Developmental Biology
Unit -5. Implications of Developmental Biology

Teratogenesis: Teratogenic agents and their effects on embryonic development

Teratogenesis:

Teratogenesis or teratogenicity is the process by which congenital birth defects occur by some biological infections (viral, protozoan etc.), physical agents (ionizing radiations, excessive heat etc.), pharmacological drugs (thalidomide, corticosteroids, antiepileptic or antimalarial drugs etc.), industrial pollutants (toluene, cadmium etc.), tipsiness of mother (alcohols, nicotine etc.), maternal health problems (diabetes mellitus, rheumatoid arthritis etc.).

Teratology is the science that investigates the congenital malformations and their causes (how environmental agents disrupt normal development).

Teratogenic Agents:

The agents which are responsible for causing congenital malformations are called Teratogenic Agents.

1) Infectious agents:

Some infections during pregnancy are teratogenic like viral infections (e.g. **rubella, herpes simplex and cytomegalovirus**), spirochetal infections (e.g. **syphilis**), and protozoal infestations (e.g. **toxoplasmosis**). First trimester maternal **influenza** exposure is associated with raised risk of a number of non-chromosomal congenital anomalies including neural tube defects, hydrocephalus, congenital heart anomalies, cleft lip, digestive system abnormalities and limb defects.

2) Physical agents:

Radiation is teratogenic and its effect is cumulative. The degree of ionizing radiation needed for health testing (checking) procedures is very close to the threshold for teratogenicity, especially in the first trimester. There is a basic assumption that risk prediction for human radiation exposure is proportional to the total radiation dose.

3) Chemical agents:

 Placental transporter proteins are involved in the pharmacokinetics of drugs and have an effect on drug level and foetal drug exposure. There is an association between P-glycoprotein polymorphisms and the risk of foetal birth defects induced by medications during pregnancy. Six underlying teratogenic mechanisms are stated to be associated with medication use. They include folate antagonism, neural crest cell disruption, endocrine disruption, oxidative stress, vascular disruption and specific receptor- or enzyme-mediated teratogenesis.

 Antiepileptic drugs (AEDs) are frequently used to treat epilepsy, headaches, and psychiatric disorders in women of childbearing age. In many instances, clinicians are obliged to stop these drugs or switch to another category of medications. Discontinuation of AEDs during pregnancy is not advised due to the risk of seizures that may be fatal to both mother and fetus. Hepatic mixed oxidase system and other systems like epoxide hydrolase, glutathione reductase and superoxide dismutase as well as toxin-scavengers are important modifiers that lower the teratogenic risk of the drug. In utero exposure to some AEDs can lead to significant cognitive and behavioural teratogenic risks for the foetal outcome. Valproate obviously induces impaired cognitive development and increased risk rate of autism incidence.

 Retinoic acid (RA) or **retinol** is the active metabolite of vitamin A and is responsible for all of the bioactivity associated with this vitamin. It plays essential signalling roles in mammalian embryogenesis. Excess intake of vitamin A often results malformations to foetus's skulls, faces, limbs, eyes and central nervous system. The RA catabolic CYP26 enzymes prevent the teratogenic consequences caused by uncontrolled distribution of RA particularly on the RA-sensitive tissues like the limbs and the testis.

 Isotretinoin is a very effective oral medicine (a byproduct of vitamin A) for the treatment of severe acne. In greater amount intake, it can cause foetal abnormalities including cleft lips, ear and eye defects, and mental retardation.

 Mefloquine (MQ) is a potent effective antimalarial drug against Plasmodium falciparum. It is safe during the second and third trimesters. In early gestation in Wistar rats, MQ induce minimal extension of lateral brain ventricles and renal pelvis together with delayed ossification in the foetuses.

 Miltefosine a drug used in the treatment of visceral leishmaniasis but its use is hampered by its potential teratogenicity. Its excess use hamper the development of foetus.

 Ritodrine is a drug used to stop premature labor. Its excess use cause cardiovascular problems in foetus. **Nifedipine** (Adalat) is used to manage angina, hypertension, Raynaud's phenomenon and premature labour. Its overdose can cause vascular dilation in both the uterus and the placenta. However, ritodrine + nifedipine combination had reduced the toxic and teratogenic effects of nifedipine alone on embryos.

4) Environmental pollutants:

 Toluene is an organic solvent necessary for industry. Many women of childbearing age are increasingly exposed to toluene in occupational settings (i.e. long-term, low-concentration exposures) or through inhalation abuse (e.g. episodic, binge exposures to high concentrations). High levels of toluene exposure may lead to retardation of mental health and growth in foetus.

 Cadmium (Cd) is a heavy metal pollutant and teratogen. Cd mediated teratogenicity, in a chick embryo, had occurred because of impaired endogenous nitrous oxide (NO), increased oxidative stress, and activated apoptotic pathways. Cd

significantly decreases foetal body weight, forelimb and hindlimb bone lengths. It also may cause abortion.

5) Tipsiness of mother:

 Alcohol (prenatal) is considered as a teratogenic agent. Genetic factors seem to influence foetal alcohol spectrum disorders in both humans and animals. Micro RNAs and their target genes are involved in the pathogenesis of foetal alcohol syndrome. Some sociobehavioral risk factors (e.g. low socioeconomic status) are permissive for foetal alcohol syndrome (FAS). These permissive factors are related to biological factors (e.g. decreased antioxidant status) which together with alcohol, provoke FAS/ alcohol-related birth defects (ARBDs) in vulnerable foetuses.

 N**icotine** consumption by mother is teratogenic leading to increased incidence of attention hyperactivity disorder. There is a correlation of teratogenic effects of alcohol and tobacco, and the risk of anorectal atresia. Smoking increases risk for SIDS (Sudden Infant Death Syndrome) which is the sudden and unexpected death of an infant under 12 months of age that occurs typically while sleeping, also related to failure of auto-resuscitation, normal heart rate and breathing.

 Cocaine abuse significantly reduces foetal weight, increases the malformation rate, and augments the stillbirth rate due to abrupt placentae. Cocaethylene or ethylbenzoylecgonine is formed in the liver when cocaine and alcohol are simultaneously ingested; each is a potent stimulant and dopamine uptake blocker that is more toxic to myocardial cells than cocaine alone.

6) Maternal health problems:

 Diabetes mellitus of mother are of great concern during pregnancy. Teratogenesis is associated with pre-existing and gestational diabetes. The risk of congenital anomalies increases in the offspring of obese diabetic women.

 Oral antihyperglycemic agent use is not recommended during pregnancy. Healthy diet and regular exercise may help optimize pre-pregnancy weight and reduce the risk of congenital anomalies. Cardiac and neural tube defects are the most common malformations observed in foetuses of pre gestational diabetic mothers.

 Multiple sclerosis (MS) of pregnant mother should carefully consider the risks and benefits of ongoing therapy for the health of both the mother and the foetus. The immunosuppressant **mitoxantrone** and **fingolimod** are teratogenic and should be prescribed only with strict effective contraception.

 For pregnant women suffering from **rheumatoid arthritis (RA)**, the use of immune modulating medications has low risk allowing for optimal outcomes. Some women with RA may have a risk of miscarriage or low-birth-weight babies.

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Amniocentesis

What is Amniocentesis? (Amniotic Fluid Test)

Medical procedure used for prenatal genetic testing for chromosomal abnormalities (presence or absence of certain chromosomes, genes or enzymes), fetal infections as well as for sex determination to obtain a sample of amniotic fluid from a pregnant woman. A long sterile needle is inserted through the abdominal wall into the amniotic sac to obtain the fluid.

Amniotic fluid—the substance that fills the amniotic sac and surrounds the developing fetus—contains fetal cells (e. g. multipotent mesenchymal, hematopoietic, neural, epithelial, and endothelial stem cells) that can be used for genetic testing.

Why is an Amniocentesis performed?

This process is performed to look for certain types of birth defects. Because amniocentesis presents a small risk for both mother and her baby, the prenatal test is generally offered to women who have a significant risk for genetic diseases, including those who:

- Have an abnormal ultrasound or abnormal lab screens
- Have a family history of certain birth defects
- Have previously had a child or pregnancy with a birth defect
- Had an abnormal genetic test result in the current pregnancy

Amniocentesis does not detect all birth defects, but it can be used to detect the following conditions if the parents have a significant genetic risk of:

- Down syndrome
- Sickle cell disease
- Cystic fibrosis
- Muscular dystrophy
- Tay-Sachs disease

Procedure:

Amniocentesis is routinely performed as an outpatient procedure either with or without the use of a local anaesthetic.

- Ultrasonography is used to locate the position and movements of the fetus, location of placenta, and characteristics of the amniotic fluid in the uterus.
- With the aid of ultrasound guidance, a long, sterile needle is inserted through the abdominal wall of the uterus at an angle through the muscle, then through the wall of uterus and finally into the amniotic sac.

 The physician then punctures the sac in an area away from the fetus and extracts approximately 20ml of amniotic fluid. This procedure can be performed with a single needle and double needle technique. These techniques have their own variations in how they are performed including guidance of needle insertion location, and angle of needle insertion.

 From the 20ml of amniotic fluid, the first 2ml is typically discarded due to mixture with maternal blood cells to ensure high quality fluid sampling.

 Fetal cells are separated from the amniotic fluid and placed in a culture medium that stimulates them to grow and divide, then fixed and stained. Under a microscope, the chromosomes are examined for abnormalities.

 After the procedure, the puncture seals and the amniotic sac replenishes the liquid over the next 24-48 hours.

Medical uses:

1) Genetic diagnosis:

Early in pregnancy, amniocentesis is used for diagnosis of chromosomal and other fetal problems such as:

 Down syndrome (Trisomy 21)

 Patau syndrome (Trisomy 13)

 Edwards' syndrome (Trisomy 18)

 Sex chromosome aneuploidies

 Neural tube defects (diseases where the brain and spinal column don't develop properly) – anencephaly (a baby born with an underdeveloped brain and an incomplete skull) and spina bifida/split spine (a birth defect in which there is incomplete closing of the spine and the membranes around the spinal cord during early development), by alpha-fetoprotein levels

 Rare metabolic disorders

2) **Infection*:***

This process can detect infections via decreased glucose level, a Gram stain showing bacteria, or abnormal differential count of WBCs.

3) Lung maturity:

This can predict fetal lung maturity, which is inversely correlated to the risk of infant respiratory distress syndrome. Several tests are available, including the-

 Lecithin-sphingomyelin ratio (L/S ratio): if the result is less than 2:1, the fetal lungs may be surfactant deficient.

 The presence of phosphatidylglycerol (PG): indicates fetal lung maturity.

 The surfactant/albumin ratio (S/A ratio): the result is given as mg of surfactant per gm of protein. An S/A ratio <35 indicates immature lungs, 35-55 is intermediate, and >55 is mature surfactant production.

4) Decompression of polyhydramnios:

Polyhydramnios (the accumulation of amniotic fluids) can be relieved via decompression amniocentesis. Amniocentesis can also be used to diagnose potential causes of polyhydramnios.

5) Rh incompatibility:

This process can be used to diagnose Rh incompatibility, a condition when the mother has Rh-negative blood and the fetus has Rh-positive blood. Early detection is important to treat the mother with Rh immunoglobulin and to treat her baby for haemolytic anemia.

Risks:

Amniocentesis is performed between the 15th and 20th week of pregnancy; performing this test earlier may result in fetal injury. The term "early amniocentesis" is sometimes used to describe use of the process between weeks 11 and 13.

Complications of amniocentesis include preterm labor and delivery, respiratory distress, postural deformities, chorioamnionitis or intra-amniotic infection (an inflammation of the fetal membranes – amnion and chorion, due to a bacterial infection), fetal trauma and alloimmunisation or rhesus disease (an immune response to foreign antigens after exposure to genetically different cells or tissues) of the mother. Amniotic fluid embolism or AFE (a very uncommon childbirth emergency in which amniotic fluid enters the blood stream of the mother to trigger a serious reaction and results in cardiorespiratory collapse and massive bleeding) has also been described as a possible outcome. Additional risks include amniotic fluid leakage and bleeding. These two are of particular importance because they can lead to spontaneous abortion in pregnant patient.

 The first amniotic stem cells bank in the US is active in Boston, Massachusetts.

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In vitro Fertilization (IVF)

What is IVF?

In vitro fertilisation (IVF) is a process of fertilisation where an egg is combined with sperm outside the body, in vitro ("in glass"). The process involves monitoring and stimulating a woman's ovulatory process, removing an ovum or ova (egg or eggs) from the woman's ovaries and letting sperm fertilise them in a liquid in a laboratory. After the fertilised egg (zygote) undergoes embryo culture for 2–6 days, it is implanted in the same or another woman's uterus, with the intention of establishing a successful pregnancy.

Why it's done?

IVF is a treatment for infertility or genetic problems. Sometimes, IVF is offered as a primary treatment for infertility in women over age 40. IVF can also be done for certain health conditions:

 Fallopian tube damage or blockage:

This problem makes it difficult for an egg to be fertilized or for an embryo to travel to the uterus.

 Ovulation disorders:

If ovulation is infrequent or absent, fewer eggs are available for fertilization which reduces the chances of pregnancy.

 Endometriosis:

It occurs when the uterine tissue implants and grows outside of the uterus – often affecting the function of the ovaries, uterus and fallopian tubes.

 Uterine Fibroids:

Fibroids are benign tumours in the wall of the uterus and are common in women in their 30s and 40s. These can interfere with implantation of the fertilized egg.

Previous tubal sterilization or removal:

If one had have tubal ligation, in which her fallopian tubes are cut or blocked to permanently prevent pregnancy – and want to conceive, IVF may be an alternative to tubal ligation reversal.

 Impaired sperm production or function:

Below-average sperm production, weak movement of sperm, or abnormalities in sperm size & shape can make it difficult for sperm to fertilize an egg. If semen abnormalities are found, it also affect in fertilization.

 A genetic disorder:

If one and his/her partner is at risk of passing on a genetic disorder to the child, he/she may be candidate for pre-implantation genetic testing – a procedure, that involves IVF. After the eggs are harvested and fertilized, they're screened for certain genetic problems. Embryos that don't contain identified problems, can be transferred to the uterus.

 Fertility preservation for cancer or other health conditions:

If one is about to start cancer treatment (radiation or chemotherapy), that could harm her fertility, IVF may be an option. Women can have eggs harvested from their ovaries and frozen in as unfertilized state for later use, or the eggs can be fertilized and frozen

as embryos for future use. Women, who don't have a functional uterus or for whom pregnancy poses a serious health risk, might choose IVF using another person to carry the pregnancy (gestational carrier).

Steps involved in IVF:

IVF involves several steps – ovarian stimulation/induction, egg retrieval, sperm retrieval, fertilization and embryo transfer. One cycle of IVF can take about two to three weeks.

 Ovulation induction:

If a woman using her own eggs during IVF, at the start of a cycle she'll begin treatment with synthetic hormones to stimulate her ovaries to produce multiple eggs – rather than the single egg that normally develops each month. Multiple eggs are needed because some eggs won't fertilize or develop normally after fertilization.

In this aspect, some medications are –

- For ovarian stimulation - An injection containing FSH, LH or a combination of both. They stimulate more than one egg to develop at a time.
- For oocyte maturation - When the follicles are ready for egg retrieval (generally after 9-14 days), she has to take HCG to help the eggs mature.
- To prepare the lining of the uterus - On the day of egg retrieval or at the time of embryo transfer, the doctor might recommend that she begin taking progesterone supplements to make the lining of uterus more receptive for implantation.

Typically, she'll need 1-2 weeks of ovarian stimulation before the eggs are ready for retrieval. To determine when the eggs are ready for collection, doctor will likely perform i) vaginal ultrasound (to see the development of follicles), ii) blood tests (to measure the response to ovarian stimulation medications – oestrogen levels increase as follicles develop, progesterone levels remain low until after ovulation).

 Egg retrieval:

It can be done 34-36 hours after the final injection and before ovulation.

- During egg retrieval, she'll be sedated and given pain medication.
- Trans-vaginal ultrasound aspiration is the usual retrieval method. An ultrasound probe is inserted into the vagina to identify follicles. Then a thin needle is inserted through the vagina and into the follicles to retrieve the eggs.
- The eggs are removed from the follicles through a needle connected to a suction device. Multiple eggs can be removed in about 20 minutes.
- Mature eggs are placed in a nutritive liquid (culture medium) and incubated. Eggs that appear healthy and mature, will be mixed with sperm to attempt for fertilization. However, not all eggs may be successfully fertilized.

 Sperm retrieval:

If the woman using her partner's sperm, he'll provide a semen sample at the doctor's clinic in the morning of egg retrieval. Other methods such as testicular aspiration (use of a needle to extract sperm directly from the testicle) are sometimes required. Donor sperm can also be used. Sperms are separated from the semen fluid in the lab.

 Fertilization:

Fertilization can be attempted using two common methods:

- Conventional insemination – during this process, healthy sperm and mature eggs are mixed and incubated overnight.
- Intra-cytoplasmic sperm injection (ICSI) – in this process, a single healthy sperm is injected directly into each mature egg. It is often used when semen quality or number is a problem.

 Embryo transfer:

Embryo transfer is done 2-5 days after egg retrieval:

- The woman might be given a mild sedative.
- The doctor will insert a long, thin, flexible tube (catheter) into patient's vagina through the cervix and into the uterus.
- A syringe containing one or more embryos suspended in a small amount of fluid, is attached to the end of the catheter.
- Using the syringe, the doctor places the embryo(s) into the uterus
- If successful, an embryo will implant in the lining of the uterus about 6-10 days after egg retrieval.

 Result:

About 12days – 2weeks after egg retrieval, the doctor will test a sample of patient's blood to detect the pregnancy.

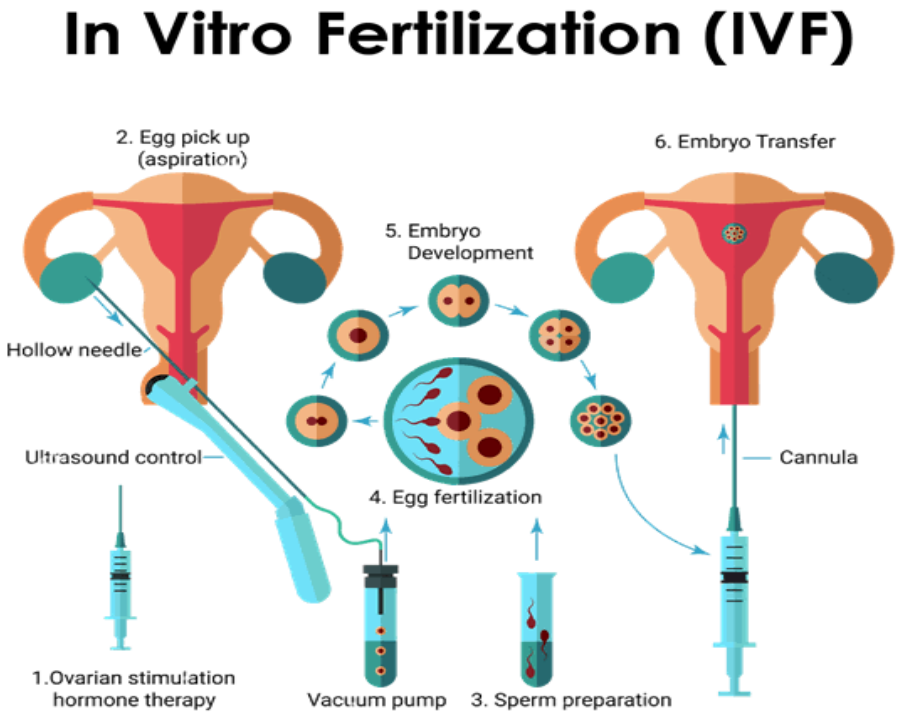
- If pregnancy occurs – the doctor will refer her to other pregnancy specialist for prenatal care.
- If pregnancy doesn't occurs – she'll stop taking progesterone. The doctor might suggest steps she can take to improve the chances of getting pregnant through another cycle of IVF.

Risks:

Risks of IVF includes –

- *Multiple Births:* IVF increases the risk of multiple births if more than one embryo are transferred to the uterus.
- *Premature delivery and low birth weight:* IVF slightly increases the risk that the baby will be born early or with a low weight.

- *Ovarian hyperstimulation syndrome:* Use of injectable fertility drugs (i.e., HCG) to induce ovulation can cause ovarian hyperstimulation syndrome, in which the ovaries become swollen and painful. Symptoms include mild abdominal pain, bloating, nausea, vomiting and diarrhoea.
- *Miscarriage:* The rate of miscarriage for women who conceive through IVF with fresh embryos is similar to that of women who conceive naturally – about 15% - 25%, but the rate increases with maternal age.
- *Egg-retrieval procedure complications:* use of an aspirating needle to collect eggs could possibly cause bleeding, infection or damage to the bowel, bladder or blood vessel. Risks are also associated with sedation and anaesthesia, if used.
- *Ectopic pregnancy:* About 2%-5% of women who use IVF will have an ectopic pregnancy (the fertilized egg implants outside the uterus, usually in the fallopian tube). The fertilized egg can't survive outside the uterus and there's no way to continue the pregnancy.
- *Birth defects:* The age of the mother is the primary risk factor in the development of birth defects (i.e. septal heart defect, cleft lips with or without cleft palate, oesophageal atresia, anorectal atresia etc.). IVF, including ICSI, is associated with an increased risk of imprinting disorders including Prader – Willi syndrome and Angelman syndrome.
- *Spread of infectious diseases:* Hepatitis-B, HIV etc. may be transmitted through IVF when sperms are from unknown donor.



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Stem Cells

What are Stem Cells?

Stem cells are raw cells found within the human body from which all other cells with specialized functions are generated. In proper experimental conditions, these stem cells can be divided to create daughter cells. These daughter cells can either be new stem cells themselves, by the way of self-renewal, or end up being functional cells (i.e. blood cells or brain cells).

Where do these stem cells come from?

Researchers have discovered several sources of stem cells –

 Embryonic stem cells: These stem cells come from embryos that are 3 – 5 days old. They are by far the most versatile and can thus be used for both regenerative and cell replacement purposes.

 Adult stem cells: These stem cells are found (in small numbers) in most adult tissues (such as bone marrow or fat). Compared with embryonic stem cells, these have a more limited ability to produce various cells found in the body. These can be versatile, but not to the extent found in embryonic cells.

 Adult cells altered to have properties of embryonic stem cells: Scientists have successfully transformed regular adult cells into stem cells using genetic reprogramming. Through altering the genes in the adult cells, researchers can reprogram the cells to act similarly to that of embryonic stem cells.

 Perinatal stem cells: Researchers have discovered stem cells in amniotic fluid as well as within umbilical cord blood. These also have the ability to change into specialized cells when required.

Embryonic Stem: ESCs are pluripotent stem cells derived from the inner cell mass of a blastocyst, an early-stage pre-implantation embryo. Human embryos reach the blastocyst stage 4–5 days post fertilization, at which time they consist of 50 – 150 cells. Isolating the embryoblast or inner cell mass (ICM) results in destruction of the blastocyst, a process which raises ethical issues, including whether or not embryos at the pre-implantation stage should have the same moral considerations as embryos in the post-implantation stage of development.

Properties:

Embryonic stem cells (ESCs), derived from the blastocyst stage of early mammalian embryos, are distinguished by their ability to differentiate into any embryonic cell type and by their ability to self-renew. It is these traits that makes them valuable in the scientific and medical fields. ESCs have a normal karyotype, maintain high telomerase activity, and exhibit remarkable long-term proliferative potential.

1) Pluripotent:

Embryonic stem cells of the inner cell mass are pluripotent, meaning they are able to differentiate to generate primitive ectoderm, which ultimately differentiates during

gastrulation into all derivatives of the three primary germ layers: ectoderm, endoderm and mesoderm. These germ layers generate each of the more than 220 cell types in the adult human body. When provided with the appropriate signals, ESCs initially form precursor cells that in subsequently differentiate into the desired cell types. Pluripotency distinguishes embryonic stem cells from adult stem cells, which are multipotent and can only produce a limited number of cell types.

2) Self-renewal and repair of structure:
Under defined conditions, embryonic stem cells are capable of self-renewing indefinitely in an undifferentiated state. Self-renewal conditions must prevent the cells from clumping and maintain an environment that supports an unspecialized state.

Uses:

Due to their plasticity and potentially unlimited capacity for self-renewal, embryonic stem cell therapies have been proposed for regenerative medicine and tissue replacement after injury or disease. Pluripotent stem cells have shown promise in treating a number of varying conditions, including but not limited to: spinal cord injuries, age related macular degeneration, diabetes, neurodegenerative disorders (such as Parkinson's disease), AIDS, etc. In addition to their potential in regenerative medicine, ESCs provide a possible alternative source of tissue/organs. ESCs can also be used for research on early human development, certain genetic disease, and in vitro toxicology testing.
Human embryonic stem cells have the potential to differentiate into various cell types, and, thus, may be useful as a source of cells for transplantation or tissue engineering:

 Cell replacement therapies (CRTs)–
Some of the cell types that are differentiating from ESCs, have or are currently being developed include cardiomyocytes (CM), neurons, hepatocytes, bone marrow cells, islet cells and endothelial cells. These are in current research.
 Clinical potential –

- ESCs have been differentiated to natural killer (NK) cells and bone tissue.
- Researchers have differentiated ESCs into dopamine-producing cells with the hope that these neurons could be used in the treatment of Parkinson’s disease.
- Studies involving ESCs are underway to provide an alternative treatment for diabetes. For example, researchers were able to differentiate ESCs into insulin producing cells to produce large quantities of pancreatic beta cells from ES.
- An article describes a translational process of generating human embryonic stem cell-derived cardiac progenitor cells to be used in clinical trials of patients with severe heart failure.

 Drug discovery – Besides becoming an important alternative to organ transplants, ESCs are also being used in field of toxicology and as cellular screens to uncover new chemical entities (NCEs) that can be developed as small molecule drugs.

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