Reproductive/developmental toxicology

Study of the recurrence, causes, manifestations, and sequalae of adverse effects of exogenous agents on reproduction

Reproductive toxicity -Effects on sexual behavior and fertility in males and non-pregnant females

Developmental toxicity-abnormal structure or functional development following exposure of pregnant or lactating female

The contamination can come from:

- Food/packanging
- Environment
- Drugs

Environmental Chemical Exposure Associated with Reproductive Function	
Males	Females
Carbon disulfide	Anesthetic gas (OR personnel)
Chlordecone (Kepone)	Aniline
Chloroprene	Benzene
Dibromochloropropane (DBCP)	Carbon disulphide
Ethylene dibromide	Chloroprene
Ethylene oxide	Ethanol consumption
Ethanol consumption	Ethylene oxide
Glycol ethers	Glycol ethers
Hexane	Formaldehyde
Inorganic lead (smelter emissions)	Inorganic lead (smelter emissions)
Organic lead	Organic lead
Pesticides (occupational exposure)	Methyl mercury
Vinyl chloride	Pesticides (occupational exposure)
	Phthalic acid esters (PAEs)
	Polychlorinated biphenyls (PCBs)

Drugs that Are Gonadotoxic in Humans	
Males	Females
Bisulfan	Bisulfan
Chlorambucil	Chlorambucil
Cyclophosphamide	Cyclophosphamide
Nitrogen mustard	Nitrogen mustard
Adriamycin	
Corticosteriods	
Cystosine arabinoside	
Methotrexate	
Procarbazine	
Vincristine	
Vinblastine	

When?

Occurrence of adverse effects on the developing organism occurring anytime during the lifetime of the organism that may result from exposure to environmental agents prior to conception (either parent), during prenatal development, or postnatal until the time of puberty

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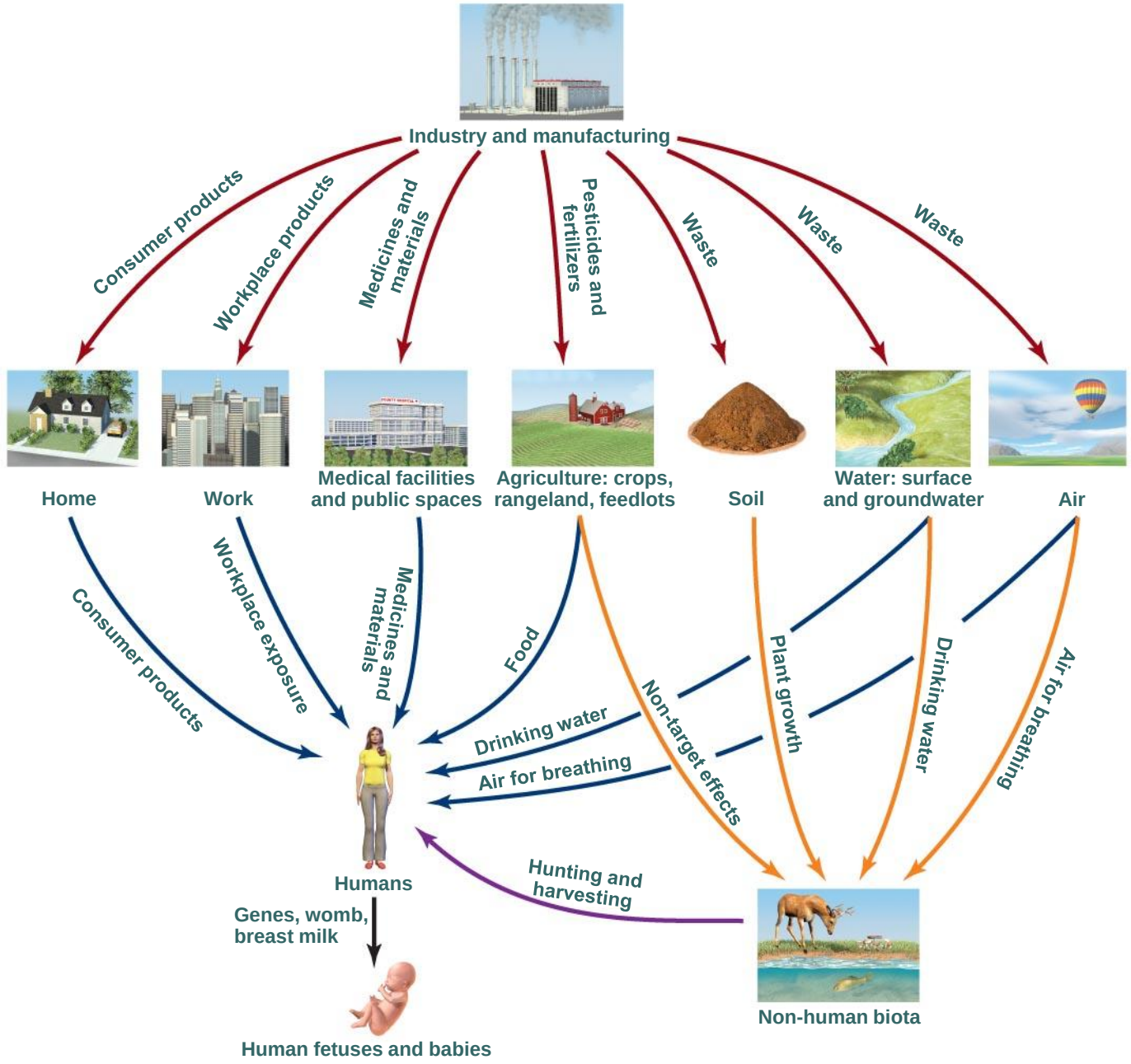


How Chemicals Affect Your Health?

- any adverse effect on any aspect of male or female sexual structure or function, or on the conceptus or on lactation, which would interfere with the production of development of normal offspring which could be reared to sexual maturity, capable in turn of reproducing the species.

Ancient Awareness

- Many ancient cultures had fertility goddess
- Malformations rich aspect of mythology (
- 6500 BC – Turkey - figurine of conjoined twins
- 4000-5000 BC – Australia drawings of twins
- 2000 BC - Tablet of Nineveh – describes 62 malformations and predicts the future

Historical Awareness

- 15th-16th centuries malformations caused by the devil, mother and child killed
- 1830's - Etienne Geoffroy Saint-Hilaire experimented with chicken eggs and created the scientific field of teratology
- 1900's began acceptance of malformations related to genetics
- 1940's - Josef Warkany – environmental factors affect rat development

Historical Events

- 1941 – Human malformations linked to rubella virus
- 1960's – Thalidomide (a sedative and anti-nausea drug) found to cause human malformations
- 1950's – Methylmercury recognized as developmental toxicant
- 1970's – Alcohol related to developmental effects – Fetal Alcohol Syndrome (FAS)

The chemicals can act during:

- Reproduction – issues associated with the egg and sperm
- Pregnancy – the critical environment of early development
- Development of the infant.

All life depends on reproduction and development.

Reproductive toxicology involves

- Effects on sexual behavior and fertility in males and non-pregnant females
- Effects on the offspring

Developmental toxicology involves

- Toxicity-abnormal structure or functional development following exposure of pregnant or lactating females

Teratogenicity → the production or induction of malformations especially of a developing embryo or fetus

Reproductive toxicology involves

Effects on sexual behavior and fertility in males and non-pregnant females

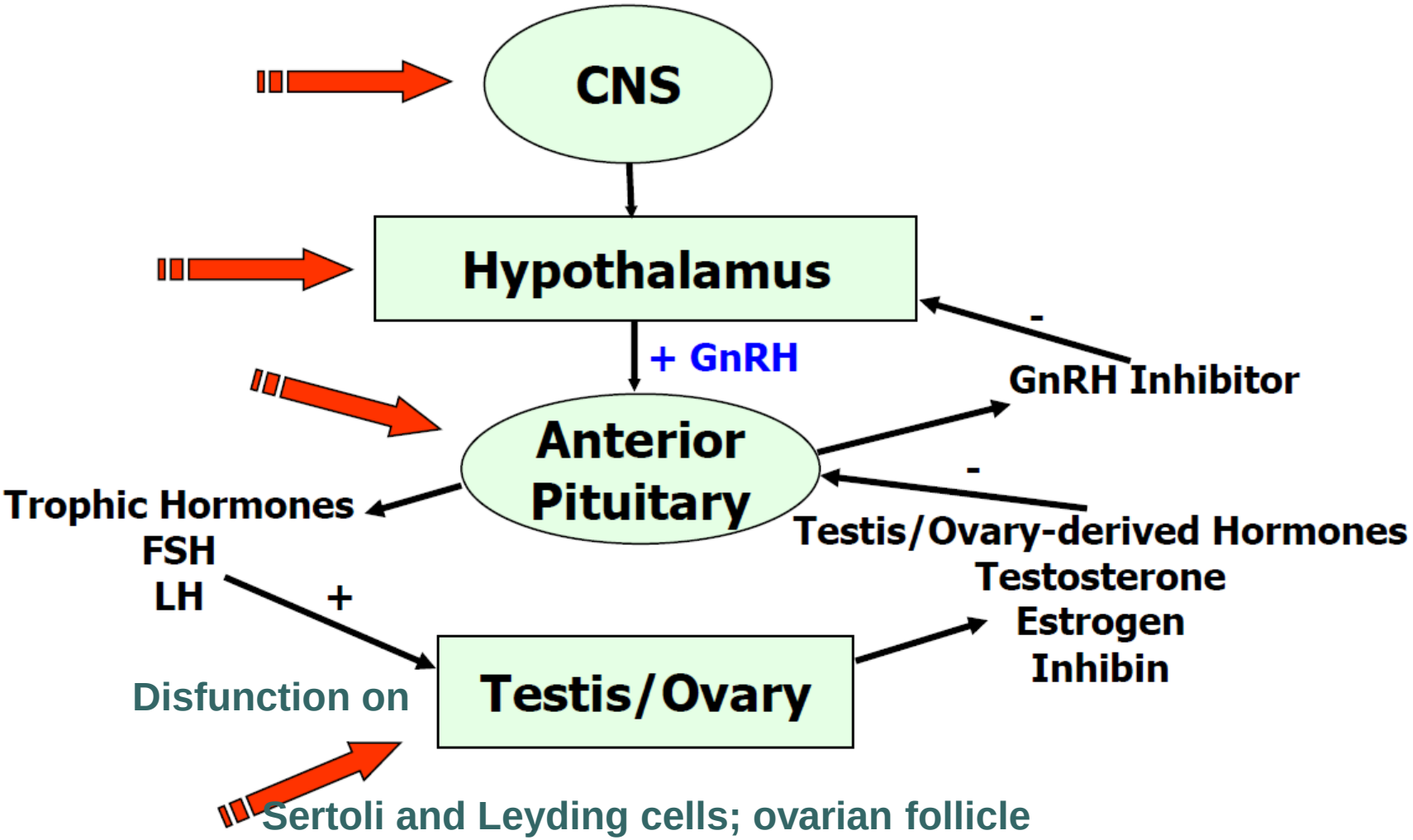
Effects on the offspring

The toxic effects on reproductive system are caused at very low concentrations of pollutants and generally these compound interfere with different ways

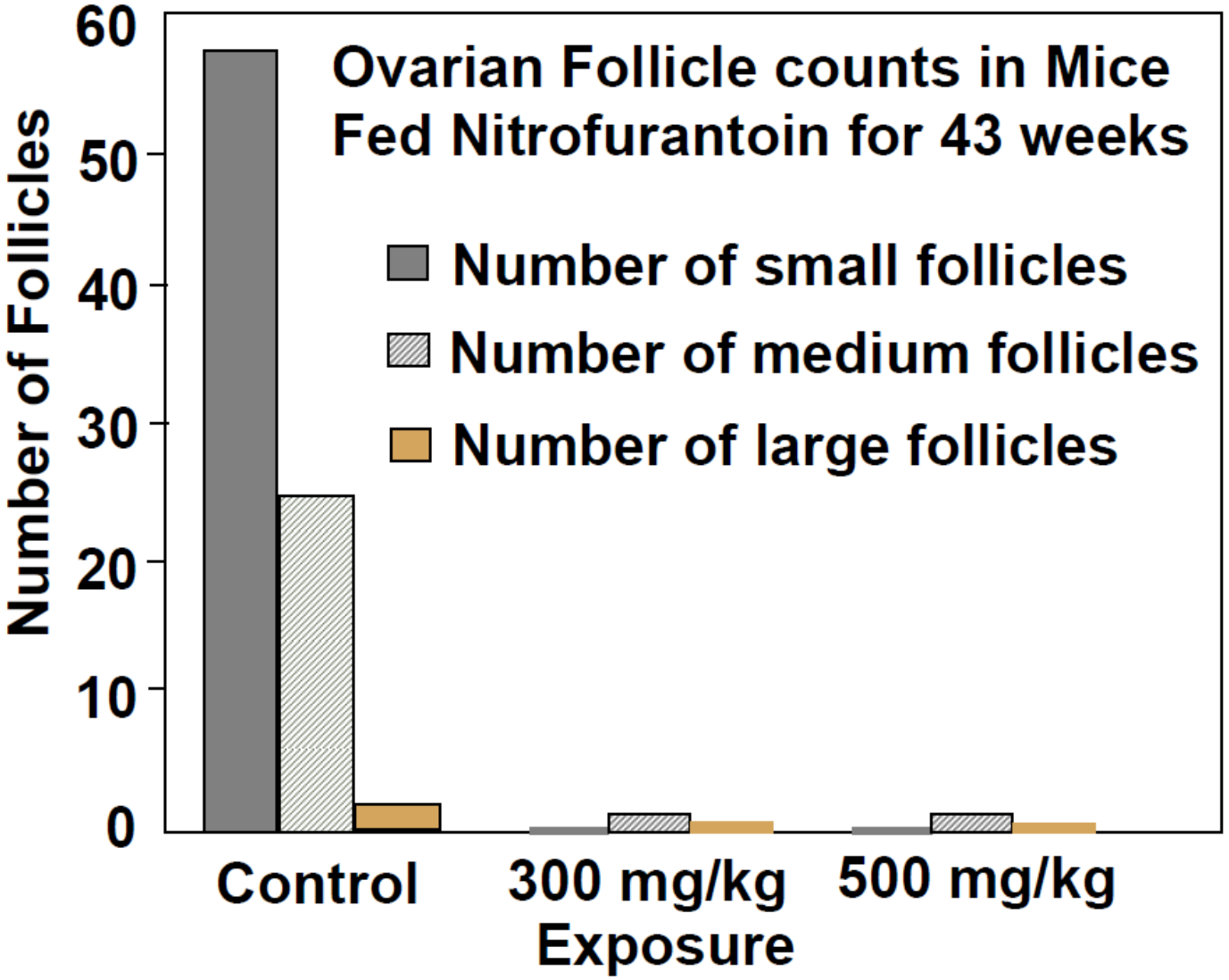
- 1. Agents that interfere with the activity of hormones at their receptors
  - Clomiphene and tamoxifen
  - Oral contraceptives
  - Xenoestrogens (genistein and other isoflavones in clover, soybeans, alfalfa, fruits and vegetables)
  - Pesticides (DDT, PCBs, dioxin, kepone)
- 2. Agents that interfere with steroid hormone metabolism
  - Inhibitors: danazol, ketoconazole, metyrapone, aromatase inhibitors
  - Inducers: methoxychlor, heptochlor, chlordane, DDT, and other organochlorine pesticides, dioxin

- 3. Agents that affect Sertoli cells in the testes
  - Dibromochloropropane
  - Monoethylhexylphthalate
  - n-Hexane
  - Tetrahydrocannabinol
- 4. Agents that affect Leydig cell function
  - Cadmium
  - Inhibitors of androgen synthesis
- 5. Agents that affect germ cell chromosomes/DNA
  - Mercury, lead, cadmium
  - Alkylating agents and other cytotoxic agents (cyclophosphamide, chlorambucil, busulfan, methotrexate, adriamycin, cytosine-arabinoside, vincristine, vinblastine)

Neuroendocrine targets for reproductive toxicology



Reproductive Tissue - Ovary



Common reproductive dysfunctions in human

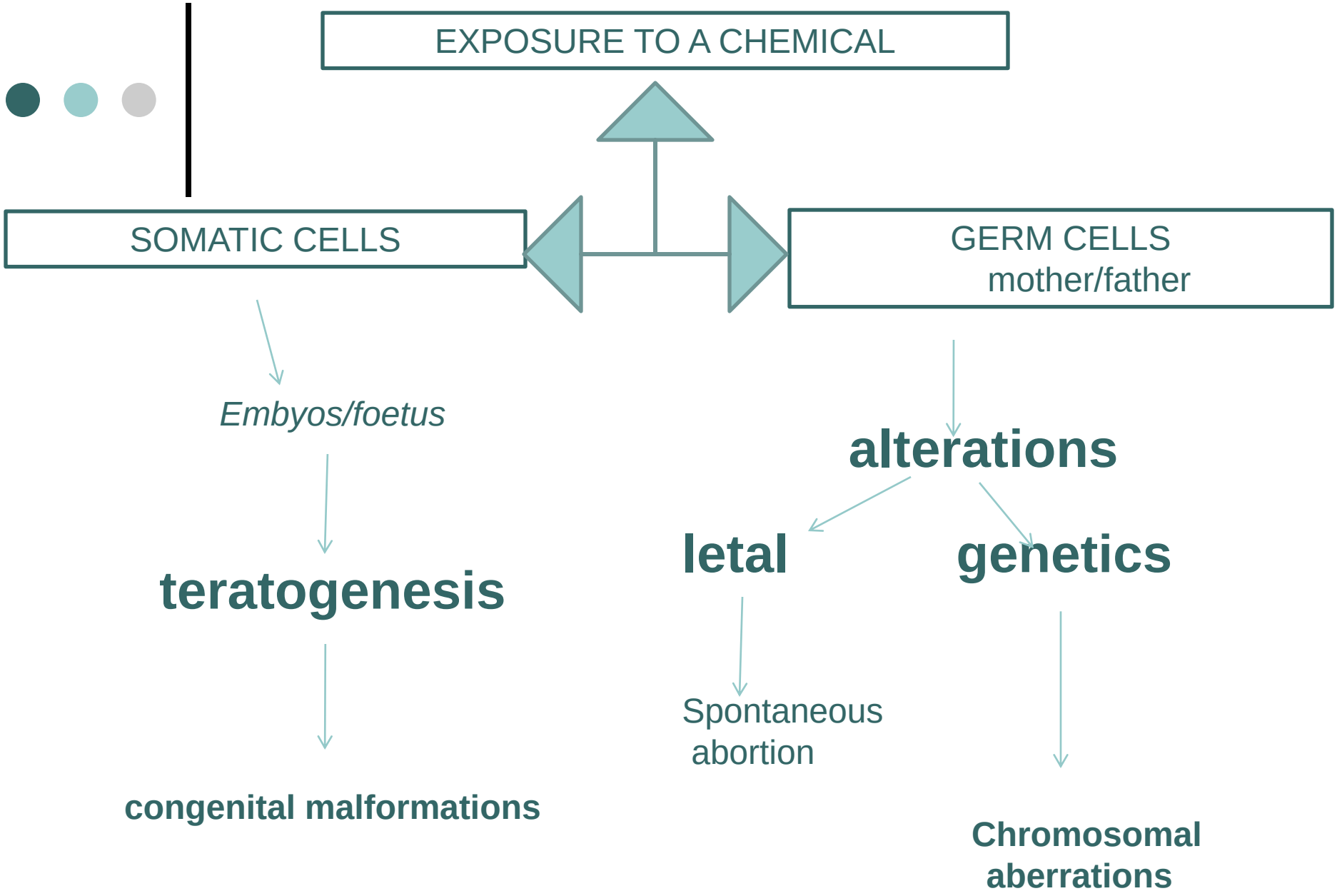
1. **Decrease of libido:** impotence
2. **Sperm aberrations :**

in morphology; decreased of number or motility.

3. **Subfecundity:** Aberrations in external genitalia;

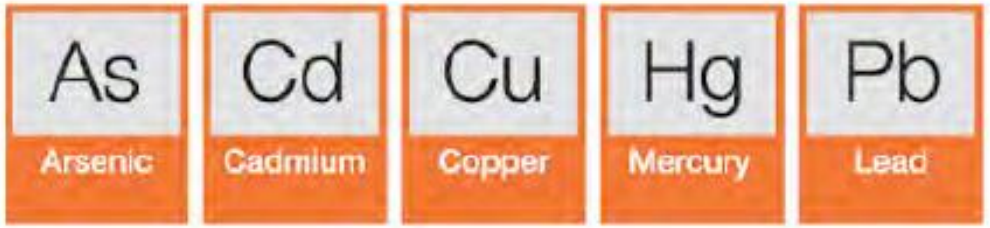
Infertility;
Amenorrhea;
Anovulatory cycles;
Delay in conception

4. **Illness during pregnancy:** hemorrhage
5. **Decreased birth weight**
6. **Early or late fetal loss**
7. **Chromosome abnormalities**
8. **Gestational age at delivery as prematurity or postmaturity**
9. **Intrapartum death**
10. **Birth defects**
11. **Infant death**


EXPOSURE TO A CHEMICAL
SOMATIC CELLS
GERM CELLS
mother/father
Embyos/foetus
teratogenesis
congenital malformations
alterations
letal
genetics
Spontaneous
abortion
Chromosomal
aberrations

Contaminants

1. Heavy metals



2 Methylmercury

3. Dioxins

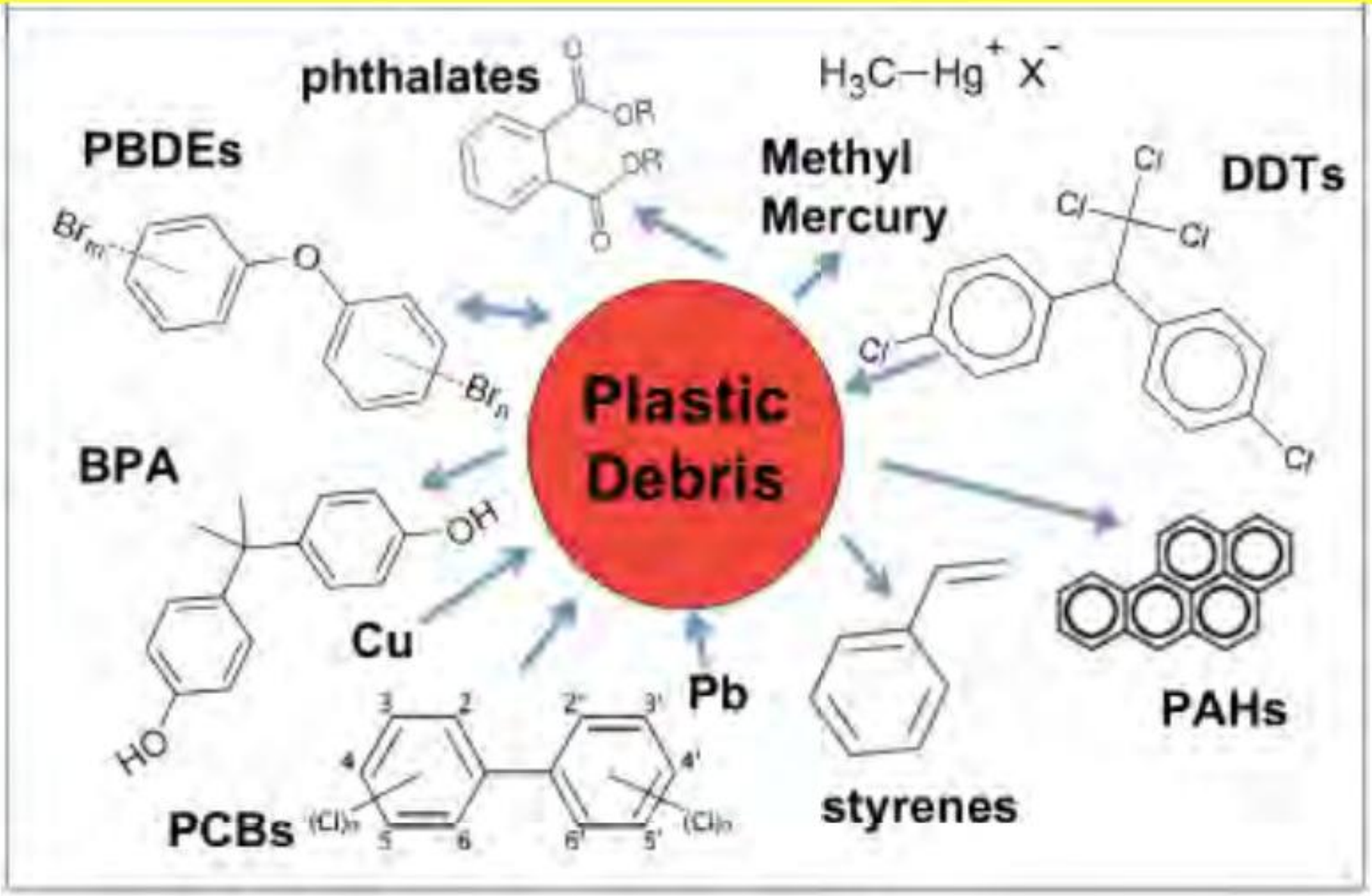
4. Mycotoxins

5. Pesticide residues

6. Packaging materials

7. Ionizing radiation

Cocktail of Chemical Contaminants



Developmental toxicology

The study of the effects of toxic poisoning on development of embryos. Developmental toxicology looks at substances the embryo may have been exposed in the uterus.

FACTS

- About 150,000 babies are born each year with birth defects.
- The parents of one out of every 28 babies receive the frightening news that their baby has a birth defect
- **There are over 4,000 known birth defects**
- **Birth defects are the leading cause of death in the first year of life.**

Teratology

- Teratology is the science that studies the causes, mechanisms, and patterns of abnormal development.
- Developmental disorders present at birth are called congenital anomalies, birth defect or congenital malformation.
- Congenital anomalies are of four clinically significant types: malformation, disruption, deformation and dysplasia.

Teratology - terms

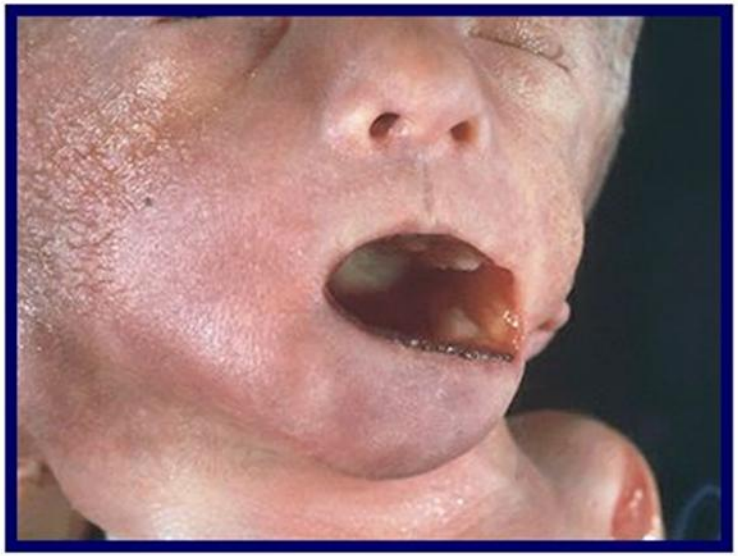
- **Malformation** is a primary structural defect resulting from a localized error of morphogenesis
- **Disruption** is specific abnormality that results from disruption of normal developmental processes. It depends on time not on agent
- **Deformation** is an alteration in shape / structure of previously normally formed part
- **Syndrome** is a recognized pattern of malformations with a given etiology.

Malformation

- Defect of morphogenesis in an organ or structure due to an intrinsically abnormal problem with formation, growth, or differentiation of an organ or structure
  - hypoplasia of an organ or structure (microtia), incomplete closure (NTDs, cleft palate), incomplete separation (syndactaly)

Disruption

- Defect resulting from a destructive breakdown of, or interference with, a normally developing structure resulting in death of cells or tissue destruction.
- May be secondary to mechanical forces, infections, or even vascular events.
  - Loss of digit due to amniotic band constriction, lack of normal limb development due to intrauterine vascular accident



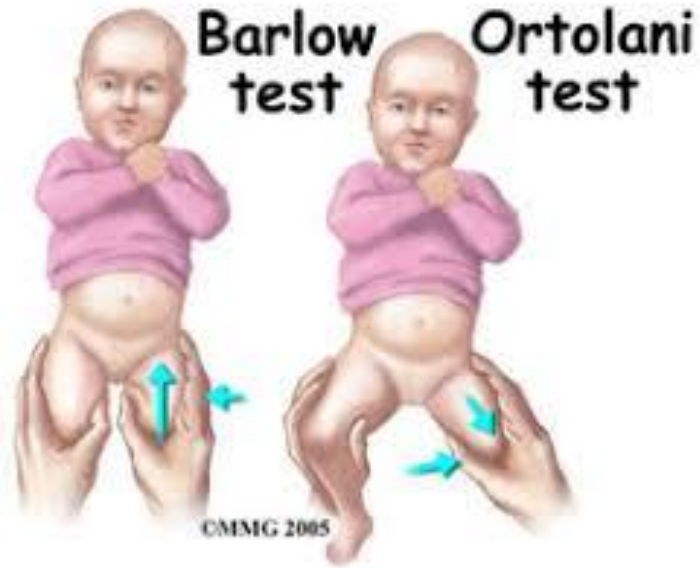
Disruption of lip formation due to amniotic bands

Deformations

- are due to an abnormal form or position of a body region caused by non-disruptive mechanical forces

Examples: clubfoot, congenital hip dislocation

Deformations often involve the musculoskeletal system and can be reversible postnatally



Histpric Events in Modern Teratology

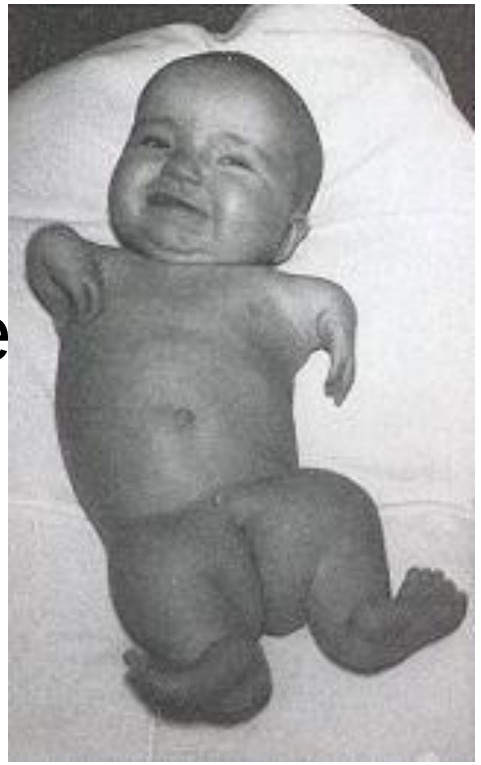
1800's	Experimental teratogenesis in chick embryos (St. Hillaire, Dareste)
1905	1st experimental developmental toxicity in a mammal – Embryolethality in kittens with x-irradiation (Tousey)
1921	1st experimental teratogenesis in a mammal – Limb defects from fatty diet (Zilva et al.)
1929	1st exogenously caused malformations in humans – microcephaly with pelvic x-rays (Goldstein and Murphy)
1935	Teratogenesis by dietary deficiency – Vitamin A deficiency in sows (Hale)

Histpric Events in Modern Teratology

1937	Masculinization of female mouse fetuses with androgen (Raynaud)
1941	Virus-caused malformations in humans reported – congenital defects from maternal rubella (Gregg)
1940s	Experimental teratogenesis in rats from deficiencies and toxicants (Warkany)
1952	1st reported human malformations by a drug – malformations in abortuses with aminopterin (Thiersch)
1959	1st reported human malformations by environmental chemical – methylmercury (Kitamura et al.)
1961	Thalidomide embryopathy (Lenz And McBride)
1960s	Beginnings of regulatory testing for developmental toxicity

The Damage

- 10,000-12,000 thalidomide babies
- 46 affected countries
- Drawn-out legal battle
- Disrupted families



The birth defects usually seen in **babies** exposed to **thalidomide** during **pregnancy** are : very short or missing arms and legs, missing parts of the ears, and deafness.

Symptom Pattern

- Phocomelia, flippers, or missing limbs
- Abnormal number of digits
- Missing/malformed eye(s) and ear(s)
- Anal atresia
- Brain damage/autism

Negative Family Dynamic

- Divorce
- Abandonment
- Suicide (rare, but occurred)
- Sibling Resentment
- Infanticide (Belgium case)

Drug development stages

1
Drugs are tested in test tubes and using computers to try to assess if they may be dangerous to humans.

2
Drugs are tested in two animal species to check that they are not toxic and to find the best dosage

3
Drugs are tested on a few healthy young men to ensure they are safe and to check for side effects.

4
Drugs are tested on patients to check that the drug works to treat the disease in people.

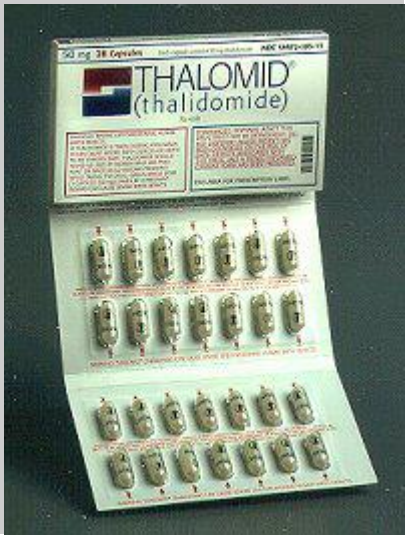
5
Drugs are tested on large numbers of patients. Half are given the new drug, half receive a placebo.

6
Drugs receive a license so doctors can prescribe them to patients

Thalidomide case

Thalidomide was tested safe in animals, however pregnant animals were not used because scientists did not, then, believe that drugs taken by the mother would have an effect on the unborn foetus. As a result the side effects were not predicted.

Thalidomide does show teratogenicity (causing birth defects) in many animals and the laws on drug testing were rewritten after the tragedy.



Thalidomide has recently been made available to doctors to treat certain cancers.

Dr Frances Kelsey worked at the Food and Drug Administration in America in the 1960s. Her first job was to approve thalidomide. Looking at the drug, she was concerned that there might be side effects, especially when reports from Europe suggested women were giving birth to children with physical defects.

Despite pressure from pharmaceutical companies, she refused to approve the new drug. When the connection between thalidomide and birth defects was proved, the drug was banned and America avoided the thalidomide tragedy. Dr Kelsey was heralded as a national hero.

Biological Characteristics of Thalidomide

- Inhibitory activity on tumour necrosis factor (TNF)-α production). Thalidomide decreases TNF-α production by accelerating the degradation of the encoding mRNA

  - Cell growth
  - Suppression of apoptosis
  - Metastasis
  - Immune and inflammatory responses

Current Uses

- Cancer treatment
  - Inhibit tumors directly
    - Drug will stop blood vessels from forming in and around tumors
  - Activate immune system
  - Anti-inflammatory
- Promising results seen in most intractable cancers

Birth defects

A birth defect is "any anomaly, **functional or structural,** that presents in **infancy or later in life and is caused by** events preceding birth, whether inherited, or acquired."

The placenta is an imperfect barrier

- Amnion sac originally thought of as a completely protected environment

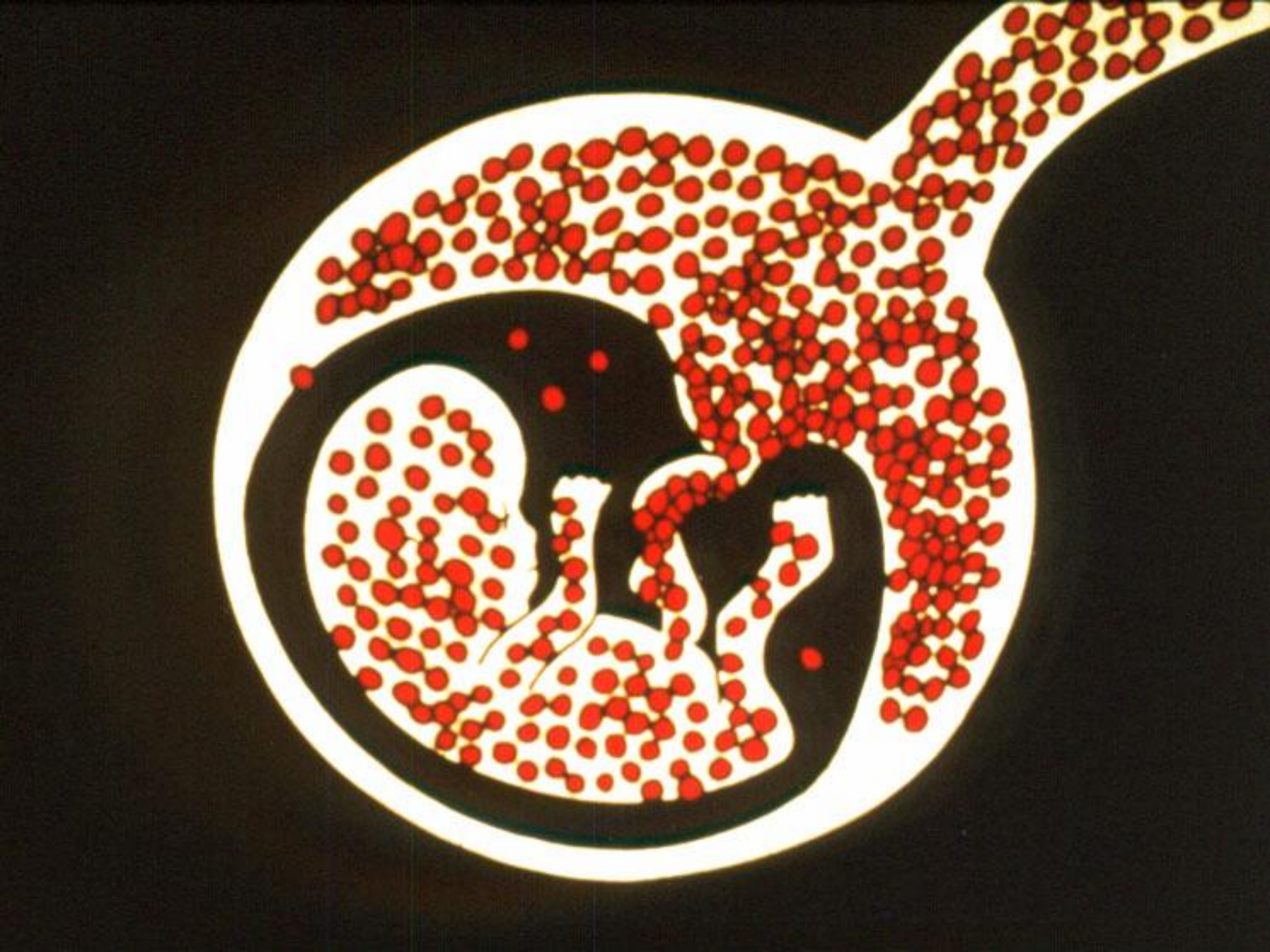
- Now realised that materials/chemicals can cross placenta to a greater or lesser degree

- Small non-polar molecules cross easily

- Large polar molecules cross poorly but rate still may be significant

    Example 1: Acetyl salicylate (aspirin), mostly charged at pH 7 but uncharged crosses placenta rapidly

    Example 2: Heparin, used as an anti-coagulant in pregnant women because size and polarity limit placental transfer. It replaces warfarin which cross readily and is a potent teratogen in first trimester (nasal hypoplasia)



Prenatal Alcohol Exposure

Alcohol is a teratogen

Effects have been demonstrated in animals and humans

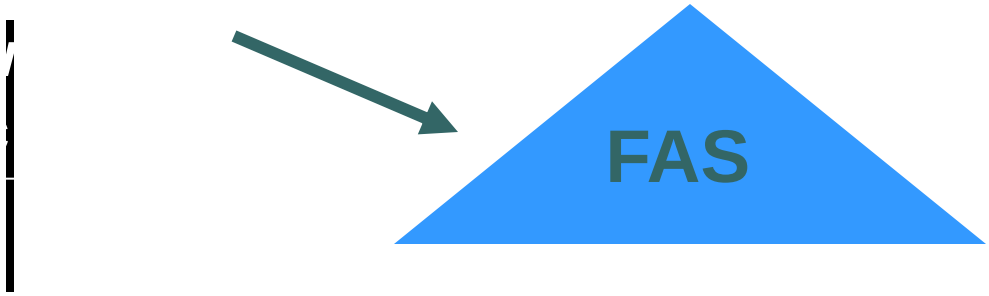
Neurobehavioral effects have been found to be more injurious and long-lasting than cocaine and other drugs abused prenatally.

Teratogenic Effects of Prenatal Alcohol Exposure

- Direct toxic effect of alcohol on cells
- Hypoxia (inadequate oxygenation of blood) due to impaired placental/fetal blood flow
- Effect on cell migration in the brain
- Effect on apoptosis (a natural process of programmed cell death)

Fetal Alcohol Syndrome

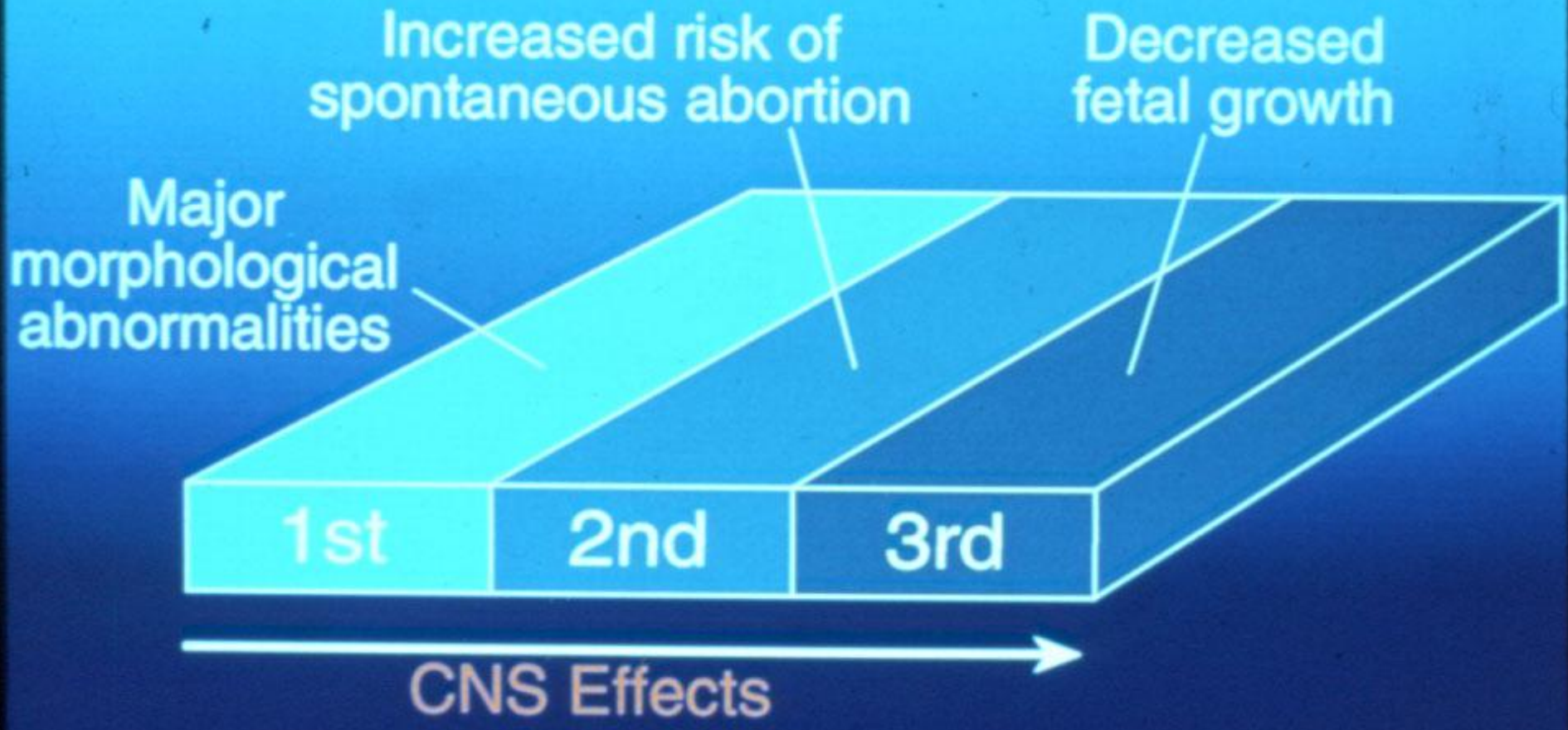
- A permanent birth defect caused by maternal alcohol use during pregnancy.
- The leading preventable cause of mental retardation in the Western world.
- Annually: 40,000 infants born with FASD (more common than Muscular Dystrophy, Cystic Fibrosis, Downs Syndrome and Spina Bifida combined).

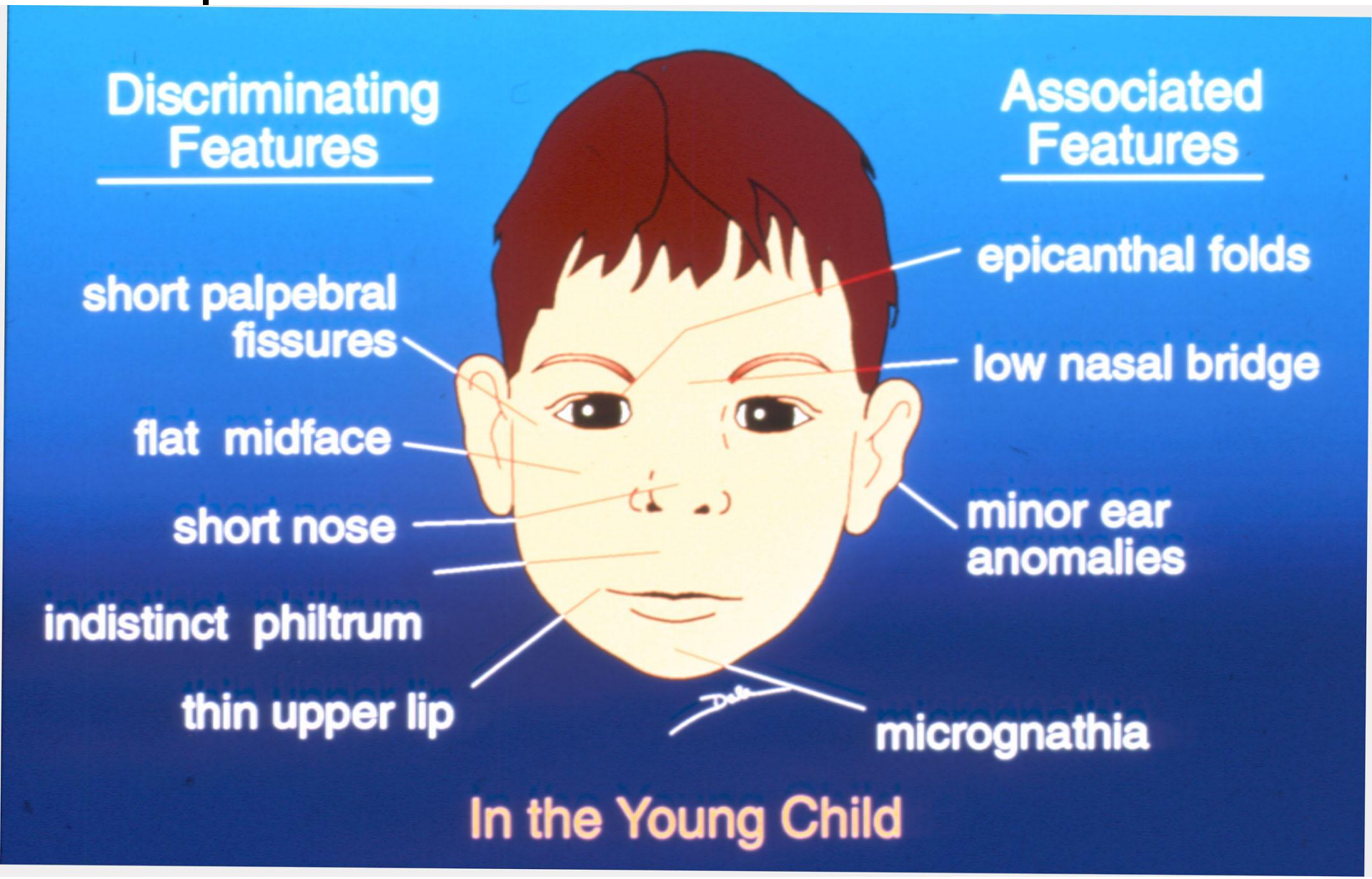


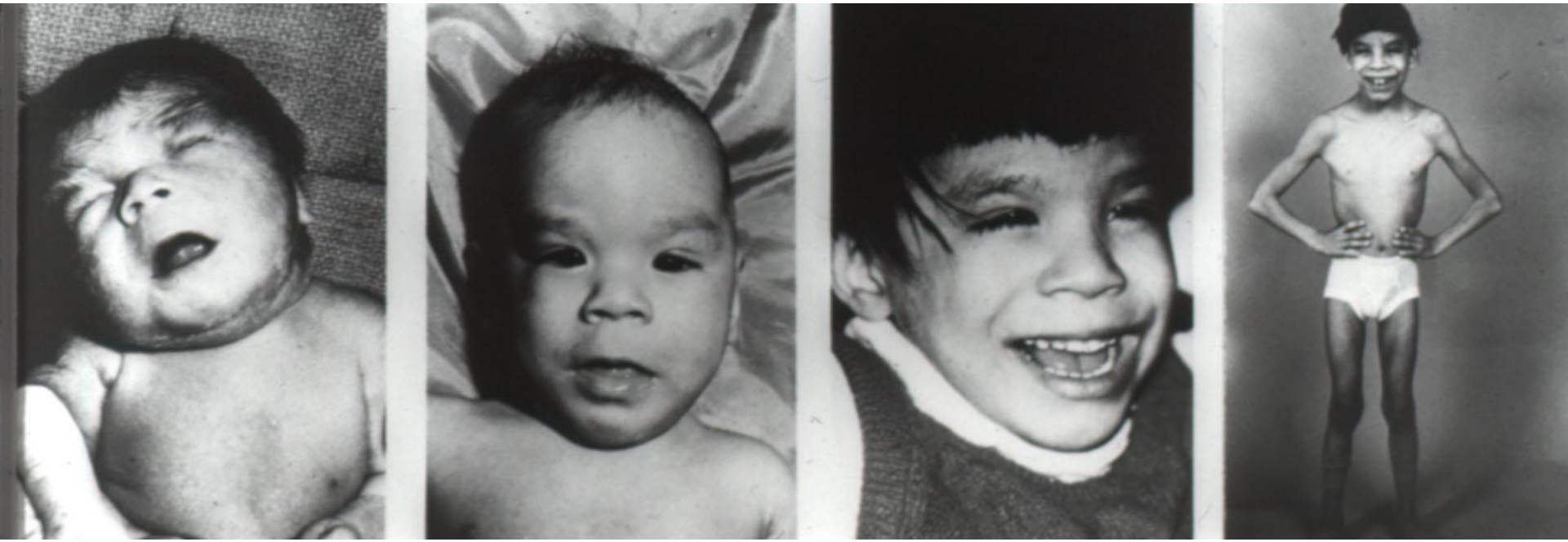
Central Nervous System Dysfunction
Organic Brain Damage

- Hyperactivity, attention deficits
- Intellectual deficits, learning disorders
- Problems with memory, language & judgment
- Developmental delay, microcephaly
- Fine & gross motor problems, seizure disorder
- Mental retardation, structural brain damage

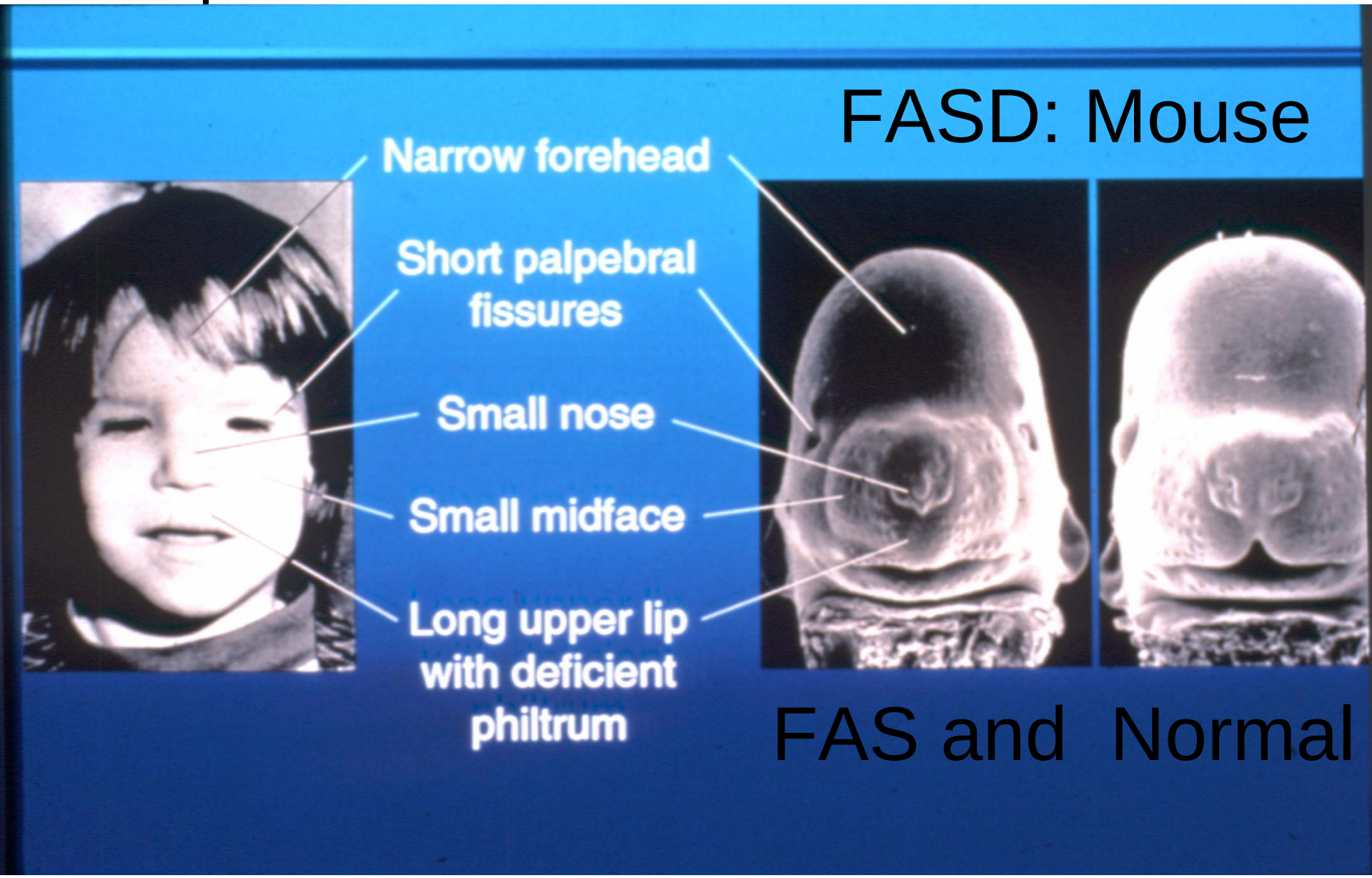
Major Effects of Ethanol by Trimester of Pregnancy



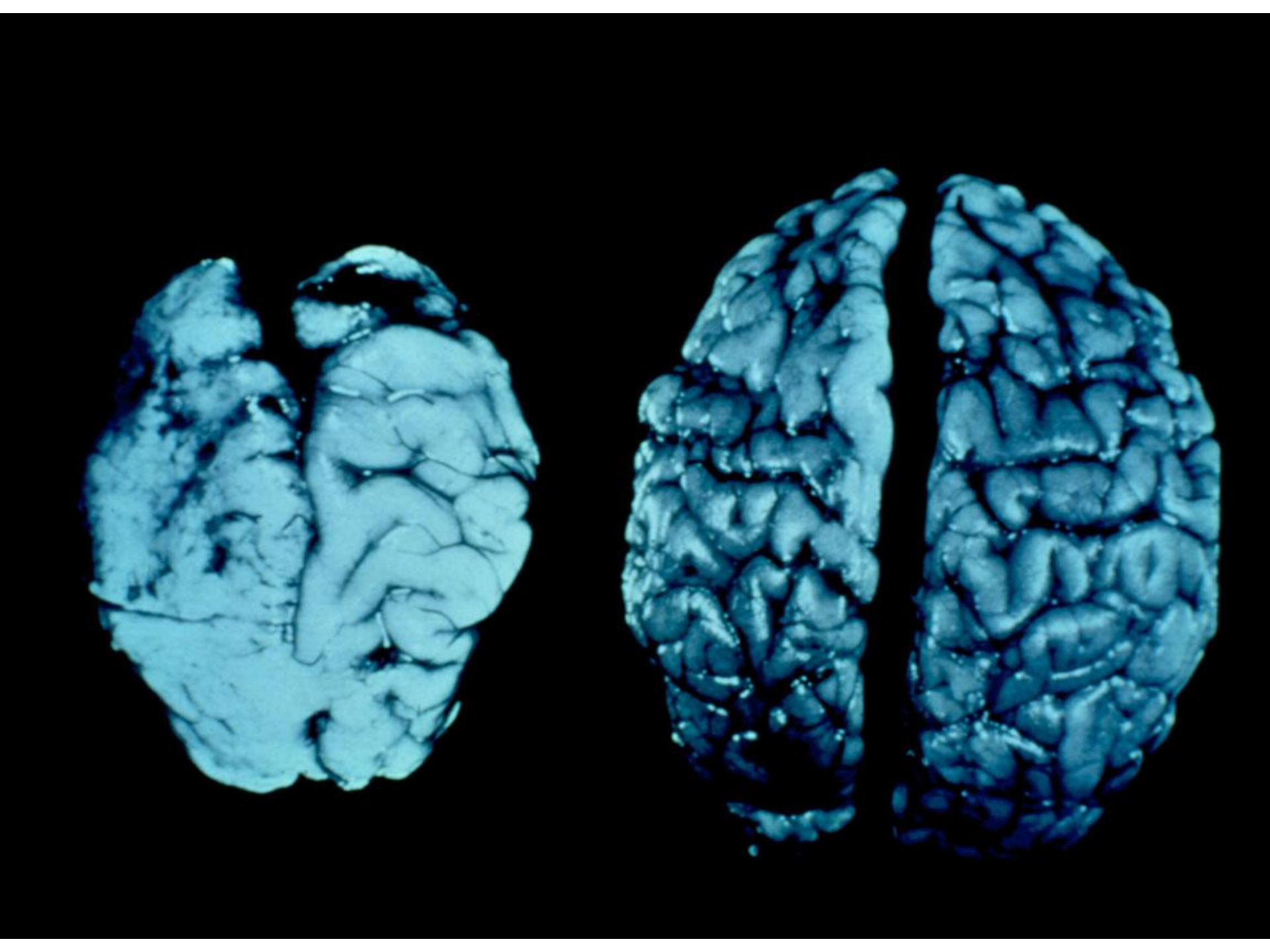

Discriminating Features
short palpebral fissures
flat midface
short nose
indistinct philtrum
thin upper lip
Associated Features
epicanthal folds
low nasal bridge
minor ear anomalies
micrognathia
In the Young Child

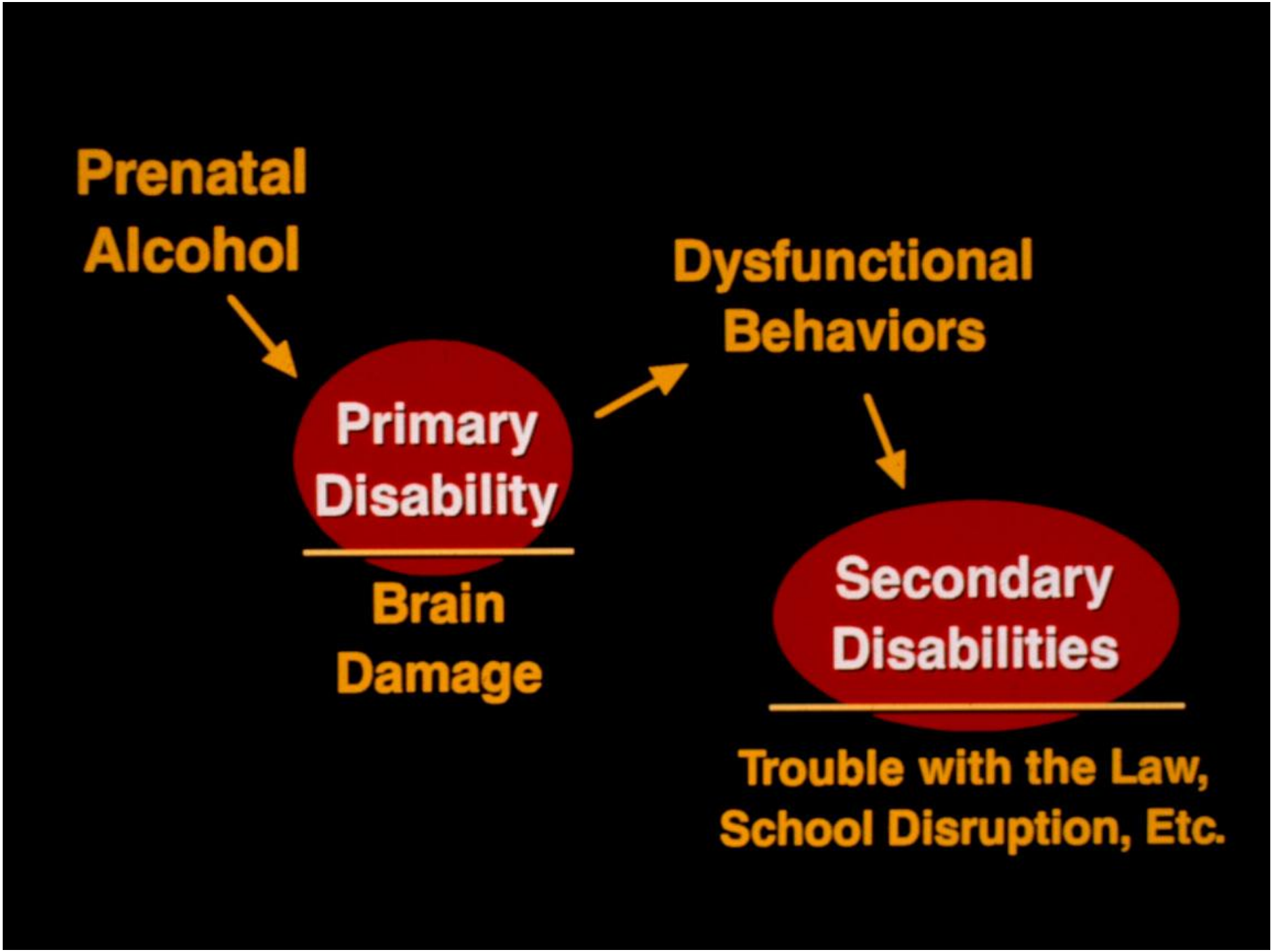


FASD: Mouse



FAS and Normal




Prenatal
Alcohol
Primary
Disability
Brain
Damage
Dysfunctional
Behaviors
Secondary
Disabilities
Trouble with the Law,
School Disruption, Etc.

FASD: Clinical Implications

Poor judgment …… Easily victimized

Attention deficits …… Unfocused / distractible

Arithmetic disability ….. Can't handle money

Memory problems ….. Doesn't learn from experience

Difficulty abstracting …. Doesn't understand consequences

Disoriented in …… Fails to perceive social signals time and space

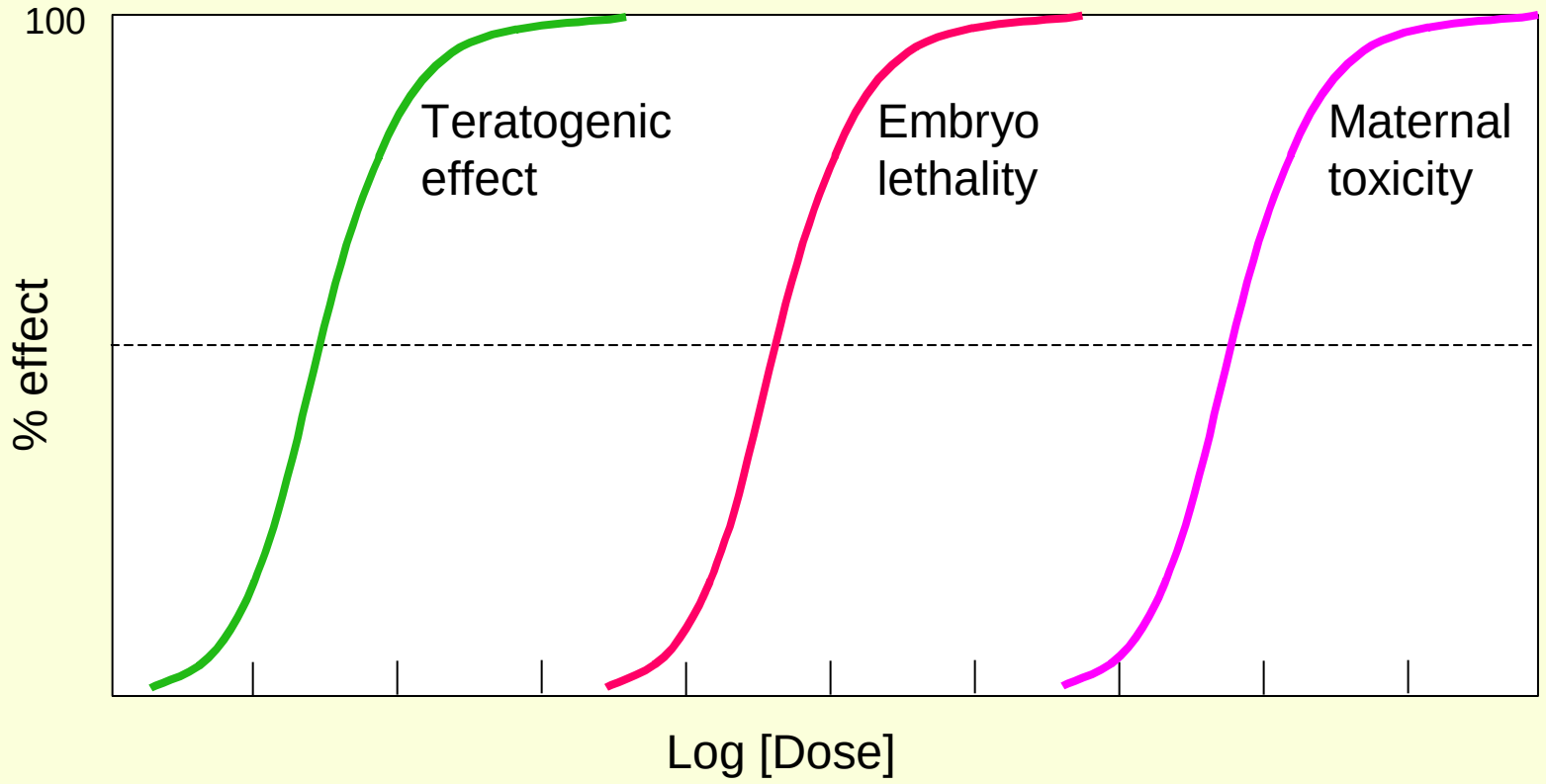
Poor frustration …….. Quick to anger tolerance

If I'm Pregnant, Can I …

…Have a beer?
The Centers for Disease Control says "no level of alcohol…has been determined safe," but some doctors feel limited drinking – no more than a pint a day, suggests Dr. Gibb – after the first trimester is okay.

- People Magazine, April 17, 2006, pp 102-107

Embryos can be vulnerable to low concentrations of teratogen



Footnotes:

1. Teratogens usually have a general toxicity
2. But: distinguish between general toxicity and specific formation of defects
3. Maternal toxicity can be much lower. In some cases (eg thalidomide in humans), there is no maternal toxicity
4. Teratogenicity can be very variable in different mammals

Teratogen

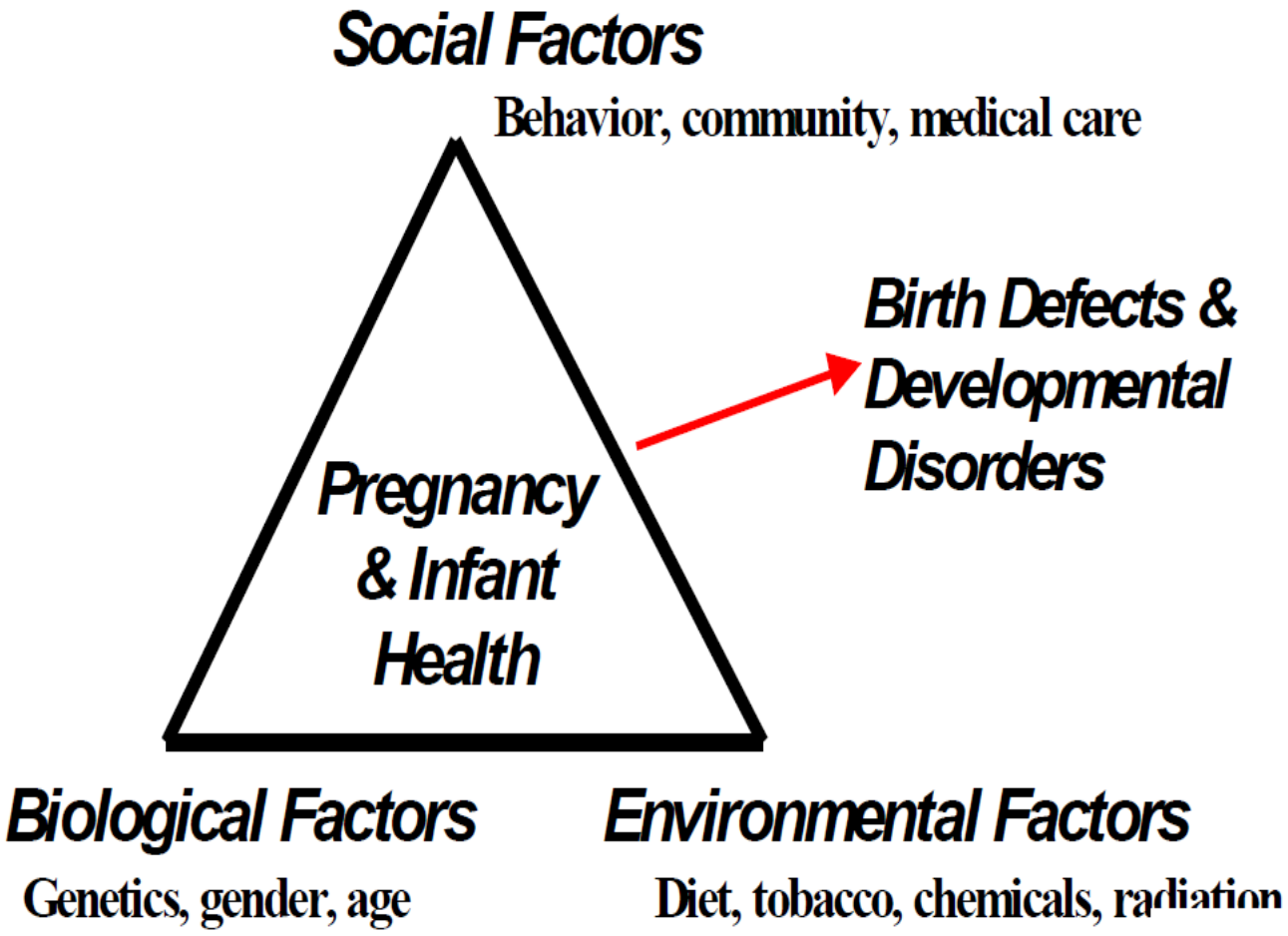
- Act during a critical narrow period of development
- Often a small concentration sufficient to cause damage
- Variable effect in different species*

** - This makes the use of model animals for testing teratogencity a problem (some laboratory rodent strains were completely resistant to massive doses of thalidomide).*

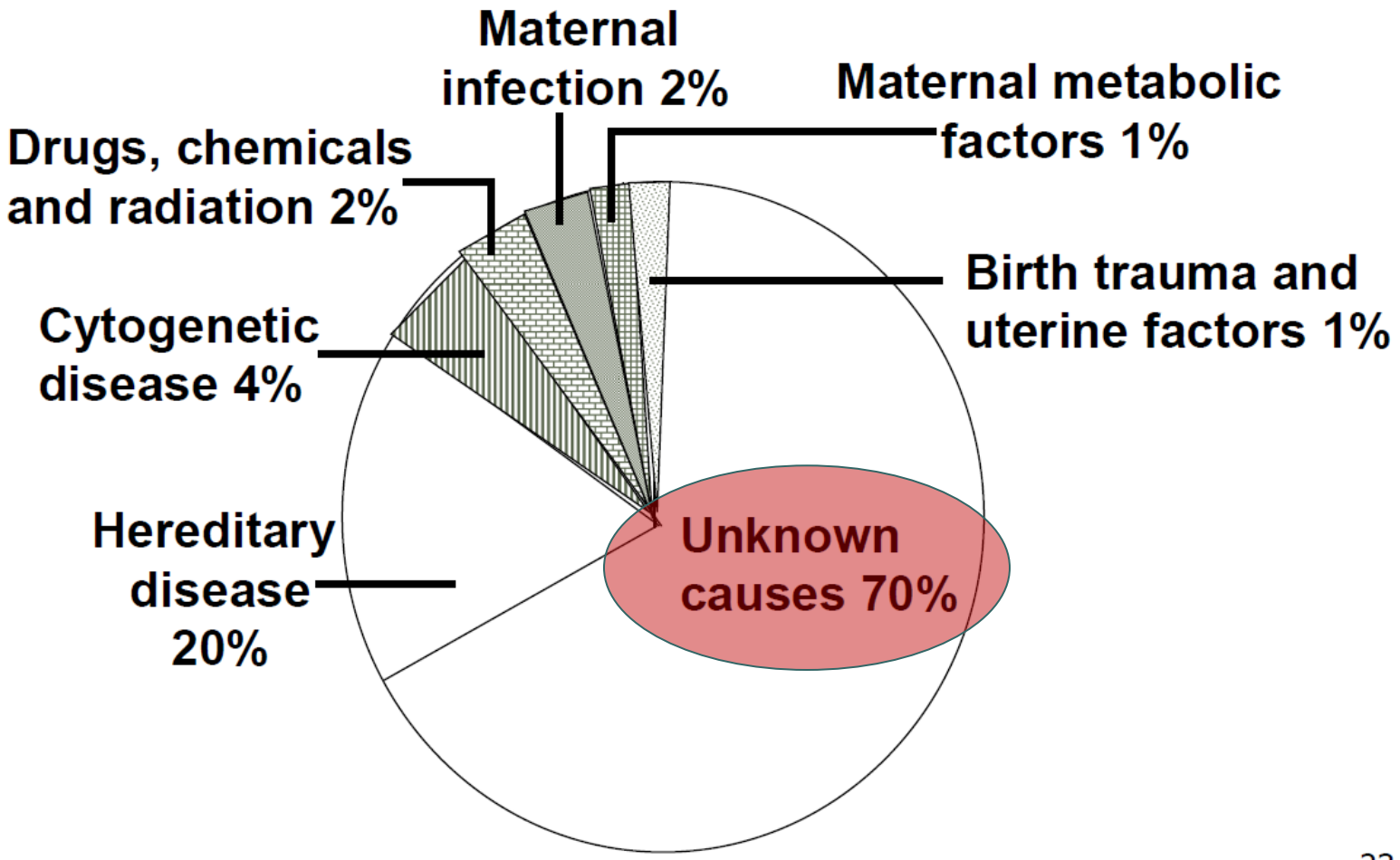
Birth defects

- 3% of all live-born infants have a major anomaly
- Additional anomalies are detected during postnatal live – about 6% at 2 year-olds, 8% in 5year-olds, other 2% later
- Single minor anomalies are present in about 14% of newborns
- Major anomalies are more common in early embryos (up to 15%) than they are in newborns (3%). Most severely malformed embryos are spontaneously aborted during first 6 to 8 weeks.

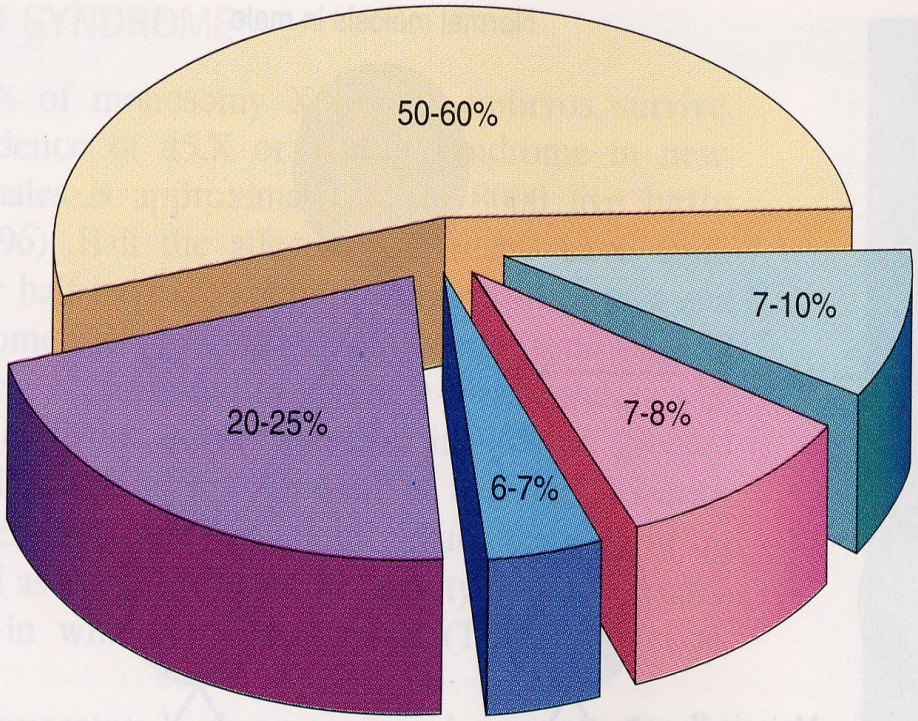
Causes of Birth Defects



Causes of Birth Defects



Causes of congenital anomalies



Genetic and environmental factors

Unknown etiology

Multifactorial inheritance

Chromosomal abnormalities

Mutant genes

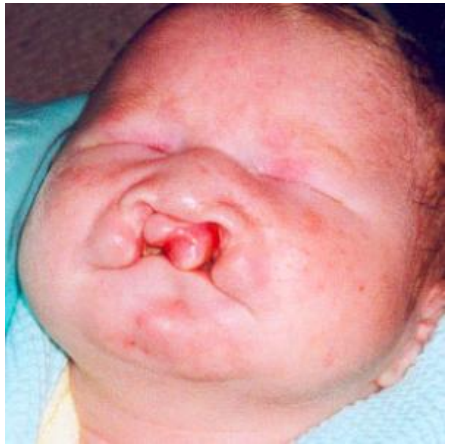
Environmental agents

Figure 9–1. Graphic illustration of the causes of human congenital anomalies. Note that the causes of most anomalies are unknown and that 20 to 25% of them are caused by a combination of genetic and environmental factors (multifactorial inheritance).

Anomalies caused by genetic factors

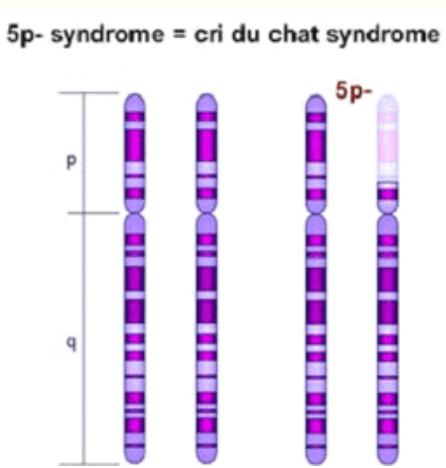
- Chromosomal aberrations are common and are present in 6 to 7% of zygotes – (result =abort)
- **Numerical chromosomal abnormalities** – usually non-disjunction- error in cell division

  Down syndrom (21) Edwards (18) Patau (13) Turner (X0), Klinenfelter (XXY)



Anomalies caused by genetic factors

- **Structural chromosomal abnormalities** – chromosome breaks = translocation, deletion (cri du chat syndrome), duplication, inversion.
- **Mutant genes** – achondroplasia, fragile-X syndrome



Anomalies caused by environmental factors

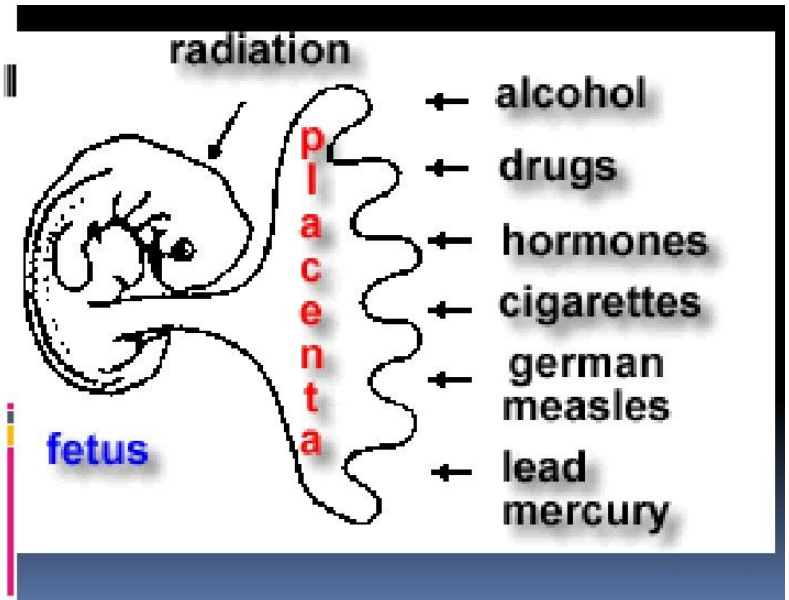
- **Teratogens** are exogeneous agents that may cause developmental defects:
- *Drugs* (warfarin, valproic acid, phenytoin, vitamin A, thalidomide, cytostatic drugs – cyclophosphamide, lithium carbonate)
- *Chemicals* (PCBs, methylmercury, alcohols)
- *Infections* (rubella, cytomegalovirus, herpes, toxoplasma, syphilis)
- *Ionizing radiation* (RTG)
- *Maternal factors* (diabetes mellitus, hyperthermia, phenylketonuria, hyper-/hypo-thyreosis)

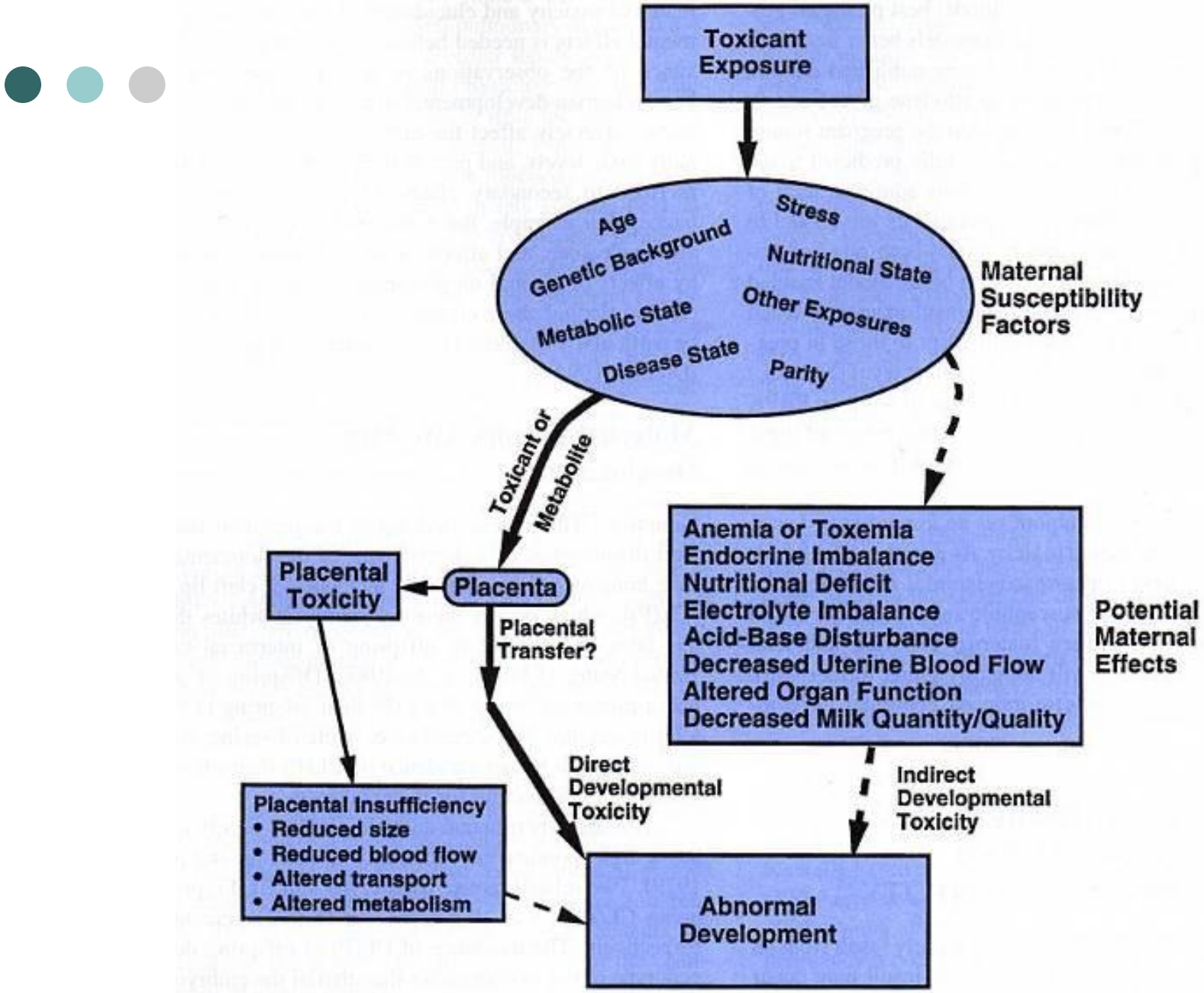
Six principles of teratogenicity (Wilson, 1973)

- 1. Teratogenic susceptibility is determined by the genotype of the conceptus and the interaction of this genotype with the environment
- 2: Susceptibility to teratogenic agents depends on the developmental stage of the embryo or fetus at the time of exposure
- 3: Teratogenic agents work by specific mechanisms on developing cells and tissues to initiate pathogenesis
- 4: Perturbations of developmental processes can result in death, malformation, growth retardation, and/or functional disorders
- 5: The nature of the influence (or agent) determines the extent of the interaction between the environmental agent and the conceptus
- 6: A dose response relationship exists in the occurrence of birth defects induced by a chemical or physical agent, from the no effect level to the totally lethal level

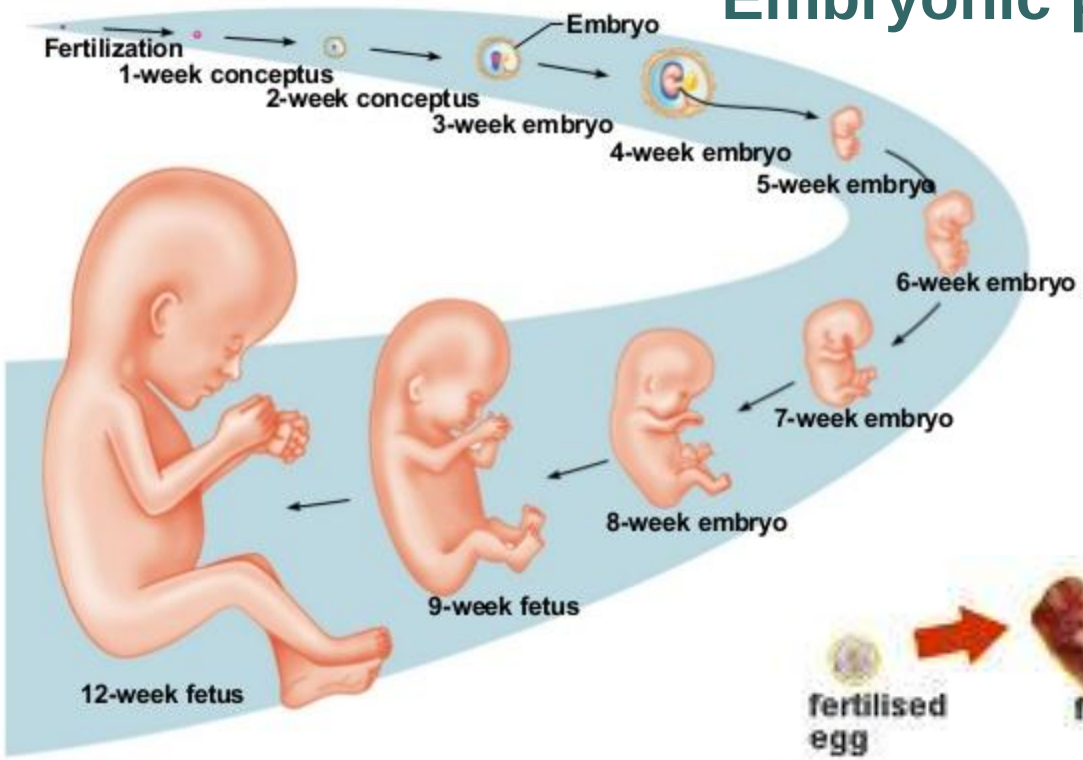
Basic principles in teratogenesis

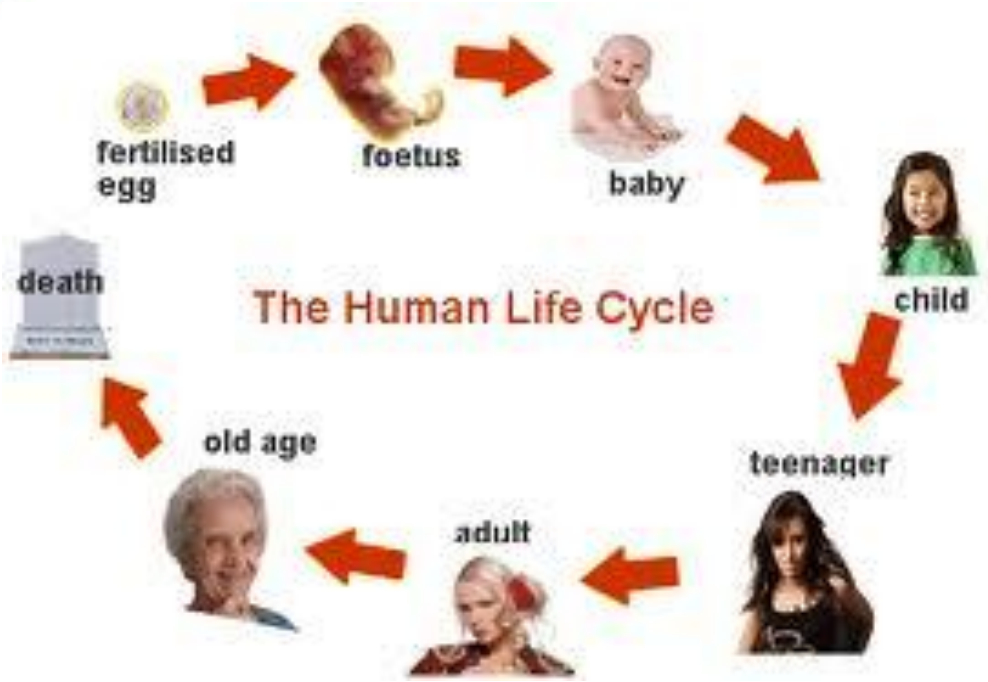
- Genotype (genetic constitution) of the embryo and mother
- Critical periods of development
- Dosage of the drug or chemical



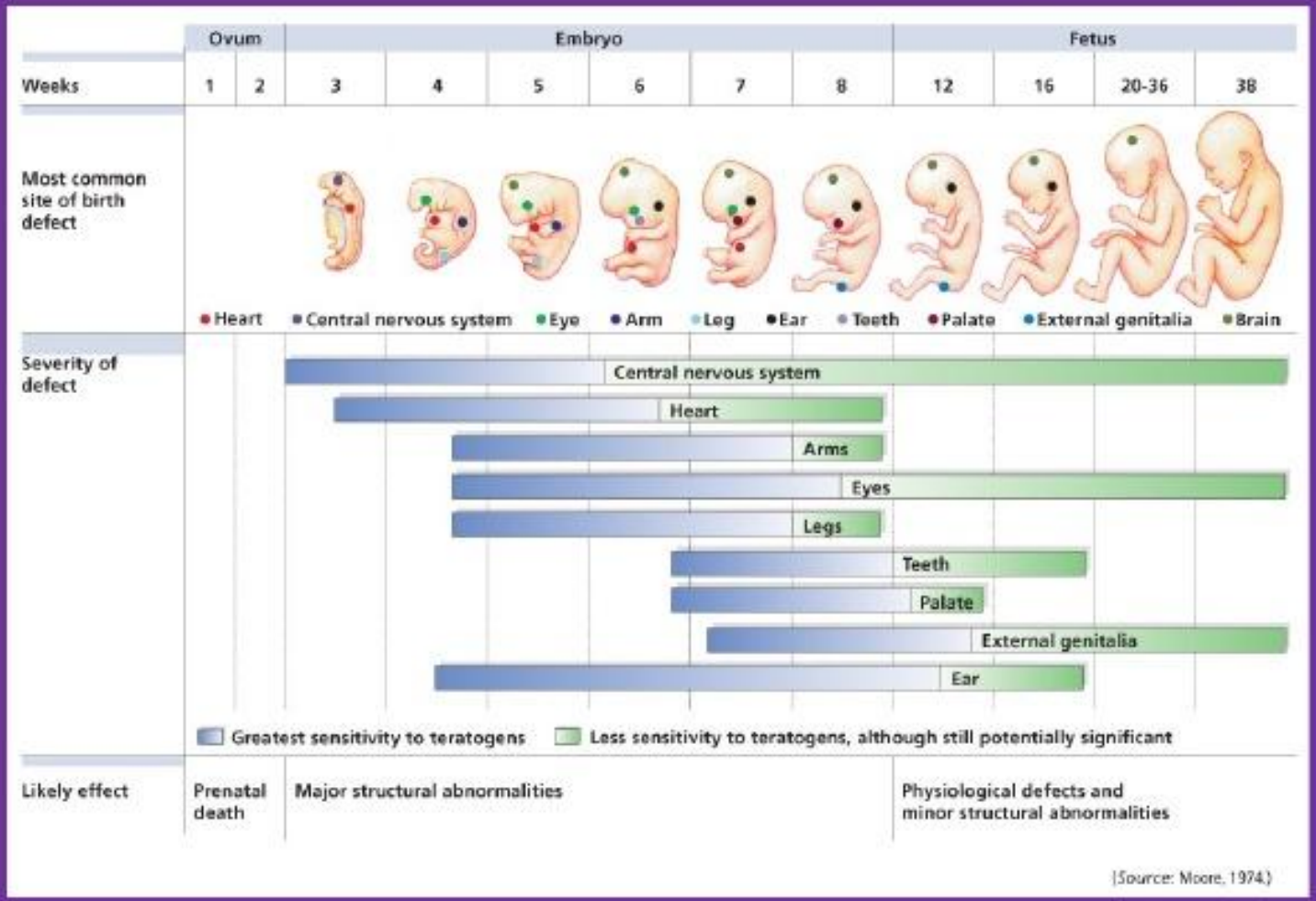

Toxicant Exposure
Age
Genetic Background
Stress
Nutritional State
Metabolic State
Other Exposures
Disease State
Parity
Maternal Susceptibility Factors
Toxicant or Metabolite
Placental Toxicity
Placenta
Placental Transfer?
Anemia or Toxemia
Endocrine Imbalance
Nutritional Deficit
Electrolyte Imbalance
Acid-Base Disturbance
Decreased Uterine Blood Flow
Altered Organ Function
Decreased Milk Quantity/Quality
Potential Maternal Effects
Direct Developmental Toxicity
Indirect Developmental Toxicity
Placental Insufficiency
• Reduced size
• Reduced blood flow
• Altered transport
• Altered metabolism
Abnormal Development

Embryonic period and fetal period in human

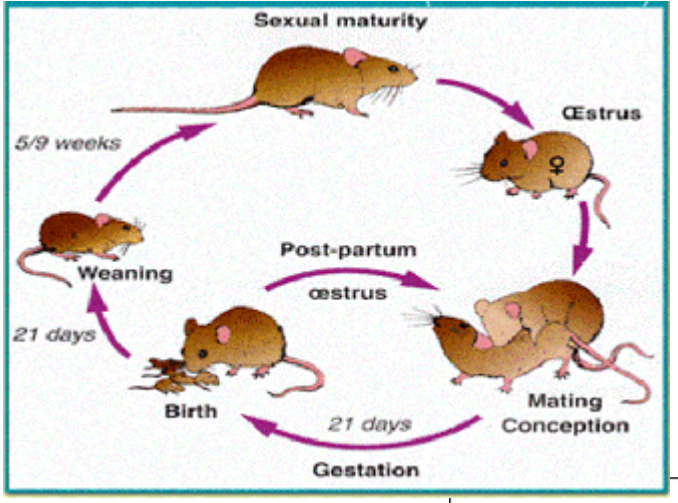


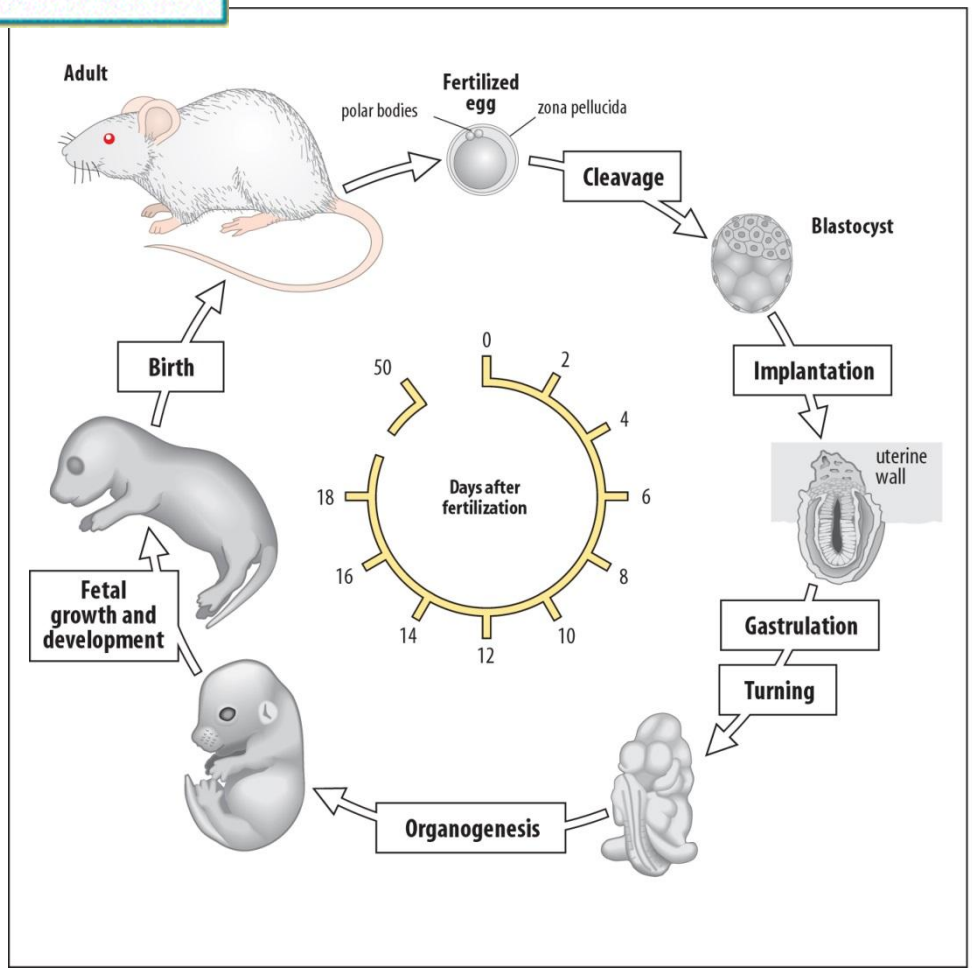


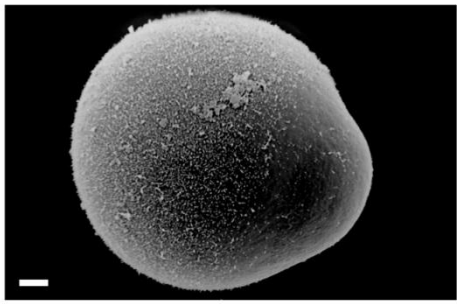
Teratogen Sensitivity Timeline

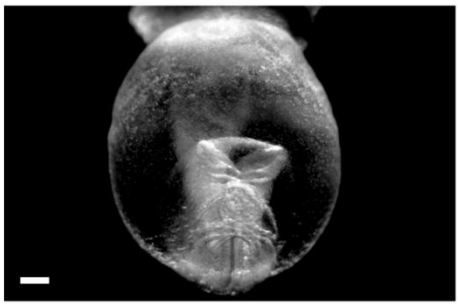


Embryonic period and fetal period in rat

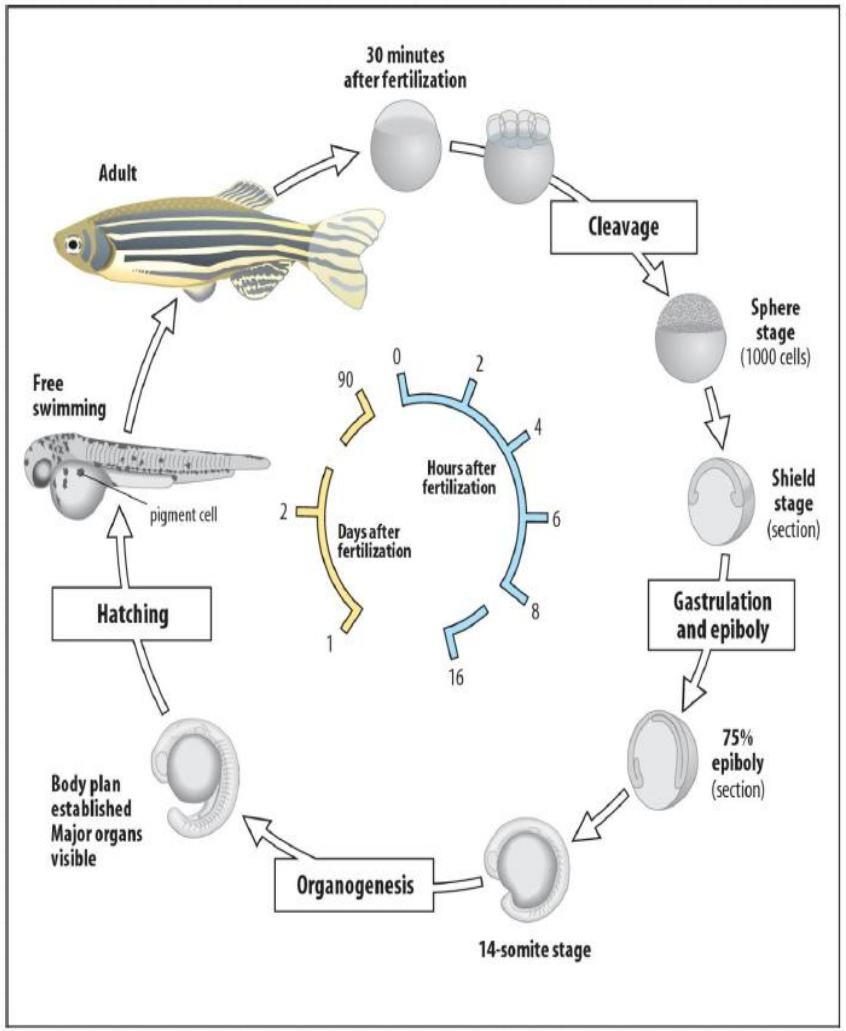


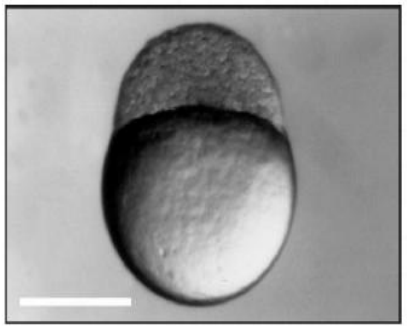


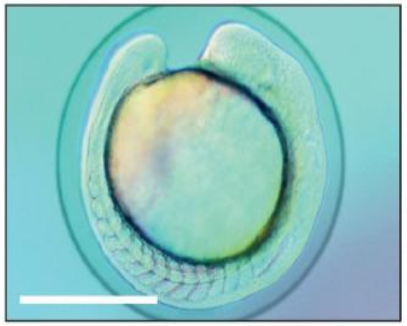




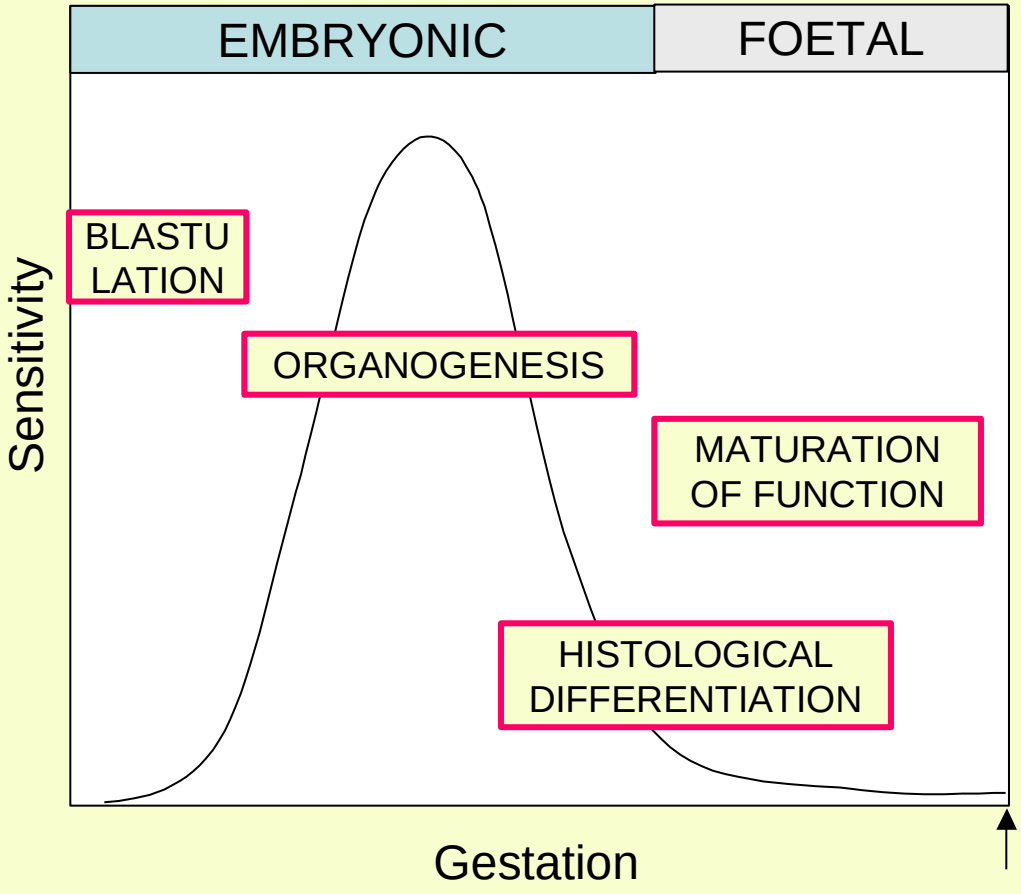
Sviluppo di Zebrafish







Organogenesis is susceptible to teratogensis

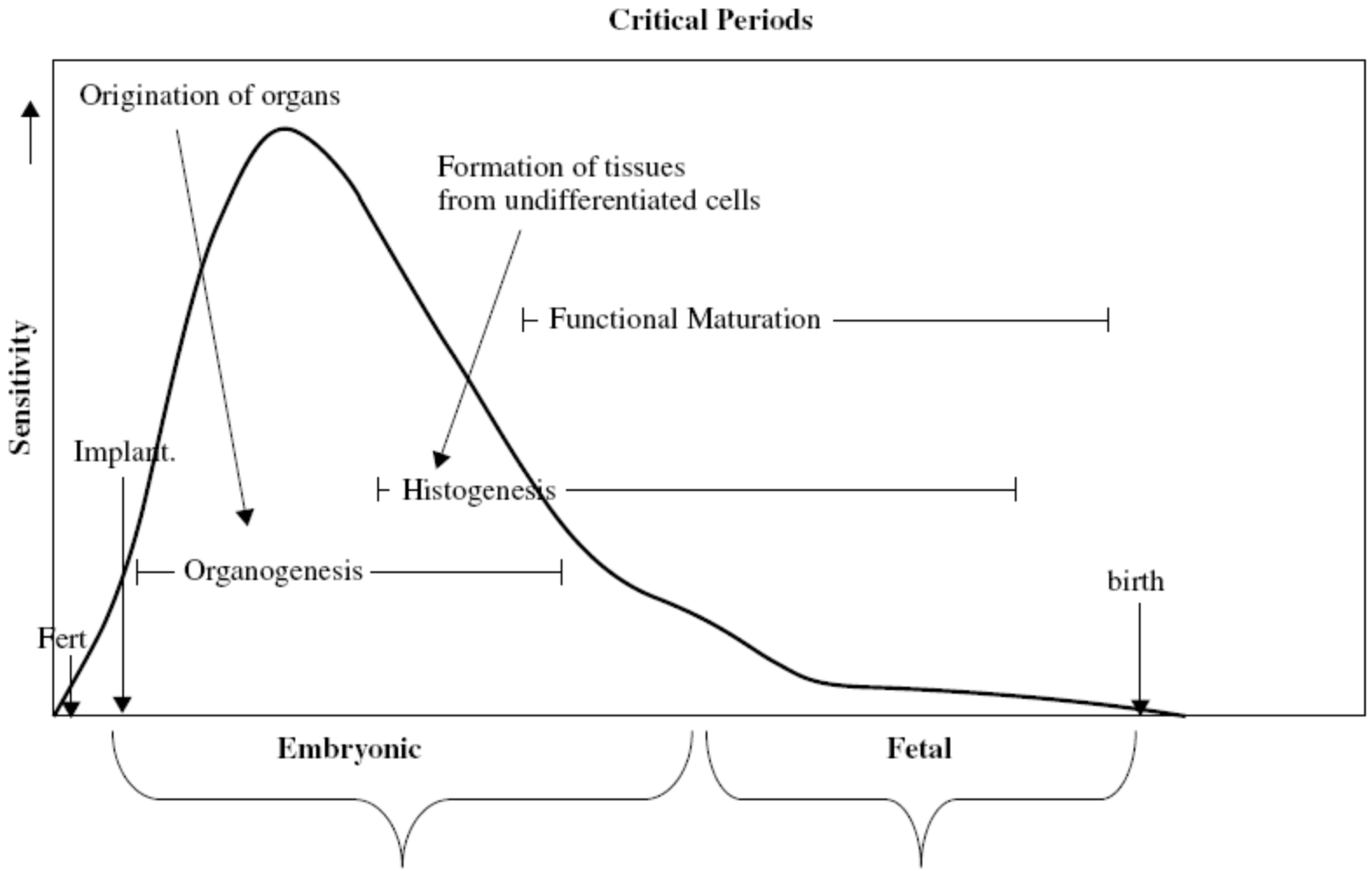


At early stages, cells are pluripotent and cells can replace damaged cells

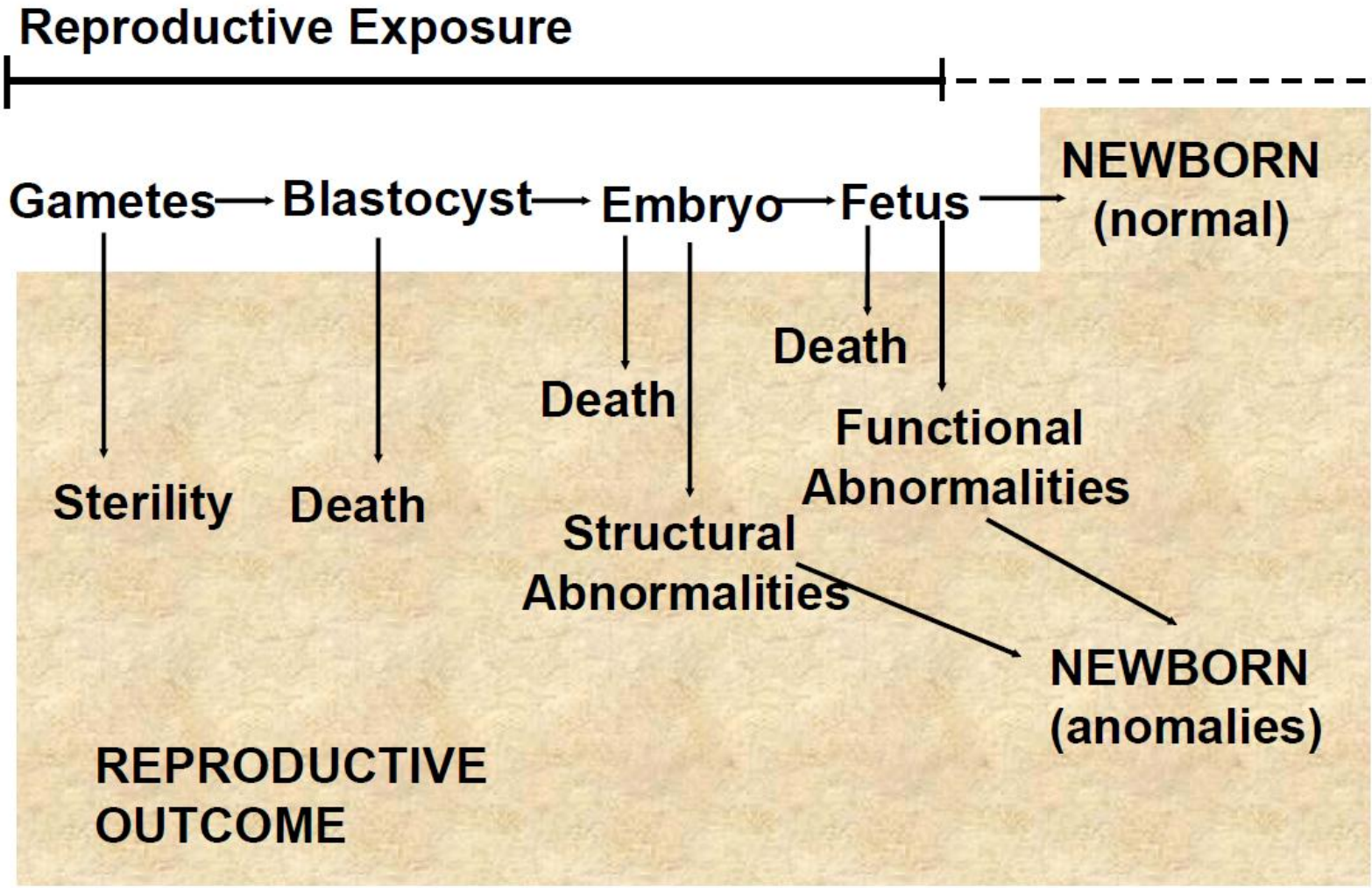
At late stages, organ systems have formed and cellular differentiation is occurring

Footnote: Some organogenesis is late and some teratogens act late eg cerebellum, palate, urinary and reproductive

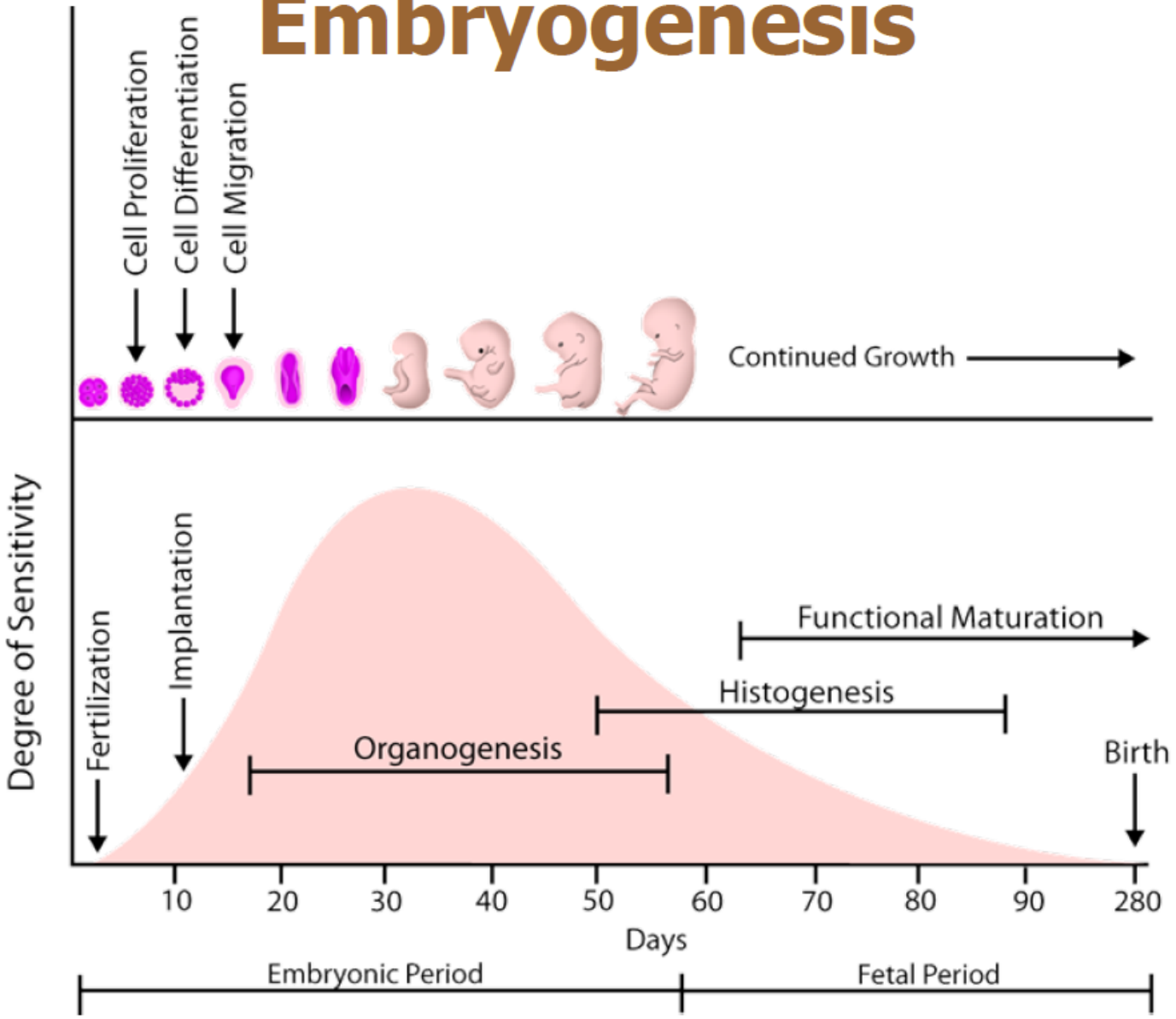
Critical periods in developmental toxicology



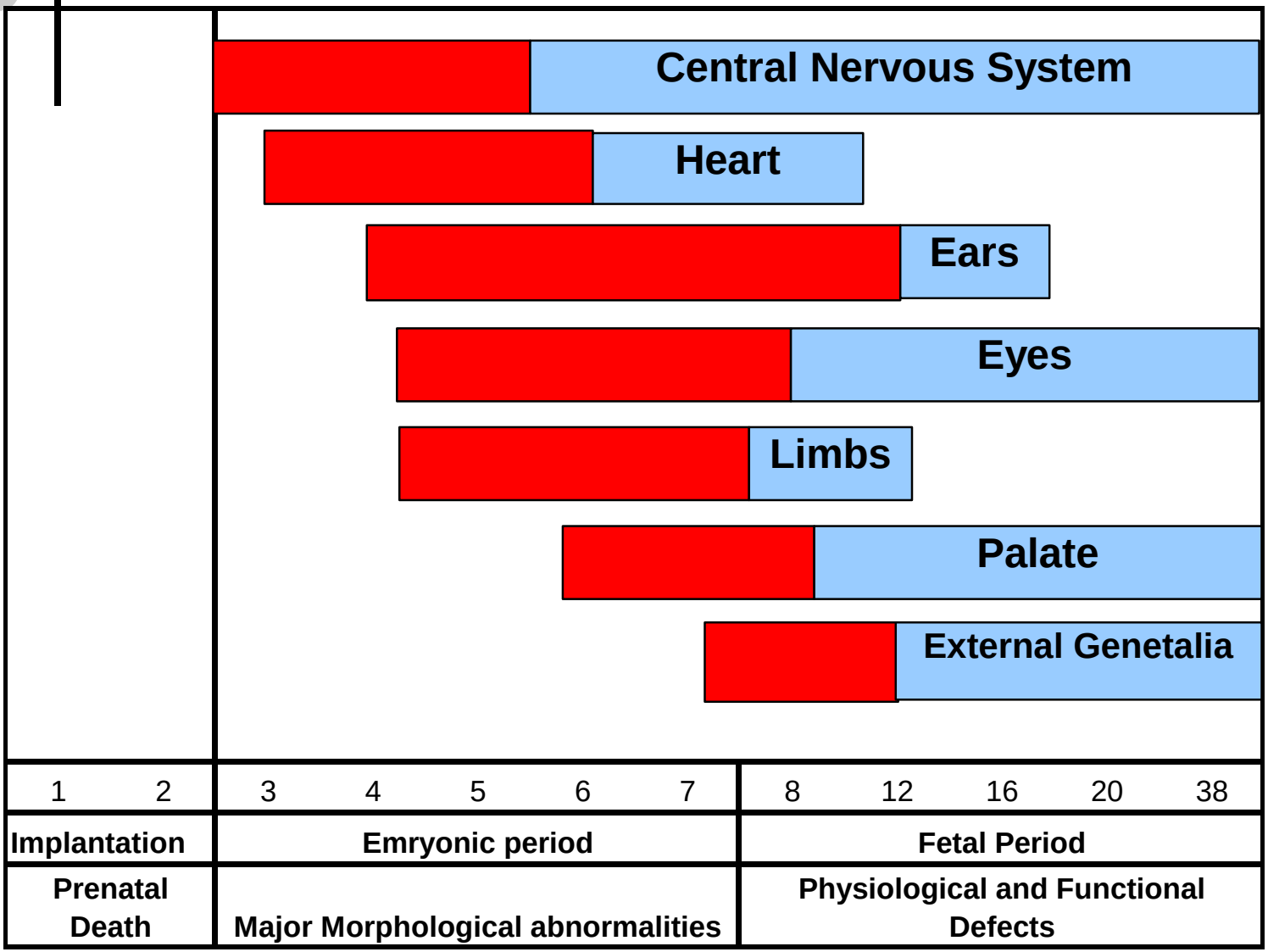
Toxic Windows



Toxic Windows: Stages of Embryogenesis



Sequence of Human Development



Red - most sensitive, Gray - Less

Pattern of effects depends on precise date in the organogenesis calender

'Abruptly, as organogenesis begins, the embryo becomes susceptible to teratogenic agents, usually reaching a peak corresponding to the structural formation of the target organ' (Wilson 1973)