The Mouse Placenta

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Today’s Discussion Outline
• Comparative anatomy & histology
• Embryonic development of the mouse placenta (extra-embryonic tissue)
• Normal histology
• Histopathology - lesion classification
• Developmental abnormalities
• Infectious diseases
• Toxicologic pathology
• Tumors
• Resources

Origin of the Terminology “placenta”
Modern Latin from L, literally, a cake from Classical Greek *plakounta*
Πλακούντας
What is a Placenta?

Classification of The Placenta by Gross Appearance
Satoshi Furukawa et al, Nissan Chemical Industries, J Tox Path 27: 11, 2014

Horses, pigs
Ruminants

Carnivores

Primates, rodents

Classification of The Placenta by Histology
S Furukawa et al, J Tox Path 27: 11, 2014

Horses, pigs
Carnivores

Primates
Rats, mice

Ruminants

Rabbits
Extra-embryonic Tissues

Uterus & Embryo Necropsy Sheet
From Ward & David-Henneman 2000

Placenta Dissection & Trimming
use formalin fixation

SC Pang et al.
In BS Coy et al.
Guide to Investigation of Mouse Pregnancy 2014
Fixation and Trimming of The Placenta

IHC, ISH: paraformaldehyde or formalin
Imaging: formalin or other fixatives

The intact placenta is fixed in formalin (48 hrs), not Bouin’s

The Pregnant Mouse Uterus

E 8.5
E 12.5
E 14.5

Mouse Placental Development
The Mature Mouse Placenta E12.5

Embryonal & Maternal

Decidua

Yolk Sac

Chorionic plate

Labyrinth

Embryonic erythroid cells

Embryonic Labyrinth

Trophoblasts (LTB)

Maternal endothelium

Dam's rbc

Maternal rbc

Embryonic rbc

Labyrinth E10

Labyrinth E11
Placental Vascularization – micro-CT

Expansion of the uteroplacental arterial circulation during pregnancy imaged by micro-CT.

Glycogen Cells - E14
a trophoblast cell of unknown origin with unknown functions
Placental Aging/Degenerative Changes

Membranes

Decidua
Immune Functions of The Placenta

The Metrial Gland in The Rat
C Picut et al. Tox Path 37: 474, 2009

Perforin IHC
NK marker

Mouse Granulated Metrial Gland?
D. Bulmer et al, Cell Differentiation 20: 77, 1987
Mouse Placental Gene Expression

DG Simmons, In Guide to Investigation of Mouse Pregnancy
BA Croy et al, Eds

Table 1. Mouse Placental Gene Expression

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Marker</th>
<th>Developmental Stage/Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Labyrinth TBs</td>
<td>Endothelium - MECA-32, CD31</td>
<td></td>
</tr>
<tr>
<td>Labyrinth and Junction TBs</td>
<td>Spongiotrophoblasts - SOS-2</td>
<td></td>
</tr>
<tr>
<td>Decidual cells</td>
<td>MAC2</td>
<td></td>
</tr>
<tr>
<td>TB giant cells</td>
<td>Prolactin</td>
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</tbody>
</table>

Genes Expressed in Mouse Placenta

Endothelium - MECA-32, CD31
Spongiotrophoblasts - SOS-2
Decidual cells – MAC2
TB giant cells - Prolactin
The Placenta Is Important For Embryonic Growth From E9-12

If it develops abnormality or fails, the embryo will die due to lack of normal nutrition received from the dam through the placenta.

Possible Causes of Embryonic Mortality E9-12

- Placental failure
- Membrane abnormalities
- Cardiovascular abnormalities
- Abnormal embryonal blood vessel development
- Heart failure
- Abnormal erythropoiesis (in yolk sac/embryo)
- Loss of normal cell cycle regulation
- Cell adhesion/germ layer defects/patterning
Placental Failure As A Cause of Embryonic Lethality

- Inactivation of a gene important for placental development and function
- Toxic or other changes to specific placental anatomical components
- Functional changes in the placenta that interfere with nutrition and oxygenation of embryo
- Changes that interfere with normal embryo development
- Most placental changes cause embryonic lethality prior to birth

How to Evaluate Placental Causes of Embryo Lethality

- Determine patterns of gene expression in placenta, membranes and embryo at various stages of gestation – ISH/IHC/Northern blotting/other methods
- Determine cells and anatomic placental/embryonal structures normally expressing the gene
- In null mice, these cells may not function normally for placental development
- Null mutation → abnormal placenta development → embryonic death

Jackson Laboratory Mammalian Phenotype Browser
http://www.informatics.jax.org/vocab/mp_ontology/MP:0001711
558 genotypes
1214 annotations
Which Comes First?
Placental Lesions or Embryonic Lesions
or Are They Concurrent?

Patterns of Lesions in the Placenta

Developmental Abnormalities in The Mouse Placenta

- Labyrinth blood vessel formation (94 genotypes) - Vhl,
  Tfeb, Dlx3, Pparγ, Mash2 (Ascl2), Hgf, Egfr, Lifr, Il11ra, Err-
  1, JunB, Hsp90β, Cyr61, Rap250 (Ncoa6)
- Labyrinth trophoblasts - Vhl, Egfr, Ascl2, Rara, Arnt, Lifr, Err-
  1, Linfra (Mdfi), Hsd17b2, Cx26 (Gjb2)
- Spongiotrophoblasts - Arnt
- Giant cell trophoblasts - Mdfi, Cdkn1c
- Giant placenta – cloned mice, Cdkn1c
- Decidua - Il11ra
Blood Vessels of the Placental Labyrinth

- Angiogenesis – the formation of new blood vessels from pre-existing blood vessels (endothelium)

- Vasculogenesis - the formation of new blood vessels when there are no pre-existing ones; formation of new blood vessels from mesenchyme, in mouse embryo from the chorionic plate (CP)

No Nulls At Birth
DNA Analyzed From Yolk Sac
+/+, +/-, but no -/- (no yolk sac found grossly or in HE slides)

Vascular Lesions in the Placenta

+/

Vhl -/

Dead Embryo

Hemorrhagic placental sites
3/12 were KO

E12.5
Lack of normal vasculogenesis
disruption of normal placental development
Laser Capture Microdissection Performed

16 yolk sac laser capture microdissection-procured DNA analyzed
14 were -/-
2 were +/-

Abnormal Vasculogenesis of The Labyrinth
CD31 IHC

CD31 (formalin, Santa Cruz M-20, goat anti-mouse, 1:500, antigen retrieval)

Trophoblast Degenerative Lesions

Necrosis, Dysplasia, Hyperplasia
Syncyial Labyrinth Trophoblasts

Eosinophilic Droplets
Abnormalities of Cell Cycle Regulation

- Increased apoptosis
- Cell proliferation (Rb1 in placenta)
- Imbalance between apoptosis and cell proliferation
- Brca1, Brca2, Rad51, Braf, Kras, Ski, p130 (Nolc1), DNA ligase IV (Lig4)
- Cell cycle abnormalities were usually reported in the embryo and not placenta

Trophoblast Hyperplasia from Rb loss in Tb stem cells

Placentomegaly of Cloned Embryos

Expansion of spongiotrophoblasts with increased glycogen cells
Abnormalities of The Yolk Sac

- Mesodermal defects - alpha-5-integrin (Itga5), Fn1
- Endodermal defects – Fn1, Hand1
- Vascular hypoplasia - Itga5, MEF2C
- Vascular dysgenesis - Myc, Hand1, Np1/Np2
- Dysgenesis of erythropoiesis - Tf, Scl, Tgf-β1
- Hypoplasia of vitelline vessels - Tf, c-myc, Scl

Yolk Sac lesions

Defects in Vasculogenesis & Hematopoiesis

No vessels or no blood?

Ballooning degeneration, apoptosis – E16

Defective haematopoiesis and vasculogenesis in transforming growth factor-beta 1 knock out mice

MC Dickson Development 121: 1845, 1995

MC Dickson
Development
121: 1845, 1995
Abnormalities of the Chorion & Allantois

- Lack of or abnormal fusion - Vcam1, alpha-4 integrin (Itga), Rbp-Jk, Smad1, Lpp (Ppap2b)
- Mesodermal defects - Mash2 (Ascl2)
- Allantoic ballooning - alpha-4 integrin (Itga)
- Chorionic ectoderm defects - Hand1
- Allantoic defects - Myc

Absence of Chorioallantoic Fusion

VCAM-1 null, GC Gurtner et al, Genes Dev 9:1, 1995

Infectious Disease

- Bacteria (Brucella, Streptococcus, Listeria, Mycobacterium, Treponema)
- Fungi (Candida)
- Viruses (CMV, Zika, Herpes, HIV, others)
- Protozoa (Trypanosomes, Plasmodium)
Placental Pathology of the Pregnant Mouse Inoculated with *Brucella abortus Strain 2308*


BALB/c mice, ip at E9, sac at E18, necrosuppurative placentitis

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Massive Decidual Necrosis: *Plasmodium chabaudi*


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Zika Virus Infection during Pregnancy in Mice

Causes Placental Damage and Fetal Dementia


Normal mice – clear virus

Kos -Ifnar1-/- mice (lack of Type I interferon signaling)

develop placental infection (trophoblasts, endothelial cells), and brain infection
Toxicology - Rat Placenta
Chlorpromazine-induced placental toxicity

Tumors of the Placenta
- Choriocarcinoma
- Yolk sac carcinoma
- Deciduoma
- Granular cell tumor?

Summary
How to Determine if Placental Failure Occurs
- E9.5-10.5 determine if embryo is grossly normal
- Determine if there are any histopathological embryonic lesions that could cause death
- Check especially placenta, heart, blood vessels, yolk sac, and any tissues where gene is expressed
- Placenta – determine if lesions found occur prior to early lesions of embryonic death
- Embryonic lethality may occur from lesions in both placenta and embryo
Mouse Placenta References

- Comparative Placentation (K. Benirschke)
  http://placentation.ucsd.edu/

Mouse Embryo References