What are Common Birth Defects in Humans, and How are They Diagnosed?

CHAPTER 1

A major malformation is defined as a structural abnormality that is either lethal, requires treatment (medical or surgical), or is of cosmetic importance. These abnormalities occur with similar frequency in all species studied, including humans, mice, rats, dogs, cats, pigs, cows, and horses. Structural abnormalities are a major factor in the survival of an embryo or fetus; in every species, the frequency of malformations is highest in pregnancies that result in miscarriages or stillbirths.

About two percent of newborn infants are identified as having a major malformation at birth. Examples of common major malformations include:

• Heart defects, such as a ventricular septal defect
• Down Syndrome (Trisomy 21, characterized by mental retardation, a sloping forehead, low-set ears, a flat nose, and short, broad hands)
• Spina bifida (defective development of the spinal cord and a failure of the overlying skin and vertebrae to close properly, producing weakness or paralysis below the defect)
• Polydactyly (an extra finger, usually next to the fifth finger; occasionally there is an extra thumb)
• Fetal alcohol syndrome (a growth deficient infant with a distinctive pattern of facial features)

Newborn infants are examined at the time of delivery by the birth attendant, and then more thoroughly by the baby’s health care provider a few hours later. If a fetal abnormality was detected during pregnancy, by prenatal screening or ultrasound, a pediatrician may be present in the delivery room to assess whether the infant requires intensive care.

The newborn examination includes looking for abnormal facial features, an abnormally large or small head, extra fingers, webbing between fingers, genital abnormalities and a club foot. Examining the abdomen and listening to the heart may detect other defects.
Despite the routine examination of babies, not all birth defects are detected at birth. Kidney abnormalities, heart defects, hip dysplasia and brain abnormalities may not be detected until months or years later. If several malformations are identified, chromosome analysis is done on white blood cells and a geneticist can be consulted.

The geneticist examines the infant and interviews the parents to review the pregnancy and family history to find out, for example, whether other relatives have similar malformations, or whether the mother had a chronic disease, such as diabetes, or exposure to a drug that could have affected the infant. The geneticist determines whether the baby has a recognized pattern of problems and advises the parents about probable causes and the options for treatment. Additional tests, such as DNA studies, may be ordered.

Recognized causes of major malformations include:

- A Dominant Gene
  An infant with a skeletal dysplasia, such as achondroplasia, could have inherited the dominant gene that causes this condition from an affected mother or father. The infant could also be affected as the result of a spontaneous mutation, in which case the infant would be the first affected member of the family.

- A Recessive Gene
  In infantile polycystic kidney disease, a child is affected because both parents carry the same rare recessive gene. Parents who carry the gene are unaffected, but their children have a one in four chance of being affected.

The website www.genetests.org can be used to identify laboratories which provide analyses for gene mutations that have been identified for about 10% of the recognized disorders due to either dominant or recessive genes. Disorders due to these genes are listed in the website Mendelian Inheritance in Man.

- Chromosome abnormalities
  Having an extra chromosome causes specific syndromes. The presence of an extra number 21 chromosome causes Down Syndrome, also called trisomy 21. An extra number 18 chromosome (trisomy 18) or number 13 chromosome (trisomy 13) are less common than Down syndrome; all of these syndromes cause mental retardation but each syndrome is associated with a different set of malformations. Other chromosome abnormalities include the presence of an extra X chromosome (47,XXX) or the absence of one X (45, X Turner Syndrome), which is associated with a webbed neck and, in newborns, swelling of the hands and feet. Adults with Turner’s syndrome never develop breasts or other secondary sexual characteristics.

In the 1990s it became possible to detect a missing chunk of chromosome material in cases where the number of chromosomes was normal. These deletions are the basis for disorders like the Prader-Willi Syndrome (deletion in chromosome 15, which is associated with short stature, small hands and feet, obesity, overeating, and underdeveloped gonads) and Williams Syndrome (deletion in chromosome 7), which is associated with an elf-like face, mental retardation, short stature, and cardiac abnormalities.

### Common Birth Defects

- Multifactorial inheritance
  Both genetic and non-genetic factors contribute to many birth defects. Genetic factors may affect the occurrence of spina bifida, but the intake of folic acid by the mother at the time of conception is also very important.

- Environmental causes of birth defects include teratogenic exposures to physical, chemical or biological agents and conditions. For example, smoking cigarettes or using cocaine can affect the fetus and cause harm. Drinking an excessive amount of alcohol causes abnormal embryofetal development and affects 1 in every 500 to 1,000 humans.

- Twinning
  The frequency of malformations is higher in identical than in non-identical twins. In fact, identical twinning, which is a splitting apart of a zygote or early embryo, can itself be considered a developmental abnormality.

  Education can help prevent some major malformations. If all women of childbearing age took before becoming pregnant a daily vitamin containing at least 0.4 mg folic acid, the rate of spina bifida and some other types of birth defects would decrease. Diabetics with well-controlled glucose levels before conception have half the risk of having a malformed child compared to diabetics with poorly controlled blood sugars. Women who plan their pregnancies can stop smoking cigarettes and drinking alcohol before they conceive. Women who take anti-seizure medications can discuss changing to a drug that poses the least risk during pregnancy.

### Suggested Reading


What Are Some Of The Important Events Occurring During Embryogenesis That May Be Disrupted By A Teratogen?

CHAPTER 2

Most major structural malformations triggered by environmental exposure to teratogens are initiated during the embryonic period, which, in the human, extends between the third and eighth week of gestation. This critical six-week period sees the conceptus change from a relatively simple bilayered disk of cells into a distinctly human fetus of marvelous intricacy. The speed and complexity of this extraordinary metamorphosis makes the fact that the process normally proceeds without incident the more remarkable.

During embryogenesis, the definitive germ layers along with the foundations for all of the organ systems are established. These definitive germ layers are derived from the epiblast (the primordial germ layer forming the amniotic cavity) during gastrulation, which takes place during approximately the first 2 weeks of the embryonic period (weeks 3 and 4 post fertilization).

During gastrulation, some cells from the epiblast migrate towards a forming midline groove called the primitive streak. Some of these cells change shape, detach, and burrow underneath (invaginate) the epiblast. Some of these invaginating cells displace the old hypoblast (a cell layer beneath the epiblast) creating the embryonic endoderm. Some of these invaginating cells come to lie between the newly forming endoderm and the overlying epiblast. These cells constitute the embryonic mesoderm. The remaining epiblast cells that have not invaginated are now termed embryonic ectoderm.

Interfering with gastrulation may have not invaginated are now termed embryonic ectoderm. Interfering with gastrulation may cause malformations in the embryo. The degree of effect depends upon the timing and duration of the teratogenic insult. Thus, an exposure may primarily affect only one subgroup of gastrulating cells leaving the rest with only a minor disturbance or no effect at all.

Specialized ectodermal cells form the neural plate. The subsequent folding and fusion of this neuroectoderm to form the neural tube is termed neurulation. Neurulation starts while gastrulation is still underway. Union of the neural folds occurs first at the future back of the head and then proceeds both cranially and caudally. The anterior neuropore (the opening between the closing neural tube and the amniotic cavity) typically closes complete-

Important Events During Embryogenesis

ly late in the fourth week of development, while the caudal neuropore closes a few days later. Interference with neurulation causes such neural tube defects as anencephaly (absence of portions of the brain and overlying skull), exencephaly (protrusion of brain tissue through a defect in the skull), and spina bifida (split spine: failure of vertebrae to close).

The sensory and autonomic components of the peripheral nervous system derive from two progenitor cell populations. The first is called neural crest because it originates at the margin of the neuroectoderm. Neural crest cells leave the margins of the folding neuroectoderm around the time the neural tube is closing. They migrate throughout the embryo and give rise to most of the nerves outside the brain and spinal cord. The second progenitor cell population derives from the cranial placodes (thickenings in the surface ectoderm that are associated with the future nose and ear as well as other craniofacial regions). Some of the cranial sensory neurons develop from these cranial placodes.

Neural crest cells contribute importantly to a number of other embryonic components, as well. These include the facial skeleton and connective tissue. Therefore, it is easy to see that the presence of a particular teratogen at a specific “sensitive” time might dramatically affect a variety of forming structures. For example: should those neural crest cells entering the forming facial region be delayed, disrupted in some fashion, or even killed, the face might develop devoid of some or most skeletal and connective tissue elements as well as exhibiting abnormalities in its innervation.

Teratologists often single out “critical events”, which are key developmental steps that are completed by a particular developmental stage. Such events include gastrulation, neural tube closure, septation of the heart into separate chambers, and fusion of the secondary palatal shelves. Although an embryonic organ may be damaged by an environmental insult after its development is essentially complete, damage from such insults usually occurs earlier in the morphogenesis of that organ. For example, an open neural tube may result from interference with the actual process of fusion of the neural folds. Earlier interference might prevent the neural folds from ever meeting. Virtually all of the steps toward achieving the definitive structure of the conceptus are important.

Over the years, developmental milestones have been defined based on the tools available to the embryologist. When only crude hand lenses were available for studying chick development, only gross changes could be detected. For example, looping of the simple heart tube could be seen to result in a structure more closely resembling the adult heart. With the advent of the microscope, scientists could define milestones based on changes in cell numbers, size, shape, and location. More recently, genes and gene products that allow cells to signal one another and to influence cell interactions have been identified. So, molecular embryologic milestones can be defined based on the appearance and disappearance of key signals.

Teratogenesis can be due to interference with embryonic milestones at any of these levels. Much current research in teratology focuses on how errors in chemical signaling can occur and how they may lead to abnormalities in cellular events and, ultimately, to abnormal organogenesis.
Can Exposure to Chemical or Physical Agents in the First Week after Conception Cause Malformations?

CHAPTER 3

Most experimental teratogens and all established human teratogenic exposures affect the embryo in the interval between two key events in embryo development: gastrulation, the formation of an embryo with three cell layers, which begins to occur about two weeks after fertilization, and closure of the palate, which occurs about two months after fertilization. Chemical or radiation damage can cause mutations and chromosomal abnormalities in germ cells, but these kinds of germ cell damage often do not result in human malformations. Reasons include decreased viability of the germ cells, repair of mutations, or a splicing out of the damaged DNA.

Between fertilization and implantation, during the first week of development, the embryo transforms itself from a single cell zygote to a multicellular, histologically undifferentiated ball of cells called a blastocyst. Traditional teaching holds that damaging a portion of these cells causes no permanent harm to the embryo, because all cells in the blastocyst are similar and can compensate for their damaged or missing cells. If the number of dead or damaged cells is too great to be compensated by the remaining cells, the whole embryo dies. This period of embryonic development has come to be called the all-or-none period, because damage to the embryo either destroys the whole embryo, or is completely compensated for by the remaining normal cells.

In contrast to traditional teaching, experimental results in rodents suggest that embryonic death is not an inevitable result of uncompensated damage. Exposure even at this extremely early stage can result in a malformed embryo that continues to develop.

Zygote

The mouse has been the most popular animal model for testing effects of chemicals in the early embryo. In vitro exposure of the mouse embryo within a few hours after fertilization, but before cleavage to the 2-cell stage, causes loss of some zygotes. Others survive, at least temporarily. Some survivors die prior to implantation; some die soon after implantation, and some succumb later in gestation. Those that survive until late pregnancy have a variety of abnormalities, including fetal edema (swelling), open abdominal wall defects, open eyes, and bent limbs.

Many agents disrupt organogenesis, but only a few agents are known to produce defects after zygotic treatment. So far, only powerful mutagens, most of which cause chromosome breakage or increase point mutations, have shown an effect; these mutagens include x-rays, ethylene oxide, acrylamide, and ethylnitrosourea. Ethylene oxide and other highly reactive toxic agents have been used as model mutagens in a variety of rodent experiments. A few chemicals, including ethanol and ethylene glycol, that are known to produce an increase in birth defects, do not seem to affect the mouse zygote. Most chemicals have not been tested.

The mechanism by which some agents can induce changes in the zygote that are manifested only many, many cell generations later has not been established. It is known that damage is inflicted directly on the zygote, rather than indirectly through the pregnant female. In embryo transfer experiments, if only the female, but not the zygote, is exposed, nothing happens to the zygote. The types of anomalies induced by zygotic exposure are not specific, but resemble some common human birth defects thought to have multifactorial causes.

Multi-cell embryo

Just 24 hours after fertilization, the 2-cell embryo responds quite differently to exposure. The predominant effect is embryonic loss or late fetal death, but morphologic defects also occur. Some of the same mutagens (e.g., methylmethanesulfonate) that cause abnormalities after zygote exposure, cause microcephaly (small head), cleft lip and palate, open neural tubes, and late fetal death when exposure occurs on gestational days 2 through 5. These results beg for an explanation. The agents employed are all rapidly metabolized and should not be found in any significant concentrations in an embryo after a few hours, much less after several days of logarithmic growth.

Certain novel agents give even more interesting results after pre-implantation exposure. Retinoic acid, the best-studied of the chemicals that instruct cells in the embryo on how to develop, induces changes in the multi-cell embryo that result in marked anatomic anomalies after organogenesis. The most dramatic malformations occur after exposures in a 24-hour window, beginning on day 4-5 of the mouse 18-day gestational period. Otherwise normal embryos exhibit bizarre caudal anomalies, including duplications of the entire pelvis with extra sets of limbs, genital abnormalities, single limb duplications, and an occasional misplaced limb.

It is known that a localized excess of retinoic acid can induce genes necessary for limb formation, and that limb duplication can result only at the time the limb bud forms, but the entire set of malformations caused by retinoic acid is very difficult to explain embryologically. Although similar anomalies are observed very rarely in humans, these malformations have not been reported among human pregnancies exposed to retinoic acid.

Exposing mouse embryos on gestational day 2.5 to 5-azacytidine, a nucleotide analog that inhibits DNA methylation, produces a very different set of unique anomalies, characterized by a lack of cranial structures and severe frontalonasal dysplasia. Methylation of DNA is thought to be important in epigenetic regulation of gene expression.

Effects of these agents on humans

It is almost impossible to obtain information about the effects of exposures on human embryos before implantation. The window of action of teratogenic agents in animal experiments is narrow, and the agents tested so far are unlikely to be encountered in humans except as acciden-
Suggested Reading


Is the Fetus Susceptible to Adverse Effects of a Chemical or Physical Agent after the First Trimester?

It is a common myth that birth defects occur only after exposures in the first trimester. While classic birth defects, such as cleft palate, can arise only during the period commonly called organogenesis (corresponding to the first trimester of human pregnancy), serious defects can originate much later in fetal life, and even after birth. The only requirement for expression of an adverse effect is that a teratogenic exposure coincides with a critical developmental event, and critical developmental events are not restricted to the first trimester of human pregnancy. While most organ systems become resistant to teratogenic influences after organogenesis is complete, the urogenital system and the CNS (central nervous system, consisting of the brain and spinal cord) are examples of systems that are very sensitive to late injury.

The ovaries, testes, and their associated ducts, including the uterus and fallopian tubes, are formed early, but much of the development of the external genitalia occurs after the period that is classically called organogenesis. In males, hypospadias is a common defect of late development. Hypospadias results from incomplete fusion of the genital folds in the ventral midline; in its most severe form, the urethra is splayed open along the whole extent of the ventral surface of the penis; the division of the two sides may extend posteriorly into the scrotum. More typically, only a small distal defect is present. Visually, this form of hypospadias is far less dramatic, but this defect may interfere with normal urination.

The descent of the testes from the abdominal cavity into the scrotum is not complete until close to the time of birth, and delays or failures of the process are not uncommon. Exposure of female fetuses to diethylstilbestrol in midgestation is associated with a variety of anomalies of the reproductive tract, including a T-shaped uterus and vaginal and cervical tissue that is more susceptible to developing cancer. In both sexes, the brain control of reproductive functions is dependent on normal sexual differentiation of the brain in the fetal period. Abnormal exposure to sex hormones during this stage of development can have permanent effects on reproductive physiology and on behavior in rodents.
Prenatal exposure. Prenatal exposure to some chemicals can even accelerate age-related neuronal loss. Anatomic and behavioral deficits that are minor in young adult animals may be much more severe when the animals reach old age. The effects of prenatal exposures on function in adults is a fascinating and largely unexplored area of research.

Suggested reading


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Can Prenatal Exposure to Chemicals have Delayed Long-term Effects on Behavior?

Having a baby is a momentous occasion, and it’s always a relief to parents when a newborn appears normal. For many years, scientists thought about birth defects only in terms of abnormal physical structures. However, normal intellectual and emotional development cannot be determined until long after birth. The study of neurobehavioral teratogenesis or developmental neurotoxicity is an area that some scientists view as separate from teratology; others subsume the field under the heading birth defects, teratogenesis, or developmental toxicity.

In the 1970s, scientists demonstrated long-term effects on postnatal behavior in animals exposed prenatally to a variety of drugs. The first clear example of functional abnormalities in humans was seen after methylmercury poisoning in Minamata Bay, Japan. People living around the bay who ate bay fish exhibited mental retardation, cerebral palsy, and blindness. Accidental methylmercury poisoning has since been reported several more times around the world, and each time the most severely injured are the babies born to women who ingested contaminated food during pregnancy. Even women who ate fish with only moderate amounts of methylmercury had children with subtle mental and neurological symptoms. Surprisingly, these effects occur even though the mothers show no symptoms and even when the fish they eat were not in the past officially classified as contaminated.

Since Minamata disease was first identified, many other examples of neurobehavioral teratogenesis have been found. Lead is perhaps the best known of the chemicals producing this kind of effect in the industrialized world. Over the last 30 years, research has shown increasing numbers of children with reduced mental abilities because of lead exposure, and these changes have been found at lower and lower doses. Lead is an important example of a developmental neurotoxicant for several reasons. Lead not only affects brain development after prenatal exposure, it also affects brain development after childhood exposure. As we’ve already discussed (see Chapter 4), the brain develops gradually over a long period of time, and so is vulnerable over a wide interval. While susceptibility to injuries slowly diminishes as development progresses, there remain vulnerable windows of brain development after birth that are as important as those that occur prenatally.

Other exposures that may produce neurobehavioral teratogenesis include alcohol abuse and recreational use of drugs including heroin, cocaine, and methamphetamine. Methadone also affects brain development. Fetal alcohol syndrome (FAS) and associated syndromes often cause intellectual impairment (see Chapter 21).

Cigarette smoking is associated with intrauterine growth retardation, but new studies show that children exposed prenatally to smoking grow up with impairments of IQ of approximately 10 points. The IQ reductions are seen primarily on verbal functions.

Although prenatal cocaine use was once thought to be a devastating cause of fetal injury, early fears were not substantiated in carefully controlled studies, which suggested that cocaine had few or no effects on newborns and infants. However, these early studies were too short-term to predict later effects. Studies that have followed children for longer periods and that compare the cocaine-exposed children to unexposed children of similar socioeconomic background show that cocaine-exposed children do in fact have lasting effects, including impaired attention, delayed language development, and IQ reductions. Although the IQ reductions are small, there is little doubt that the impact on society as a whole will be substantial, with thousands more children requiring costly special and remedial education programs.

A few human studies and a growing number of experimental animal studies suggest that methamphetamine (also called meth, crystal, or ice) causes intellectual impairments in the offspring without causing structural malformations. Methamphetamine use is spreading rapidly as people discover that it can be smoked like crack cocaine. Information from the National Institutes of Health indicates that methamphetamine use is now as common as the use of crack cocaine. In animal experiments, methamphetamine causes more severe effects on brain development and behavior than cocaine, raising concern about the long-term developmental impact.

Opioids, including heroin and methadone, can also affect babies. Neonatal withdrawal has been recognized in such babies for many years; good medical management prevents the most severe effects. However, opioid-exposed infants have been found to have behavioral abnormalities.

Marijuana has often been thought of as the least dangerous drug of abuse. However, when smoked during pregnancy, marijuana has been linked to long-term changes in the behavioral development of children as they reach mid-school age. Marijuana does not affect IQ, but is associated with reductions of visual processing and impulse control.

Prescription medications are another source of long-term postnatal neurobehavioral effects. The most striking example is 13-cis-retinoic acid (Accutane), used to treat acne. Accutane is a retinoid, one of a group of compounds that includes vitamin A. Within a few years of marketing Accutane, cases of children with multiple birth defects appeared; this syndrome was termed retinoid embryopathy. Many of these children had low IQs. In animal experiments, Vitamin A was known to cause neurobehavioral deficits and malformations, so low IQs associated with severe birth defects was not surprising. What was most surprising about Accutane is that effects on intelligence did not occur only in children with severe birth defects. Some children who had subtle, barely detectable birth defects showed large impair-
ments in intellectual function.

The idea that neurological and behavioral impairments only occur when there are visible birth defects is wrong and outmoded. The old “guilt by association” approach of screening for birth defects in the hope of uncovering functional defects at the same time cannot be relied upon; rather, searching for functional effects is a whole separate enterprise.

Many antiepileptic medications cause developmental defects. First linked to birth defects, these drugs were later found to be associated with syndromes similar to fetal alcohol syndrome (FAS). Phenytoin causes fetal hydantoïd syndrome, a syndrome similar to FAS. The syndrome consists of facial changes, shortened digits, and reduced intellectual ability. The antiepileptic drugs trimethadione, valproic acid, phenobarbital, and carbamazepine are also associated with syndromes that include reduction in intellectual development; for some drugs, the effect on intelligence is the principal feature.

The brain is not the only system exhibiting pre- and postnatal development, but is unique in the wide diversity of ways that the brain can be injured and the length of time that it is vulnerable to injury. The idea that neurological and behavioral impairments only occur when there are visible birth defects is wrong and outmoded.

It is not only the first trimester of pregnancy that matters. Today, we recognize that developmental toxicity is a phenomenon that extends from conception through adolescence. After the first trimester, effects are no longer seen as malformations, but are instead seen as changes in function. The most common alterations are in neurobehavioral development, and such effects are almost always long-lasting.

Suggested Reading


Can the Exposure of Germ Cells affect Pregnancy Outcome?

CHAPTER 6

We all know that certain exposures during pregnancy can damage the developing embryo or fetus. But could the exposure of spermatogonia or oocytes prior to conception also result in adverse effects?

Almost all of the studies in this area have been done on sperm, not oocytes. It is difficult to obtain eggs from an ovary, and only a few can be obtained at a time. This clearly is an area that needs more research. Scientists have been unenthusiastic about studying oocyte exposure because it is assumed that non-dividing cells are resistant to damage. Female gametes are thought not to divide after birth. All of the eggs that a woman will ovulate during her life are produced late in her own fetal life. Oocytes are suspended at a specific point in meiosis, only undergoing the final stages on a monthly basis during a woman’s reproductive years.

Spermatogonia are much easier to study. They are abundant, replenished daily, and easy to obtain. Male germ cells undergo several mitotic and meiotic divisions, which is important because division is the time when cells are most vulnerable to the action of chemicals and altered environmental conditions.

We don’t know the extent to which exposures of men contribute to infertility and abnormal pregnancy outcomes. In some studies, being a welder, painter, auto mechanic, or fireman has been associated with an increased risk of male infertility and adverse effects on progeny outcome, including increases in spontaneous abortions, birth defects, and childhood cancer. However, researchers have not been able to pin down specific exposures that cause specific paternal adverse effects on pregnancy outcome.

One problem is that people are usually exposed to a combination of chemicals, and, most of the time, little information is available on dose, duration of exposure, or potential chemical interactions. Also, infertility and pregnancy loss are very frequent in humans even without environmental exposures. Detection of small changes requires very large studies. Finally, the only information that we have in humans comes from accidental, environmental or therapeutic
exposures, because intentionally exposing humans to potentially harmful substances is unethical. There are three mechanisms by which male exposure to a drug theoretically adversely affect progeny outcome. During sex, seminal fluid could carry chemicals directly to the embryo or developing fetus. In animal experiments, high doses of several drugs, including methadone, morphine, and several anticancer drugs, including cyclophosphamide, given to males before mating adversely affect progeny outcome. Cyclophosphamide given to male rats was found in seminal fluid after systemic administration. The drug was transmitted to the female during mating, and caused a dose-dependent decrease in the number of subsequent embryos. This effect could be due to direct exposure of the early conceptus or through an effect on sperm function.

Since normal testicular function depends on hormonal signals coming from the pituitary, which is in turn controlled by the hypothalamus, interference with communication between the hypothalamic-pituitary complex and the testis can have adverse effect on spermatogenesis. p,p'- DDE, a metabolite of the pesticide DDT, causes this type of toxicity. Although any disruption of the hypothalamic-pituitary-testicular axis can affect male fertility, this does not necessarily directly affect offspring. Ethane dimethanesulphonate targets Leydig cells and epididymal principal cells, and Sertoli cells may be targeted by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) or hexachlorobenzene. Disrupting the functions of these cells may alter germ cell quantity or quality.

The third mechanism, and the one that is of most interest to researchers, is a direct effect of the toxicant on the male germ cell, either during spermatogenesis in the testis or sperm maturation in the epididymis. Damage that stops sperm maturation cannot affect offspring because the germ cells never achieve the ability to fertilize oocytes. But when spermatogonia are affected by drugs or chemicals and still retain their ability to fertilize eggs, trouble may arise.

In the testis

Rat spermatogonia undergo several mitotic cell divisions to become spermatocytes, and then two meiotic cell divisions to form spermatids. Then they condense their nuclear elements, develop a propulsion mechanism, shed most of their cytoplasm, and finally graduate as spermatids. We can deduce the stage of spermatogenesis affected by a toxicant based on the time from treatment of the male animal to adverse effects after mating that animal.

For example, in rats, an effect on progeny outcome during the first week after exposure of males to a drug or X-rays means that spermatogonia in the epididymis were affected.

Exposure of Germ Cells

Exposure 2-4 weeks prior to conception represents an effect on germ cell first exposed as spermatid, and exposure 5-6 weeks before conception represents an effect on germ cells first exposed as spermatocyte. Exposures more than seven weeks prior to conception represent an effect on germ cells first exposed as spermatogonia.

Spermatogonia are susceptible to exposure to certain drugs, including procarbazine; these exposures reduce sperm numbers and increase the percentage of abnormal sperm among survivors. In mice, exposure to chlorambucil, a cancer drug, causes a peak in mutations in offspring when germ cells are exposed as spermatids. Spermatids and spermatozoa are most sensitive to mutations induced by acrylamide and lethality induced by ethyl-nitrosourea.

Cyclophosphamide has very different effects that depend on both the timing of exposure and the dose. When spermatogonia are exposed to cyclophosphamide, a small increase in external malformations and growth retardation is seen in developing fetuses. But when spermatids or spermatozoa are exposed to chronic low-dose cyclophosphamide, postimplantation losses are increased, but no malformations or growth retardation is seen.

Cyclophosphamide is particularly interesting because effects on postimplantation loss and malformations, and even some behavioral abnormalities, occur not only in offspring but also in the second generation, that is, the grandchildren of the treated rats.

In the epididymis

Spermatogonia acquire the ability to fertilize an egg while travelling through the epididymis. Either radiation or drugs can affect spermatogonia in the epididymis and vas deferens. Methyl chloride increases embryo death, possibly by causing inflammation in the epididymis; damage may be partially repaired, in which case spermatogonia in the epididymis, and even some behavioral abnormalities, occur not only in offspring but also in the second generation, that is, the grandchildren of the treated rats.
Will Alterations In Maternal Metabolism Increase The Risk Of Birth Defects?

CHAPTER 7

Illness in a pregnant woman can dramatically affect embryonic growth and development. Disruptions in circulating maternal metabolites produced by disease states can produce nutrient deficiencies or toxic metabolites that adversely affect embryogenesis. In some cases, the period of greatest sensitivity for the embryo is when organ systems are forming, from the 3rd-8th weeks of gestation. However, later stages, when the central nervous system is continuing its differentiation, may also be susceptible. It is very difficult to study these conditions because altering one metabolic process can cause multiple biochemical changes that are potentially detrimental. Results obtained from in vitro models, where many factors are disrupted simultaneously, are difficult to interpret. Consequently, in vivo techniques, such as rodent whole embryo culture (see Chapter 17), which permit testing each factor independently, have provided the greatest insights into factors responsible for adverse effects.

Two of the best-studied maternal metabolic diseases are insulin dependent diabetes mellitus (IDDM) and phenylketonuria (PKU). Both disease states are associated with altered, potentially harmful, factors. For example, diabetes is characterized by decreased insulin levels, episodes of hyper- and hypoglycemia, ketoacidosis, and changes in growth factors and their inhibitors. Children of diabetic mothers have a higher incidence of many different birth defects, including heart and neural tube malformations. Determining which aspect of diabetes affects development has important clinical implications. For example, is it more important for pregnant women to avoid hyperglycemia or hypoglycemia during pregnancy?

Although animal models have been developed to study the disease, trying to determine which of the many factors might be teratogenic in vitro has been virtually impossible. Whole embryo culture, in which normal growth and development of rat and mouse embryos can be maintained during gastrulation and neurulation stages (3rd-8th weeks of human development), has been used to investigate the teratogenic potential and mechanisms of action of individual diabetic factors. Each factor can be added to the culture medium and its effects on embryogenesis determined without modification by maternal metabolism.
The whole embryo culture system has shown that hypoglycemia is a highly teratogenic factor, even short-term exposures to moderate decreases in glucose result in growth retardation and malformations. The heart is particularly sensitive, in part because of its high energy demands. At these early stages, embryos rely on glucose as an energy source and most of this substrate is metabolized via glycolysis to lactic acid.

In contrast, hyperglycemia can produce neural tube defects, but only at concentrations 4-6 times higher than normal levels in humans. Beta-hydroxybutyrate, the major ketone produced during ketoadidosis, is also teratogenic, but only at high concentrations. Furthermore, embryos have a great capacity to recover from a ketoacidotic insult. Somatomedin inhibitors are small molecular weight molecules that are elevated in serum from IDDM mothers and are highly toxic to embryos in culture. These factors produce severe craniofacial and neural tube defects and growth retardation, perhaps by targeting the yolk sac, where they disrupt embryonic nutrition provided by this organ. Insulin, free fatty acids, and branched chain amino acids, which also accumulate in the serum of diabetics, are not toxic to embryos in culture.

In vitro, of course, these factors act in concert. Some of the factors that are toxic only in very high concentrations may interact synergistically, increasing the risk to the embryo. Thus, diabetic embryopathy is probably a multifactorial entity with a combination of factors having an adverse impact.

PKU (phenylketonuria), a hereditary metabolic disorder in which a defective enzyme prevents conversion of the amino acid phenylalanine to tyrosine) is another example of altered maternal metabolism that can produce congenital malformations, including microcephaly, heart defects, and mental retardation. Phenylalanine and its metabolites, phenylactic acid, phenylpyruvic acid, phenylacetic acid, 2-hydroxyphenylactic acid, and phenylethylamine, accumulate in maternal blood due to a deficiency of phenylalanine hydroxylase. Using rodent whole embryo culture, phenylalanine and each of its metabolites were tested for embryotoxicity during the period of neural tube closure. Phenylalanine was teratogenic only at concentrations far higher than that observed in vitro. Each of the metabolites was also embryotoxic, but only phenylethylamine produced defects at relevant concentrations. All of the acid metabolites caused abnormalities at higher concentrations. Evidence suggests that the acid metabolites are neurotoxic in humans. The fact that all of the metabolites produced some toxicity suggests that combinations may act in synergistic or additive fashions to increase risks to the embryo.

Because the types of defects induced by maternal diabetes and PKU have their origins at early stages of embryogenesis, particularly during the 3rd-5th weeks post fertilization, it is important to introduce prevention strategies prior to conception. Most women do not know that they are pregnant at the 3rd and 4th weeks of gestation and the majority do not seek their first prenatal visit until the 10th week, e.g., after the time of peak sensitivity to the teratogenic effects of some metabolic disorders. In the case of IDDM and PKU, studies have shown that if a woman’s medical treatment can be optimized prior to conception, birth defects can be reduced to the levels present in the general population.

Suggested Reading


CHAPTER 8

Does the Placenta Protect Against Insult Or Is It a Target?

The placenta is the anchor, the conduit, and the controller of pregnancy – and it can also be a target for toxicant action. The placenta attempts to protect the embryo and fetus from insult, but, like a valiant bodyguard who takes a bullet for his or her employer, the placental protector can be compromised.

The placenta is not just a barrier, but has many functions that are vital to the health of the embryo/fetus. The placenta encompasses not only the chorioallantoic placenta but all of its extraembryonic membranes (chorion / amnion) and the yolk sac. The placenta and its membranes secure the embryo and fetus to the endometrium (uterine lining) and release a variety of steroid and protein hormones that characterize the physiology of the pregnant female.

Alterations of any of these functions can lead to pregnancy loss (miscarriage). Of particular concern is the relative hypoxic environment that is normally present during early embryonic development and the abnormal maternal blood flow, which can produce higher levels of oxygen and lead to miscarriage and pre-eclampsia/eclampsia. The placenta transports nutrients to the embryo/fetus and waste products away from the embryo/fetus, but during this exchange, foreign compounds can hitchhike across the placenta. The placenta stores chemicals and also serves as a site for biotransformation. Substances that may be benign to the pregnant woman can be transformed by metabolizing enzymes in the placenta to agents toxic to the fetus. So the functions of the placenta that nourish and support the fetus can be compromised or hijacked to result in placental toxicity, fetal nutrient deprivation, and production of toxic chemicals.

As for other organs, patterns of placental development are similar among different animal species, but time schedules of development may differ. The placentas themselves vary widely among species. Marsupials, for example, have only a yolk sac, while sheep have multicytotrophoblastic placentation. Rats, mice, and rabbits, the principal species studied in teratology, all have yolk sac placentas, as do humans, but the function of the yolk sac is very different. In rodents and rabbits, the yolk sac everts (turns inside out) and becomes an important port of entry for molecules into the embryo and fetus throughout pregnancy, although a choriosalpinotic placenta develops during the last half of pregnancy. In contrast, the yolk sac shrinks and becomes vestigial (nonfunctional remnant) in humans during the latter part of pregnancy.
the first trimester.

In laboratory species, immunoglobulins, critical for immune function in the neonate (IgG), are transported only through the yolk sac; in humans transport occurs via receptors in the chorioallantoic placenta. Both the yolk sac of the rodent as well as the chorioallantoic placenta of the human are important sites for breaking down proteins and providing amino acids to the embryo and fetus. Trypan blue and other agents that interfere with protein degradation can kill the rodent embryo or fetus, or cause birth defects.

Many agents can alter placental function (see Table). Injecting cadmium in a pregnant rat close to giving birth, for example, causes the fetus to die and the placenta to break down within 24 hours. Directly injecting fetuses with cadmium near term doesn’t kill them, although they do develop hydrocephalus (head enlargement due to excess cerebrospinal fluid in the ventricles of the brain). Cadmium was previously thought to affect primarily the kidney, but it turns out that, at least in rats, cadmium is even more highly concentrated in the placenta. Human placentas definitely concentrate cadmium and can break down under its effects. Early on in pregnancy, cadmium’s effects on the placenta can cause miscarriage, and women affected by cadmium can have repeated pregnancy losses. The placenta’s ability to sequester cadmium protects the fetus or embryo for a while, but when a pregnant woman is exposed to a lot of cadmium, the placenta—and with it, the fetus—may die.

One source of cadmium is cigarette smoke. Cadmium may be one reason why smoking causes problems during pregnancy. Smoking is associated with pregnancy loss, premature delivery, and decreased birth weight. Smokers’ placentas have very high levels of cadmium compared with those of nonsmokers and these levels may contribute to the adverse outcomes that are associated with cigarette smoke.

The placenta can prevent, or at least delay the transmission of viruses that infect the mother. Experiments have shown that a variety of viruses, including cytomegalovirus (CMV), human immunodeficiency virus (HIV), Coxsackie B, and Echo 11 cannot directly cross the placenta. However, these viruses may infect certain cells within the placenta. Whether or not the placenta is infected determines whether the embryo/fetus eventually becomes infected.

After delivery, the baby gets all the attention. The placenta is neglected and discarded. But during pregnancy, the placenta is the star of the show. Teratology would be incomplete as a science without attention to this important organ.

Suggested Reading


Miller RK, Ebbesen P, Popek E, Nahumias A, Pollioti B, Shielch A, Zachar V, Roberts D,


### SOME XENOBOTICS OBSERVED TO ALTER PLACENTAL AND YOLK SAC FUNCTION

<table>
<thead>
<tr>
<th>DRUGS OF ABUSE</th>
<th>EFFECTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cocaine</td>
<td>in humans, reduces amino acid uptake and causes reperfusion injury...</td>
</tr>
<tr>
<td>Ethanol (alcohol)</td>
<td>In humans, alters membrane fluidity and inhibits nutrient transport; in animals, ethanol induces immunosuppression</td>
</tr>
<tr>
<td>Methadone</td>
<td>In humans, antagonizes dopamine and decreases dopamine turnover</td>
</tr>
<tr>
<td>SMOKING</td>
<td>Smoking, in humans alters histology, induces mononuclear cells, and increases cytokine levels, and doubles or triples the rate of FISH and histological abnormalities of the placenta</td>
</tr>
<tr>
<td>NARKOTIKS</td>
<td>Narkotik</td>
</tr>
<tr>
<td>DRUGS</td>
<td>Opiates, amphetamines, and other psychoactive substances</td>
</tr>
<tr>
<td>Opiates</td>
<td>In humans, reduces amino acid uptake, inhibits differentiation. In animals, inhibits phosphocytidylinositol</td>
</tr>
<tr>
<td>Nicotine</td>
<td>In animals, causes a pathologically hypertrophied small placenta</td>
</tr>
<tr>
<td>Verapamil</td>
<td>In humans, increases GAPD (glutamic acid decarboxylase) activity</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>In humans, stimulates pregnancy completion</td>
</tr>
<tr>
<td>Aspirin (NSAID)</td>
<td>In humans, inhibits phospholipid synthesis, decreases the activity of prostaglandins, and increases the activity of the prostaglandin synthetase activity</td>
</tr>
<tr>
<td>Chloroform</td>
<td>In animals, causes placental necrosis.</td>
</tr>
<tr>
<td>METALS</td>
<td>In humans, decreases GARP activity</td>
</tr>
<tr>
<td>Cadmium</td>
<td>In humans, causes placental necrosis, loss of placental integrity, decreased release of placental hormones, inhibition of trophoblast proliferation, and induction of metallothionein. In animals, cadmium causes placental necrosis, increased mitotic/carcinoma calcium, and altered enzymes. Cadmium accumulation radiates in both humans and animals, the effect is irreversible, with zinc.</td>
</tr>
</tbody>
</table>

Compounds presented in this table have been shown to adversely affect either the chorioallantoic placenta or the visceral yolk sac. Limitations in the data set are that often high concentrations of the compounds are used, few doses are used and the stage of gestation may vary. Thus, this table is presented to the reader as a compilation of responses; however, the reader is referred to the original research.
research for specific responses and doses. This table was adapted and updated from Skidmore W and Miller RK. Metabolism and Transfer: Role in Developmental Toxicology. In: Rimarck C and Wachter-Saloner J (Eds) Developmental Toxicology. 2nd edition. Raven Press, New York: 1994: 245-283.

Why Do Individuals Respond Differently To Exposure To A Chemical Or Physical Agent?

CHAPTER 9

A key concept in teratology is that there are individual differences in sensitivity to teratogenic exposures. Only a small percentage of infants with known teratogenic exposures display any adverse effects. For example, fewer than 10% of the infants exposed as fetuses to phenytoin have congenital defects. Similarly, fewer than 20% of thalidomide-exposed infants are adversely affected. What puts some infants at increased risk and not others? Most of us believe that differences stem from the inheritance of a gene or set of genes that interacts with the teratogen, altering normal pathways and leading to the development of a birth defect.

In the case of phenytoin, it has been suspected that the drug was converted to a toxic intermediate within the embryo, damaging DNA or protein. The embryo could protect itself from the toxic intermediate using an enzyme called epoxide hydrolase. To determine whether low levels of epoxide hydrolase increased the risk of fetal malformation after hydantoins exposure, a study at the University of Nebraska Medical Center tested epoxide hydrolase activity in amniocytes, which are cells in the amniotic fluid that have come from the skin of the fetus. In a random sample of amnionocytes from non-epileptic women, enzyme activity ranged widely, from 8% to 116% of standard enzymatic activity. In contrast, the epoxide hydrolase activity in 16 children diagnosed as having the prenatal hydantoins syndrome had values that were tightly clustered between 9.6% and 30% of the standard. A comparison revealed that the mean epoxide hydrolase activity in the general population was more than twice that observed for the children with the hydantoins syndrome.

In a prospective study of 19 phenytoin-exposed pregnancies, 15 fetuses were found to have normal enzymatic activity, while four infants had low enzyme activity. When the infants were examined at term, all four of the children with low enzyme activity had clinical features compatible with hydantoins toxicity. Not one of the 15 infants with normal enzyme activity had any physical or developmental problems. These data suggest that the four affected infants, who were stressed in utero by anti-epileptic drugs, were unable to readily detoxify the intermediary oxidative metabolites produced, making them significantly more susceptible to the teratogenic effects than the 15 exposed, una-
fected infants, whose high enzymatic activity enabled them to successfully detoxify the teratogenic intermediate.

Another example of genetically determined susceptibility to a teratogenic exposure is cigarette smoking and the risk for having an infant with an orofacial cleft. A case-control study involving over a half-million births identified 348 isolated cleft lip and palate and 141 isolated cleft palate cases. Shaw and colleagues determined that in mothers who smoked more than 20 cigarettes a day, the odds for having a child with a craniofacial defect were 5.7 times higher when the fetuses had the uncommon allele for TGFα (transforming growth factor alpha) than when the fetuses had the common allele for TGFα. Children with the common TGFα allele and mothers who smoked heavily also had increased risk.

The more cigarettes the mother smokes, the greater the risk is to the fetus. But this risk is not the same across the entire population of smokers, because of the interaction between the TGF locus and the risk for clefting. In some situations, knowing the maternal genotype at a particular locus can better predict risk of birth defects from a teratogenic exposure.

Neural tube defects (NTDs) are among the most common congenital malformations, occurring in approximately 1 per 1,000 liveborn infants. NTDs have both an environmental and a genetic component to their development. While they sometimes cluster in families, for the most part they do not follow a pattern of simple Mendelian inheritance.

To learn more about the genes responsible for conferring sensitivity or resistance to teratogens-induced NTDs, several mouse models have been developed. Exposing different inbred mouse strains to known tuber teratogens, it was apparent that some strains were genetically resistant to the induction of NTDs, while other strains were more susceptible. Because the experimental environment was carefully held constant between the animals of the different inbred strains, underlying genetic differences are the most likely explanation for why some strains had higher NTD frequencies than others. Although the complete story is still unfolding, there is increasing evidence that genes involved in folate transport and metabolism can explain some of the differences.

**Suggested reading**


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**Do Teratogenic Exposures Act through Common Pathways or Mechanisms of Action?**

Although exposure to teratogens can cause spectacularly diverse effects, there appear to be only a few methods by which chemical or physical agents can perturb development. Mechanisms of action refer to the chemical interactions of an agent with the organism. Agents can interact with a receptor, bind to DNA or protein, degrade cell membranes or proteins, inhibit an enzyme, or modify proteins by interference with sulfhydryl groups. Any of these interactions can lead to changes in cell function or to cell death, and, if the damage is extensive, abnormal development results. Teratogens with entirely different molecular actions can ultimately produce the same effect.

**Receptor interactions**

Receptors are molecules, usually proteins, within or on the surfaces of cells, which are targeted by hormones or other signaling molecules. Receptors perform the same function for cells as our senses perform for our bodies: they inform the cell about its environment and, when activated, lead to changes in cell function. Some agents become toxic by interacting with receptors. Examples include retinoic acid (the biologically active form of vitamin A) and DES (diethylstilbestrol, a potent estrogen once given to pregnant women in an effort to prevent miscarriage). Retinoic acid is essential for normal development, but too much causes defects in numerous structures. DES binds to estrogen receptors and causes defects in male and female reproductive organs, as well as a rare form of vaginal cancer in about one of every thousand women whose mothers took DES during pregnancy.

**Covalent binding to DNA or protein**

Some agents are chemically reactive or are metabolized by the body to chemically reactive forms. These reactive forms create covalent bonds to important biomolecules, changing the function of these molecules. For example, cyclophosphamide, a drug used to treat cancer, is metabolized to phosphoramid mustard, a reactive intermediate that covalently binds DNA and other important molecules in the cell.
Peroxidation

Chemicals that generate highly reactive substances like hydrogen peroxide can oxidize molecules, particularly the lipids that form the foundation for cell membranes.

Enzyme inhibition, interference with thiol groups

Enzymes are proteins with many functions. Inhibiting the function of an enzyme that supplies critical building blocks for development may have teratogenic consequences.

For example, methotrexate, a folic acid antagonist used to treat cancer, psoriasis, rheumatoid arthritis, and ectopic pregnancy, will decrease the synthesis of the nucleotides needed to make DNA, and interfere with other metabolic processes.

Sulfhydryl groups, which contain sulfur and hydrogen and are found on the amino acid cysteine, are important in creating the three-dimensional structure of proteins: two sulfur atoms that are distant from each other link together to form a disulfide bridge, creating a loop in the protein. Sulfhydryl groups are also used to hold essential minerals like zinc in place in proteins. Sulfhydryl groups are also important in caspases and other enzymes involved in programmed cell death, a normal developmental process. Cadmium, mercury, or other toxic heavy metals can interact with sulfhydryl groups, disrupting the function of the proteins that contain them.

Different exposures can cause the same cascade of events that result in abnormal development. For example, the edema syndrome results when embryos are exposed to low oxygen levels. Heart rate and blood pressure drop, sodium and potassium concentrations in the plasma change, and fluid seeping out of blood vessels causes hollow organs to swell and blisters to form in solid structures. The distortions caused by fluid accumulation disrupt development. But other agents can cause the edema syndrome as well: trypan blue (a biological stain) and other agents that affect the nutrition of the early embryo cause similar effects.

Apoptosis is a form of programmed cell death that occurs in embryos during normal development as a means of sculpting limbs and other structures, to get rid of extra cells or cells that have served their purpose, and to remove damaged cells. Apoptosis in normal cells that have not completed their useful lifespan can cause big problems. Zinc deficiency and ethanol (the form of alcohol found in beer, wine, and liquor), for example, extend the size of areas undergoing cell death beyond what is normal. Other chemicals, such as deoxycoformycin, produce apoptosis in areas where it does not normally occur. Cyclophosphamide does both.

Common Pathways

Research into teratogenic mechanisms and pathogenesis is advancing as great strides are made in our understanding of the molecular processes that control embryonic development. Teratologists have only begun to study how chemical agents interact with these processes.

Suggested Reading

Is There a Safe Dose of a Teratogen?

CHAPTER 11

The dose makes the poison.

Paracelsus

Almost any agent may be harmful to the embryo if the dose is high enough. Establishing a safe level of exposure to a teratogenic agent is an important area of research, both for counseling women inadvertently exposed during pregnancy, and for determining which drugs are appropriate for use in pregnancy. It is not feasible to simply exclude drugs during pregnancy. Chronic conditions such as asthma, high blood pressure, and diabetes can have adverse effects on pregnancy (See Chapter 7), so withholding drugs during pregnancy can be bad for both the woman and the embryo or fetus.

The idea that safe doses of teratogens may exist is based on the threshold concept. This theory holds that embryotoxicity depends on multicellular injury and that there is a threshold dose below which no risk exists. So for a teratogen given at high doses, there will be a significant risk of embryo or fetal toxicity. However, the same drug given at a low dose would produce a lower risk. For example, thalidomide, a well-known teratogen, affects human embryos when a dose of 50 mg is administered to a pregnant woman during the susceptible period. However, a dose of 0.5 mg thalidomide given at the same critical period would have no observable effect. This example supports the threshold concept, but a key question is not answered. Were enough patients studied to detect a rare outcome? In other words, if the number of women exposed to the low dose of thalidomide were increased 10 or 100 fold, would any embryos be harmed?

If one assumes repair mechanisms are functioning to correct damage to embryonic cells, then it makes sense that an insult to the multicellular embryo can be overcome. Indeed, apoptosis, or programmed cell death, is a normal and necessary part of development. Early on in fetal life, humans have webbed fingers; it is apoptosis that eliminates this webbing. The knowledge that cells can be repaired or replaced supports the theory that there may be a threshold dose, below which no ultimate adverse effect occurs.

Here’s the argument against the idea of a threshold dose. Only about one third of the malformations that occur in humans can be traced to genetic or environmental causes. Most defects are categorized as spontaneous malformations, meaning that we don’t know what causes them. But if unknown internal factors can cause birth defects by unknown mechanisms, and environmental or drug induced factors affect the same mechanisms, then a drug-induced influence on that mechanism could tip the balance in the direction of embryo malformation. In this scenario, no safe level of exposure exists; instead, there is a continuum of probability for malformation, no matter how miniscule the drug exposure.

If the relationship between dose of an agent and its response is continuous, can there be a safe exposure to a teratogenic agent? The threshold concept is broadly accepted for most other types of toxicities caused by exposure to an agent (for example, liver toxicity) provided the drug or agent does not directly interact with genetic material (a non-genotoxic mechanism of action). In the case of pregnancy, if the relationship between dose and outcome is known from animal studies or from human cases of accidental exposure, then the risk of malformation can be reduced tremendously by administering lower doses that are unlikely to cause embryotoxicity. Doses may be calculated by using safety factors (reducing problematic doses by multiples, usually of 10) or by using quantitative biologically-based dose-response models to mathematically estimate doses with a very low risk for the developing embryo.

We can also use our knowledge about vulnerable periods to reduce or eliminate exposure during a susceptible period. The vulnerable period, however, is not the same for all exposures. For example, thalidomide teratogenicity appears to require exposure during the first trimester of pregnancy, while the teratogenic effects of angiotensin converting enzyme inhibitors (a class of blood pressure medicines) occur during the second two-thirds of pregnancy.

Protecting the embryo or fetus from the effects of toxic agents requires knowledge of the target site of the agent, the dose at which toxicity occurs, and the mechanisms by which toxicity occurs. If we know enough about these three areas, safe use of potentially dangerous medications may be possible.

Suggested Reading
What Methods are Available for Prenatal Testing? Are they Useful?

CHAPTER 12

One use of a screening test is to identify people in the general population who have a higher than average risk of a disease of interest. Diagnostic tests are different, because they address the question of whether a particular individual is affected. Prenatal screening tests can identify patients who are at increased risk for an abnormal pregnancy outcome, but these tests do not diagnose specific conditions. The ability of these tests to predict abnormalities varies depending on the incidence of the condition and the reliability of the test. Ideally, the test should have few false positive results (predicting that a normal pregnancy is abnormal) and few false negatives (predicting that an abnormal pregnancy is normal). A positive screening test is followed by a diagnostic test. Most diagnostic tests in pregnancy are invasive and are associated with a risk for pregnancy complications and fetal loss.

The background risk for birth defects and mental retardation in liveborn babies is 3.5%. No prenatal screen or diagnostic test that is currently available would identify all of this risk. Prenatal screening tests may be offered to all pregnant patients or to those with specific indications, such as a potential teratogenic exposure in utero, or a family history of risk.

The available prenatal screening and diagnostic tests utilize ultrasound, maternal serum, amniotic fluid, chorionic villi, and fetal blood. Each test has specific indications and risks (See Table).

Ultrasound

Ultrasound, also called sonography, uses the reflection of sound waves to make an image of tissue interfaces. These images can be highly detailed, almost photographic depictions of the embryo and fetus. Ultrasound can confirm a live pregnancy, establish gestational age, and identify twins and other multiple gestations. Ultrasound can also detect fetal anomalies and is often the only useful prenatal test following a potential or known teratogenic exposure. Ultrasound is useful for evaluating fetal growth and development, but cannot determine the underlying cause of an abnormality. Ultrasound also cannot provide much information about neurological functioning of the fetus.

Ultrasound can be used for both screening and diagnosis. For example, during the first trimester, thickening of the tissues of the embryo’s neck is associated with an increased risk of certain chromosome abnormalities. The test is not diagnostic, because some embryos with thickened neck tissues are normal. If ultrasound is used to evaluate the thickness of the neck tissues, this abnormal screening test can be followed by a diagnostic test for chromosome abnormalities (discussed below). In other cases, ultrasound can be diagnostic. For example, if the fetal brain has not developed, a condition called anencephaly, ultrasound will detect the abnormality with about 100% accuracy.

Maternal serum screening

Maternal serum alpha-fetoprotein (MSAFP) measurement at 15-20 weeks’ gestation is used to determine if the fetus is at risk for an open neural tube defect. Alpha-fetoprotein is secreted by the fetal liver and excreted in the fetal urine, but some AFP crosses the placenta and can be measured in maternal serum. The median values of AFP in amniotic fluid and maternal serum change with gestational age, so results are expressed as multiples of the median, or, cutely enough, MoM.

Elevated MSAFP can be due to any of several factors. An abnormal fetal condition such as an open neural tube defect or abdominal wall defect can cause excessive AFP in the amniotic fluid, or an abnormal maternal-placental interface could allow excessive AFP to cross into the maternal circulation. Because AFP rises throughout pregnancy, inaccurate gestational age could cause an MSAFP level to seem high. Multiple gestations can also increase MSAFP, because more than one fetus is generating AFP. A less common reason for elevated amniotic fluid and maternal serum AFP levels are some rare inherited renal and skin diseases. Even if no reason can be found for an elevated MSAFP, the pregnancy would be considered at increased risk for preterm delivery or other adverse outcomes.

Nowadays, a “triple screen” or “quadruple screen” is often done, which combines a test for MSAFP with measurement of two or three other chemicals. These chemicals currently include estriol, human chorionic gonadotropins (HCG), and inhibin A, but the search for new and more predictive analytes or combinations of analytes is an area of active research. Initially designed to identify pregnancies at increased risk for trisomy 21 (Down syndrome), these multiple-analyte screens are also useful for detecting trisomy 18 and other less common aneuploidies (abnormalities of chromosome number). For the multiple-analyte screens, maternal blood is drawn between 15-20 weeks of gestation, at which time median values for
Diagnostic Testing

Because diagnostic testing in pregnancy is usually invasive, it is reserved for situations where the risk of an abnormal result exceeds the risk associated with the procedure.

Three different procedures can diagnose chromosomal abnormalities. The earliest test that can be done is chorionic villus sampling (CVS), which is performed between 10 and 12 weeks of gestation. CVS involves suctioning bits of placental tissue, called chorionic villi, through a needle or a thin tube. These bits of placental tissue usually have the same chromosomes as the embryo. The cells from the chorionic villi are grown in culture and their chromosomal complement is analyzed. CVS is performed under ultrasound guidance; samples can be obtained either through the cervix or through the abdomen, depending on the operator’s preference and location of the embryo in the uterus. The risk of miscarriage is generally described as 1/100, but is considerably lower with more experienced operators. The advantage of CVS is that it can diagnose an abnormality earlier in pregnancy than any other test. However, CVS can only be used for chromosomal abnormalities, as we will see, amniocentesis can also be used for evaluating open neural tube and other defects.

Amniocentesis, the sampling of amniotic fluid, is commonly performed in the second trimester; chromosomal analysis is performed on amniocytes, which are cells that originated in the fetal skin and have the same chromosomes as the rest of the fetus. AFP levels in amniotic fluid are used to test for open neural tube and a few other kinds of defects. Amniocentesis performed under continuous ultrasound guidance is described to patients as having an approximate 1/200 risk for miscarriage, although the actual risk in experienced hands is much lower. First trimester amniocentesis is possible, but carries a greater risk of miscarriage. Amniocytes or amniotic fluid can also be used for DNA-based mutation analysis or enzyme analysis in the diagnosis of many inherited diseases. Tests for unusual genetic diseases are only done when the fetus has been determined by genetic counseling/history taking to be at risk for a specific condition.

The third way to do a chromosomal analysis is by taking a blood sample directly from the fetus after 16 weeks of gestation. This procedure involves the removal of blood from the umbilical vein, preferably close to the placental insertion site, and is associated with a 1.3% risk of fetal loss.

Fetal cells are present normally in the maternal circulation. If isolated, these cells have potential to be useful in prenatal diagnosis, as a method that involves no risk to the fetus. However, the volume of fetal cells in the maternal circulation is very small, and there is some evidence that fetal cells may persist in maternal cells for many years, significantly beyond the pregnancy. Fetal cells sampled in a woman’s second pregnancy, then, might be contaminated with cells from the woman’s first child. A better alternative to harvesting fetal cells from maternal blood may be the identification of fetal DNA in maternal blood. DNA is shorter-lived than whole cells. Harvesting of fetal DNA offers an opportunity to test the fetus by drawing blood from the mother, a procedure that carries little if any risk. This test is not yet in clinical use, but may be a common practice in the future.

Are these tests useful?

The usefulness of a test depends on what you want the test to tell you. Prenatal screening tests are limited in terms of the conditions detected and cannot be expected to give yes or no answers; they simply identify a population at greater than average risk of a given disorder. For example, chromosome abnormalities occur in about 1 in 1000 pregnancies in the general population. A woman with a triple screen result showing a 1 in 250 chance of an affected pregnancy has a higher than average risk and she may choose to have diagnostic testing. Notice, however, that the screening test does not give a normal/abnormal result. After all, 99.6% (240/250) of women with this “abnormal” result will have an unaffected pregnancy.

Prenatal diagnostic screens and tests

<table>
<thead>
<tr>
<th>Sample</th>
<th>Tests</th>
<th>Indications</th>
<th>Risks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal serum</td>
<td>AFP Multiple-analyte screens</td>
<td>Open HTD, abnormally large defects, Down syndrome, Trisomy 18, aneuploidy</td>
<td>AFL testing (1000 x 7), incompatible sex</td>
</tr>
<tr>
<td>Amniotic fluid (amniocentesis)</td>
<td>Karyotype AF, DNA, enzyme, hormone analysis</td>
<td>Maternal age ≥ 35, Abnormal MSP or triple screen indicated as result of genetic counselling</td>
<td>≥ 200 risk for miscarriage</td>
</tr>
<tr>
<td>Chorionic villi (CVS)</td>
<td>Karyotype DNA, enzyme, hormone analysis</td>
<td>Maternal age ≥ 35, indicated as result of genetic counselling carrier diagnosis desired</td>
<td>≤ 100 risk for miscarriage</td>
</tr>
<tr>
<td>Fetal blood (fetal blood sampling)</td>
<td>Karyotype DNA testing</td>
<td>1 to 3 in 100 risk for fetal loss</td>
<td></td>
</tr>
</tbody>
</table>

Even diagnostic testing is limited to chromosome analysis and AFP testing, and cannot guarantee a perfect baby. An anomaly that is detected early gives a pregnancy and her partner the option to continue or terminate the pregnancy. When a decision is made to continue the pregnancy, medical management may be altered, and fetal surgery is sometimes possible for certain structural anomalies. Knowing about a problem in advance may be helpful to the family.
Can We Use Medication Labels To Tell Us About Pregnancy Safety?

CHAPTER 13

When health care practitioners have questions about medications during pregnancy, they usually consult the product label. Most physicians use the Physicians Desk Reference (PDR), a collection of product labels for many marketed drugs. Pharmaceutical companies pay to have their labels reproduced in the PDR. Other sources of product labels are the Internet sites of drug companies or of the FDA. Regardless of the source, it’s the same product label, written by the manufacturer and approved by the FDA. A clinician who calls a drug company with a question about the effects of a medication in pregnancy will get the same information that is in the label.

The pregnancy section currently contains a standardized rating system for pregnancy effects with five categories, each of which is given a letter (A, B, C, D, and X) [See Table]. These categories, introduced by the FDA in 1979 in an effort to make pregnancy information easier to understand by practitioners, have been a dismal failure. The category system doesn’t help practitioners because it doesn’t communicate accurately what is known about the effects of a medication on pregnancy, nor does it tell practitioners or patients what action to take.

The first problem is the use of codes. In communication, the use of code requires that the person sending and the person receiving both attach the same meaning to the code element. For example, in Morse code, everyone on the sending and receiving end of the message understands that dot-dot-dot means S. With the FDA code, the people on the sending end and the people on the receiving end may attach different meanings to the code elements.

If you look closely at the definitions, you can see why the gradation-of-risk idea doesn’t work. Category B might be assigned to a drug that has not been adequately tested in experimental animals, rather than a drug that has been shown to be safe. Category X might be assigned to a drug that does not cause birth defects but simply has no conceivable use during pregnancy. Birth control pills are a great example of a Category X drug that has been shown not to increase the risk of birth defects. Birth control pills have been much better tested than some drugs in Category B or C and are likely to be much safer than some drugs in Category B or C. Oral contraceptives are in Category X only because there is no reason to take birth control pills during pregnancy.

Just as the categories do not describe a gradation of risk, the severity of effect is not indicated by the category level. For example, phenytoin, a Category D drug, is associated with birth defects in about 10% of exposed pregnancies, while lovastatin, a Category X drug, is associated with birth defects in about 0% of exposed pregnancies. The difference between D and X may be simply whether there could be a reason to prescribe the drug during pregnancy, in spite of the presumed risk.

Human data are available for only about 60% of Category X drugs. In other words,
the Category X listing was awarded for presumed risk based on experimental animal studies plus a lack of a reason to ever use the medication during pregnancy. In some cases, human data exist but show the opposite of what the category implies. The best example is birth control pills, which do not increase the risk of malformation. Benzodiazepines (Category D) are another example. These medications may be associated with withdrawal symptoms in newborns after pregnancy exposure, but they do not increase birth defects.

Knowing a drug’s category status doesn’t necessarily give a clinician information about what to do. Certainly, a clinician counseling a pregnant woman about a drug she is considering, would want to avoid medications known to increase the risk of fetal harm, but what if you are counseling a woman who has been inadvertently exposed? The pregnancy categories will not help. For example, alprazolam and valproic acid both are labeled Category D. We have just seen how the Category D designation for benzodiazepines is misleading. A woman exposed to alprazolam woman can be told to relax and enjoy her pregnancy. But valproic acid is associated with a 1 to 2% incidence of lumbar meningomyelocele. Even then, of course, 98 to 99% of exposed fetuses are unaffected, and prenatal testing can diagnose lumbar meningomyelocele. The Category D designation, however, is mute on the option of testing.

In 1994, the Teratology Society issued a recommendation that the Teratology system be abandoned in favor of a plain-text explanation of the available information on toxicity during development or on reproduction. Society members viewed the Categories as hazardous to the fetus in potentially causing the termination of wanted pregnancies through inaccurate and incomplete information. The Society was not taking an anti-abortion stance; rather, the Society’s view was that women who wanted to have a baby should not be tricked into terminating their pregnancies because of faulty labels.

Anxiety about liability may cause a health care provider to rely on the Categories, but the Categories don’t help with liability for the following reasons:

- The labels may not be up-to-date. Although the PDR appears each year with a new date on its cover, many of the labels have never been updated.
- The manufacturer, not the FDA, owns the label. The FDA approves the label when it is issued but the label cannot be regarded as either an official government document or as a peer-reviewed source.
- Bad advice is bad advice, no matter what the source. A doctor was sued for recommending abortion to a woman who was inadvertently exposed to birth control pills. The woman lost her uterus secondary to a procedure-related injury. The doctor’s defense that the product label said Category X was not successful given the substantial literature showing that there is no increase in birth defects with oral contraceptive exposure.
- The label is a conservative statement made by drug companies to limit liability.
- Withholding important medication because of pregnancy fears may result in an adverse outcome due to untreated maternal illness. Without necessary medication, epileptics can have seizures and asthmatics can become hypoxemic. These outcomes are not good for either the mother or the baby.

In providing the best care for patients, there is no substitute for good information—single letter codes just don’t work. The good news is that the FDA recognizes that the category system doesn’t work, and is engaged in finding a more effective way to communicate important information in the product label. We can hope that a new system is in place before too much longer.

The categories

Category A is the easiest, because virtually nothing is Category A. Category A means that adequate controlled studies in women have failed to show a risk to the fetus.

Category B means either (1) experimental animal studies show risk but human studies do not, or (2) experimental animal studies are negative and adequate human studies do not exist.

Category C means either (1) experimental animal studies show fetal risk or (2) there is no information one way or the other.

Category D means that there is evidence of risk to the human fetus (not necessarily from human studies, by the way), but that the benefit may outweigh the risk.

Category X means either (1) that there is evidence of risk to the human fetus, but the benefit would never outweigh the risk or (2) the drug has no conceivable utility in pregnancy.

Category B is a funny one, because if part (1) is fulfilled (adequate human studies do not show fetal risk), why is the drug not Category X? If part (2) is fulfilled, you have to wonder whether the experimental animal studies were adequately done. The way experimental animal studies are performed, a selection of doses is used in pregnant rodents or rabbits; the highest dose tested is supposed to result in some degree of maternal toxicity. Usually the top dose results in impairment of weight gain by the pregnant animal. In the face of impaired maternal weight gain during pregnancy, there ought to be an effect on the offspring (often a parallel impairment of offspring weight gain). This effect on offspring should count as fetal toxicity.
What Sources of Information are Available on Developmental Risks?

CHAPTER 14

Growing public awareness about birth defects has sent many worried parents to health care professionals with questions about how their exposures might affect their children. The number of papers published in teratology and developmental toxicity has increased over the years and the task of synthesizing and communicating accurate information has become more difficult. Parents need help in understanding what their risks are compared to general population risks. Approximately 15-20% of recognized pregnancies end in spontaneous abortion and between 3-5% of pregnancies result in a child with a major birth defect and/or mental retardation. Too often, counseling emphasizes the risks associated with drug therapy without balancing the discussion with the benefits to the conceptus of treating an illness in the pregnant woman.

A healthy mother helps to ensure a healthy fetus, and letting pregnant women remain ill because of fear of drug risks may not be the wisest course. To determine whether drug exposure increases a woman’s risk above background levels, health care professionals must integrate information available on the agent and medical factors. A patient’s family or medical history may carry more risk than the drug in question, and complete knowledge of the pregnant woman’s health status, pregnancy history, and family history is useful when evaluating teratogenic risk.

What sources of information are best? Many health care providers rely on FDA Pregnancy Categories, which have been criticized as incapable of providing a meaningful estimate of risk (see Chapter 13). Reliable resources available for clinicians include textbooks, computerized databases, and teratogen information services. Textbooks that provide information regarding the reproductive effects of environmental agents can be found in the Suggested Reading section that follows. Peer-reviewed journals publish original studies, review articles, editorials, and information on upcoming conferences. Computerized databases contain information on thousands of agents, including medications, and summarize information from scientific studies. These databases are available by modem, on diskette or CD-ROM, and the Internet. For links to some of these databases, see the Teratology Society web site at www.teratology.org.

Teratology Information Services (TIS) are comprehensive, multidisciplinary resources that provide up-to-date information, usually at no cost, about the reproductive effects of environmental agents to both health care providers and their patients. Most TIS have at least one full-time teratogen information specialist and are directed by individuals with a medical or doctoral degree and expertise in teratology. TIS are usually located at major medical universities or state health departments and can access a variety of resources, including medical libraries, online reproductive databases, and consultants in teratology-related fields such as toxicology, pharmacology, occupational medicine, genetics, radiation biology, infectious diseases, perinatology and epidemiology. Although TIS operate independ-
How are New Drugs Evaluated for Reproductive Risk?

CHAPTER 15

Every product label for a drug now contains a section on pregnancy. Most of the information in the rest of the drug label is derived from studies on humans, but the pregnancy section and its companion section on fertility are almost always entirely composed of non-human data. Most drugs are not tested in pregnant women, and during initial testing, women at risk of pregnancy are intentionally avoided. Only menopausal women or women who are using effective contraception are normally enrolled in drug trials before reproduction. Non-human animal models are included in large-scale clinical trials. Since the 1960’s, testing in experimental animal models has been required to estimate prenatal risk in humans. These studies are typically performed in one rodent and one non-rodent species. Rats and rabbits are almost invariably used, unless it is known that one or the other is not considered an appropriate model.

Worldwide, the International Conference on Harmonization (ICH) guidelines for testing pharmaceuticals are followed. Similar guidelines and regulations from the US Environmental Protection Agency (EPA) and the European Organization for Economic Co-operation and Development (OECD) govern the testing for potential exposures to chemicals in the environment.

Developmental toxicity studies involve pregnant female animals during the period of organogenesis, typically defined as the period from implantation to palate closure. They are dosed on gestational days 6–17 and the fetuses are examined on day 21, just prior to term. Rabbits are dosed on gestational days 7–19 and the fetuses are examined on gestational days 29 or 30. The external parts, viscera (internal parts), and skeleton of the fetuses are examined for malformations. Growth is usually evaluated by body weight and in some cases by crown-rump (body) length.

The doses for these studies are carefully selected to cover a range of concentrations including exposures at or above expected human exposures. Animals are exposed to at least three different doses and outcomes are compared with a control group, exposed only to water or another inactive vehicle. The highest dose of the test chemical is usually chosen as one that will produce minor toxicity, for example, a small decrease in pregnancy weight gain in the mother. The lowest dose of the chemical is one that is close to the anticipated human exposure level. Middle doses are chosen between these two levels. In this way, the chemical is tested over a range of doses up to a dose that stresses the system by producing some degree of maternal toxicity.

The lowest dose of the test chemical that produces abnormal development is called the LOAEL (lowest observed adverse effect level). The next lower dose is called the NOAEL (no observed adverse effect level). These levels can be compared to the anticipated human exposure level to see how close they are. In general (in the absence of other modifying information about the toxicity of the chemical), if the anticipated human exposure level is 100 times lower than the NOAEL, adverse effects on human development are considered unlikely. Some researchers believe that this 100-fold “safety margin” is unnecessarily high, and that much smaller margins, perhaps between 4-fold and 100-fold, would be just as protective. In instances where no abnormal effects on the offspring occur in an animal, even when the test chemical is given at maternally toxic levels, we can feel reassured that abnormal effects on human development are unlikely.

Developmental toxicity studies evaluate the potential for structural malformations and developmental delays but are not designed to assess effects on function. The pre- and post-natal study, which covers exposure during both the prenatal and early postnatal stages, is designed to assess function. Rats are dosed from gestational day 6 through lactational day 21, the day of weaning.

Physical endpoints used in developmental toxicity studies are examined in this study, but additional tests are done as well. Body weight changes in pups are measured to evaluate growth. Offspring are observed for the achievement of functional developmental landmarks, including the development of the air righting reflex (the ability of the pup to land on its feet). Vision and hearing are tested, and water mazes and other tests evaluate learning and memory. Once the animals attain sexual maturity, functional reproductive ability is evaluated by mating the offspring. The maternal animals are also observed to evaluate the potential impact of exposure on parturition and the ability of the dam to care for her young during the lactation period. The data from all of these studies form the basis for assessing risk during pregnancy.

Data from animal models are not absolutely predictive of human risk, but developmental toxicity studies provide a good assessment of potential problems. An agent’s ability to cause developmental toxicity is usually related to the concentration in blood and tissues. Concentrations that cause developmental toxicity in one species will usually cause developmental toxicity in other species — although the malformations may be quite different across species.

Study outcomes include death or malformations (cleft lip/palate, ventricular septal defect, spina bifida, etc.), but it is much more common for studies to indicate effects on overall development, such as lower fetal body weights, delayed skeletal ossification, or delayed maturation of the kidneys or other organs.

Completion of these studies is only half the task; appropriate interpretation of these data is key, and should be done by scientists trained in the concepts and principles of teratology. It is extremely important to factor in maternal toxicity when interpreting findings,
because illness in a dam will affect her pups. Malformations or significantly delayed fetal development without any effects on the dam may indicate a direct effect of the drug on the fetus.

It is also important to know the baseline incidence of specific malformations in animal models to determine the effects of an agent over the spontaneous background risk. Data are also reviewed to determine if there is a dose-response relationship, meaning that the incidence and severity of adverse outcome increases with increasing dose. Assessing patterns is important in assessing risk. A study that shows no effect of low and high doses, but shows an effect at a middle dose, is less convincing than a study that relates increasing risk to increasing dose.

Understanding the pharmacologic action of the drug and the mechanisms for toxicity are also important. If, for example, the malformations observed are unique to the species tested, then the data may not be relevant to human risk. Finally, risks must be weighed against benefits. A drug that is a member of a class known to be teratogenic in animal experiments would carry a warning contraindicating use in pregnancy, but in a pregnant woman with a chronic disease, it may be important to use the drug in spite of the possible risk. In these circumstances, testing remains an important part of the risk evaluation to characterize the margin of safety for humans.

More than 90 percent of the drugs approved for use in the United States between 1980 and 2000 have no human data on developmental toxicity. These drugs, as well as all newly approved drugs, must rely on laboratory animal data. The pregnancy portion of the drug label is often the only source for this information used by the clinician in estimating risk, but current labels are unhelpful, and the labeling system is undergoing revision (see Chapter 13). Any new label must include information on species and their predictive relevance; dosing effects on systemic or tissue exposures (including active metabolites) compared to those expected to occur in humans and appropriate endpoints within sufficient context to understand the potential for human risk. Sources for more detailed information should be provided.

In summary, animal studies for developmental toxicity provide informative, but complex, data for assessing potential risks of drug use in human pregnancy. Extrapolation of information from reproductive toxicology studies to humans requires more data than is normally available in the product label. Animal studies are designed to elicit adverse events in order to provide a margin of safety for human use. Individual factors, including genetic background and the risks of the underlying disease being treated are weighed in order to make rational decisions about the use of any drug during pregnancy.

Selected Reading


REPROTOX: http://reprotox.org


Teratogen Information System (TERIS): http://depts.washington.edu/terisweb/teris
Do Experimental Animal Studies Predict Human Risk?

CHAPTER 16

No pregnant woman wants to take chances with the health of her baby, and women expect that medications prescribed during pregnancy and exposures in the workplace have been tested for safety to the embryo or fetus. Many exposures have been tested, but in many instances, safety testing is performed only in experimental animals prior to human exposure. How well does this safety testing predict human pregnancy risk?

To answer the question, we must consider what we want experimental animal studies to tell us. Many people think that animal studies ought to be able to draw a clear line between substances that are nonteratogenic, and thus safe in humans, and those that are teratogenic, and thus unsafe.

It is not reasonable, however, to expect any testing scheme to categorize drugs and chemicals as safe or unsafe. Why? Because this expectation ignores the importance of the exposure level in determining toxicity. A chemical that is toxic at one dose will be nontoxic at another dose. Let us consider caffeine, a chemical to which many of us are exposed on a daily—or occasionally hourly—basis. Experimental animal studies have demonstrated that a single injection of 100 mg/kg caffeine given to a pregnant animal will increase birth defects in the offspring. This dose is equivalent to injecting 70 cups of coffee into the bloodstream of a pregnant woman all at once. When the dose is spread out, into, say, four separate injections of 25 mg/kg each, there is no increase in birth defects. The reason is that caffeine teratogenicity requires that a certain peak blood level be achieved in the mother. Lesser blood levels have no effect, even if the total dose is the same.

In humans, we know that caffeine does not increase birth defects under the conditions normally encountered; that is, by drinking soda, coffee, and tea. The question, then, is whether we would expect a predictive animal test to conclude that caffeine causes birth defects or does not cause birth defects? Neither answer would be ideal, because with high enough dosing, caffeine can increase birth defects, but under typical human exposure, it does not increase birth defects. In fact, all drugs and chemicals appear to follow the same pattern:

There is a dose at which toxicity occurs and a lower dose at which toxicity does not occur.

What we would like animal studies to tell us, then, is whether the anticipated human exposure to a drug or chemical may increase the risk of abnormal pregnancy outcome. We want to change the subject of the animal study from the specific drug or chemical to the exposure, that is, the drug or chemical in the context of its dose. Animal experiments are designed to test different doses (see Chapter 15).

Current testing schemes call for pregnancy testing in two kinds of experimental animals, including at least one non-rodent species (see Chapter 15). To date, there has not been a single example of a chemical that produces harm to the developing human without producing adverse effects on development in rats or rabbits at doses high enough to produce maternal toxicity. In other words, human embryos and fetuses are not uniquely sensitive to chemical insult, provided that the chemical has been tested at high enough doses in experimental animals.

The converse is not true: if a drug or chemical produces toxicity in experimental animal studies, it is not necessarily a risk at typical human exposure levels. As in the caffeine example, there are many drugs and chemicals that produce abnormal development at the high doses used in experimental animal studies but not at the exposure levels encountered by humans. Experimental animal testing, then, appears to be conservative, raising more alarms than are subsequently confirmed in humans. This conservatism results in protection and even, in the opinion of some people, in overprotection.

One final word on experimental animal testing: traditional testing often has looked only at abnormally formed structures, such as a cleft palate or a heart defect. But the toxicity for some drugs or chemicals might manifest as abnormal function (see Chapter 5). For example, a child’s brain could be anatomically normal but functionally impaired. This kind of toxicity is important, and more recently developed testing schemes have focused on testing function in the offspring of treated experimental animals.
Can In Vitro Methods Contribute to the Identification of Teratogenic Exposures?

CHAPTER 17

Teratogenic effects range from embryo death to malformations, from growth problems to long-term functional impairments such as mental retardation. The advantage of animal tests in teratogenicity risk assessment is that the pregnant animal comprises all of the potential endpoints for adverse effects of an exposure – i.e., the mother, placenta and embryo. So, how can in vitro methods contribute to the identification of teratogenic exposures?

All in vitro teratogenicity tests have a biological component that can undergo differentiation or organogenesis in vitro. This biological component could be cultured cells, an organ, or a whole embryo. Each of these levels of complexity has both advantages and disadvantages. The European Centre for the Validation of Alternative Methods (ECVAM; http://ecvam.jrc.ec.europa/index.html) has led an initiative to establish and validate in vitro tests for embryo toxicity. The ideal in vitro teratogenicity test should be rapid, reproducible, inexpensive, technically easy to perform, and not involve experimentation on animals. Cell culture involves few animals, especially if the cells have been maintained in culture over many generations as in established cell lines. A whole organ or embryo culture system, however, is more likely to involve all the processes in development that are likely to be susceptible to interference after a teratogenic exposure. No one system is perfect for every context, and a combination of several tests may be recommended. The advantage of in vitro testing is that exposure can be controlled very precisely. Species differences in how a chemical is metabolized can be overcome by adding the metabolite or an appropriate metabolizing system.

We will focus on three mammalian embryotoxicity test systems: the culture of embryonic stem cells, limb bud or limb bud micromass cultures, and whole embryo cultures. Many signalling pathways that control embryo development occur across species, so non-mammalian systems using embryos of zebrafish or Xenopus frogs may have potential, but these systems won’t be discussed here.

Embryonic stem cells

Embryonic stem cells are pluripotent cells, capable of differentiating into a wide variety of cell types, that are derived from the blastocyst inner cell mass. In the embryonic stem cell test the effects of a putative teratogen on a mouse embryonic stem cell line (3D) are compared to those on adult fibroblast 3T3 cells. The endpoints measured are the ability of the chemical to inhibit growth of the embryonic stem cells or their differentiation into cardiac myocytes (muscle cells), for example, compared to their effect on adult 3T3 cells. Thus, this test compares the effects of a test chemical on embryonic cells relative to adult cells, and also looks at the ability of the chemical to inhibit differentiation.

Limb bud or limb bud micromass cultures

In the limb bud culture system, fetal limbs, usually from mice, are removed on day 11 or 12 of gestation and cultured for 6 to 9 days, with or without the agent under study. During culture the limbs undergo extensive changes in morphology (size and shape), and biochemical differentiation, which can be assessed quantitatively. Changes in size and shape can be measured using a scoring system or image analysis. Measurable biochemical changes include DNA, RNA, and protein content as well as creatine phosphokinase (related to muscle development) or alkaline phosphatase (involved in pre-bone formation).

The limb bud micromass culture system is based on the ability of undifferentiated limb bud mesenchyme cells to form foci, or clusters, of differentiating chondrocytes (cartilage cells). Cell proliferation, differentiation, and interactions between cells, or between cells and the extracellular matrix, are all implicated in this process. Limb buds are isolated from day 14 rat embryos, and made into a cell suspension, which is placed into wells (plates or trays dotted with small round indentations). The cells are incubated for 5 days with medium and a test chemical; control wells contain only medium. The total number of viable cells, differentiated cells and foci are determined. Chemicals that reduce the number of foci, or the number of cells within foci, are considered potentially problematic.

Whole embryo cultures

In the mammalian whole embryo culture system, rat or mouse embryos with intact yolk sacs are removed during early organogenesis and cultured in rat serum. During culture, the embryos undergo tremendous growth and differentiation, advancing from the early somite stage to 30 to 45 somites by the end. The embryos turn and rotate, the neural tube closes, and the brain, heart and other organs begin to develop. The effect of chemicals on the number of dead and live embryos and the number of malformed versus normal embryos is measured.
in vitro Methods


is noted, but many other measurements are taken as well. Effects on yolk sac diameter, crown-rump length, head length, and number of somite pairs are scored. Biochemical criteria, including DNA content, gene expression, protein content and hemoglobin, are also usually assessed. Malformations induced in animal models by cadmium, retinoic acid and hydroxyurea cause very similar effects in whole embryo culture, although adding chemicals directly to an embryo’s environment may not replicate the effects of maternal exposure to and metabolism of an agent.

Role of in vitro methods in the identification of teratogenic exposures

The ECVAM international validation study concluded that the whole embryo culture test, the micromass test on limb bud cells, and the embryonic stem cell test were scientifically validated and acceptable tests for regulatory use. Test chemicals were classified correctly at rates that often exceeded 70% for agents that were strongly toxic to the embryo, these tests were almost 100% predictive. The ECVAM Scientific Advisory Committee recognized that these methods could not replace animal tests for assessing reproductive toxicity, but concluded that they could provide suitable means for reducing and refining the use of animal procedures.

Are there instances in which in vitro tests are more useful than current in vivo animal tests in identifying teratogenic exposures? Scientists have begun to acquire new information about the genes that regulate developmental events, and about the chemical messenger systems involved in establishing spatial information or position in the early embryo. In vitro systems will be particularly useful in investigating the role of a specific gene or gene family during organogenesis. Transgenic animal approaches are laborious and frustrating, especially because sometimes deleting the gene of interest kills the embryo. In vitro approaches may help us to determine the role of specific signalling molecules or pathways.

It is likely that in vitro assays will complement but not completely replace in vivo animal testing. These assays can be valuable in elucidating mechanisms by which teratogenic exposures affect development, and may be particularly useful in testing chemicals that are metabolized in a specie-specific manner. In vitro tests should be useful as screening tests to winnow compounds for further research and development.

Suggested reading

Can Molecular Endpoints Predict Abnormal Development?

CHAPTER 18

Expensive animal studies are currently the principal methods of testing agents for possible risks to the human embryo/fetus. The question has been raised whether we could identify specific molecular endpoints that could predict abnormal developmental outcome after embryonic exposure. If such endpoints could be identified, we could enhance our understanding of birth defects, reduce the number of test animals required and improve risk interpretation.

We are not there yet; though, this field is still in its embryonic stage. However, attempts have been made to correlate specific molecular endpoints with outcomes in embryos after potentially teratogenic exposures. The heat shock response, for example, is a cellular response to a variety of stresses in which specific genes are activated and specific proteins are synthesized. Heat shock proteins are not harmful and are generally believed to be protective, but their presence in embryos could be viewed as a signal that at least some cells within the embryo have been subjected to stress. The effect of about 30 teratogenic and nonteratogenic exposures on production of heat shock proteins in embryos or embryonic cells has been tested, but results have not been consistent. Although all ten nonteratogenic exposures failed to induce heat shock proteins, not all of the twenty teratogenic exposures induced the synthesis of these proteins. So far, it seems that heat shock proteins may not be suitable indicators that a specific exposure can disrupt development.

However, cells have other stress response systems, including the genotoxic stress response (a response to DNA damage), the oxidative stress response (a response to reactive oxygen species), and the activation of stress-activated protein kinases (signal transduction pathways activated by a variety of stresses). Very little is currently known about the functioning of these stress-response systems in embryos. Eventually, however, specific molecular endpoints from these response pathways could serve as a battery of end points that are predictive of the teratogenic potential of untested drugs and chemicals.

Could alterations in gene expression be used as a teratogenic end point? Until recently, the complexity of gene expression during normal development has precluded any systematic, global analysis of teratogen-induced alterations in gene expression; however, recent developments in genomics and bioinformatics have opened exciting new possibilities. Two developments in genomics are particularly noteworthy. The Human Genome Project, an international effort to completely sequence the human genome identified and sequenced approximately 30,000 protein-coding transcripts in the human genome. Similar efforts have sequenced the genomes of other species, including the roundworm, fly, frog, and mouse. Second is the development of DNA microarray chip technology, which will allow an investigator to simultaneously assay the expression of tens of thousands of genes.

Within the next 5 to 10 years, DNA chips containing all genes from the species of interest or a designer subset of genes should be available to the research community. When available, these chips will allow investigators to rapidly assess which genes are altered by known teratogenic exposures. Perhaps this global analysis will enable researchers to identify patterns of gene expression that are linked to abnormal development and therefore could be used as markers for the determination of whether an untested chemical or drug exposure is potentially teratogenic.

Because the volume of information from such analyses is expected to outstrip the ability of the human brain to find the patterns of gene expression that are casually linked to teratogenesis or other diseases, investigators in the field of bioinformatics are already developing tools to address these analyses (See Chapter 20).

The significance of these and other related developments, e.g., proteomics (analysis of protein expression and function), is that science in the future will be conducted in ways that can only be imagined at this time. Thus, although today we cannot assess the response of the human embryo to teratogenic insult using molecular end points, doing so in the near future seems almost certain.

Suggested Reading


Can Chemical Structures Predict Teratogenic Risk?

CHAPTER 19

Developmental abnormalities can be caused by unknown, genetic, or environmental factors; the latter includes many kinds of exposures, including drugs and industrial chemicals. Although only about 1%-2% of environmental causes of developmental defects are due to chemicals or drugs, it is an important area of regulatory concern because these exposures are preventable.

Mammalian assays are used to estimate risks to developing humans, but differences among species responses give these extrapolations a high degree of uncertainty. These tests can be expensive and time-consuming.

An alternative method of hazard identification involves computer-based modeling that uses what is already known about classes of compounds to predict effects for new or unknown agents. The fundamental principle underlying Structure Activity Relationships (SAR) and Quantitative Structure Activity Relationships (QSAR) is that biological activity is a function of physicochemical properties, which are a function of chemical structure. The scientific discipline of SAR, QSAR seeks to mathematically model the relationships between chemical structure and biological activity.

Physicochemical parameters and structural features about a set of compounds are used to determine relationships with observed biological activities. These relationships are expressed in mathematical terms. QSAR uses multivariate linear regression, a kind of statistical method, to model relationships when biological activities are continuous, as they are in determining LD50 (the dose lethal to 50% of the tested animals). QSAR uses linear discriminant analysis, another statistical method, to model relationships when biological activities are discrete (for example, active vs. inactive).

The pharmaceutical industry uses SAR, QSAR models in drug discovery and in the design of new medicines.

Physicochemical parameters are classified as electronic, hydrophobic, or steric.

Electronic parameters address a molecule’s potential for engaging in interactions with other molecules. Chemical reactions are an example of this kind of intermolecular interaction.

Hydrophobicity (water avoidance) is a description of where the compound can be found when it has a choice of distributing in water or in the more oil-friendly n-octanol. Hydrophobicity is important because it may correlate with how well a compound is absorbed and transported to its site of action or its site of toxicity. Steric parameters include molecular volume and surface area, measures of a molecule’s “bulkiness” that may reflect size requirements at a receptor site or target molecule. Chemicals that have similar steric parameters may activate the same receptor.

Attempts have been made to apply the principles and techniques of SAR, QSAR to developmental toxicology. Most studies have used experimental animal data to test the accuracy of the models. The SAR, QSAR validation approach starts with the assumption that chemicals can be characterized as toxic or nontoxic. Using a list of chemicals identified as developmental toxicants or nontoxicants in rats, mice, rabbits, and humans, SAR, QSAR models correctly predicted results for 77-82% of randomly constructed test sets. Species-specific differences were observed for some structurally similar fragments. For example, carboxylic acids are known to be developmentally toxic in animals. Carboxylic acid esters (e.g., COOCH3 and COOCH2) contributed to developmental toxicity in rats and mice, presumably by hydrolysis to the carboxylic acid. In humans, however, fragments referring to carboxylic acid esters were found to be benign in terms of developmental toxicity.

SAR and QSAR will never entirely replace research using animal models, and it remains to be seen whether these approaches can be reliable enough to have a place in assessing the developmental risk of new or unknown agents. But there is certainly exciting potential for further computational investigations of the relationship among chemical structure, physicochemical properties, and developmental toxicity endpoints. In the future, SAR and QSAR could be important tools for (a) achieving insights into mechanisms, (b) rapid and economical screening of compounds for hazard identification, (c) prioritizing chemicals for regulatory, research, and remedial actions, and (d) establishing guidelines for the design of industrial chemicals and pharmaceutical agents with minimal potential for human developmental toxicity.

Suggested Reading


Can Bioinformatics help to Predict which Agents will Cause Birth defects?

CHAPTER 20

Teratogenesis reflects a complex interaction between the embryo and chemical insult exposure. New technologies such as genomics and proteomics can provide information on cellular responses to a large variety of genetic and environmental factors; however, it is difficult to interpret the rapidly growing amount of available information. New advances in computer resources to handle large amounts of genomic and other data offer the hope of allowing us to understand developmental processes and toxicities. The field of managing and coordinating large amounts of data is called bioinformatics. How can teratologists take advantage of this information revolution?

Bioinformatics applies principles of information sciences and technologies to make complex life sciences data more accessible. Large amounts of the data come from what are called high-throughput technologies including DNA and protein microarray biochips. These biochips contain hundreds or thousands of minute binding areas representing, for example, different nucleotide sequences. When the chip is exposed to a sample, such as DNA from embryonic cells, binding occurs in select areas of the chip that match sequences from the embryo. The chips are processed and read by an automated method that very quickly generates numbers for each of the sequences on the chip. These chips have become standard tools for determining important cellular behaviors at the gene and protein levels. Given enough information from experimental data, these microarrays can provide a tool for comparing the impact of a large number of chemicals. Let’s say you want to compare the effect of ten different anticonvulsant medications on 1000 possible genes in the embryo. You culture embryonic cells with each of the 10 chemicals, then expose each of 10 microchips containing the 1000 candidate gene sequences to embryo DNA. You end up with 10,000 (10 x 1000) numbers (expression levels)—a large amount of information to process and interpret.

Models of toxicological events must find a connection between adverse developmental outcomes and specific exposure scenarios. The connection is through a cascade of biomolecular and physiological changes that have occurred when the toxic agent hits critical molecu-
What are the Effects of Alcohol use During Pregnancy?

CHAPTER 21

Thousands of research studies over the past 30 years have demonstrated that drinking alcohol (ethanol) during pregnancy is teratogenic. Prenatal exposure can cause growth deficiency, malformations, and neurobehavioral deficits. Whether a conceptus is affected depends on the dose, timing, and conditions of exposure. Different strains of mice respond differently to similar doses of alcohol, and it’s very likely that women also differ in how alcohol intake affects offspring. Individual variations make a difference; even twins can be differentially affected. In general, dizygotic (non-identical) twins show more disparate responses to prenatal alcohol than do monozygotic (identical) twins.

There appear to be several mechanisms for the teratogenicity of alcohol. Alcohol has a directly toxic effect on the developing brain; it can kill brain cells and interfere with the transport of amino acids (the building blocks of proteins) and glucose (the main energy source for cells). Alcohol can also impair placental-fetal blood flow, causing hypoxia or disrupting the hormonal and chemical regulatory systems in the brain that control the maturation and migration of nerve cells.

The teratogenic properties of alcohol, our most frequently used “social” drug, came to attention originally through independent clinical observations by Lemoine in France (1968) and Jones and Smith in Seattle (1973). Noting a pattern of malformation, growth deficiency, and central nervous system effects in children of alcoholic mothers, the term Fetal Alcohol Syndrome (FAS) was coined in 1973 and drew immediate attention to alcohol as a teratogen. Three decades of research have increased recognition of the types of damage caused by prenatal alcohol and the long-term consequences. Our understanding of the breadth of prenatal alcohol damage derives from studies of children with Fetal Alcohol Syndrome (FAS), experimental animal models, and long-term prospective studies of diverse human populations. These various streams of investigation lead to remarkably congruent findings.

In general, larger doses cause greater effects. Also, both animal and human studies show that a “binge” dose, or many drinks in a short time period, is damaging for the conceptus. The brain appears to be the most vulnerable organ; brain effects are widespread, and occur across a wide range of doses and patterns of exposure. In general, morphological abnormality occurs at the highest doses or highest peak doses, while behavioral deviations can occur at lower doses or lower peak doses. Direct cell death, aberrant neuronal migration and development, and disrupted neurochemical balance of the brain can result from exposure during all periods of embryonic and fetal development. The typical facial appearance of FAS, however, arises only when a fetus is exposed during a very specific time period.

Prospective epidemiologic studies link alcohol to increased risk of miscarriages, stillbirths, low birth weight, and increased neonatal morbidity. Alcohol abuse is often associated with poor nutrition, which can confound results. Experimentally, however, these adverse birth outcomes result from prenatal alcohol even when diet and rearing conditions have been tightly controlled; poor diet, however, can aggravate adverse effects.

Alcohol crosses the placenta, so that minutes after a pregnant woman consumes alcohol, the fetal blood alcohol level is similar to that of the mother. Alcohol can cause a temporary pause in fetal respirations. Autopsies of deceased children with FAS, imaging studies, and experimental animal studies have shown us that many brain regions, including the hippocampus, cerebellum, corpus callosum, and basal ganglia, are affected by prenatal alcohol. A large and compelling experimental animal literature links prenatal alcohol to disruptions in learning, memory, emotional responsiveness, and behavior. Associated brain-behavior deficits include optic nerve hypoplasia with impaired vision, reduced cerebellar size with impaired motor development and ability, decreased corpus callosum with hyperactivity, and decreased serotonin synthesis with impaired instinctive maternal behaviors. Thus, some of the behavioral aberrations of children and adults whose mothers abused alcohol during pregnancy are likely of physical origin (see Table).

The consequences of prenatal alcohol exposure can last a lifetime. Rodents exposed prenatally to alcohol have higher rates of sickness and early death. Some (but not all) studies have found that early growth deficits attenuate with time, at least in some individuals. Problems with maintaining attention and focus are not only measurable in day-old newborns, but also manifest throughout childhood, adolescence, and early adulthood. Learning prob-

First baby identified at birth with FAS, shown here as a neonate, and at 8 months, 4 years, and 9 years. Note the short palpebral fissures, smooth philtrum, thin upper lip, and microcephaly. Photo from Strangath et al., CIBA Foundation Monograph 103. London: Pitman, 1984

The discriminating features of FAS are those on the left side. However, as the facial features often coexist with polyhydramnios, not all adolescents and adults will retain these features. Graphic from Steving and Little (1994) Unit 5 of Alcohol, Pregnancy, and the Fetal Alcohol Syndrome, Second Edition, Unit 5 of the Project Cork Slade Lecture Series of Darnsworth Medical School, available from Milner-Fenwick, Baltimore (1-800-432-8433).
lems (particularly in abstract thinking and arithmetic), are not easily measurable until early childhood, but have lifelong consequences. Some of the most marked behavioral deficits in humans are observed in adaptive behavior and “executive function” as measured by communication, socialization, problem-solving, and daily living skills.

Follow-up studies show that FAS children do not fare well as they reach adolescence and early adulthood. FAS individuals often cannot live independently, are impulsive, exhibit poor judgment, do not respond appropriately interpersonally, are frequently depressed, and have socialization, communication, and problem-solving deficits. These include social or behavioral problems that stem from the primary disability of prenatal brain damage from alcohol, but may be complicated by the failure of appropriate early diagnosis, the impact of high-risk environments associated with parental alcohol abuse, and the failure of society to adequately provide appropriate services to this population of disabled adolescents and adults.

FAS and other Alcohol-Related Neurodevelopmental Disabilities (ARND) represent many deficits besides mental retardation. Epidemiologic studies from three cities in two countries have revealed an FAS prevalence of approximately 3 per 1,000 live births. The only study that attempted to measure ARND empirically across the first 7 years of life found that the rate of FAS plus ARND is almost 1 per 100 live births. Despite the impressive progress of the past 30 years in understanding the teratogenic effects of alcohol, community efforts must be intensified to identify and help alcohol-affected individuals, and to prevent additional children from being born with these preventable lifespan deficits.

Suggested Reading


Which Infections Increase the Risk of Birth Defects?

CHAPTER 22

Which infections increase the risk of birth defects?

A cold or the flu may cause a pregnant woman to be miserable, but it won’t hurt her pregnancy. A few infectious diseases, however, can kill an embryo, fetus, or newborn, cause birth defects, trigger a premature delivery, or interfere with fetal growth. Only eight infectious agents are generally regarded as increasing the risk of birth defects in humans. These include six viruses: the rubella virus, Varicella zoster virus, herpes simplex viruses, and parvovirus B19; one bacterium, Treponema pallidum; and one protozoon parasite, Toxoplasma gondii.

Rubella virus causes German measles. An exposed fetus may develop a congenital rubella syndrome that includes cardiovascular defects, microcephaly (a small head), microphthalmia (small eyes), cataracts, retinopathy, glaucoma, deafness, mental retardation, and growth impairment. A fetus exposed to rubella during the first trimester of pregnancy has a risk of birth defects that may be as high as 85 percent. Exposure between weeks 13 and 18 of pregnancy has about a 25 percent risk of birth defects; exposure after the 18th week of pregnancy carries little risk for congenital rubella defects.

Varicella zoster virus causes chickenpox (Varicella), and reactivation of the virus causes shingles, also called herpes zoster. A fetus infected during the first or second trimester of pregnancy may develop the congenital varicella syndrome, which can cause atrophy (wasting) of the limbs, scarring of the skin, brain atrophy, and eye defects. The risk for congenital varicella damage after maternal infection with the virus during the first 20 weeks of gestation is estimated to be 1 to 3 percent.

The herpes simplex viruses are DNA viruses that cause blister-like or ulcerative lesions of the skin and can infect the central nervous system, eyes, and liver. HSV Type 1 usually produces cold sores around or in the mouth, whereas HSV Type 2 generally affects the genitalia, causing genital herpes eruptions, but either type can affect either location. Most people have been infected by at least one type of herpes virus in their lives (even if they have never had symptoms) but a pregnancy is rarely affected by this very common chronic infection.

When a woman is infected with herpes for the first time during a pregnancy, however, the fetus can be severely affected. HSV can cause skin lesions, microcephaly, chorioretinitis, intrauterine growth restriction, and psychomotor retardation as well as occasional hydranencephaly (increased fluid in the brain) and brain calcification, ventricles of the brain), porencephaly, microcephaly, intracranial cleftication, and skin aplasia. The most serious effects are seen when the infection takes place in the third trimester, but abnormalities can occur after both first trimester infection and later infection; suggesting that many of the defects are due to disruption of existing structures rather than malformation. Most cases of HSV abnormalities are caused by the Type 2 virus, but some are caused by the Type 1 virus.

Parvovirus B19 is a DNA virus that causes erythema infectiosum (fifth disease). The virus causes hydrops (edema, or swelling) or fetal death in about 10 percent of the fetuses of mothers infected during the first half of pregnancy, but in less than 10 percent during the second half of pregnancy. While a few cases of major malformations have been reported in newborn infants infected during gestation, it is not known if the malformations were caused by the virus. The risk for major malformations attributable to the virus cannot be dismissed, but this risk is thought to be very low.

Treponema pallidum is a spirochete, a spiral-shaped bacterium that causes syphilis. A fetus can contract congenital syphilis at any time during pregnancy. Untreated syphilis
infection of the mother during early pregnancy causes up to 40 percent of the pregnancies to end in spontaneous abortion, fetal death, or perinatal death. Congenital syphilis can cause fetal hydrops and affects many systems in the fetus, resulting in skin lesions, enlargement of the liver and spleen, lymphadenopathy (swollen lymph nodes), osteochondritis (inflammation of the bones and cartilage), hemolytic anemia, and thrombocytopenia (low platelet count). Hydrocephaly, hemiplegia (weakness on one side), mental retardation, eighth-nerve deafness, and defects of the teeth and skeleton may occur.

The protozoan parasite Toxoplasma gondii causes toxoplasmosis. Infants infected during gestation may have hydrocephaly, microcephaly, cerebral calcification, encephalitis (inflammation of the brain), cerebral palsy, mental retardation, chorioretinitis (inflammation of the choroid), deafness, and mental retardation of the brain). Cerebral palsy, mental retardation, and myocarditis (inflammation of the heart muscle), anemia, and thrombocytopenia (low platelet count).

Is Stress a Developmental Toxicant?

CHAPTER 23

Stress is a term that means very different things under different circumstances. In toxicology, stress refers to any external challenge that disturbs the internal environment, or homeostasis. This definition encompasses the neurochemical, physiological, and behavioral reactions to a novel, dangerous, or upsetting situation. Stress isn't always bad: the endocrine and neural adaptations typically referred to as the stress response may cause a person to, for example, jump out of the path of a moving car. However, these responses can be maladaptive in certain situations, especially in cases of chronic stress.

Stressors can be environmental conditions or events, including excessive heat or cold, trauma, noise, starvation, chemical exposures or physical exertion. Stress can include domestic violence or abuse, conflicts with family members, or a hostile work situation. Psychological stressors include anxiety or depression. Although a considerable amount of animal research has been done on the topic, few human studies during pregnancy have been attempted. Women and their life situations differ widely, making such studies difficult to conduct and interpret.

Animal studies, most often with pregnant rodents, have tested the effects of physical restraint, hypothermia, electric shock, noise, visual stimuli, vibration, shipping, and crowding, on development. Deprivation of food and water has also been used as a stressor but the possible effects of malnutrition confound these studies. Studies in pregnant rodents found that certain maternal stressors could increase the developmental toxicity of chemicals known to cause malformations or developmentally toxic effects. Conversely, it is also possible that exposure to maternally toxic doses of chemicals causes stress that can, in some cases, exacerbate harmful effects on the embryo or fetus.

Numerous animal studies have examined effects of maternal stress on physiology (especially endocrine alterations) or behavior of the young at various times after birth. Others have noted mortality, decreased growth, or development of extra ribs in the embryo.
or fetus. A few studies have reported stress-related malformations. These include encephalocoele and exencephaly (different degrees of defective development of the brain and skull) due to failure of the neural tube to close normally. There are clearly significant differences in susceptibility to maternal stress during development among species and even among strains of the same species. For example, it is generally easier to induce adverse effects on growth, mortality, and anatomical development in mice than in rats. And in some strains of mice, maternal stress is associated with cleft palate, while other strains are unaffected. Several human studies have attempted to evaluate the effect of maternal stress on development. Some studies have reported increased incidences of low birthweight or preterm delivery; and a few have found diminished scores on neonatal neurological examination. However, these human studies often have methodological shortcomings, including arbitrary measures of psychological stress or inappropriate comparisons among groups. These studies also suffer from possible biases, especially recall bias. Recall bias has to do with selective memory. Women who miscarried, gave birth prematurely, or who had abnormal children may be more likely than women with normal pregnancies to remember events that they believe may have contributed to such outcomes.

Confounding factors are also common in human studies. For example, stressful life events are more common in women of low socioeconomic status, and poverty itself is a stressor. Stressed mothers may be younger, more likely to smoke, abuse alcohol, or use other drugs, or to have poor nutrition. Any of these exposures could affect results of a study on development. Thus, the human evidence for stress-induced effects on the offspring can only be considered suggestive at this point.

Although there has been much speculation, we know very little about mechanisms by which maternal stress could adversely affect pregnancy. The response to stress is a basic adaptive mechanism that is protective in times of crisis. Stress activates of the sympathetic nervous system and results in the release of hormones, including catecholamines and glucocorticoids. In mice, it is the increase in glucocorticoids that appears to be the cause of stress-induced cleft palate. Stressors may also alter activities of the immune, neural, and renal systems, as well as having additional endocrine effects. Any of these influences may, in turn, influence various aspects of a mother’s physiology and behavior.

Stress responses are complex and can vary depending on the stressor—and species—involved. Although adverse effects have been shown to occur in laboratory animals, determining possible mechanisms by which maternal stress might negatively affect embryonic or fetal development is highly problematic.

Suggested Reading
Warkany and his colleagues extended these findings by detailing the defects that vitamin A deficiency produces in virtually every organ system of the rodent. More recently, vitamin A has been shown to be critical in establishing anterior-posterior body axis patterns in the embryo. Sporadic reports in the literature have linked eye abnormalities and other adverse birth outcomes to vitamin A deficiency (the mothers in these cases had night blindness or other manifestations of severe vitamin A deficiency). Malformations induced by vitamin A deficiency in humans are rare, but in developing countries, vitamin A deficiency remains the leading cause of visual impairment and blindness.

Deficiencies in many B vitamins adversely affect development. In animals, riboflavin, niacin, folate, and pantothenic acid deficiencies cause structural malformations; pyridoxine and thiamine deficiencies increase embryonic mortality and decrease fetal growth. Folate deficiency, induced by a folic acid antagonist, causes structural malformations in animals. More importantly, folic acid supplementation significantly decreases the rate of neural tube defects in humans. The neural tube defect rate among offspring of women taking folic acid supplements at the time of conception is reduced by as much as 50% compared to that among unsupplemented pregnancies. The US Public Health Service recommends that all women receive 400 mcg of folic acid daily, and since January, 1998, all grain products are required to be fortified with folic acid at a level designed to provide additional daily intake of 100 mcg/day folic acid. It is controversial whether supplemental folic acid overcomes the effects of subclinical deficiency in the pregnant woman or a metabolic problem of the embryo.

Maternal vitamin D deficiency can cause fetal rickets in experimental animals and in humans. Vitamin E deficiency in rats produced litters in which approximately 30% of pups had brain anomalies (exencephaly or hydrocephalus). However, there is no evidence that vitamin E deficiency is teratogenic in humans. In contrast, vitamin K deficiency in humans (usually as a result of therapy with an oral anticoagulant, warfarin) results in a high percent-age of miscarriage and prematurity. Infants have characteristic bone abnormalities, optic atrophy, and mental retardation.

Zinc deficiency is teratogenic in animals, affecting the development of virtually every organ system. After only 24 hours of dietary deficiency, plasma zinc decreases by 40%, and only a few days of deficiency during the embryonic period can produce malformations. Offspring of women with acrodermatitis enteropathica, a genetic disorder of zinc absorption, have a higher rate of malformations. Epidemiological studies also suggest a relationship between zinc deficiency and central nervous system malformations in humans. Although severe zinc deficiency is uncommon in developed countries, mild deficiency is common: the average dietary intake is only about half of the recommended daily allowance (RDA) for preg-nant and lactating women. Also, some drugs, chemicals, and physiological or environmental stressors can significantly alter zinc metabolism. These two facts, along with the observation that even transitory zinc deficiency can have adverse effects, suggest that unrecognized, sub-clinical and biochemical deficiencies may play a role in some human embryonic morbidity.

The adverse effects of copper deficiency during pregnancy have been shown in numerous species, including humans. Copper-deficient lambs exhibit neonatal ataxia, blindness, and anemia. The effects of prenatal copper deficiency in humans have been reported in offspring with Menkes’ disease, an X-linked disorder of copper metabolism. These infants have mental retardation and severe cardiovascular and connective tissue defects that generally cause death by three years of age. Many of the defects can be linked to decreased activity of copper-requiring enzymes. Copper-chelating drugs, including D-penicillamine, used to treat Wilson’s disease (a genetic copper overload condition) and rheumatoid arthritis, can induce copper deficiency in humans.

Manganese deficiency during pregnancy can cause congenital ataxia, characterized by head retraction, lack of coordination, and loss of equilibrium in experimental animals. The ataxia is thought to be due to abnormal development of the stoloth in the inner ear, due to decreased activity of glycerol transferase, a manganese-requiring enzyme. The effects of manganese deficiency during pregnancy in humans have not been well documented.

Vitamins may be good for you, but more is not necessarily better. Megadoses of vita-mins may be harmful in some instances. In animal models, an excess of vitamin A is terato-genic, affecting the development of many organs. Many genes responsible for establishing the embryonic body pattern are controlled by retinoic acid, the active form of vitamin A. Vitamin A levels are tightly controlled in the embryo to regulate expression of these genes. Excess vitamin A can override the control mechanisms, leading to abnormal development. It is likely that vitamin A excess would be teratogenic in humans, but it is not clear what the minimum teratogenic dosage is. The latest thinking is that this level is much greater than 55 International Units (IU) per day; the RDA for pregnancy is 2,670 IU/day. The Teratology Society, in a position paper on vitamin A issued in 1987, reasonably advocated that vitamin A intake in pregnant women be restricted to the RDA.

Suggested Reading

Hale F. Pigs born without eyeballs. J Hered 1933; 24: 105-106.


Do Chemicals used to Purify Water Pose a Risk to Reproduction and Development?

CHAPTER 25

Access to clean water is one of the most important public health measures in the world. Countries that lack clean water suffer from epidemics of dysentery, hepatitis A, parasites, and many other water-borne diseases. The process by which municipal drinking water is disinfected, however, creates hundreds of chemical by-products, raising the question of whether disinfection by-products (DBPs) might pose a risk to human reproduction and development.

Epidemiology studies do not establish a consistent association between DBPs present in tap water and adverse pregnancy outcomes. Several studies show no association between levels of DBPs and an adverse pregnancy outcome. Other studies report an association between total or specific DBPs and decreased birth weight, spontaneous abortions and birth defects (such as neural tube and heart defects). Research is needed on genetic differences in susceptibility to estimate the risks for poor pregnancy outcome in humans. A recent study reported that DBP levels were associated with an increased risk for intrauterine growth retardation and acute lymphoblastic leukemia in children with a polymorphism of the GRPE21 gene. In addition to studies of pregnancy outcome, studies that assess reproductive function such as menstrual cyclicity and sperm production and quality are nearing completion. Therefore, based on epidemiology studies it has been difficult to determine if an association exists and if it does the relationship between DBP levels and pregnancy outcome.

One reason that epidemiology studies have come to different conclusions is that reliably estimating human exposure to DBPs is difficult. Estimating exposure is difficult because the chemicals that make up the DBPs and their concentrations can vary between cities and in different seasons. The chemical composition of DBPs is dependent upon many factors including the disinfectant used (e.g., chlorination compared to chloramination or ozoneation) and characteristics of the water being disinfected (e.g. the presence of bromide). The assessment of human exposure is further complicated because some DBPs are volatile and inhaled during showering and bathing, while other DBPs are rapidly absorbed through the skin.

Therefore, it has been difficult to accurately determine DBP exposure during pregnancy and determine if there is an association of those exposures to adverse effects. It is important to note that the risks associated with disinfected water are lower than the risk associated with drinking “unclean” water.

In order to assess the relationship between exposure and potential effects on pregnancy, animal models have been used. Many studies have focused on levels of chloroform and other halomethane levels in drinking water. Although oral administration of chloroform to pregnant experimental animals does not cause teratogenicity, it has been associated with reduced fetal weight at term and other indications of developmental toxicity. Two other halomethanes, bromoform and bromodichloromethane (BDCM), have a much more dramatic effect, causing resorptions of entire litters in Fischer 344 rats (resorptions in rats correlate with miscarriages in humans). This adverse effect occurs after exposure during the period of pregnancy that depends on luteinizing hormone-stimulated progesterone production, and BDCM does decrease progesterone production. Different strains of rats have different susceptibilities; for example, Sprague-Dawley rats are most resistant, Long-Evans rats are intermediate, and Fischer most sensitive to BDCM-induced full-litter loss. Research to better understand the relationship between strain genetic differences and sensitivity may be helpful in understanding human susceptibility.

Another category of disinfection byproducts called haloacetic acids (HAA) produce teratogenic effects in experimental animals. Chloroacetic acid, dichloroacetic acid, trihaloacetic acid, and bromoacetic acid produced heart malformations in animal models. Bromochloroacetic acid is embryolethal and produces postnatal death when administered in drinking water to rats. In whole-embryo culture, conceptuses directly exposed to HAAs developed cranofacial and heart abnormalities; the brominated- and bromochloroacetic acids were more potent than the chlorinated acids. When rat embryos were exposed to a mixture of two or three HAA, an exposure that would more closely approximate drinking water exposure, the adverse effects of these compounds were additive. A subset of the HAA, disinhibited haloacetic acids, alters spermatogenesis in animal studies. It is not known whether these compounds will have the same effect in humans at exposures normally encountered.

Thus experimental animal data suggest that the trihalomethanes and haloacetic acids at high doses are capable of eliciting profound effects on both embryonic development and spermatogenesis. To evaluate human risk, it is critical to compare the concentrations of DBPs to which humans are exposed and the resulting blood levels of the DBPs and their metabolites to that in animal studies. The “margin of exposure” based on administered dose is one estimate of the possible risk. Margin of exposure is roughly calculated by the highest dose that causes no adverse effect in animal studies divided by the concentration to which humans are exposed in drinking water. For the DBP the margins of exposure are very large (e.g., 15,000 for dichloroacetic acid effects on cardiac development). In other words, based on the doses that produce adverse effects in rodents, the levels of DBP present in drinking water are not likely to be a major risk to human pregnancy outcome.
Can Hormonally-active Chemicals in the Environment Disrupt Development?

CHAPTER 26

Many hormones, including estrogen and androgen, regulate the expression of genes that play critical roles in guiding the development of organ systems in the embryo. For example, humans with a defect in the androgen receptor gene have androgen insensitivity syndrome; although they are genetically male, they look female, because androgens cannot activate the receptor to masculinize the reproductive tract.

Changes in either the amount or the timing of hormone exposure can lead to altered development. Hormonally-active chemicals were first shown to have profound effects on development in humans in the early 1970s, when a dramatic increase in vaginal adenocarcinoma, a previously rare cancer, was shown to be linked to fetal exposure. During the 1950s, the mothers of these young women had been given diethylstilbestrol (DES), a synthetic estrogen, in an effort to prevent miscarriage and preterm delivery.

Since then, many hormonally active chemicals have been demonstrated to cause abnormalities of the reproductive tract in experimental animals exposed during critical developmental periods. In Taiwan in 1979, many people consumed rice oil contaminated with high levels of polychlorinated biphenyls and dibenzofurans. The offspring of exposed women were smaller at birth and had delays in neurological development. Several more recent studies on PCBs have shown neurological effects in other populations exposed to lower amounts. Although we know that PCBs and related compounds can interact with various components of the endocrine system, the exact cause for the developmental disorders observed after these exposures is not known.

There is more certainty about the mechanisms of action of other chemicals. For example, in experimental animals, the DDT isomer o,p'-DDT binds to the estrogen receptors and feminizes hormonally sensitive tissues, while the DDT metabolite, p,p'-DDE, demasculinizes development by binding to, and inhibiting, the androgen receptor. In laboratory studies, other chemicals that have been implicated in disrupting development by endocrine-based mode of action include the fungicides vinclozolin, the plasticizers dibutylphthalate, a group of surfactants called alkylphenols, and 2,3,7,8-tetrachlorodibenzo-p-dioxin (commonly called dioxin or TCDD).

Chemicals that affect development though hormonal processes are variously called endocrine disruptors, endocrine modulators, or, simply, hormonally active substances. The International Programme on Chemical Safety, part of the World Health Organization, adopted the term endocrine disruptors, and defined them as “exogenous substances that alter function(s) of the endocrine system and consequently cause adverse health effects in an intact organism, or its progeny, or (sub)populations.” Worldwide attention to the question of whether endocrine disruption is responsible for a variety of adverse health effects in humans grew rapidly in response to a 1992 publication of the proceedings of a conference organized by Theo Colborn that concluded, “We are certain of the following...: A large number of man-made chemicals that have been released into the environment...have the potential to disrupt endocrine systems of animals, including humans.” Evidence supporting this hypothesis is derived from studies in wildlife living in contaminated environments, studies of livestock foraging on phytoestrogen-containing plants, laboratory animal studies, and reports of increasing rates of health effects in hormonally sensitive tissues, including tumors of the testes, breast, and prostate, birth defects that affect the reproductive tract (hypospadias and cryptorchidism), and diminished semen quality.

The most convincing evidence to support the endocrine disrupter hypothesis has been obtained in laboratory experiments. Female rodents exposed as embryos or fetuses to a sufficient dose of an estrogenic chemical typically develop accelerated puberty; males have reduced accessory sex gland weights, delayed preputial separation, and reduced sperm production. Anti-androgenic exposures affect primarily male offspring, causing reduced anogenital distance, hypospadias, retained nipples, reduced testes and accessory gland weights, and reduced sperm counts. Exposures that alter the function of the thyroid gland during development affect the central nervous system and sensory organs (e.g., hearing), which require appropriate levels of thyroid hormone for normal differentiation. In rodents, testis size is inversely proportional to the thyroid status in the neonate. In all three examples, it is clear that many of the effects of hormone disruption are not obvious at birth, but become apparent on functional level as the individual matures.

Organizations that have reviewed the evidence concerning the impact of endocrine disruptors in the environment on human health (see Selected Readings) have generally concluded that additional research is needed before cause-and-effect determinations can be made with any degree of confidence. The link between exposure to endocrine disruptors and effects in humans is largely based on the concurrent rise in the use of many chemicals with hormonal activity and the increase over time in certain health outcomes that have at least a partial endocrine basis. There is, however, a general lack of conclusive evidence that human development is affected by environmental levels of endocrine disrupting chemicals.

Nevertheless, the United States Congress was sufficiently concerned that it included in the Food Quality Protection Act of 1996 a provision that the US Environmental Protection Agency begin a program to screen and test chemicals for endocrine activity. When completed, this program will provide a much more comprehensive evaluation of the chemicals that can interfere with endocrine mediated processes. The latest information on the status of this requirement can be found at: http://www.epa.gov/ottopnto/otsproashes/index.htm.
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Suggested Reading


Do Assisted Reproductive Techniques Increase the Risk of Birth Defects?

CHAPTER 27

About 15% of couples meet the clinical definition of infertility by not becoming pregnant after trying for one year. In some cases, these couples are normal and just need more time, but in other cases, there is a problem for which medical attention may be helpful.

Causes of infertility affect three general features of the reproductive process:

• Normal ovum may not be ovulated, or ovum may not be ovulated normally.

• Normal sperm may not be introduced into the female genital tract or may not survive once they are introduced.

• There may be an obstruction in the genital tract, preventing the ovum and the sperm from reaching one another.

The following are some of many therapies in use for these abnormalities:

• Hormones or hormone-like compounds that induce ovulation.

• In vitro fertilization (IVF). This technique harvests ovum from the ovaries through a needle placed into the ovary through the vagina. The ovum are mixed with sperm in a laboratory dish. The resultant embryos are placed into the uterus through the cervix.

• Gamete intrafallopian transfer (GIFT) and Zygote intrafallopian transfer (ZIFT). These techniques are similar to IVF; however, in GIFT, the ovum and sperm meet in the fallopian tube, rather than a laboratory dish before fertilization. The fallopian tube is where the ovum and sperm would join under normal circumstances, but in GIFT, the ovum and the sperm are injected into the fallopian tube together, through a thin instrument inserted into the belly through a tiny incision. In ZIFT, the fertilized ovum, called a zygote, is placed into the fallopian tube. Thus, fertilization has occurred in the laboratory, but early development happens in the fallopian tube, which may be a more natural place for it to start out.

Photo: courtesy of Shady Grove Reproductive Science Center, Rockville, MD.
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- Intracytoplasmic sperm injection (ICSI). A spermatozoon obtained by masturba-
tion is injected directly into the ovum using a microscopically tiny pipette. The technique
is otherwise exactly like IVF. ICSI eases the job for the sperm by ferrying it across the
membrane that surrounds the ovum.

There is concern that so-called assisted reproductive techniques may increase the
risk of an abnormal pregnancy. These concerns are based on two possibilities: that the med-
ications or physical procedures could injure a normal gamete or embryo, or that an abnor-
gal gamete, ordinarily incapable of fertilization, will be helped to achieve fertilization and
will give rise to a child with birth defects.

The good news for couples using these techniques is that no
increase in birth defects has been proven to occur in the resulting children, although not all
researchers agree on this point. Experimental animal studies sup-
port the safety of these techniques; also, animal breeding programs
have used these assisted reproduc-
tive methods extensively for many
years without an apparent increase
in birth defects. The news is not
entirely reassuring, however. Even
if these technologies do not turn
out to increase birth defects, there
may be other reproductive conse-
quences.

Possibilities include:

Miscarriage

It has been suspected that some assisted reproductive techniques might increase mis-
carriages, but it is very difficult to determine this. Accurate miscarriage rates are almost
impossible to determine because many miscarriages occur so close to the time of an expect-
ed period that a woman would never have suspected that she was pregnant. Miscarriage
occurs in at least 30% of all pregnancies, but only about half of those pregnancies are recog-
nized. In a group of women undergoing fertility treatments, detailed monitoring of hormone
levels will catch even very early pregnancies, and an episode of bleeding will be correctly
identified as a miscarriage. So the reason that miscarriage rates among women undergoing
fertility treatments appear higher than in the general population may be because it is closer
to the true rate.

This argument notwithstanding, at least one ovulation inducing medication, called
clophemine, may increase the miscarriage rate slightly. Clomiphene stimulates maturation
of the oocyte in the ovary. During maturation of the oocyte granulosa cells that surround the
ovocyte produce primarily estrogen. After ovulation, these cells produce primarily proges-
tosterone. Both estrogen and progesterone prepare the lining of the uterus for implantation of
the fertilized egg. Clomiphene may alter the function of the granulosa cells so that hormone
production is inadequate to support implantation. Under these circumstances, a fertilized
egg might fail to implant or might implant poorly.

How often clomiphene causes early miscarriage due to faulty implantation is
unknown, but we do know that only half of women who ovulate in response to clomiphene
are recognized to be pregnant. Correcting ovulation with clomiphene clearly does not com-
pletely restore the reproductive system to normal.

Ectopic pregnancy

Sometimes an embryo implants outside the uterus, usually in the fallopian tube. In
early pregnancy, fingerlike projections called chorionic villi dig into the tissue where the
embryo is implanted, in order to set up the placenta. When a pregnancy occurs outside the
uterus, the invading villi can erode into blood vessels, causing bleeding that can be life-threat-
ening. Ectopic pregnancies may result from scarred fallopian tubes that trap the fertilized
ovum before it can pass freely into the uterus. It is not clear whether assisted reproductive
techniques increase the risk of ectopic pregnancies, or whether these techniques are used in
women who have a higher rate of tubal damage to begin with.

Chromosome abnormalities

Abnormal chromosome numbers occur commonly in early pregnancies. In pregnan-
cies that miscarry, about half have an abnormal number of chromosomes, which is presumed
to be the reason for the subsequent miscarriage. A naturally-conceived pregnancy that mis-
carries very early would not be checked for chromosome abnormalities. It has been noted
that chromosomal abnormalities appear to be more common in embryos created through
assisted reproductive techniques that involve fertilization in the laboratory, compared to the
general population; however, when embryos are placed into women without pre-testing, chro-
mosome abnormalities at birth are not increased. It appears likely, then, that chromosomal-
ly abnormal embryos are largely eliminated by natural processes.

Imprinting defects

Case reports have associated ICSI with an unusual group of genetic abnormalities
called imprinting defects. Imprinting refers to the methylation of certain parts of the DNA,
and can change the way the DNA is read. Imprinting defects include Angelman syndrome,
associated with severe mental retardation, ataxia, and seizures, or Beckwith-Wiedemann syn-
drome, associated with macrosomia (an abnormally large body), omphalocele (a defect in the
abdominal wall at the umbilicus) and other clinical features. DNA methylation occurs during
the development of sperm and oocytes, and ICSI techniques are believed by some
researchers to interfere with normal methylation. It is not known, however, whether imprint-
ing abnormalities are increased by assisted reproductive techniques, or whether they are
associated with the underlying infertility problems.

Multiple gestations

Hormone-like medications that induce ovulation may cause more than one egg to be
released in a cycle, resulting in twins, triplets, and higher-order multiple gestations. The rate
of twins in the general population is about 1%, but between 8 and 25% of pregnancies
...
achieved with ovulation induction result in twins. The rate of triplets in the general population is about 0.01%. With some ovulation induction medications, this rate can reach a few percent. Quadruplets and higher do not occur very often with optimal monitoring of the induced ovulation cycle. IVF, GIFT, and ZIFT have the potential for producing high order multiples (more than two babies at once) if more than one embryo or zygote is placed in the woman’s reproductive tract. Just one embryo may not implant successfully. If four embryos are placed into the uterus, the chances are improved that at least one of them will implant. If all of them happen to implant, the result is quadruplets. Many fertility doctors now recommend implanting no more than one or two embryos at a time, in order to avoid multiples of a higher order than twins.

Although having lots of babies at one time may sound like an efficient way for a couple to complete their family quickly, multiple gestations are associated with pregnancy risks, including increased birth defects. Perhaps the most important risk is prematurity, because the more babies there are in the uterus, the earlier in pregnancy the uterus will sense that it is overfull. Labor may start weeks before the babies are due. Prematurity is an important cause of death and disability in children arising from these pregnancies.

Suggested Reading


Is Herb use During Pregnancy a Reason for Concern?

CHAPTER 28

Women commonly use medicinal herbs during pregnancy. A tea made from raspberry leaf (Rubus idaeus) is used as a tonic throughout pregnancy in many cultures, and teas made from ginger (Zingiber officinale), peppermint (Mentha piperita), or spearmint (Mentha spicata) are common folk remedies for morning sickness. Some midwives incorporate herbs into their practices, perhaps including oral or topical oil of evening primrose (Oenothera biennis) to speed cervical ripening, or a mixture of blue cohosh (Caulophyllum thalictroides) and the unrelated black cohosh (Cimicifuga racemosa) to treat stalled labor.

The most common herb intentionally ingested by pregnant women is red raspberry leaf. There is no evidence that raspberry leaf causes morning sickness or labor, however, a placebo-controlled clinical trial of raspberry leaf extract administered from 32 weeks gestation until labor found no significant differences between groups in pregnancy outcomes.

Dried ginger root, commonly used for morning sickness, has been tested in clinical trials in doses up to 1 g/day and appears to be effective. No adverse pregnancy outcomes have been linked to ginger, and reproductive toxicology studies of ginger in rats have identified no problems.

Evening primrose oil, a source of gamma-linolenic acid, is more commonly topically applied than ingested orally during pregnancy; it is usually used in an effort to soften the cervix. In a clinical trial, orally ingested evening primrose oil from the 37th gestational week until birth did not shorten gestation or decrease the overall length of labor.

No adverse events have been associated with the herbs most commonly ingested during pregnancy, but not all herbs are benign. Licorice, an herb that is used medicinally but is consumed most commonly in the form of candy appears to shorten gestation in humans. A questionnaire study in Finland, where licorice candy consumption is so common that participants could be separated into low, medium, and high-exposure groups, found that heavy exposure to licorice more than doubled the risk of delivering a baby before 38 weeks. Isolated case reports have associated other herbs with adverse pregnancy outcomes.
Maternal use of blue cohosh in high doses for a month prior to birth was associated with heart attack and congestive heart failure in an infant. Severe hypoxic-ischemic symptoms were noted in the baby of a woman who took a mixture of blue cohosh and black cohosh (Cimicifuga racemosa) to induce labor. Blue cohosh rhizomes contain cimicifugin and cimicifugoside, chemicals that can constrict blood vessels and are toxic to the heart.

Lack of adequate regulation of herbal products in the United States complicates the use of herbs. Herbal products may contain different herbs than are stated on the label, be adulterated with other drugs, or be contaminated with heavy metals or bacteria, any of which could cause adverse effects during pregnancy.

Misidentification of an herb was associated with neonatal hirsutism in a baby born with hair on its forehead, pubic hair, swollen nipples and enlarged testes. The mother had taken a product that purportedly contained eleuthero, also called Siberian ginseng (Eleutherococcus senticosus) throughout pregnancy and during lactation. Subsequent analysis showed that the herb consumed was actually Chinese silk vine (Periploca sepium), which is contraindicated during pregnancy. Eleutherococcus senticosus caused no adverse effects on reproductive performance in a two-generation rat study.

The belief that herbs are natural and thus harmless is certainly inaccurate, but this view is not widely held among either pregnant women or midwives. Surveys of both groups have not revealed reckless use of problematic herbs. Some alarmist articles in the medical literature include lists of plants to avoid in pregnancy, but these lists are padded with plants never used in pregnancy, never used medicinally, or never ingested intentionally except by those bent on suicide. These lists are not helpful to clinicians.

On the other hand, the safety of many herbs commonly used during pregnancy has not been established. Even where some data in humans exist, no studies specifically designed to pick up adverse reproductive effects have been performed to date. There are many unknowns about the safety of herbs in pregnancy. The popularity of some herbs makes further research an important public health issue.

Selected Reading

Lessons from Thalidomide: Should Drugs Known to Harm the Fetus be Marketed?

The story of thalidomide is filled with tragic twists and turns. What other drug has gone from vilified deformity of babies to coveted black market item to approved pharmaceutical? Thalidomide was first synthesized in Germany in 1954 and was marketed there as a sedative beginning in 1957. The drug was subsequently sold in at least 46 countries throughout the world, but not in the US. A medical officer, Frances Conley, at the U.S. Food and Drug Administration, was concerned about some of the animal studies and delayed its approval; during this delay, the teratogenic effects of thalidomide were recognized.

An epidemic of phocomelia, a severe malformation that caused limbs to be sometimes no more than flipperlike appendages (see figure), was noted in the early 1960s. In 1961, two alert clinicians, Professor Widukind Lenz, a German human geneticist, and Dr. William McBride, an Australian obstetrician, associated a mother’s use of thalidomide early in pregnancy with the occurrence of these birth defects. The recognition that a medication that was reasonably safe for the mother could produce such severe malformations in her baby revolutionized the way that physicians, scientists, government regulatory agencies, and the public at large viewed the use of medicines by pregnant women.

After its devastating effect on fetal development was recognized, thalidomide was withdrawn from general distribution in the countries that had approved it. However, physicians could still obtain the drug by special request for individual patients who required treatment. Thalidomide has a variety of interesting therapeutic effects, and research on the drug revealed that it was helpful in treating a complication of leprosy called lepromatous leprosy and some complications of AIDS. In the 1980s and 1990s, the drug was sold on the black market to people with AIDS. On July 16, 1998, after tumultuous hearings on the subject, the US Food and Drug Administration approved an application for marketing thalidomide for use in moderate to severe skin manifestations of leprosy. Strict restrictions on prescribing the drug were implemented to prevent the accidental use of the drug by women.
at risk of pregnancy.

The FDA’s decision was very controversial. Some people argued that the FDA gave in to political pressure and that greater limitations on the use of thalidomide are necessary to prevent the birth of thalidomide babies in the US. This group believes that allowing physicians to prescribe thalidomide for conditions in which its efficacy has not been rigorously proven poses an unacceptable risk. The counterargument was made that the growing availability of thalidomide on the black market posed a much greater danger than restricted availability by prescription. Another argument holds that the unprecedented restrictions FDA placed on the use of thalidomide were an unjustifiable, paternalistic constraint of individual freedom. These critics argue that some of the FDA’s prescribing requirements were highly unreasonable; for example, requiring women who state that they are sexually abstinent to identify two methods of contraception while taking thalidomide.

New uses of thalidomide

The first serious disorder for which thalidomide was shown to be effective was erythema nodosum leprosum. In addition, thalidomide has been shown to be useful in the treatment of Behcet syndrome (recurrent painful mouth ulcers and other inflammatory symptoms) and severe mouth ulcers associated with HIV infections. Preliminary studies suggest that thalidomide may also be useful in the treatment of a variety of other conditions, including chronic graftversus-host disease (a debilitating disease of bone marrow transplant recipients), prostate cancer, advanced breast cancer, malignant melanoma, and Kaposi’s sarcoma (a vascular cancer often associated with AIDS).

The FDA approved marketing thalidomide only for treatment of erythema nodosum leprosum, a condition that affects no more than a few hundred patients in the US each year. However, now that the drug is available on the American market, thalidomide can be prescribed for many other, more common conditions.

Thalidomide was approved under a set of restrictions that are more stringent than those for any drug previously marketed in the U.S. These restrictions are designed to limit the risk of fetal exposure to thalidomide, provide positive confirmation of universal compliance with pregnancy avoidance measures, and eliminate underground dispensing of the drug, which poses an uncontrolled threat to pregnant women. The restrictions include:

- Registration of all prescribing physicians;
- Registration of all dispensing pharmacists;
- Patient education;
- Informed consent;
- Maintenance of a database on all patients for followup; and
- The ability to intervene if all components of the program are not followed in a particular case.

Fertile women can receive thalidomide under this program only if they use two methods of contraception and undergo mandatory pregnancy testing before and each month during treatment. Further information on FDA’s approval, restrictions on distribution, and patient information on thalidomide is available at www.fda.gov/der/news/thalinfo.

A Second Generation of Thalidomide Babies?

In practice, it is probably impossible to make thalidomide available without some risk of fetal damage. Providing thalidomide to patients who need it therefore requires balancing the benefits to adults against the risks to embryos. Issues of profit and social responsibility confound the situation further. If children with severe birth defects are born as a result of their mothers taking thalidomide during pregnancy, should the pharmaceutical company, the prescribing physician, the family, the FDA, or the public at large shoulder the cost? How many babies with thalidomide-induced birth defects will it take to justify a change in the marketing or distribution requirements for the drug?

Thalidomide is not the only teratogenic medication that is widely available by prescription. Maternal use during early pregnancy of isotretinoin, a drug given to treat severe acne, causes brain, eye, facial, and heart malformations in a developing embryo. Treatment during pregnancy with lithium, which is used for patients with manic-depressive illness, may cause heart malformations in an infant, and maternal treatment with valproic acid, a drug used to prevent seizures, can cause spina bifida. Treatment of pregnant women with several other medications occasionally causes birth defects in their babies, but none of these drugs causes severe problems as frequently as thalidomide.

Thalidomide’s pharmacologic mechanisms of action are not fully understood, and the mechanisms by which thalidomide interferes in embryonic development are unknown. We do not know whether it will be possible to design alternative medications that are equally effective but that can be used without endangering fetal development. The discovery of chemical variants of thalidomide that retain its efficacy but lack its teratogenic effect would be an effective resolution of the thalidomide problem.

Suggested Reading


Teratogens and Litigens: What are the Legal Considerations of Exposures to Chemicals and Physical Agents during Pregnancy?

CHAPTER 30

Birth defects, cancer, mental retardation, psychiatric diseases and hereditary disease have been viewed as stigmatizing by both ancient and modern societies. All of these conditions have at some time or another in history been viewed as an affliction or punishment. In ancient Babylon mothers who delivered a child with some types of birth defects were prosecuted, because it was believed that the malformed child was punishment for misbehavior. Historically, the father or mother of a malformed infant was open to ridicule, criticism, persecution, or criminal penalties. Folklore and superstition dominated the field, and malformations were attributed to evil spirits, virginity with animals, foul thoughts, or other immoral acts. Hundreds of trials involving blaming a party for the birth of a malformed infant have been recorded. Even today, emotional reactions to a child with birth defects continue to trump scientific plausibility in courtrooms.

Although parents and attorneys may believe that many infants are born with congenital anomalies or other defects caused by in utero exposure to drugs or medications, less than 1% of congenital malformations are due to drugs prescribed by physicians or chemicals in the environment. More importantly, serious congenital malformations are diagnosed in approximately 3% of live births in the general population, regardless of drug or chemical exposure. Since exposure to drugs, chemical and physical agents occur quite commonly, it is easy for the parents of an abnormal baby to assume that an exposure caused a bad outcome. Physicians or employers may be blamed, even if there is no evidence that the exposure at issue causes congenital anomalies.

The specialty of obstetrics and gynecology is particularly vulnerable to negligence lawsuits because obstetrician-gynecologists deliver most of the babies in North America. There has also been an increase in personal injury lawsuits alleging that congenital anomalies in the newborn are the result of medications taken during pregnancy, or exposures in the workplace. These suits are being directed at physicians, pharmaceutical companies, and employers.

Unlike criminal law, in which there must be strict proof of cause and effect (i.e., absence of a reasonable degree of doubt), civil law is based on preponderance of the evidence, i.e., more than 50% certainty. Although not validated by scientific evidence, drugs or medications may be declared as teratogens by the legal system, resulting in large monetary awards. Lawsuits have prompted some pharmaceutical companies to discontinue the manufacture of useful and harmlessly medications, or generally to advise patients that “this drug has not been proven safe for pregnant women and children.” These disclaimers by the pharmaceutical company place the entire burden of using the medication on the physician’s shoulders.

Both the drug industry and the U.S. Food and Drug Administration have adopted this approach. Drugs that are not proven to cause birth defects but that have been the subject of lawsuits are sometimes referred to as “litigens” or “teratogens.” The best-known example of a litigens is Bendectin (a combination of doxylamine, pyridoxine and thalidomide), marketed from 1956 to 1963 to alleviate nausea of pregnancy. Many lawsuits linked Bendectin with birth defects despite the fact that the increased risk of congenital anomalies was associated with its use. In fact, at the time of its removal from the market, numerous published studies showed that Bendectin did not increase the risk of birth defects.

Contraceptive spermicidal jelly was another agent characterized as a litigens. Significant scientific data indicate that spermicides do not cause congenital anomalies, yet spermicidal jelly was ruled a teratogen by the court, resulting in an 8.1 million verdict. It is interesting that both Bendectin and contraceptive jelly appeared in the courts because attorneys were able to locate physicians and scientists who were willing to testify “with a reasonable degree of certainty” that these agents were responsible for a child’s congenital malformation.

In summary, many consumers, as well as attorneys, believe that most congenital malformations are caused by an exposure during gestation. Counseling of the parents of a child with a birth defect requires a significant degree of knowledge and skill. Erroneous counseling by inexperienced health professionals is one of the reasons for frivolous litigation.

Suggested Reading


Glossary

A
Adduct – A compound formed by an addition reaction.
Allele – Alternative form of a gene.
Alpha fetoprotein – A protein produced by fetal tissues; an abnormally high amount of this protein in the amniotic fluid or maternal serum may signal a neural tube defect, or other abnormal opening in the fetus.
Amino acid – One of a group of organic compounds, containing an amino group and a carboxyl group, which are the building blocks of protein.
Aminoaceteosis – A procedure in which a small amount of amniotic fluid is removed and analyzed to detect genetic abnormalities of the unborn baby.
Amniotic cavity – The fluid-filled cavity that surrounds the developing embryo.
Anencephalus – Congenital absence of the upper part of the brain and the flat bones of the skull. See also Exencephaly.
Angiostatin converting enzyme inhibitors – A class of drugs that inhibit the proteolytic enzyme that converts angiostatin I into angiostatin II, used to treat high blood pressure.
Anaphylaxis – An abnormal number of chromosomes.
Anovia – Congenital absence of the ears.
Antimitotic – Inhibits cell division.
Apoptosis – Programmed cell death; a type of cell death in which the cell uses its own specialized machinery to kill itself.
Ataxia – A loss of voluntary muscle coordination.
ATPase – An enzyme that hydrolyzes ATP into ADP and phosphate.
Atrophy – Wasting.

B
Basal ganglia – Several large clusters of nerve cells, including the corpus striatum and the substantia nigra, deep in the brain below the cerebral hemispheres, participate in the regulation of motor performance.
Bioinformatics – The science of managing and analyzing large amounts of biological data using advanced computing techniques, especially in genomics.
Biotransformation – The conversion of a compound from one form to another by the actions of enzymes.
Blastocele – An early stage of the embryo; a fluid-filled cavity surrounded by a single cell layer, the trophoblast, and containing the inner cell mass, which will become the embryo.

C
Caspase – A member of a group of protease enzymes that mediate apoptosis.
Cataract – Partial or complete opacity of the lens of the eye; a common cause of blindness; curable by surgery.
Catecholamine – One of a group of hormones that are derivatives of catechol and affect the sympathetic nervous system. Epinephrine is an example.
Caudal – Towards the tail; inferior.
Central nervous system – The brain, spinal cord, olfactory bulbs, and optic nerves.
Cerebellar agenesis – Failure of development of the cerebellum.
Cerebellum – A part of the brain that is important for a number of cognitive and motor functions, including balance and coordination of movement; it is located behind and below the cerebrum.
Cerebral cortex – The layer of unmyelinated neurons (the gray matter) forming the cortex of the cerebrum.
Cerebral palsy – A condition resulting from brain damage before, at, or shortly after birth, marked by lack of muscle control. Also called spastic paralysis.
Cerebrospinal fluid – The fluid that fills the spaces in and around the brain and spinal cord.
Chondrocyte – A cartilage cell.
Chorioldendritic placenta – The placenta developed from the allantoid and chorion; establishes a nutritive and circulatory connection between the blood of the fetus and that of the mother.
Chorion - The outermost membrane enclosing the fetus.
Choriocarcinoma – Any of the tiny tufts from the chorion that contain fetal blood vessels and combine with the uterine tissue to form the placenta.
Chorioamnionitis – Inflammation of the chorion layer behind the retina.
Cleft palate – A congenital fissure along the midline of the hard palate.
CMV (cytomegalovirus) – A DNA virus; maternal infection during pregnancy can cause severe problems in the fetus.
CNS – Central nervous system.
Conceptus – An embryo or fetus.
Congenital – Present at birth.
Corpus callosum – A band of white neural tissue that joins the left and right hemispheres of the cerebrum.
Coxsackie virus – A group of viruses that occur in the human intestinal tract, causing a variety of diseases, including one resembling polioymyelitis but without paralysis.
Cranial – Relating to the cranium, or skull.
Cranial placodes – Thickening in the surface ectoderm of the embryo associated with the future eye and ear regions.
Craniopagus – Premature fusion of the cranial bones, usually present at or shortly after birth, and leading to
abnormal head shape.

Cretinism - A developmental disorder caused by deficiency of thyroid hormone, and characterized by severe mental retardation, sometimes resulting from maternal iodine deficiency.

Cyotrophoblast - The inner cellular layer of the trophoblast (trophoblast); part of the mammalian placenta.

D
Dental-skeletal ossification - A developmental delay in the formation of bones.

Developmental neurotoxicity - Adverse effects on the development of the nervous system.

Dextro - Farther or farthest from the center or trunk.

Down Syndrome - A disorder caused by an extra chromosome 21 (trisomy 21) and characterized by mental retardation and distinguishing physical features.

E
Ectoderm - The outermost layer in an embryo, which will develop into the skin and nervous system.

Encapheptasis - Inflammation of the brain.

Encaphalocele - Prolapse of brain tissue through a fissure in the skull.

Endocrine - Belonging to the endocrine glands or their secretions.

Endocytosis - A process by which extracellular materials are taken up by cells.

Endoderm - The innermost layer of an embryo that will develop into the lining of the digestive tract and respiratory tract.

Endometrium - The inner lining of the uterus that is shed during menstruation.

Embryo - The developing organism from the stage after the long axis appears until all major anatomical structures are present. In humans, this is from about the second week after fertilization until about the end of the seventh week after fertilization.

Epiblast - The primitive ectoderm of the early embryo.

Epidermis - The tightly-compact, thickest-tissue that conducts sperm from the testis to the vas deferens.

Epigenetic - Refers to changes in gene expression that are stable and potentially heritable, but do not result from changes in the DNA sequence.

Epoxide Hydrolase - An enzyme that modifies steroids by adding a molecule of water.

Epstein-Barr Virus - The herpes virus that causes infectious mononucleosis.

Estriol - A human estrogen.

Ethanol - Ethyl alcohol, the most common human intoxicant.

Exencephaly - An open brain resulting from failure of the neural tube to close. In humans this is followed by degen-

G
External genitalia - The external sex organs.

Extraembryonic matrix - A mixture of proteins on the outside of a cell that help the cell attach to a surface on which it can grow.

Extraembryonic membranes - Membranes that surround the embryo, e.g. the chorion, yolk sac, allantois and amnion.

F
Fetal alcohol syndrome - Characteristic facial changes, and impaired mental development, resulting from maternal alcohol intoxication during pregnancy.

Fetal hydantoin syndrome - Facial changes, shortened digits, and reduced intellectual capacity, associated with maternal ingestion of phenytoin, an antiepileptic drug.

Fetus - An unborn baby from the end of the 8th week after conception until birth.

Folic acid - A B vitamin involved in DNA synthesis that is essential for growth and reproduction.

Frontonasal dysplasia - Also known as midline cleft face syndrome; a rare craniofacial disorder.

G
Genetic - A sex cell. In higher animals, a sperm or an egg.

Gametogenesis - A stage of embryo development in which a two-layered embryo (ectoderm and endoderm) develops a third layer (mesoderm) through the movement of specific cells. The name comes from the embryos of lower animals in which the primitive stomach is created in this way.

Gene - The DNA segment that codes for a single protein. Each human chromosome contains many thousands of genes. It has recently been discovered that a single gene can code for a family of related proteins.

Genital folds - The embryonic structure that will differentiate into part of the penis in boys or the labia in girls.

Genome - All the genetic material in the chromosomes of an organism.

Genomics - The study of genes and their function.

Genotype - The genetic makeup of an individual, in contrast to phenotype, which is how the individual looks. In the case of a recessive gene, such as that for albinism, persons who carry one albino gene and one normal allele and persons who carry two normal alleles, have the same (normal) phenotype, but different genotypes.

Germ cells - Sperm and egg cells and their precursors.

GIFT (Gamete Intratubal Transfer) - A technique to treat infertility by placing an egg and sperm together into the fallopian tube. The embryo is expected to travel through the fallopian tube and implant in the uterus much as it would have had natural fertilization occurred.

Glucoma - A disease caused by increased pressure of the fluid within the eye, resulting in damage to the optic nerve; advanced disease is a common cause of blindness.

Glia cell - A kind of connective tissue cell in the brain and spinal cord. Glia cells provide structural support and nourishment to nerve cells.
Growth hormone releasing factor - A hormone made in the hypothalamus (a part of the base of the brain) that causes the pituitary to release growth hormone. Growth hormone is involved in growth and in energy metabolism.

**K**

Karyotype - A picture of an individual's chromosomes, arranged in order from largest to smallest, to make it easier to look for extra, missing, or rearranged chromosome material.

Ketonacidosis - Abnormally high levels of ketones and acids in the blood; may occur in a diabetic person who does not get enough insulin.

**L**

Leprosy - A disease caused by infection with the bacterium Mycobacterium leprae, often affecting the skin and nerves and causing body parts to become deformed.

L2AEL (Lowest Observed Adverse Effect Level) - In toxicology, the lowest tested dose that produces detectable damage.

Laminar meningomyelocele - A meningomyelocele in the lower back.

Lutamaining hormone - A chemical made by the pituitary gland at the base of the brain that is important in controlling egg maturation and in triggering ovulation.

Lymphocytosis - A disease caused by infection with the bacterium Borrelia burgdorferi, often affecting the skin, nervous system, heart, and joints.

Lysosome - A cell organelle that contains destructive enzymes.

**M**

Macrosomia - an abnormally large body or body part

Malformation - A structural defect due to abnormal development.

Meliosis - The kind of cell division used to make germ cells from body cells. The diploid number of chromosomes is reduced to a haploid number; for example, in humans with 46 chromosomes, meliosis results in germ cells with 23 chromosomes.

Membrane - A thin layer of tissue.

Mendelian inheritance - Passing of genetic traits from parents to offspring, as expected if they were determined by single genes.

Meningomyelocele - A birth defect following failure of the neural tube to close, resulting in protrusion of a sac of nerve tissue and its covering membranes. Muscle weakness or paralysis below the level of the defect is common.

Mesoderm - A middle layer of cells in the embryo, lying between the ectoderm and the endoderm.

Metabolism - The chemical processes occurring in the body.

Metalloproteinase - A protein in the body that binds metals such as zinc.

Metylation - Attachment of a methyl group.

Micronemy - A two dimensional array, typically on a glass, fiber, or silicon wafer, upon which hundreds of DNA or protein samples are deposited or synthesized in a high-density matrix, in a predetermined spatial order, allowing...
them to be made available to labelled probes in a high-throughput, parallel manner. Used to study how large numbers of genes interact with each other and how a cell’s regulatory networks control vast batteries of genes simultaneously.

Microarray culture - A laboratory technique in which disaggregated cells of an embryonic organ such as the brain are allowed to reaggregate in culture.

Mikrozia disease - A syndrome of mental deficiency and neurologic impairment caused by exposure of a fetus to methylmercury.

Mitochondria - Cellular organelles in which energy is generated.

Mitosis - A cell division that creates two genetically identical daughter cells by duplicating the genetic material of a parent cell.

Microcephaly - A small head.

Microphthalmia - A small eye.

Morphogen - A chemical message that signals a tissue to take shape.

Morphological - Pertaining to structure or form.

MSAFP - Maternal serum alpha-fetoprotein. Alpha-fetoprotein is a protein made in the fetus that normally leaks, in small amounts, into the mother’s circulation. If there is an abnormal opening in the fetus, such as a neural tube defect, larger amounts appear in the mother’s serum, providing a screening test for such fetal anomalies.

Multifactorial inheritance - The transmission of a trait from parents to offspring, determined by multiple genetic and environmental factors, each with a small effect.

Mutagen - An agent that increases the mutation rate.

Mutant - A gene altered by mutation, or an organism bearing such a gene.

Mutation - A permanent change in the genetic material.

Mycoplasm - A kind of minute microorganism that sometimes causes disease in humans.

Myelination - Coating of certain nerve fibers with a fatty sheath that enhances nerve signal transmission

Myocarditis - Inflammation of the heart muscle.

Myositis - Inflammation of muscle.

N

Necrosis - Abnormal cell or tissue death.

Neural - Pertaining to nerves.

Neural crest - A band of cells on either side of the neural tube from which cells migrate to the nervous system, face, skin, and heart.

Neural plate - A flat area in the middle of the early embryo that will roll up to make the neural tube.

Neural tube - The embryonic tube that becomes the brain and spinal cord.

Neurobehavioral - Pertaining to the function of the nervous system as it relates to behavior.

Neuroendocrine - Pertaining to the nervous and endocrine systems in anatomical or functional relationship.

Neuron - A nerve cell.

Neuropore - An opening at the front or back end of the neural tube before it completes closure.

Neuralization - The formation of the neural plate and its rolling up into the neural tube.

NOAEL (No Observed Adverse Effect Level) - In toxicology, the highest dose used that fails to produce evidence of damage.

Nucitride - One of the basic building blocks of DNA and RNA, consisting of a nitrogenous base, a phosphate group, and a sugar molecule.

O

Occipital meningocoele - An outpouching of the brain and its coverings (meninges) through a gap in the back of the skull, resulting from failure of the upper neural tube to close.

Omphalocoele - A hernia of abdominal contents through the umbilical cord. It results from failure of the normal withdrawal of the intestines from the cord.

Ovary - A female germ cell in the ovary; precursor of the ovum.

Organogenesis - Formation and development of organs.

Orficial cleft - The failure of the lip or palate to fuse properly.

P

Pan (secondary) - The roof of the mouth, consisting of the hard palate, soft palate, and uvula.

Parvovirus B19 - A DNA virus that causes erythema infectiosum (fifth disease).

Phenylketonuria - A recessively inherited condition in which metabolism of an amino acid, phenylalanine, is blocked; increased phenylalanine in the infant causes nerve and brain cell damage, and mental retardation.

Pheochromocytoma - Gross underdevelopment of limbs, but with hands and feet present; this and other severe malformations are associated with maternal thalidomide exposure.

Pleurosis - The engulfment of liquid droplets by a cell through minute invaginations of its membrane.

Placenta - The organ within the pregnant uterus through which the fetus derives its nourishment.

Pluripotent - Able to differentiate into a variety of cell types, e.g., the ovum and embryonic stem cells.

Polysaccharide - The presence of extra fingernails or toes.
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Polyomorphism – The occurrence of two or more genetically different forms of a gene in the same population, where the less frequent form has a frequency of 1% or more.

Polyadactyly – Polydactyly; combined with fusion or webbing of two or more fingers or toes.

Foreencephaly – A cystic cavity in the brain; may result from brain tissue destruction or maldevelopment.

Posterior – Situated at the back.

Post-implantation – Occurring after the early embryo embeds into the lining of the uterus.

Prader-Willi syndrome – A condition resulting from a deletion in chromosome 15; it is associated with short stature, mental retardation, small hands and feet, obesity, overeating, and underdeveloped gonads.

Pre-implantation – Occurring before the early embryo embeds in the lining of the uterus.

Progestosterone – A steroid hormone produced in the ovary by the corpus luteum, and essential for the maintenance of pregnancy.

Proteolysis – An enzyme catalyzing protein phosphorylation; often involved in the signal transduction pathways activated by stressors.

Proteomics – Analysis of protein expression and function.

Psychomotor retardation – Retardation of both mental and motor development.

Q

QSR – Quantitative structure activity relationship; the study of the relationship of the structure of a chemical to its biological effect.

R

Receptor – A cell component that combines with a drug or other substance and thereby alters cell function.

Retinoid – A group of compounds that includes many metabolites of vitamin A.

Retinopathy – Disease of the retina, the innermost layer of the eye that receives and transmits images.

S

Salmonella – Gram negative rod shaped motile bacteria, some of which cause intestinal inflammation.

SARS – Severe acute respiratory syndrome; a respiratory illness caused by a coronavirus.

Sertoli cells – Somatic cells within the seminiferous tubule which support germ cell development and form tight junctions to create the blood-testis barrier.

SNRAs – Short or small interfering RNA molecules that decrease the expression of a specific gene by degrading its messenger RNA.

Somatic – Pertaining to the body (excludes reproductive cells).

Somatoedin – A growth factor produced by the liver upon stimulation by somatotropin that acts directly on cartilage cells to stimulate skeletal growth.

Somatic – One of paired, segmental blocks of mesodermal cells on either side of the neural tube of the embryo which give rise to connective tissue, bone, muscle, and the dermis of the skin.

Spermatid – A haploid male germ cell resulting from the division of a spermatocyte; the precursors of sperm.

Spermatocyte – A male germ cell arising from the division of a spermatogonium during meiosis.

Spermatogonium – An undifferentiated male germ cell located close to the basement membrane of the seminiferous epithelium in the testis, gives rise to spermatocyte.

Spinous bulia – A defect in which part of the vertebral column is absent, allowing the spinal membranes and sometimes the spinal cord to protrude; a result of failure of the neural tube to close.

Steroid – One of the cholesterol-based hormones that regulate body functions.

Structure activity relationship – The relationships between chemical structure and biological activity.

Synectrothrophoblast – The multinuclear layer of trophoblast cells that invades the endometrium, during implantation.

Syphilis – A sexually transmitted infection caused by the bacterium Treponema pallidum.

T

Tay-Sachs disease – A recessively inherited disease, in which a deficiency of hexosaminidase A causes abnormal storage of a ganglioside. There is progressive mental deterioration and early death.

Teratogen – An agent that may induce abnormal embryo development when administered during pregnancy.

Teratology – The study of abnormal development.

Teratogenesis – The process by which birth defects arise.

Teratogenesis – The study of how genes and teratogens interact to cause birth defects.

Tetralogy of Fallot – A complex congenital heart disease involving four abnormalities: a ventricular septal defect, pulmonary stenosis, right ventricular hypertrophy, and an overriding aorta, which means that the aorta lies directly over the ventricular septal defect.

Thalidomide – A sedative, antinematode, and hypnotic drug; causing abnormalities of limbs, heart, ear, and craniofacial structures when taken by pregnant women.

TGF – Transforming growth factor alpha.

Threshold dose – The dose at which the agent has just begun to have an effect.

Thrombocytopenia – An abnormally low number of platelets in the blood.

Tourette syndrome – A disease caused by the protozoan Trypanosoma gondii. Infants infected during gestation may have hydrocephaly, microcephaly, encephalitis, cerebral palsy, mental retardation, loss of vision, deafness, and other problems.

Transcripts – Messenger RNAs that carry the genetic information from DNA to protein.
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Transgenic organism – An organism derived by the transfer of one or more genes from another organism.

Trimester – A period of three months; human pregnancy is divided into three trimesters.

Triple screen – A combination of three tests (levels of maternal serum alpha fetoprotein, estriol and human chorionic gonadotropin) which, if abnormal, indicate that the health of the fetus may be at risk.

Trisomy 18 - The presence of an extra chromosome 18, causing Edwards syndrome.

Trisomy 21 - The presence of an extra chromosome 21, causing Down syndrome.

Trophoblast – The outer layer of flattened cells forming the wall of the blastocyst.

U

Ultrasound – Sound waves of frequency higher than the range audible to the human ear used to delineate body structures by measuring the reflected waves.

Urogenital – Relating to the organs of the urinary and genital tracts.

V

Vas deferens – The tube that conveys sperm from the epididymis to the ejaculatory duct.

Ventricular septal defect – A defect in the wall dividing the two ventricles of the heart.

W

Whole embryo culture – A technique in which embryos undergoing organogenesis are cultured in vitro.

Williams syndrome – A syndrome resulting from a deletion in chromosome 7, which is associated with an elf-like face, mental retardation, short stature, and cardiac abnormalities.

X

X-linked – Refers to a gene that is located on one of the sex chromosomes, which is carried by the female in a double dose (XX) and the male in a single dose (XY).

Y

Yolk sac – A fluid-filled sac on the ventral side of the early embryo.

Z

ZIFT (Zygote Intracytoplasmic Transfer) – The transfer of an in vitro fertilized zygote into the fallopian tube.

Zygote – The fertilized ovum.