Microbes: *The Final Frontier*

PRODUCTION OF β-HEMOLYSIN

by Group B Streptococci increases risk of preterm labor, microbial invasion of the amniotic cavity and fetal injury

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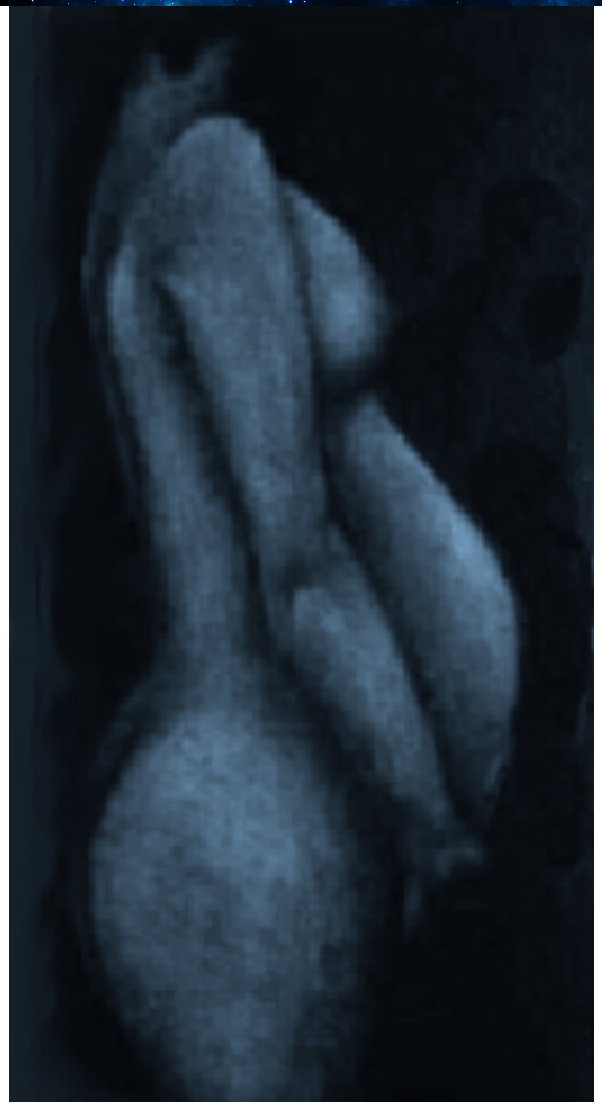
Preterm Births



~1 in 9 births in U.S. are preterm

- Leading cause of neonatal morbidity and death
- Infection and inflammation are major causes of early preterm birth, which impart the greatest risk of fetal injury
- Infected amniotic fluid often contains organisms colonizing the lower genital tract such as Group B Streptococcus (GBS)

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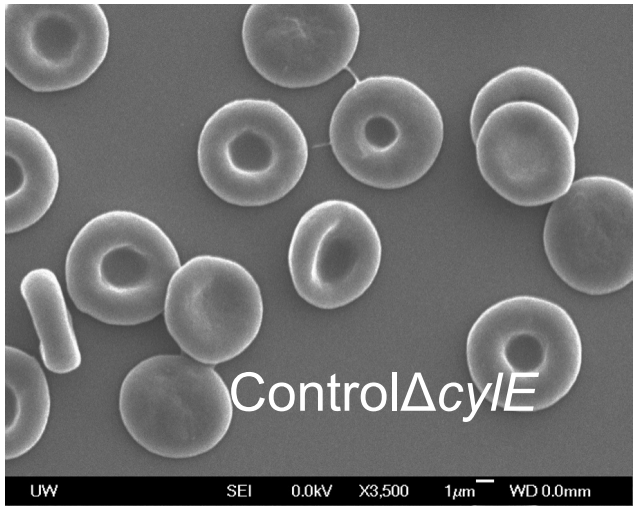
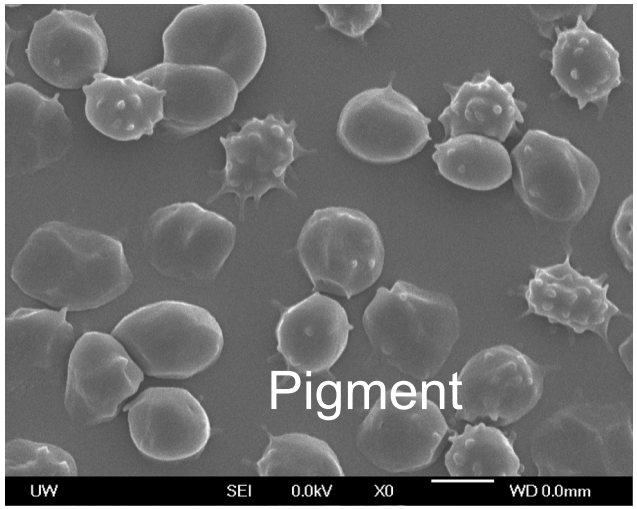
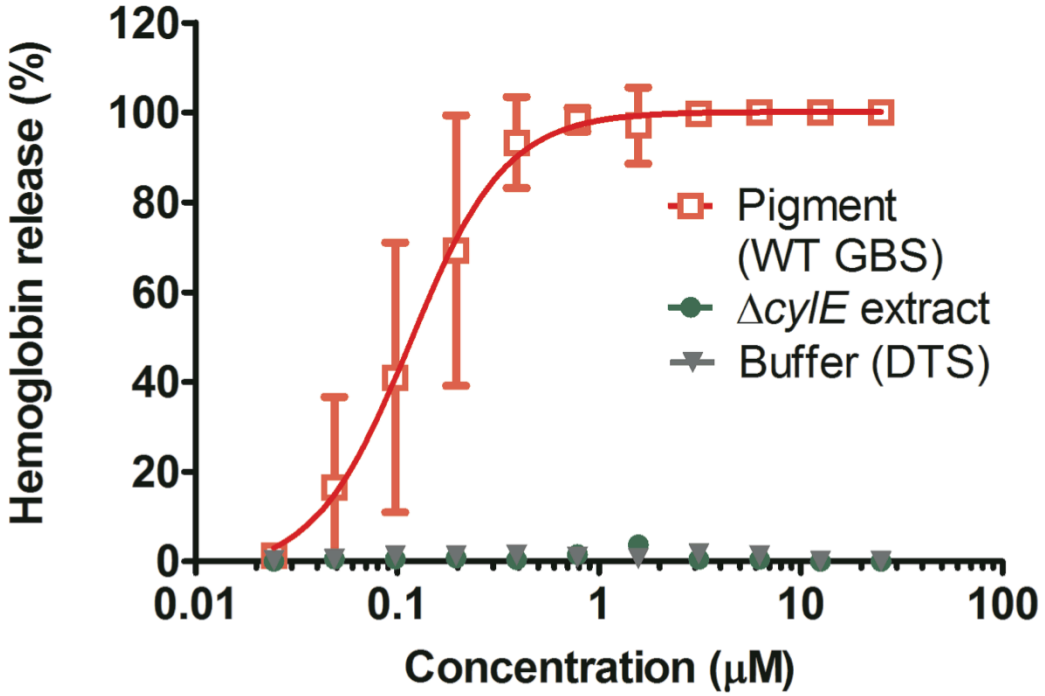
GBS PIGMENT IS HEMOLYTIC







Whidbey et al.
J Exp Med. 2013; 210: 1265

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Women in PTL with Hyperpigmented GBS Strains (covR/S mutations)

Table 1. GBS clinical isolates associated with preterm labor and mutations in *covR/S* locus

Isolate number[a]	Source	Mutation in *covR/S* loci[b]	Gestational age at birth[c]
1	Amniotic fluid	Deletion of adenine residue at position 658 in *covS* resulting in truncation of CovS at amino acid 220 (CovS 220Stop)	30 wk
1	Chorioamnion	Same as above *i.e.* CovS 220Stop	30 wk
2	Amniotic fluid	Deletion of 4 nucleotides 'ATTT' spanning -110 to -107 upstream to the ATG start codon in the promoter region of *covR/S*	34 wk
3	Amniotic fluid	Substitution of adenine instead of guanine resulting in amino acid substitution from valine to methionine in CovS (CovS V343M)	26 wk
3	Blood	CovS V343M	26 wk
4	Chorioamnion	CovS V343M	36 wk
5	Chorioamnion	None	36 wk
6	Chorioamnion	None	28 wk

Identification of many GBS mutations inducing overexpression of the pigment (β-hemolysin) isolated from the amniotic fluid, blood and chorioamnion in women in PTL

Whidbey, et al. J Exp Med 2013; 210 (6): 1265

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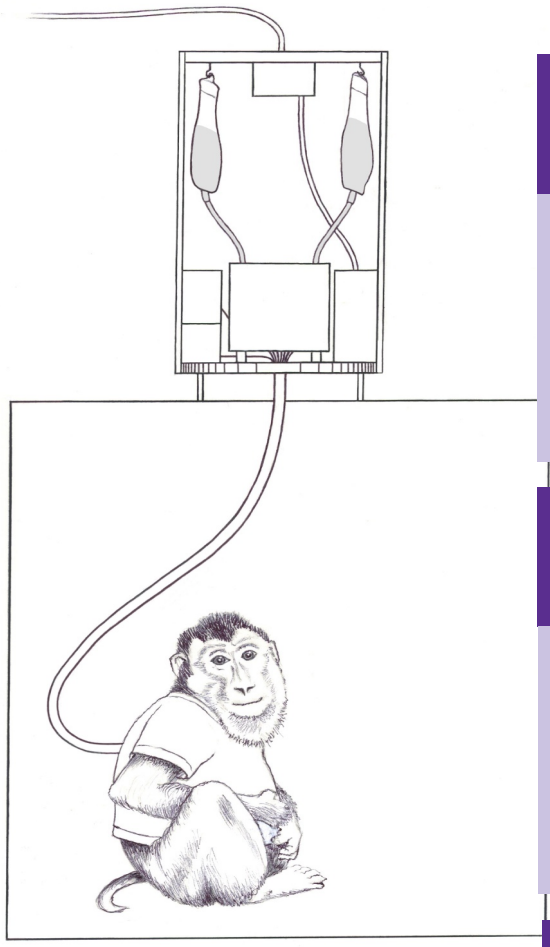
Study Objective

To determine if choriodecidual inoculation with hyperpigmented GBS increases the incidence of:

- preterm labor
- microbial invasion of amniotic cavity
- fetal injury

Experimental Model



Pigtail macaque
(*Macaca nemestrina*)
Term gestation ~172 d

Surgery (118–125 d)

Catheterization of:

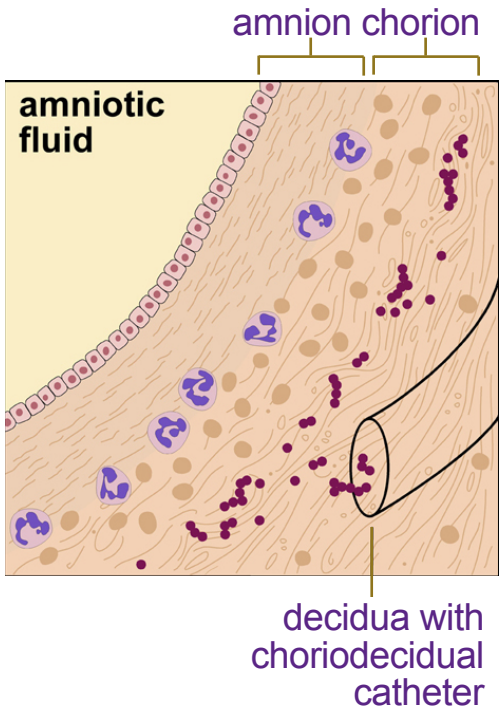
- Maternal vein
- Choriodecidual space
- Amniotic fluid

Intervention (~10–14 d postop)

Choriodecidual inoculation of either:

- GBSΔ*covR* 1 x 10^8 CFU (N=6), or
- GBSΔ*covR*Δ*cylE* 1 x 10^8 CFU (N=5), or
- Saline (N=5)

Delivery 4 days post-GBS inoculation
7 days after saline infusion



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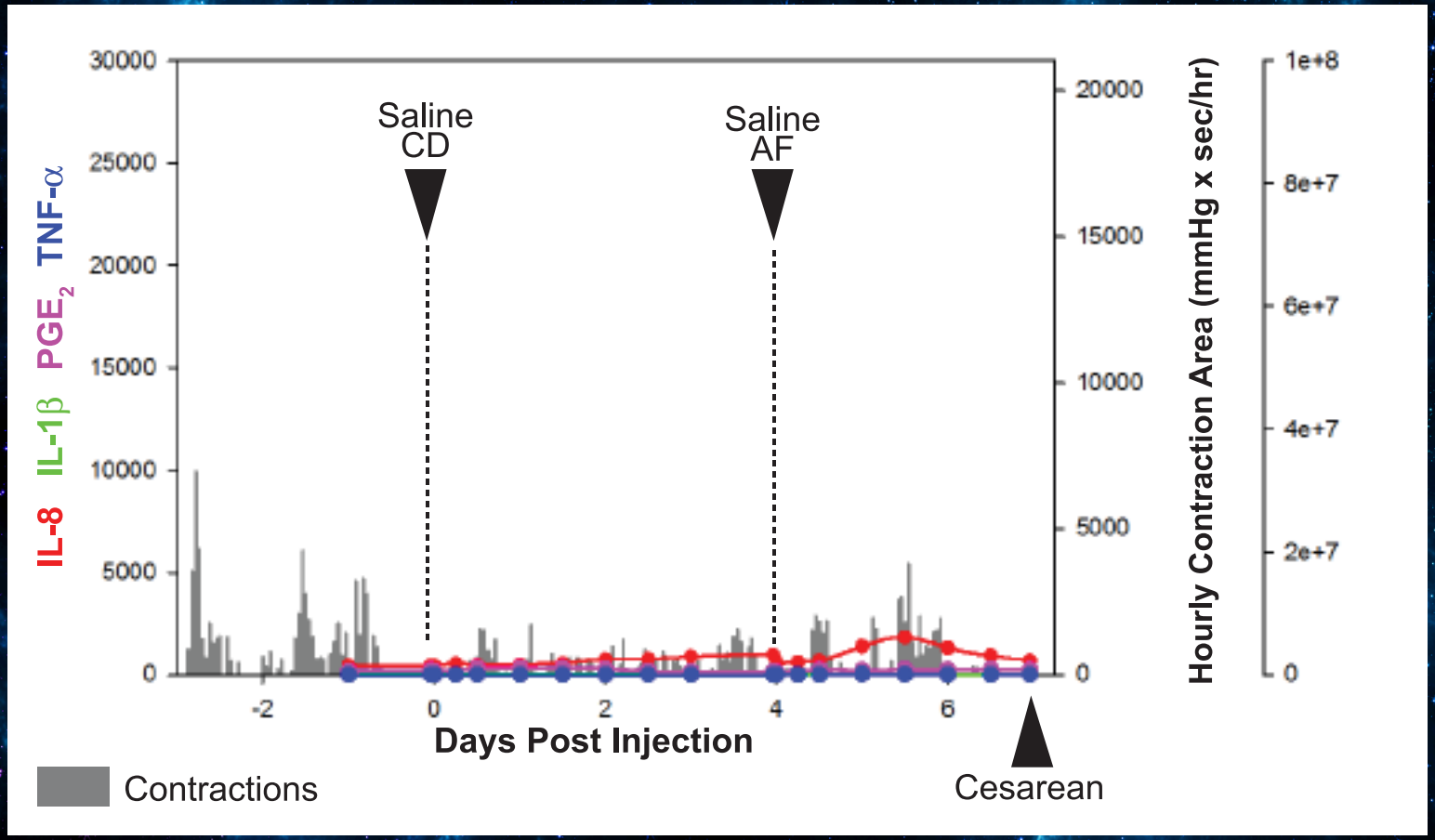
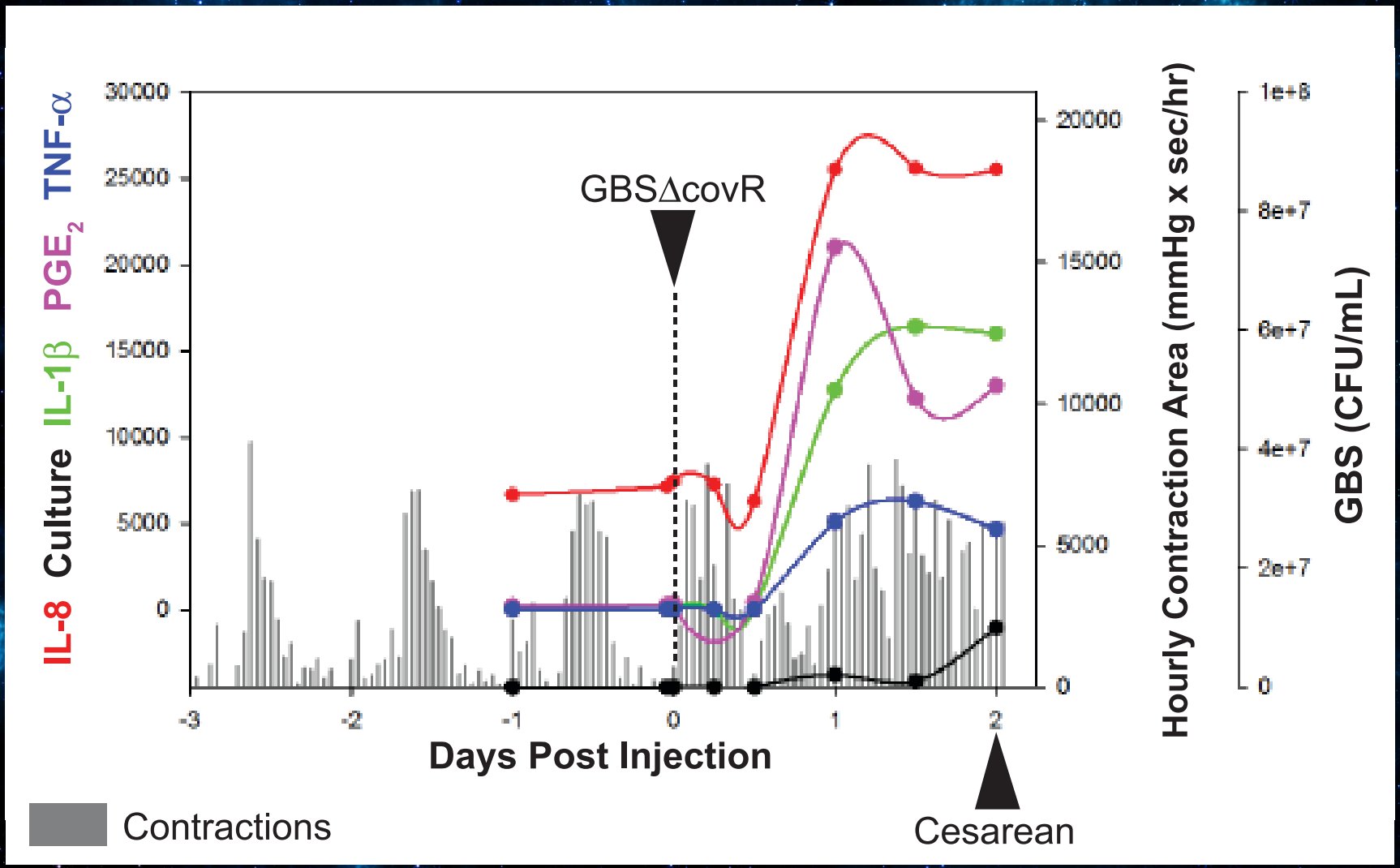
Methods

- Luminex/ELISA – quantitate cytokines
- Placental and fetal histopathology
- Culture of amniotic fluid and fetal organs
- Scanning electron microscopy
- “Neutrophil killing” assay

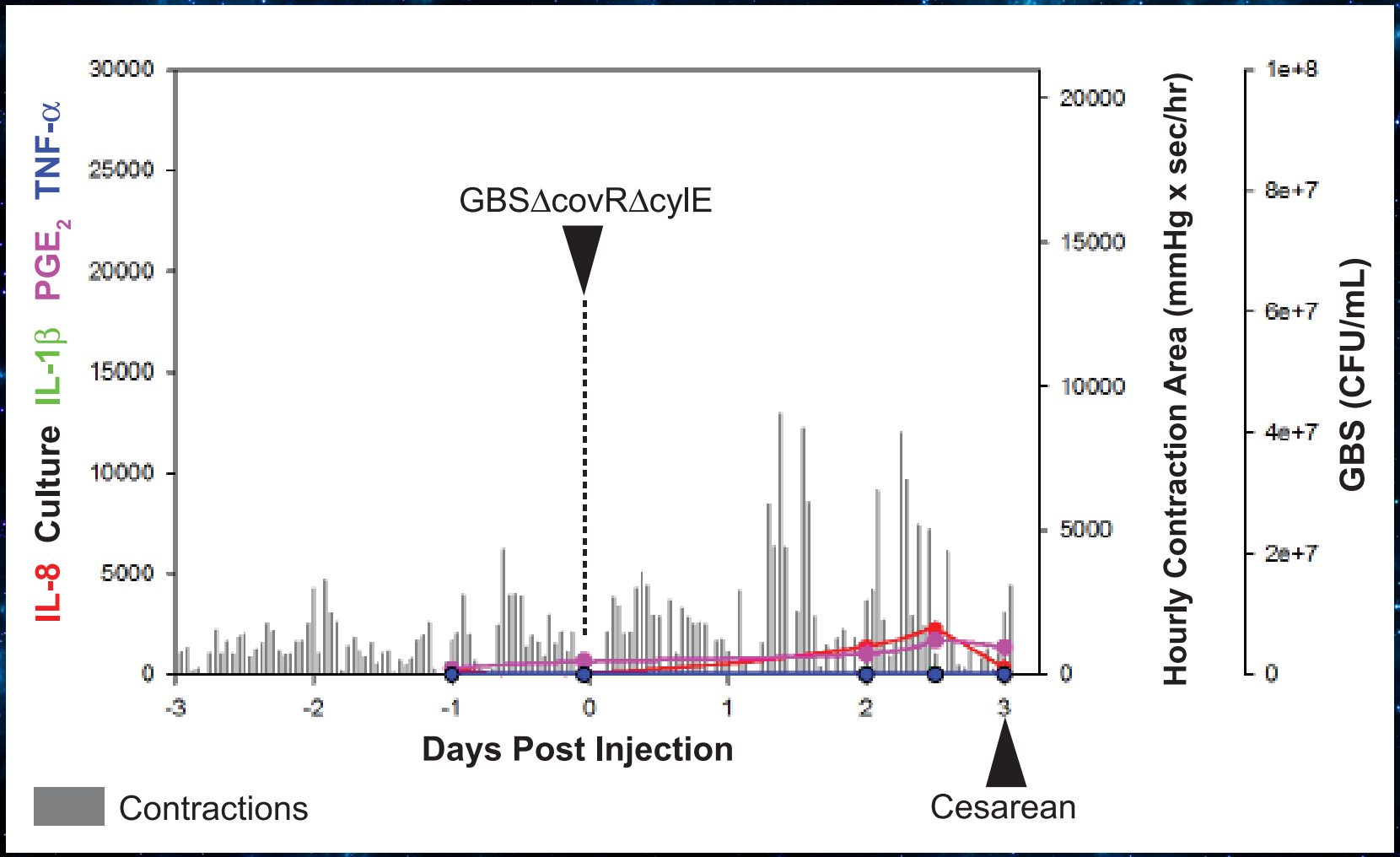
Saline Inoculation Does Not Induce Labor or Amniotic Fluid Cytokines



GBSΔ*covR* Choriodecidual Inoculation Induces Rapid Labor and MIAC



GBSΔ*covR*Δ*cylE* Inoculation – Lower Rate of PTL and MIAC



Primary Outcomes

	Saline (N=5)	GBSΔ*covR* (N=6)	GBSΔ*covR*Δ*cylE* (N=5)	P value Saline vs. GBSΔ*covR*
Preterm Labor	0 (0%)	5 (83%)	2 (40%)	P=0.006
Microbial Invasion of Amniotic Cavity	0 (0%)	4 (67%)	1 (20%)	P=0.02
FIRS (Fetal plasma IL-6 >11 pg/mL)	0 (0%)*	5 (83%)	2 (40%)	P=0.006

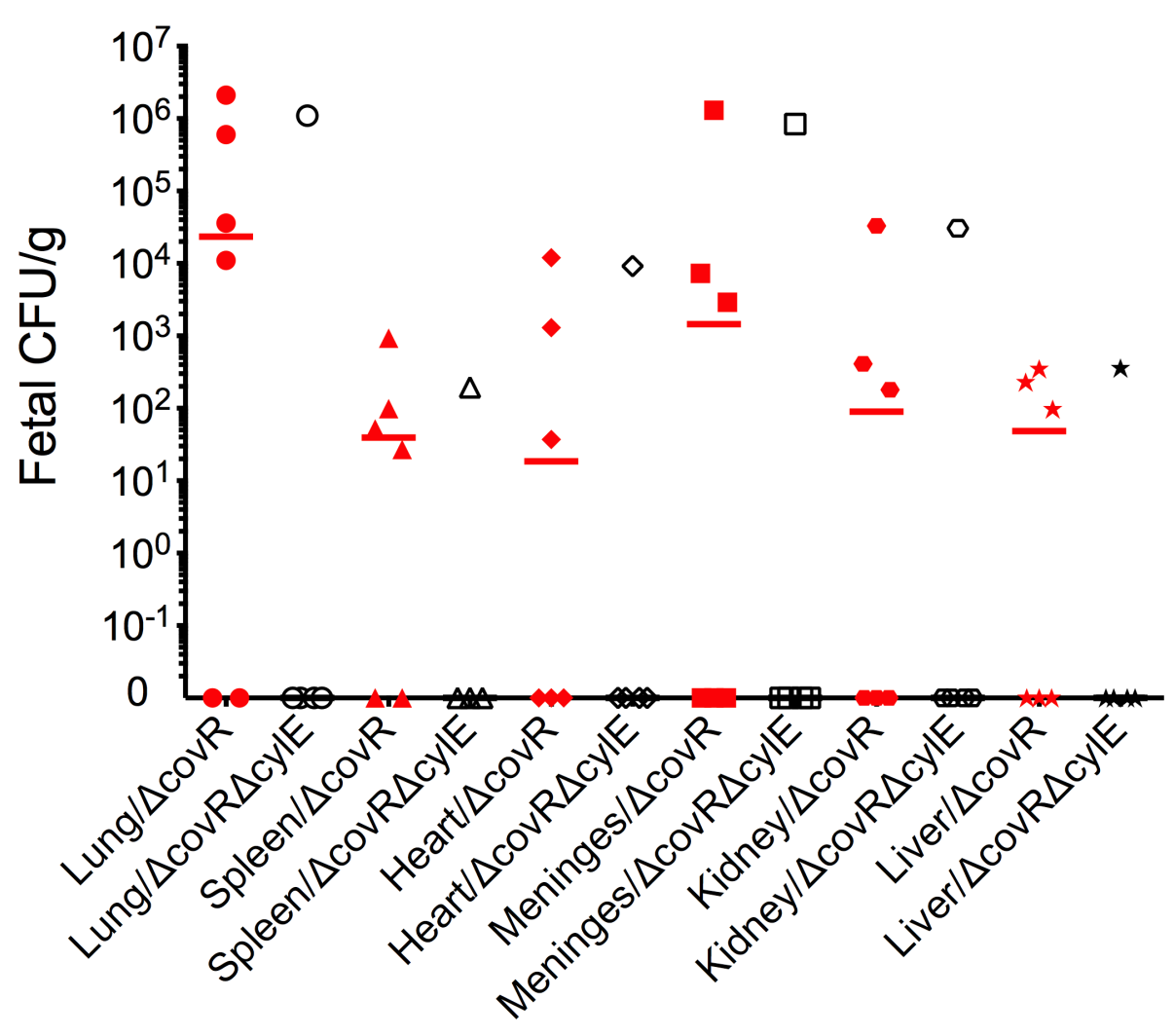
* In 2 cases, fetal blood could not be obtained, but no AF inflammation and no fetal lung inflammation. Assigned value of no FIRS.

Peak Cytokine and Prostaglandin Levels

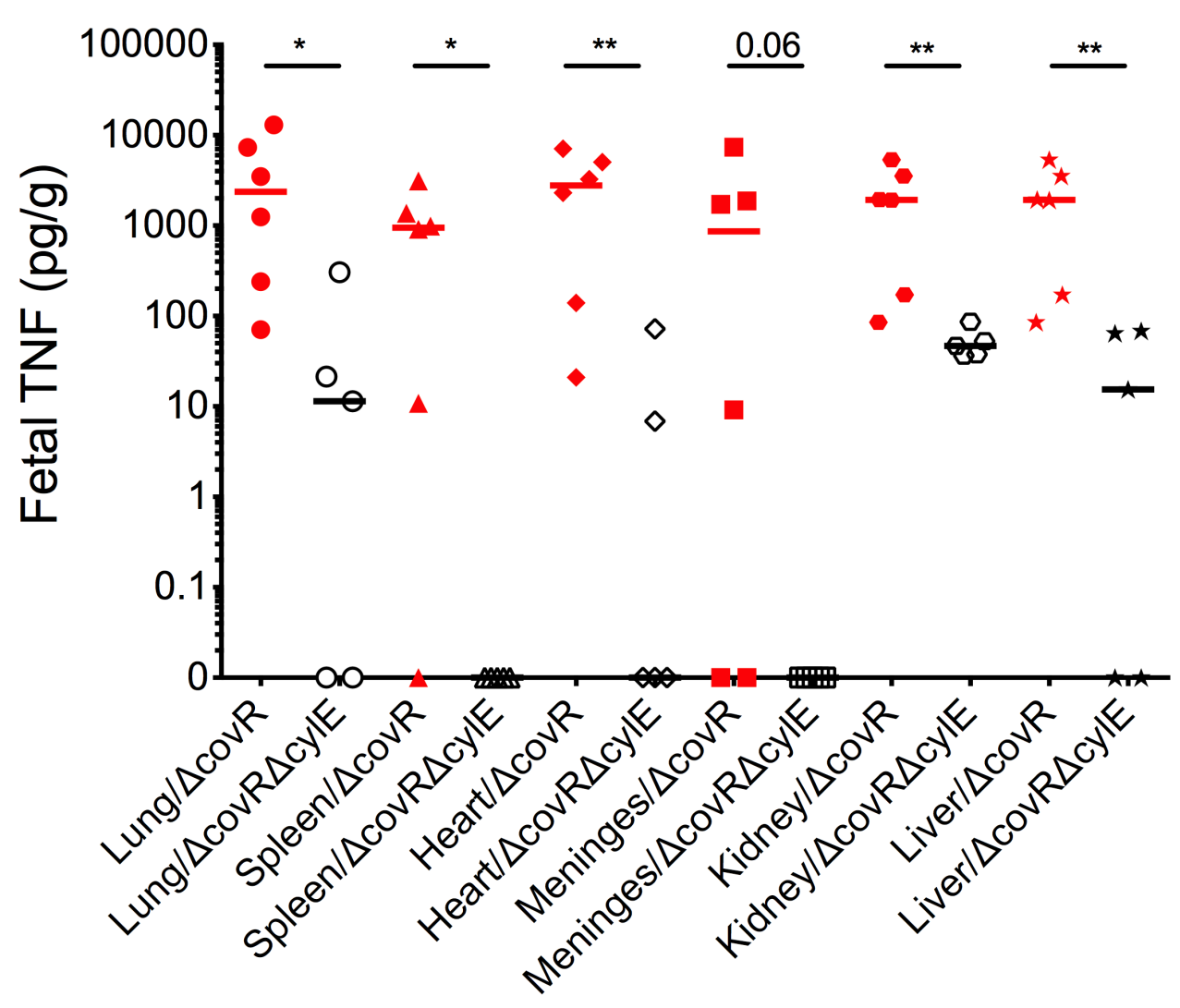
	Saline	GBSΔ*covR*	GBSΔ*covR* Δ*cylE*	P value Saline vs. GBSΔ*covR*
IL-1β	0.005 (0.003)	5.1 (2.8)	0.5 (0.4)	0.03
TNF-α	0.02 (0.01)	1.9 (1.1)	0.07 (0.04)	0.06
IL-6	10.7 (2.2)	20.1 (2.3)	6.2 (4.3)	0.05
IL-8	1.5 (0.3)	14.1 (4.3)	5.3 (2.3)	0.001
PGE2	3.0 (2.0)	12.1 (5.7)	24.3 (15.1)	NS
PGF2α	0.7 (0.5)	3.7 (3.5)	0.7 (0.3)	NS

*Values are shown as mean peak (SEM) in ng/mL

GBS Colony Forming Units in Fetal Organs

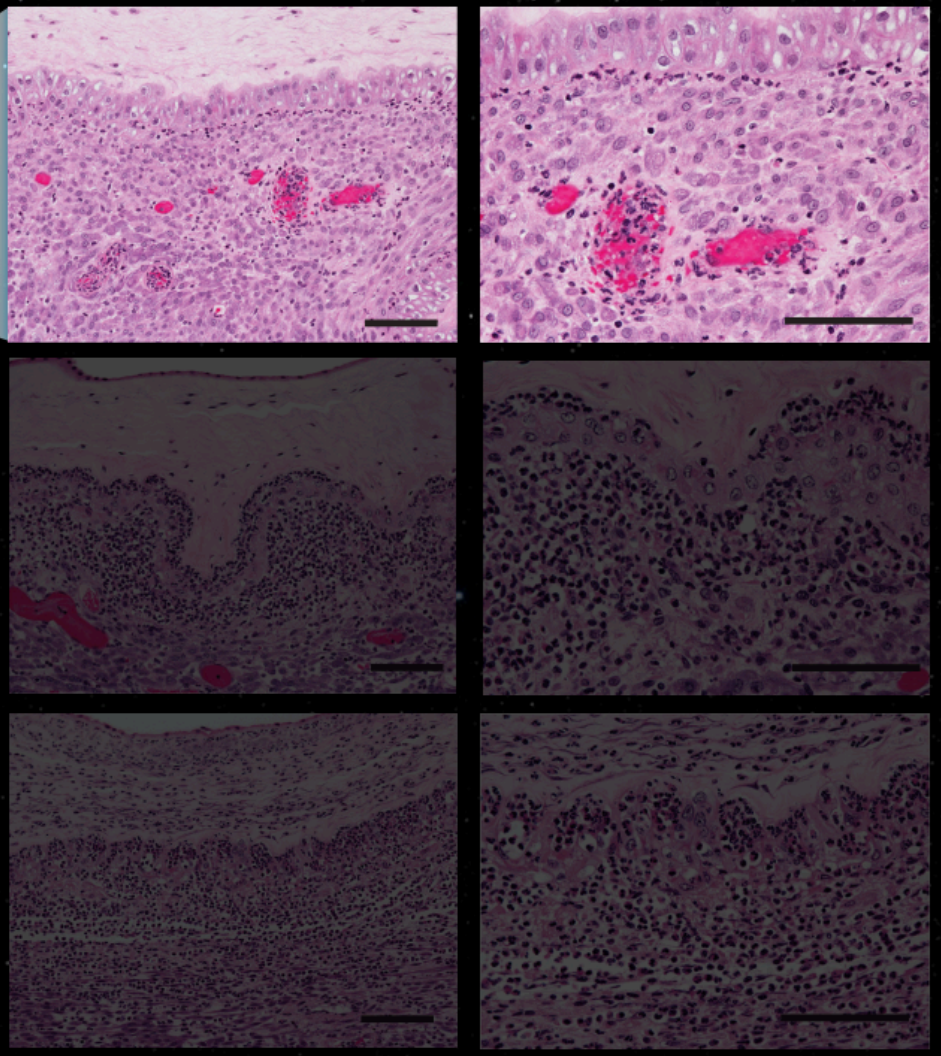


GBS*ΔcovR*: ↑ TNF-α in Fetal Organs



Temporal Progression of Chorioamnionitis

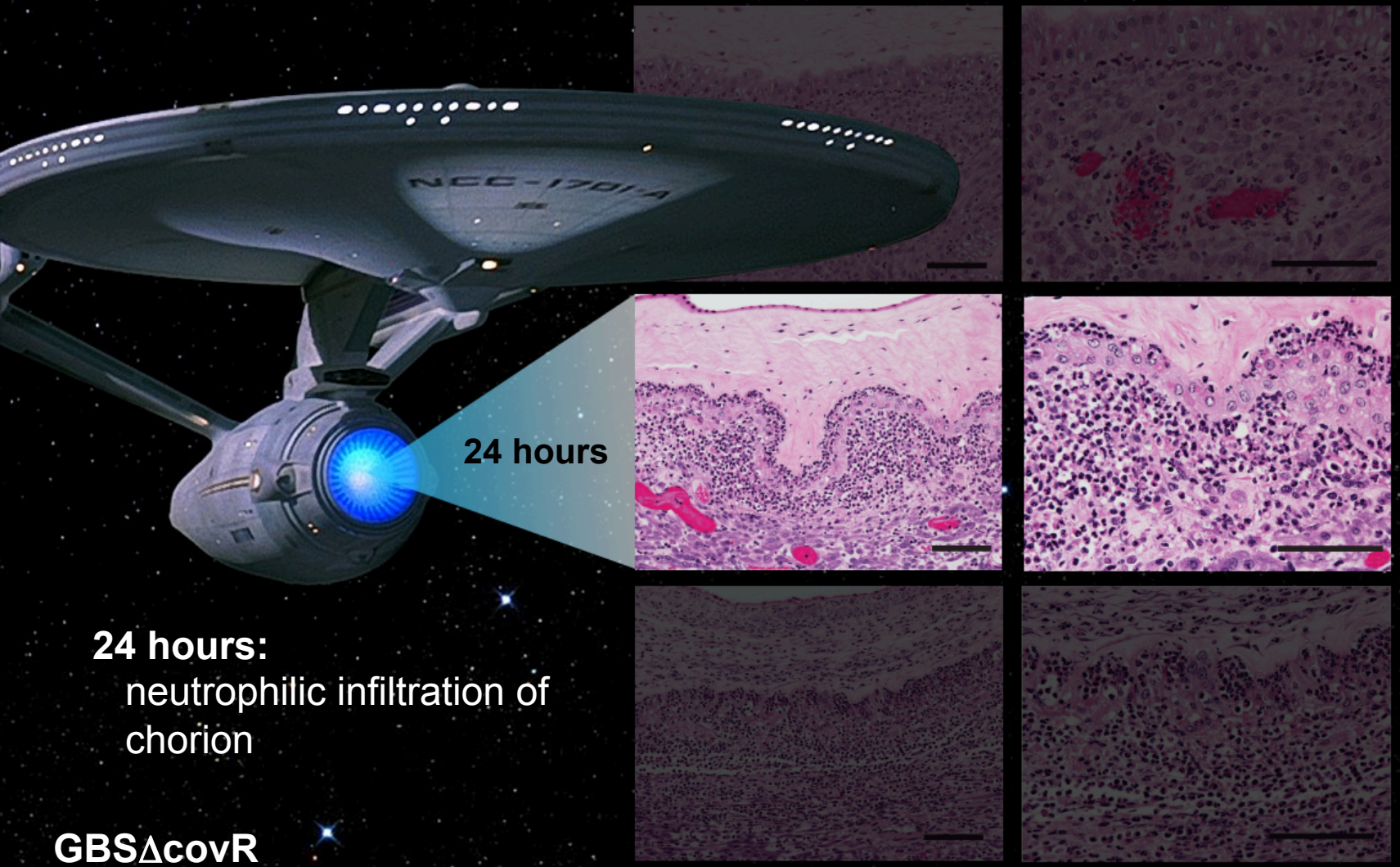
6 hours



6 hours:
chorionic vasculitis

GBSΔcovR

Temporal Progression of Chorioamnionitis



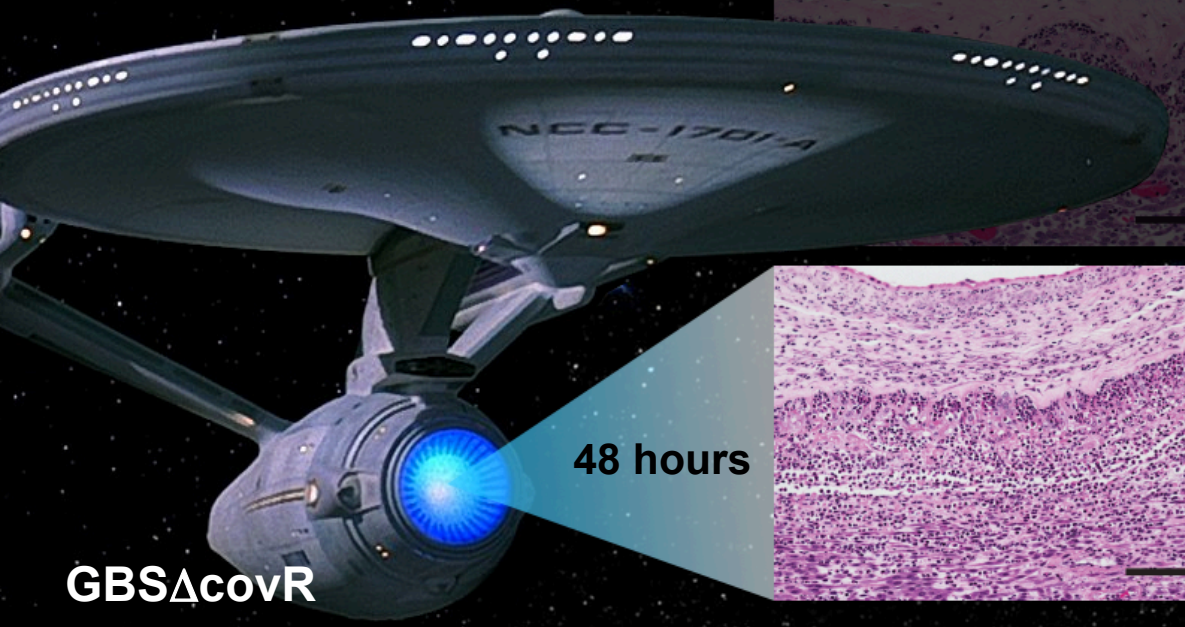
24 hours:
neutrophilic infiltration of chorion

GBSΔcovR

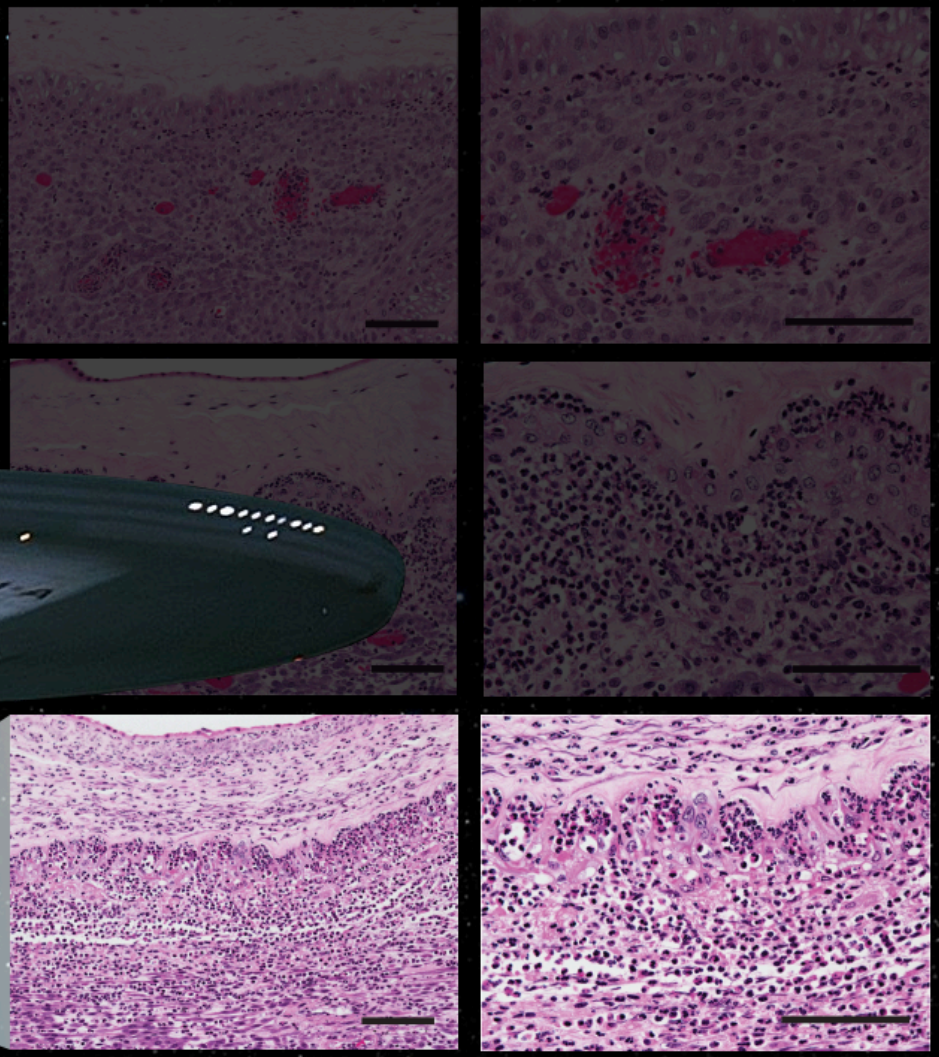
Temporal Progression of Chorioamnionitis

48 hours:
neutrophilic infiltration of chorion AND amnion

>72 hours:
Inflammatory injury and degradation of amnion

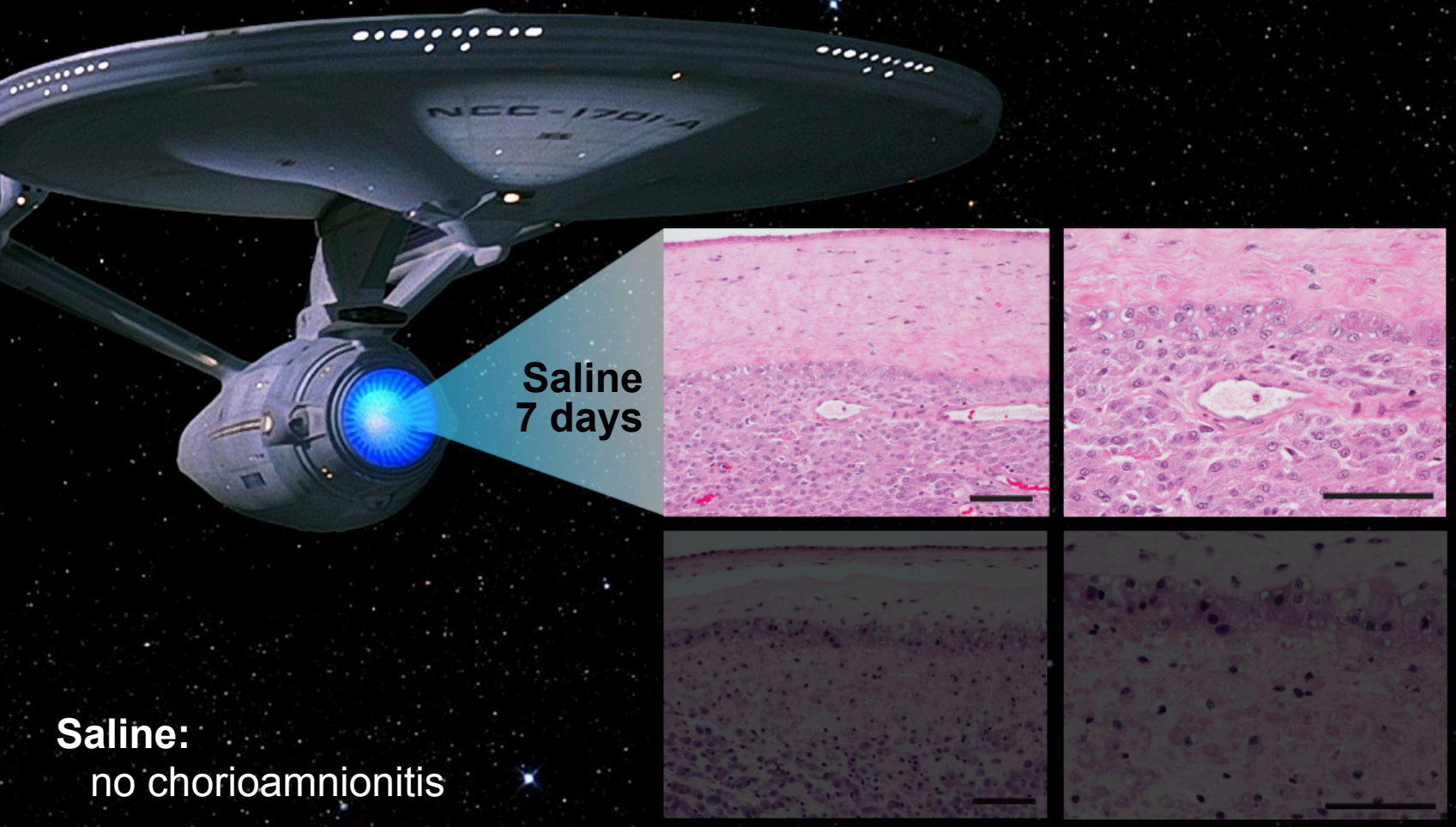


48 hours



GBSΔcovR

Temporal Progression of Chorioamnionitis



Saline:
no chorioamnionitis

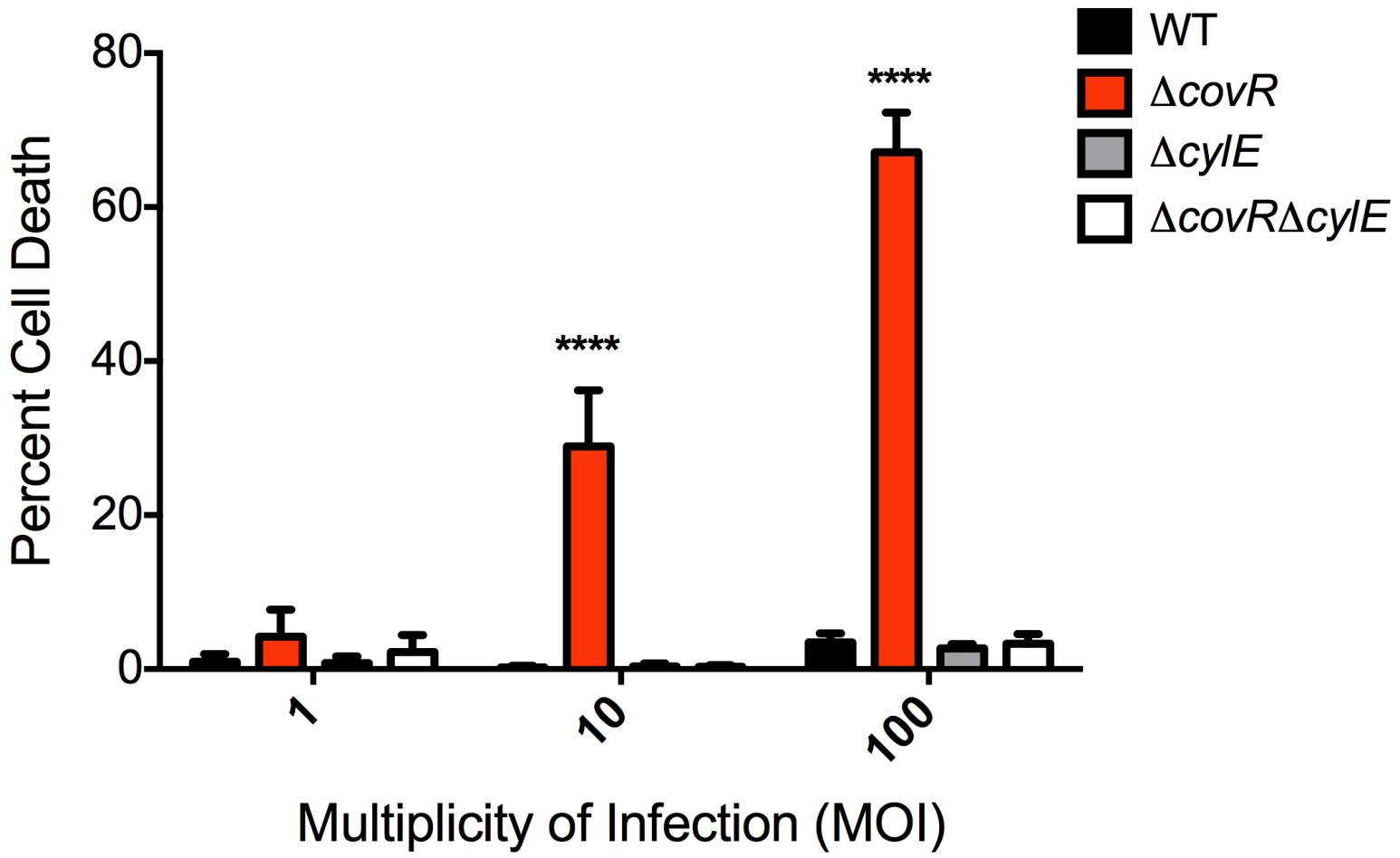
Saline

Temporal Progression of Chorioamnionitis

3 days:
few neutrophils in one case

GBSΔcovRΔcylE

GBS*ΔcovR* INDUCES FETAL NEUTROPHIL DEATH



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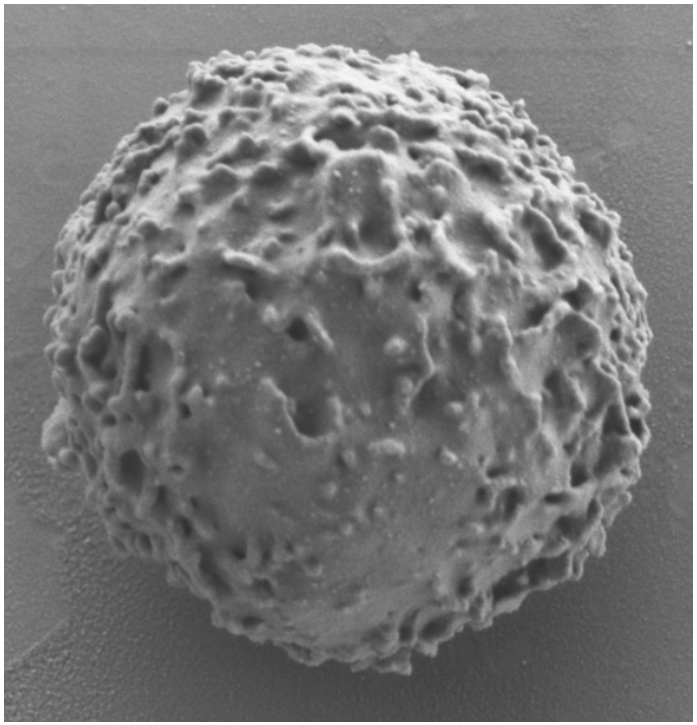
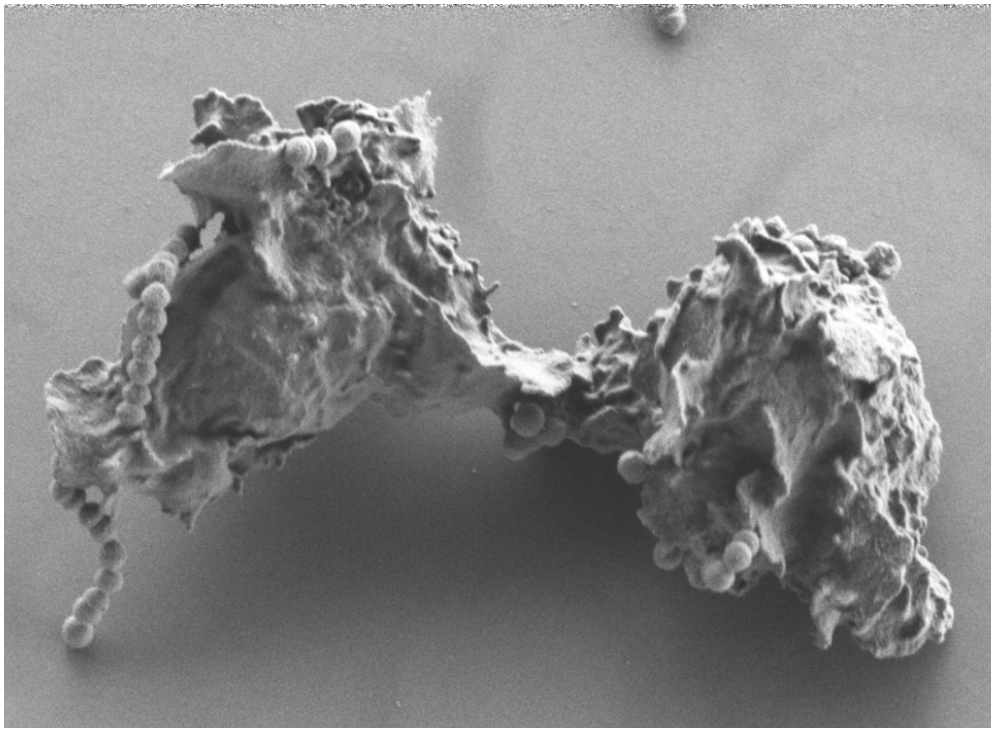
FETAL NEUTROPHILS: TOO SLOW? KILLED BY GBS?



Adult Neutrophil



GBS (Cocci in Chains)
Attacking Adult Neutrophils

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Conclusions

- Production of β-hemolysin by GBS confers a greater risk of preterm labor, microbial invasion of amniotic cavity and fetal injury in our unique nonhuman primate model.
- Placental histopathology revealed a clear temporal progression in development of chorioamnionitis beginning with chorionic vasculitis (+6 hr) and progressing to neutrophilic infiltration of the chorion (+24 hr) and then amnion (+48 hr) with degradation of the amnion (>72 hr)
- GBS β-hemolysin kills fetal neutrophils necessary to control placental infection

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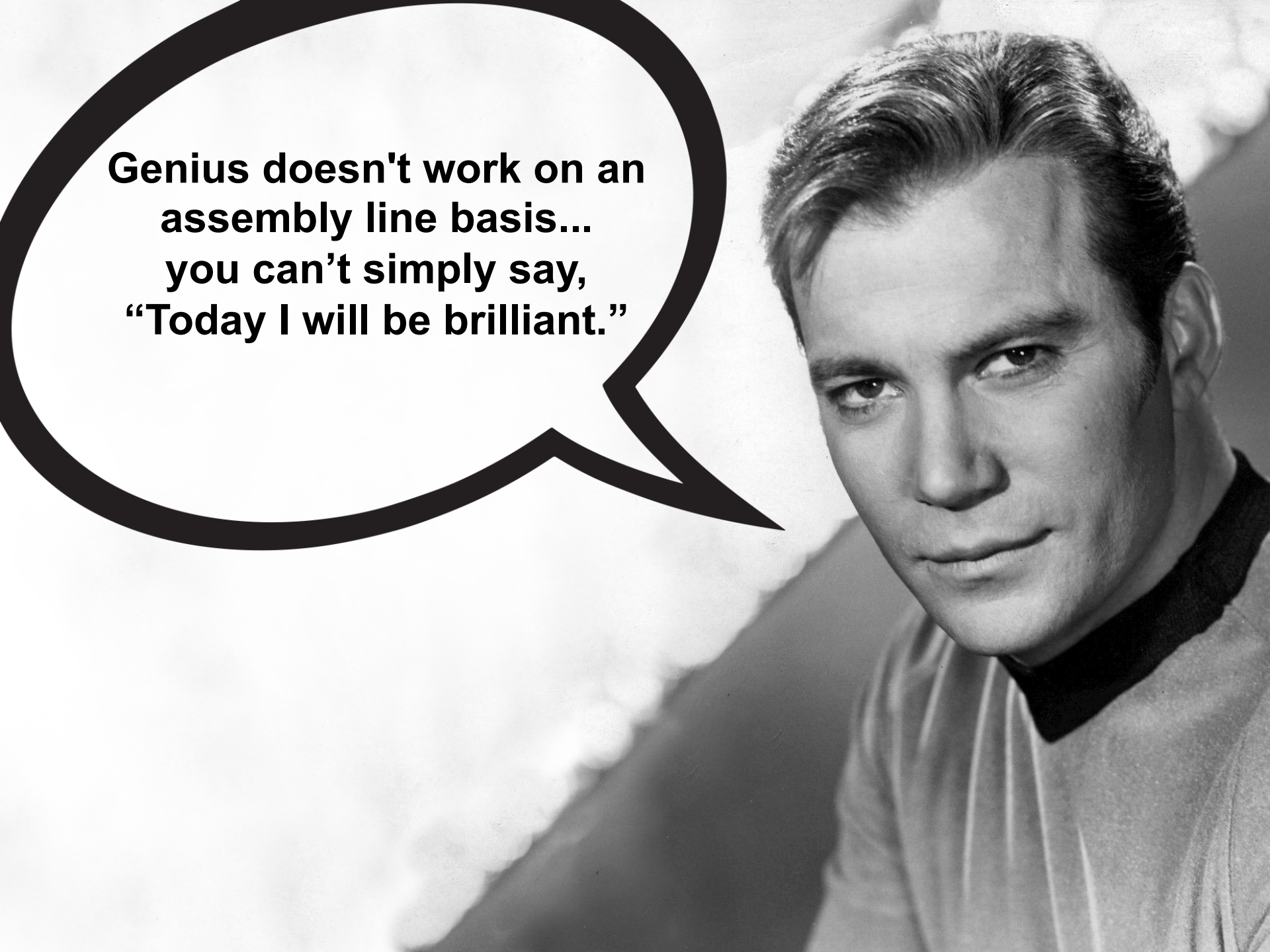
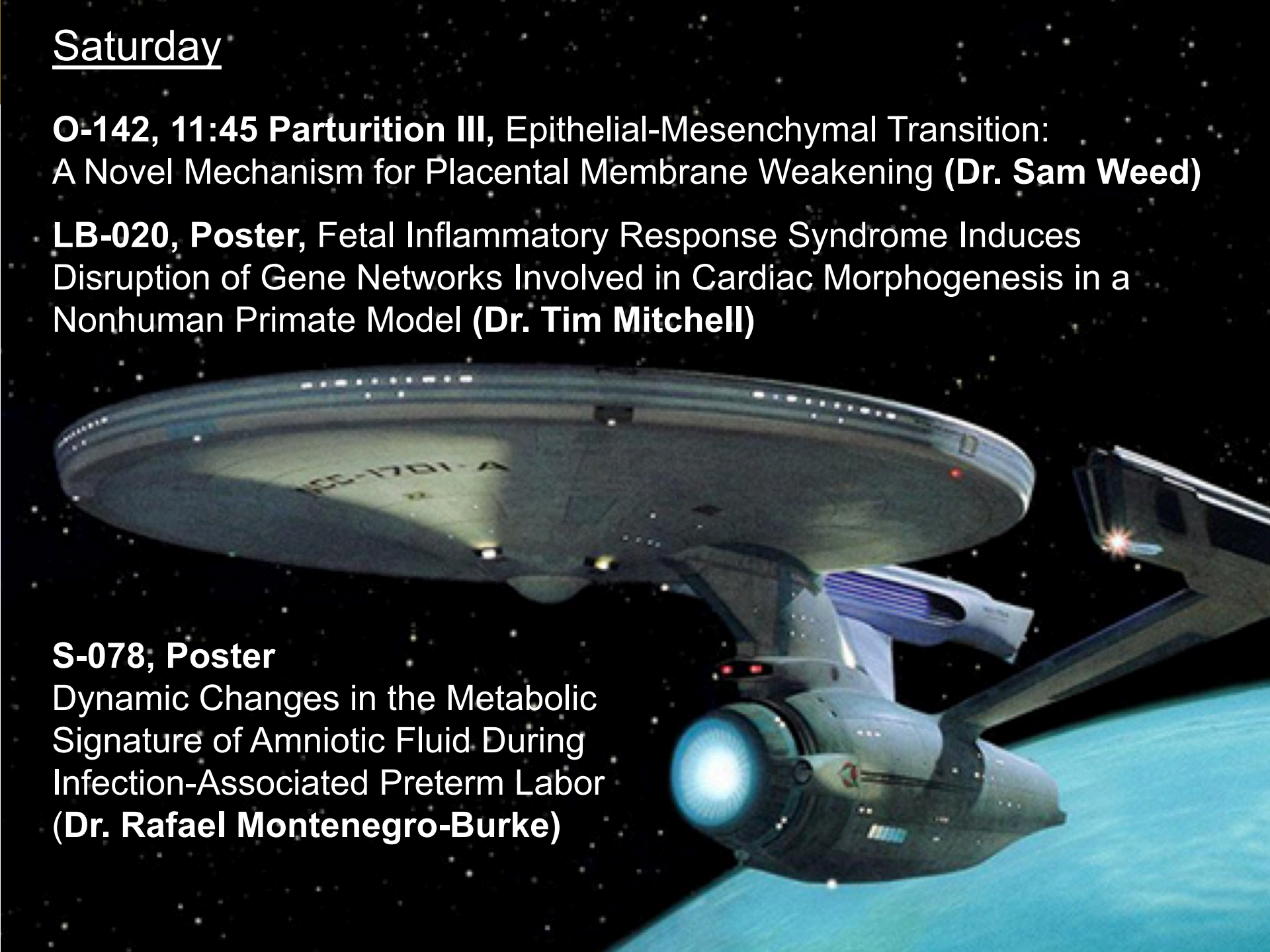

Genius doesn't work on an
assembly line basis...
you can’t simply say,
“Today I will be brilliant.”

Saturday

O-142, 11:45 Parturition III, Epithelial-Mesenchymal Transition: A Novel Mechanism for Placental Membrane Weakening **(Dr. Sam Weed)**

LB-020, Poster, Fetal Inflammatory Response Syndrome Induces Disruption of Gene Networks Involved in Cardiac Morphogenesis in a Nonhuman Primate Model **(Dr. Tim Mitchell)**



S-078, Poster
Dynamic Changes in the Metabolic Signature of Amniotic Fluid During Infection-Associated Preterm Labor (**Dr. Rafael Montenegro-Burke**)