PRRS pathology and control

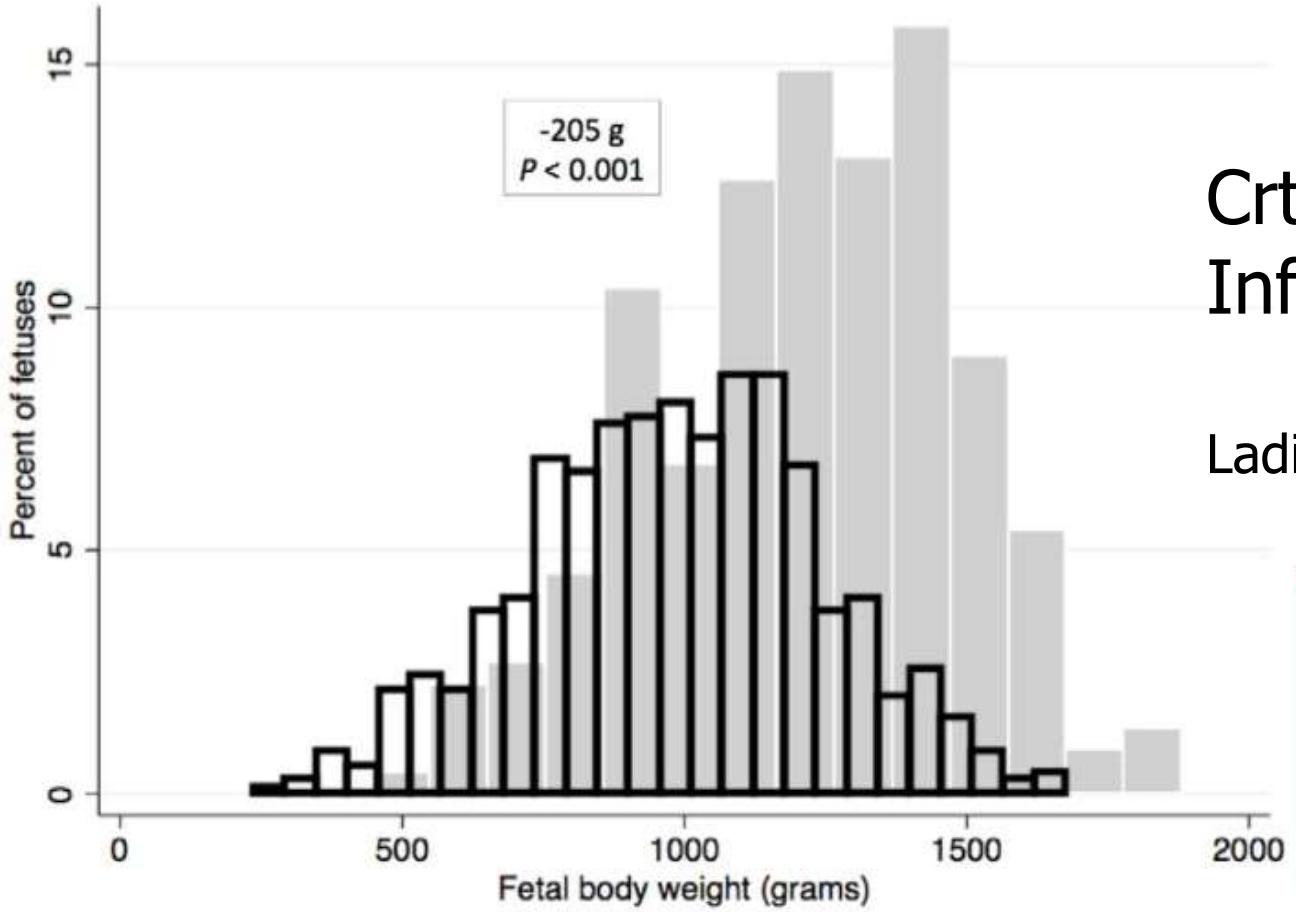
Reproductive pathology

- Early gestation
  - embryonic death, infertility return to estrus
- Late gestation
  - transplacental infection – abortions, early farrowing, fetal death, weak, congenitally infected piglets

Reproductive pathology

- Focal detachment/degeneration of the placenta (Karniychuk et al. 2009, 2013)
  - Sn/CD169 cell numbers increase in the placenta during late gestation
  - Activated NK cells induce placental damage
- Fetal infection – THYMUS!! (Ladinig et al. 2014, 2015)
  - Inoculation of 114 pregnant gilts at 85 DOG – extermination at 21DPI: >95% of dead fetuses were infected

Bodyweight of live piglets

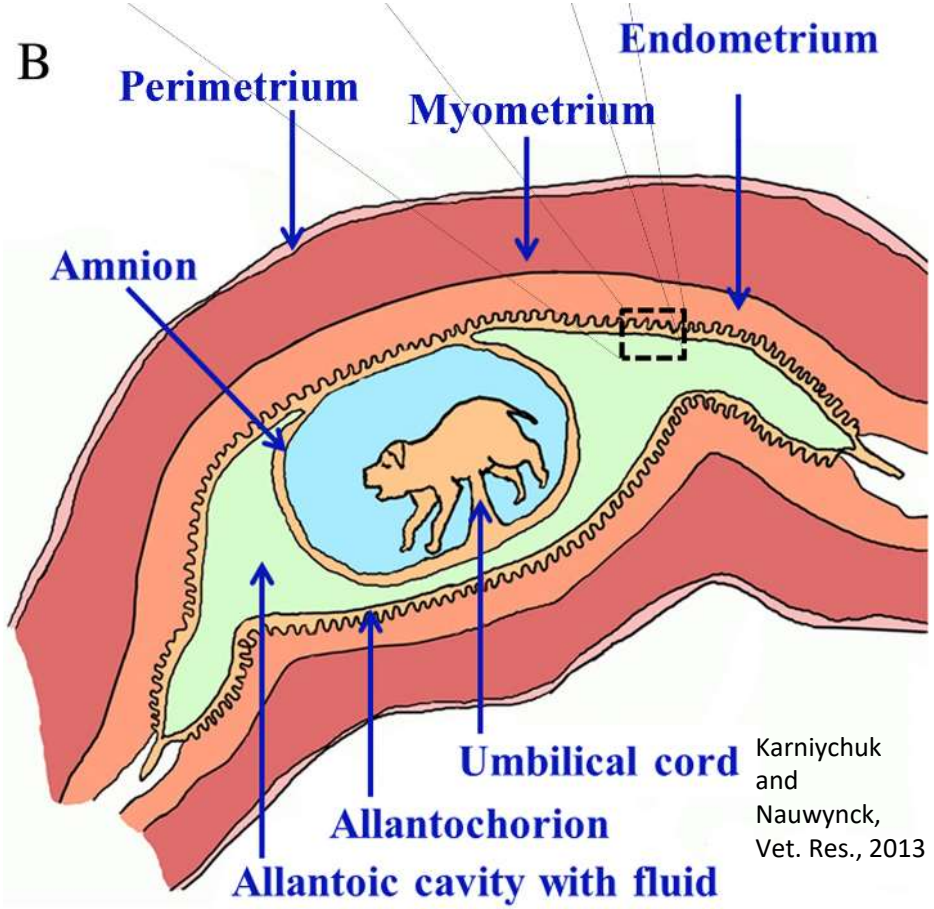


Crtl: 1204 (±36) g
Infect: 999 (±21) g

Ladinig et al. 2014

Viable

Reproductive PRRS Pathology

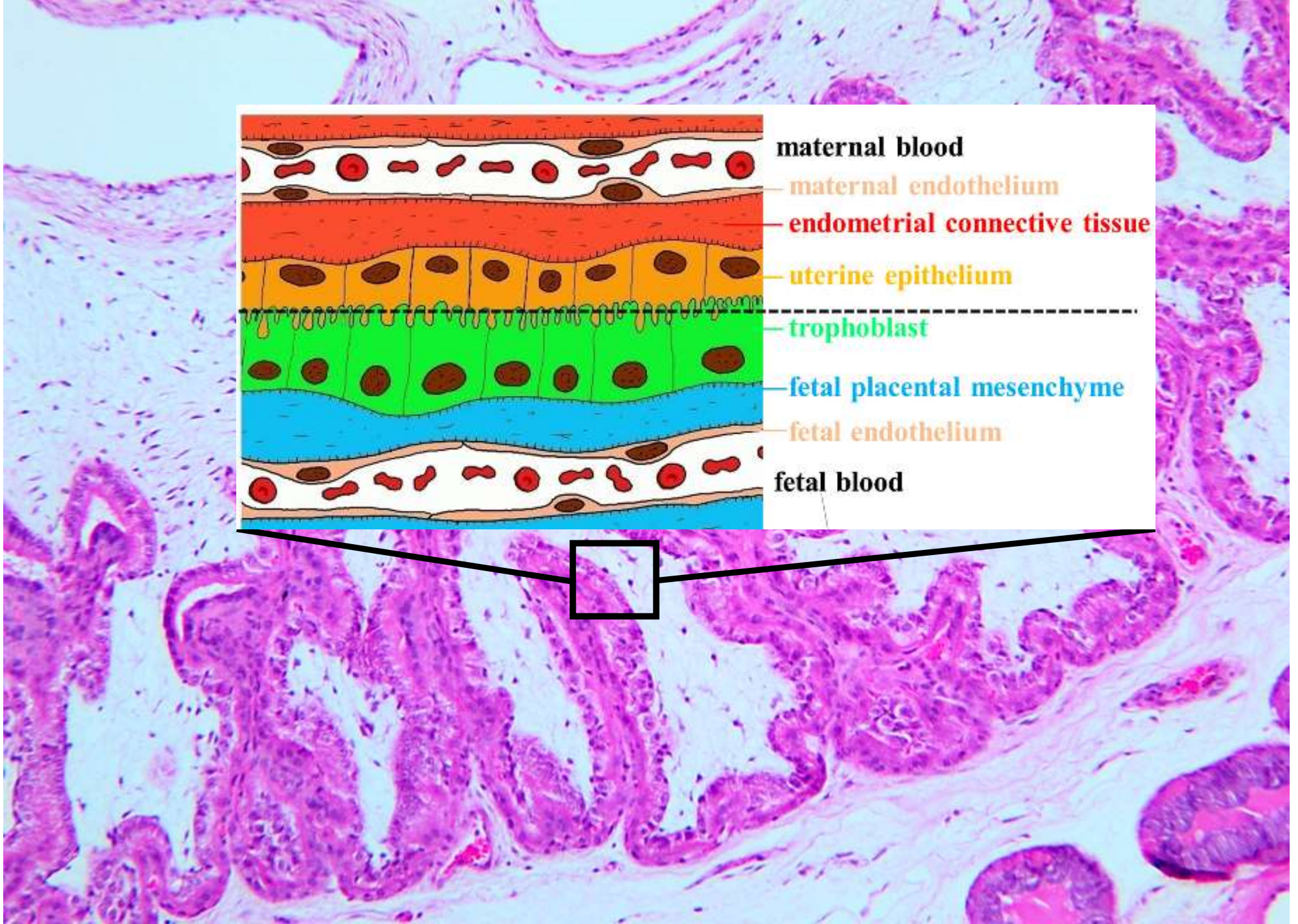


Karniychuk and Nauwynck, Vet. Res., 2013

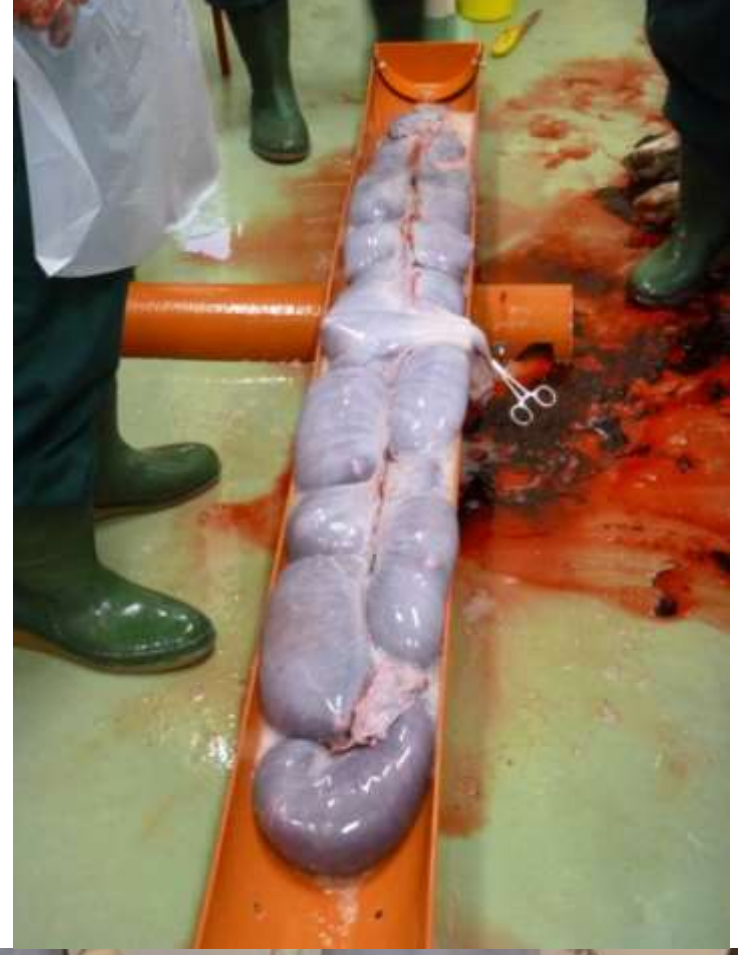
Andrea Ladinig,
Susan Detmer,
John Harding et al.
2014, 2015, 2016

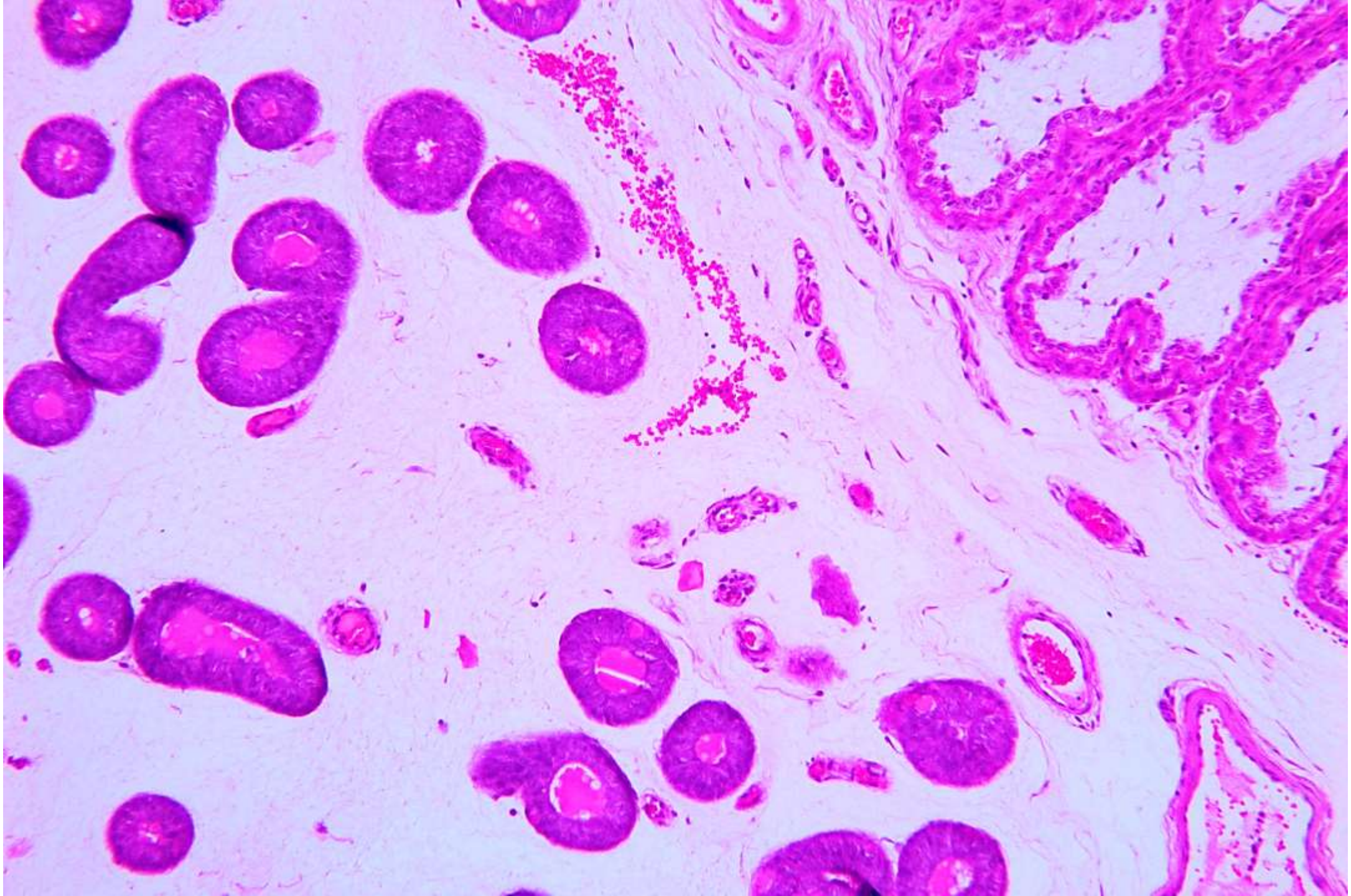
Incomplete diffuse epitheliochorial placenta

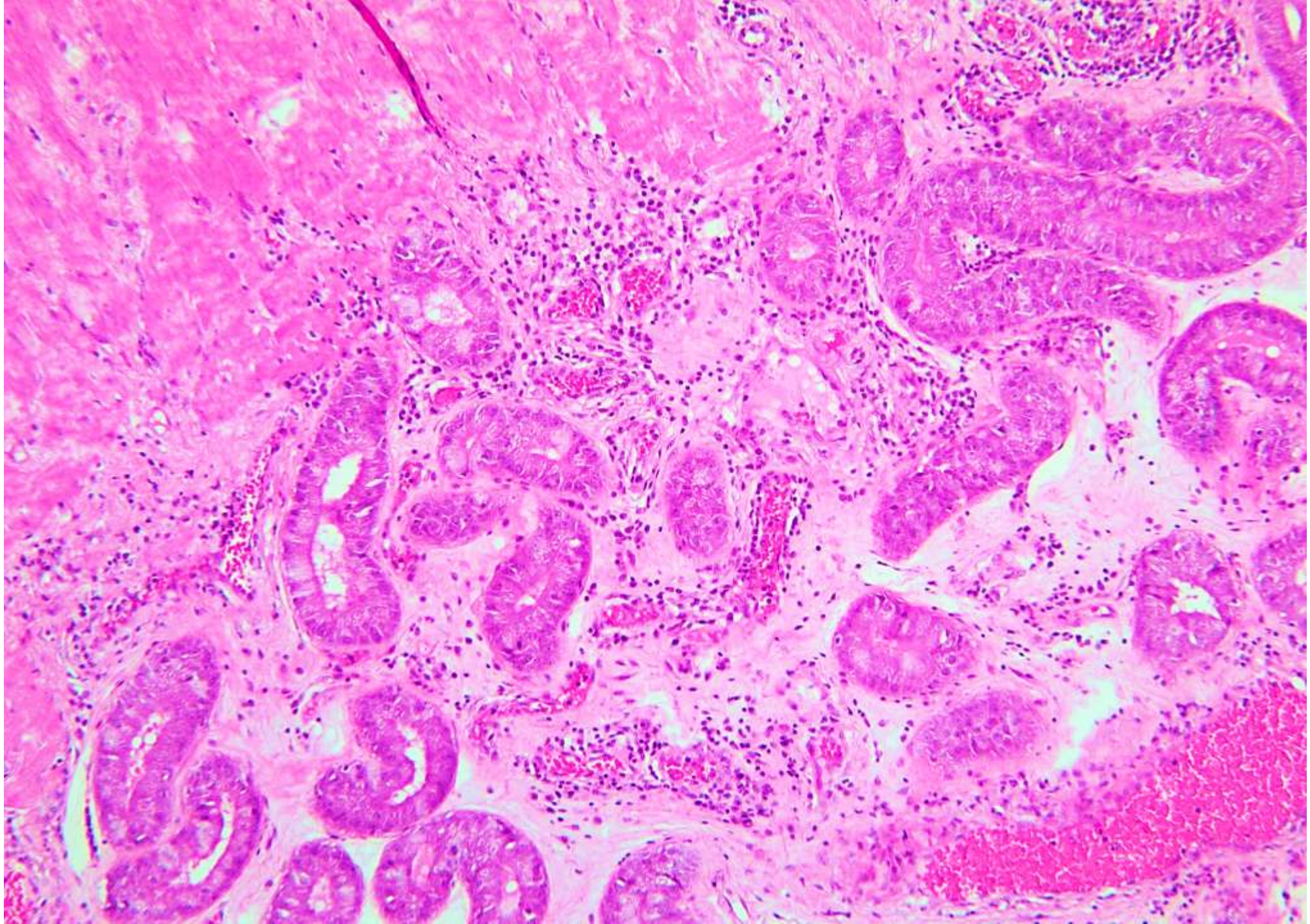
with atrophy at the peripheral tips without invasion of fetal tissue into the maternal endometrium, nor endometrial decidualization

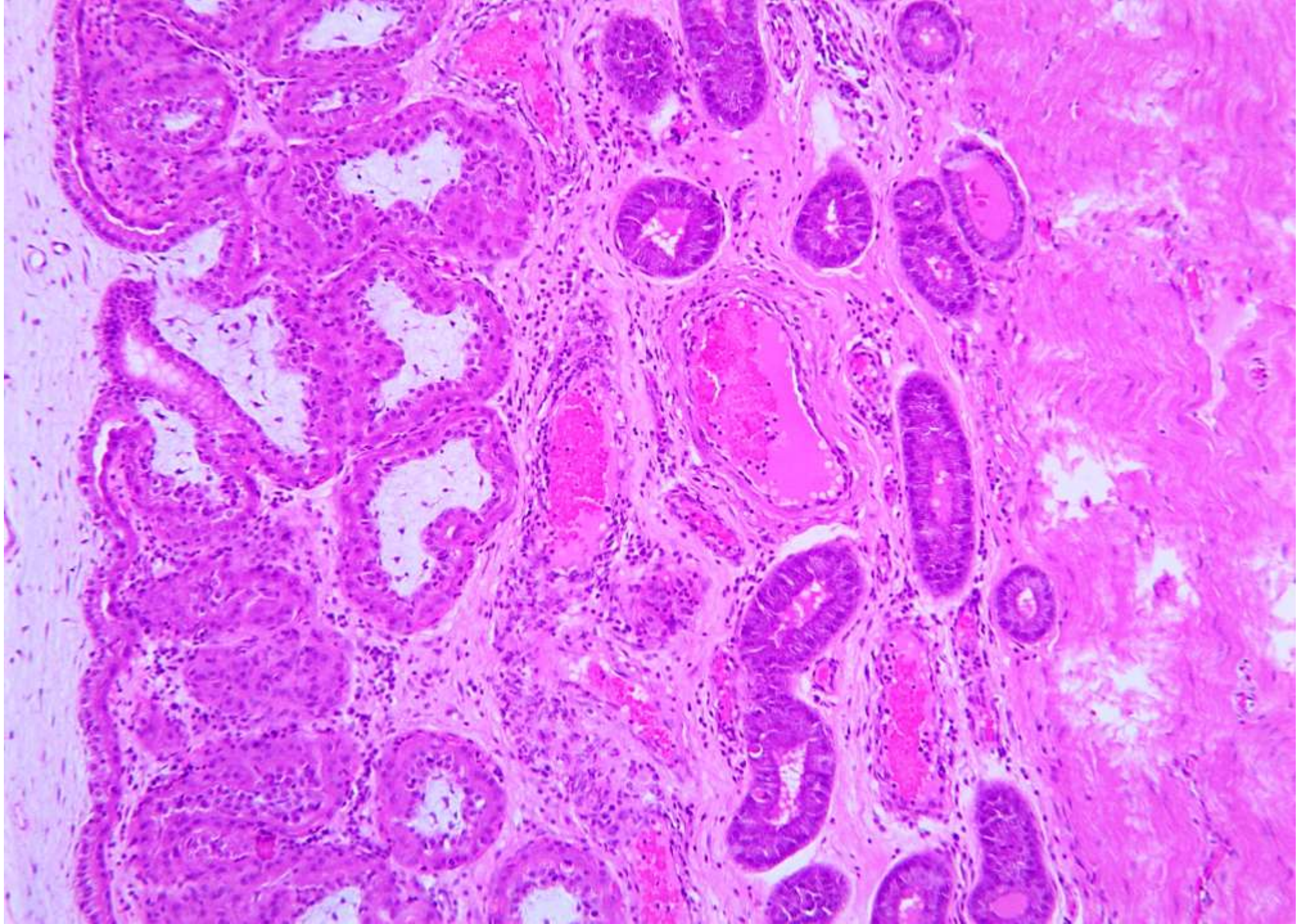

maternal blood
maternal endothelium
endometrial connective tissue
uterine epithelium
trophoblast
fetal placental mesenchyme
fetal endothelium
fetal blood

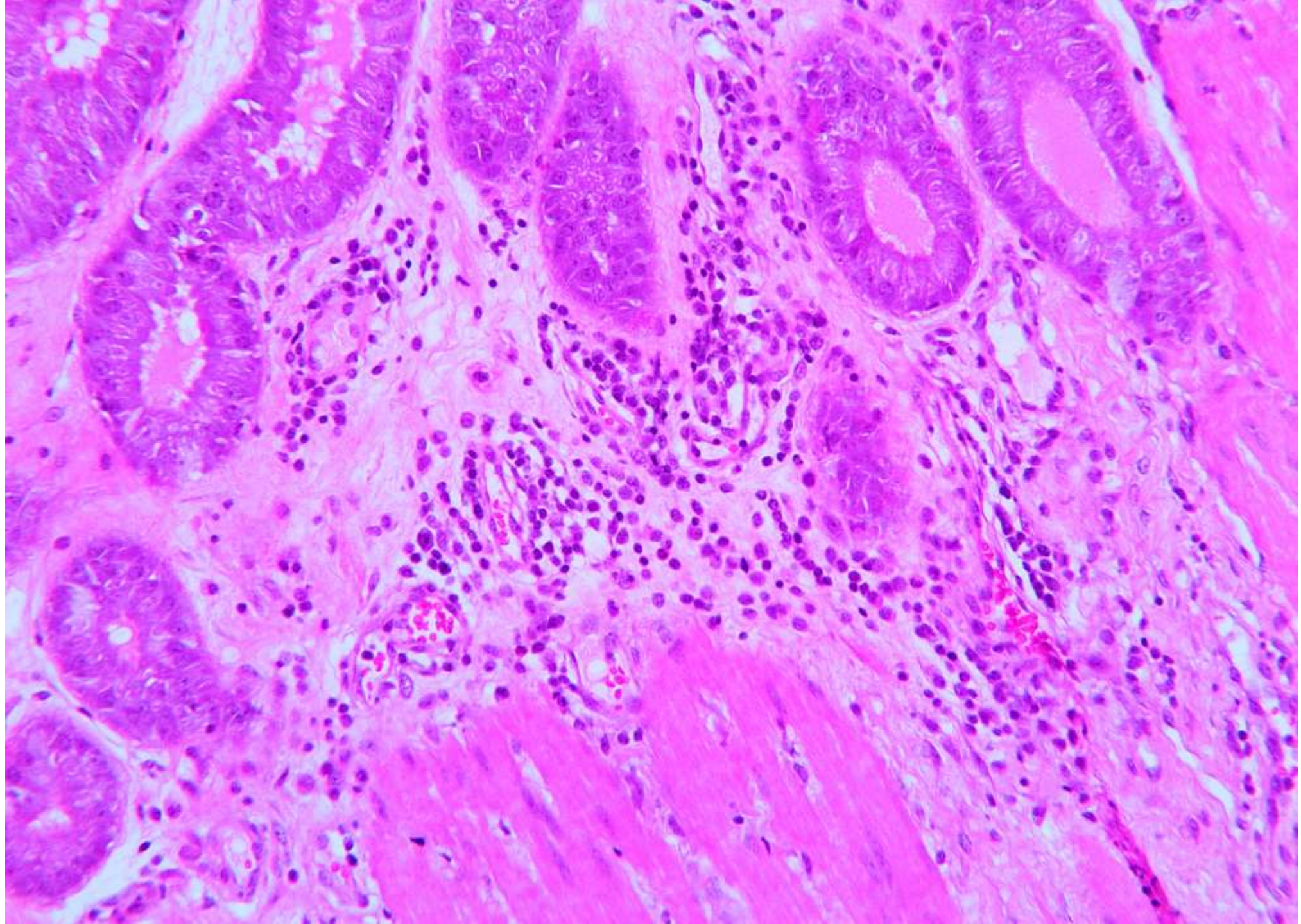
Fetal evaluation

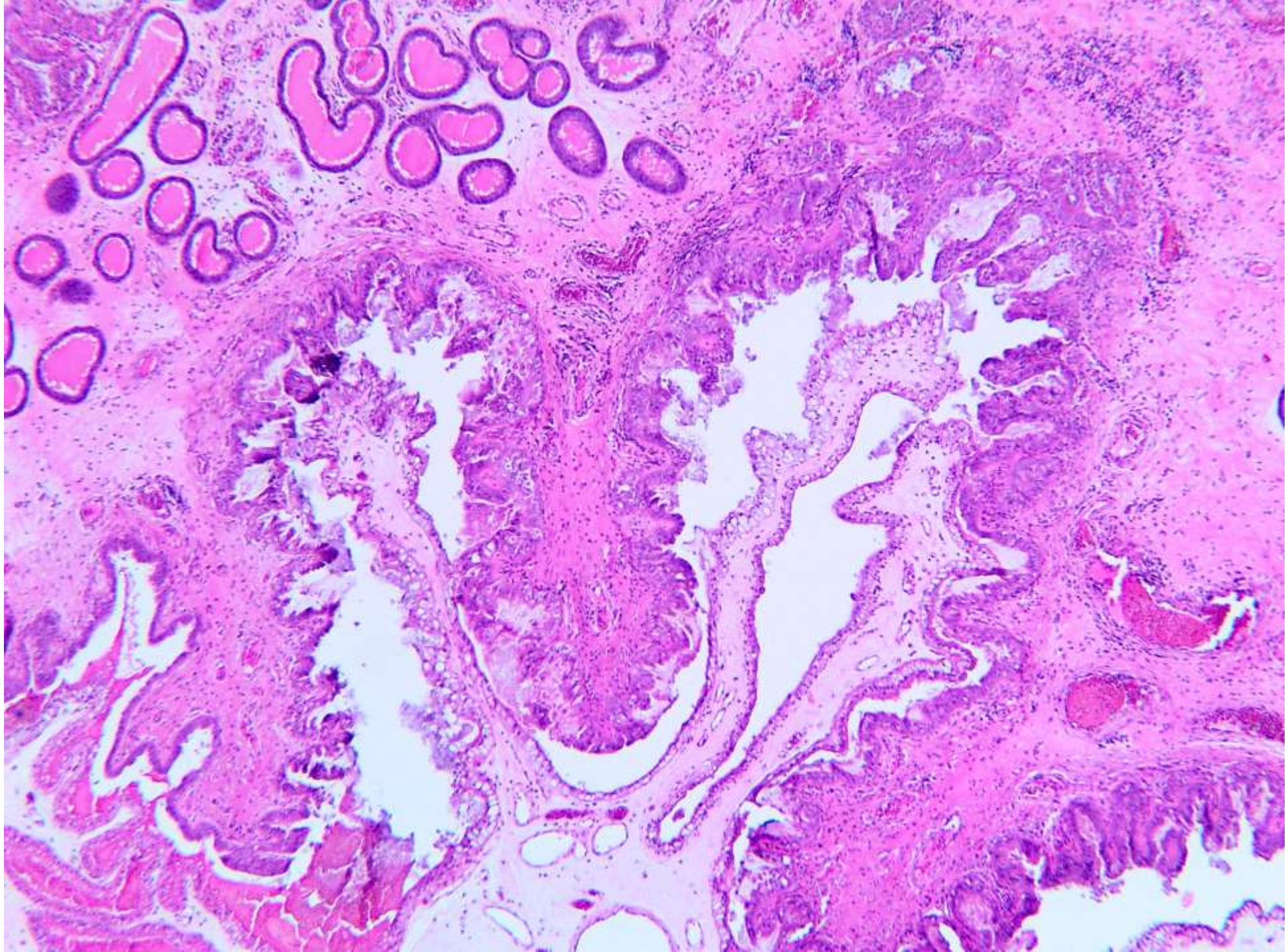


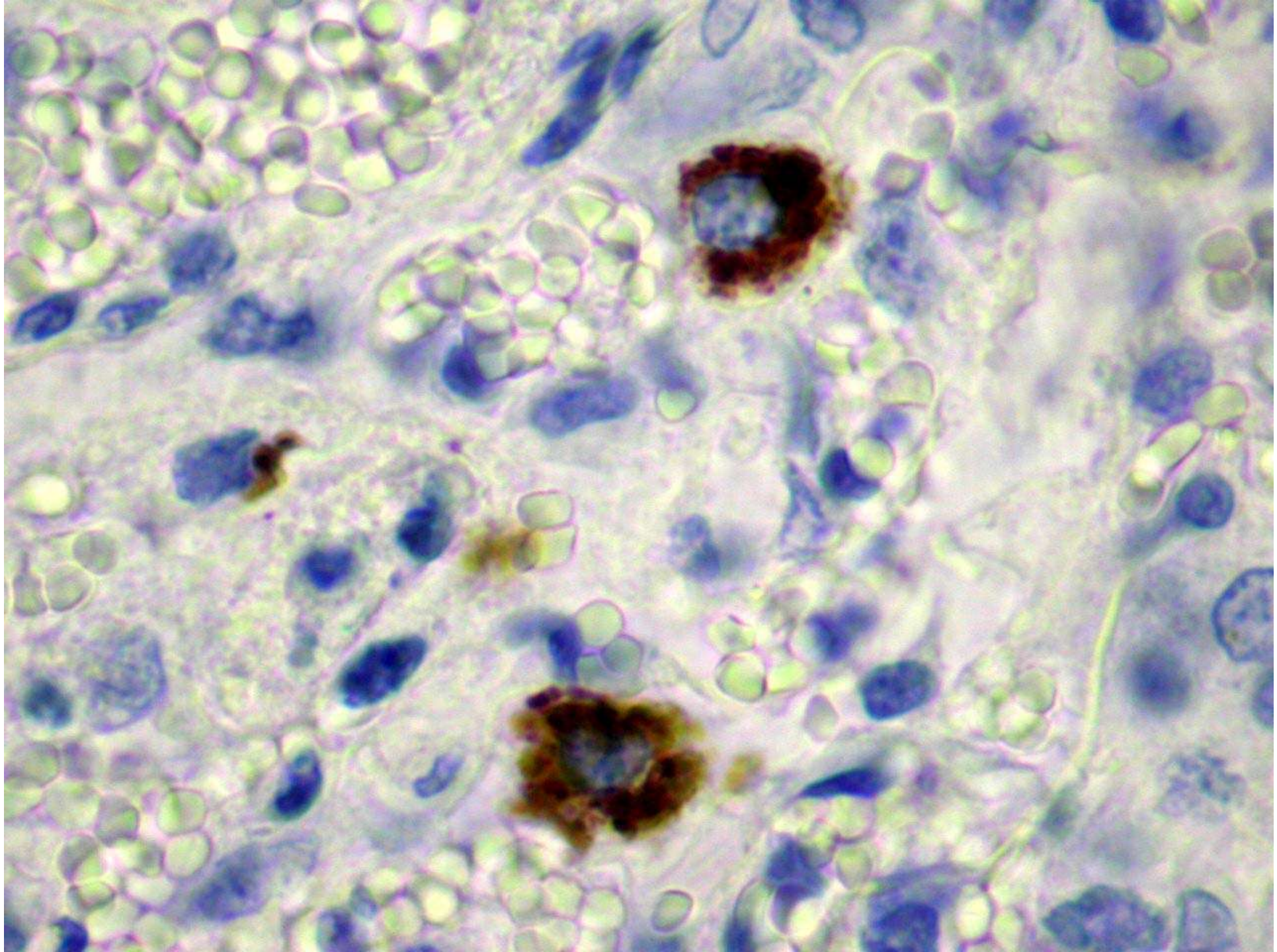






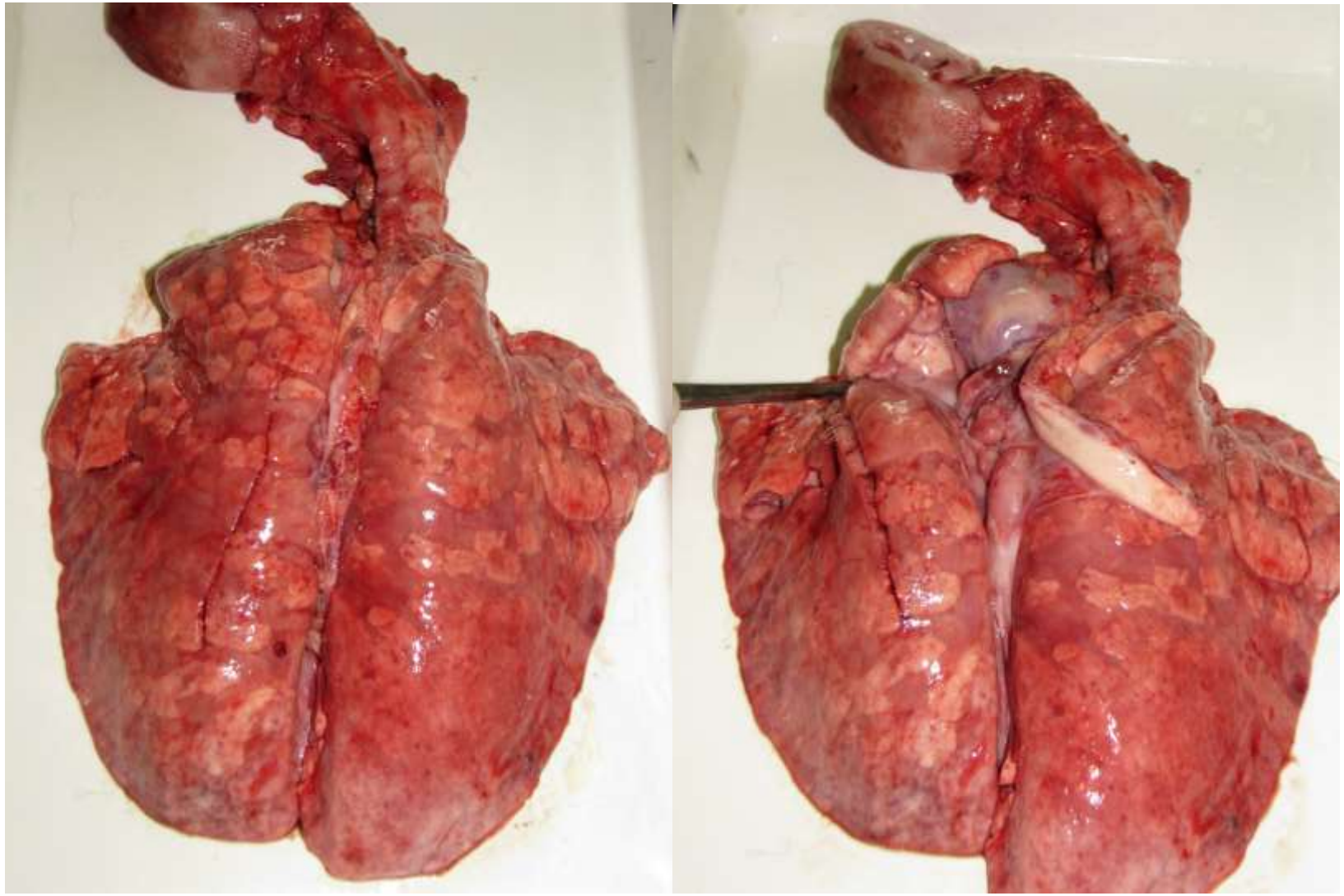


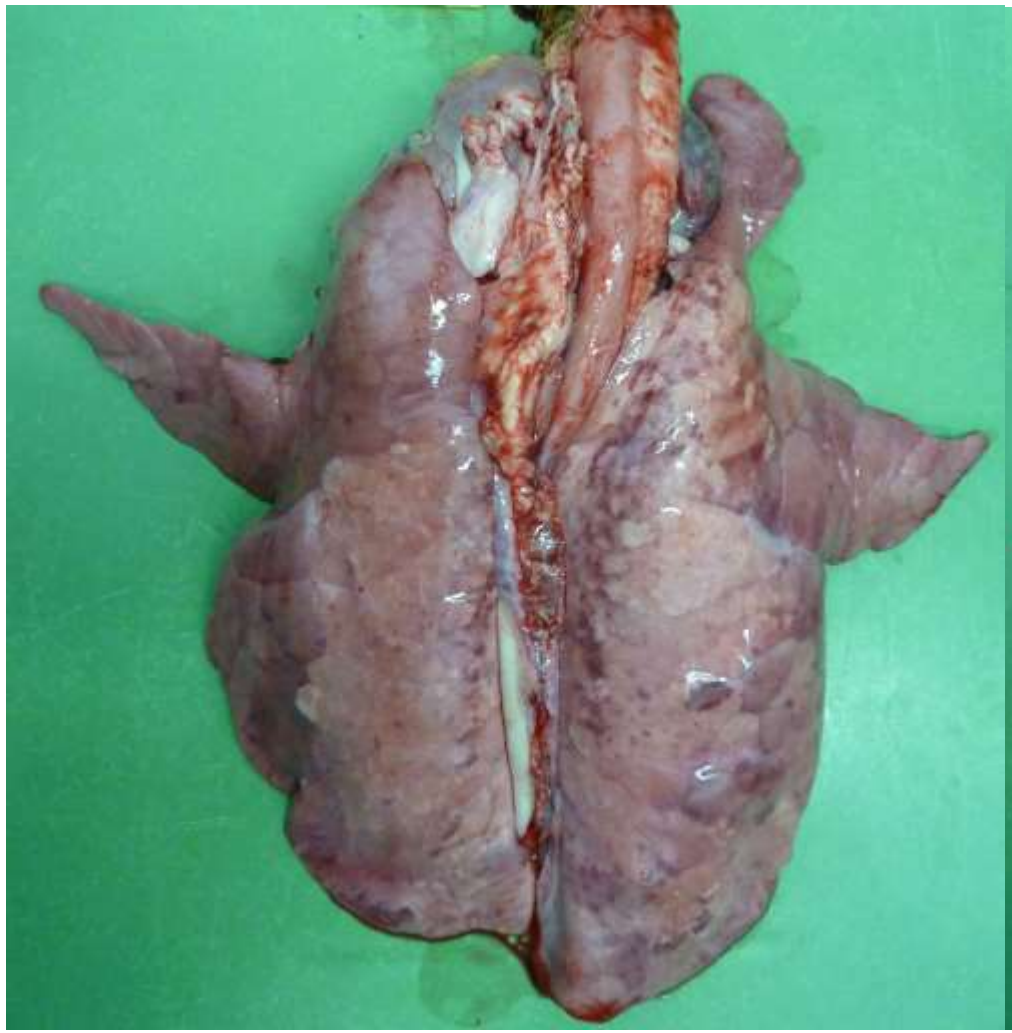


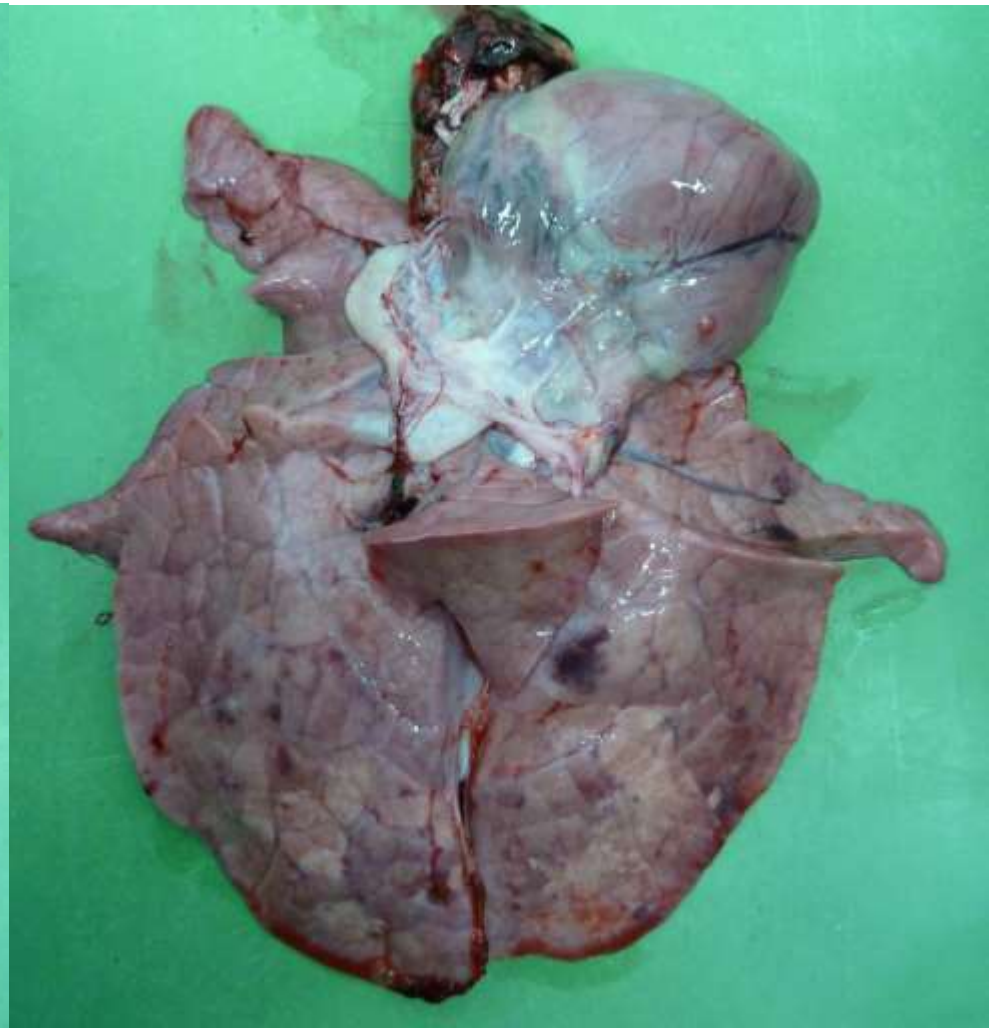


Immunopathogenesis in the lungs
(Gómez-Laguna et al., 2013)

- Pro inflammatory cytokine upregulation (TNF-α, INF-β)
- Anti inflammatorry cytokine downregulation (IL-10, IL-4)
- Haptoglobin levels increase
  - CD163 receptor upregulation helps virus internalization, replication
  - Decrease the bactricide activity of macrophages → **SECONDARY INFECTIONS**



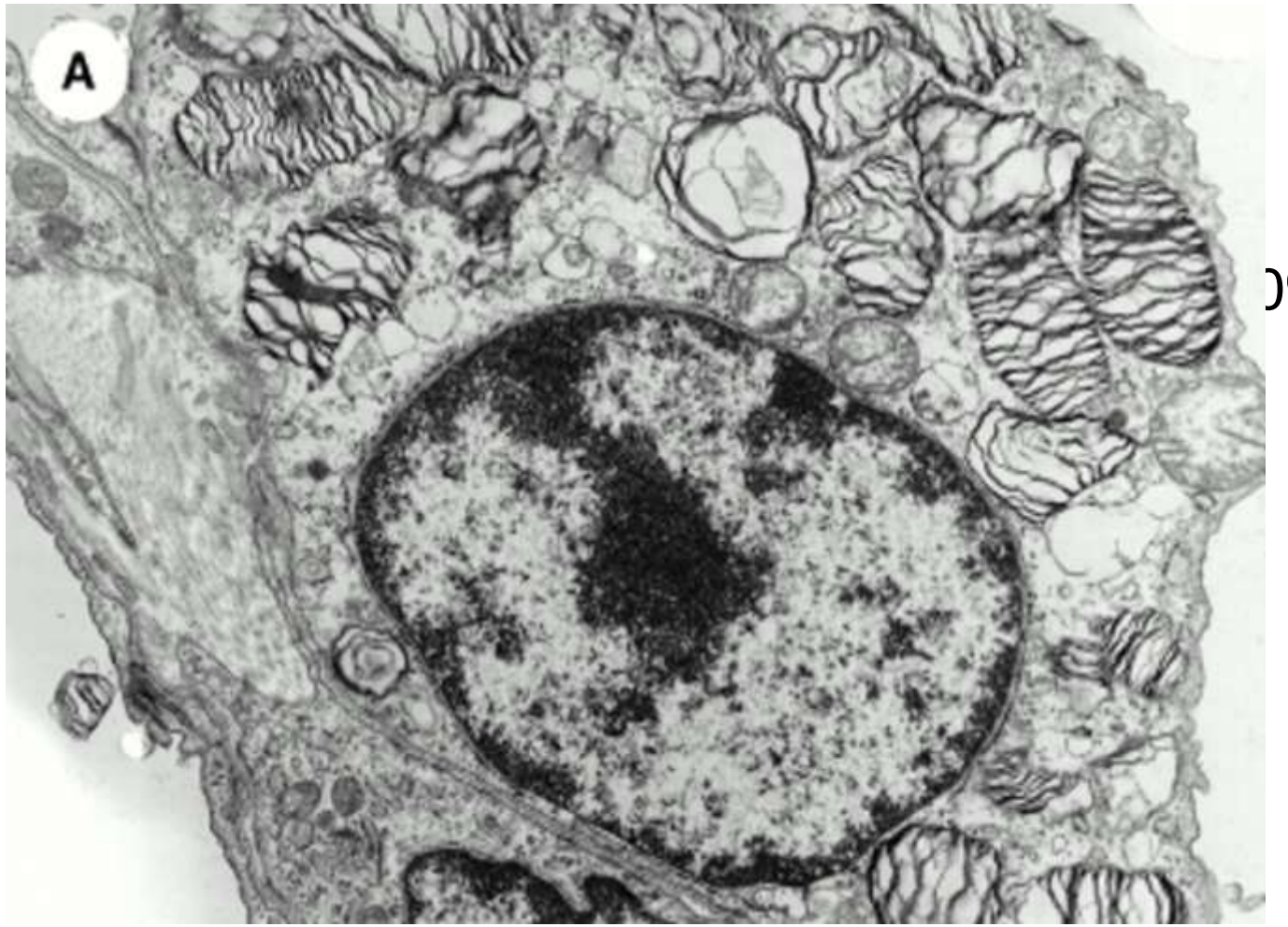




Microscoipc lung lesions in PRRS

- Intralobular interstitial pneumonia
- Septal infltration by mononuclear cells
- Intraalveolar necrotic debris
- Intraalveolar cellular infilration
- Type 2 pneumocyte hypertrophy and hyperplasia

Type 2 pneumocytes



)%

Aims

- Identification of type 2 pneumocytes in pigs
- Characterize PRRSV induced lesions
  - H&E
  - IHC
- Establish a new scoring system
- Find a more objective scoring method by counting IHC positive cell

PRRSV infection model

- 9-week-old piglets
- 2.2×10^5 $TCID_{50}$/ml, subtype 1 „wild” isolate, from Germany
- Euthanasia at 10 DPI (n = 7) and 21 DPI (n = 5), parallely from PBS treated control group
- Necropsy and histopathology from all 7 lung lobes
- FFPE
- H&E
- IHC (left middle lobe only): TTF-1, Ki67, MAC387, panCK
- Blinded analysis

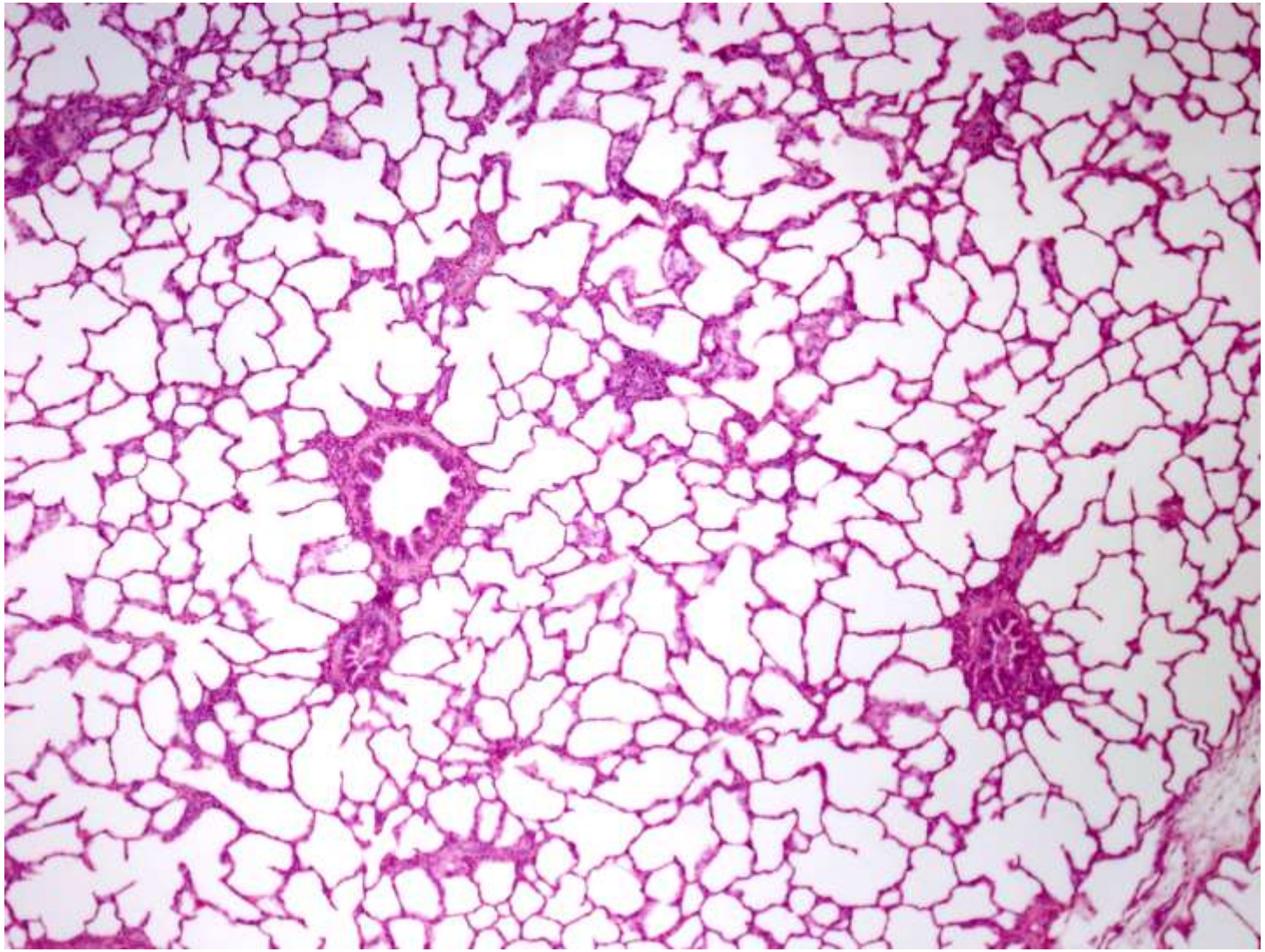
Scoring of H&E slides

Severity (0–3) and extention (0–3)

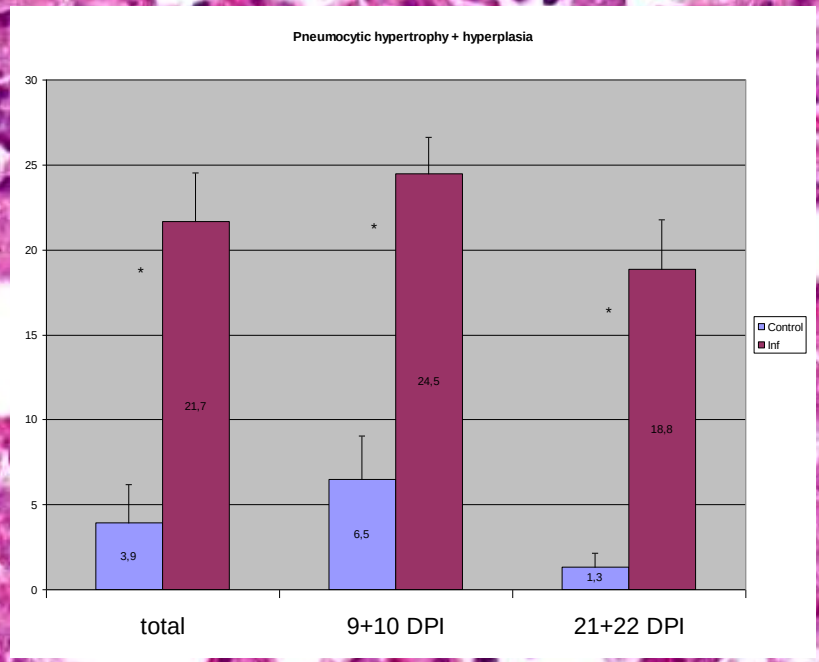
- pneumocyte hypertrophy and hyperplasia
- septal mononuclear infiltration
- intraalveolar necrotic debris accumulation
- intraalveolar inflammatory cells
- perivascular inflammatory cells

Scoring IHC slides

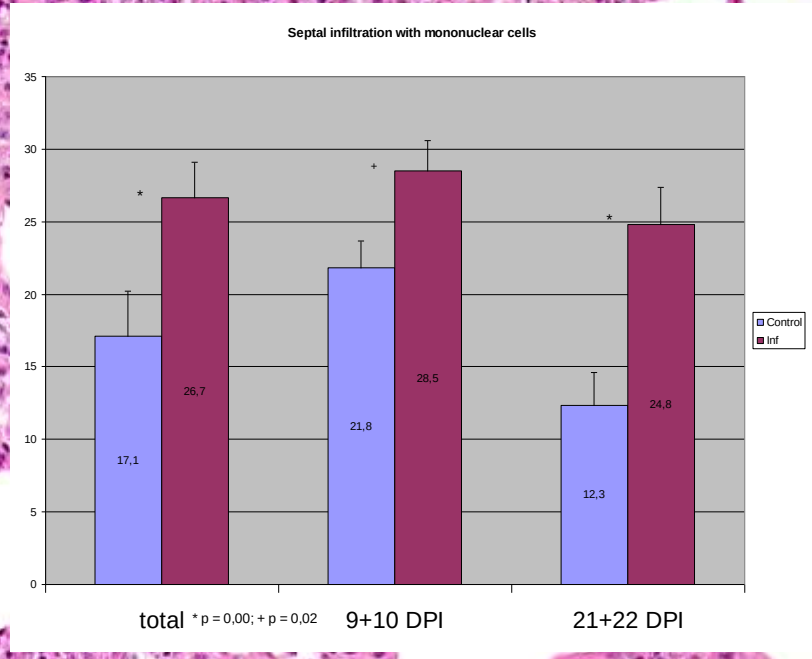
- TTF-1, Ki67, MAC387, (panCK)
- IHC positive cells on 50, non overlapping 0.20 mm^2 fields
- SPSS:
  - Significance calculation: Student’s T test
  - Correlation analysis IHC and H&E scores: Pearson’s X^2-test



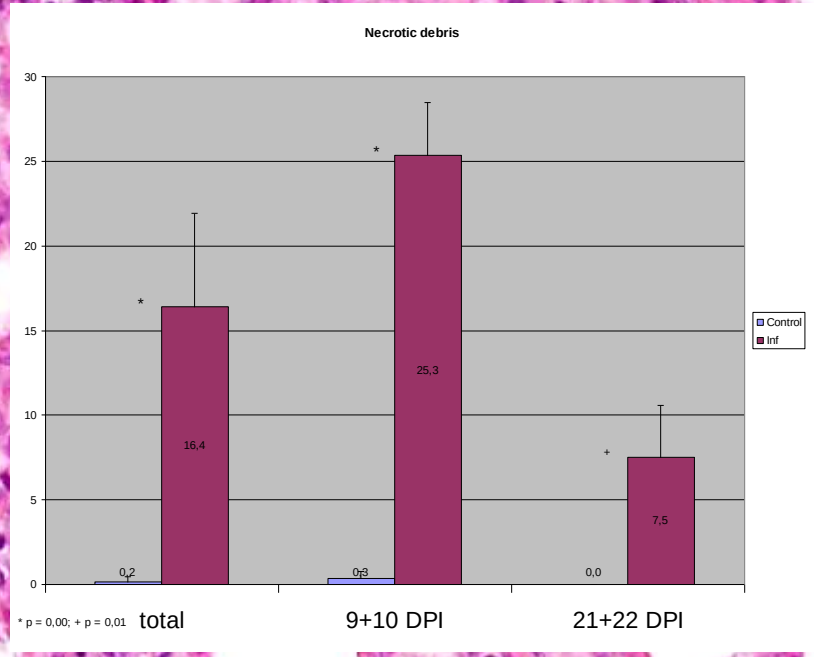
pneumocyte hypertrophy and hyperplasia



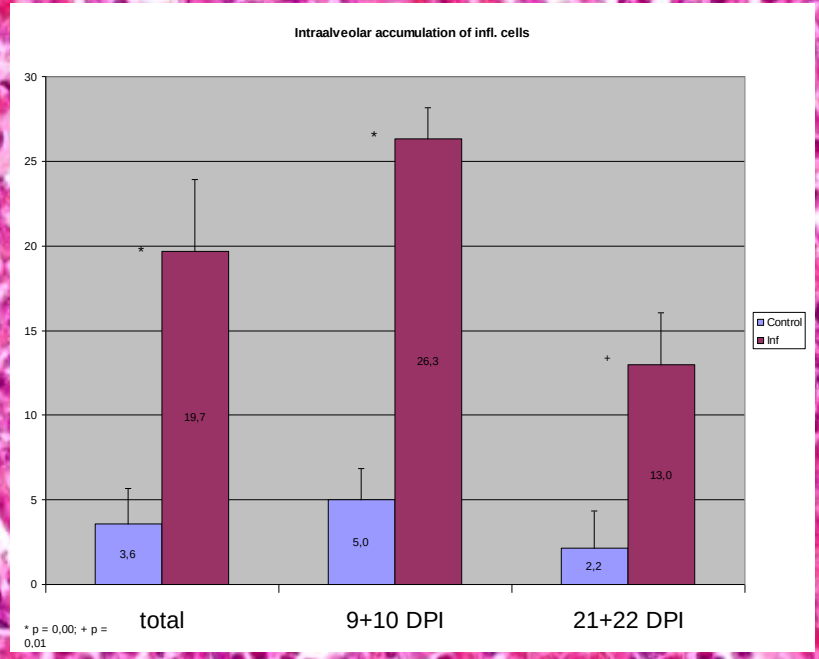
septal mononuclear infiltration



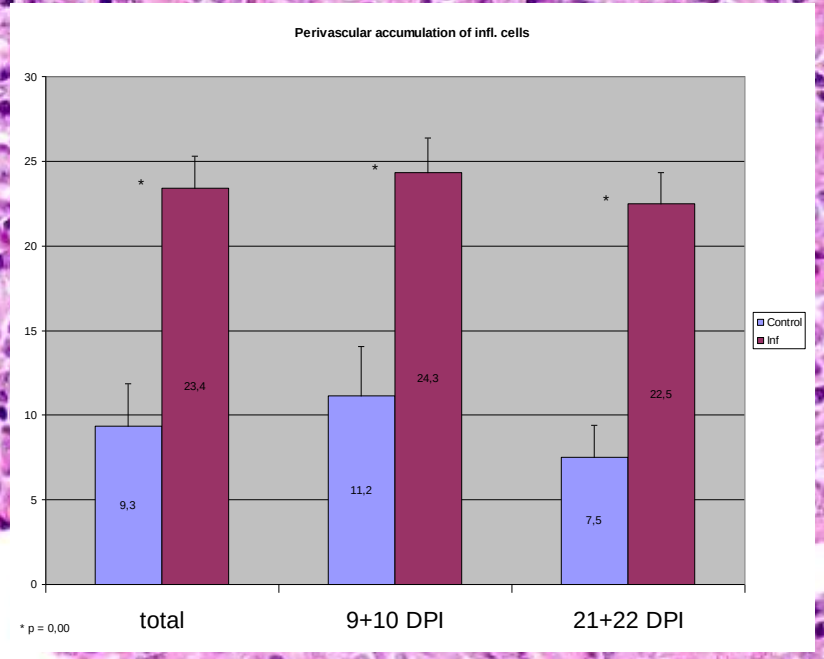
intraalveolar necrotic debris accumulation

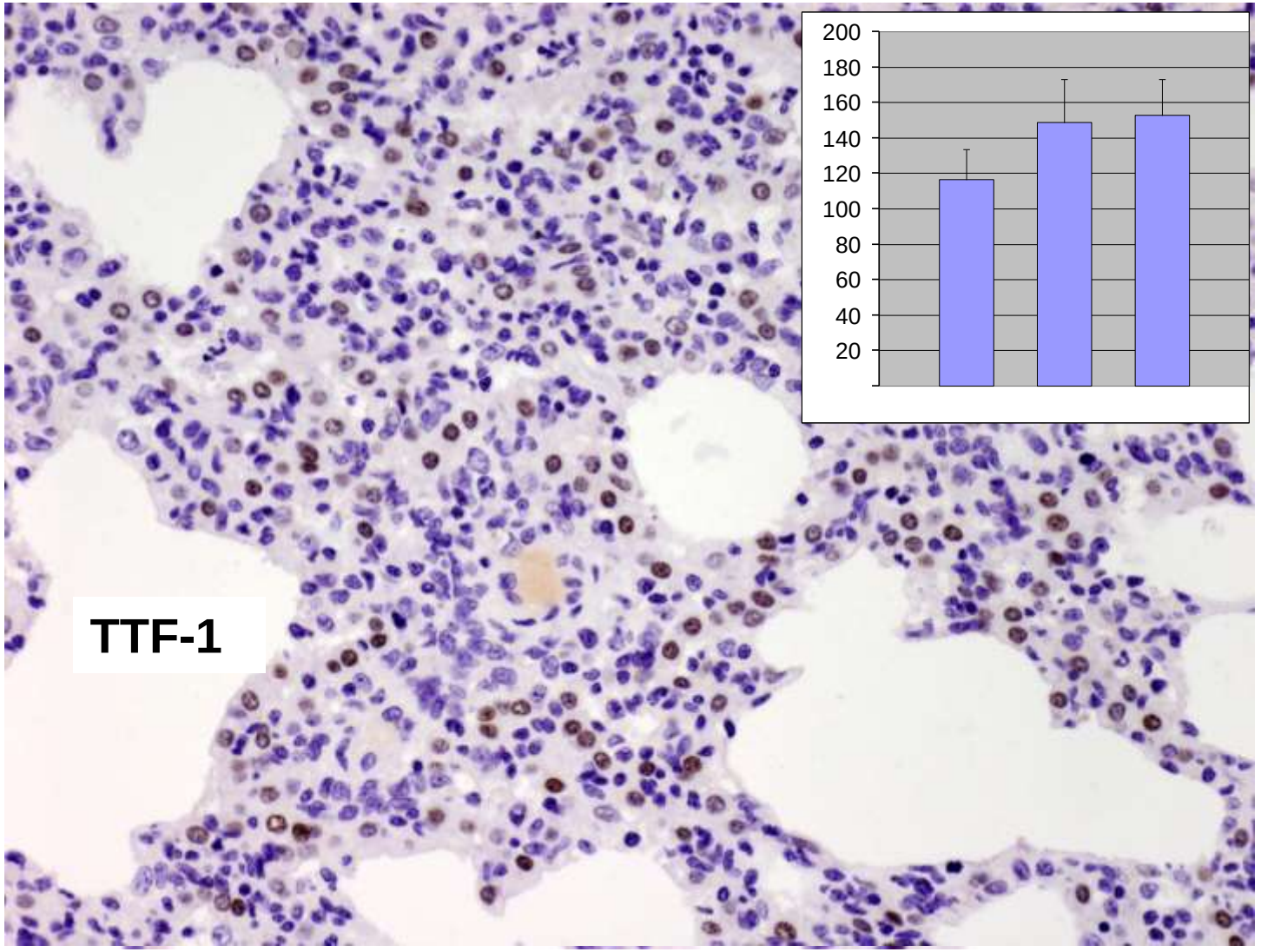


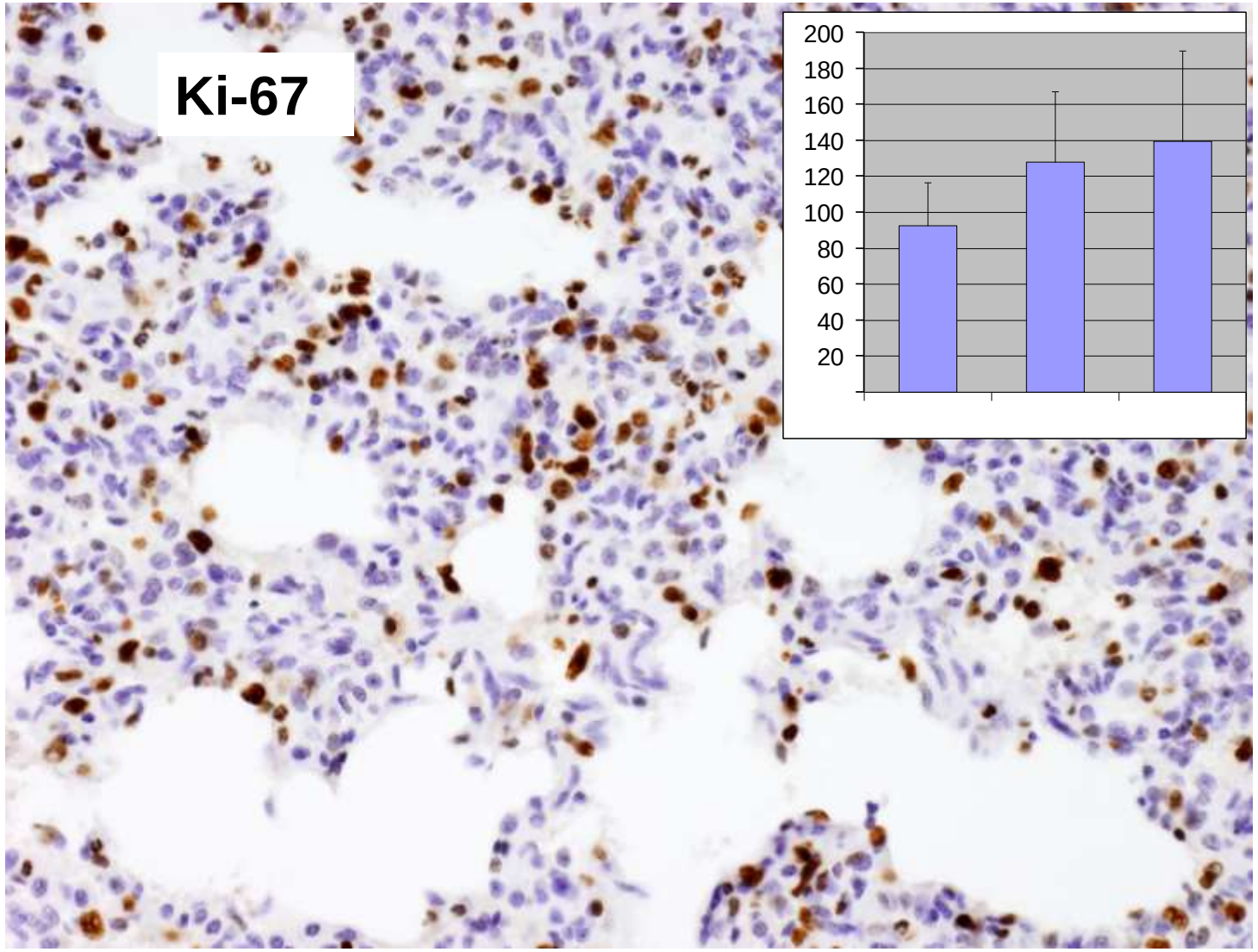
intraalveolar inflammatory cell inflitration

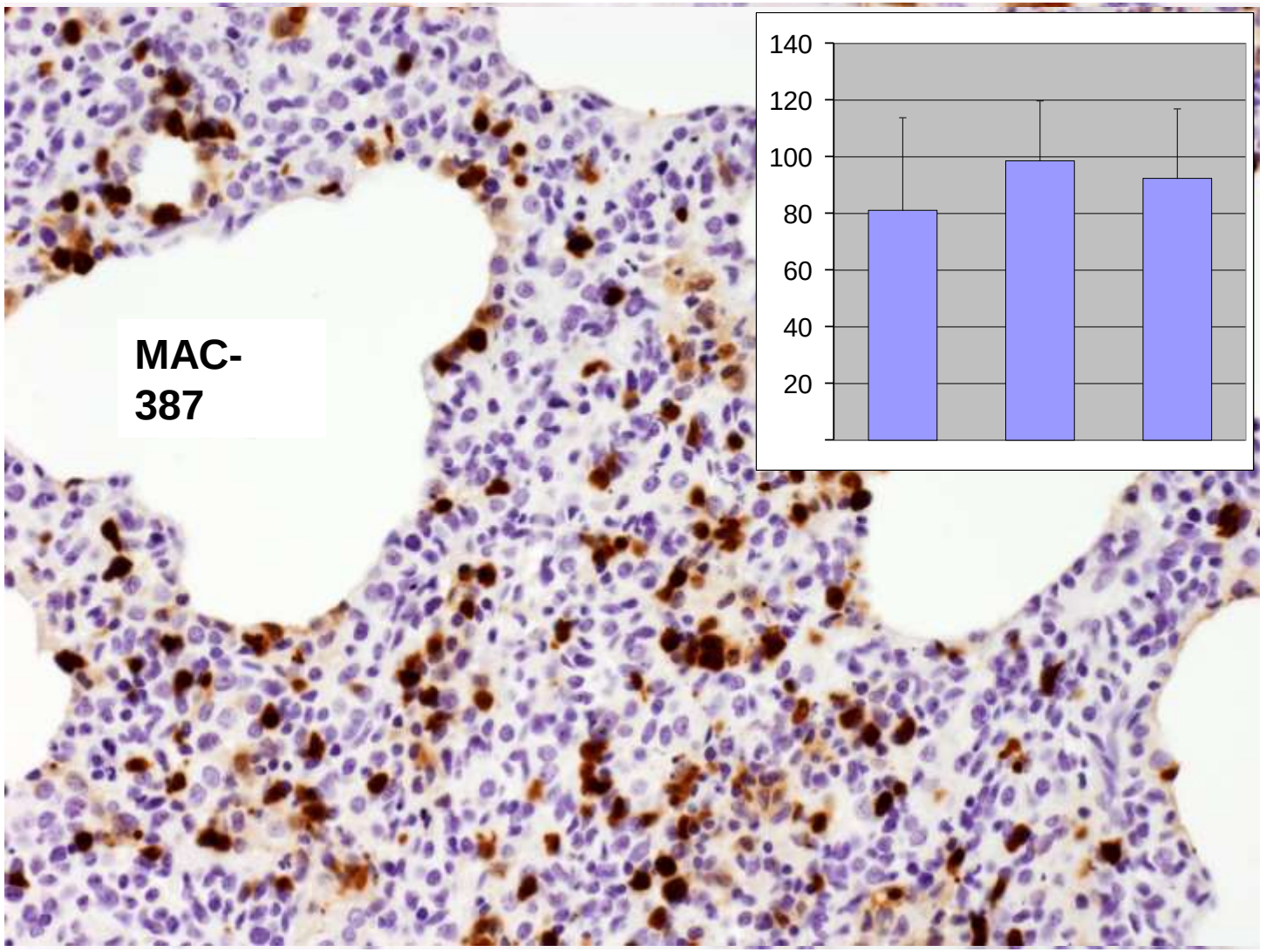


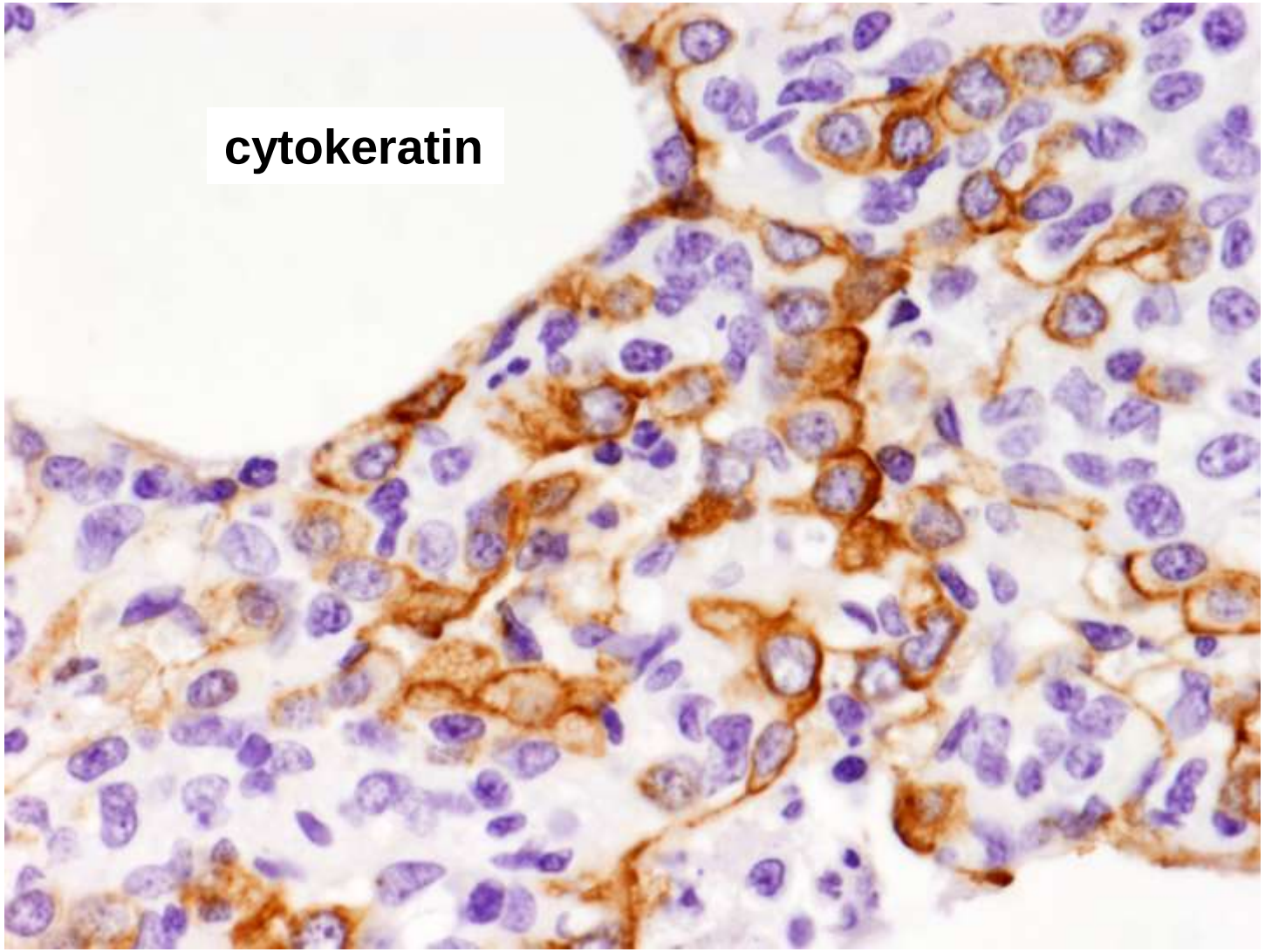
perivascular inflammatory cells

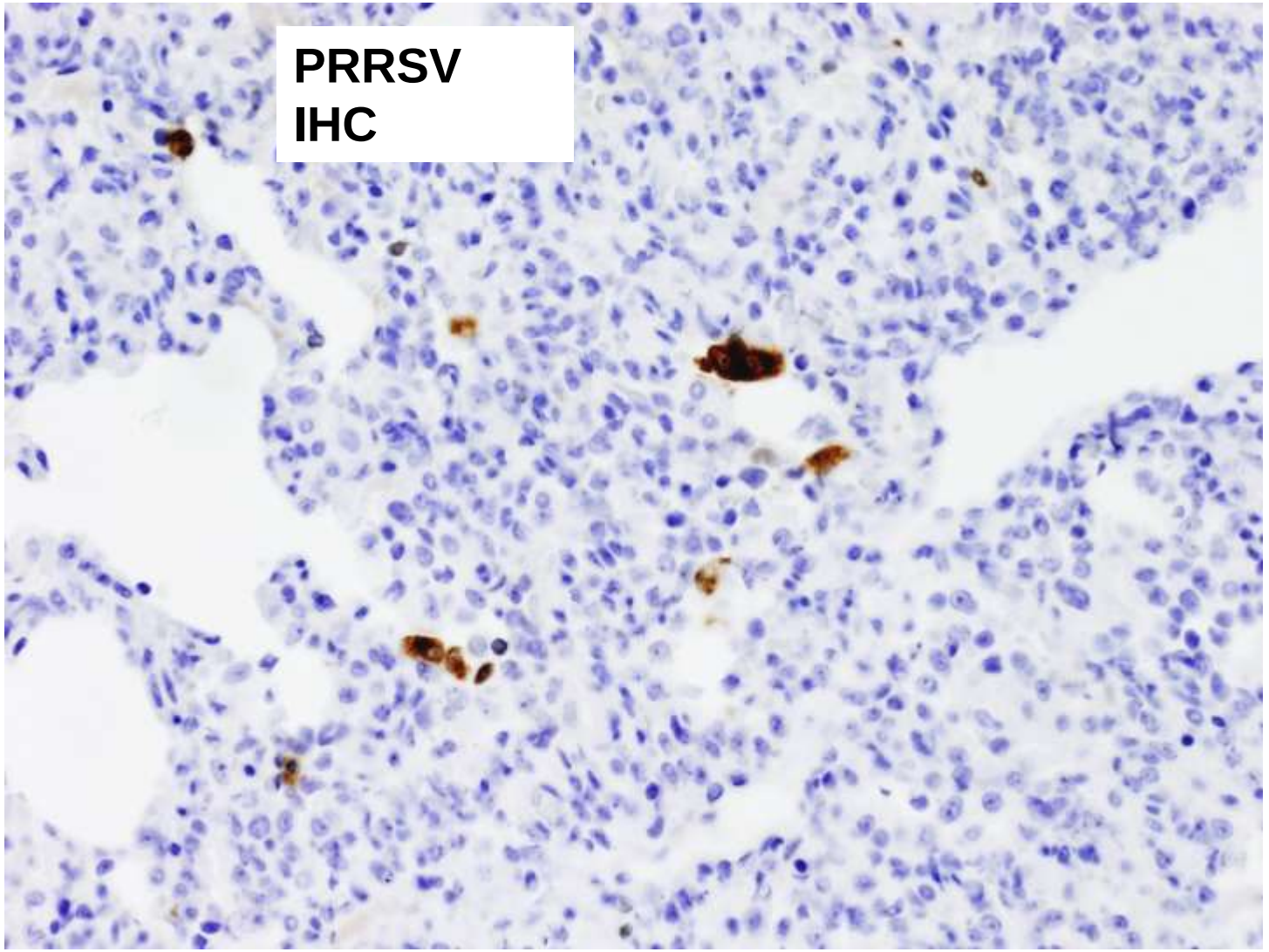



TTF-1
200
180
160
140
120
100
80
60
40
20


Ki-67
200
180
160
140
120
100
80
60
40
20


MAC-
387
140
120
100
80
60
40
20


cytokeratin


PRRSV
IHC

J. Comp. Path. 2013, Vol. 149, 322–330

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EXPERIMENTALLY INDUCED DISEASE

Immunohistochemical Characterization of Type II Pneumocyte Proliferation after Challenge with Type I Porcine Reproductive and Respiratory Syndrome Virus

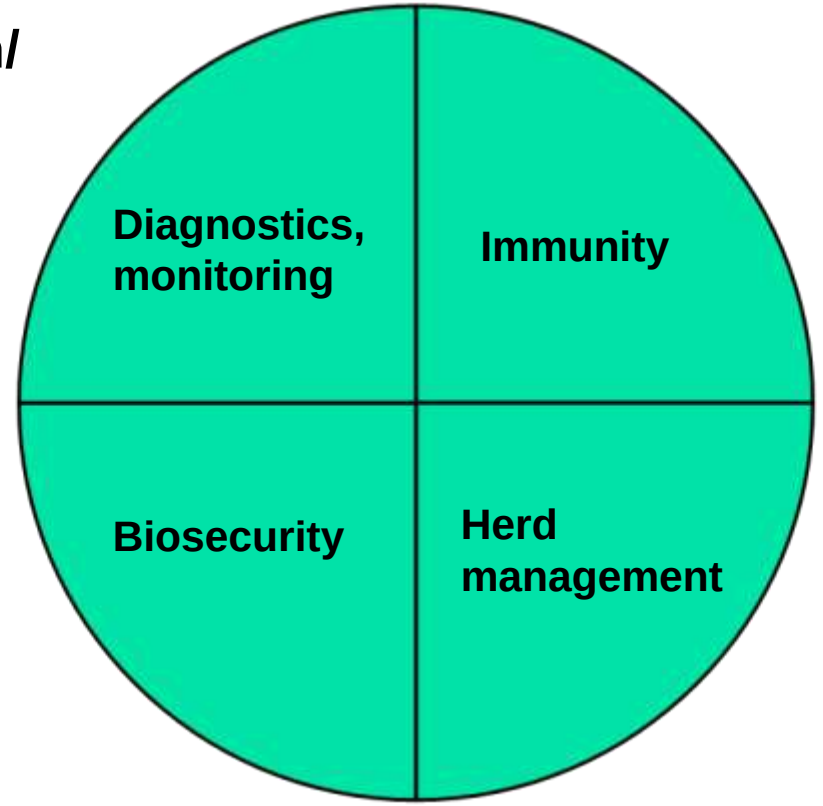
G. Balka[*], A. Ladinig[†], M. Ritzmann[†,‡], A. Saalmüller[§], W. Gerner[§], T. Käser[§], C. Jakab[*], M. Rusvai[*] and H. Weißenböck[¶]

** Department of Pathology and Forensic Veterinary Medicine, Faculty of Veterinary Science, Szent István University, Budapest, Hungary, † Clinic for Swine, Department for Farm Animals and Veterinary Public Health, University of Veterinary Medicine, Vienna, Austria, ‡ Clinic for Swine, Ludwig-Maximilians University, Oberschleissheim, Germany, § Institute of Immunology, Department of Pathobiology and ¶ Institute of Pathology and Forensic Veterinary Medicine, University of Veterinary Medicine, Vienna, Austria*

Control of PRRS

Control – eradication

Characterisation/ Early diagnosis



Gilt acclimatization Vaccination

External, Internal

Reduction of transmission, reduction of the economic impact

After Mateu 2017

Control – eradication

- The key of control: decrease or stop virus excretion/circulation among the sows
  - homogenous immune status of the breeding animals (gilts introduction)
  - Piglet vaccination to reduce shedding
- Eradication: LOAD-CLOSE-EXPOSE
  - Min. 210 days, mass vaccination two times at least, then loading with naive gilts
  - Thorough examination of suckling piglets (PCR), then the sentinels (ELISA)
- Prevent reinfection – biosecurity (air filtration)
- Vaccination + management!!!

Linhares et al. 2013

- **TTPN** (time to produce negative piglets)
- **TTBP** (time to baseline production)

LVI (local/live virus inoculation) vs. **MLV**

- With LVI negative piglets earlier
- With LVI earlier herd stability

BUT!!!

The use of MLV was significantly more cost effective, less additional losses (abortions, medication, loss of income, etc.)

Vaccines

- **MLV**: strong reaction, horizontal and vertical spread
- **KV**: no AB reaction in naïve animals; booster effect after infection or MLV;

  harmless, no shedding, no spread
- Marker, subunit, peptide vaccines, genome shuffling

Vaccines

- Genetic heterogenicity has a negative impact on vaccine efficacy (Labarque et al. 2004), BUT
- Degree of (ORF5) similarity can NOT predict vaccine efficacy (Prieto et al. 2008)
- Different strains (vaccines) induce differet cytokine production pattern (Díaz et al. 2006)
- Vaccines do not prevent infection and viraemia after heterologous challenge, BUT in a natural infection model they can provide 70% clinical protection (Martelli et al. 2009)

Suvaxyn PRRS challenge trial

- 41 piglets involved in the study born to 4 sows
- Piglets were randomly cross fostered after birth, then 2 litters got vaccinated

Treatment Group	Test Material	Dose Volume per Admin (mL)	Day of Admin	Challenge	Day of challenge	Day of necropsy**
T01	Saline solution (CP)	2 mL IM	D0	PRRSV-1 subtype 1 strain AUT15-33 by IN route at $1x10^6$ cfu total in 5ml	D28	D41/42
T02	Suvaxyn PRRS MLV (IVP)					

- At weaning (D25) former littermates were put back together

Suvaxyn PRRS challenge trial

- Challenge at day 28 with highly pathogenic PRRSV-1, Subtype 1 „ACRO” strain AUT15-33
- Clinical observation, recteal temperature, body weight
- Serum samples, nasal swabs, oral swabs
  - ELISA and PCR

- Euthanasia at day 41/42
- Necropsy
  - Macroscopical evaluation (% affetced)
  - Lung tissue samples for PCR and histopathology

Suvaxyn PRRS challenge trial

- Histopathology (Balka et al. 2013, J. Comp. Pathol.)

BLINDED ANALYSIS

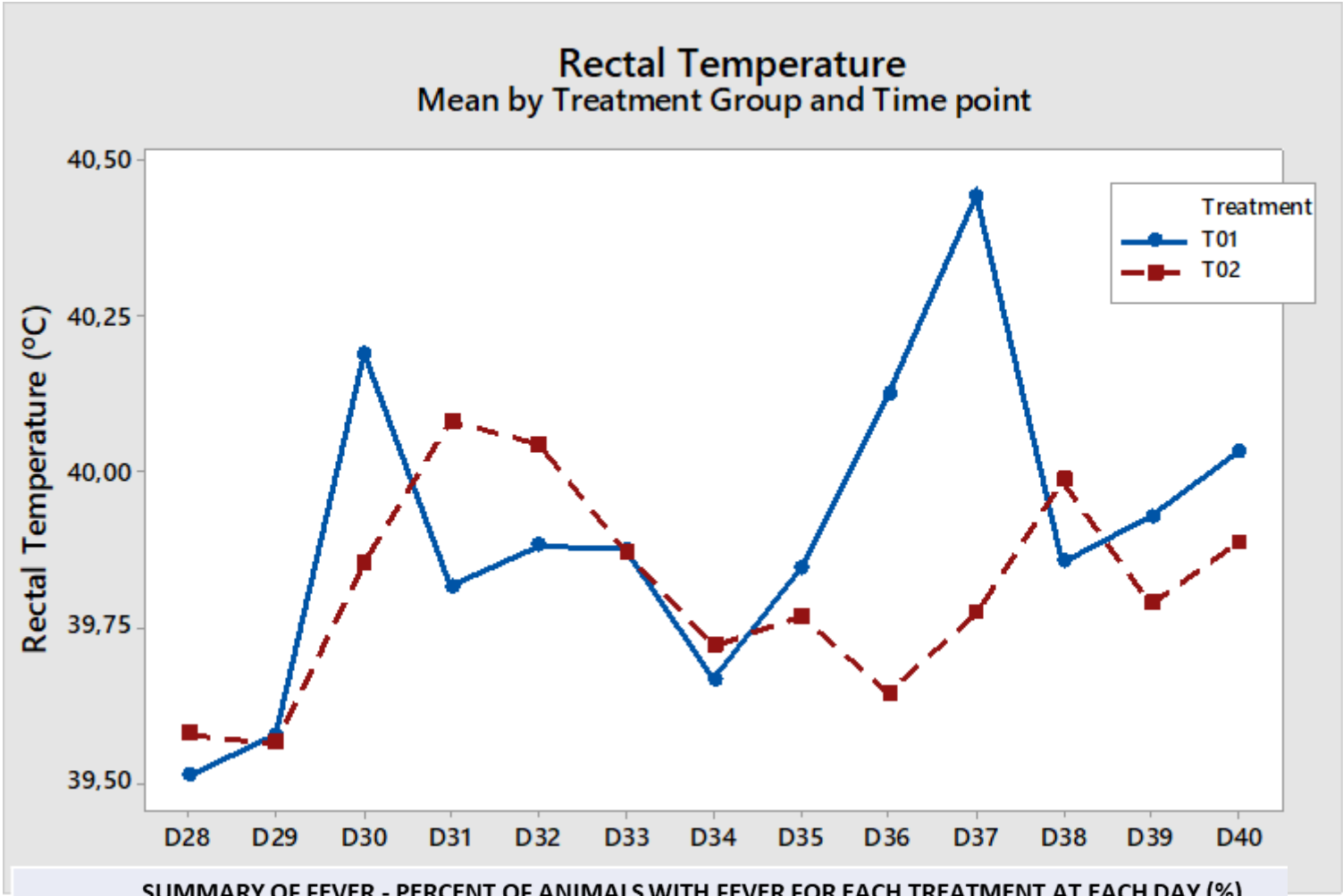
  - Pneumocytic hypertrophy and hyperplasia
  - Septal infiltration with mononuclear cells
  - Intraalveolar necrotic debris
  - Intraalveolar accumulation of inflammatory cells
  - Perivascular accumulation of inflammatory cells

Suvaxyn PRRS challenge trial RESULTS

ANALYSIS OF BODY WEIGHT - COMPARISONS OF AVERAGE DAILY GAIN BETWEEN TREATMENTS

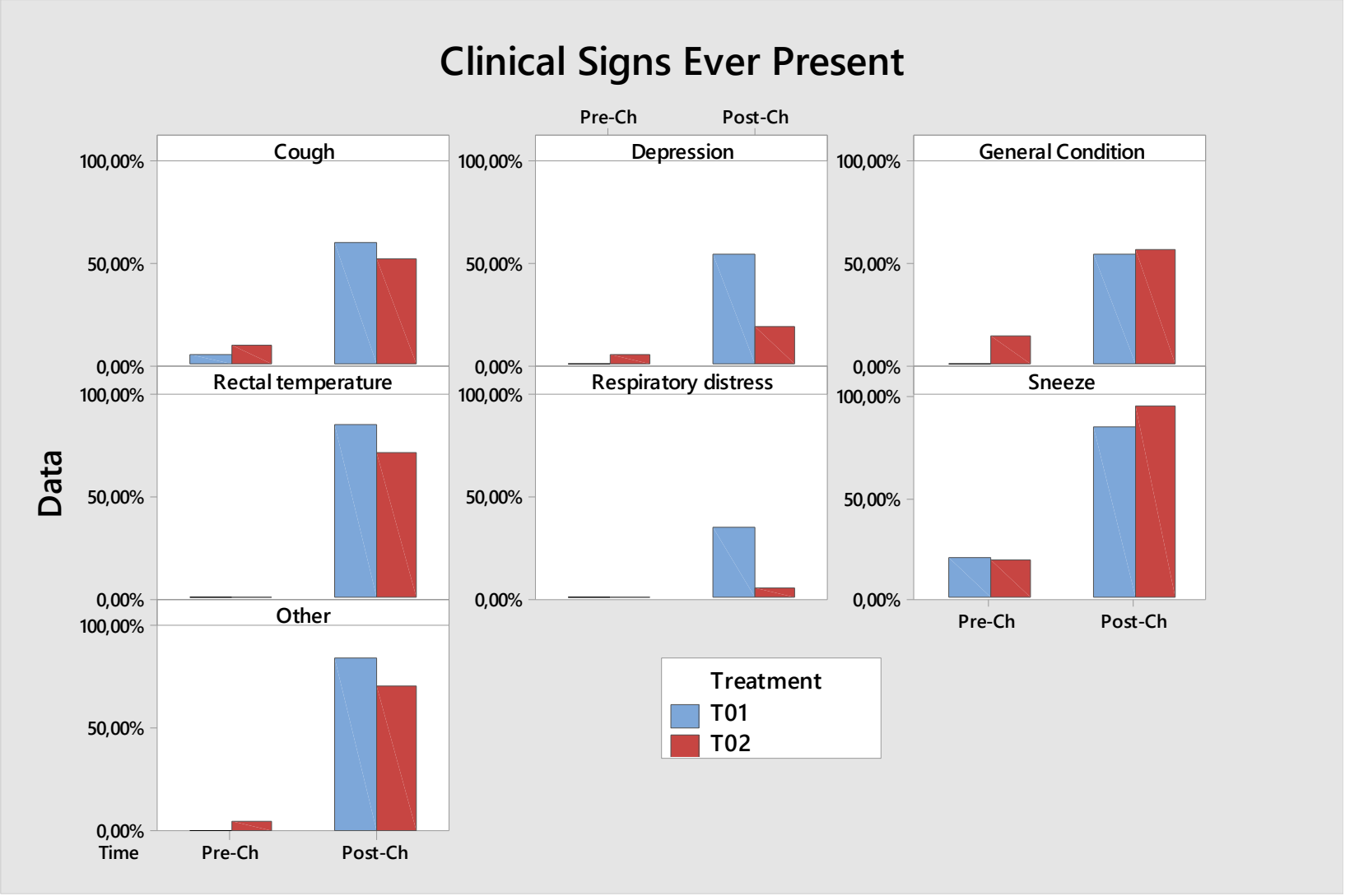
Label	Difference in average daily gain	std error of diff. in average daily gain	2-tailed p-value (1)	Significance of 2-tailed p-value (2)
28 to 35 ADG T01 v T02	**0.07**	0.02	0.0013	*
28 to 41/42 ADG T01 v T02	**0.07**	0.03	0.0173	*
35 to 41/42 ADG T01 v T02	0.07	0.04	0.0971	N.S.

Suvaxyn PRRS challenge trial RESULTS

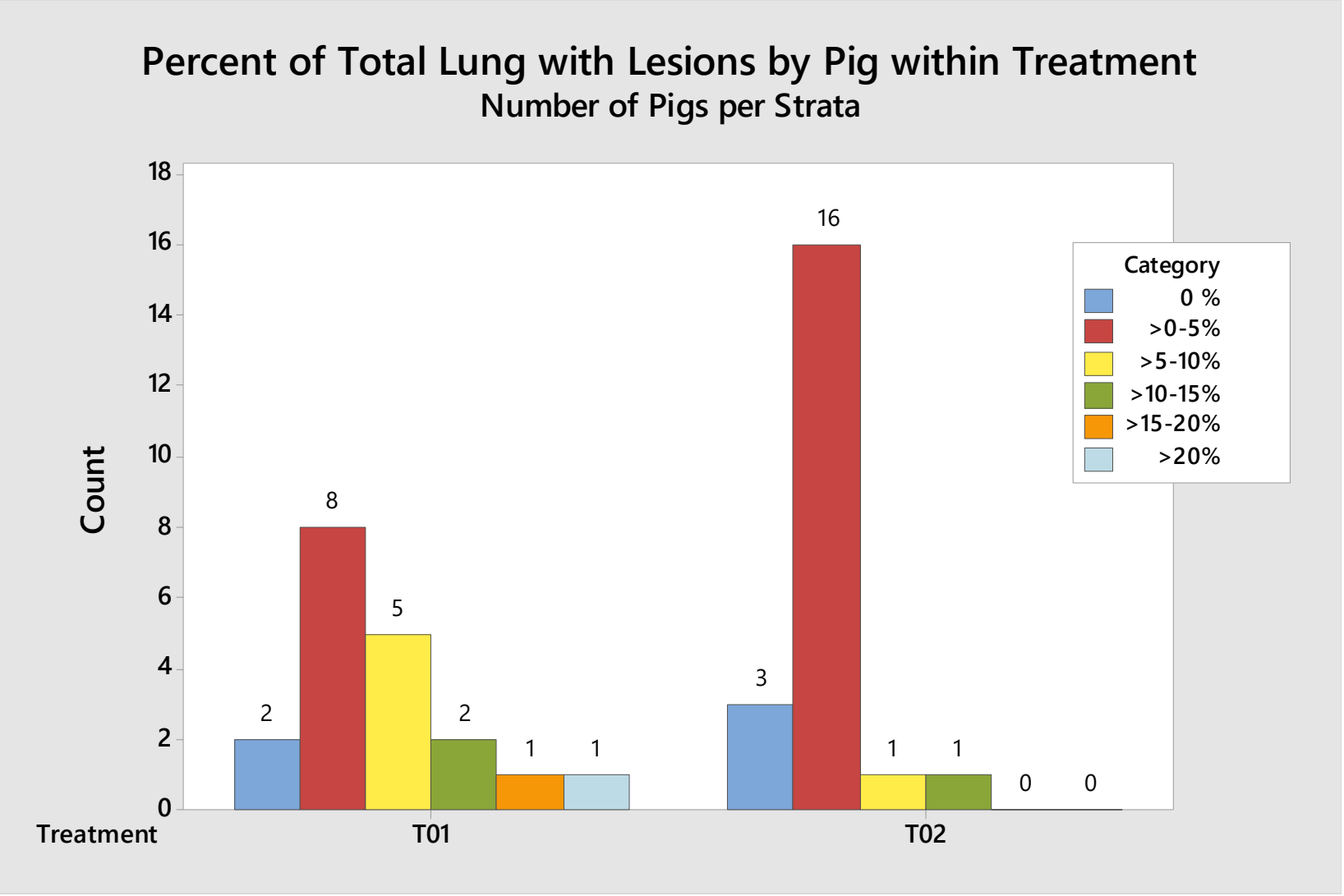


SUMMARY OF FEVER - PERCENT OF ANIMALS WITH FEVER FOR EACH TREATMENT AT EACH DAY (%)													
T01	0.0	0.0	30.0	15.0	5.0	5.0	0.0	0.0	15.0	55.0	5.0	15.8	21.1
T02	0.0	4.8	0.0	28.6	28.6	4.8	0.0	9.5	4.8	4.8	4.8	4.8	9.5

Suvaxyn PRRS challenge trial RESULTS



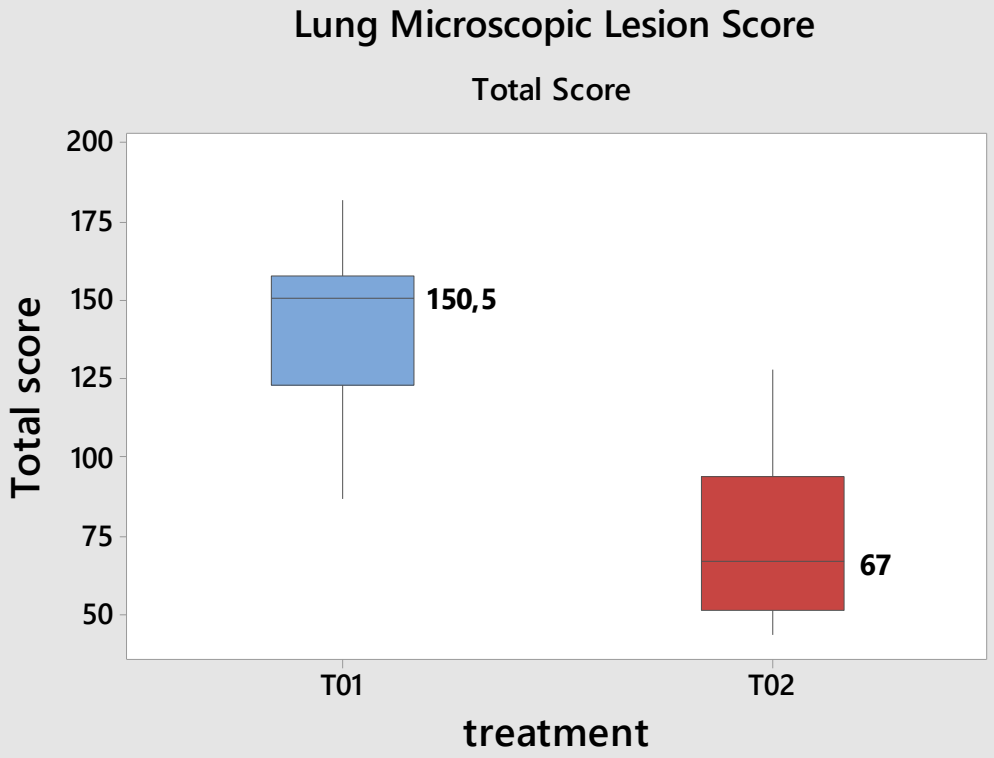
Suvaxyn PRRS challenge trial RESULTS

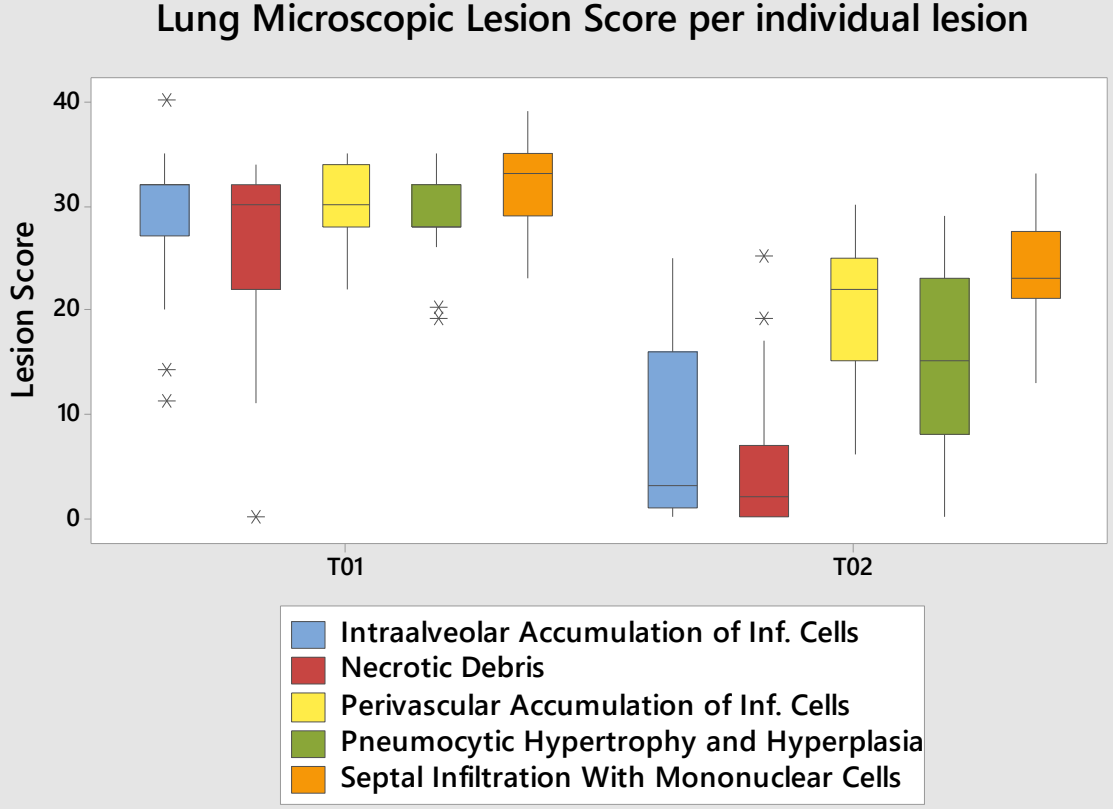


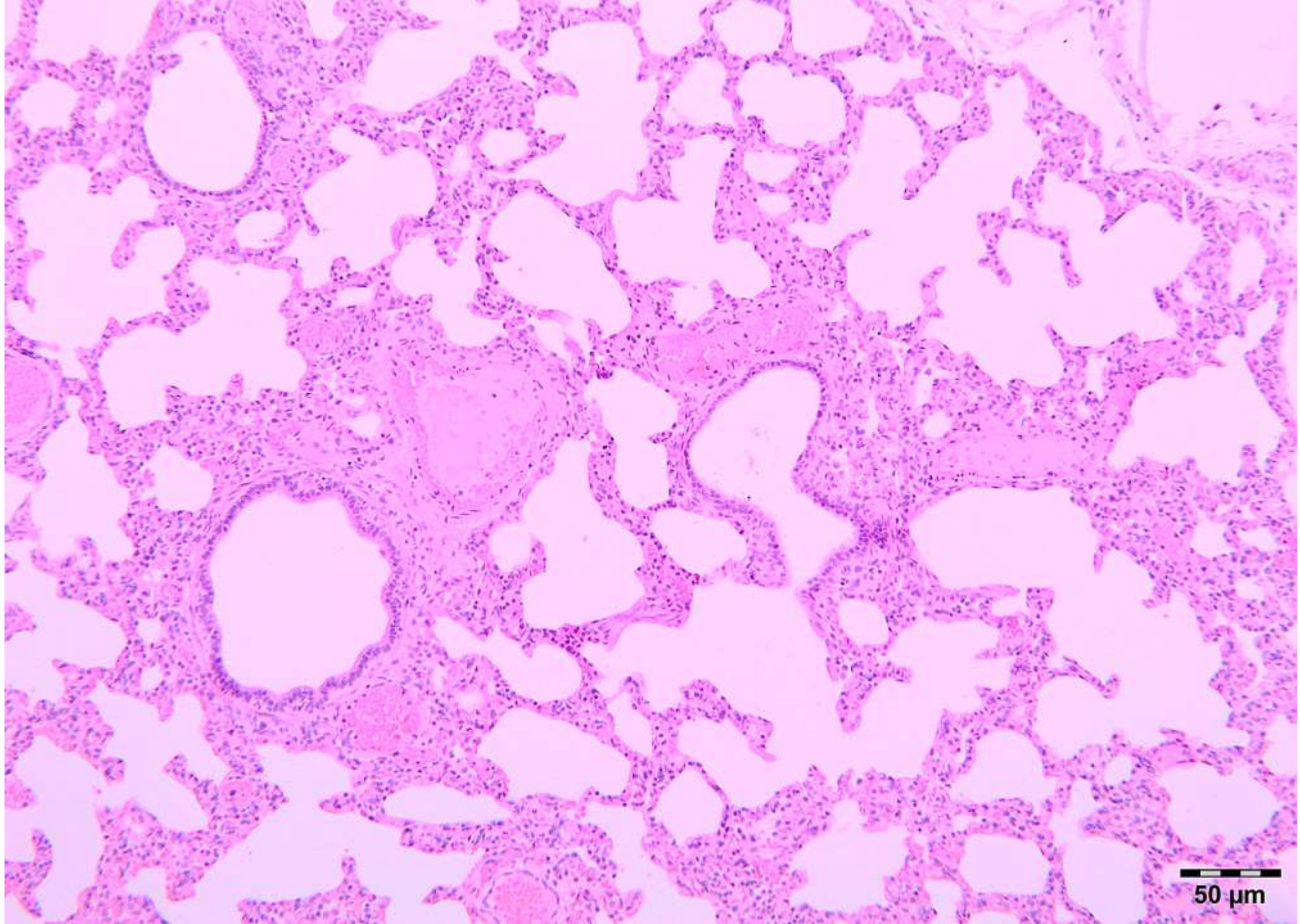
Suvaxyn PRRS challenge trial
RESULTS – histological scoring sheet

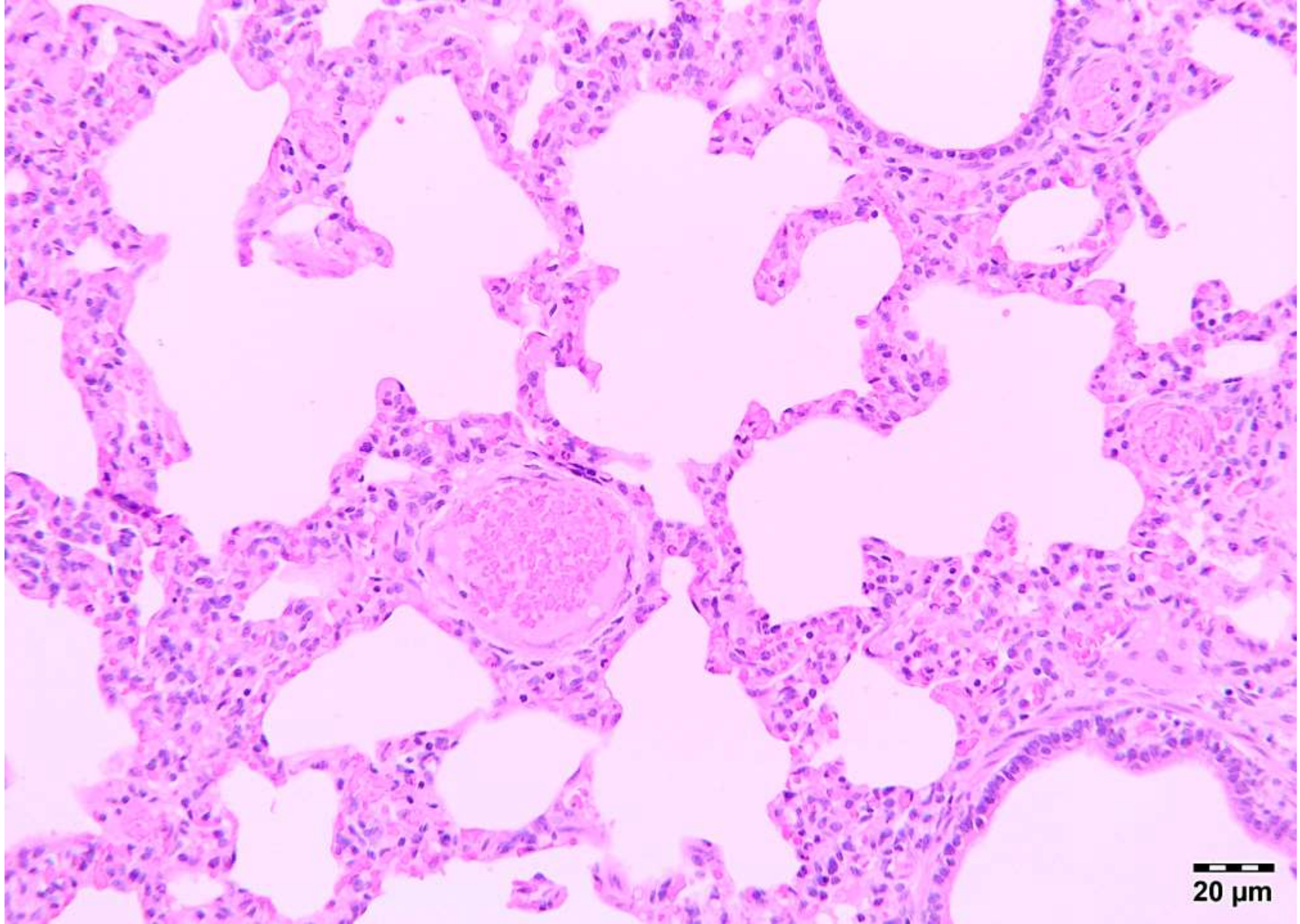
Microscopic Examination of the Lung			**Nr.: 5886**													
			L cran		L mid		L caud		R cran		R mid		R caud		acc	
lesions			S	E	S	E	S	E	S	E	S	E	S	E	S	E
pneumocytic hypertrophy and hyperplasia	0	not present					0	0					0	0	0	0
	1	mild	1	1	1				1		1	1				
	2	moderate				2				2						
	3	severe														
septal infiltration with mononuclear cells	0	not present					0	0					0	0	0	0
	1	mild	1								1	1				
	2	moderate		2	2	2			2	2						
	3	severe														
necrotic debris	0	not present	0	0	0	0		0			0	0	0	0	0	0
	1	mild							1	1						
	2	moderate														
	3	severe														
intraalveolar accumulation of infl. cells	0	not present	0	0			0	0			0	0	0	0	0	0
	1	mild			1	1			1							
	2	moderate								2						
	3	severe														
perivascular accumulation of infl. cells	0	not present					0	0					0	0	0	0
	1	mild	1	1							1	1				
	2	moderate			2	2			2	2						
	3	severe														
		total														

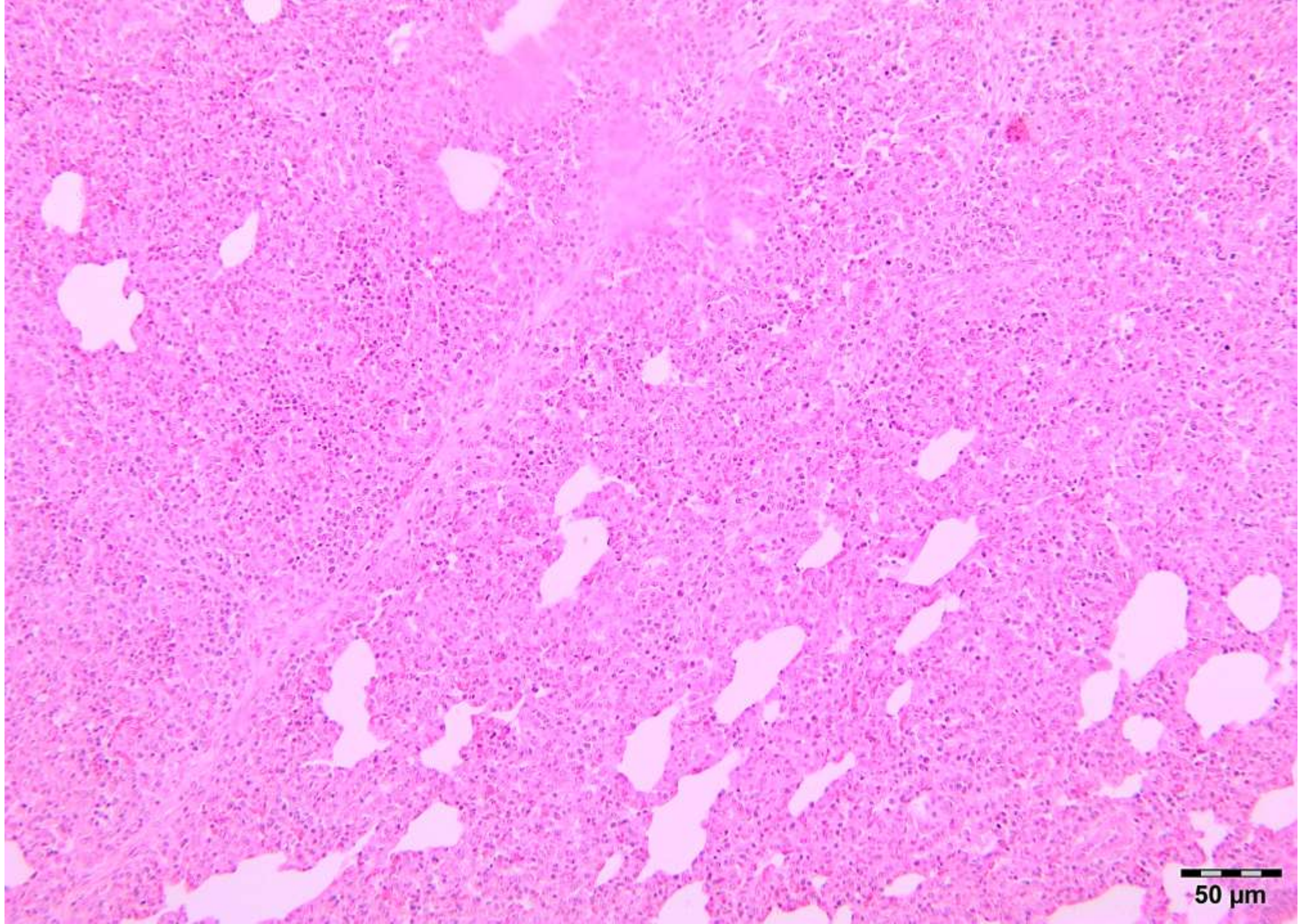
Suvaxyn PRRS challenge trial
RESULTS – summary of histological data

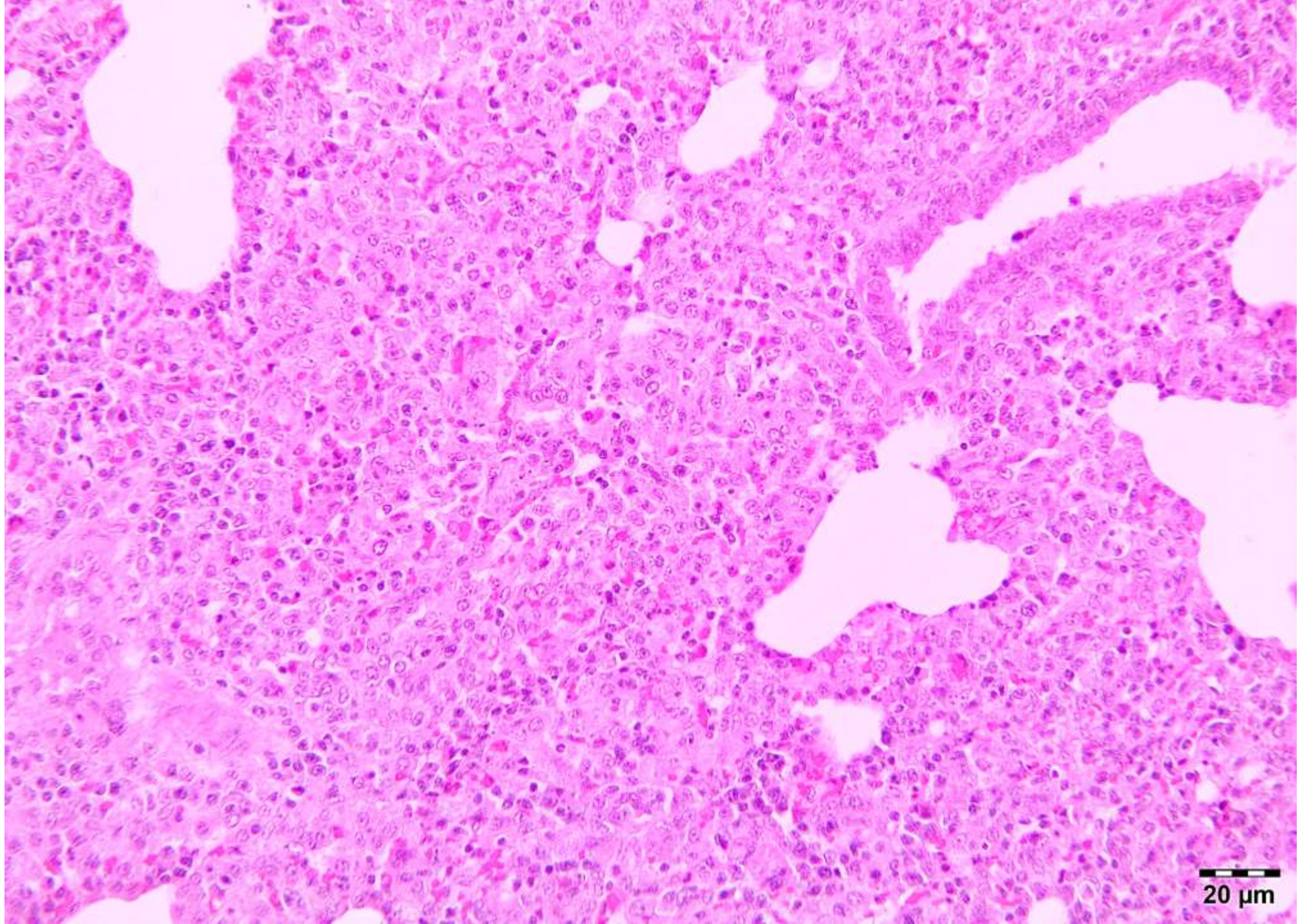


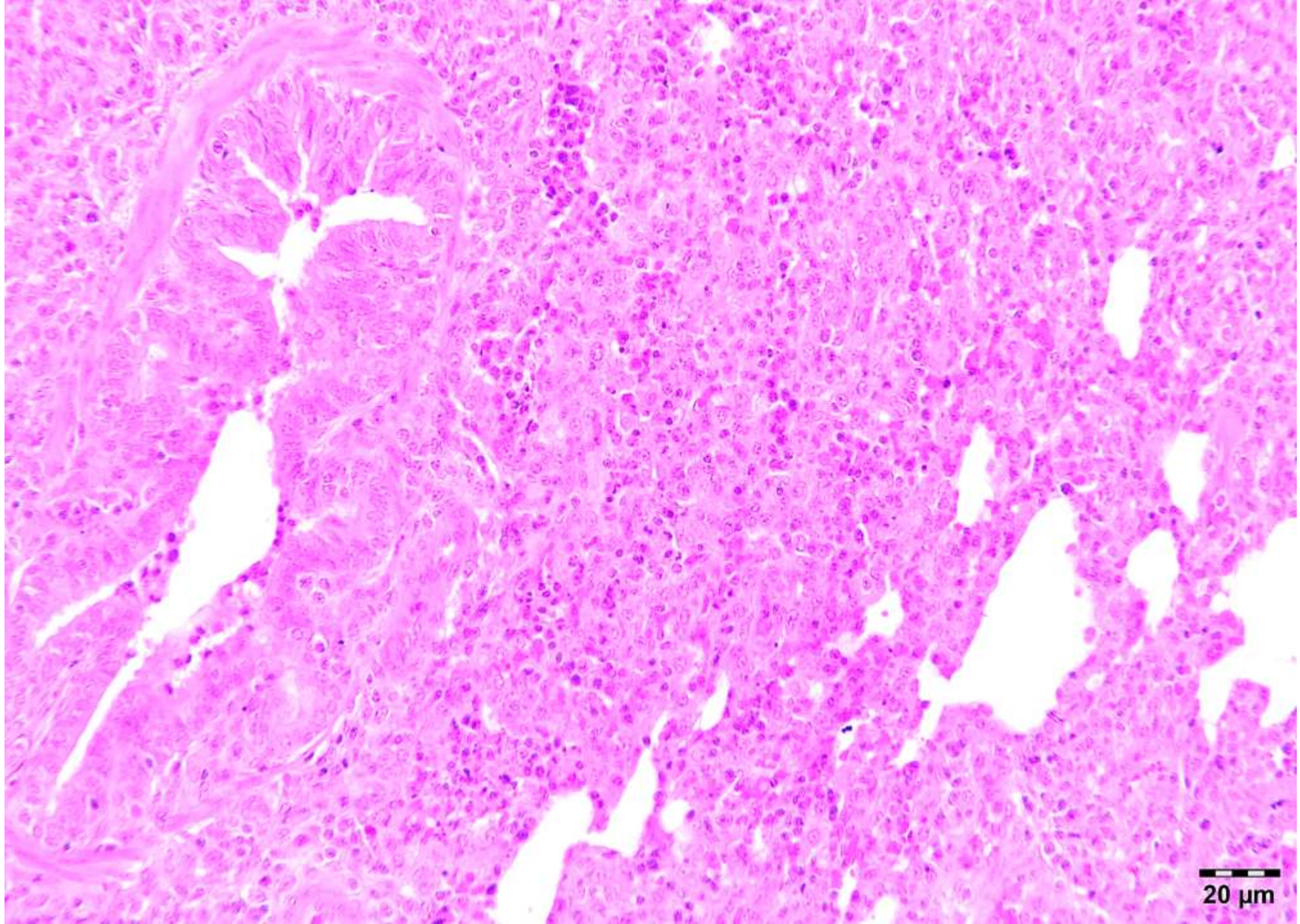


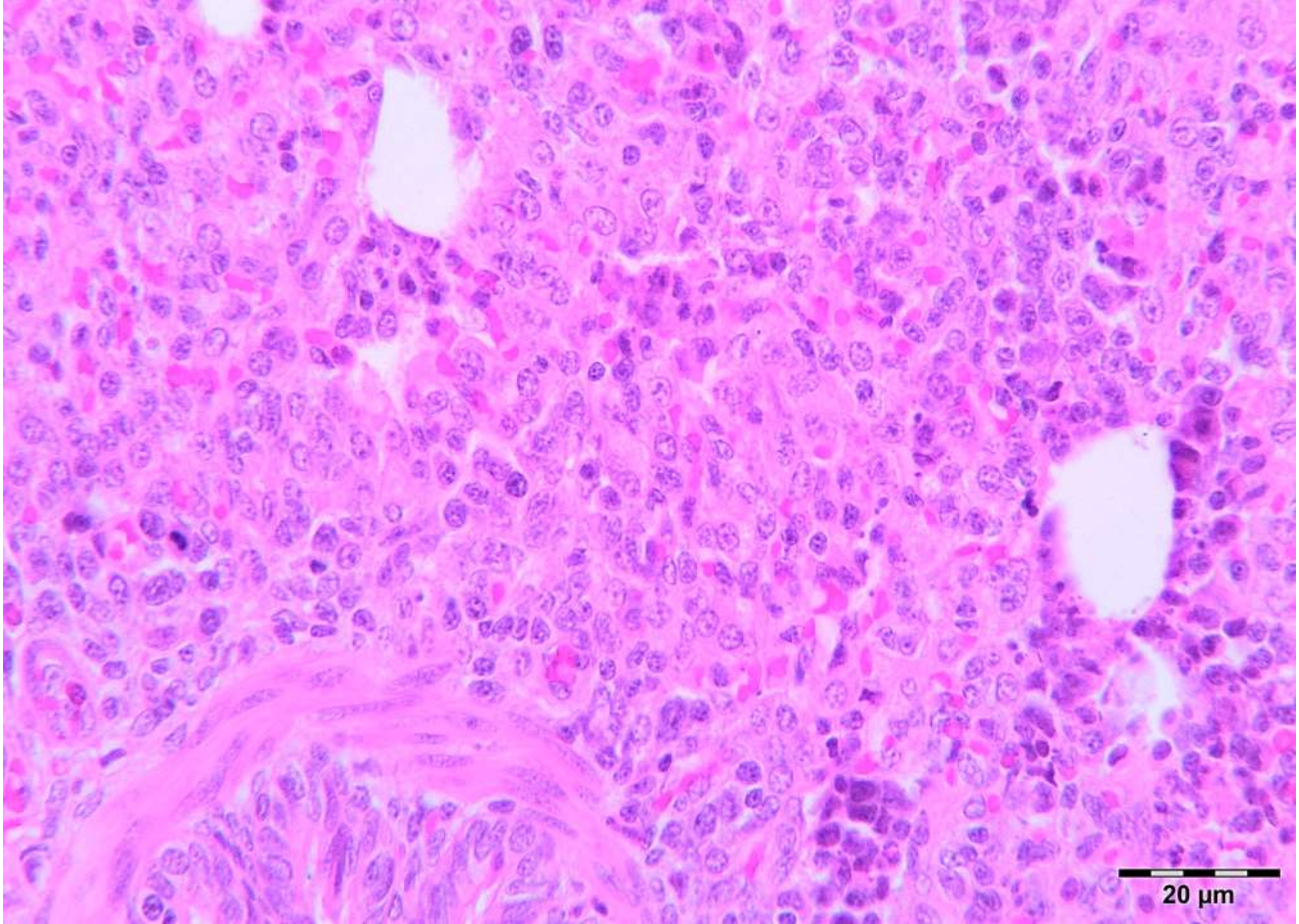

50 µm


20 µm


50 µm


20 µm


20 µm


20 µm

Thank you for your attention!