



UNIVERSITI PUTRA MALAYSIA

**HISTOPATHOLOGY AND IMMUNOHISTOCHEMISTRY (IHC) STUDY OF
AFRICAN SWINE FEVER (ASF) IN DOMESTIC PIG TISSUE**

HUAM ZE SHIUN

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FPV 2022 81**

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FACULTY OF VETERINARY MEDICINE

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SERDANG, SELANGOR

2022/2023

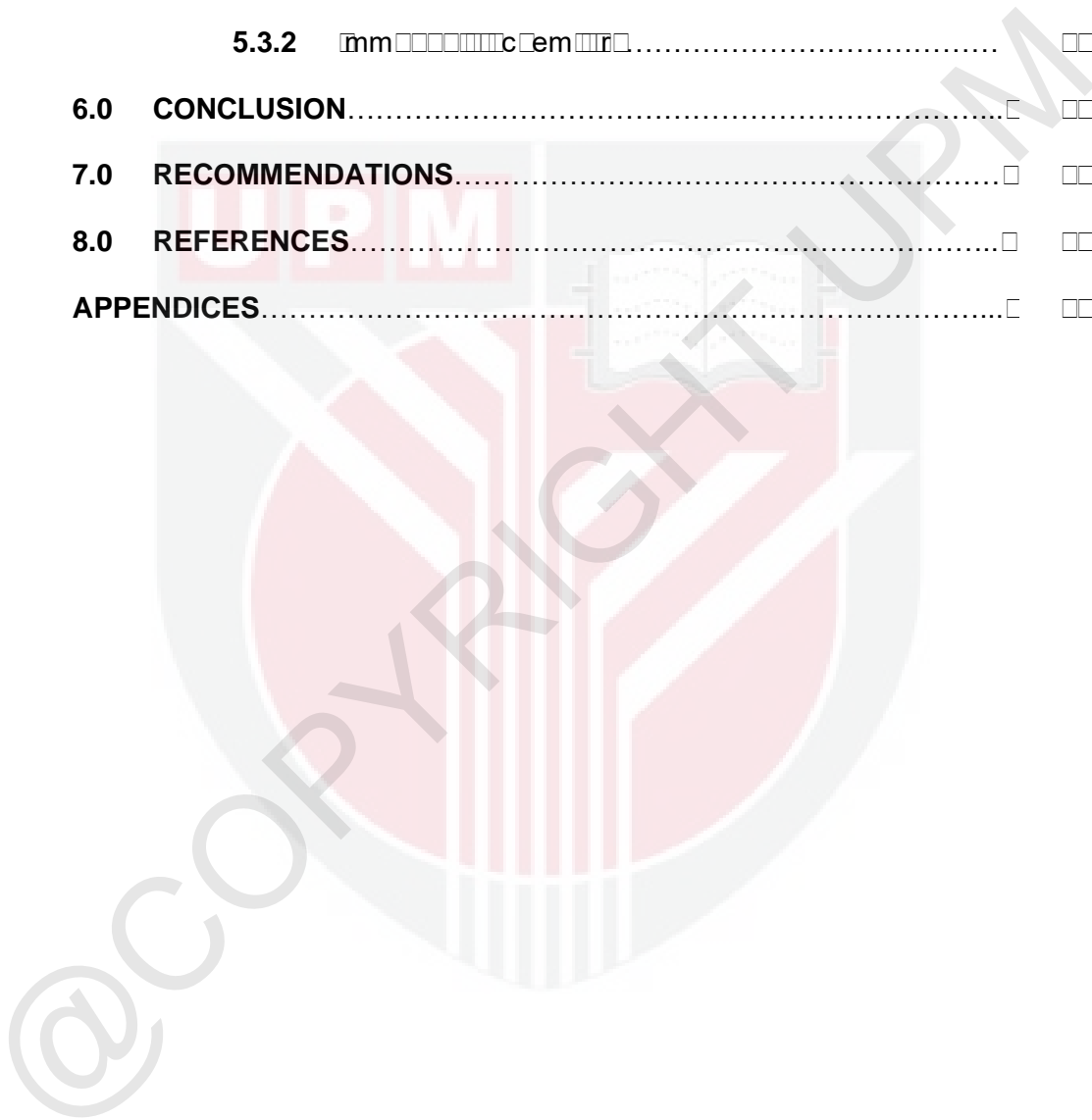
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4.2.1.2	100
4.2.2	100
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ABSTRAK

Abstrak mengenai kajian histopatologi dan immuno-histokimia penyakit demam babi Afrika (ASF) dalam tisu babi domestik.

□

KAJIAN HISTOPATOLOGI DAN IMMUNOHISTOKIMIA PENYAKIT DEMAM BABI

AFRIKA (ASF) DALAM TISU BABI DOMESTIK

□
□ e □ □

Huam Ze Shiun

2022

Penyelia: Dr. Nurul Izzati Uda Zahli

Penyelia bersama: Profesor Madya Dr Ooi Peck Toung, Dr Michelle Fong Wai Cheng

Abstrak mengenai kajian histopatologi dan immuno-histokimia penyakit demam babi Afrika (ASF) dalam tisu babi domestik. Kajian ini bertujuan untuk mengenalpastikan perubahan histopatologi dan imuniti selular dalam tisu babi domestik yang terdedah kepada ASF. Hasil kajian menunjukkan bahawa terdapat perubahan histopatologi yang signifikan dalam tisu babi domestik yang terdedah kepada ASF, termasuklah nekrosis epitelium, infiltrasi selular, dan perubahan dalam imuniti selular. Kajian ini juga menunjukkan bahawa terdapat perubahan dalam imuniti selular dalam tisu babi domestik yang terdedah kepada ASF, termasuklah peningkatan dalam sel-sel T dan sel-sel B. Kajian ini menunjukkan bahawa terdapat perubahan dalam imuniti selular dalam tisu babi domestik yang terdedah kepada ASF, termasuklah peningkatan dalam sel-sel T dan sel-sel B.

embedded in the membrane of the cell. The cell membrane is a phospholipid bilayer with hydrophilic heads and hydrophobic tails. The membrane is selectively permeable, allowing some substances to pass while blocking others. The cell membrane is also involved in cell signaling and communication.

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Keywords: cell membrane, phospholipid bilayer, hydrophilic heads, hydrophobic tails, selectively permeable, cell signaling, communication.

1.0 INTRODUCTION

1.1 Epidemiology

The first case of African Swine Fever (ASF) was reported in Italy in 1921. The disease was caused by the ASF virus (ASFV), a large DNA virus belonging to the *Asfarviridae* family. ASFV is a highly contagious disease that affects domestic and wild pigs. The disease is characterized by high mortality rates, especially in young piglets. The virus is transmitted by soft-bodied arthropods, such as ticks and blood-sucking insects. The most common vector is the tick *Ornithodoros erraticus*, followed by *O. moulata* and *procinus*. The virus can also be transmitted through direct contact with infected animals or their secretions. The disease has since spread to other parts of Europe, Africa, and Asia. In Asia, ASF is a major threat to the pig industry, with significant economic losses. The disease is currently present in several countries in Asia, including Cambodia, Laos, and Vietnam. The ASF virus is highly resistant to heat and drying, and can survive in the environment for several years. The disease is a zoonotic disease, but it does not affect humans. The ASF virus is a double-stranded DNA virus with a genome size of approximately 190,000 base pairs. The genome contains 195 genes, including 150 structural genes and 45 non-structural genes. The structural genes encode the capsid proteins, the glycoproteins, and the DNA polymerase. The non-structural genes encode the proteins involved in the replication and pathogenesis of the virus. The ASF virus is a member of the *Asfarviridae* family, which also includes the African swine fever virus and the African swine fever virus-like virus. The ASF virus is a highly contagious disease that affects domestic and wild pigs. The disease is characterized by high mortality rates, especially in young piglets. The virus is transmitted by soft-bodied arthropods, such as ticks and blood-sucking insects. The most common vector is the tick *Ornithodoros erraticus*, followed by *O. moulata* and *procinus*. The virus can also be transmitted through direct contact with infected animals or their secretions. The disease has since spread to other parts of Europe, Africa, and Asia. In Asia, ASF is a major threat to the pig industry, with significant economic losses. The disease is currently present in several countries in Asia, including Cambodia, Laos, and Vietnam. The ASF virus is highly resistant to heat and drying, and can survive in the environment for several years. The disease is a zoonotic disease, but it does not affect humans. The ASF virus is a double-stranded DNA virus with a genome size of approximately 190,000 base pairs. The genome contains 195 genes, including 150 structural genes and 45 non-structural genes. The structural genes encode the capsid proteins, the glycoproteins, and the DNA polymerase. The non-structural genes encode the proteins involved in the replication and pathogenesis of the virus. The ASF virus is a member of the *Asfarviridae* family, which also includes the African swine fever virus and the African swine fever virus-like virus.

1.2 ASF in Asia

The first case of ASF in Asia was reported in Cambodia in 1960. The disease was caused by the ASF virus (ASFV), a large DNA virus belonging to the *Asfarviridae* family. ASFV is a highly contagious disease that affects domestic and wild pigs. The disease is characterized by high mortality rates, especially in young piglets. The virus is transmitted by soft-bodied arthropods, such as ticks and blood-sucking insects. The most common vector is the tick *Ornithodoros erraticus*, followed by *O. moulata* and *procinus*. The virus can also be transmitted through direct contact with infected animals or their secretions. The disease has since spread to other parts of Asia, including Laos and Vietnam. In Asia, ASF is a major threat to the pig industry, with significant economic losses. The disease is currently present in several countries in Asia, including Cambodia, Laos, and Vietnam. The ASF virus is highly resistant to heat and drying, and can survive in the environment for several years. The disease is a zoonotic disease, but it does not affect humans. The ASF virus is a double-stranded DNA virus with a genome size of approximately 190,000 base pairs. The genome contains 195 genes, including 150 structural genes and 45 non-structural genes. The structural genes encode the capsid proteins, the glycoproteins, and the DNA polymerase. The non-structural genes encode the proteins involved in the replication and pathogenesis of the virus. The ASF virus is a member of the *Asfarviridae* family, which also includes the African swine fever virus and the African swine fever virus-like virus.

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1.2.1 ASF in Malaysia

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ere d e e de e d r e ce cre ed
r e e re er e r bre M

1.3 Histopathology of ASF

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er em c red e re ed er r c e ce debr d e e
c be ee er e Be de em r r c be ee
r d e e ed c r m cr e e e d e
e e e e re red re c e e d e ce d r e e
e d e e b em r r e m c c r e e r e e C r r c e
e re c e d e ce m be ee

1.4 Immunohistochemistry of ASF

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e e C rre e m e e e b r r me d de ec
R CR r D c be de e d r m e e b d r er m e
ec ed m er e r e ec b R CR b C e

can be used here to determine the relationship between the variables. The results of the regression analysis can be used to determine the relationship between the variables. The results of the regression analysis can be used to determine the relationship between the variables. The results of the regression analysis can be used to determine the relationship between the variables.

1.5 Objective

The objective of this study is to determine the relationship between the variables. The objective of this study is to determine the relationship between the variables. The objective of this study is to determine the relationship between the variables. The objective of this study is to determine the relationship between the variables.

1.6 Hypothesis

Null hypothesis There is no relationship between the variables. There is no relationship between the variables. There is no relationship between the variables. There is no relationship between the variables.

Alternative hypothesis There is a relationship between the variables. There is a relationship between the variables. There is a relationship between the variables. There is a relationship between the variables.

1.7 Justification

The relationship between the variables is important because it can help us understand the underlying mechanisms. The relationship between the variables is important because it can help us understand the underlying mechanisms. The relationship between the variables is important because it can help us understand the underlying mechanisms. The relationship between the variables is important because it can help us understand the underlying mechanisms.

2.0 LITERATURE REVIEW

2.1 Clinical form

Definitely, the clinical form of acute subarachnoid hemorrhage (ASAH) is characterized by a sudden onset of severe headache, vomiting, and loss of consciousness. The clinical form of acute subarachnoid hemorrhage (ASAH) is characterized by a sudden onset of severe headache, vomiting, and loss of consciousness. The clinical form of acute subarachnoid hemorrhage (ASAH) is characterized by a sudden onset of severe headache, vomiting, and loss of consciousness.

2.1.1 Peracute ASF

Peracute subarachnoid hemorrhage (ASF) is characterized by a sudden onset of severe headache, vomiting, and loss of consciousness. The clinical form of acute subarachnoid hemorrhage (ASAH) is characterized by a sudden onset of severe headache, vomiting, and loss of consciousness. The clinical form of acute subarachnoid hemorrhage (ASAH) is characterized by a sudden onset of severe headache, vomiting, and loss of consciousness.

2.1.2 Acute ASF

Acute subarachnoid hemorrhage (ASF) is characterized by a sudden onset of severe headache, vomiting, and loss of consciousness. The clinical form of acute subarachnoid hemorrhage (ASAH) is characterized by a sudden onset of severe headache, vomiting, and loss of consciousness. The clinical form of acute subarachnoid hemorrhage (ASAH) is characterized by a sudden onset of severe headache, vomiting, and loss of consciousness.

2.1.3 Subacute ASF

Subacute subarachnoid hemorrhage (ASF) is characterized by a sudden onset of severe headache, vomiting, and loss of consciousness. The clinical form of acute subarachnoid hemorrhage (ASAH) is characterized by a sudden onset of severe headache, vomiting, and loss of consciousness. The clinical form of acute subarachnoid hemorrhage (ASAH) is characterized by a sudden onset of severe headache, vomiting, and loss of consciousness.

□

em rre Be de ce c d d r c r e
 d c m c e m c be ee e bc c m be
 d e e ec d r b c er ec d e e m be mm re ed
 me e

2.2.4 Chronic ASF

e e c r c re m re ed ec d r b c er ec r
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 d e e c e e

2.3 Pathogenesis

2.3.1 Pathogenesis of Lymphoid Depletion

e e e e c m e c ed m e c be
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 r e r b e b e e ced c e r re c e
 r m de B me e d re c e ec d r r
 d d e e re d e er r me e
 r re c e e c m m c e m cr e d e re c
 e d e cr e r e ec ed ce e e ce re
 e cr ed r r re re e ed e c e c e m er B d
 m c e d m cr e re r ce de e me me

“Cytokine storm” is the main mechanism that induces apoptosis in lymphocytes

me e

2.3.2 Pathogenesis of vascular changes

e c r b membr e e ed d e e e d e ce
 c c e e e d ere e c r b membr e e e

de...e...e...r...b...membr...e...e...e...e...
 c...em...d...d...ce...d...em...ed...r...c...r...c...D...C...
 me...e...e...e...c...e...b...c...e...r...mb...c...e...
 ee...D...r...em...d...e...e...b...c...e...r...c...r...c...e...
 me...r...c...e...e...b...e...m...r...r...c...e...e...de...e...me...em...r...e...
 e...m...B...e...

2.4 Diagnostic methods

M...d...ere...e...be...ee...d...c...c...e...e...er...C...e...re...
 m...m...r...e...erm...c...c...d...m...r...em...e...
 C...re...em...e...e...b...r...r...me...d...de...ec...re...me...
 mer...e...c...re...c...R...CR...e...n...D...c...be...de...ec...ed...er...e...r...
 e...ec...R...CR...c...c...m...c...be...de...ed...
 ec...e...m...e...c...ee...b...d...d...e...d...m...de...d...
 be...ed...e...e...de...ec...e...e...r...e...CR...r...e...
 em...d...r...red...b...d...ce...e...r...ce...ec...ed...ce...c...be...ee...d...r...
 r...e...b...e...m...r...r...er...er...b...d...e...c...e...c...re...me...
 r...r...d...e...em...d...r...er...e...c...e...c...e...ec...
 e...c...e...Be...de...e...e...c...be...de...ec...ed...b...
 mm...re...ce...ce...e...e...r...b...e...de...ec...
 d...d...rec...mm...re...ce...ce...c...be...ed...de...ec...e...b...d...e...b...
 c...e...c...e...e...m...d...e...be...re...e...b...d...e...re...r...d...ced...

□

3.0 METHODOLOGY

3.1 Samples

There are several reasons why the research is needed. The first reason is that the current research is very limited. The second reason is that the current research is very limited. The third reason is that the current research is very limited. The fourth reason is that the current research is very limited. The fifth reason is that the current research is very limited. The sixth reason is that the current research is very limited. The seventh reason is that the current research is very limited. The eighth reason is that the current research is very limited. The ninth reason is that the current research is very limited. The tenth reason is that the current research is very limited.

3.2 H&E Staining

3.2.1 Materials

The materials used in this study are as follows: 1. Formalin, 2. Hematoxylin, 3. Eosin, 4. Glycerin, 5. Alcohol, 6. Xylene, 7. Paraffin, 8. Glass slides, 9. Coverslips, 10. Microscope.

3.2.2 Procedure

The procedure for H&E staining is as follows: 1. Fixation, 2. Dehydration, 3. Clearing, 4. Embedding, 5. Sectioning, 6. Staining, 7. Mounting, 8. Microscopy.

... cre B... ce... N... e... m... M... M... m... d...
r... m... b... d...

D... B... b... e... b... er...

D... B... c... r... m... e...

m... d... c... m... b... er...

Me... e... e... b... e...

3.3.2 Procedure

... m... ed... r... embedded... e... b... c... e... c... ec...

... e... ec... m... e... e... b... e... ed... b... e... de... re... c... ed...

... e... ce... e... c... r... c... er... c... b... e... e... e... e...

c... r... ed... e... ce... m... e... m... b... r... e... e... c... r... ed... r... ce...

... c... m... e... c... r... e... c... re... r... ce...

... e... de... re... e... dr... er...

De... r... b... m... b... m... er... de... e... e... r... m... e... d...

e... re... dr... e... de... c... d... r... m... e...

re... ec... e...

... e... de... r... er... er... re... dr... d... r... e... m... m...

d... ed... er...

... b... re... e... m... e... e... b... d... e... e... e... r... e... d... e... b... e...

m... c... e... r... m... e... c... e... de... re... m... m... e... d... c... r... e...

b... er... m... C... m... d... ed... er...

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the Birminghame

the b d d ed e e B

the m r b d d r ed e de d e red e b d c
ered e e m e e de

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b c b e r m e d B r m e e

e D B d b r e e m D ed d e D B
ced e de

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e de d ed e r

C e r e de e e b e r m e

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4.0 RESULTS

4.1 Histopathology lesion

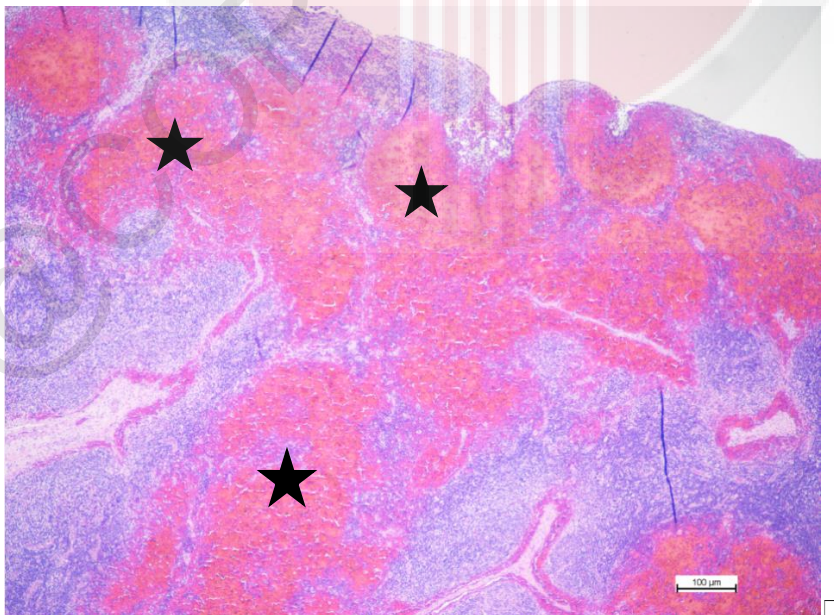
	id e	ee	er		m de
	em rre	em rre			em rre
	em rre				em rre
	em rre	em rre	em rre	C e	C e

be Re

m e c e d

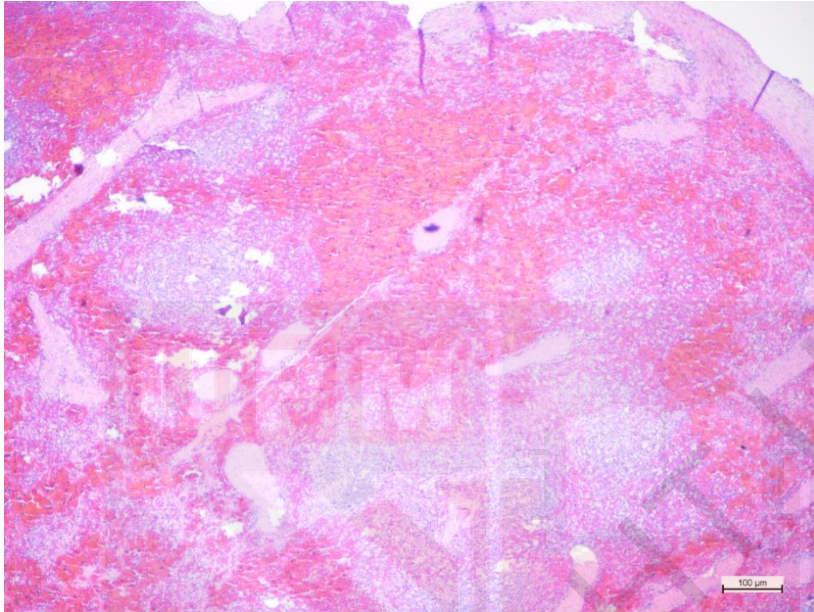
4.1.1 Pig 1

4.1.1.1 Lymph node



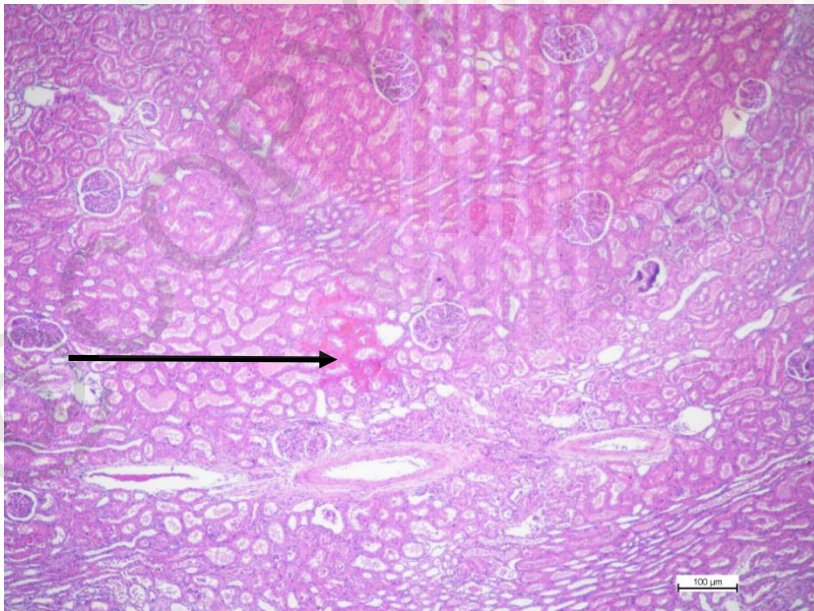
re em rre e m de

4.1.1.2 Spleen



□□□re □□□□□□□□□□ □em □rr □□□e □□□□□e red □□□□□□□□□□ ee □□□□□□□□

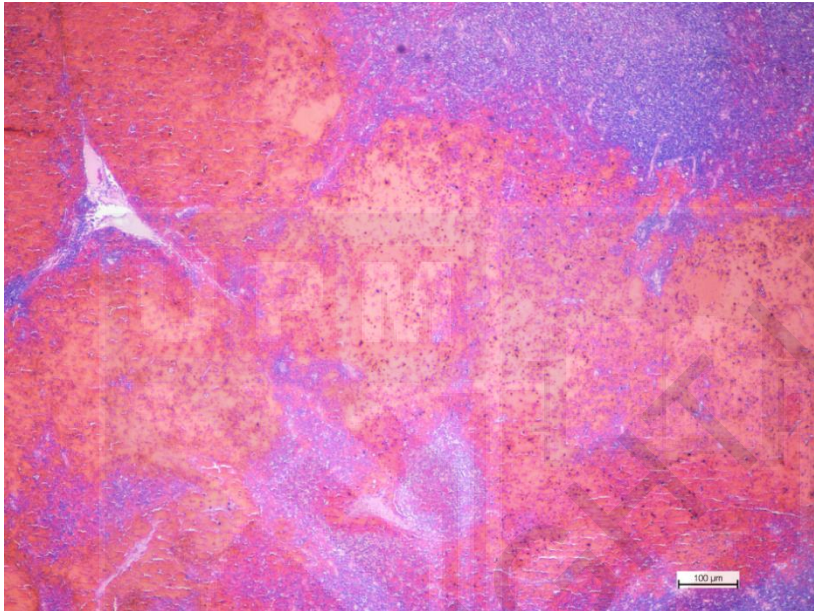
4.1.1.3 Kidney



□□□re □□□□□□□□□□ □em □rr □□□e □□□□□e bb □e □□□□□e dd □e □□□□□□□□

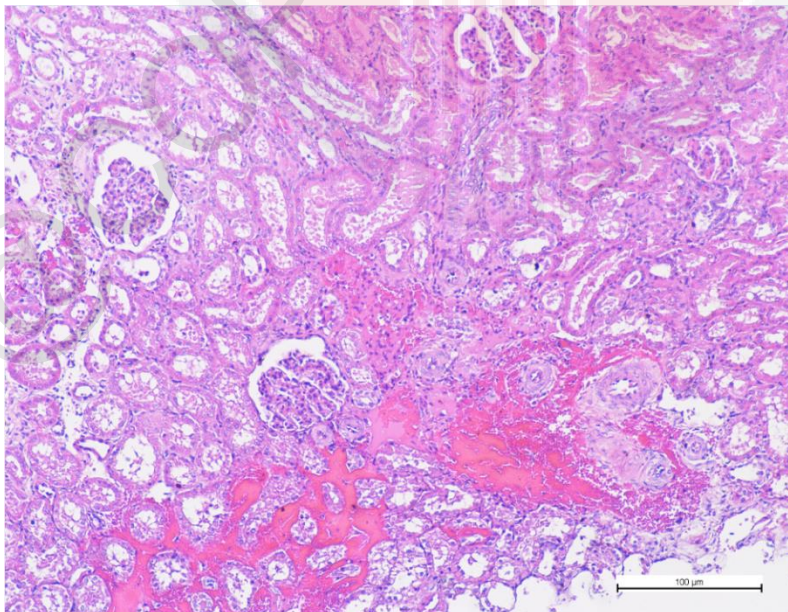
4.1.2 Pig 2

4.1.2.1 Lymph node



□□□re □□□□□□□□□□e□ere □□em□rr□□□e□□□□□m□□□□□de□□□□□□□□

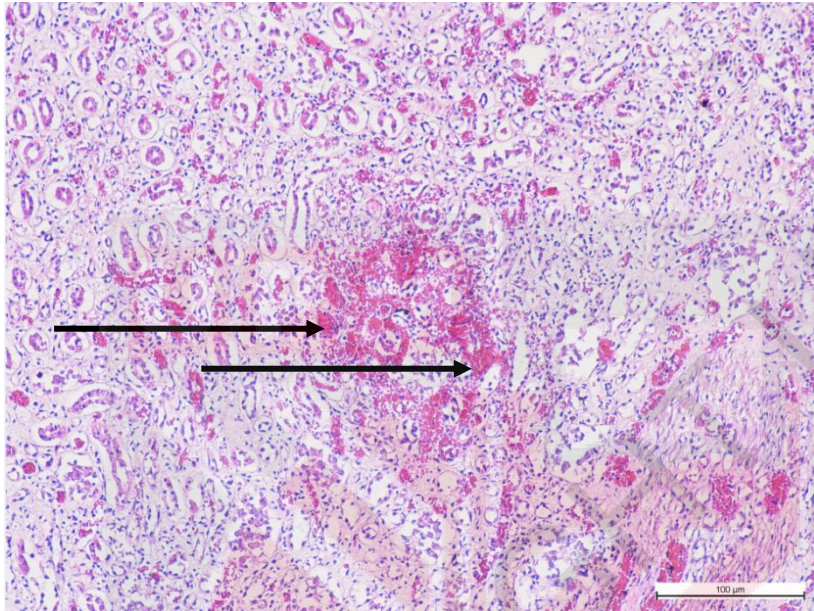
4.1.2.2 Kidney



□□□re □□□□□□□□□□c□□□re □□□□□em□rr□□□e□□□□□□□□

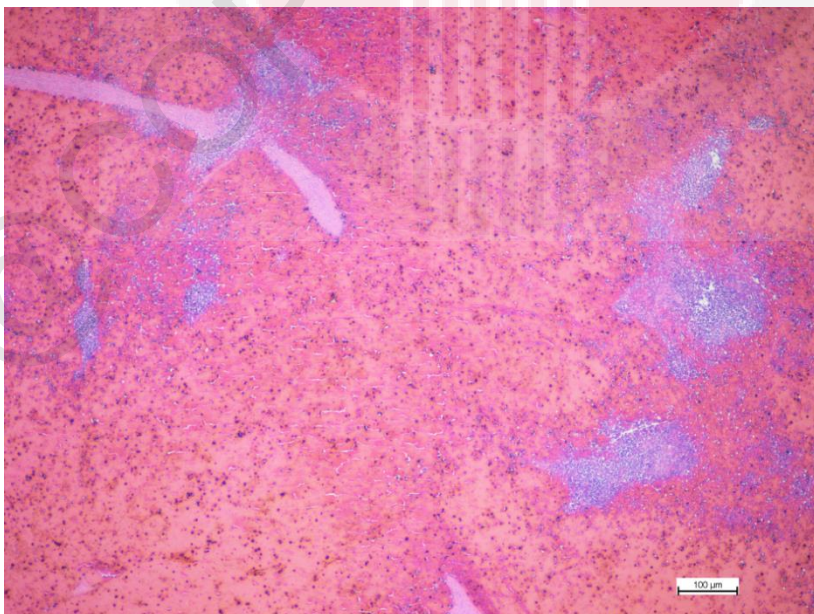
4.1.3 Pig 3

4.1.3.1 Kidney



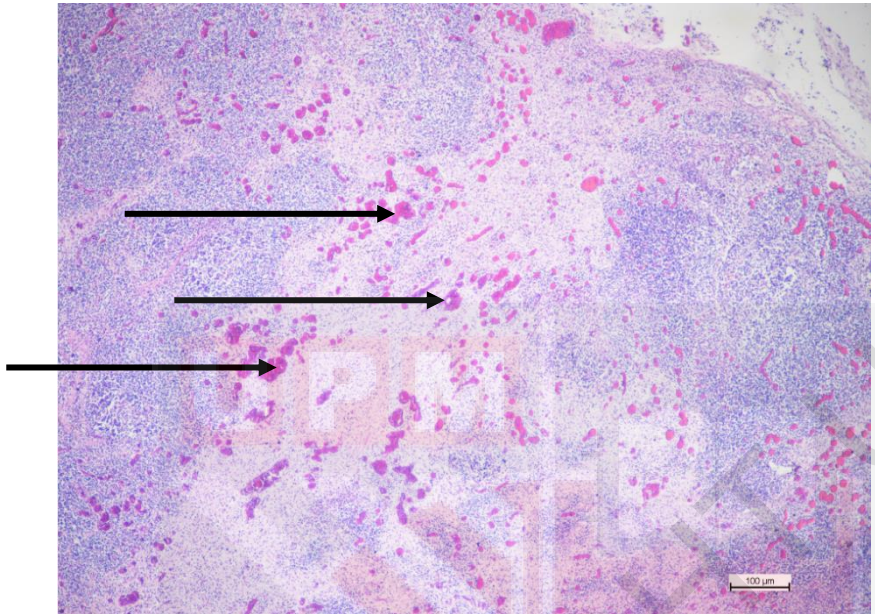
re c re em r e

Figure 4.1.3.2: Spleen



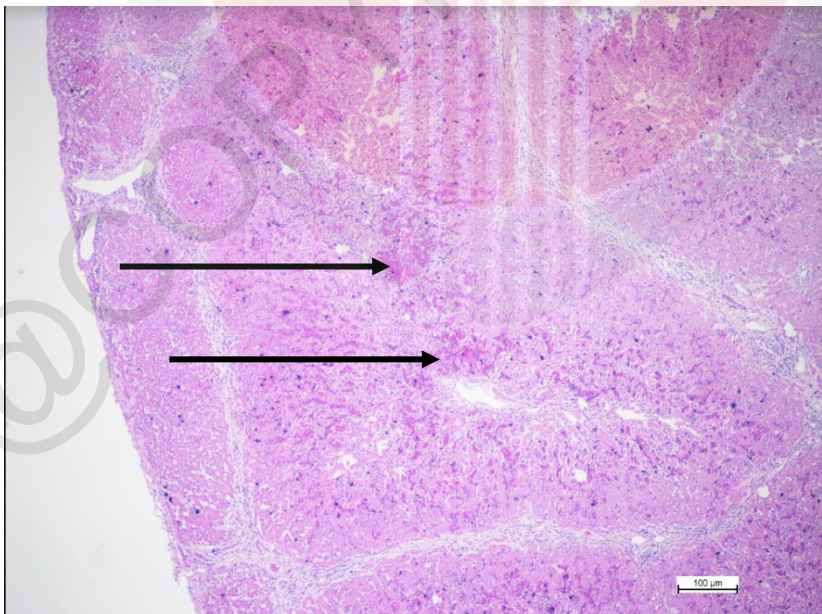
re e ere em r e e ee

Figure 4.1.3.3: Lymph node



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Figure 4.1.3.4: Liver



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4.2 Immunohistochemistry

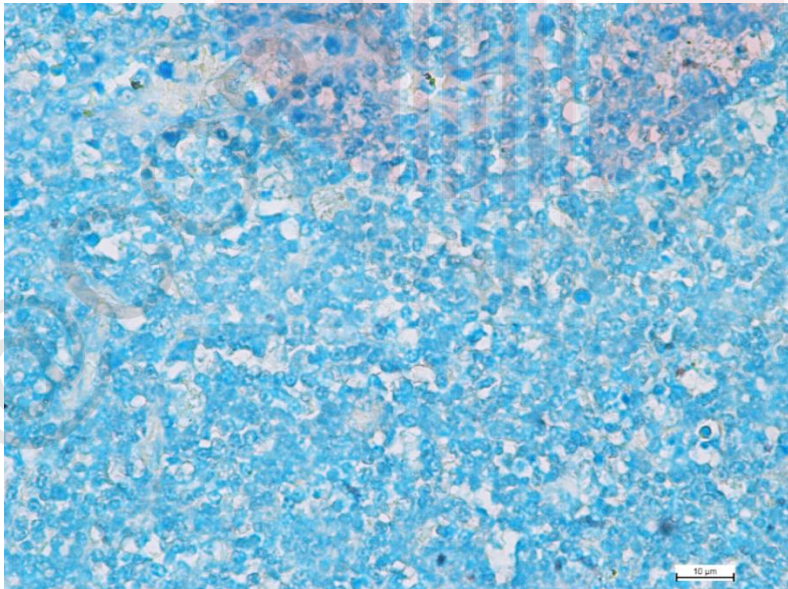
	Kidney	Spleen	Liver	Lung	Tonsil	Lymph Node
Pig 1	+	+	Nil	Nil	+	+
Pig 2	+	Nil	Nil	Nil	+	-
Pig 3	+	+	+	+	+	+
Negative Control (B22/573)	Nil	Nil	Nil	Nil	Nil	Nil

Receptor-mediated immunocytochemistry

+ immunoreactive = immunoreactive Nil = immunoreactive

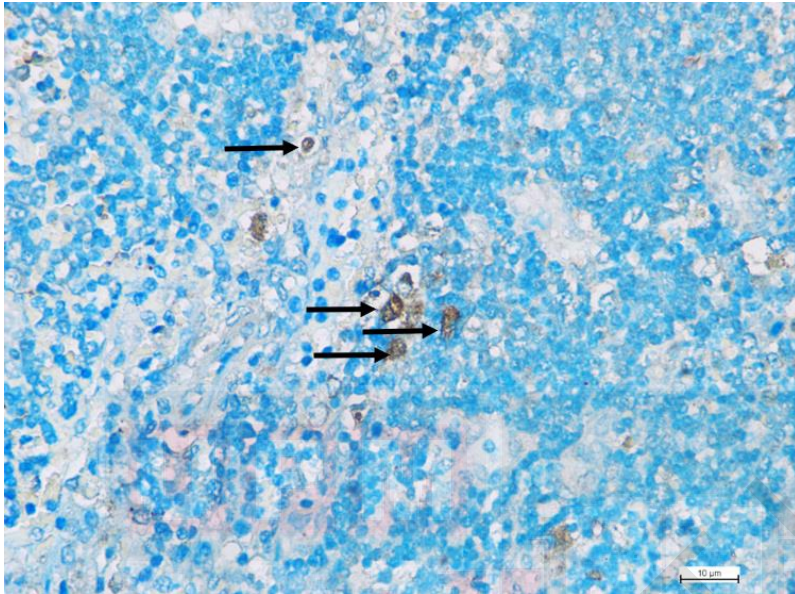
4.2.1 Negative control

4.2.1.1 Negative tissue control



receptor-mediated immunocytochemistry

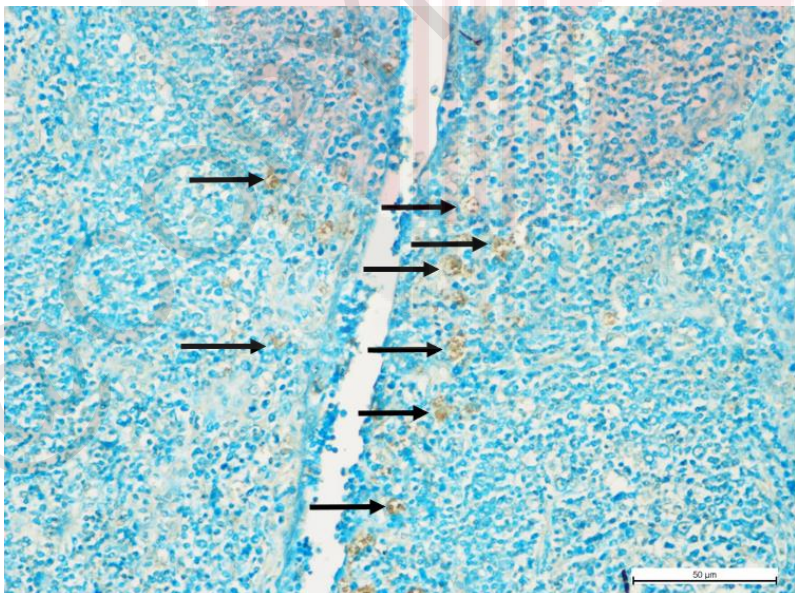
receptor-mediated immunocytochemistry



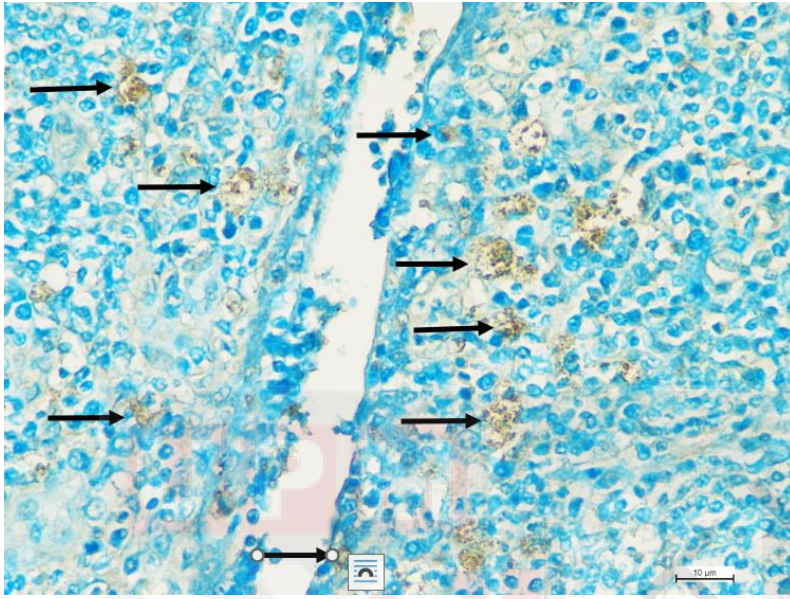
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4.2.3 Fig 2

4.2.3.1 Tonsil



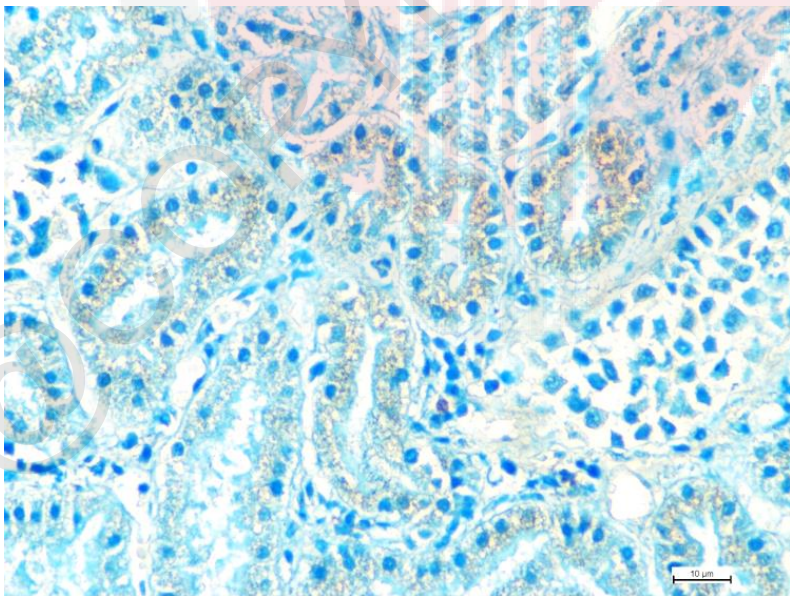
re mer e e ce e r cr



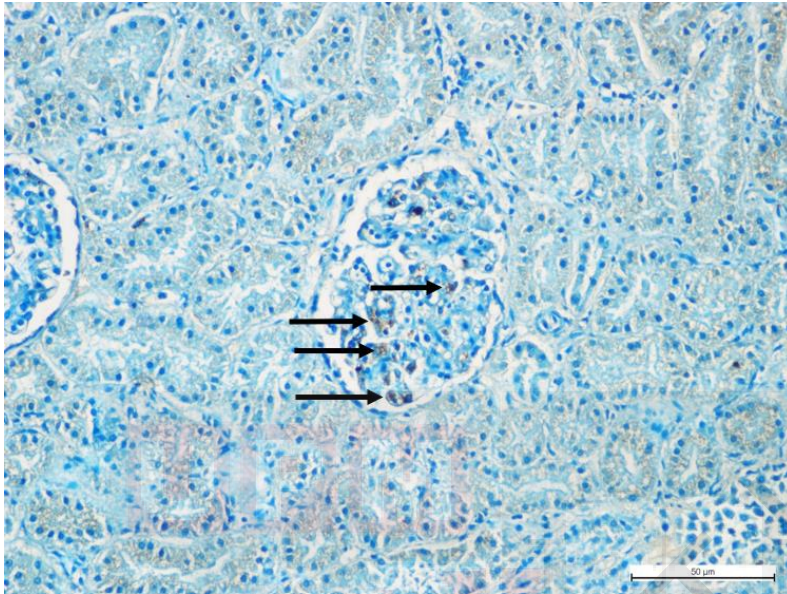
re b c e ce

4.2.4 Fig 3

4.2.4.1 Kidney

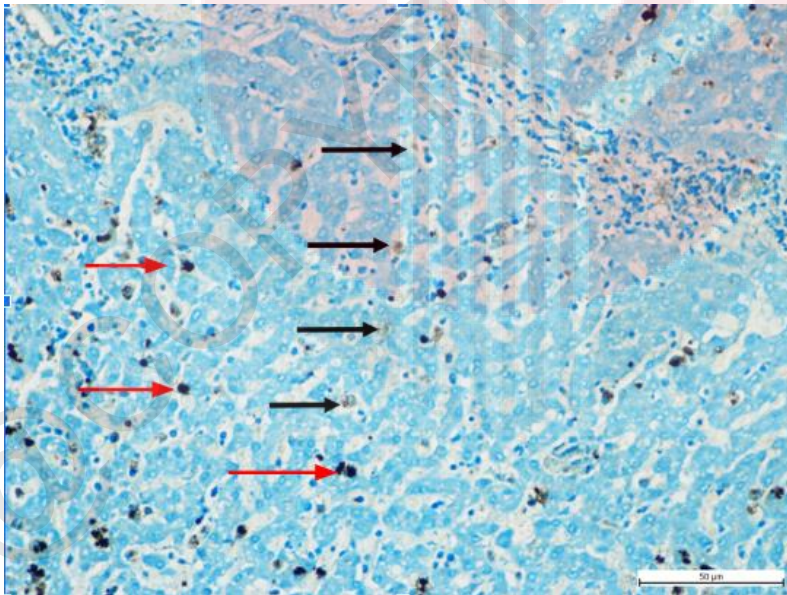


re br e b e d e re b r



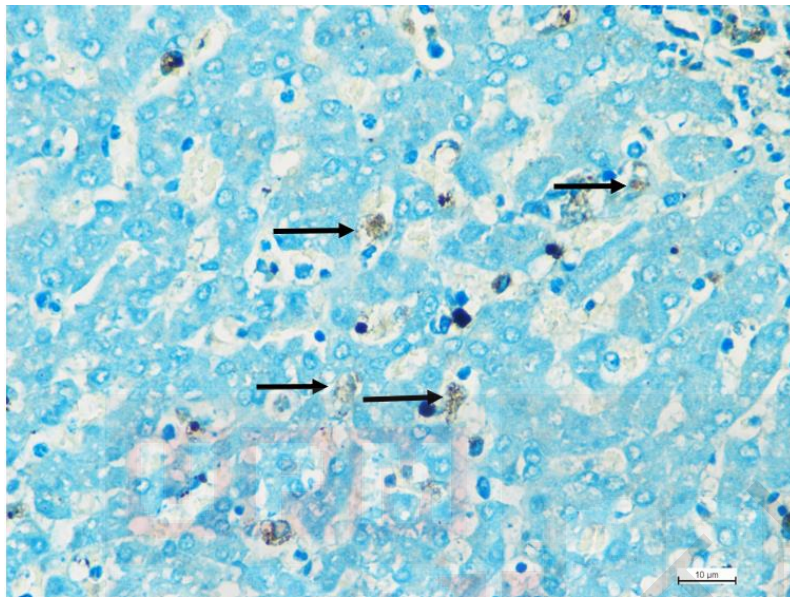
re b mer e ce e mer

4.2.4.2 Liver



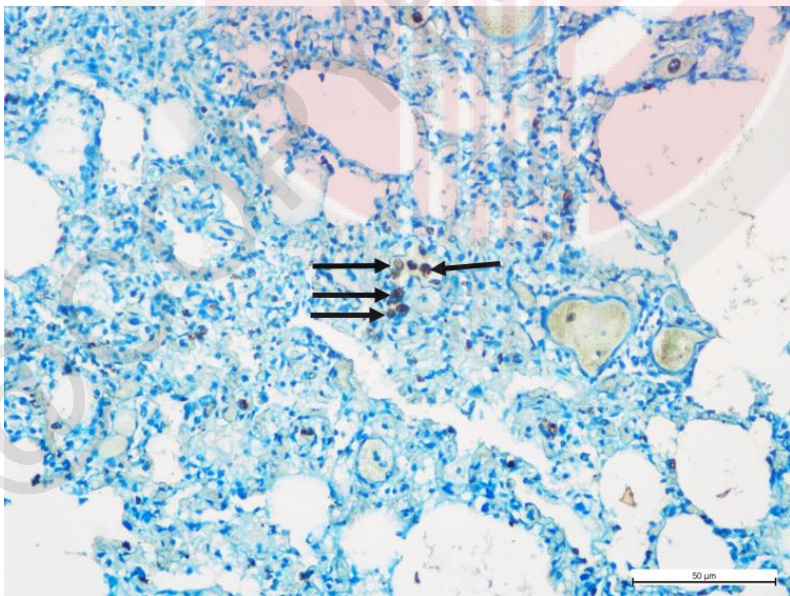
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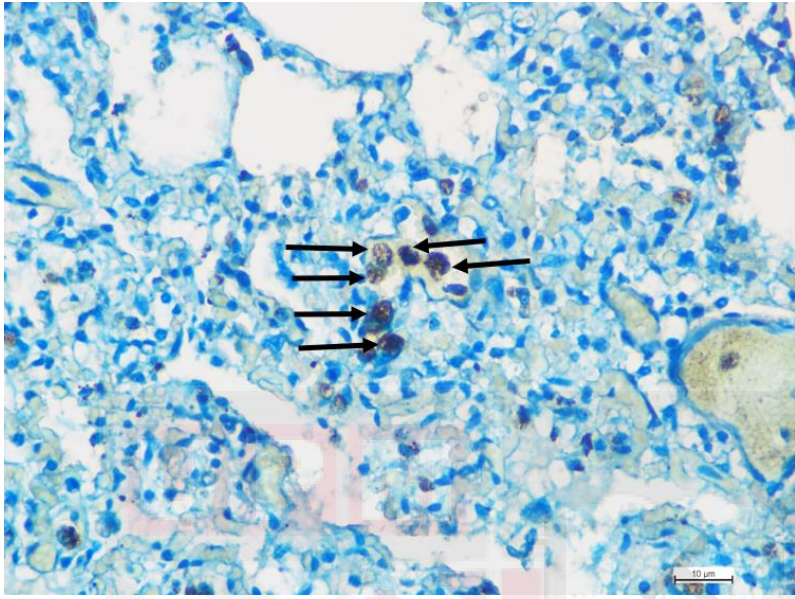


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4.2.4.3 Lungs



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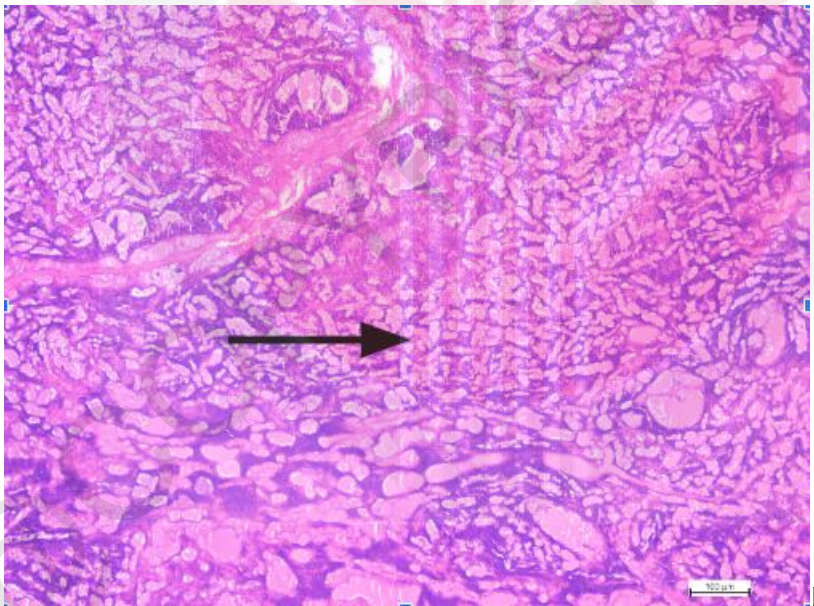
re b c e r c r m cr e

@COPYRIGHT UPM

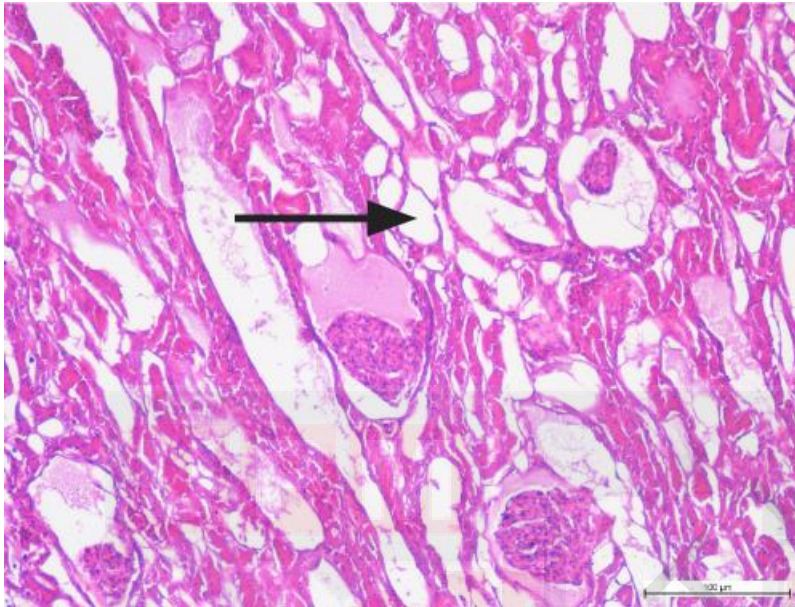
5.0 DISCUSSION

5.1 Freezing artefacts

Freezing artefacts are common in cryostat sections and can be identified by characteristic features such as ice crystals, cellular distortion, and loss of fine detail. These artefacts are often seen in the form of clear, irregular spaces or clefts within the tissue, which are surrounded by a thin layer of cytoplasm. The presence of these artefacts can be minimized by using appropriate freezing techniques and handling procedures.



The presence of these artefacts can be minimized by using appropriate freezing techniques and handling procedures.



re b c e e d e e e m e

5.2 Optimization for Immunohistochemistry

Microwave setting	50W for 15 minutes		100W for 10 minutes	
Primary antibody concentration	e	e c r	e	e c r
1:500				
1:1000				
1:1500				
PBS				

b e m m c e m r

5.2.1 Primary antibody concentration

cc rd e m c r r c e r m e d e r m r b d

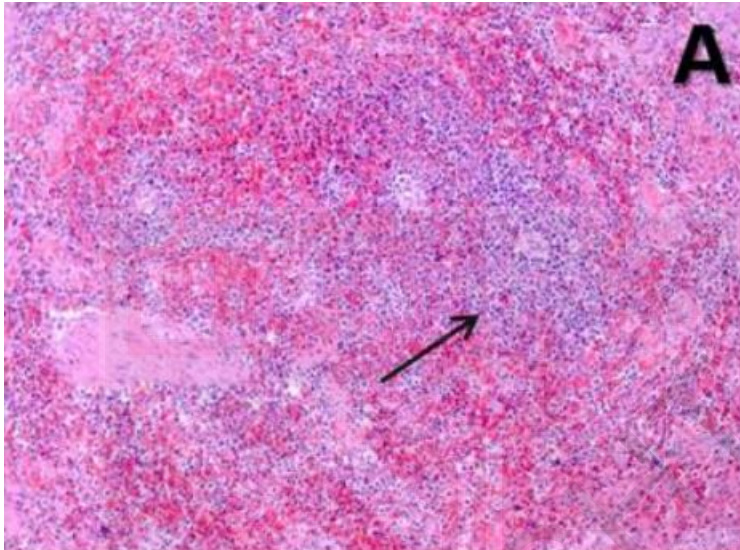
c ce r B e r c b e d e m r b d

c ce r e e e e e d e r b r d

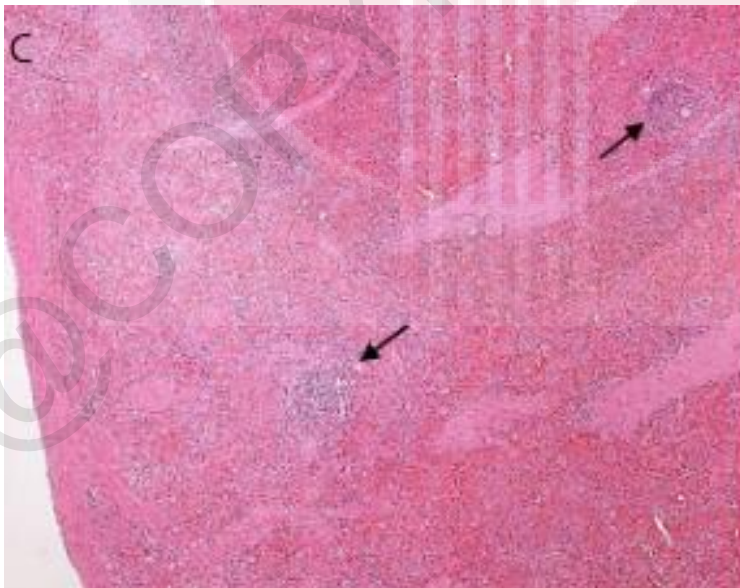
e r d c d e r m e e e e e e e

5.3 Comparison of results

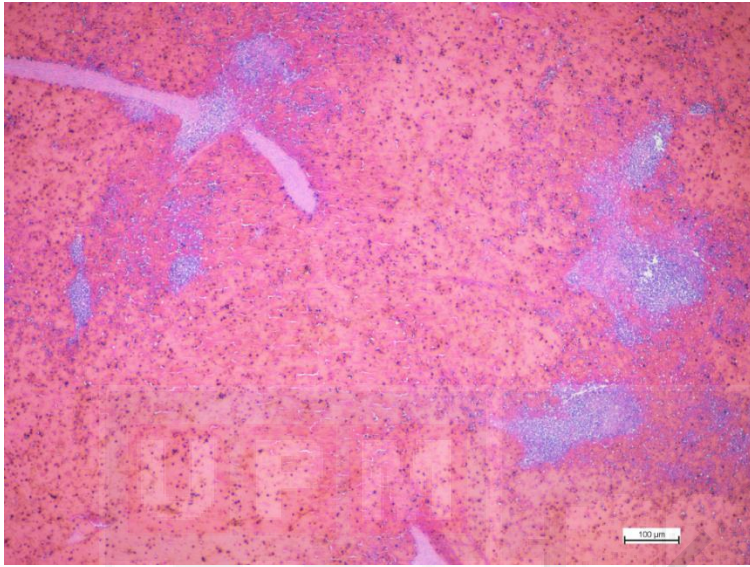
5.3.1 Histopathology



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red er



re e c red red b d ce d e ee
c e

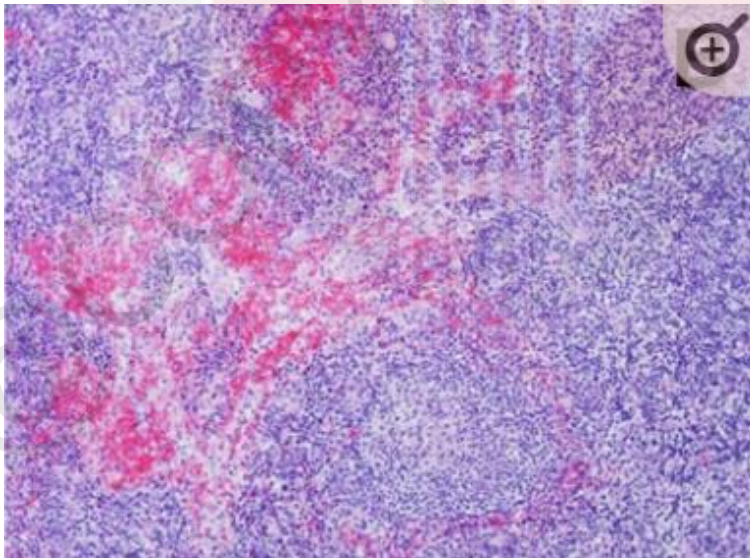


Micrograph showing a dense population of cells with purple nuclei and pink cytoplasm/extracellular matrix. There are some lighter, amorphous areas scattered throughout. A scale bar in the bottom right corner indicates 100 micrometers.

Micrograph showing a dense population of cells with purple nuclei and pink cytoplasm/extracellular matrix. There are some lighter, amorphous areas scattered throughout. A scale bar in the bottom right corner indicates 100 micrometers.

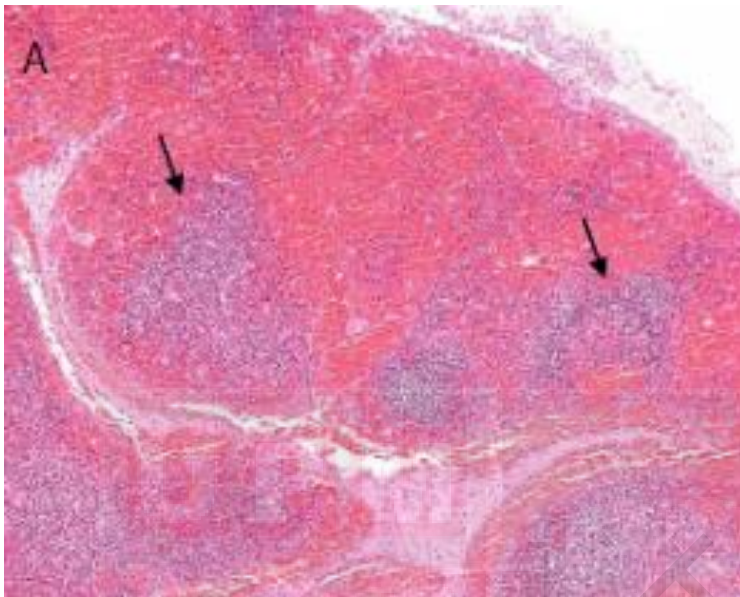
Micrograph showing a dense population of cells with purple nuclei and pink cytoplasm/extracellular matrix. There are some lighter, amorphous areas scattered throughout. A scale bar in the bottom right corner indicates 100 micrometers.

Micrograph showing a dense population of cells with purple nuclei and pink cytoplasm/extracellular matrix. There are some lighter, amorphous areas scattered throughout. A scale bar in the bottom right corner indicates 100 micrometers.

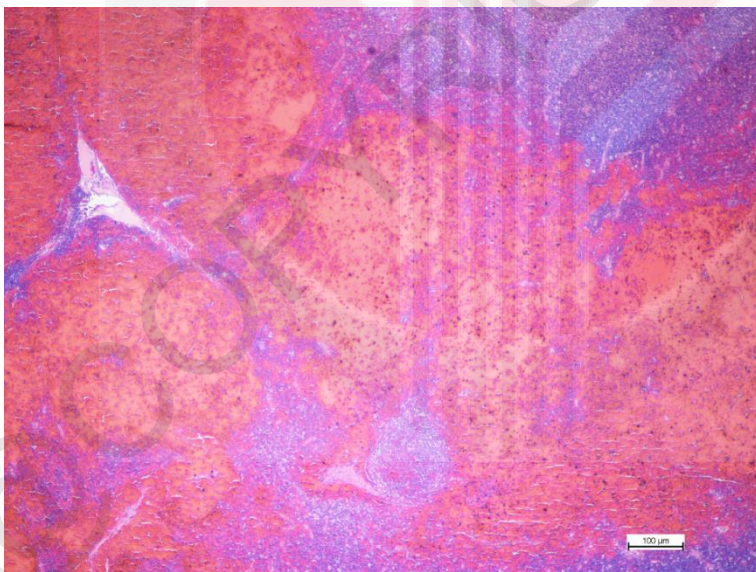


Micrograph showing a dense population of cells with purple nuclei and pink cytoplasm/extracellular matrix. There are some lighter, amorphous areas scattered throughout. A magnifying glass icon is visible in the top right corner.

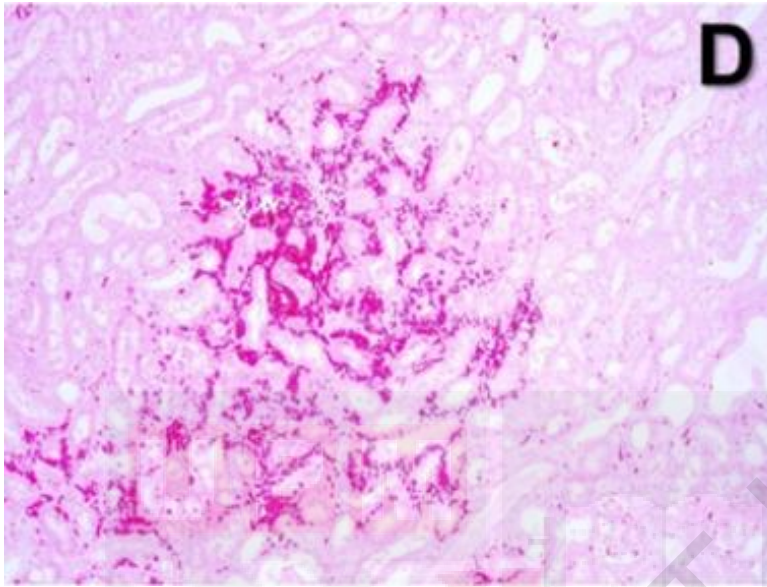
Micrograph showing a dense population of cells with purple nuclei and pink cytoplasm/extracellular matrix. There are some lighter, amorphous areas scattered throughout. A magnifying glass icon is visible in the top right corner.



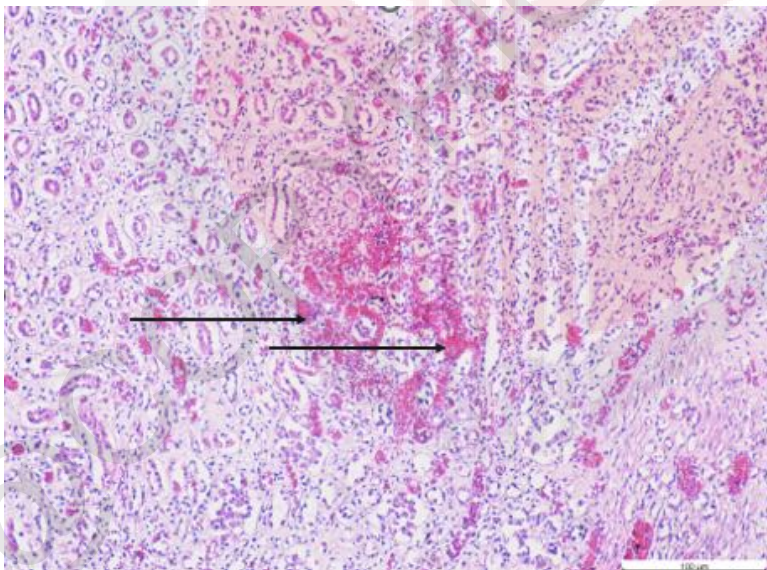
Micrograph A shows a low-magnification view of a brain slice. The central area, indicated by two black arrows, is a pale, irregularly shaped region, likely representing a lesion or a specific anatomical structure. The surrounding tissue is stained pink and purple. A small white box is present in the bottom right corner.



Micrograph B shows a high-magnification view of a brain slice. The central region is a dense, purple-stained area, likely representing a lesion or a specific anatomical structure. A scale bar in the bottom right corner indicates 100 μm. A small white box is present in the bottom right corner.

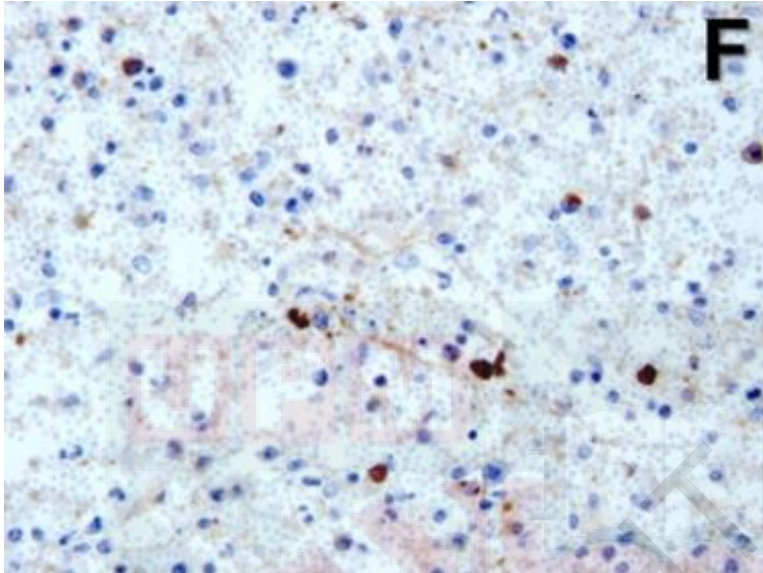


revascularization of the placenta is essential for the development of the fetus. The process involves the formation of new blood vessels in the chorionic plate and the decidua. This process is regulated by various factors, including hypoxia, hypoxia-inducible factor (HIF), and angiogenic factors such as vascular endothelial growth factor (VEGF).

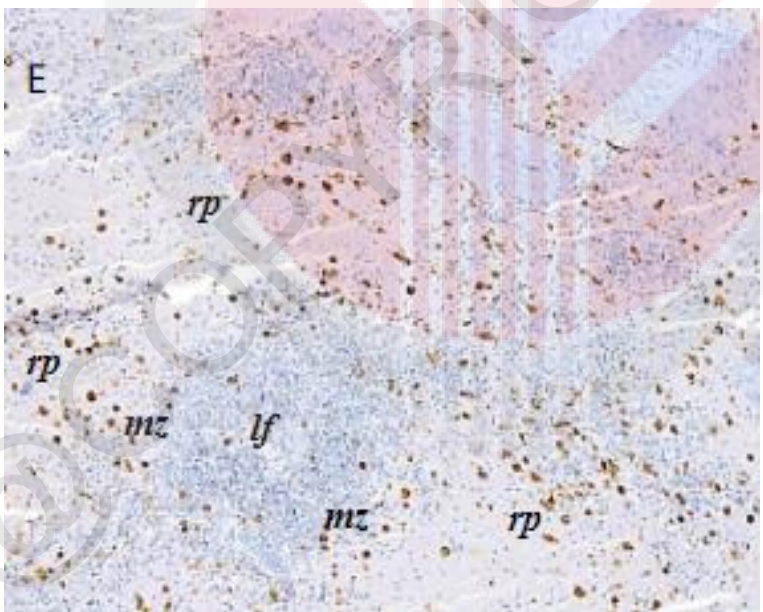


revascularization of the placenta is essential for the development of the fetus. The process involves the formation of new blood vessels in the chorionic plate and the decidua. This process is regulated by various factors, including hypoxia, hypoxia-inducible factor (HIF), and angiogenic factors such as vascular endothelial growth factor (VEGF).

5.3.2 Immunohistochemistry

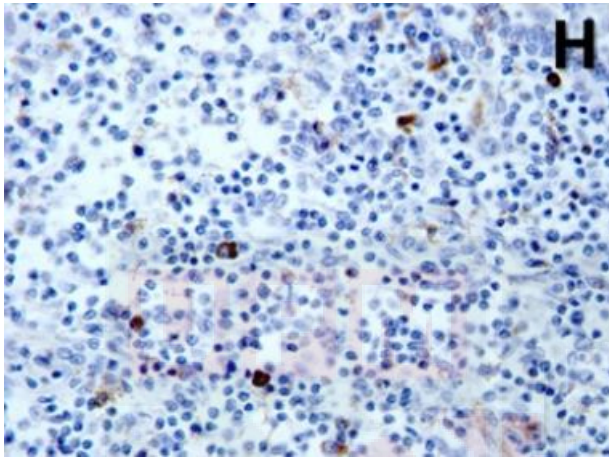


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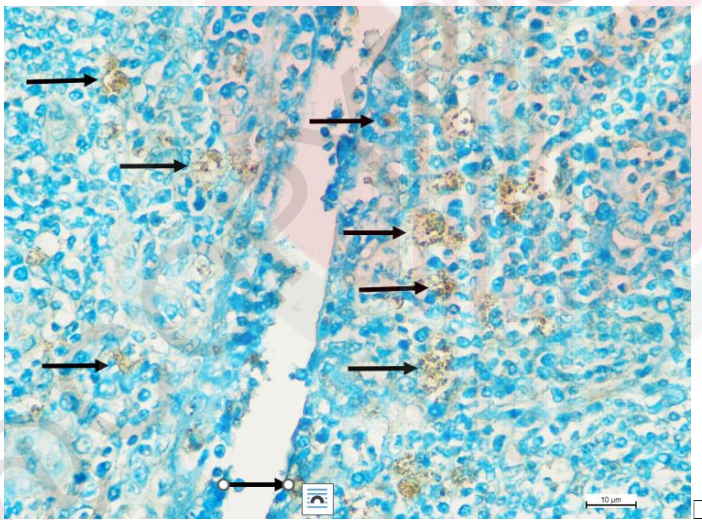


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c be ee



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re b c e e ce

re r e re c m cr e re b

c m r re B re r e ce

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6.0 CONCLUSION

The research has shown that the use of the proposed system can significantly reduce the time and cost of the design process. The system is easy to use and can be integrated with existing design tools. The results of the study show that the system can be used by a wide range of users, from novice designers to experienced professionals. The system can be used to create a wide range of designs, from simple 2D drawings to complex 3D models. The system can also be used to generate technical specifications and bills of materials. The system can be used to create a wide range of designs, from simple 2D drawings to complex 3D models. The system can also be used to generate technical specifications and bills of materials.

7.0 RECOMMENDATIONS

It is recommended that the system be used by a wide range of users, from novice designers to experienced professionals. The system can be used to create a wide range of designs, from simple 2D drawings to complex 3D models. The system can also be used to generate technical specifications and bills of materials. The system can be used to create a wide range of designs, from simple 2D drawings to complex 3D models. The system can also be used to generate technical specifications and bills of materials.

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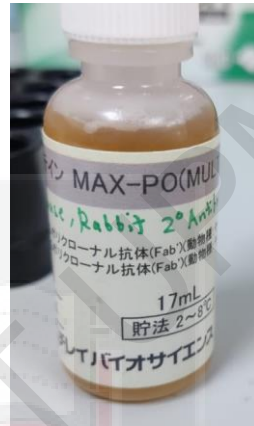
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APPENDIX

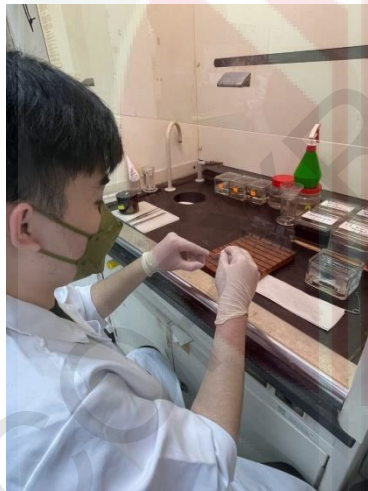
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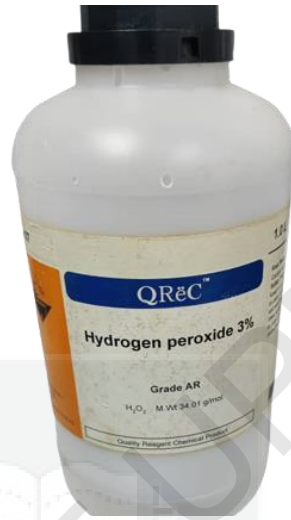
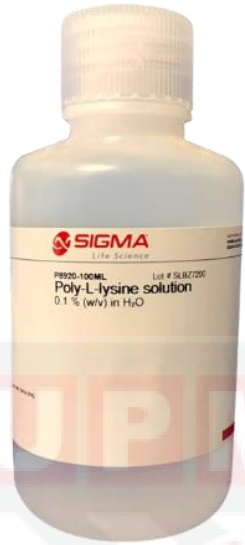


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