Comparative Aspects of the Placenta in Animals Used in Developmental and Reproductive Toxicology Studies

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Outline

- Introduction
- Comparative aspects of the placenta
- Classification of the placenta
- Placenta as an endocrine organ
- Placenta - Immune adaptations in pregnancy
- Placental transfer of xenobiotics
- Extrapolation of animal studies to human

Introduction

- **Placental physiological functions**
  - Anchoring the developing fetus
  - Controlling maternal-to-fetal exchanges
  - Protecting the embryo/fetus against xenobiotics
  - Immunological function
    - mediating maternal immune tolerance
  - Hormonal function

- **Reproductive toxicity**
  - female fertility
  - parturition
  - lactation
- **Developmental toxicity**
  - mortality
  - dysmorphogenesis
  - alterations to growth
  - functional impairment.

Introduction

- 3 species used predominantly in regulatory DART
  - Rats, Rabbit, NHP
- 3 species utilize different strategies for maternal-embryonic exchange
- Rats and rabbits are the default species (ICH S5 (R2)
- The NHP is not the default species for biopharmaceuticals
- NHPs should be only considered when rodents and/or rabbits are not pharmacologically or biologically relevant species

Introduction

+ **Effect of Xenobiotics on Developmental Toxicity**

Direct embryo/fetal toxicity

Placental unit toxicity

- Direct placental toxicity
  - pathologic changes in the trophoblast eg cadmium
- Diminished uteroplacental blood flow
  - maternal exposure to hydroxyurea
- Poisoning of transport mechanisms
  - azo dyes
- Immunotoxicants

It is possible, although it has not yet been shown, that biopharmaceuticals might compromise placental function

Comparative Aspects of the Placenta

There is a broad range of morphologies and functional differences within species

- **Anatomical differences**
  - Arrangement of fetal blood vessels
  - Duration and function of the yolk sac placenta
  - Relationship to the maternal circulation
  - Number of placental layers
  - Developmental schedules
- **Functional differences**
  - Metabolic and bio-transformational capabilities
  - Endocrine function
  - Genotypic susceptibilities
  - Carrier mediated mechanisms across the placenta

There is modification of the placental structure and function throughout gestation

Classification of Placenta

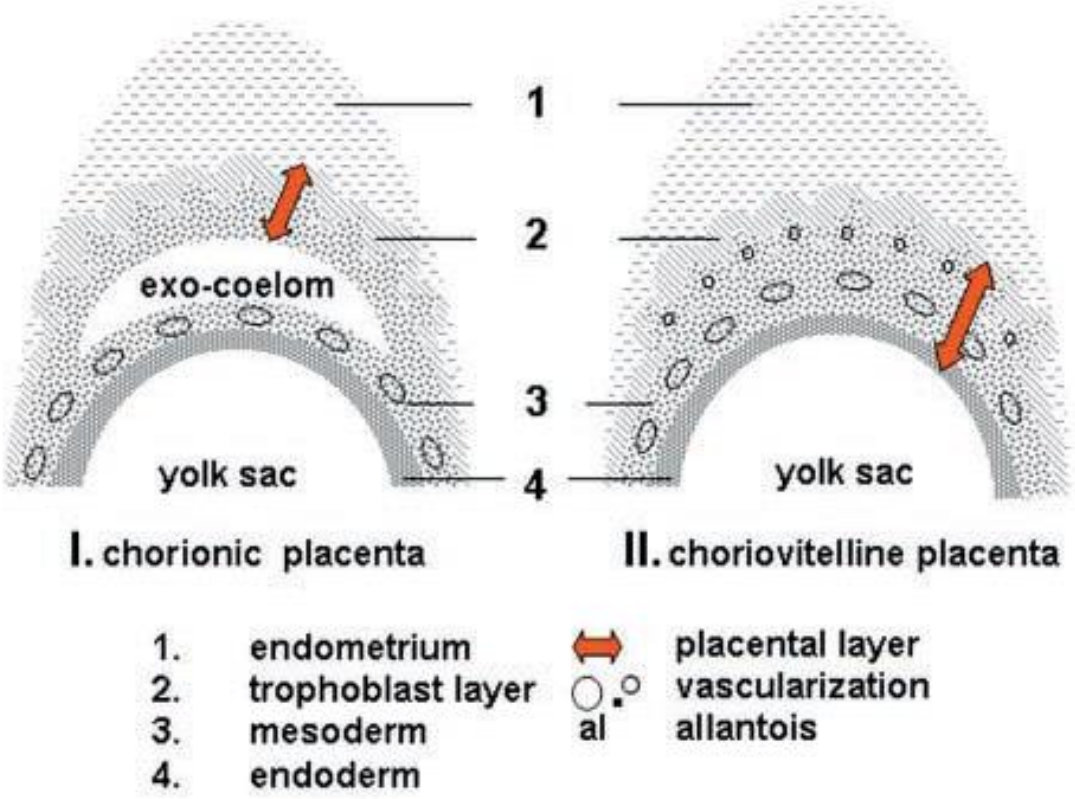
1. Origin of the Embryonic Membranes
2. Gross Shape
3. Internal Structure
4. Relation to Maternal Tissues
5. Fetal-Maternal Interhemal Barrier
6. Blood Flow

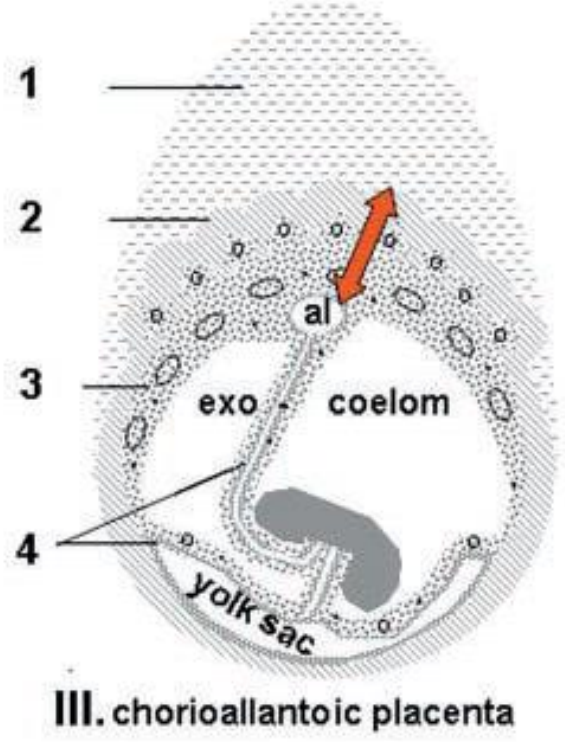
Classification According to Origin Fetal Membranes

Many mammals have 2 of the placenta types at some time during pregnancy

- **Chorionic placenta =** transient
  - most primitive type -lacks vessels
  - formed by the fusion of maternal endometrium with the trophoblast
- **Yolk sac placenta (choriovitelline) =** transient
  - early post implantation
  - elements of the yolk sac are added to the chorion membrane
  - Humans, NHP - becomes vestigial after the 1st trimester
- **Inverted Yolk Sac Placenta**
  - yolk sac "inverts" forming a special placenta
  - Rodents and Rabbits
- **Chorioallantoic placenta**
  - contains allantois constituents
  - the principal placenta in mammals during middle to late-gestation
  - different shapes and histomorphology between species

Classification According to Fetal Membranes





Toxicol Pathol 2008 36:108S-118S

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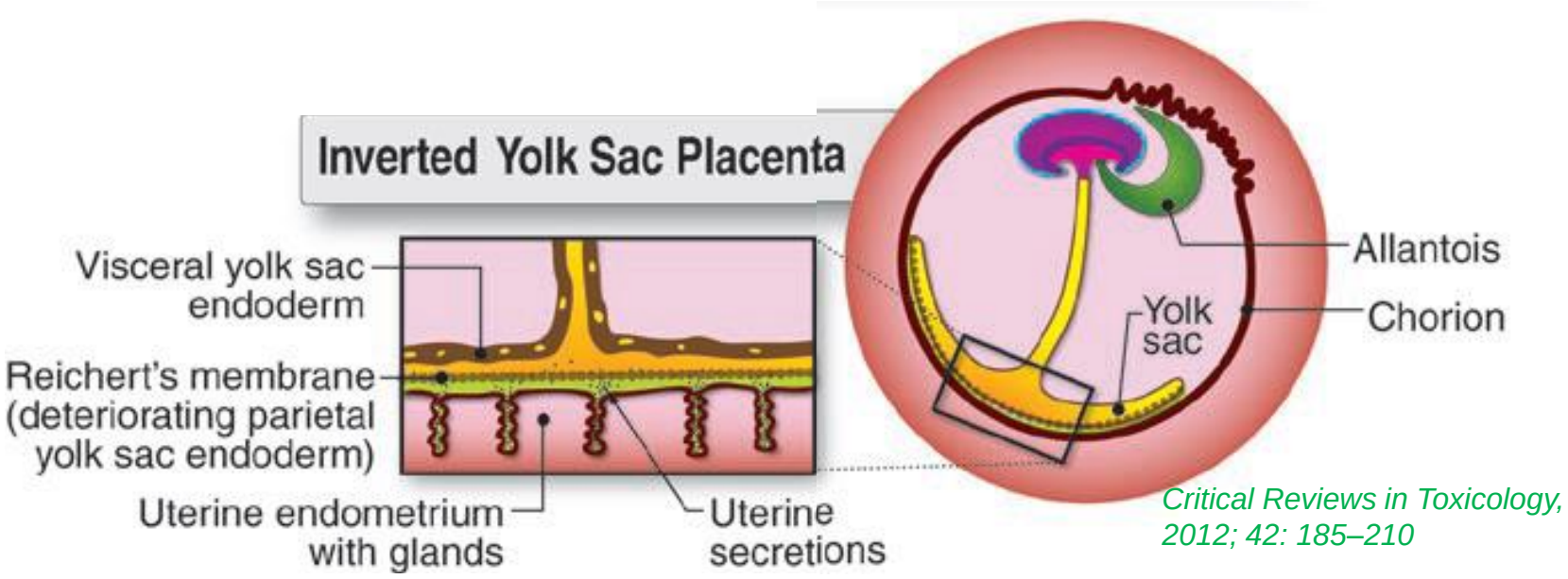
Classification According to Fetal Membranes

Inverted yolk sac placenta – Rodents and Rabbits



Critical Reviews in Toxicology, 2012; 42: 185–210

It is the inverted yolk sac placentae of rodents and rabbits that are the major conduits for transfer of IgG and Fc-containing biopharmaceuticals.

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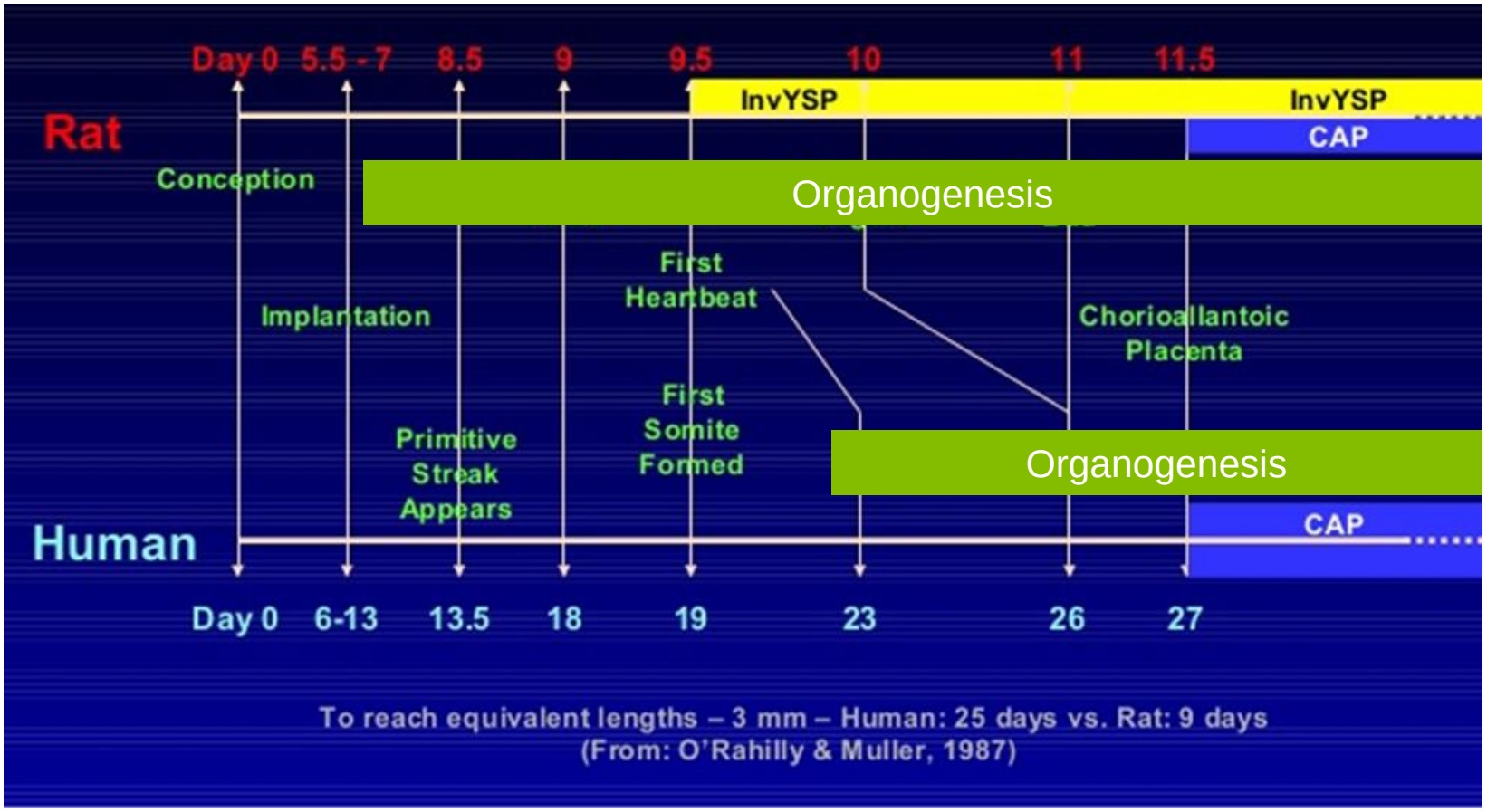
Classification According to Fetal Membranes

Inverted yolk sac placenta



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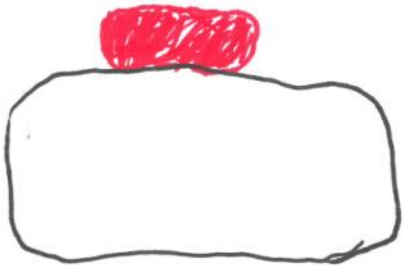
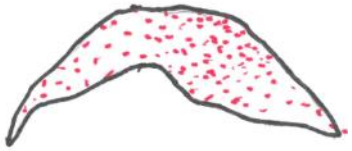
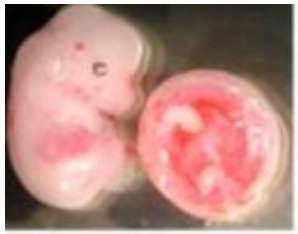
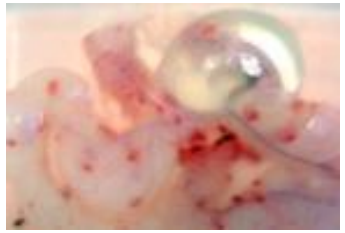
Classification According to Gross Shape

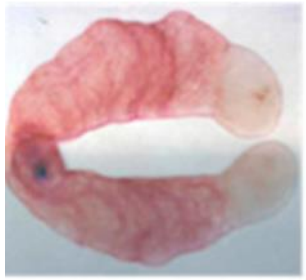
Discoid	Zonary	Cotyledonary	Diffuse
Humans, NHP, Rat, Rabbit	*Dog*	*Ruminants*	*Minipigs*











Classification According to Internal Structure

- **Labyrinthine**

  Rodents, Rabbit, Dog

- Trophoblasts form an intercommunicating network with maternal capillaries

- **Villous**

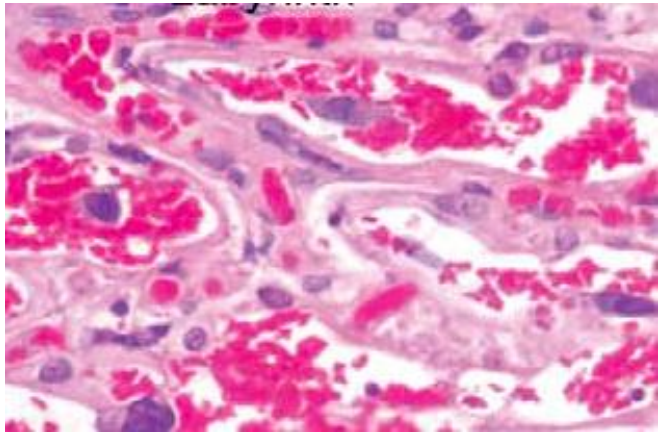
  Human, NHP

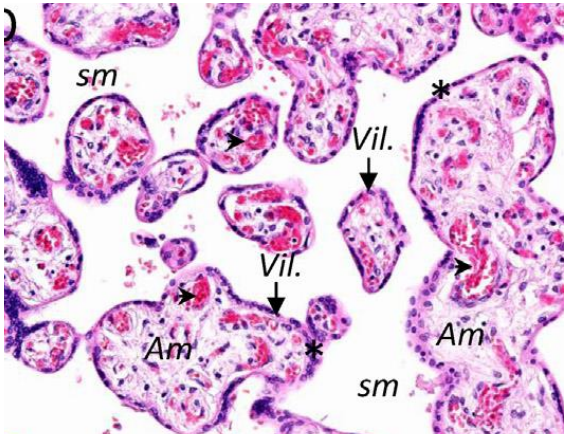
- Branched chorionic villous protrusions interdigitate with maternal crypts

- **Folded**

  Minipigs

- Macroscopic undulations of fetal tissues interlock with corresponding infoldings in the endometrium





Significance – Surface area

Classification According to Relation to Maternal Tissues

- **Deciduate placenta**

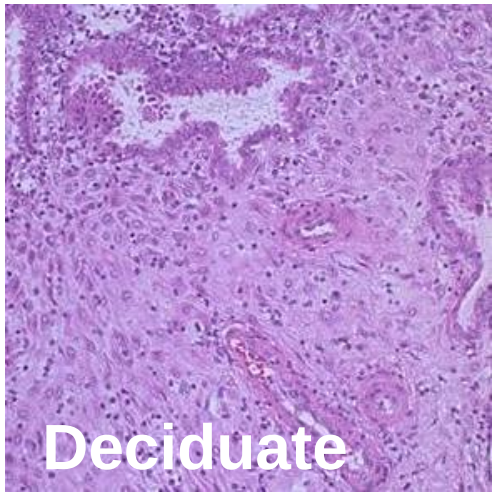
  Humans, NHP, Rabbit, Rodents

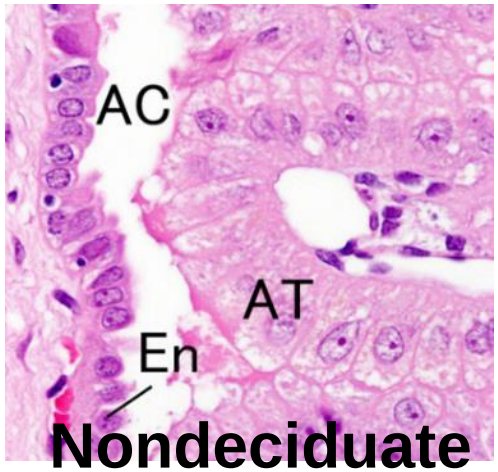
  - trophoblasts invade and destroy superficial endometrium
  - decidual cell formation
  - at parturition there is loss of maternal tissue
  - dogs and cats have mildly deciduate placentas

- **Non-deciduate placenta**

  Minipig

  - Trophoblasts do not significantly invade or destroy the endometrium
  - At parturition there is virtually no loss of maternal tissue





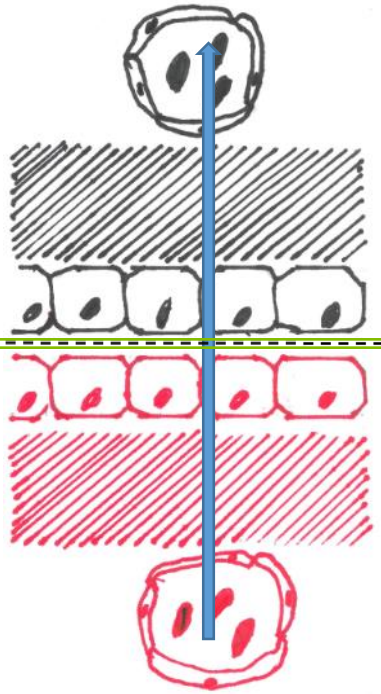
Classification Based Upon the Fetal-Maternal Interhemal Barrier

Classification of placentas is based on degree of removal of the maternal layers

Placenta: Key Characteristics

6 layers of tissue separating maternal and maternal circulation

Fetal tissues



1. allantochorionic capillary endothelium
2. allantochorionic connective tissue
3. chorionic epithelium (trophoblast)

Maternal tissues

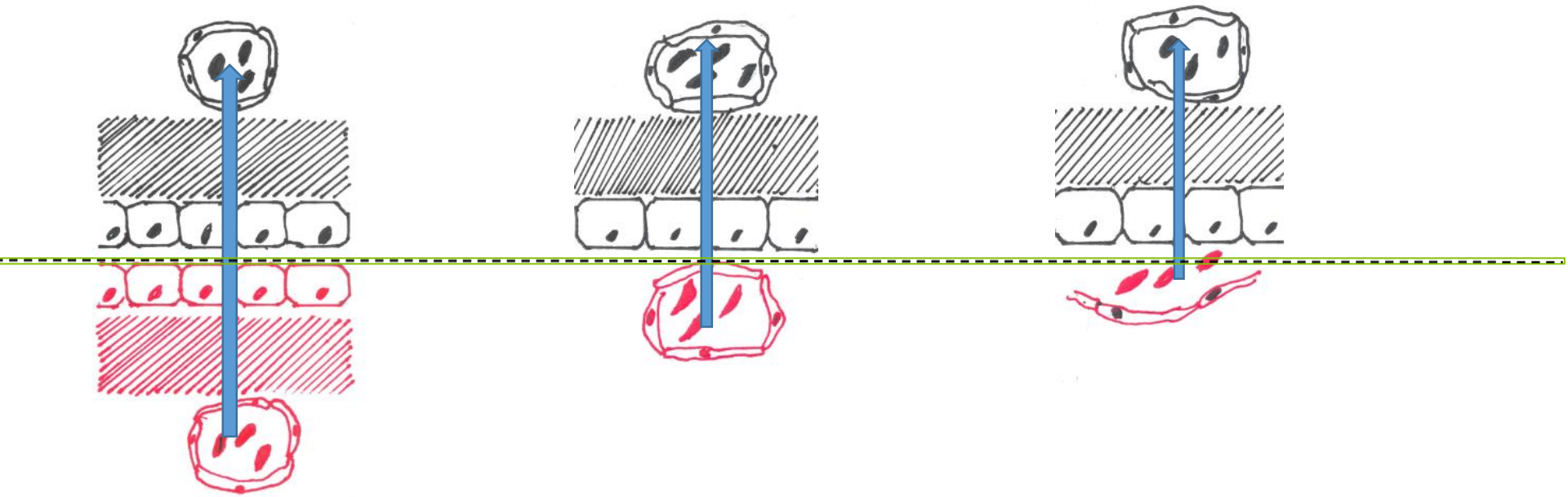
4. uterine epithelium
5. maternal connective tissue
6. maternal capillary

Classification Based Upon the Fetal-Maternal Interhemal Barrier

Epitheliochorial
- 6 layers present
Minipig

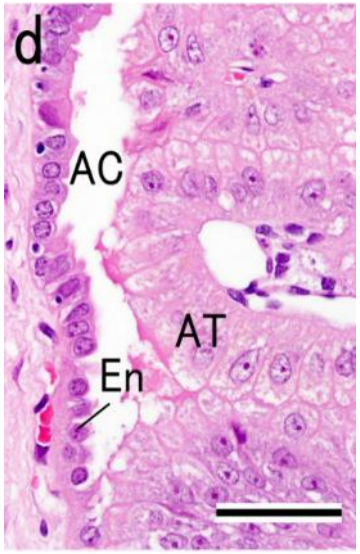
Endotheliochorial
- 4 layers present
Dog

Hemochorial
- All maternal layers eroded
Humans, NHP, Rabbit, Rodent



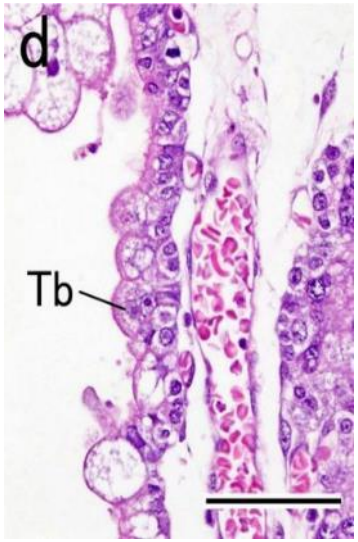
Classification Based Upon the Fetal-Maternal Interhemal Barrier

Epitheliochorial



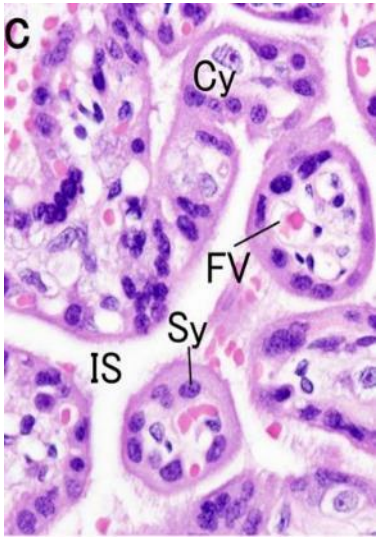
Minipig

Endotheliochorial

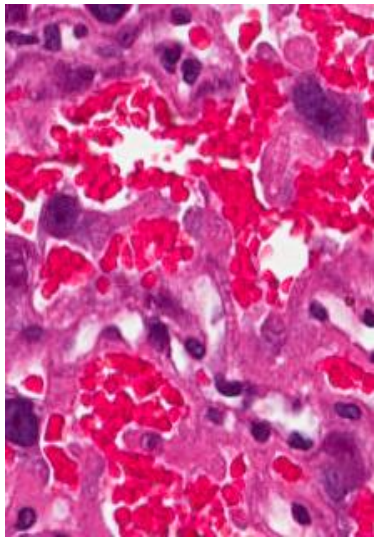


Dog

Hemochorial



Primate



Rat

J Toxicol Pathol 2014; 27: 11–18

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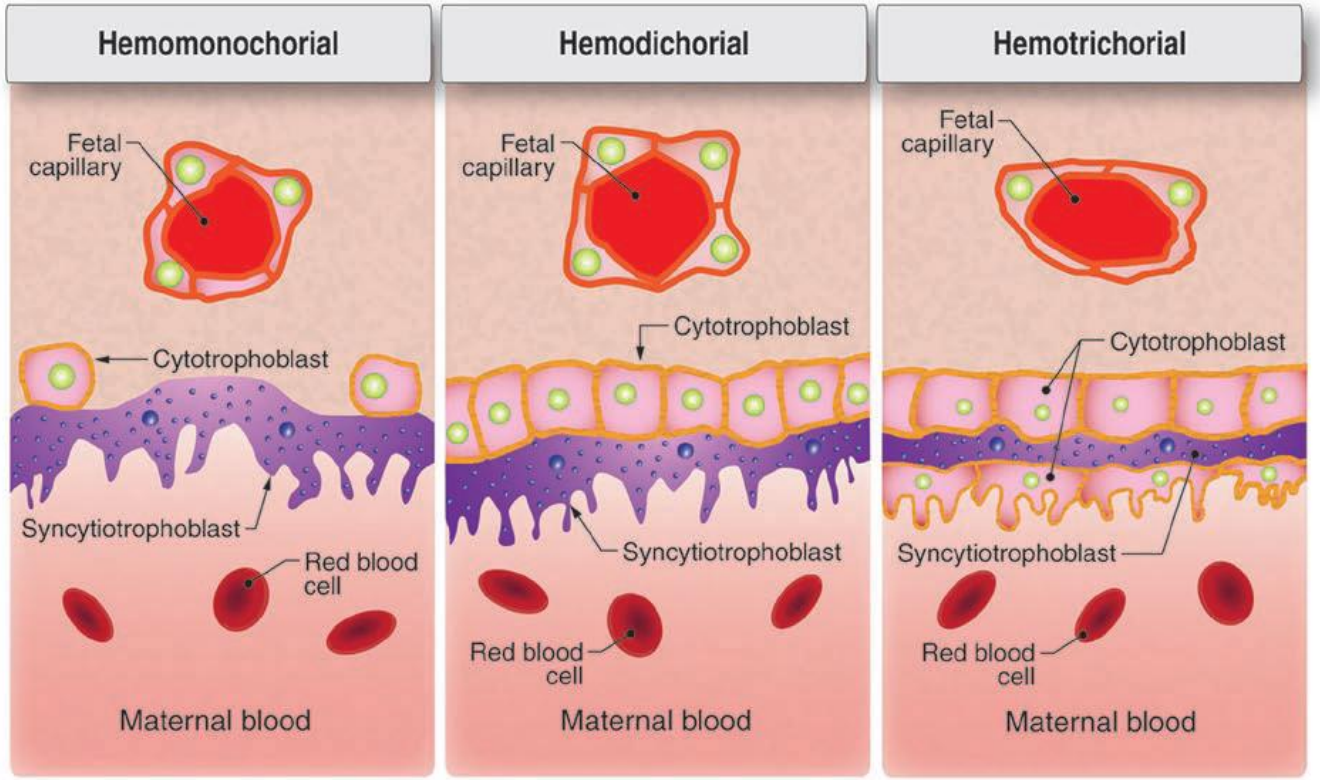
Classification Based Upon the Fetal-Maternal Interhemal Barrier

Hemochorial –further classification



Human, Non-Human Primate, Guinea Pig | **Rabbit** | **Rat, Mouse, Hamster**

Critical Reviews in Toxicology, 2012; 42: 185–210

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Classification Based Upon the Fetal-Maternal Interhemal Barrier

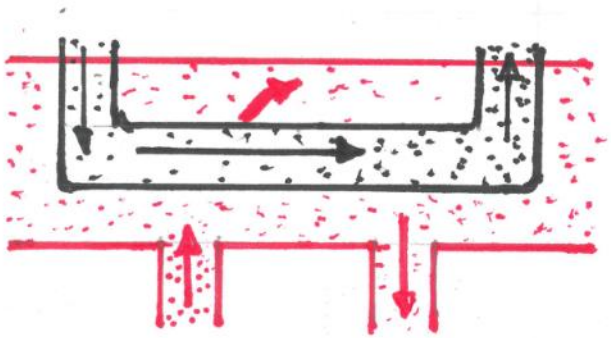
- **Hemochorial placenta**
  - **Advantages**
    - direct access to maternal blood for oxygen–CO_2
    - contact with maternal blood facilitating maternal-fetal transport
    - active receptor-mediated transfer of IgG
    - direct access to the maternal organism for hormones derived from the fetus and trophoblast
  - **Disadvantages**
    - extensive bleeding at parturition
    - greater chance of passage of cells between fetus to the maternal organism organisms
      - microchimerism
      - erythroblastosis fetalis
    - easy passage of xenobiotics from maternal blood

Classification Based Upon the Fetal-Maternal Interhemal Barrier

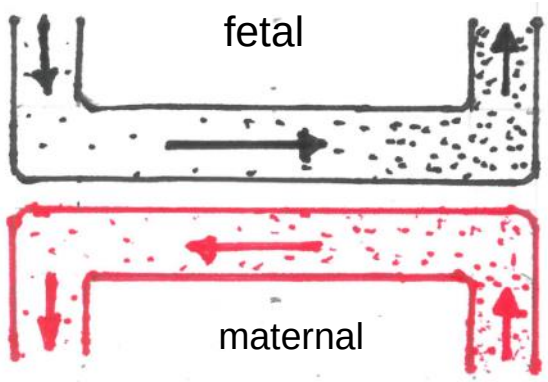
Type of Placentation	Animals	Layers of tissue separating fetal and maternal circulation	Placental IgG Antibody Transmission
Hemochorial	*Humans, NHP*	3	+++
	Rabbit	3	+++
	Rat	3	+
Endotheliochorial	*Dog*	4	+
Epitheliochorial	*Minipig*	6	0

Classification According to Blood Flow

- **Multivillous** ***Humans, NHP***
  - Maternal blood circulates in blood channels



- **Counter-current** ***Rodents, Rabbit***
  - Fetal and maternal capillaries arranged in parallel
  - Most efficient arrangement for passive diffusion



Summary of Placenta Classification

	Yolk Sac Dev	Gross Shape	F-M Interface	Interhemal Barrier
Human	+	Discoid	Villous	Hemo(mono)chorial
NHP	+	Discoid	Villous	Hemo(mono)chorial
Rabbit	+++	Discoid	Labyrinthine	Hemo(di)chorial
Rat	+++	Discoid	Labyrinthine	Hemo(tri)chorial
Dog	+++	Zonary	Labyrinthine	Endotheliochorial
Minipig	+	Diffuse	Folded	Epitheliochorial

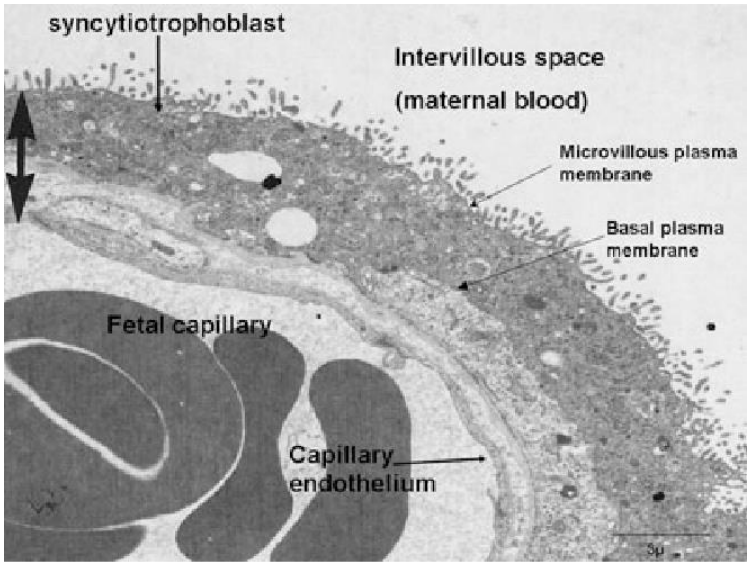
Placental Function- The Trophoblast

Extravillous anchoring trophoblast

Undifferentiated cytotrophoblasts

Invasive intermediate trophoblasts

Syncytiotrophoblasts
Giant trophoblasts
Spongiotrophoblasts



- Transport/exchange
- Endocrine function
- Mediating immune tolerance

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Placenta as an Endocrine Organ - Species Differences

Placental Hormones

- **In all animals trophoblasts produces hormones**
  - steroid hormones
  - protein/peptide
- **Type and quantity of hormones varies between species**
  - *Humans, NHP*
    - substitute for the ovaries in the maintenance of gestation at various times during pregnancy
    - placenta functions as a transient H-P axis during pregnancy
  - *Rats and Rabbits*
    - placenta eclipses the pituitary in the maintenance of ovarian function
- **Endocrine function may be modulated by endocrine disrupters**
  - eg. Chlorpyrifos - deregulation of placental estrogen production

Placenta as an Endocrine Organ - Species Differences

Steroid Hormones

- **Progesterones**
  - The placenta of all mammals produce progesterones
  - Quantity of PG depends on the species
    - *Humans, NHP*
      - Sufficient PG is secreted by the placenta to maintain pregnancy
    - *Rodents, Rabbits*
      - Luteal PG is necessary -placenta does not produce sufficient amounts
- **Estrogens**
  - *In Humans, NHP*
    - placental estrogens are synthesized from androgens
    - maternal estrogen levels are often a useful indicator of fetal well being
  - *Rat, Rabbits*
    - Placenta lacks aromatase (estrogen synthase)
    - Estrogens are produced by the ovary

Placenta as an Endocrine Organ - Species Differences

Dependence of pregnancy on maternal ovarian and pituitary secretion in different species

Species	Duration of pregnancy	Days of pregnancy when hypophysectomy without effects	Days of pregnancy when ovarioctemy without effects
Human	280	?	40
NHP (CM)	155	29	21
Rabbit	31	Term	Term
Rat	20-21	11	Term
Pig	114	Term	Term
Dog	61	Term?	Term

Placenta - Immune Adaptations in Pregnancy

Immune Adaptations in Pregnancy

- **Local Immune Responses (placenta + uterine mucosa)**
  - Trophoblasts
  - Decidual cells?

- **Systemic Immune Responses**
  - Innate
  - Adaptive

Species differences

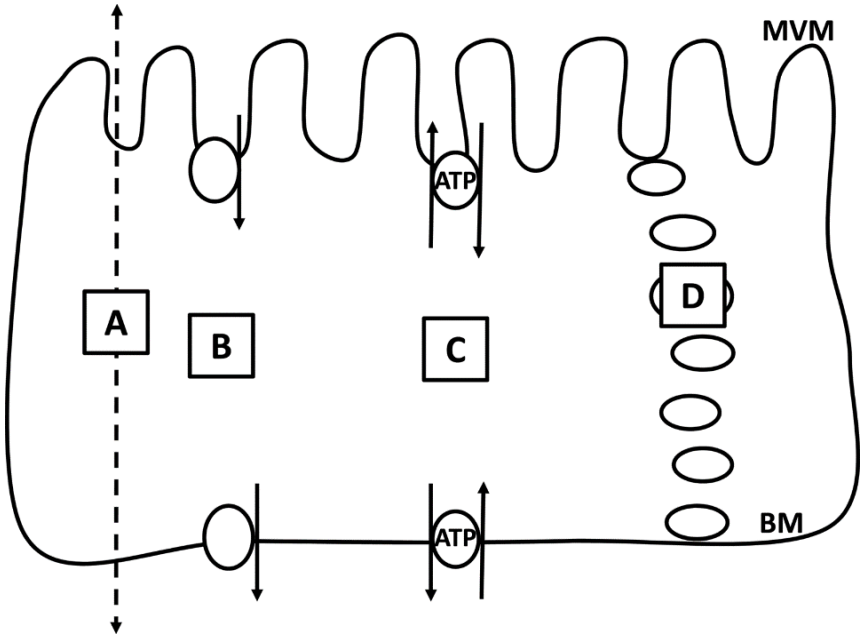
- Humans, NHP, Rodents, Rabbits (Hemochorial placenta)
  - Intimate contact between the maternal immune system and fetal tissue
- Dog (Non-hemochorial placenta)
  - No intimate contact

Placental Transfer of Xenobiotics

- **Considerations**
  - **Type of molecule**
    - Small molecular weight drug or chemical (<1000 Da)
    - Non-antibody biopharmaceuticals (>1000 Da)
    - Fc-containing antibodies or IgG-like biopharmaceuticals (>1000 Da)
  - **Species differences**
    - Humans v Lab animals used in DART studies

Placental Transfer of Small Molecules

- **Passive diffusion**
- **Carrier-mediated diffusion**
- **Assumption**
  - small molecules diffuse passively across the placenta
  - fetal blood concentrations are similar to those of the mother

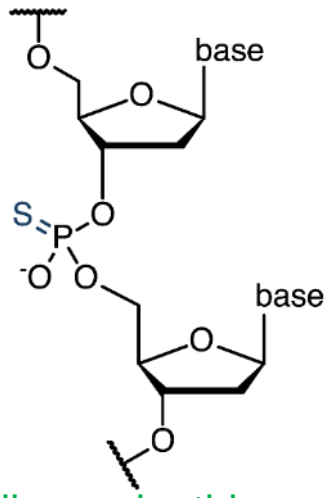


The typically used experimental animals are adequate models to assess the likelihood and extent of placental transport

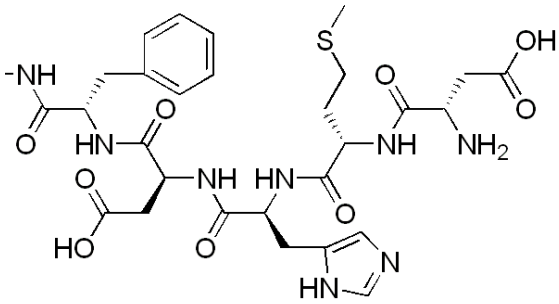
Potential supplemental transfer by means of the inverted yolk sac placenta in rodents and rabbits during period of organogenesis?

Placental Transfer of Non-Antibody Biopharmaceuticals

- Molecules cannot be efficiently transported by simple diffusion across intact epithelia
- Developmental toxicity unlikely to involve direct exposure to the conceptus
- Species- and molecule-specific differences in placental physiology unlikely to affect the risk assessment.



Oligonucleotides



Peptides

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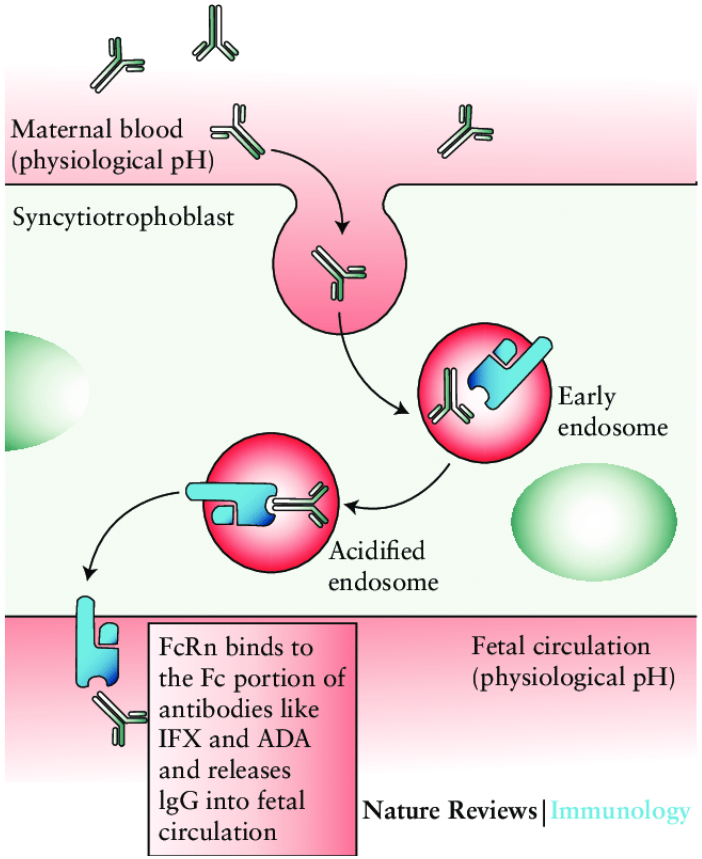
Placental Transfer of IgG-like Biopharmaceuticals

- A common physiological means for placental transfer of IgG exists
  - via FcRn-mediated active transport
    - Hemochorial placenta
    - Yolk Sac Placenta
    - Endothelial cells??
- Important differences
  - species
  - gestational stage



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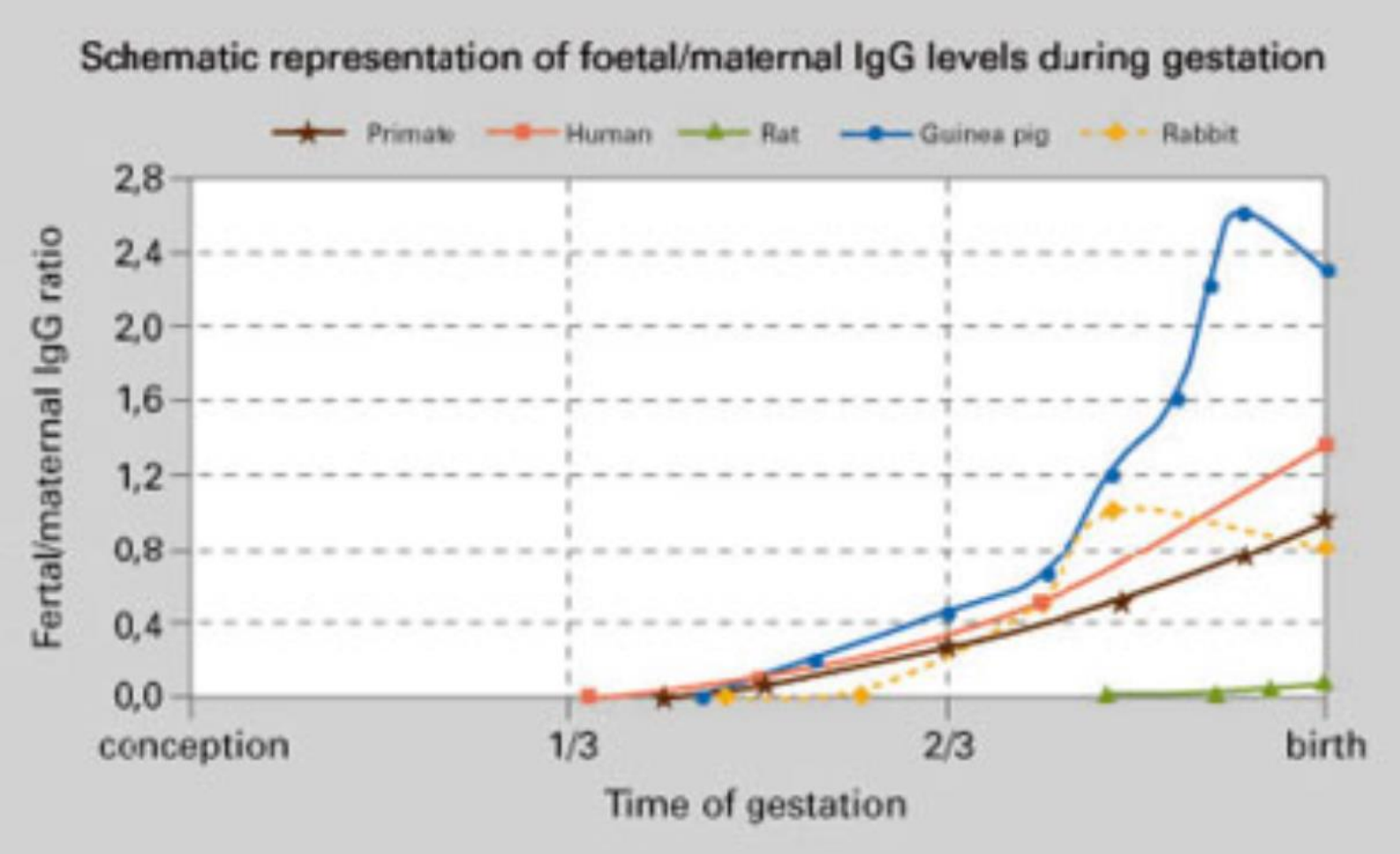
Placental transfer of Ig and Ig-like biopharmaceticals

Pre- and Post-Natal IgG Transfer in Various Species		
	Absorption by the young	
	Prenatally	**Post-natally**
Rodents (rats, mice)	Inverted yolk sac, FcRn	Proximal small intestine, FcRn
Guinea pigs	Inverted yolk sac, FcRn	No significant transfer
Rabbits	Inverted yolk sac, FcRn	No significant transfer
Nonhuman primates	Placenta, FcRn	Fetal gut (1–2 days after birth), FcRn
Humans	Placenta, FcRn	Fetal gut (1–2 days after birth), FcRn

Species selection for Ig-like biopharmaceticals



Birth Defects Research 86:328–344 (2009)

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Conclusions

- **Choice of animal model**
  - Placentae exhibit wide interspecies differences in morphology and function
    - Rat, Mouse, Rabbit - Inverted visceral yolk sac placenta
  - Ideal animal model should be similar to the human placenta
    - Fetomaternal interdigitation
    - Trophoblast invasion and placental blood flow
    - Placental endocrine and immunological function

Choice of experimental animal should depend on the aspect of placental function that should be most similar to the human placenta

Conclusions

- **Accurate extrapolations of test animal data to the human**
  - **Case-by-case basis**
    - Knowledge of the mechanisms underlying species-specific embryonic disposition
    - Knowledge of the pharmacokinetic characteristics of the test compound
    - Knowledge of the critical periods of susceptibility
  - '*The physico-chemical properties of the test substance, and when available, TK data, including placental transfer and milk excretion, should be taken into consideration when evaluating the test results. – '(OECD TG 443)'*

Thank you for listening.....