RhCMV Fetal Infection: A Nonhuman Primate Model for the Treatment of Congenital HCMV Infection

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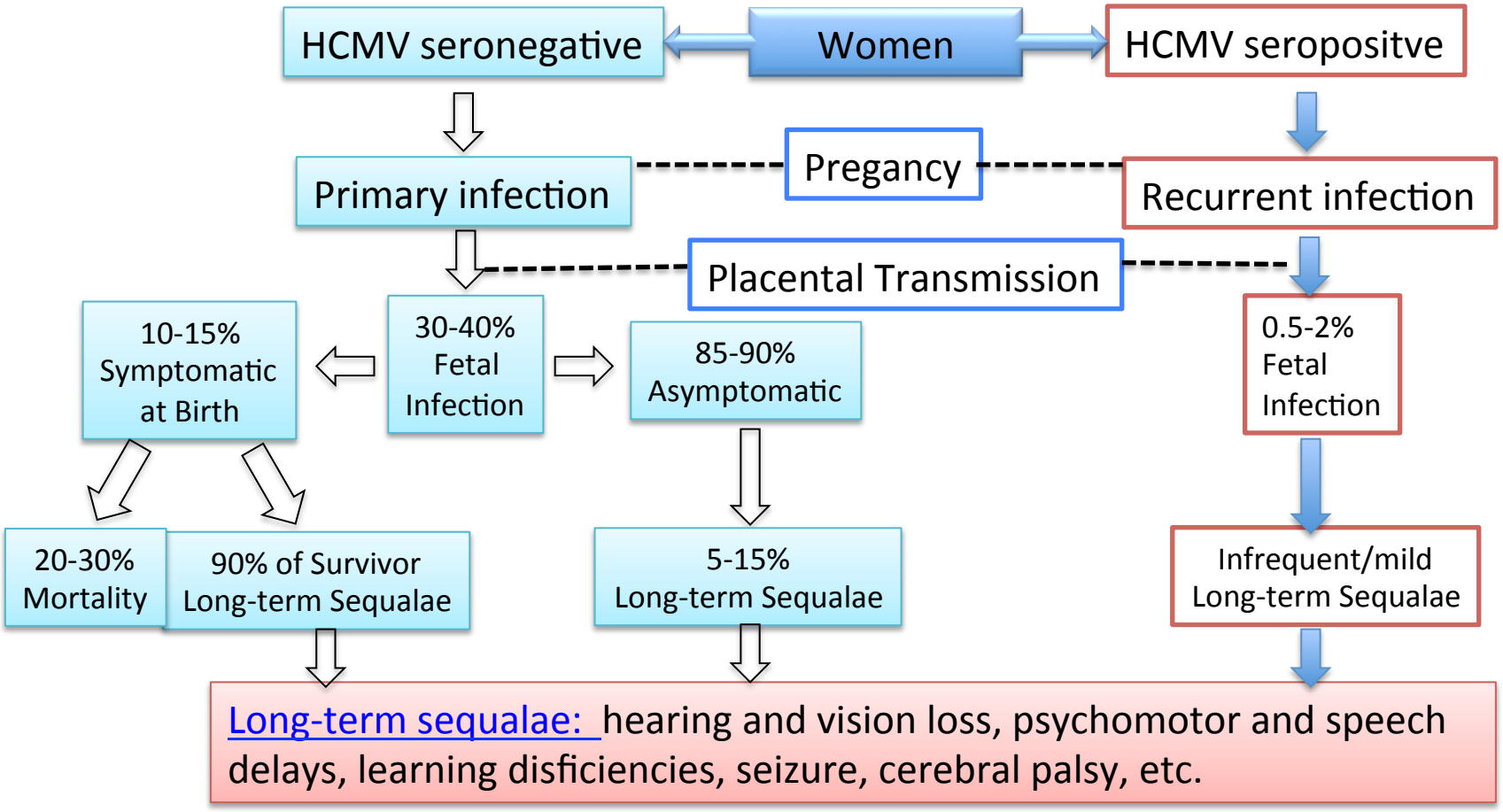
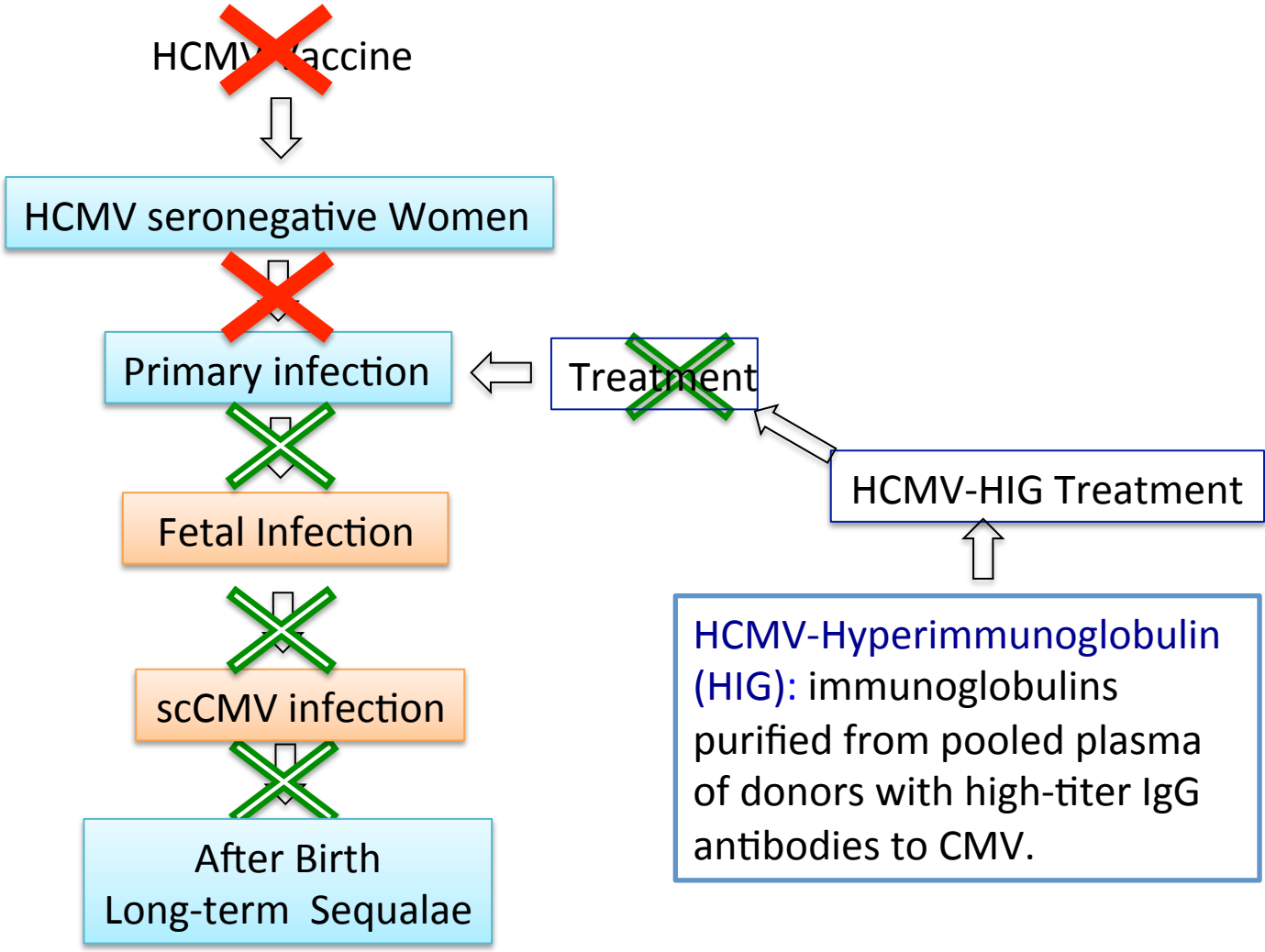
I. Congenital HCMV Infection

HCMV is the most common infectious cause of congenital viral infection, affecting 0.4-2.3% of all liveborns.



-Yinon Y Obstet Gynecol Surv. 2010 65(11):736-43

HCMV Prevention and Treatment



Dilemmas with Regarding to the effectiveness of HCMV-HIG Treatment

Non-random controlled/uncontrolled clinical trials

- Well-tolerated.
- Nigro, et al. 2005 :
  - **Maternal HIG prophylactic treatment: Reduced cCMV rate** 16% (treated) vs 40% (untreated control)
  - **HIG therapeutic treatment :**
    - **Reduced scCMV rate** 3% infants of treated women(1/31) VS 50% of untreated women (7/14).
- Visentin, et al. 2012:
  - **Maternal HIG therapeutic treatment: improved** the outcome of infants at 1-year age: 13% (4/31) infants of treated patients and 43% (16/37) infants of non-treated patients.

A randomized clinical trial (Revello, et al. 2014)

Maternal HIG prophylactic treatment:

- **Did not** significantly decrease the rate of congenital infection:

  30% in the treated group vs 44% in the placebo group (a difference of 14 percentage points; 95% confidence interval, -3 to 31; P=0.13)
- **Did not** improve the clinical outcome of congenital infection at birth
- **Higher number** of obstetrical adverse events. (13% in the treated group vs. 2% in the placebo group) .

Issues related to HCMV-HIG Human Clinical Studies

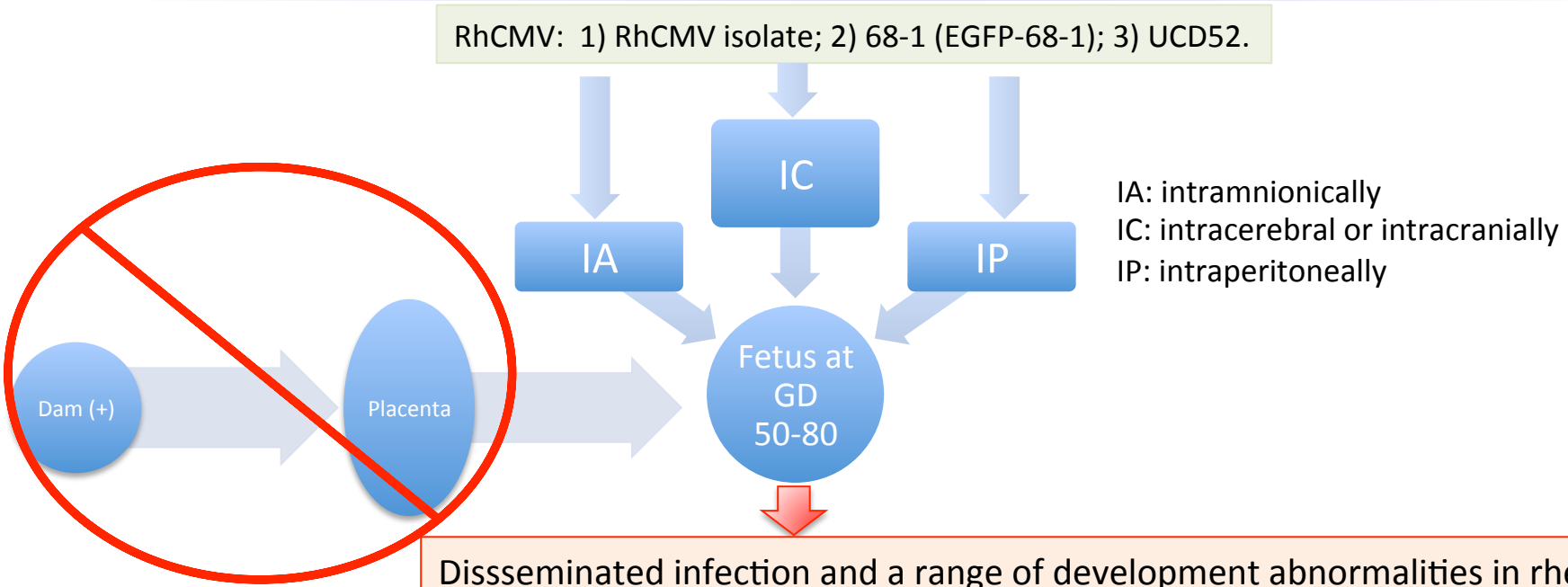
1. Variables :
   - Timing of maternal primary HCMV infection.
   - Maternal viral loads and duration of viremia.
   - Levels of maternal anti-HCMV immunity
   - Timing of fetal infection
   - Fetal viral loads.
2. HIG-treatments:
   - Time, mode, dose and route have not been optimized
   - Differential specificities of CMV-HIG preps.

To address these issues, a relevant model is needed.=> Rhesus Macaque Model?

II. RhCMV Fetal Infection: A Nonhuman Primate Model for HCMV Fetal Pathogenesis

- Similarities between macaques and humans, especially, the reproductive similarity.
- Similarities between RhCMV and HCMV in the aspects of genome, biology, immunity and pathogenesis, including fetal pathogenesis.

RhCMV Fetal Infection

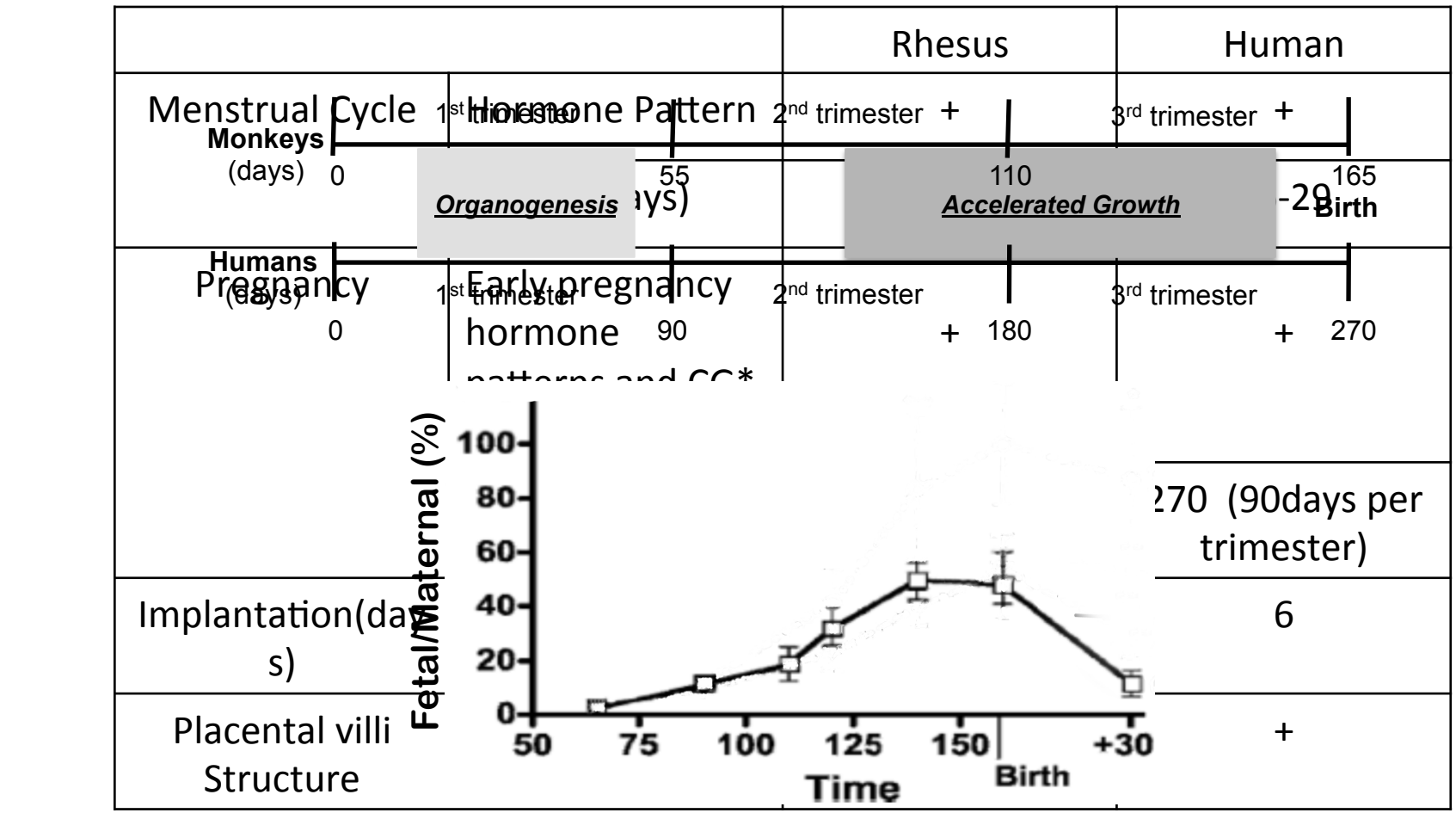


Dissseminated infection and a range of development abnormalities in rhesus fetuses:

1. CNS damage: lepomeningitis, ventriculomegaly, microcephaly, lissencephaly and periventricular calcification.
2. Sensorineural abnormalities: Loss of cellularity within the spiral ganglia, spiral ligament, stria vascularis and organ of Corti, RhCMV positive inclusion-bearing cells, and lymphocytic infiltration
3. Placental abnormalities: deciduitis, infarction, calcification, and lymphocytic infiltration.
4. Multiorgan involvements: splenomegaly, lymphadenopathy.
5. IUGR and hydrops.

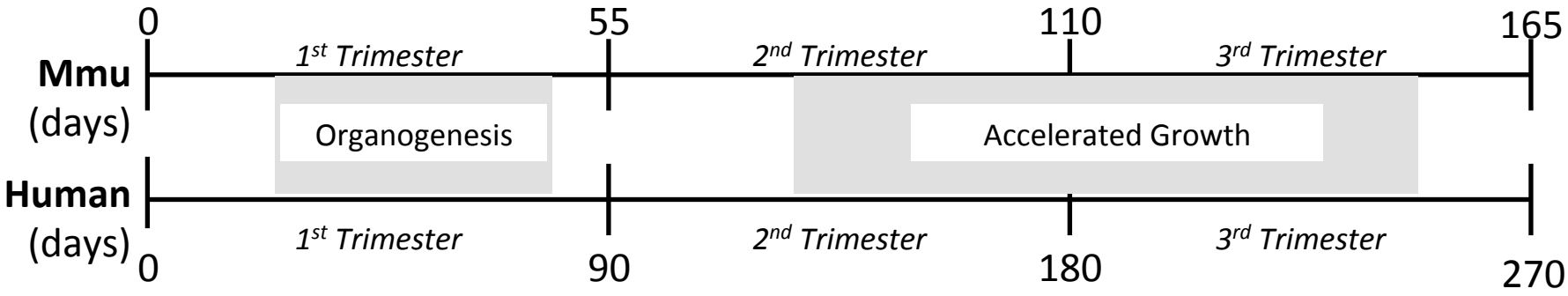
Similarities between Rhesus Macaque and Human in Reproductive Physiology and Endocrinology

	Rhesus	Human
Menstrual Cycle	Hormone Pattern	
Pregnancy	Early pregnancy hormone patterns and CG*	270 (90days per trimester)
Implantation(days)		6
Placental villi Structure		+



*CG: Chorionic Gonadotropin

Fetal Outcome is related to fetal age at the Time of RhCMV fetal Inoculation



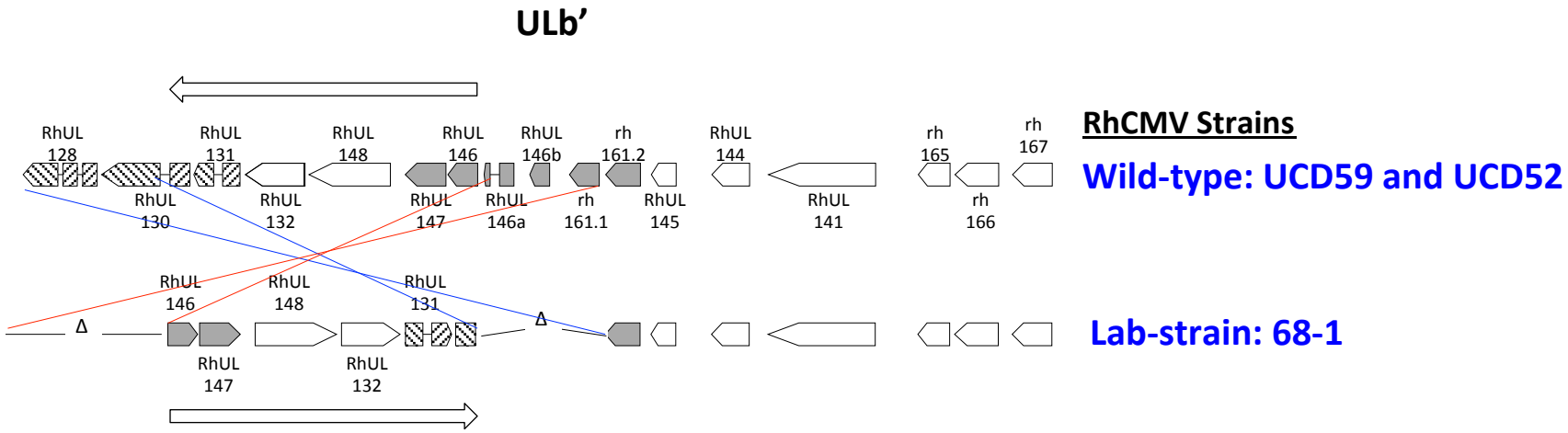
RhCMV 68-1 Fetal Inoculation (IP)

Outcomes following Fetal Inoculation with RhCMV 68-1 (~6X10^5 pfu)	Inoculation Time points	
Outcomes (%)	GD50	GD65
Non-viable fetus	54 (7/13)	14 (1/7)
Fetuses with RhCMV sequelae	77 (10/13)	57 (4/7)

Barry *et al ILAR 2006*

Revello J Clin Virol 2004

Issues with RhCMV 68-1

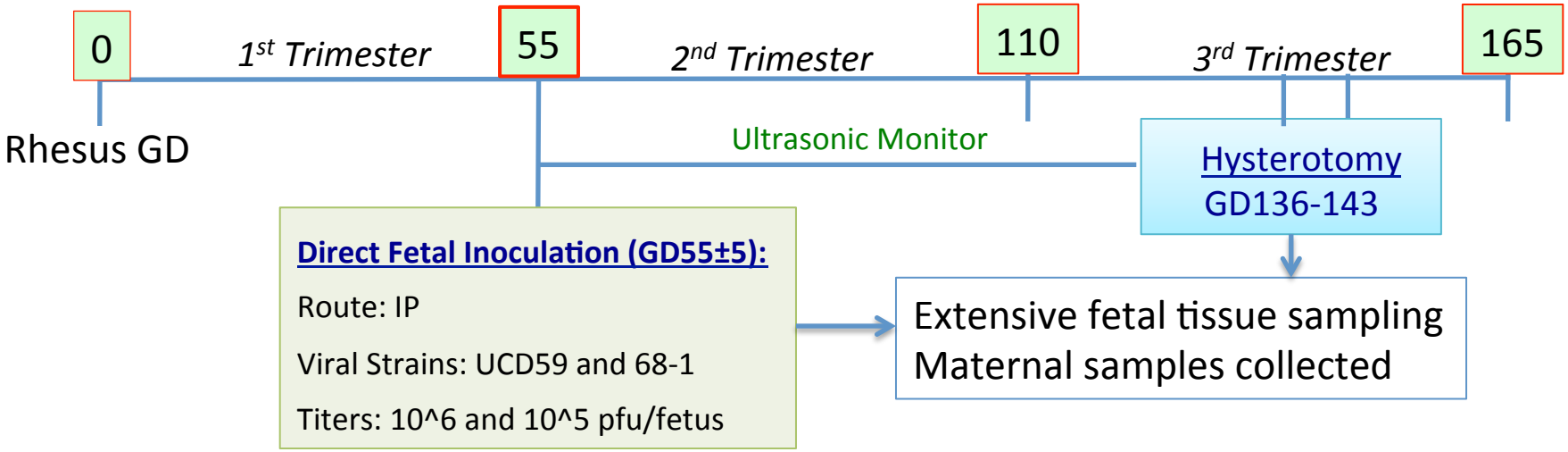


- A fibroblast-adapted lab strain.
- Lost RhUL128 and RhUL130, the two important components for endothelial and epithelial tropism, and three CXC chemokine-like ORF.
- Less pathogenic.

Effects of Different RhCMV Strains and Titers on Fetal Pathogenesis

Experimental Design:

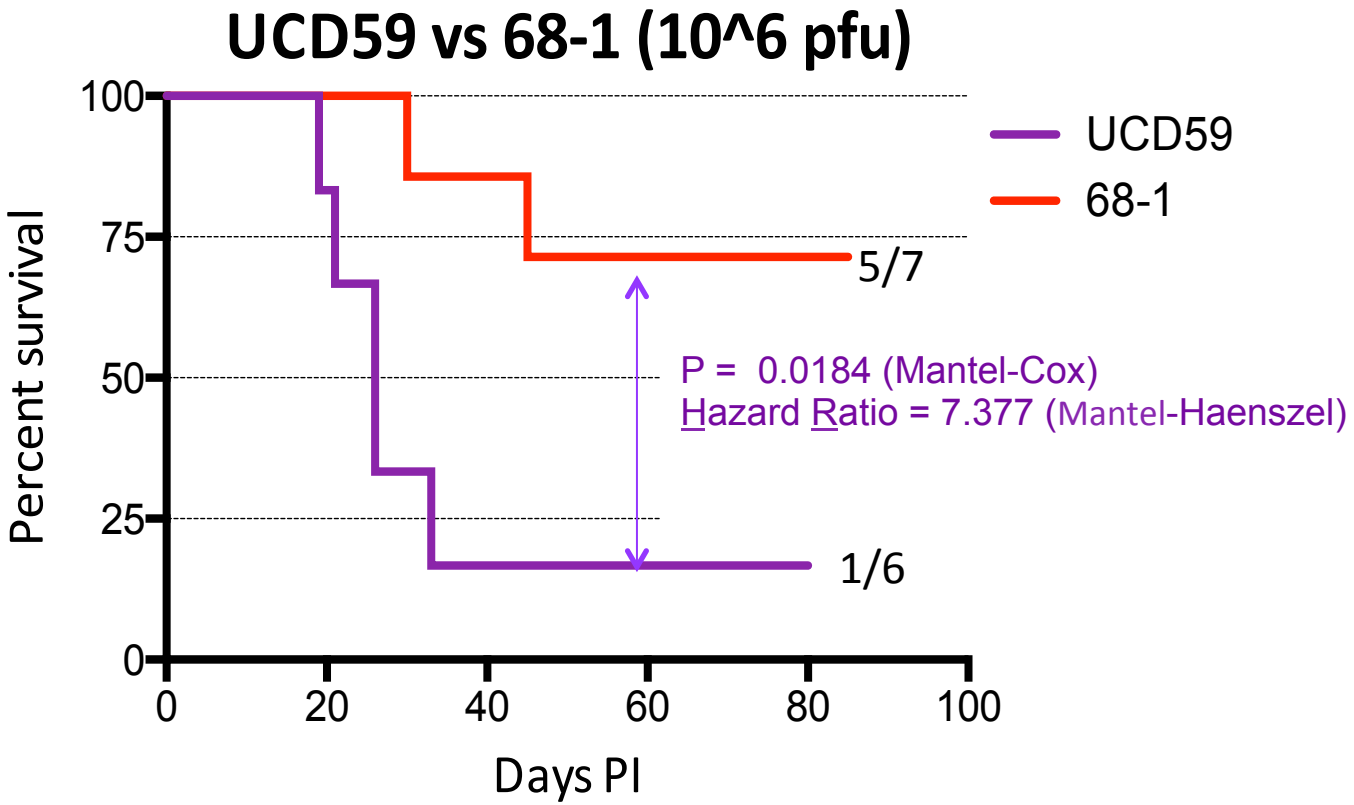
Animals: Time-mated RhCMV-seropositive gravid dams.



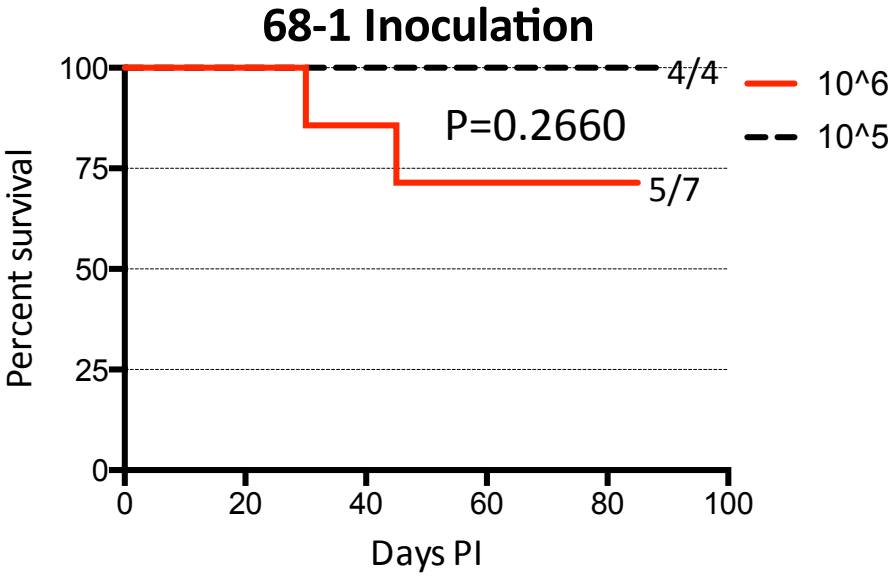
Fetal Pathogenesis:

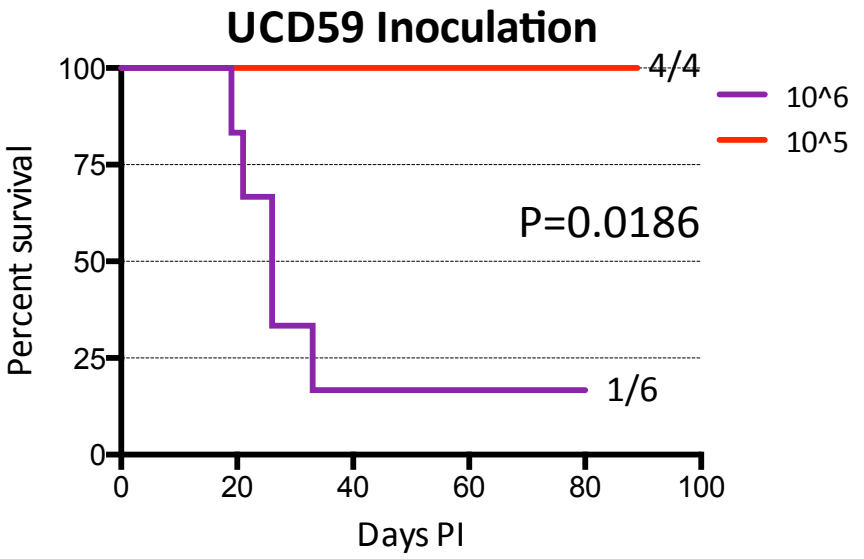
- Survival & Developmental defects
- Gross pathology at Nx
- qPCR (100 ng in triplicate): Limit of Detection: 1-10 copies per reaction
- RhCMV histopathology and IHC

Fetal Survival Analysis



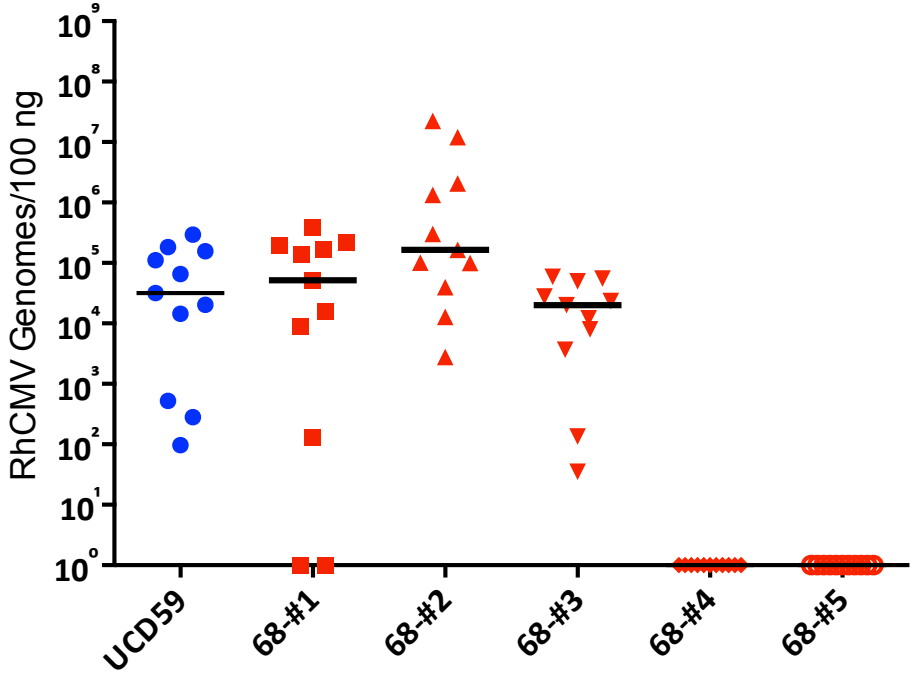
Fetal Survival Analysis



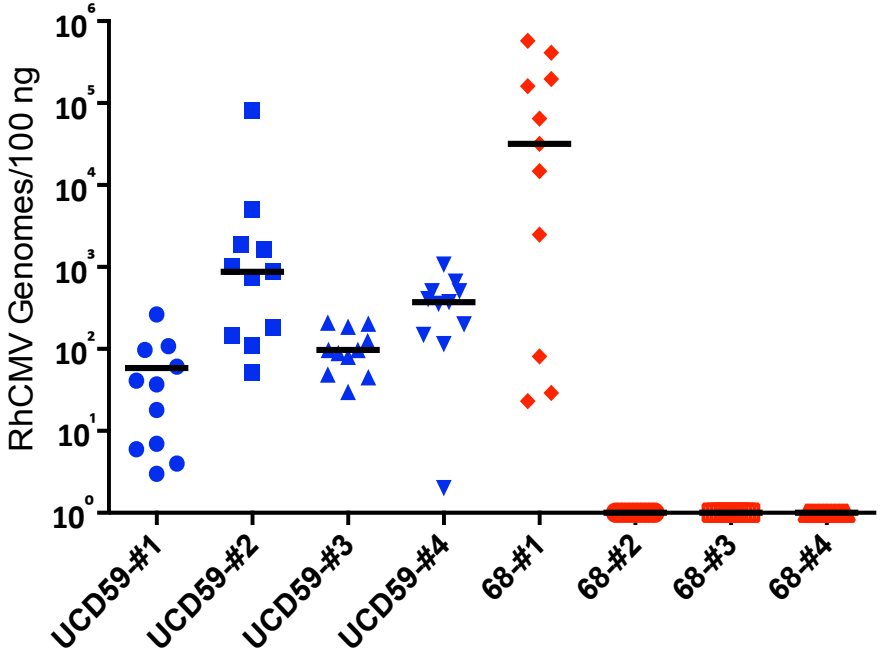


Viral DNA in Fetal Brain

10^6 pfu Inoculation



10^5 pfu Inoculation



Specimen: (n=10)

Parietal Lobe-R & L

Occipital Lobe-R & L

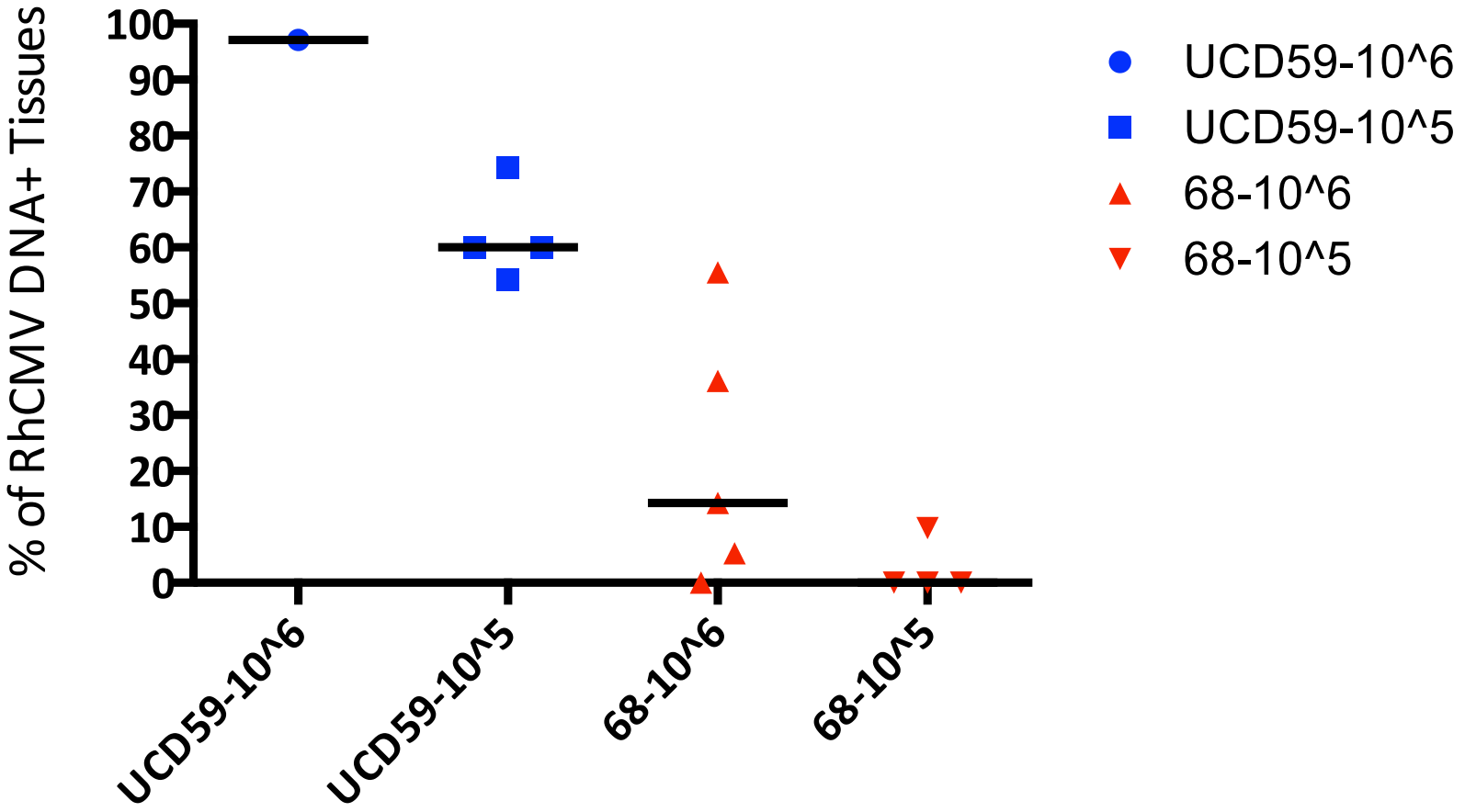
Ventricle-R & L

Frontal Lobe-R & L

Temporal Lobe-R & L

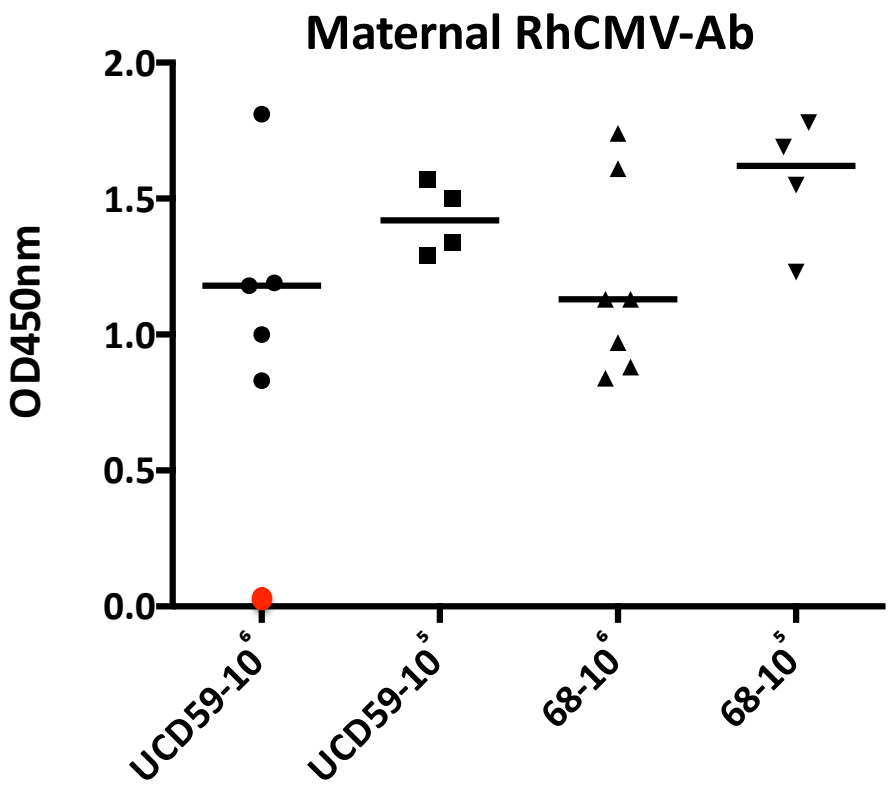
Cerebellum

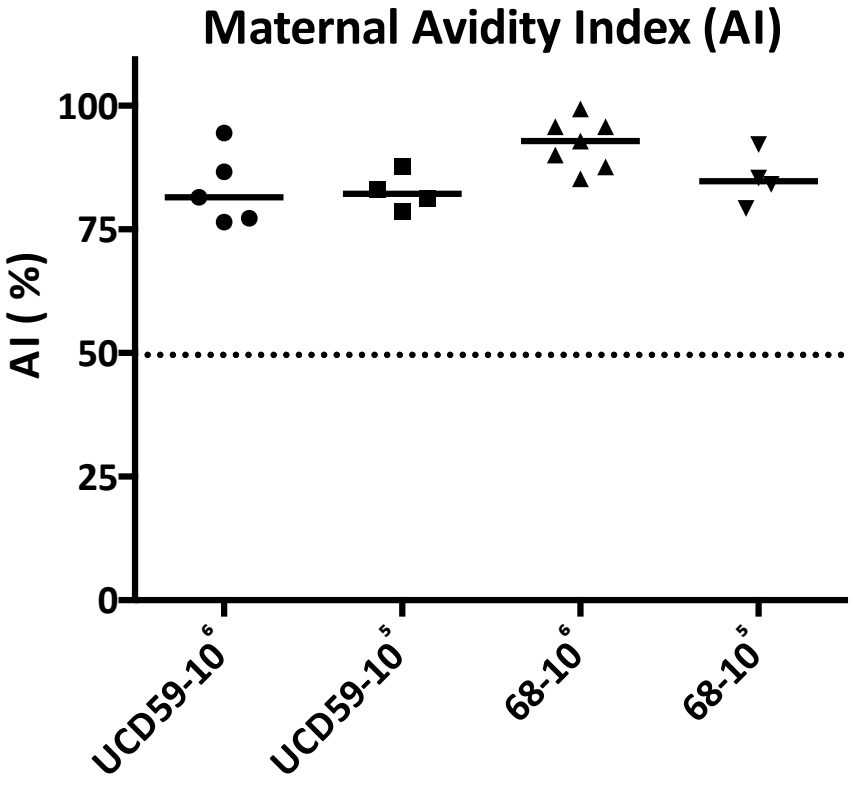
Viral DNA in non-brain Fetal Tissues



Fetal tissues: 35-38
RhCMV genomes: ≥ 10 copies/100ng

Maternal RhCMV-Ab at the Time of Inoculation

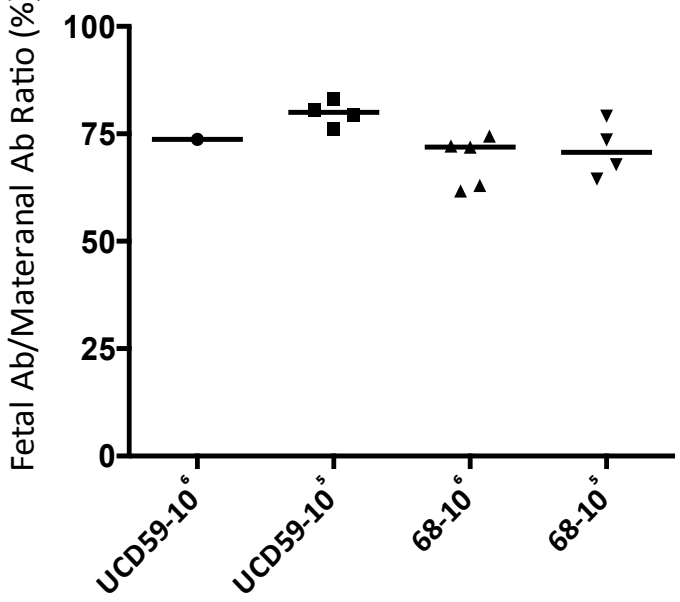




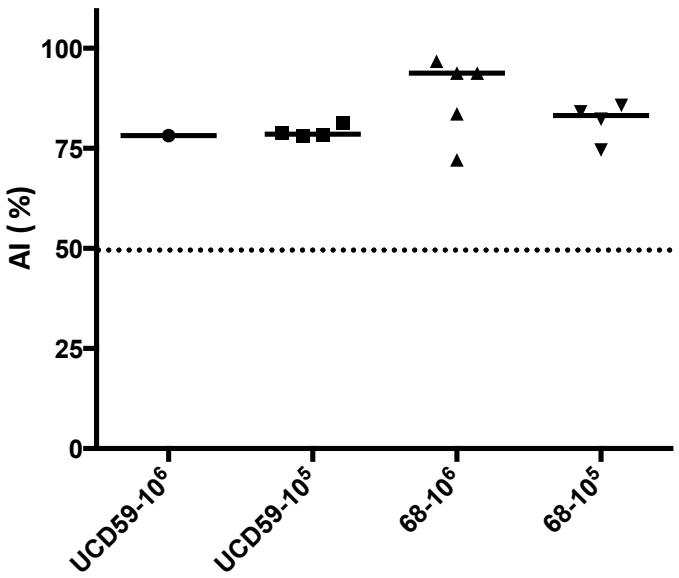
The observed differential fetal pathogenesis are associated with the inoculated viral strains and titers.

Fetal RhCMV-Ab at the Time of Hysterotomy (GD136-143)

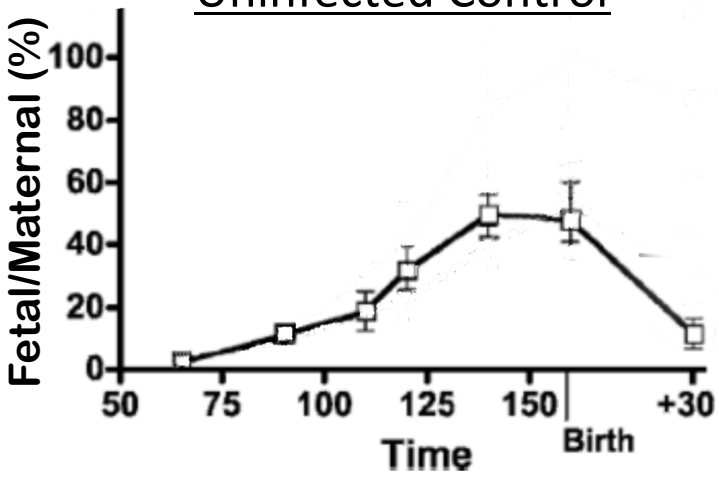
Fetal /Maternal Ab Ratio



Fetal Ab Avidity (AI)



Uninfected Control



Summary

RhCMV fetal infection:

- Recapitulates the broad tissue tropism and pathology observed during congenital HCMV infection.

RhCMV fetal pathogenesis are associated with:

- Time of fetal infection
- Viral infectious dose
- Virulence of infected RhCMV stain

Maternal RhCMV specific Ab:

Certain levels of protection- low viral titers and low pathogenic strains.

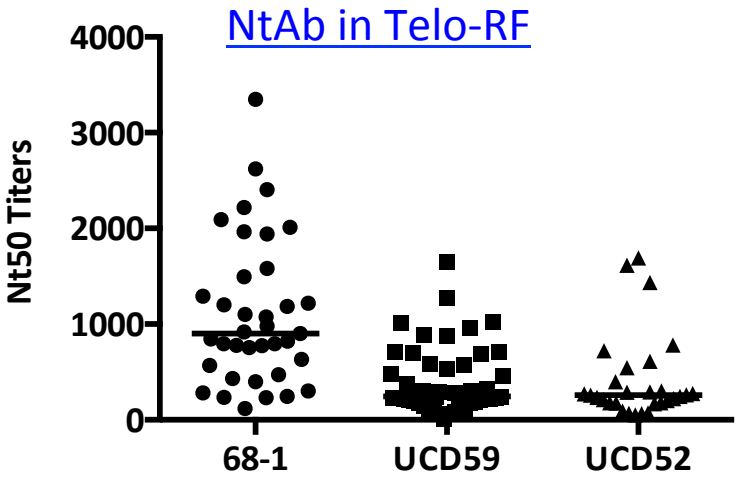
III. Future Study: RhCMV-HIG Treatment of Fetal RhCMV Infection

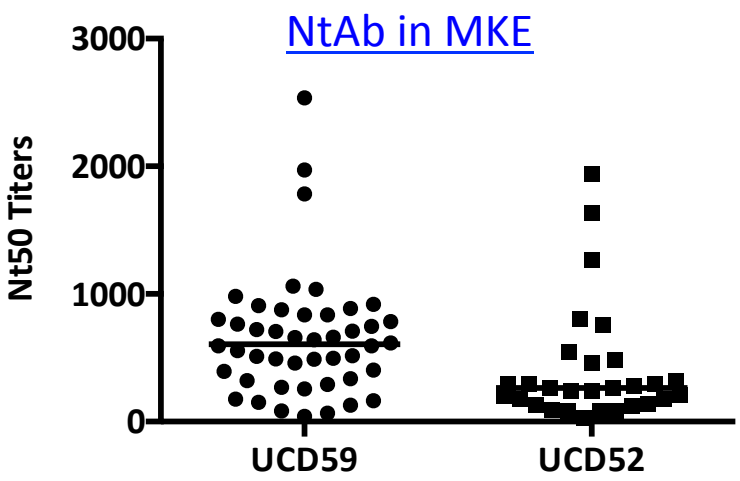
Hypothesis:

With defined fetal viral infection time and dose and maternal immunity, the outcome of fetal RhCMV infection will be largely related to the treatment of RhCMV-HIG either to gravid dams or to fetuses.

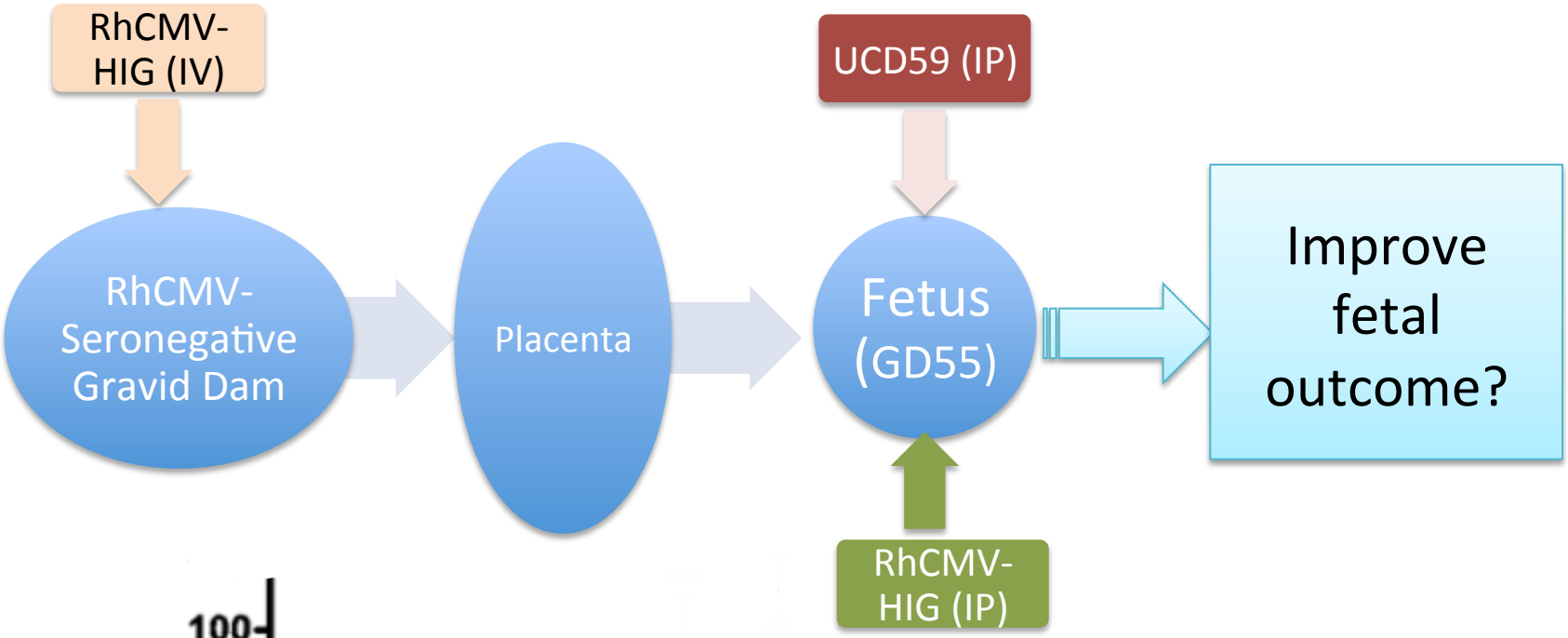
RhCMV-HIG Preparation

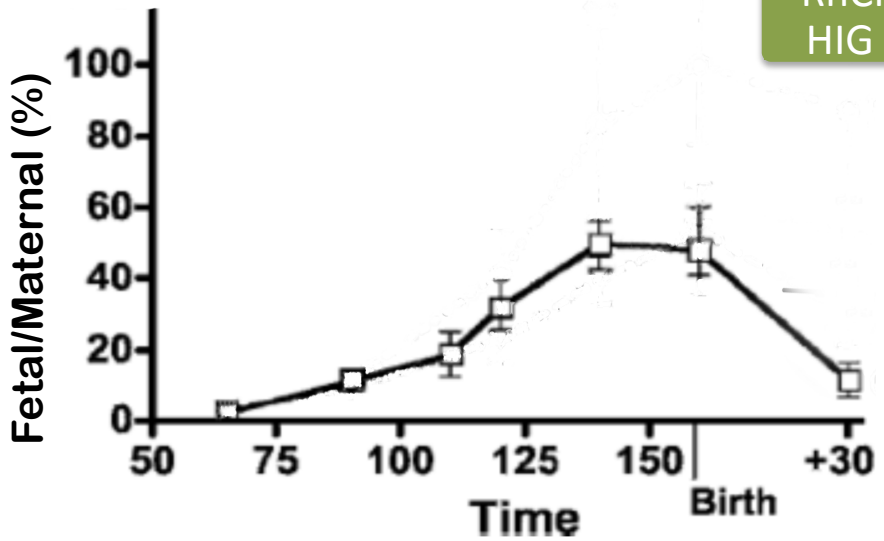
- RhCMV infection is ubiquitous in rhesus macaques.
- Natural infection induced neutralization antibodies (NtAb) to different RhCMV strains (68-1, UCD59 and UCD52) in different cell-types, including fibroblast (F) and epithelial cells (E).
- RhCMV-HIG: prepared from the pool plasma with high NtAb to RhCMV.





A Proposal of RhCMV-HIG Treatment of Fetal RhCMV Infection





Conclusion

- RhCMV fetal infection is a relevant nonhuman primate model for congenital HCMV infection.
- It will be possible to direct evaluate the effectiveness of RhCMV-HIG on improvement of fetal pathogenesis.

Acknowledgement

UC, Davis

Barry's Lab:

Kim Schmidt

Andrew Lehman

Nancy Ngyen

Dr. Alice Tarantal

OHSU

Dr. Louis Picker

Dr. Klaus Früh

NIH Grants

AI094417 AI063356 AI095113 OD011107

Bill and Melinda Gates Foundation

OPP1033121