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Human Data for assessment of Developmental Effects

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- Over 2,000 agents have been shown to be embryotoxic/teratogenic in one or more animal species.

- However, only a limited number of those agents have been proven to be embryotoxic/teratogenic in humans.

- Many human teratogens were identified by clinicians when they observed a small number of patients with birth defects.

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The value of preclinical laboratory testing is sometimes challenged.

Identification of Human Teratogens

1920s	Radiation
1930s	Endemic cretinism
1940s	Toxoplasmosis, Rubella
1950s	Virilizing tumors
	Cytomegalovirus, Syphilis
	Aminopterin
1960s	Herpes II virus
	Methylmercury
	Diabetes mellitus, Phenylketonuria
	Methotrexate, Cyclophosphamide
	Thalidomide, Busulfan, Progestins
1970s	Venezuelan encephalitis virus, Varicella, Herpes I virus
	Polychlorobiphenyls
	Diethylstilbestrol, Warfarin, Phenytoin, Trimethadione
	Alcohol
	Hyperthermia
1980s~	Parvovirus B19, HIV virus
	Retinoids, Valproic acid, Anti-inflammatory drugs
	Angiotensin-converting enzyme (ACE) inhibitor
2016	Zika virus

Comparison of Teratogenicity in the Human and Laboratory Animals*

Agents teratogenic in humans (N=38)		Agents not teratogenic in humans (N=165)	
Species	Teratogenic (Correctly positive)	Species	Not teratogenic (Correctly negative)
Mouse	58%	Mouse	35%
Rat	80%	Rat	50%
Rabbit	60%	Rabbit	70%
Hamster	45%	Hamster	35%
Nonhuman primate	30%	Nonhuman primate	80%
Two species or more	80%	Two species or more	50%
Any species	97%	All species	28%

*Compiled by US FDA (1980).

Extremely high doses are given to pregnant animals under experimental conditions.

Developmental toxicity in humans may have been prevented by preclinical toxicological studies using laboratory animals.

Therefore, the value of preclinical studies using laboratory animals cannot be underestimated.

Possible causes of species difference in teratogenesis

1) Condition of exposure

    Dose of exposure

    Timing of exposure

2) Different **susceptibility** of embryonic tissues to the exogenous agent

3) Phylogenetic difference in reproduction and pregnancy

4) Species difference in pharmacokinetics in the mother-placenta-embryo complex

    Absorption, tissue distribution, metabolism and excretion

Is the human less susceptible to teratogenic agents?

	Teratogenic dose (mg/kg/day)				
Teratogen	**Human**	**Mouse**	**Rat**	**Rabbit**	**Nonhuman primate**
Alcohol	**400**		**1500**		
Aminopterin	**0.02**	**0.15**	**0.15**		
DES	**0.02**				**0.2**
Methylmercury	**0.005**	**2**	**0.25**		
Thalidomide	**1**			**150**	**5**
Trimethadione	**12-24**	**600**			**60**
X-ray	**20-50R**	**200R**	**30R**		**250R**

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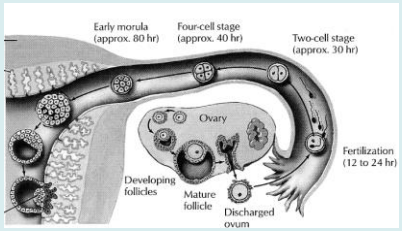
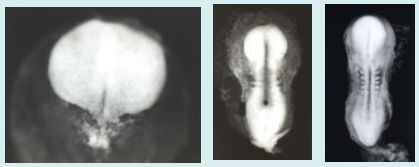
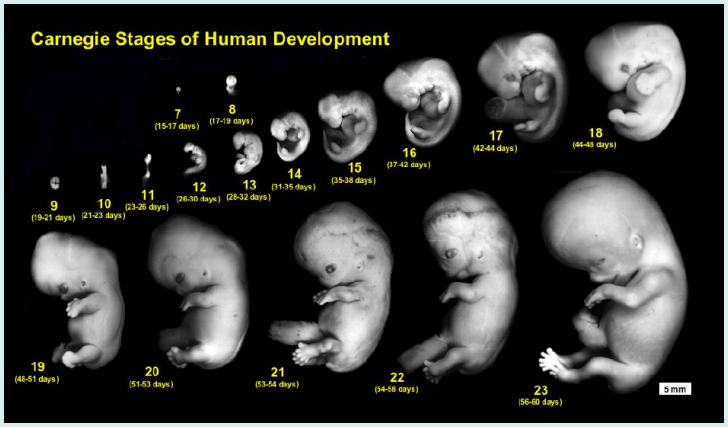
Gametogenesis
Fertilization
Preimplation period
Early development
Major organogenesis
Fetal period
Perinatal period



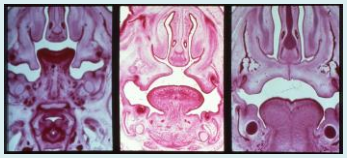
Gene mutations
Chromosomal aberrations



Imprinting disorders



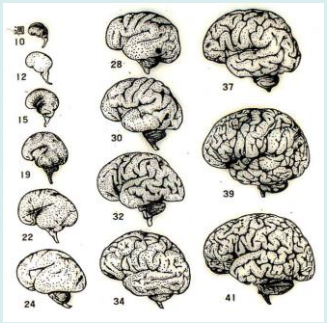
Malformations



Palate

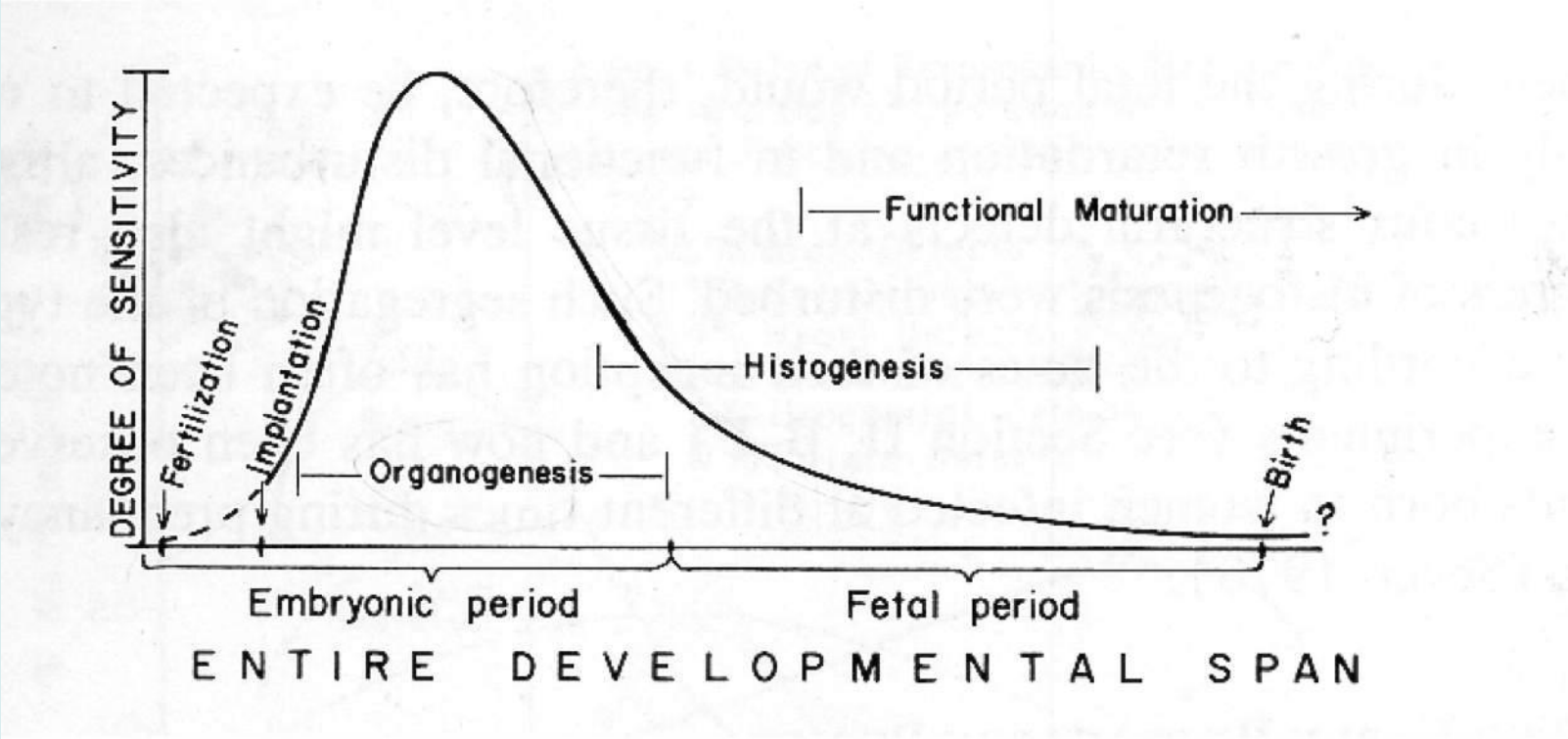
Genital organs

Abnormal histogenesis



Brain

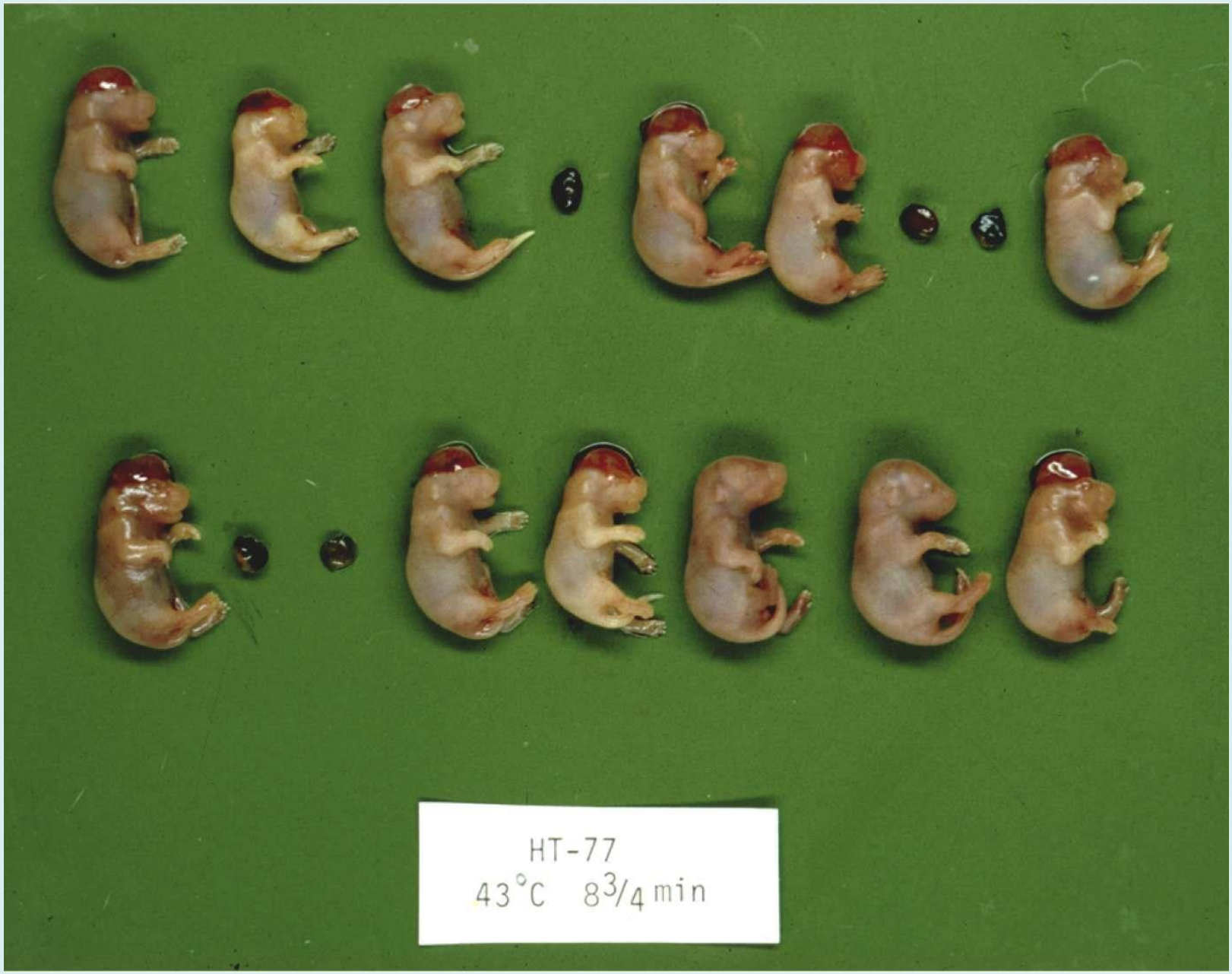
Functional deficits



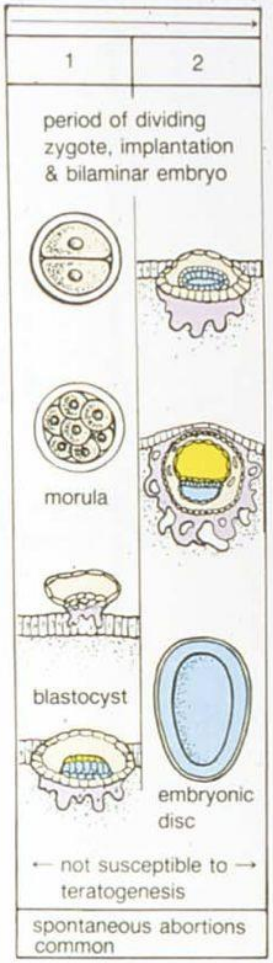
Sensitivity of the embryo/fetus to teratogens
(Wilson, 1971)

Species Characteristics of Reproduction

Species	Length of gestation (days)	Duration of reproductive cycle (days)	Critical period of organogenesis (days)
Mouse	19	4-5	7-15
Rat	22	4-5	9-17
Hamster	16	4-15	7-14
Guinea pig	68	13-20	11-25
Rabbit	30	15-16	7-20
Rhesus monkey	165	24-38	20-45
Human	266	26-29	18-55



Littermates from a female mouse exposed to hyperthermia at a critical period of teratogenesis



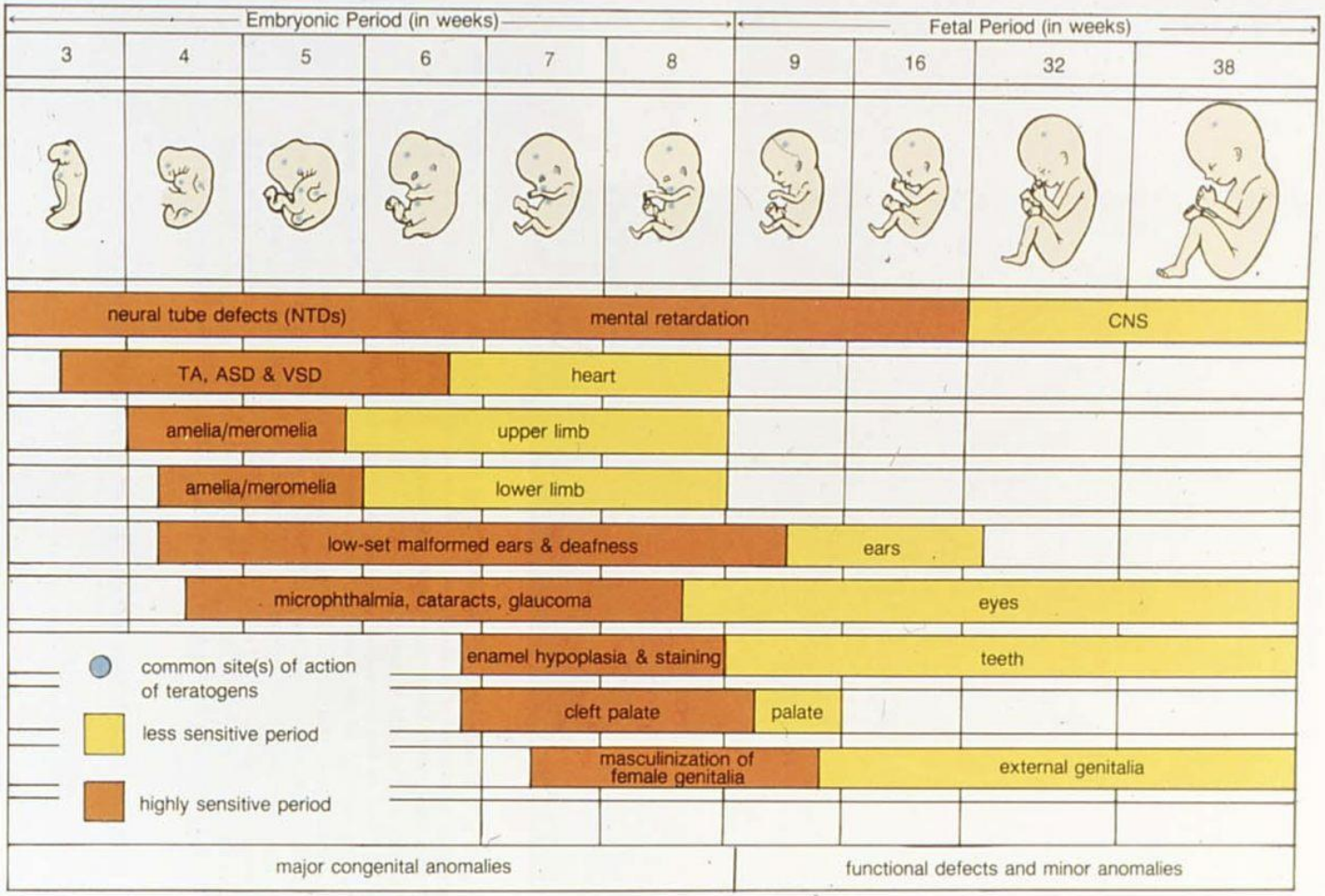


Figure 5–2. Schematic illustration showing the critical periods of human development. Note that each part or organ of the embryo has a critical period when development may be disrupted, resulting in major congenital anomalies. Thereafter is a period when environmental agents (e.g., drugs and viruses) may cause minor anomalies and functional disturbances (e.g., mental retardation). TA, Truncus arteriosus; ASD, atrial septal defect; VSD, ventricular septal defect; NTDs, neural tube defects, e.g., spina bifida (see Figs. 13–14 to 13–18).

Critical period of induced teratogenesis in human embryos/fetuses (Moore 2007)

Estimated critical phases of thalidomide embryopathy in the human

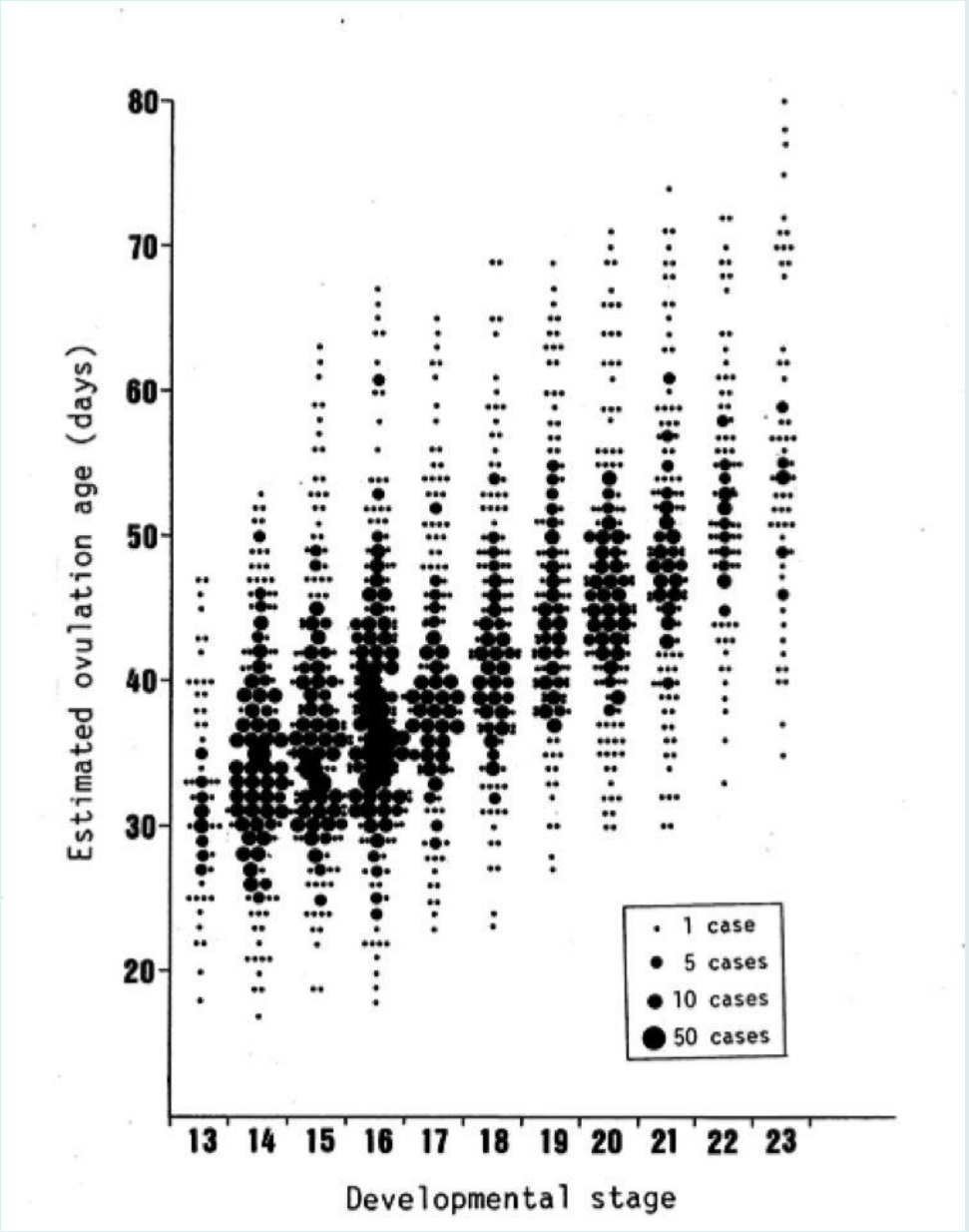
26 - 39 days after conception (Lenz & Knapp, 1962)

16 - 39 days after conception (Hielscher, 1968)

>40% of the mothers of thalidomide babies took the drug between 3rd and 6th weeks of gestation
>20% of the mothers did so before the 3rd week or after the 6th week
(Weicker et al., 1962)

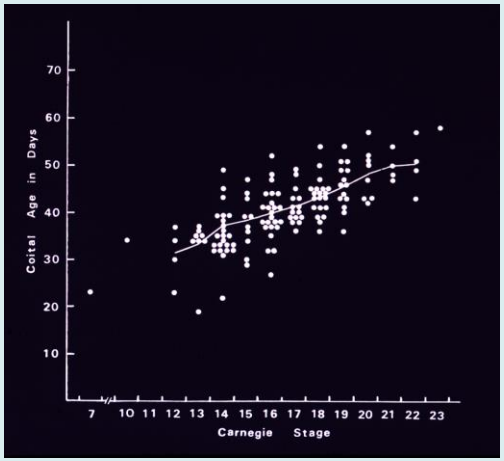
Out of 7 babies born to women who took the drug between 34th and 50th days LMP, only 3 were malformed
(Kajii et al., 1973)

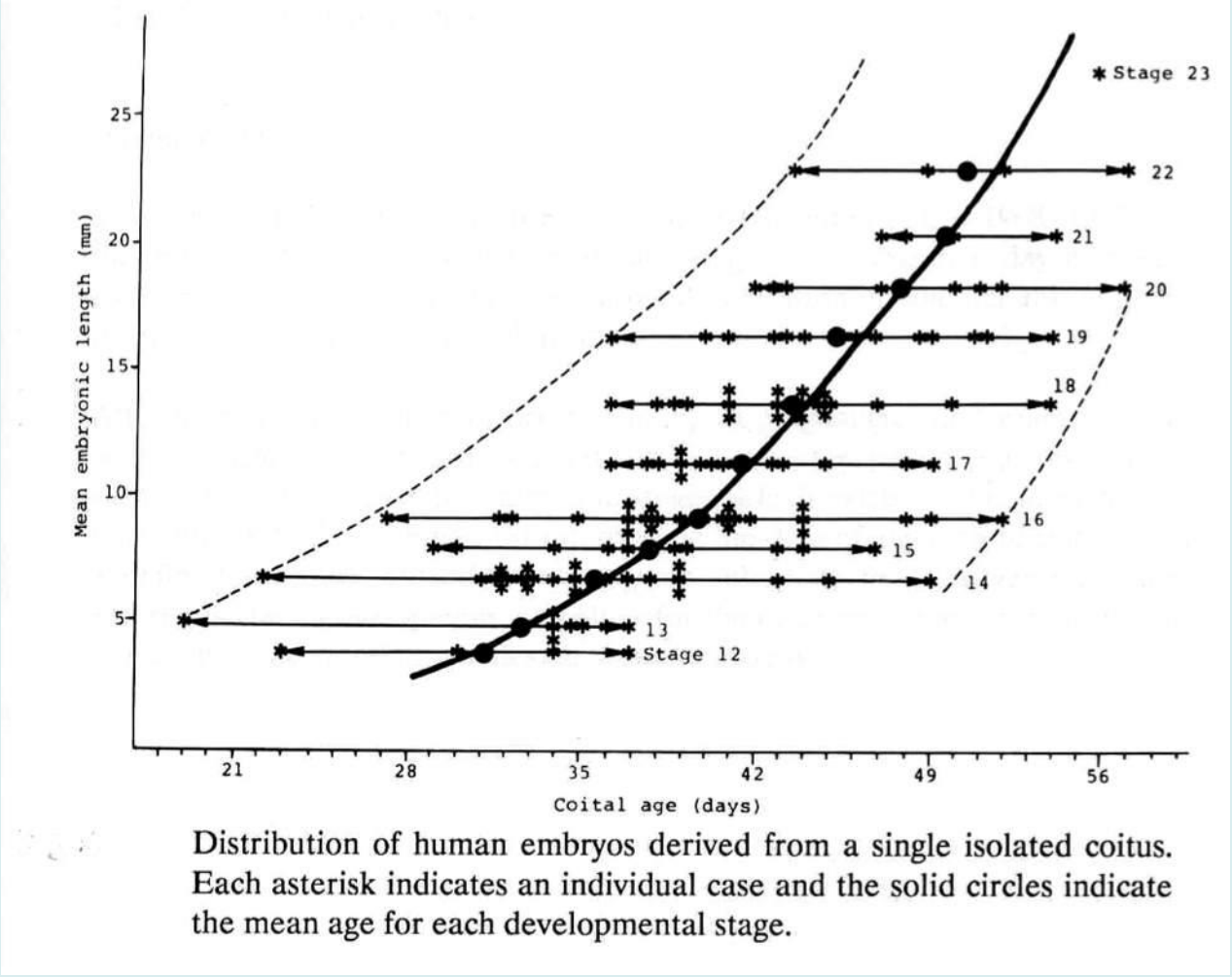
The susceptibility of human embryos/fetuses to induced teratogenesis seems to be variable, and the teratogenic risk may not be easily predicted from the gestational age.



Developmental variability in human embryos

The variability in human embryonic development is so enormous that for individual case, it is difficult to estimate the developmental stage based on Its gestational age (LMP).





Distribution of human embryos derived from a single isolated coitus. Each asterisk indicates an individual case and the solid circles indicate the mean age for each developmental stage.

(Shiota et al., 1988).

- Considerably large variability exists in the developmental stage of human embryos at a given gestational age. Therefore, it is not easy to precisely estimate the teratogenic risk of the embryo based on the gestational age.

- It seems that the developmental variability is larger in human embryos than in other animal species.

- Variability in embryonic development may partly be biological in nature.

Possible causes of developmental variability in human embryos

1) Timing of fertilization and implantation

2) Intrauterine environmental factors (implantation site, nutrition, oxygen, placental function, etc.)

3) Speed of embryonic development (cell cycles, etc.)

4) Loss of blastomeres

5) Unreliable maternal memory (human factor)

Abortion rate in humans

- It is generally estimated that 10-15% of recognized pregnancies end in clinical abortion.

- 15% of the total 6,835 pregnancies ended in spontaneous abortion.

(Warburton and Fraser, 1964)

- However, recent studies show that a substantial proportion of human conceptions are lost at such early stages of pregnancy that the mothers are not aware of abortion (subclinical or unrecognized abortion)

Prevalence of chromosomal abnormalities in human gametes, embryos and fetuses

Sperms	8 - 10 %
Zygotes	60 %
8-cell embryos	65 %
Therapeutic abortuses	3 - 6 %
Spontaneous abortuses	50 - 60 %
Newborns	0.6 %

Prevalence rates of major malformations (X1,000)

Anomaly	Newborns	Therapeutic abortuses	Spontaneous abortuses
Neural tube defects	0.5-1.0	2.4	13.4
Holoprosencephaly	0.1	4.0	-
Cleft lip +/- cleft palate	1.0-2.7	4.3	8.0
Polydactyly	0.5-1.4	2.8	1.3
Syrenomelia	0.02	-	4.0
Turner syndrome	0.05	-	5.4

In humans, approximately 10-15% of clinically recognized conceptions end in spontaneous abortions.

A considerably large proportion of human conceptions seem to end in subclinical abortions.

About half of spontaneous abortuses are morphologically and/or chromosomally abnormal.

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Spontaneous abortion is an important screening device for abnormal conceptuses.

Estimated proportion of embryos/fetuses with major malformations at the beginning of each gestational

Gestational interval	Total malformed embryos (%)	Neural tube defects (%)	Holoprosen-cephaly (%)	Cleft lip (%)	Polydactyly (%)
5th week	7.0	1.1	2.6	-	-
6th week	6.5	1.0	2.4	1.2	2.8
7th week	5.7	0.9	1.8	1.2	2.9
8th week	4.7	0.8	1.3	1.0	2.1
Fetal period	2.4	0.1	0.3	0.6	1.5
Newborns	1.0	0.08 0.01	0.2	0.1	

(Shiota, unpublished)

Cumulative intrauterine mortality rate of normal and malformed human embryos

Gestational interval	Normal embryos (%)	Neural tube defects (%)	Holoprosen-cephaly (%)	Cleft lip (%)	Polydactyly (%)
5th week	1.3	11.4	10.4	-	-
6th week	1.7	25.4	33.8	-	-
7th week	2.5	35.1	54.2	16.8 28.6	
8th week	4.5	88.6	90.0	54.9 50.0	
Fetal period	10.2	93.9	99.6	87.6 71.4	

(Shiota, unpublished)

Estimated fate of human ova (Hertig et al., 1967)

Out of 100 ova exposed to sperm:

16	fail to be fertilized
15	die during the first week
27	die during the second week
8	die during the third and sixth weeks
3	die during the following months
31	born alive (excluding 1 case with anomalies)

Two-thirds of mankind die before birth.

The reproductive loss rate is extremely high in the human.

It seems that defective development occurs frequently early in human development and more than 90% of malformed embryos die in utero.

Spontaneous abortion is a natural screening device that reduces the birth of abnormal babies.

Prenatal mortality and reproductive efficiency in various animal species

Species	Prenatal mortality (%)	Reproductive efficiency (%)
Mouse	25	75
Rat	28	72
Chinese hamster	14	86
Golden hamster	23	77
Sheep	30	70
Cow	37	63
Pig	40	60
Human	69	31

Compared with other animals species,
the human appears to have:

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1. A wider variability in embryonic development
2. A lower fertility rate
3. A higher prenatal mortality rate
4. More frequent pathological embryonic development

Evaluation of Reproductive Risks based on Laboratory Studies

1. Is the reproductive toxicity observed in two or more species?

2. Are the reproductive effects tested in appropriate animal species?

3. Are specific effects (phenotypes) induced by the agent concerned?

4. Are the reproductive effects dose-related?

5. Is the embryotoxic dose far below the maternal toxicity dose?

6. What are the embryotoxic threshold dose and the NOAEL?

7. What is the difference between the embryotoxic dose in laboratory animals and the human clinical dose?

8. How serious could the possible effects be in humans?

9. What kind of human populations could be at risk?

Proof of Teratogenesis in the Human

1. Majority of epidemiological studies demonstrate an increased incidence of a particular group of malformations in exposed populations.

2. The incidence of patients prenatally exposed to the agent is significantly higher in the population having the particular group of malformations.

3. An animal model is developed which mimics the human situation.

4. The embryotoxic effects are dose-related.

5. The critical period and mechanism of teratogenesis are biologically plausible.

Modified after Shepard (1994).

- **The Berlin Workshops** have significantly contributed to deeper understanding of fetal findings in Dextox studies and to establishing international agreement for assessment.

- Each laboratory may have its own criteria for fetal findings and may produce different assessment.

- Findings that are not always clear (such as “misshapen” or “grey zone” findings) should be minimal so as to minimize the discrepancy among laboratories.

Thank you
for your Attention!