Teacher Background

Pregnancy – What is Normal, What Can Go Wrong, and How Can We Assist Infertile Couples?

Note: The Teacher Background Section is meant to provide information for the teacher about the topic and is tied very closely to the PowerPoint slide show. For greater understanding, the teacher may want to play the slide show as he/she reads the background section. For the students, the slide show can be used in its entirety or can be edited as necessary for a given class.

When Does Pregnancy Occur?

A pregnancy can occur when the oocyte (egg) is released during ovulation and sperm is present. The ovulated oocyte in the female reproductive tract can survive for about 24 hours. Ideally, the sperm fertilizes the oocyte in the fallopian tubes so the developing embryo has time to mature before reaching the uterus for implantation. However, fertilization can occur ectopically (in the abdomen) and also in the uterus; these do not usually result in successful pregnancies.

The haploid (monoploid) oocyte is one of the largest human cells. At 0.15 to 0.20 mm in diameter, it is just visible to the naked eye. It is about the size of a period at the end of the sentence (.) and can fit into the eye of a needle. An entire ovary (which contains hundreds of immature oocytes) measures about 4.0 cm in length, 2.0 cm in width, and 0.8 cm in thickness.

The haploid sperm is one of the smallest human cells. Its head measures 0.005 mm by 0.003 mm, and with the tail included, the sperm is 0.05 mm in length. It has the ability to swim the entire length of the female reproductive tract. Sperm can live in a female's cervical mucus and upper genital tract for about 72 hours (about 3 days), but sperm ejaculated outside the body might survive in the semen for only up to a few hours. (1, 2, 3, 15, 16)

How Do Oocytes and Sperm Cells Develop?

The ovary contains a large number of small primordial oocytes located in primordial follicles. These are present at the birth of the female and will be all of the primordial oocytes she will ever have. A small number of these primordial oocytes will develop into primary oocytes which are arrested in prophase I of meiosis I. Clinically this stage is called a germinal vesicle (GV) when the nucleus and nucleolus are present.

Beginning at puberty in response to specific levels of estrogen and progesterone achieved during a monthly cycle, one primary oocyte, clinically called Metaphase I (M1) when the nucleus and nucleolus disappear, will complete its first meiotic division to form two unequal cells; one is the secondary oocyte and the other is the 1st polar body. Clinically, this stage containing the secondary oocyte and its polar body is called Metaphase II (M2). The secondary oocyte and its 1st polar each contain 23 chromatid pairs (or 46 chromatids).

At the end of the meiosis I the follicle containing the secondary oocyte with its 1st polar body will be stimulated by hormones to burst open and release the secondary oocyte and its polar body – a process called ovulation. The ovulated secondary oocyte in the female reproductive tract can survive for about 24 hours.

Meiosis II division is triggered by the entry of a sperm into the secondary oocyte as it is fertilized. The secondary oocyte divides during meiosis II (the 1st polar body does not divide) forming two unequal cells: the haploid (monoploid) oocyte and a haploid 2nd polar body. Both the 1st polar body and the second polar body remain beside the oocyte. After the sperm enters the oocyte, the 2PN (2 pronucleus) stage occurs when the oocyte pronucleus and the sperm pronucleus exist separately within the cytoplasm of the oocyte. The haploid oocyte pronucleus fuses with the sperm haploid pronucleus to form the zygote (fertilized oocyte). The zygote has 46 chromosomes – 23 from the oocyte after meiosis II and 23 from the mature sperm cell. The 1st and 2nd polar bodies will eventually go through apoptosis.

If no sperm is present to trigger meiosis II in the secondary oocyte, it is sloughed out of the body through the uterus and vagina, usually unnoticed. A primary oocyte will mature through this process and be ovulated once a month, in response to changing levels of estrogen and progesterone, until the female reaches menopause when she is 40-50 years old and the hormone levels begin to substantially decrease. (18)

Spermatogenesis begins at puberty in response to testosterone and continues the entire lifetime of the male. Millions of sperm are made at a time. Spermatogenesis begins with spermatogonia (plural for spermatogonium) are undifferentiated Type A(d) stem cells in the testes which divide by mitosis to produce Type A(p) cells and more Type A (d) cells. Type A (p) cells divide by mitosis to produce Type B cells which divide by mitosis to produce primary spermatocytes. The primary spermatocytes each undergo meiosis I to form two equally sized secondary spermatocytes. They in turn undergo meiosis II to form four equally sized haploid (monoploid) spermatids. These spermatids go through cell differentiation to develop into four haploid sperm cells flagellated for motility. A sperm has the ability to swim the entire length of the female reproductive tract. Sperm can live is a female's cervical mucus and upper genital tract for about 72 hours (about 3 days). Sperm ejaculated outside the female body might survive in the semen for only up to a few hours.

The mature sperm is one of the smallest human cells - its head measures 0.005 mm by 0.003 mm and with the tail included, the sperm is 0.05 mm in length. During that time, the DNA is condensed to fit into the small head of the spermatid by removing the nucleosomes (histones) and wrapping the DNA around transition proteins which are later replaced with protamines. Protmines are small and highly basic so bind tightly to the DNA with high affinity into a toroidal or doughnut shaped ring. After fertilization, there is a rapid protamine-to-nucleosome exchange in the earliest stages of the zygote and most of the histones that assemble the paternal pronucleus are of maternal origin. (19)

What Types of Genetic Errors Can Occur As Oocytes and Sperm Develop?

As the primary oocyte or spermatocyte undergoes meiosis, there is a possibility of errors in the cell division that can have dramatic effects on the developing zygote, embryo, and fetus if fertilization takes place. These errors include aneuploidy in which the chromosome number is abnormal. Aneuploidy is caused by nondisjunction during meiosis I or II.

In humans, the normal number of chromosomes is 2n = 46. Aneuploidies are further classified by the number of chromosomes that are in excess or missing: If the chromosome number is 2n – 2 = 44, it is called nullisomy and there are no known live births in humans that are examples of nullisomic aneuploidy. If the number is 2n - 1 = 45, it is called monosomy. The only human live birth that is the result of a monosomic aneuploidy is Turner's syndrome, 45XO, present in 1 in 5000 female births. A Turner's female is sterile, short in stature, and often has a webbed neck but is of normal intelligence.

If 2n + 1 = 47, it is called trisomy. There are several human live births that are examples of trisomic aneuploidy including Klinefelter's syndrome, 47XXY, (1 in 1000 male births), in which the male is sterile, has a lanky build, and is intellectually disabled. Others are the 47XYY male and 47 XXX female who are usually fertile and of normal build and intelligence. The most common type of live human trisomic aneuploidy is Down's syndrome, 47trisomy 21. The incidence of trisomy 21 increases with the age of the mother. For example, the incidence is 1 in 1400 births in mothers who are ages 20 – 24, 1 in 700 births in mothers who are ages 30 – 34, and from age 35 on the incidence dramatically increases from 1 in 350 at age 35, 1 in 225 at age 37, 1 in 140 at age 39, 1 in 85 at age 41, 1 in 50 at age 43, and 1 in 25 at age 45+. Down syndrome children have multiple issues including intellectual disability, diminished muscle tone, growth failure, and heart defects. Two other human trisomy aneuploidy syndromes, Patau's syndrome, 47 trisomy 13 (1 in 25,000 births) and Edward's syndrome, 47 trisomy 18 (1 in 8000 births) have multiple and very severe abnormalities at birth and usually don't live more than a few months or years.

Other chromosomal mutations can take place during of meiosis I. These include crossing over which is the exchange of genetic material between homologous pairs of chromosomes and translocation in which a piece of one chromosome is transferred and attached to a non-homologous chromosome. An

example of translocation is the Philadelphia chromosome abnormality BRC/ABL created by a reciprocal translocation between chromosome 9 and 22 which results in coding for a new protein that leads to chronic myelogenous leukemia (CML). Another type of chromosomal mutation is a deletion of a large section of a chromosome, such as $5p^-$ in which a section of the tip of the short (p) arm of chromosome is missing. Sometimes that segment may be added to another chromosome, making it longer but balanced in terms of a complete complement of genes. If that person has a child, however, the child may inherit only one of those two abnormal chromosomes, and in one case would have $5p^-$ and in the other $5p^+$. Either way, the child would have too few genes or too many genes, respectively, which both can cause serious defects. For example, $5p^-$ (1 in 20,000 to 50,000 births) is known as Cri du Chat syndrome, or Cat's Cry syndrome, since the baby mews like a kitten at birth rather than crying. These children often have severe intellectual disability with some ability to learn enough verbal skills to communicate and many physical complications, such as low or incomplete development of motor skills. Other chromosomal mutations include inversions, where a region of the DNA on a chromosome inverts its orientation, and duplications, where some genes are duplicated and displayed twice on the same chromosome.

Prior to implantation, the 1st and 2nd polar bodies can be used to check for some genetic abnormalities in the oocyte. If *in vitro* fertilization is being done, removing one cell from the 4-8 cell embryo can be done to detect genetic abnormalities, such as aneuploidies, deletion, and some translocations, through karyotype analysis. After implantation, using amniocentesis or chorionic villus sampling, a karyotype of the fetus can be compiled and used to detect genetic abnormalities. From there, decisions can be made by the parents as to how to proceed. (9, 10, 11, 12, 24)

<u>What Happens When An Oocyte and Sperm Meet?</u>

As a human oocyte is released from the ovary and is drawn into the fallopian tube, it is ready to be fertilized. If sperm are present, many of them will surround the oocyte and try to pass through the *zona pellucida* which surrounds the oocyte. Fertilin proteins in the sperm plasma membrane have a hydrophobic region that mediates the fusion of the sperm membrane with the oocyte membrane. As one sperm enters the oocyte, it brings with it a haploid nucleus and a centriole. Generally, a series of events follows fertilization that makes it impossible for additional sperm to enter the egg including a *zona pellucida* reaction in which both glycoproteins ZP2 and ZP3 are altered by proteases so they no longer bind to sperm. In addition, as the sperm enters the oocyte, the intracellular calcium ion concentration of the oocyte greatly increases. In this high calcium concentration, the cortical granule membranes fuse with the oocyte plasma membrane releasing their contents. Once this membrane fusion begins at the point of sperm entry, a wave propagates around the cortex to the opposite side of the oocyte.

If this series of events is disrupted, polyspermy, where multiple sperm enter the oocyte, may result. In polyspermy, there is the possibility of 2 sperm pronuclei fusing with the oocyte pronucleus to give a 3n or triploid nucleus; if 3 sperm enter a 4n or tetraploid nucleus will form. Nuclei with 3N and 4N do not produce live births. In addition, as the sperm's centriole divides to form the two poles of mitotic division, the triploid chromosomes may be divided into as many as four cells rather than the normal two cells with a bipolar mitotic spindle. (13)

When the sperm enters the oocyte, meiosis II begins in the oocyte. Then the sperm pronucleus and the oocyte pronucleus fuse to form the zygote (fertilized egg) and from there mitosis can begin. In humans from the point of fertilization, it will take approximately 40 weeks for the human zygote to develop into a fetus and be born. However, births can often occur between 37 to 42 weeks. (13)

In the sea urchin, meiosis I and II have already taken place in the egg and sperm when they meet. Fusion of the sperm and egg membranes is mediated by "fusogenic" proteins. In order to prevent polyspermy, within 1 – 3 seconds after the binding of the sperm the membrane potential shifts from its resting potential of -70 mV to +20 mV due to influx of seawater into the egg. This is not enough to prevent polyspermy if sperm are bound to the vitelline envelope, however. In this case, the removal of extra sperm is accomplished by the cortical granula reaction. As a sperm enters the egg, the cortical granules fuse with the egg plasma membrane and release their contents into the perivitelline space between the egg plasma membrane and the vitelline layer. Mucopolysaccharides released by the cortical granules produce an osmotic gradient that causes water to rush into the perivitelline space, causing it to expand and to form the fertilization envelope. The sperm pronucleus and egg pronucleus fuse to form the zygote and then mitosis begins to occur. From the zygote stage on until the 32-cell blastula stage, the human and sea urchin development look very similar. (13)

<u>After Fertilization, What Occurs?</u>

In humans, as the zygote moves along the fallopian tube toward the uterus, it begins to undergo mitotic division. By day 2 after fertilization, the zygote has divided once into two cells. By day 3, the two cells have divided to form the 4-cell stage. Transcription of the embryonic genome first begins at the 4-cell stage. By day 4, the 4-cells have divided to form 8-cells, at which time the genome transitions from maternal to embryonic gene control. The 8-cells then go through cellular changes including cell-to-cell adhesion to form a compacted solid mass of 12-16 cells called the morula which enters the uterus.

By day 5, after rapid cell division, the early 200-250 cell blastocyst has formed which contains an outer layer of cells surrounding the blastocyst called the trophectoderm which forms the placenta, a hollow cavity called the blastocoel which will form the body cavity, and at one end of the blastocoel, the inner cell mass, a group of about 30 cells that forms the 3 germ layers (ectoderm, mesoderm, and endoderm) of the embryo and fetus. Embryonic stem cells are derived from the inner cell mass and are pluripotent with the ability to make cells of any of the three germ layers.

From the 2-cell to the 8-cell stage, the blastomere cells in the developing offspring are totipotent and are able to develop into complete human beings if the cells are separated. This is the case of monozygotic twins; if the blastomere cells at the 2-cell stage become separated, they can develop into two genetically identical twins. The twins can develop into two blastocysts or into one blastocyst with two inner cell masses. Each inner cell mass would develop into one of the twins. If the two inner cell masses fail to fully separate, then Siamese twins develop as two individuals attached to each other by some point on their bodies. (17) (See Chapter 10 on Stem Cells for more details).

How Does Implantation in the Uterus Occur and How Does the Placenta Form?

By day 6-7, the *zona pellucida* is extruded and the blastocyst attaches to the uterine wall with the inner cell mass located closest to the epithelium of the uterus in preparation for implantation. This attachment is followed by the invasion of the uterine wall.

The inner cell mass then divides into two layers. One layer is the hypoblast which is next to the blastocoel and will give rise to the primitive endoderm. Later this tissue will become the outer layer of the yolk sac. The other cell layer is the epiblast which will give rise to the cells of the embryo. At this point, the epiblast cells succeed the inner cell mass as the only pluripotent cells in the developing zygote. Implantation is completed by day 9 or 10 with the entire developing zygote and extraembryonic tissue embedded into the uterine wall and the entry hole plugged with a fibrin plug.

The extraembryonic cells form the syncytiotrophoblast which form lacunae or cavities which will develop toward maternal blood vessels by day 10. The formation of the placenta is critical during human embryogenesis since it anchors the embryo to the uterine wall and connects it to the mother's blood supply. While the mother and developing embryo do not share blood, the mother supplies the offspring with ions, metabolites, and waste removal through diffusion. Later, the umbilical cord will develop and contain fetal arteries and veins with soluble substances passing between mother and fetus via villi. (14, 17)

If the placenta does not form properly, it can trigger a spontaneous abortion (miscarriage). This is fairly common during this first 2 week period of development. During that first 14 days of the pregnancy, the dividing zygote, implantation, and bilaminar embryo are not susceptible to teratogenesis (a teratogen is any agent that can disturb the development of an embryo or fetus).

Implantation takes about a week and is usually completed by day 14 of the pregnancy. The corpus luteum has maintained the lining of the uterus through production of progesterone and estrogen and through negative feedback lowering levels of follicle stimulating hormone (FSH) and luteinizing hormone (LH), thus inhibiting a new follicle and ovulation from occurring. If fertilization had not taken place, the corpus luteum would have atrophied and decreasing levels of progesterone and estrogen would have triggered a sloughing of the uterine lining and onset of menstruation. In order to maintain production of

progesterone and estrogen by the corpus luteum, a LH-like hormone called human chorionic gonadotropin (hCG) is secreted by the trophoblast cells of the blastocyst. The ovarian-pituitary controls are bypassed and the corpus luteum continues to make progesterone in response to hCG. The placenta takes over the role of producing progesterone and estrogen by the 2nd or 3rd months of fetal development and the corpus luteum atrophies. Because of the continuing production of progesterone and estrogen by the placenta, the ovaries continue to remain dormant until after birth. Pregnancy can be determined by testing the mother's blood with an ELISA test for the antigen hCG by the 3rd week of gestation. (25)

What Occurs as the Epiblast Becomes an Embryo?

At the end of the second week of development (14 days), the developing epiblast begins to be called an embryo. However, the term embryo is used from as early as the 4 cell stage (day 3-4) until the end of the 8th week (56 days or 2 months).

The cells of the epiblast begin to migrate and differentiate. This process is called gastrulation and begins between days 14 and 16 by forming a primitive streak along the posterior axis of the embryo. The epiblast cells spread laterally and induce the formation of the mesoderm and the notochord (future backbone). The anterior region of the mesoderm will give rise to the heart by the middle of the 3rd week and the anterior epiblast cells will generate the neuroectoderm and ectoderm that covers the surface of the embryo. The ectodermal tissue dorsal to the notochord will generate the neural plate, the precursor of the brain and spinal cord.

By the end of the third week of development (21 days), the epiblast tissue of the post implantation blastocyst has generated ectoderm, mesoderm and endoderm layers of the gastrula. The ectoderm will become skin, neurons, eyes, ears, the pituitary gland and the gastrointestinal tract. The mesoderm will become the skeletal, smooth, and cardiac muscles, bone marrow, kidney tubules, heart, blood vessels, and reproductive organs. The endoderm will become the pancreas, lung, bladder, thyroid, and liver.

By the end of the 4th week (28 days), the embryo is 5 -7 mm in length and the upper and lower limb buds have begun to form as well as the ears and eyes. The third and fourth weeks of pregnancy are a highly sensitive time for congenital abnormalities in the central nervous system including the optic lens, heart, limb buds, and somites (future muscles, bones, connective tissue). During these highly sensitive periods for congenital abnormalities, diseases and some prescribed drugs can affect the embryo's development. Many over the counter drugs, illegal drugs, alcohol, poor diet, and smoking can affect the development of the embryo and fetus. If a mother were to have measles during this time, for example, the embryo has a high possibility of being born blind since the virus attacks the eyes, which are not covered yet by an eyelid. Thalidomide taken by mothers as a sedative for

morning sickness just when the limb buds were being formed led to children who were born without arms and/or legs. After the correlation was determined between thalidomide use and birth defects over a 3 year period of time, it was taken off the market in 1961 until just recently when it began to be used again to treat other illnesses, with a strict warning not to be taken by pregnant women.

At 37 days, the embryo is 11- 14 mm in length and goes through another highly sensitive period for congenital abnormalities for development of the retinal pigment, pharyngeal arch, and thigh, foot, and leg. At 44 days (1.5 months), the embryo is 13 – 17 mm in length and has another highly sensitive period for development of lumbar flexure, hand notches, elbow, teeth, and eyelid folds.

At 56 days (almost 2 months), the embryo is 25 – 27 mm in length and is highly susceptible to abnormalities of the ear, hand extension, fingers, and eyelids. The development of the embryo in the first two months of pregnancy looks very similar across the Phylum Chordata, so an embryo of a person, a pig, and a bird would look very similar. After that time around the 8^{th} or 9^{th} week of pregnancy, the more human features begin to form. (14)

When Does the Naming Transition from Embryo to Fetus Occur?

Beginning at week 9 (56 days or almost 2 months old) until birth, the embryo begins to be called a fetus and at birth, the fetus is then called a baby. By the end of 10 weeks (70 days or 2.5 months old) in the womb, alcohol abuse is particularly harmful and can lead to fetal alcohol syndrome which leaves the baby intellectually disabled and with ocular, joint, and growth abnormalities. With illegal drug abuse, the fetus can be born addicted to the drug. Smoking leads to low birth weight babies due to the constriction of the mother's vascular tissue by the components of the smoke.

By six months, the fetus has a reasonable chance of living to birth. Premature babies born at this time or later can survive with few or no adverse long-term side effects if they have access to neonatal hospital care. Many of their tissues are still developing in that last few months before a full term birth so premature babies need to be cared for as they continue to develop those tissues outside the womb. For instance, many have issues with breathing since the surfactants in their still lungs are one of the last things to develop before birth. (14)

What Can Cause Infertility?

Infertility can happen for many reasons. For females, the follicle containing the oocyte may not be capable of ovulation. The cause could range from anorexia to a variety of illnesses, including pelvic inflammatory disease. Another cause of female infertility is that the oocyte is abnormal. In men, infertility could be due to a low sperm count, immotile sperm, and/or abnormal sperm. Tight clothing, denim jeans in particular, can cause low sperm count. Other causes of fertility in both sexes could be genetic, age, and environmental toxins.

How Is Infertility Treated?

Assisted reproductive technologies (ART) can help treat couples who haven't had success in having an offspring on their own. Methods include artificial insemination, *in vitro* fertilization (IVF), and intra-cytoplasmic sperm injection (ICSI).

Artificial insemination is the placement of sperm into a female's uterus for the purpose of achieving a pregnancy by means other than sexual intercourse. The sperm can be collected fresh or it could be frozen-thawed. The sperm are deposited into the woman's uterus when she is about to ovulate. This method is used for couples where there is male infertility and in cases where a single woman wants to have a child. In addition, this is a common practice in animal breeding, such as in cattle, horses, and zoo elephants.

In vitro fertilization (IVF) is a laboratory technique that can be used to help people who are unable to have their own children naturally due to problems in their reproductive systems. IVF is a procedure in which the offspring is produced by combining in a Petri dish the egg and sperm harvested from the couple, growing the zygote to the 4- or 8-cell stage, and then implanting it into the uterus of the mother to further develop. Often called test tube babies, the offspring is actually developed in a Petri dish rather than a test tube. The first IVF baby, Louise Brown, born in on July 25, 1978 in England, from an 8-cell embryo that was developed in a Petri dish. Her mother had blocked fallopian tubes so couldn't conceive naturally. Louise has a younger sister, Natalie, who also was an IVF baby. Natalie was the first IVF baby to herself give birth to a child – her daughter was born in 1999. Louise later had naturally conceived children of her own. By 2013, there had been 5 million IVF babies born, with IVF used in 3% of all live births in developed countries. (20, 21)

British biologist Robert G. Edwards worked to get oocytes and sperm to successfully unite outside the body and, with his colleague, Dr. Patrick Steptoe, (a gynecologist who pioneered laparoscopic surgery in order to extract the oocytes), collaborated on the first live birth in 1978. In 2010, Robert G. Edwards won the Nobel Prize in Physiology or Medicine "for the development of *in vitro* fertilization". Unfortunately, the Nobel Prize is not awarded posthumously and Dr. Steptoe died in 1988.(22)

The IVF procedure contains 3 parts – retrieval of oocytes, combining oocytes with sperm, and inserting the developing zygotes into the woman's uterus to grow. First, the woman is given extra estrogen and progesterone over the course of a month in order for her to produce multiple oocytes. Then the metaphase II oocytes are aspirated from the ovaries in a laparoscopic procedure, and the eggs are cleaned of extra tissue and placed in a Petri dish. The man's sperm is added to the Petri dish

and the cells are incubated for 3-4 days. Then the two or three best 4- to 8-cell stages are chosen and inserted into the woman's uterus for further development. The procedure is about 20-30% effective in humans. (22)

Research is being done to determine the most viable one embryo to reinsert into the uterus because multiple embryos crowd each other causing each to develop in the womb less well. More recently the timing of the mitotic cell divisions has been done and the developing cells are ranked. The most perfectly dividing one is implanted first, then the second if the first doesn't continue to develop, then the third, and so on. Sometimes, IVF development will stall out at the 8-cell stage, so in some cases, the embryo has been allowed to develop to the blastocyst stage before implantation to ensure a greater chance of a live birth.

Eventually, those developing human embryos that are not implanted will be frozen in liquid nitrogen until a later time. 4- to 8-cell stages have been successfully unfrozen and implanted to produce live births. It was found in 1989 with non-human primates that the IVF 4-cell stage could be frozen and then thawed, implanted in the uterus, and produce a live birth. The first Rhesus monkey IVF baby in the world was born on October 31, 1989 at the Oregon National Primate Research Center (ONPRC) and was named Shiver. The first Rhesus monkey twins, Arnold and Danny, were born from *in vitro* fertilization, frozen-thawed 4-cell stage embryos at ONPRC.

Intra-Cytoplasmic Sperm Injection (ICSI) is similar to IVF but involves injecting a single sperm directly into an oocyte in order to fertilize it, rather than mixing the oocyte and sperm together in a Petri dish and waiting for the oocyte to be fertilized. The zygote then divides to the 4- to 8-cell stage and is transferred to the woman's womb. ICSI is used if there is a low sperm count, sperm have poor motility, previous IVF attempts have failed, or sperm need to be collected from the testicles or epididymis because of a vasectomy.(23)

These infertility treatments can be used with non-human primates and other mammalian endangered species to help preserve their populations. Animals in zoos and rescued animals can be treated with Assisted Reproductive Technologies (ART) including artificial insemination, *in vitro* fertilization, and intra-cytoplasmic sperm injection to try to increase the numbers of their species to prevent extinction. For example, Timu was the first Western lowland gorilla born through *in vitro* fertilization at the Cincinnati Zoo in 1966. She now lives in the Henry Doorly Zoo, Omaha, Nebraska and had babies of her own in 2003 and 2005.

How Can Pregnancy Be Prevented?

Methods of birth control fall into two categories – a) preventing ovulation and b) preventing the oocyte and sperm from meeting.

a) **Preventing Ovulation** - Birth control pills, skin patches, implants, and vaginal rings use both estrogen and progestin to prevent ovulation by changing the levels of the natural hormones the body makes. Progestin helps prevent sperm from entering the uterus by making the mucus around a woman's cervix thick and sticky. Progestin alone, such as Depo-Provera, can be given by injection or pill.

   Since conception typically doesn't occur immediately after sex and may occur up to several days later, the morning-after pill can prevent pregnancy. The morning-after pill does not end a pregnancy that has implanted in the uterus but acts to delay or prevent ovulation or block fertilization. FDA-approved morning-after pills include Plan B One-Step and Next Choice which are available over the counter at pharmacies and Ella which is available by prescription. The morning-after pill is not the same as mifepristone (Mifeprex) also known as RU-486 or the abortion pill which terminates an implanted established pregnancy.

b) **Preventing the Oocyte and Sperm From Meeting** - Methods used to prevent the oocyte and sperm from uniting include barrier methods such as the male and female condoms (also the best methods of preventing sexually transmitted diseases), diaphragm, cervical cap, spermicidal jelly, cervical sponge, intrauterine device (IUD), vasectomy, and tubal ligation. Other methods include abstinence and natural family planning (NFP), which is an awareness of the monthly cycle and when ovulation (fertility) is occurring.

The most effective methods of birth control are vasectomy, tubal ligation, IUD, and hormone implants where there is less than 1 pregnancy per 100 women each year when used correctly. The least effective are the spermicidal jelly and natural family planning which result in about 25 pregnancies per 100 women each year. (4, 5, 6, 7, 8)

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