Q 1 Multiple Choice questions (Tick the appropriate answer )

(i) During fetal life the outer surface of the ovaries covered by: (b) Germinal Epithelium

(ii) In which phase of female sexual cycle the plasma Concentrations of the Gonadotropic hormone (FSH & LH) are increase: (b) Follicular Phase

(iii) Folliculogenesis occurs within: (c) The cortex of the ovary

(iv) The first major event in folliculogenesis (c) Recruitment

(v) Beginning of the cycle of menstruation called: (a) Menarche

(vi) In which phase corpus luteum secretes large quantities of progesterone, estrogen and inhibin (c) Postovulatory phase

(vii) The force for sperm propulsion is provided by (d) Dynein

(viii) The volume of sea urchin egg is about (b) 200 Picoliters

(ix) Ranging between the of implantation of a fertilized ovum is (a) 6 to 12 days

(x) Embryo is attached to the surface of uterine lining and projects freely into uterine cavity called (b) Central implantation

SECTION B

Long Answer Type

Q2. What is puberty give the graphical representation of estrogen secretion in female sexual life

Puberty is the process of physical changes by which an early stage child's body matures into an adult body capable of sexual reproduction to enable fertilisation. It is therefore defined as the onset of sexual maturity and is remarked by the development of secondary sexual characters

Graphical representation of estrogen secretion in female sexual life

Oogenesis - The formation, development, and maturation of an egg = ovum; a long and complex process which (1) begins in the fetal period with the development of oogonial stem cells by mitosis, and (2) is followed by the initiation of meiosis division I which produces primary oocytes which are arrested in development in meiotic prophase I; (3) at puberty, in response to increasing blood levels of FSH and LH from the anterior pituitary, meiosis continues in certain follicles each month during the menstrual cycle, and (4) one egg and its surrounding follicle develop to the point of being mature follicle called Graafian follicle, (5) this ovum completes meiosis division I at the time of ovulation, and then meiosis is arrested until further external stimuli are provided.
Hormonal control of puberty can be explained on the basis of two hypothesis

1. Missing link hypothesis- suggests that some component of brain- pituitary –gonadal axis is missing or non – functional
2. Gonadostat hypothesis- nstates about the lesions of hypothalamus which resultd in enhanced gonadotropin secretion and initiation of pubertal process (Hadley, 2000)

**Actions of Estradiol**

- Estradiol also has important actions in a number of other tissues:
  - causes proliferation of uterine endometrium
  - increases contractility of uterine myometrium
  - stimulates development of mammary glands
  - stimulates follicle growth (granulosa cell proliferation)
  - effects on bone metabolism, hepatic lipoprotein production, genitourinary tract, mood, and cognition
- Effects are mediated through the intracellular estrogen receptors (alpha and beta), and possible membrane effects.

_Effect of Estrogens on the Uterus and External Female Sex Organs._ During childhood, estrogens are secreted only in minute quantities, but at puberty, the quantity secreted in the female under the influence of the pituitary gonadotropic hormones increases 20-fold or more. At this time, the female sex organs change from those of a child to those of an adult. The ovaries, fallopian tubes, uterus, and vagina all increase several times in size. Also, the external genitalia enlarge, with deposition of fat in the mons pubis and labia majora and enlargement of the labia minora.

In addition, estrogens change the vaginal epithelium from a cuboidal into a stratified type, which is considerably more resistant to trauma and infection than is the prepubertal cuboidal cell epithelium. Vaginal infections in children can often be cured by the administration of estrogens simply because of the resulting increased resistance of the vaginal epithelium.

During the first few years after puberty, the size of the uterus increases twofold to threefold, but more important than the increase in uterus size are the changes that take place in the uterine endometrium under the influence of estrogens. Estrogens cause marked proliferation of the endometrial stroma and greatly increased development of the endometrial glands, which will later aid in providing nutrition to the implanted ovum. These effects are discussed later in the chapter in connection with the endometrial cycle.
Puberty Phase

Level of Estrogen in urine at pubertal stage
Q2. What is photoperiod? Define Estrous cycles and their phases in females

Recurring cycle of light and dark periods. The natural photoperiod is approximately 24 hours, and the ratio of light to dark hours slowly changes over the course of a year. In controlled experiments, the photoperiod is usually (but not necessarily) retained at 24 hours, and the light:dark ratio is typically constant. The amount of hours in a day where the environment is either light or dark.

Photoperiodism is the physiological reaction of organisms to the length of day or night. It occurs in plants and animals. Photoperiodism can also be defined as the developmental responses of plants to the relative lengths of the light and dark periods. Here it should be emphasized that photoperiodic effects relate directly to the timing of both the light and dark periods.

### Estrous cycles and their phases in

Reproductive processes in female mammals are characterised by cyclic alterations in the female tract and in sexual receptivity.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Ovary</th>
<th>Uterus</th>
<th>Vagina</th>
<th>Smear</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diestrus</td>
<td>Small follicles only are present with motility, lumen small and slit-like. Corpora lutea from the previous cycle. Ovulation. Cells of the uterine mucosa columnar, very short time unless pregnancy or pseudopregnancy intervenes.</td>
<td>Small and anaemic, low epithelial, lumen small and slit-like. Endometrial glands become vascularized.</td>
<td>Epithelium thin, mitotic figures infrequent. Leucocytes abundant in stroma, migrate through the epithelium into the lumen.</td>
<td>Stringy mucus in which are entangled many leucocytes and a few nucleated epithelial cells.</td>
</tr>
<tr>
<td>Proestrus</td>
<td>Some follicles grow rapidly.</td>
<td>Become more vascular, water content increases, organ distends. Contractility more pronounced. Epithelial cells become higher (continuing into estrus). Leucocytes disappear from mucosa.</td>
<td>Epithelium thickens, numerous mitoses in inner layers. Old layers of epithelium line the lumen. Leucocytes no longer nucleated epithelial cells, migrate through the lining as singly or in sheets. None to few leucocytes. Superficial few leucocytes.</td>
<td>Epithelial cells slough off into the lumen.</td>
</tr>
<tr>
<td>Estrus</td>
<td>Ovulation in the rat is spontaneous and occurs about 10 hours after the end of dioestrus. &quot;Heat&quot; vasculatisation. (Receptivity) lasts about 13 hours. Cells reach maximum size. Usually 10-20 eggs ovulated each cycle. No leucocytes.</td>
<td>Epithelium continues vascularization. Many corpora lutea, which secrete degeneration and replacement only for a very short time, and small leucocytes in stroma.</td>
<td>Epithelium continues vascularization. Many corpora lutea, which secrete degeneration and replacement only for a very short time, and small leucocytes in stroma.</td>
<td>Many leucocytes and a few nucleated epithelial cells.</td>
</tr>
<tr>
<td>Metestrus</td>
<td>Many corpora lutea, which secrete degeneration and replacement only for a very short time, and small leucocytes in stroma.</td>
<td>Decrease in size and vascularity.</td>
<td>Reduction of mitotic activity. Leucocytes in stroma and migrating through the epithelium into the lumen.</td>
<td>Many leucocytes and a few nucleated epithelial cells.</td>
</tr>
</tbody>
</table>
The recurrent period of receptivity, or "heat" is called Estrus. The estrous cycle has been most extensively studied in laboratory rodents (mice and rats). Rats kept separate from males in the laboratory repeat the Estrous cycle throughout the year at intervals of about five days, unless subjected to pregnancy, pseudo-pregnancy (after a sterile mating), or disease. The cycle involves the whole of the reproductive tract, and it is possible to determine the sexual status of the female rat by examination of smears prepared from the vaginal fluid.

Rats and mice are examples of polyestrus mammals (as are cats which are seasonally polyoestrus). Monestrus forms (most wild animals - foxes, bears, wolves etc.) complete a single estrous cycle annually. In the wild, rats and mice probably suspend the cycle for a period during the winter;
the reproductive organs are in a state of quiescence, called anestrus.

Q4. What is recruitment a primordial follicle?

1. Brief of Follicular growth

**Ovarian follicles** - containing a primary oocyte surrounded by one or more layers of supportive cells; they develop during embryonic life, but remain dormant until puberty; at puberty, under the influence of FSH and LH from the anterior pituitary, they begin a sequential developmental cycle, the ovarian cycle, and a few each month, begin the cycle leading to ovulation; the developmental stages consist of the primordial follicle, primary follicle, secondary follicle, and mature follicle = vesicular follicle = Graafian follicle.

**Follicular cells** - Any of the cells forming protective, supportive layers around the egg = ovum in the cortex of the ovary; they provide nutrients to the egg = ovum and secrete estrogens and progesterone in response to blood levels of FSH and LH from the anterior pituitary; their shape and number vary with the phase of the ovarian cycle and are major criteria for defining the the developmental stages of the ovarian follicles; once a follicle has begun development during a menstrual cycle, beginning with the primary follicle stage and onward, its supporting cells enlarge, develop a granular cytoplasm, and begin secreting estrogens and progesterone, and these active secretory follicular cells may also be called granulosa cell

**Granulosa cells** - The lining cells of all ovarian follicles except the primordial follicle, and of the corpus luteum; the granularity of the cytoplasm of these follicular cells is due to the increase in the production and secretion of estrogens and progesterone; their endocrine activity is regulated by FSH from the anterior pituitary.
STAGES for recruitmentment

1. 3 to 5 million OOGONIA differentiate into PRIMARY OOCYTES during early development
2. OOCYTES becomes surrounded by squamous (follicular) cells to become PRIMORDIAL FOLLICLES
3. Most PRIMORDIAL FOLLICLES undergo atresia leaving 400,000 at birth

(1) PRIMORDIAL FOLLICLES
(2) GROWING FOLLICLES a) early primary follicle
- follicular cells still unilaminar but now are cuboidal in appearance
- oocyte begins to enlarge
(b) late primary follicle
multilaminar follicular layer; cells now termed granulosa cells
- zona pellucida appears; gel-like substance rich in GAGs
surrounding stromal cells differentiate into theca interna and theca externa
(b) secondary (antral) follicle
cavities appear between granulosa cells forming an antrum
- follicle continues to grow
- formation of cumulus oophorus and corona radiate

(3) MATURE (GRAAFIAN) FOLLICLES

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primordial</td>
<td>Dormant, small, only one layer of flat granulosa cells</td>
<td>Primordial follicles are about 0.03-0.05 mm in diameter.</td>
</tr>
<tr>
<td>Primary</td>
<td>Mitotic cells, cuboidal granulosa cells</td>
<td>Almost 0.1 mm in diameter</td>
</tr>
<tr>
<td>Secondary</td>
<td>Presence of theca cells, multiple layers of granulosa cells</td>
<td>The follicle is now 0.2 mm in diameter</td>
</tr>
<tr>
<td>Early tertiary</td>
<td>The early tertiary follicle is arbitrarily divided into five classes. Class 1 follicles are 0.2 mm in diameter, class 2 about 0.4 mm, class 3 about 0.9 mm, class 4 about 2 mm, and class 5 about 5 mm.</td>
<td></td>
</tr>
<tr>
<td>Late tertiary</td>
<td>Fully formed antrum, no further cytodifferentiation, no novel progress</td>
<td>Class 6 follicles are about 10 mm in diameter, class 7 about 16 mm, and class 8 about 20 mm. It is common for non-dominant follicles to grow beyond class 5, but rarely is there more than one class 8 follicle.</td>
</tr>
<tr>
<td>Preovulatory</td>
<td>Building growth in estrogen concentration, all other follicles atretic or dead</td>
<td></td>
</tr>
</tbody>
</table>

Q5 Explain folliculogenesis with suitable diagram

Folliculogenesis is the maturation of the ovarian follicle. Folliculogenesis constitutes the progression of a number of small primordial follicles into large preovulatory follicles that enter the menstrual cycle. The process of folliculogenesis ends when the remaining follicles in the ovaries are incapable of responding to the hormonal...
cues that previously recruited some follicles to mature. This depletion in follicle supply signals the beginning of menopause.

**FEMALE REPRODUCTIVE SYSTEM**

- **OVARY**

  THREE STAGES OF OVARIAN FOLLICLES CAN BE IDENTIFIED FOLLOWING PUBERTY:
  (each follicle contains one oocyte)

  1. **PRIMORDIAL FOLLICLES**
     - very prevalent; located in the periphery of the cortex
     - a single layer of squamous follicular cells surround the oocyte

  2. **GROWING FOLLICLES**
     - three recognizable stages:
       (a) early primary follicle
       (b) late primary follicle
       (c) secondary (antral) follicle

  3. **MATURE (GRAAFIAN) FOLLICLES**
     - follicle reaches maximum size

**FEMALE REPRODUCTIVE SYSTEM**

- **HORMONAL REGULATION OF OOGENESIS AND OVULATION**

  **FOLLICULAR PHASE**

  - 19-20 primordial follicles begin to develop in response to FSH and LH levels
  - FSH and LH stimulate theca and granulosa production of estrogen and progesterone
  - surge of LH induces ovulation

  **OVULATION**

  **LUTEAL PHASE**

  - theca and granulosa cells transform into the corpus luteum and secrete large amounts of progesterone
  - if fertilization does not occur: corpus luteum degenerates... If fertilization does occur: HCG released from the embryo maintains corpus luteum
However in primates the process depends upon the maturation of the neuroendocrine control system which directs the pulsatile secretion of GnRH from the hypothalamus.

The primary role of the follicle is oocyte support. From birth, the ovaries of the human female contain a number of immature, primordial follicles. These follicles each contain a similarly immature primary oocyte. After puberty and commencing with the first menstruation, a clutch of follicles begins folliculogenesis, entering a growth pattern that will end in death or in ovulation (the process where the oocyte leaves the follicle).

During post-pubescent follicular development, and over the course of roughly a year, primordial follicles that have begun development undergo a series of critical changes in character, both histologically and hormonally. Two-thirds of the way through this process, the follicles have transitioned to tertiary, or antral, follicles. At this stage in development, they become dependent on hormones emanating from the host body, causing a substantial increase in their growth rate.

With a little more than ten days until the end of the period of follicular development, most of the original group of follicles have died (a process known as atresia). The remaining cohort of follicles enter the menstrual cycle, competing with each other until only one follicle is left. This remaining follicle, the late tertiary or pre-ovulatory follicle, ruptures and discharges the oocyte (that has since grown into a secondary oocyte), ending folliculogenesis. Folliculogenesis lasts for approximately 375 days. It coincides with thirteen menstrual cycles. The process begins continuously, meaning that at any time the ovary contains follicles in all stages of development, and ends when a mature oocyte departs from the preovulatory follicle in a process called ovulation.
Q6. Explain postovulatory secretion of the ovarian hormone and pre-ovulatory surge of LH and FSH causes ovulation.

Feedback Oscillation of the Hypothalamic-Pituitary-Ovarian System

Now, after discussing much of the known information about the interrelations of the different components of the female hormonal system, we can attempt to explain the feedback oscillation that controls the rhythm of the female sexual cycle. It seems to operate in approximately the following sequence of three events.

1. Postovulatory Secretion of the Ovarian Hormones, and Depression of the Pituitary Gonadotropins. The easiest part of the cycle to explain is the events that occur during the postovulatory phase—between ovulation and the beginning of menstruation. During this time, the corpus luteum secretes large quantities of both progesterone and estrogen, as well as the hormone inhibin. All these hormones together have a combined negative feedback effect on the anterior pituitary gland and hypothalamus, causing the suppression of both FSH and LH secretion and decreasing them to their lowest levels about 3 to 4 days before the onset of menstruation.

GnRH (FSH and LH) Level during preovulatory and

Negative Feedback Effects of Estrogen and Progesterone in Decreasing Both LH and FSH Secretion

Estrogen in small amounts has a strong effect to inhibit the production of both LH and FSH. Also, when progesterone is available, the inhibitory effect of estrogen is multiplied, even though progesterone by itself has little effect.

These feedback effects seem to operate mainly on the anterior pituitary gland directly, but they also operate to a lesser extent on the hypothalamus to decrease secretion of GnRH, especially by altering the frequency of the GnRH pulses.

Positive Feedback Effect of Estrogen Before Ovulation—The Preovulatory LH Surge

The mechanism by which anterior pituitary secretes a large amount of LH for 1-2 days at the beginning of 24-48 hrs before ovulation and the much smaller preovulatory surge as well.

The cause of this abrupt surge in LH secretion is not known. However, several possible explanations are as follows: (1) It has been suggested that estrogen at this point in the cycle has a peculiar positive feedback effect of stimulating pituitary secretion of LH and, to a lesser extent, FSH; this is in sharp contrast to its normal negative feedback effect that occurs during the remainder of the female monthly cycle. (2) The granulosa cells of the follicles begin to secrete small but increasing quantities of progesterone a day or so before the preovulatory LH surge, and it has been suggested that this might be the factor that stimulates the excess LH secretion.

Without this normal preovulatory surge of LH, ovulation will not occur.
Sharp surge in LH with simultaneous increase in FSH

Positive and negative feed back mechanism for the control of hypothalamus over the FSH & LH
Q7. Explain fertilization and structure of gametes with diagram.

**Fertilization** is a complex process which involves the fusion of male and female gametes followed by the fusion of their cytoplasm. The process of fertilization has dual independent functions

(i) to cause the egg to start developing ….. **ACTIVATION**
(ii) to inject a male haploid nucleus into the egg cytoplasm……… **AMPHIMIXIS** or (intermingling of paternal and maternal hereditary characters in the cytoplasm)

**Mechanism of fertilization:** It constitutes five stages

1. **Encounter of spermatozoa and ova**
   a) **External fertilization**
   (In liquid medium outside the body e.g. Fishes, amphibians, fresh water invertebrates)
   b) **Internal Fertilization** (In oviparous forms like reptiles, birds where the eggs are completely inside impermeable membrane, in ovoviviparous and in viviparous)

2. **Capacitation and contact**: the capacity of spermatozoa to fertilize eggs of the same species but not the other.
   Fran Lillie was first to show that this happens under the influence of chaemotaxis where sperm responding to the specific jelly like chemical substance which surround the egg. This process consists
   a) **Agglutination**: The adhesion of spermatozoa or clumping.
   b) **Fertilizin-antifertilizin reaction: to block polyspermy**
   Fertilizin – is mucopolysaccharide or glycoprotein present in egg
   Anti-fertilizin in sperms

3. **Acrosome reaction and penetration**
   When the acrosome reaction occurs, a number of proteolytic enzymes are exposed or released.
   One or more of these enzymes is responsible for digesting the hole through the zona pellucida through which the sperm enters the perivitelline space.
When sperm arrives at zona pellucida with the acrosome still intact. This time the sperm has **hyaluronidase** activity. **Events when the sperm gets to the zona pellucida?**

1. Attachment - loose association
2. Binding - strong attachment
3. Acrosome reaction - release of enzymes
4. Penetration of the zona pellucida by the sperm

**Zona pellucida is composed of 3 glycoproteins ZP1, ZP2, ZP3**

Repeating subunits of ZP2 and ZP3 form filaments that are bound together by ZP1
4) **Activation of Ovum.** Constitutes seven events

1. **Release of Ca++** (calcium) stored in the egg endoplasmic reticulum a critical step in the process.

2. **Cortical reaction** - rupture of cortical granules that occurs concurrently with the Ca++ release. Contents of granules are released into perivitelline space and cause “hardening” of the vitelline membrane or zona pellucida. Causes vitelline/fertilization membrane to rise away from surface of egg in some species.

3. An **influx of Na⁺ (sodium) into the egg cytoplasm** causes a change in membrane potential - block to polyspermy.

4. In some species a **reorganization of the egg cytoplasm**.

5. In most cases, **completion of meiosis by the egg**.

6. An **efflux of H⁺ (hydrogen) ions** causing an increase in cytoplasmic pH - this activates previously inhibited synthetic pathways.

7. **Increase in metabolism** - zygote gears up for development

---

**A. Cascade for Egg activation**

**B. Signaling Pathway during Egg activation resulting Intracellular Release of Ca**

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5) Migration of pronuclei and amphimixis

Events that occur soon after egg activation:

a. DNA replication as male and female pronuclei approach each other
b. Male and female pronuclei merge
c. Preparation for first cleavage

The male nucleus enters the egg cytoplasm and becomes the male pronucleus.

2. As a result of the sperm fusing with the egg plasmalemma, the oocyte nucleus, which is at metaphase of the second meiotic division, completes that division giving rise to another polar body.

3. Following the second meiotic division, what is now the nucleus of the ovum becomes the female pronucleus.

4. The haploid male and female pronuclei move toward one another, meet, and fuse to form the diploid nucleus of the zygote.

Structure of Gametes

Female Gamete

Male Gamete
8. Describe molecular aspects of Implantation

Embryo implantation represents the most critical steps of the reproductive processes in many species. It consists of unique biological phenomenon by which the blastocyst becomes intimately connected to the maternal endometrial surface to form the placenta. The process of implantation may be classified into three stages: apposition, adhesion and invasion. During blastocyst apposition, trophoblast cells adhere to the receptive endometrial epithelium. The blastocyst will subsequently anchor to the endometrial basal lamina and stromal extracellular matrix (ECM). This is followed by the invasive blastocyst penetration through the luminal epithelium. Implantation involves a complex sequence of signaling events that are crucial to the establishment of pregnancy. A large number of identified molecular mediators, under the influence of ovarian hormones, have been postulated to be involved in this early feto–maternal interaction. These mediators embrace a large variety of inter-related molecules including adhesion molecules, cytokines, growth factors, lipids and others.

**Pinopods**
Pinopods are bleb-like protrusions found on the apical surface of the endometrial epithelium. Pinopod expression is limited to a brief period of maximum 2 days in the menstrual cycle corresponding to the putative window of implantation. The pinopod-regulated expression pattern throughout the menstrual cycle advocates their use as markers of implantation. Although the role of pinopods remains unknown, it seems that they are the preferred sites of embryo–endometrial interactions.

**Cellular adhesion molecules family**
The cell adhesion molecule (CAM) family is composed of four members known as integrins, cadherins, selectins and immunoglobulins. Their classical functions include maintenance of tissue integration, wound healing, morphogenic movements, cellular migrations and tumor metastasis.

**Integrins**
Integrins are a family of transmembrane glycoproteins, formed by the association of two different, non-covalently linked, and subunits. A large variety of integrins have been described within the luminal and glandular endometrial epithelium. Whereas the majority of the integrins are constitutively expressed throughout the entire menstrual cycle, others exhibit an interesting regulated pattern within the cycle. Three cycle-specific integrins are co-expressed by the human endometrium defined histologically on days 20–24 of the human menstrual cycle: 1 1, 4 1 and V 3, but only the 3 mRNA subunit expression was shown to increase after day 19 and is not detected beforehand. In regard to its expression pattern along with its epithelial localization, V 3 has been proposed as a potential receptor for embryonic attachment. Integrins are also expressed by the human trophoblast at the time of implantation.

**Selectins**
Selectins are glycoproteins which also belong to the CAM family. They include P-selectin, L-selectin and E-selectin. The human L-selectin, which is of importance in the implantation process, consists of a large, highly glycosylated extracellular domain, a single spanning transmembrane domain and a small cytoplasmic tail. The selectin adhesion system is well established at the maternal–fetal interface. On the blastocyst side, strong L-selectin staining has been observed over the entire embryo surface. On the maternal side, the expression of selectin oligosaccharide-based ligands, such as MECA-79 or HECA-452, is up-regulated during the window of implantation. The physiological importance of the interaction between L-selectin and its oligosaccharide ligands was investigated in the human endometrium. In conclusion, very little is known about the involvement of
selectins in embryo implantation. It appears, however, that selectins take part in the very early stages of blastocyst interactions with the uterine wall.

**Cadherins**

Cadherins constitute a group of glycoproteins responsible for the calcium-dependent cell-to-cell adhesion mechanism. In regard to implantation, E-cadherin represents the most studied subclass. E-cadherin is expressed by a variety of tissues and plays an important role in embryogenesis formation during gastrulation, neurulation, and organogenesis. Studies on mouse embryo implantation have shown that targeted mutations in the E-cadherin gene result in defective pre-implantation development. Role of E-cadherin in human embryo implantation is not known, but based on its expression pattern, we suspect that it is of importance for this process. E-cadherin mRNA levels were shown to be significantly higher during the luteal phase (Fujimoto et al., 1996). Nevertheless, these menstrual cycle variations were not detected at the protein level by immunohistochemical studies.

**Immunoglobulins**

Among the CAMs family, the immunoglobulins superfamily is the most extensive. Intercellular adhesion molecule-1 (ICAM-1 or CD54) is a transmembrane glycoprotein that belongs to the immunoglobulin superfamily and is constitutively expressed within the endometrium. Stromal cell expression of ICAM-1 is up-regulated at the time of menstruation. It was recently shown that endometrial cells in culture are able to constitutively express ICAM-1 mRNA and protein without hormonal supplement.

**Mucins**

Mucins are high molecular weight (MW) glycoproteins, which contain at least 50% of carbohydrate O-linked to a threonine/serine rich peptide core. Among the 14 cloned human mucins, only Mucin-1 (MUC1) and to a lesser extent MUC6 have been found in the human endometrium. In the endometrium, MUC1 extends beyond the glycocalyx and is probably the first molecule that the embryo encounters on its route to attachment. One could contemplate the possibility that endometrial MUC1 repels the blastocyst until it finds the correct time and place for implantation. High-progesterone levels presumably reduce MUC1 expression, therefore, facilitating embryo–epithelial interactions by unmasking CAMs on the endometrial surface (Surveyor et al., 1995). Hence, MUC1 inhibits implantation and its down-regulation could contribute to the achievement of endometrial receptivity. Indeed, human in vitro implantation models indicate that MUC1 is lost at the site of embryo attachment. TNF, a proinflammatory cytokine, secreted both by the endometrium (Hunt et al., 1992; Tabibzadeh et al., 1995; Bischof et al., 2000) and by the human blastocyst (Witkin et al., 1991), could play a role in locally removing the repelling MUC1 (Thathiah et al., 2004). Interestingly, TNF has a dual effect. On the one hand, it increases MUC-1 gene expression. This stimulation seems to be mediated by the binding of nuclear factor B to its site in the MUC1 gene promoter (Thathiah et al., 2004). On the other hand, TNF was shown to markedly stimulate MUC1 shedding in human uterine epithelium. In conclusion, MUC1 appears to be a negative factor for embryo implantation. Indeed, in the area where implantation takes place, MUC1 disappears.

**Cytokines**

Cytokines comprise a group of proteins that separately or in concert modulate a variety of cellular functions, such as cellular proliferation and differentiation. They play a major role in the reparative and inflammatory-like processes occurring every menstrual cycle in the human endometrium, but they are also implicated in critical reproductive events such as ovulation and implantation.
LIF
LIF is an IL-6 family pleiotropic cytokine which also includes oncostatin M (OSM), ciliary neurotrophic factor (CNTF) and cardiotrophin 1. LIF expression has been demonstrated in the uterus of a variety of mammals. Although LIF mRNA expression in the proliferative to early-secretory phase is controversial, its expression at high levels is well established in the mid- to late-secretory phase. In endometrial biopsies obtained from women of proven fertility, LIF mRNA expression was observed from day 18 to 28 with a peak at day 20 of the menstrual cycle. The pivotal role of LIF in human embryonic implantation has been established based on abnormal LIF levels in infertile patients and especially in those with RIFs.

IL-6
Within the human endometrium, IL-6 expression follows a regulated temporal pattern with highest detected levels during the luteal phase. Endometrial IL-6 mRNA expression increases progressively during the mid-to late-secretory phase and decreases in the late-secretory phase. The IL-6 receptor was found to be expressed by the blastocyst, the trophoblast and the endometrium. The fact that IL-6 is maximally expressed during the window of implantation and that its receptor is found both in the blastocyst and in the endometrium suggests a paracrine/autocrine role for IL-6 in the peri-implantation period.

IL-1
The family members of IL-1, key mediators of the inflammatory and immunological response, include three polypeptides: IL-1α, IL-1β and a natural inhibitor, IL-1 receptor antagonist. IL-1 was detected in the human endometrium throughout the menstrual cycle, both in stromal and glandular cells, although macrophages of the mononuclear phagocytic system (MPS) have been suggested to be an important reservoir of this cytokine. IL-1Rα mRNA and protein are localized in the human endometrial epithelium and reach maximal levels during the luteal phase of the menstrual cycle.

Prostaglandins
It has long been speculated that prostaglandins (PGs), as vasoactive factors, play an important role in ovulation, fertilization and in late-pregnancy processes leading to the onset of labour (Espey, 1994). Moreover, PGs were recently demonstrated to be crucial for successful embryo implantation. Human PGT expression is elevated in the proliferative and early-secretory phase and low in the mid- to late-secretory phase, as shown by quantitative RT-PCR. Their role consists in timing the window of implantation. Delayed timing of blastocyst implantation has a ripple effect that presents in mice as embryo crowding near the cervix, abnormal placentation and fetal resorption. Whether PGs have a similar role in human implantation should be further explored.

Human embryo implantation in the uterus:

(A) Endometrium proliferates under estrogen enhancement.
(B) Progesterone from luteinized follicles leads to endometrial differentiation.
(C) The blastocyst enters the uterus through the ostia and rolls freely over the endometrium under signals by L-selectin.
(D) Mucin-1 (MUC-1) repels the blastocyst and prevents its adhesion to endometrial areas with poor chances of implantation.
(E) Chemokines and cytokines attract the blastocyst to the optimal implantation spot.
(F) Adhesion molecules (e.g. integrins and cadherins) firmly attach the blastocyst to the endometrial pinopods to ensure further successful implantation.
**Stages**

A. Endometrium proliferates under estrogen enhancement
B. Progesterone leads to endometrial differentiation
C. Blastocyst enters to the uterus, rolls freely over endometrium under the influence of L-selectin
D. Mucin – (MUC-1) repels the blastocyst, prevents adhesion to endometrium areas with poor chance of implantation
E. Chemokines and cytokines attract the blastocyst to the optimal implantation spot
F. Adhesion molecule – integrin and cathepin firmly attach the blastocyst to the endometrial pinopods to ensure further successful implantation