binding fluorescent dye (H33342) was used to observe the migratory behavior of foreign leukocytes. The fluorescent cells were placed into the vagina of secretory phase Balb/C mice and at different intervals, from injection, the mice were dissected. Since the vagina is drained lymphatically into the iliac lymph nodes of mice, the iliac nodes were dissected out and observed for H33342 dyed cells. The vagina was also removed, fixed, and later sectioned. The 5μm sections were observed for evidence of blue cell penetration into vaginal/cervical epithelium.

## Materials

Avertin
Balb/C Mice, Female, 40-100 days old @ ordering (Harlan)
Cell Strainer 70μm Nylon (Becton Dickinson Labware)
Depo-Provera® (Upjohn) in PBS
Estradoil in peanut oil
H33342 (Hoescht 33342, Sigma Chemical Company)
Metofane™ (METHOXYFLURANE) Manufactured for Pitman-Moore
Propidium Iodide P-4170 (Sigma)

PVA mounting medium
RPMI-1640 Medium (Sigma Chemical Company)
Tissue-Tek® O.C.T. Compound (Embedding Medium for Frozen Tissue Specimens) (Miles Inc.)
2% Fetal Calf Serum in filtered Hanks Balanced Salt Solution (without Sodium Bicarbonate, GIBCO™ Laboratories) 0.05% $NaN_3$
2% Paraformaldehyde Fixative
10% Sucrose in 0.1M Pi pH 7.4 0.1% $NaN_3$ or 10% Sucrose in PBS 0.1% $NaN_3$

# Methods

## In Vivo Staining of Blue Cells

500µL of H33342 in HBSS [250µg/mL] was injected into the peritoneal cavity of donor Balb/C mice. ~18 hrs later the mice were anesthetized using Metofane, and 5mL of RPMI-1640 was injected into the peritoneal cavity. As much of the origin 5mL was withdrawn and then spun down at 1500 rpm for 6 minutes. The supernatant was poured off and the harvested peritoneal cells were resuspended in the residual supernatant (aprox 20µL).

## Injection of Blue Cells into Vaginal Lumen

7 days before blue cell injection, female Balb/C mice were treated with .001mg of Estradoil in peanut oil. 6 days before injection the same mice were treated with 2 mg of Depo-Provera in PBS. This was done so that the vaginal epithelium would be in the secretory phase at the time of fluorescent cell injection. On the day of the injection the E/DP treated mice were anesthetized using 0.28mL of Avertin injected into the peritoneal cavity. The resuspended in-vivo stained donor cells were pipetted into the vaginal lumen of the anesthetized E/DP treated recipient. The mice remained anesthetized for ~20-30 minutes.

At this point several different variables were explored; mucus washout, donor cell washout, and treatment to dissection time. One group of recipients had a mucus washout immediately before donor cells were introduced into the vagina, while another similar group did not have a mucus washout. Several different times for washing out the donor cells were explored. The time from donor cell introduction to dissection of the animal were varied to observe the frequency of donor blue cells in the recipient's vaginal and cervical epithelium, as well as in the iliac lymph node.

## Dissection

At the time of dissection the vagina with uterine horns was removed and placed in 2% Paraformaldehyde fixative for 2 hrs at 4° C. The iliac lymph nodes were removed by

gross dissection and pressed through a 75μm nylon cell strainer into 2% FCS in filtered HBSS 0.05% $NaN_3$. The lymph node cell suspension was spun down twice at 1500 rpm (6 min) and then observed using a hemocytometer. The number of fluorescent cells, both bright and dim, were noted.(see graph) After 2 hrs in Paraformaldehyde the vagina was placed into 10% sucrose at 4° C for at least 30 minutes. The vagina/uterus was further dissected into vaginal and cervical regions, imbedded in O.C.T. compound, frozen, and stored at -70°C. 5 μm sections were made in a cryostat and then stored at 4° C until ready to be viewed. Immediately before viewing, the sections were stained using 50 μL of 0.001mg/mL Propidium Iodide P-4170 for one minute; washed for one minute in distilled water; blotted dry; and mounted using PVA mounting medium. The propidium iodide was used as a couterstain to the blue fluorescing H33342. Care was taken at all times to minimize the sections exposure to light.

The PVA mounted slides of vagina and cervix were observed using an Olympus fluorescence microscope. Images were captured using a video-grabber and stored using NIH Image. The exposure time and magnification were recorded to give a rough estimate as to the relative intensities of fluorescence.

# Results

Before we could follow the migration of donor WBC's we had to eliminate the non-specific staining of the vaginal epithelium that had occurred in our initial trials. This staining was due to H33342 loss from the peritoneal cells by either diffusion out of live cells or leakage out of dead cells. It was found that by staining the cells in-vivo for ~18hrs, as opposed to in-vitro, we could allow the expected loss of dye (Durand, 1982) to occur before introducing the cells into the vagina. In an attempt to further reduce non-specific staining we washed out the injected cells at 6 hrs. The rational to this was that if any cells were going to migrate they were either adhered to the epithelium or already migrating towards the basement membrane at 6 hrs. The 6 hr washout succeeded in further reducing the non-specific staining. With the combination of in-vivo staining and a 6 hr washout, the non-specific staining was reduced to an acceptable level.

Once the problem of H33342 non-specific staining was resolved as best it could, we concentrated on looking for blue cells in two locations. One location we looked for blue cells was in frozen vaginal sections. Blue cells were sporadically found to adhered to epithelium. These cells must have resisted the vaginal washing at 6 hrs.(Figs 1.1-1.2) Although this observation was not common, we consistently observed blue cells in the vaginal and cervical epithelium (Fig 1.3-1.4) The other location we looked for blue cells was in the iliac lymph node cell suspension. At 24 hrs the greatest number of blue cells were present in the iliac lymph node. (Graph and Figs 1.5-1.6)

Fig 1.1 H33342 stained peritoneal cells adhered to recipient's vaginal epithelium.

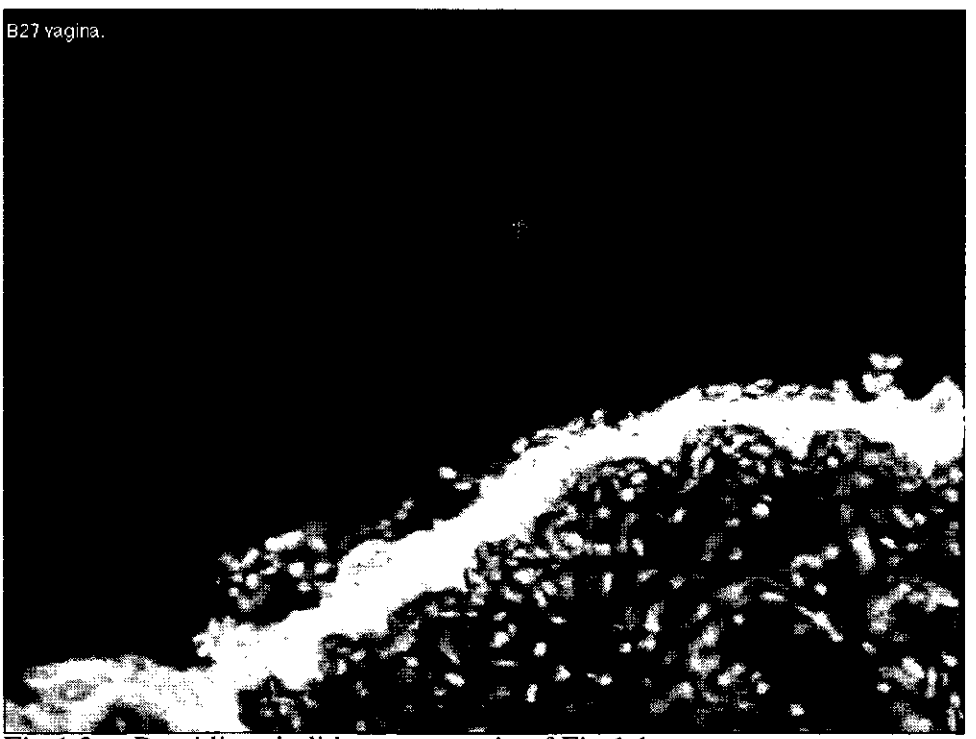


Fig 1.2 Propidium iodide counterstain of Fig 1.1

Fig 1.3 H33342 stained cell in the vaginal epithelium superficial to the basement membrane.

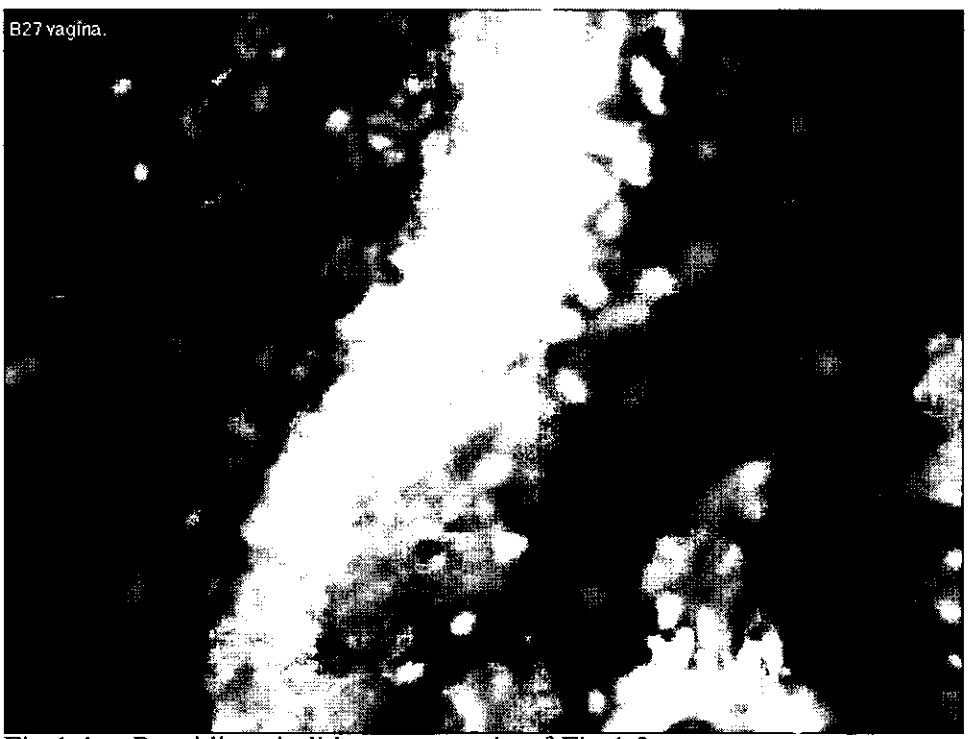


Fig 1.4 Propidium iodide counterstain of Fig 1.3

Fig 1.5 H33342 stained cell in lymph node cell suspension.

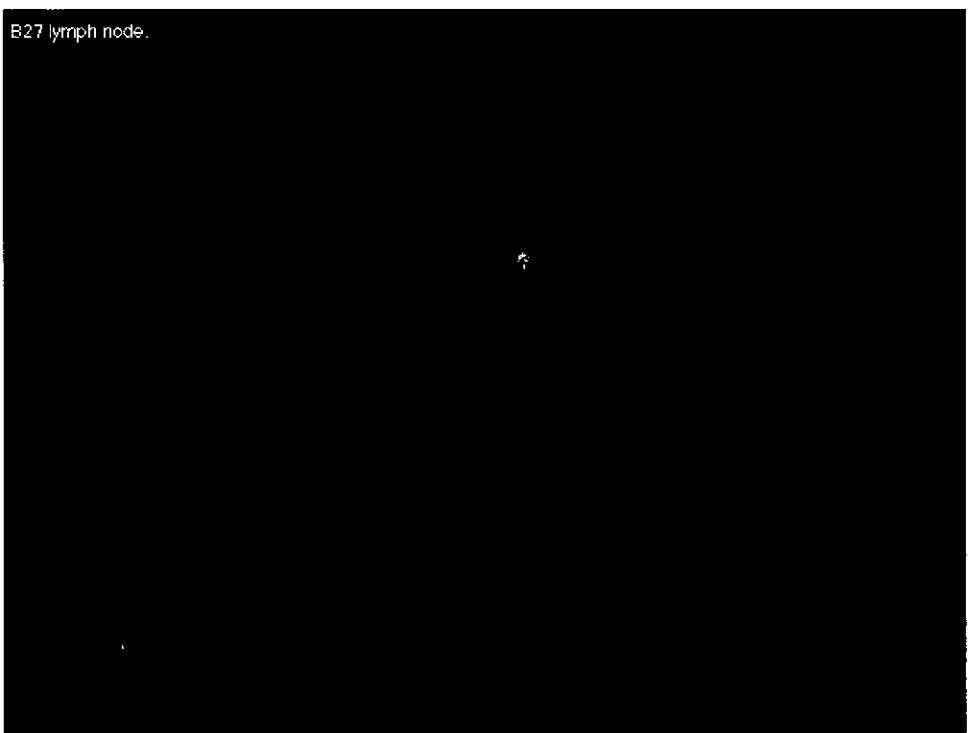
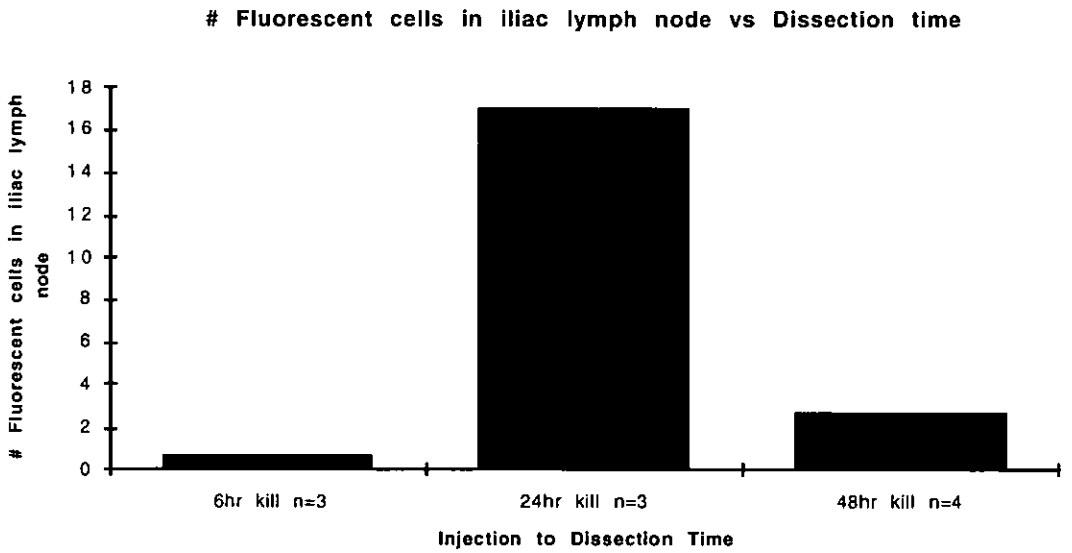


Fig 1.6 Fluorescent excited cell with background white incandescent light to show surrounding unstained cells.




# Summary

While Brenan and Parish found that labeling lymphocytes in vitro with H33342 was an effective means to observe lymphocyte migration (1984), it was found here that the problems of transfer of H33342 to recipients vaginal epithelium confuses the apparent migration of donor lymphocytes into recipient epithelium. Durand and Olive properly concluded their discussion on H33342 by stating, "[our observation] demonstrates the necessity of adequately evaluating the effects of the dye prior to its use in other systems," which is what this experiment attempted to do. The observations of the often unpredictable nature of H33342 in studying the migration of a large number of stained lymphocytes shows that H33342 may provide a valuable tool when used in conjunction with another dye or technique.

When using stained lymphocytes for our method it is preferred to stain the cells in vivo, as opposed to in-vitro, to allow for the initial loss of dye. While this significantly reduces the loss of dye, it does not entirely eliminate the loss. Placing a large number of dyed cells in a relatively isolated location like the murine vagina causes problems of non-specific staining. The benefit of using H33342, as opposed to other stains, lies in the fact that the dyed cells remain viable and able to migrate. After the cells have migrated you can use other immunolabling techniques to study the specificity of migrated cells and/or confirm the identity of the migrated cells. (King, unpublished observations)

While it does appear that injected peritoneal cells do adhere to and penetrate the vaginal epithelium, the possibility exists that the blue cells observed in the epithelium are the recipients cells that have become stained by some secondary mechanism. A way to definitively determine if injected peritoneal cells can invade the murine vagina would be to use a different strain of mouse that differs in an MHC antigen. This technique is in the

process of being explored. Until then, the observations in this experiment warrant further investigation.

It appears that foreign peritoneal cells, when injected into the vaginal lumen, can indeed invade the epithelium. Definitively proving or disproving vaginal invasion of leukocytes will prove significant in the study of male to female HIV transmission.

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# Margie Parr

# Southern Illinois University School of Medicine

## Histology of the Female Reproductive System

**(with Placenta and Breast)**

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### Objectives

i. Understand the basic organization, tissue composition and funtions of the ovary.
ii. Understand the organization and tissue composition of the female reproductive tract from the fallopian tube through the vagina.
iii. Understand the formation and organization of the placenta, including opatterns of fetal and maternal blood flow.
iv. Understand the tissue composition of the breast.

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## Histology Related Web Pages

### National Library of Medicine-Visible Human Project

If you are interested in the various imaging techniques this is a page for you. CAT, MRI, and cryosection images of representative a male and a female.

### Arizona Health Sciences Center-Human Pathology Image Libraries

An excellent page with an extensive collection of pathological images, including a few good 'normal' tissue images. A good place to quiz yourself on general H&E tissue recognition.

### LUMEN-Loyola University Medical Education Network

You must visit this site! You will find a library of histological images indexed according to tissue-organ sysems, they have an amazing collection of H& E images I strongly recommend this page for any histology study session (anyway Loyola has a nice logo).

### National University of Singapore-NUS Histonet

A WWW database for histology slides. An excellent companion to your slides and Stevens & Lowe.

### The University fo Texas-Houstan Medical School

A nice text based overview of the important components of human histology. Scroll down to Female Reproductive System.

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# Ovary

**Mesothelium (germinal epithelium)**
**Tunica albuginea**
**Medulla (including hilus)**---Contains larger blood vessels
**Cortex**---Contains all follicles
Primary oocyte
Secondary oocyte
Zona pelucida
Granulosa cells

Primordial follicle---have a single layer of squamous granulosa cells around the oocyte
Primary follicle
Secondary follicle---with theca interna (highly vascular) and theca externa (fibrous)
Tertiary (Graafian) follicle---antrum, cumulus oophorus, and corona radiata
Atretic follicle (corpus fibrosum)

Corpus luteum (yellow body)
Corpus albicans

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# Fallopian Tube

Ciliated cells
Secretory cells
Infundibulum with fimbria
Ampulla
Isthmus

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# Uterus

**Myometrium**
**Smooth muscle**
**Endometrium**
Uterine epithelium
Uterine glands
Uterine stroma
Spiral arteries
Deep layer (stratum basalis)

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Proliferative phase
Secretory phase

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# Cervix

**Cervical glands**
**Squamo-columnar junction**
Ectocervix---Nonkeratinizing, stratified, squamous epithelium
Endocervix---Columnar mucus secreting epithelium
**Internal Os**
**External Os**

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# Vagina

Mucosa---Stratified squamous epithelium
Submucosa
Smooth muscle

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# Placenta

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Intervillous space---maternal blood is within this space
Chorionic villi
Lacunae
Cytotrophoblast---dissapears in mature placenta
Syncytiotrophoblast---nonantigentic
Fetal blood vessels

**Umbilical cord**
Mucous connective tissue (Wharton's jelly)
Two umbilical arteries
One umbilical vein

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# Breast

Nipple---lactiferous ducts
Fibroadipose stroma

**Mammary gland**
Ducts
Secretory epithelium---inactive and lactating
Myoepithelial cells

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# Menstral Cycle

Proliferative phase (follicular phase)---development of spiral arteries
Ovulation---Estrogen peak
Secretory phase (luteal phase)---Progesterone peak
Menstrual phase

**Menstral Cycle** (varies)
Day 1-5 Menses
Day 5-14 Proliferative phase
Day 14 Ovulation (LH and FSH surge)
Day 14-28 Secretory phase
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Day 14-28 Secretory phase<BR>
Day 28      Menses<BR>
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# Self Quiz for the Histology of the Female Reproductive System

This quiz is designed to be open book (Stevens & Lowe or equivalant). The questions are designed to force you to look up the answer. If you follow the entire quiz through you should finish with a good review of important components of the histology of the female reproductive tract.

Note: This quiz is not comprehensive nor will any of the questions appear on a real test.

Translated: Use the quiz as a tool, memorizing the questions will only waste valuable grey matter.

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Match the synonyms

a. Hypertrophy
b. Hyperplasia
c. Fallopian tubes
d. Tertiary follicle
e. Proliferative phase
f. Ovulatory phase
g. Secretory phase

i. Oviducts
ii. Oestrogenic phase
iii. Luteal phase
iv. Graafian follicle
v. Increase in cell size
vi. Follicular phase
vii. Interval phase
viii. Increase in cell number

The epithelium of the normal vaginal fornix is

a. pseudostratified columnar epithelium
b. transitional epithelium
c. stratified squamous epithelium
d. stratified columnar epithelium

Which of the following is FALSE?

a. The lactiferous sinus is superficial to the mammary duct
b. The lactiferous sinus is surrounded by longitudinal and circular smooth muscle bundles
c. The lactriferous duct is superficial to the lactiferous sinus
d. The lactiferous duct is lined with a single layer epithelium

The ectocervix has the same epithelial structure as the uterus

a. True
b. False

Remnants of the embryonic Wolffian duct are found in the

a. uterus
b. ovaries
c. lactiferous ducts
d. female lactating breasts

Which of the following is TRUE?

a. After a primordial follicle goes though atresia it becomes a shrunken mass of collagenoous tissue called the corpus albicans.
b. The FSH surge causes ovulation.
c. The ratio of ciliated cells to secretory cells decreases as the zygote travels from the ampulla to the isthmus.
d. Mickey Mouse has five fingers on each hand.

Which comes first?

a. Graafian follicle or Secondary follicle
b. Progesterone peak or Estrogen peak
c. Menarche or menopause
d. Primary oocyte or primordial follicle
e. Corpus albicans or Corpus luteum
f. Spiral arteries or chorionic villi
g. Parturition or ovulation
h. Chicken or the egg

The Graafian follicle has several distinct features. With neurosurgeon precision you manage to pierce a Graafian follicle with a pin; which answer below represents the correct order that the pin will encounter the structures?

a. Zona pellucida, theca interna, antrum, follicular cells
b. Corona radiata, zona pellucida, antrum
c. Antrum, corona radiata, zona pellucida
d. Follicular cells, antrum, theca interna, cumulus oophorus

The souce of FSH and LH is the _____ and the target tissue is the _____.

a. ovaries, vaginal mucosa
b. ovaries, uterine functional epithelium
c. adrenal glands, hypothalamus
d. anterior pituitary, ovaries

Nabothian cysts are a consequence of

a. squamous metaplasia
b. discontinuing breast feedings
c. dermatitis
d. leiomyoma

The tissue layer immediately below the ovarium (germinal) epithelium is the

a. myometrium
b. zona pellucida
c. tunica albuginea
d. theca interna

The fluid filled space in the Graafian (vesicular) follicle is the

a. antrum
b. amniotic cavity
c. graafian vesicles
d. auricle

Myoepitelial cells

a. are found only in mammary glands
b. make up the majority of the cells found in the myometrium
c. are capable of contracting
d. resemble a single layer of skeletal muscle

The stretching of Cooper's ligaments will cause

a. a prolapsed umbilical cord
b. a weak cervix
c. inferior displacement of Montgomery's tubercles
d. increased risk of a ruptured uterus

The germ cells are found within the _____ of the ovary.

a. germinal epithelium
b. medulla
c. hilum
d. cortex

Gynaecomastia is a term for

a. breast reduction
b. breast augmentation
c. breast biopsy
d. male breast enlargement

What is the stimulus which causes the secondary oocyte to complete the second meotic division?

a. FSH surge
b. fertilization
c. oxytocin
d. LH surge

The 'transformation zone' is located

a. between the ampulla and the infudibulum
b. within the cervix
c. betweeen the urethra and the vagina

Primordial Follicles are arrested in

a. interphase of the second meiotic division

b. anaphase of the first meiotic division
c. prophase of the first meiotic division
d. telophase of the second meiotic division

What hormone prevents the corpus luteum from degenerating during pregnancy?

a. FSH
b. human chorionic gonadrotropin
c. oxytocin
d. fetal estrogen

Match the following

a. sysncytiotrophoblast
b. intervilous space
c. oogenosis
d. mesovarium
e. fibroadipose
f. menses
g. trasformation zone
h. decidua basalis
i. Wharton's jelly

i. mesentery of ovary
ii. stroma of breast
iii. pooling maternal blood
iv. necrotic stratum functinalis
v. production of secondary follicle
vi. maternal contribution to placenta
vii. non-antigentic
viii. umbilical cord substance
ix. squamo-columnar junction

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<H1>Self Quiz for the Histology of the Female Reproductive System</H1>
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This quiz is designed to be open book (Stevens & Lowe or equivalant). The questions are designed to force you to look up the answer. If you follow the entire quiz through you should finish with a good review of important components of the histology of the female reproductive tract.<P> Note: This quiz is not comprehensive nor will any of the questions appear on a real test. <P> Translated: Use the quiz as a tool, memorizing the questions will only waste valuable grey matter.
<BR><BR><HR><BR>

Match the synonyms
<OL TYPE=a>
<LI>Hypertrophy
<LI>Hyperplasia
<LI>Fallopian tubes
<LI>Tertiary follicle
<LI>Proliferative phase
<LI>Ovulatory phase
<LI>Secretory phase
</OL>

<OL TYPE=i>
<LI>Oviducts
<LI>Oestrogenic phase
<LI>Luteal phase
<LI>Graafian follicle
<LI>Increase in cell size
<LI>Follicular phase
<LI>Interval phase
<LI>Increase in cell number
</OL>

The epithelium of the normal vaginal fornix is
<OL TYPE=a>
<LI>pseudostratified columnar epithelium
<LI>transitional epithelium
<LI>stratified squamous epithelium
<LI>stratified columnar epithelium

</OL><BR>
Which of the following is FALSE?
<OL TYPE=a>
<LI>The lactiferous sinus is superficial to the mammary duct
<LI>The lactiferous sinus is surrounded by longitudinal and circular smooth muscle bundles
<LI>The lactriferous duct is superficial to the lactiferous sinus
<LI>The lactiferous duct is lined with a single layer epithelium
</OL>
<BR>
The ectocervix has the same epithelial structure as the uterus
<OL TYPE=a>
<LI>True
<LI>False
</OL>
<BR>
Remnants of the embryonic Wolffian duct are found in the
<OL TYPE=a>
<LI>uterus
<LI>ovaries
<LI>lactiferous ducts
<LI>female lactating breasts
</OL><BR>
Which of the following is TRUE?
<OL TYPE=a>
<LI>After a primordial follicle goes though atresia it becomes a shrunken mass of collagenoous tissue called the corpus albicans.
<LI>The FSH surge causes ovulation.
<LI>The ratio of ciliated cells to secretory cells decreases as the zygote travels from the ampulla to the isthmus.
<LI>Mickey Mouse has five fingers on each hand.
</OL><BR>
Which comes first?
<OL TYPE=a>
<LI>Graafian follicle or Secondary follicle
<LI>Progesterone peak or Estrogen peak
<LI>Menarche or menopause
<LI>Primary oocyte or primordial follicle
<LI>Corpus albicans or Corpus luteum
<LI>Spiral arteries or chorionic villi
<LI>Parturition or ovulation
<LI>Chicken or the egg
</OL>

<BR>
The Graafian follicle has several distinct features. With neurosurgeon precision you manage to pierce a Graafian follicle with a pin; which answer below represents the correct order that the pin will encounter the structures?
<OL TYPE=a>
<LI>Zona pellucida, theca interna, antrum, follicular cells
<LI>Corona radiata, zona pellucida, antrum
<LI>Antrum, corona radiata, zona pellucida
<LI>Follicular cells, antrum, theca interna, cumulus oophorus
</OL>
<BR>
The souce of FSH and LH is the _____ and the target tissue is the _____.
<OL TYPE=a>
<LI>ovaries, vaginal mucosa
<LI>ovaries, uterine functional epithelium
<LI>adrenal glands, hypothalamus
<LI>anterior pituitary, ovaries
</OL>
<BR>
Nabothian cysts are a consequence of
<OL TYPE=a>
<LI>squamous metaplasia
<LI>discontinuing breast feedings
<LI>dermatitis
<LI>leiomyoma
</OL>
<BR>
The tissue layer immediately below the ovarium (germinal) epithelium is the
<OL TYPE=a>
<LI>myometrium
<LI>zona pellucida
<LI>tunica albuginea
<LI>theca interna
</OL>
<BR>
The fluid filled space in the Graafian (vesicular) follicle is the
<OL TYPE=a>
<LI>antrum
<LI>amniotic cavity
<LI>graafian vesicles
<LI>auricle
</OL>

<BR>
Myoepitelial cells
<OL TYPE=a>
<LI>are found only in mammary glands
<LI>make up the majority of the cells found in the myometrium
<LI>are capable of contracting
<LI>resemble a single layer of skeletal muscle
</OL>
<BR>
The stretching of Cooper's ligaments will cause
<OL TYPE=a>
<LI>a prolapsed umbilical cord
<LI>a weak cervix
<LI>inferior displacement of Montgomery's tubercles
<LI>increased risk of a ruptured uterus
</OL>
<BR>
The germ cells are found within the _____ of the ovary.
<OL TYPE=a>
<LI>germinal epithelium
<LI>medulla
<LI>hilum
<LI>cortex
</OL>
<BR>
Gynaecomastia is a term for
<OL TYPE=a>
<LI>breast reduction
<LI>breast augmentation
<LI>breast biopsy
<LI>male breast enlargement
</OL>
<BR>
What is the stimulus which causes the secondary oocyte to complete the second meotic division?
<OL TYPE=a>
<LI>FSH surge
<LI>fertilization
<LI>oxytocin
<LI>LH surge
</OL>
<BR>
The 'transformation zone' is located

<OL TYPE=a>
<LI>between the ampulla and the infudibulum
<LI>within the cervix
<LI>betweeen the urethra and the vagina
</OL>
<BR>
Primordial Follicles are arrested in
<OL TYPE=a>
<LI>interphase of the second meiotic division
<LI>anaphase of the first meiotic division
<LI>prophase of the first meiotic division
<LI>telophase of the second meiotic division
</OL>
<BR>
What hormone prevents the corpus luteum from degenerating during pregnancy?
<OL TYPE=a>
<LI>FSH
<LI>human chorionic gonadrotropin
<LI>oxytocin
<LI>fetal estrogen
</OL>
<BR>
Match the following
<OL TYPE=a>
<LI>sysncytiotrophoblast
<LI>intervilous space
<LI>oogenosis
<LI>mesovarium
<LI>fibroadipose
<LI>menses
<LI>trasformation zone
<LI>decidua basalis
<LI>Wharton's jelly
</OL>

<OL TYPE=i>
<LI>mesentery of ovary
<LI>stroma of breast
<LI>pooling maternal blood
<LI>necrotic stratum functinalis
<LI>production of secondary follicle
<LI>maternal contribution to placenta
<LI>non-antigentic

<LI>umbilical cord substance
<LI>squamo-columnar junction
</OL>

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