

Discussion of external and
visceral Grey-Zone anomalies:

Reclassification due to new
knowledge

Berlin 2011

Definitions (Berlin 1998)

- Malformation
 - A permanent structural change that is likely to adversely affect the survival or health of the species under investigation
- Variation
 - A change that occurs within the normal population under investigation and is unlikely to adversely affect survival or health

Criteria (Berlin 1998)

- Adversity (Harmfulness)
- Permanence
- Occurrence in normal population

Criteria

- **Adversity (Harmfulness)**
 - Permanence
 - some permanent changes are not harmful
 - a permanent change that is harmful to the animal might not be harmful in man
 - Occurrence in normal population
 - occurrence in controls and/or adult population ('frequency' in controls was not one the main criteria in Berlin 1998 but I think people tend to consider it because it is another way of looking a potential for adversity - if a change is seen frequently in a control population, we assume that it is less likely to be harmful)

Adversity (Harmfulness)

How do we obtain new knowledge about this ?

1. Research into post-natal consequences
 - Information from older animals
 - Information concerning extrapolation to man

2. Improved laboratory work (to provide better information for assessors)
 - Maintenance of historical data
 - Better descriptive terminology (including terms to denote severity)
 - Method development (better characterization of findings to give more information on severity)

Adversity (Harmfulness)

- Severity grading (Berlin 2007) in descriptive terminology
 - Useful tool to reduce the problems of the Grey-Zone
 - can use different words to denote different gradings of the same observation
 - *eg* dilated brain ventricle, hydrocephaly
 - can be the same term with different severity words
 - *eg* dilated brain ventricle slight, dilated brain ventricle severe

Grey-Zone findings that, with the use of severity terms, can be re-classified as malformation or variation

- External
 - Domed head
 - Malpositioned pinna
 - Misshapen nose
 - Short trunk
- Visceral
 - Retinal fold
 - Malpositioned nasal septum
 - Small aortic valve
 - Dilated aorta
 - Narrow aortic arch
 - Short intestine

Other findings that can be re-classified as malformation or variation ?

- Forelimb flexures
 - knowledge of cause and post-natal prognosis (permanence)
- Situs inversus
 - lack of adversity
- Retro-oesophageal subclavian artery
 - lack of adversity
 - also, frequency in controls (better lab recognition could be an issue here)

Adversity (Harmfulness)

How do we obtain new knowledge about this ?

1. Research into post-natal consequences (and cause)
 - Information from older animals
 - Information concerning extrapolation to man

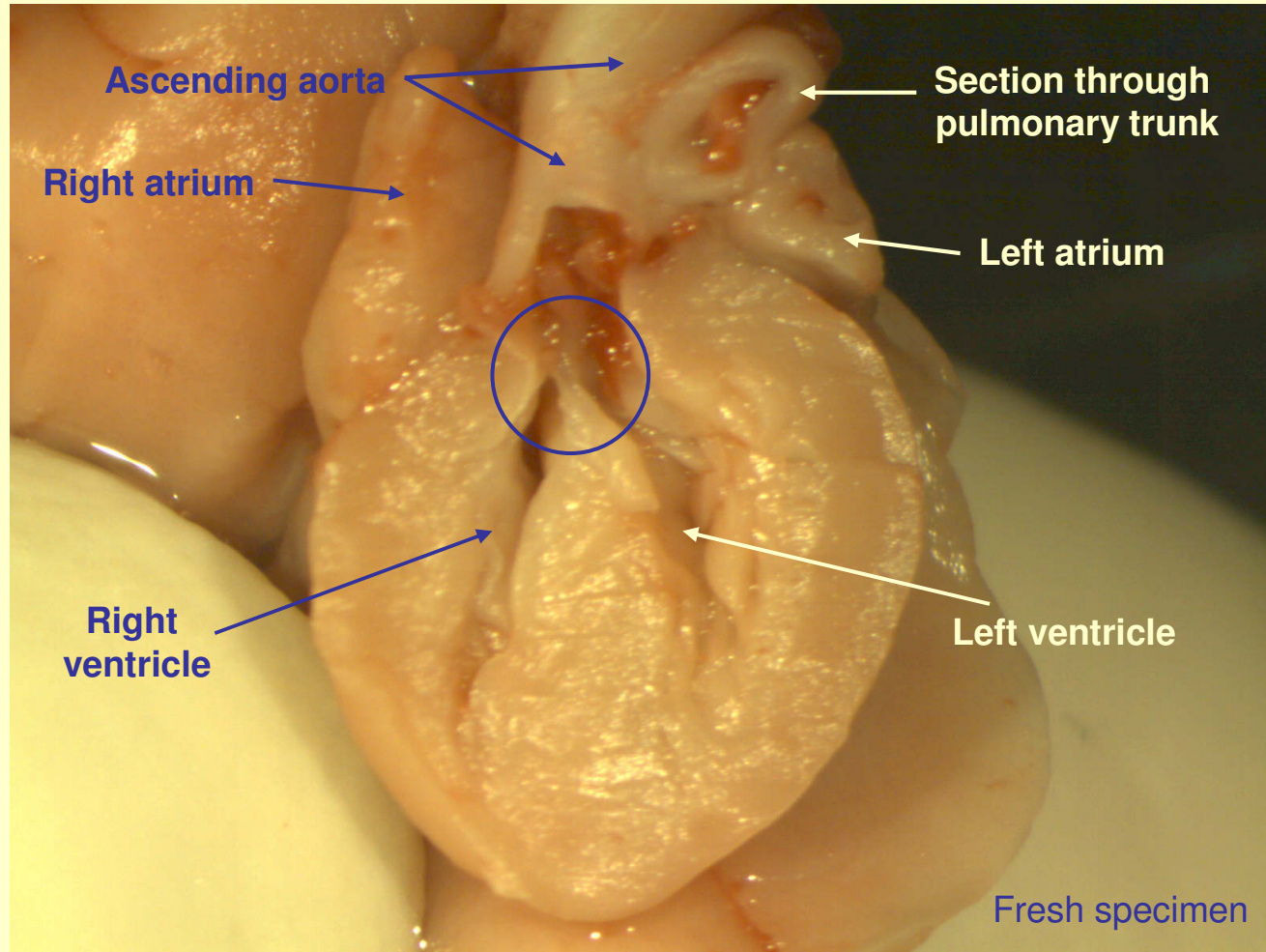
2. Improved laboratory work (to provide better information for assessors)
 - Maintenance of historical data
 - Better descriptive terminology (including terms to denote severity)
 - Method development (better characterization of findings to give more information on severity)

Method development as a tool for reducing the need to classify as Grey-Zone

Examination of the rabbit heart

Interventricular septal defect

- viewed from frontal section of unopened heart

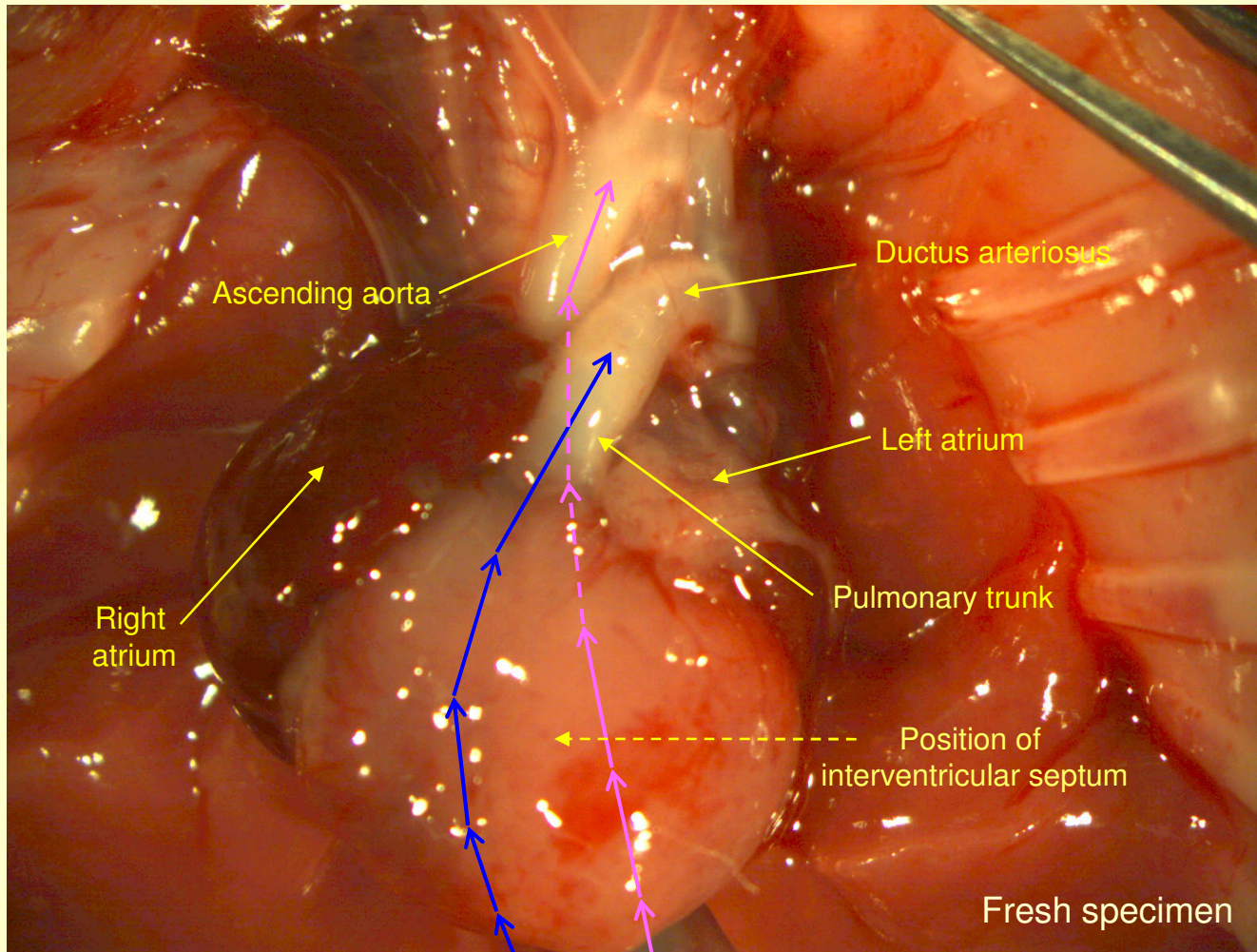


Rabbit

Rabbit, Day 28/29 of pregnancy

Routine heart sections

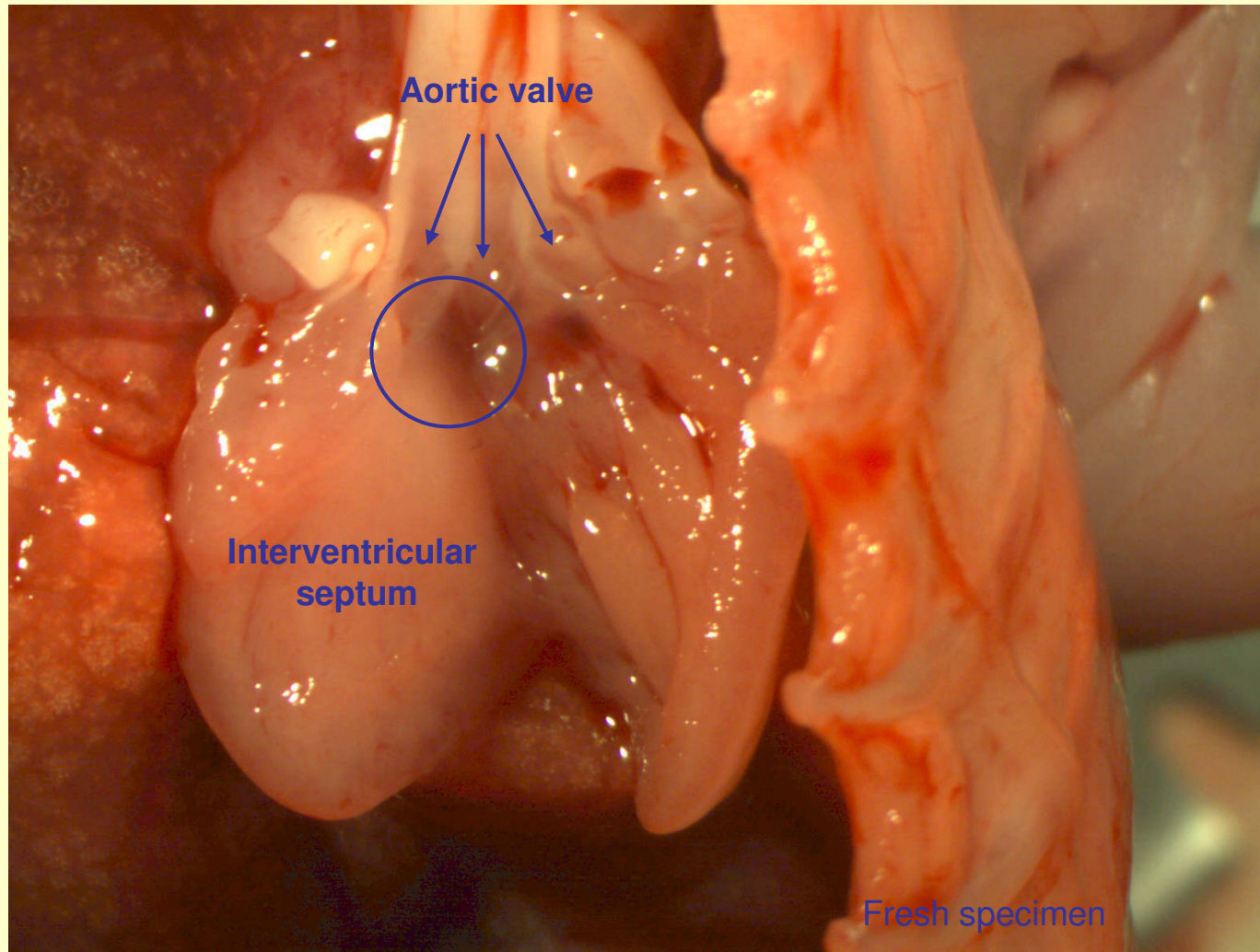
caudal → cranial direction, right ventricle opened first



- Left ventricle to ascending aorta
- Right ventricle to pulmonary trunk

Rabbit, Day 28/29 of pregnancy

Normal left ventricle - viewed at fresh dissection

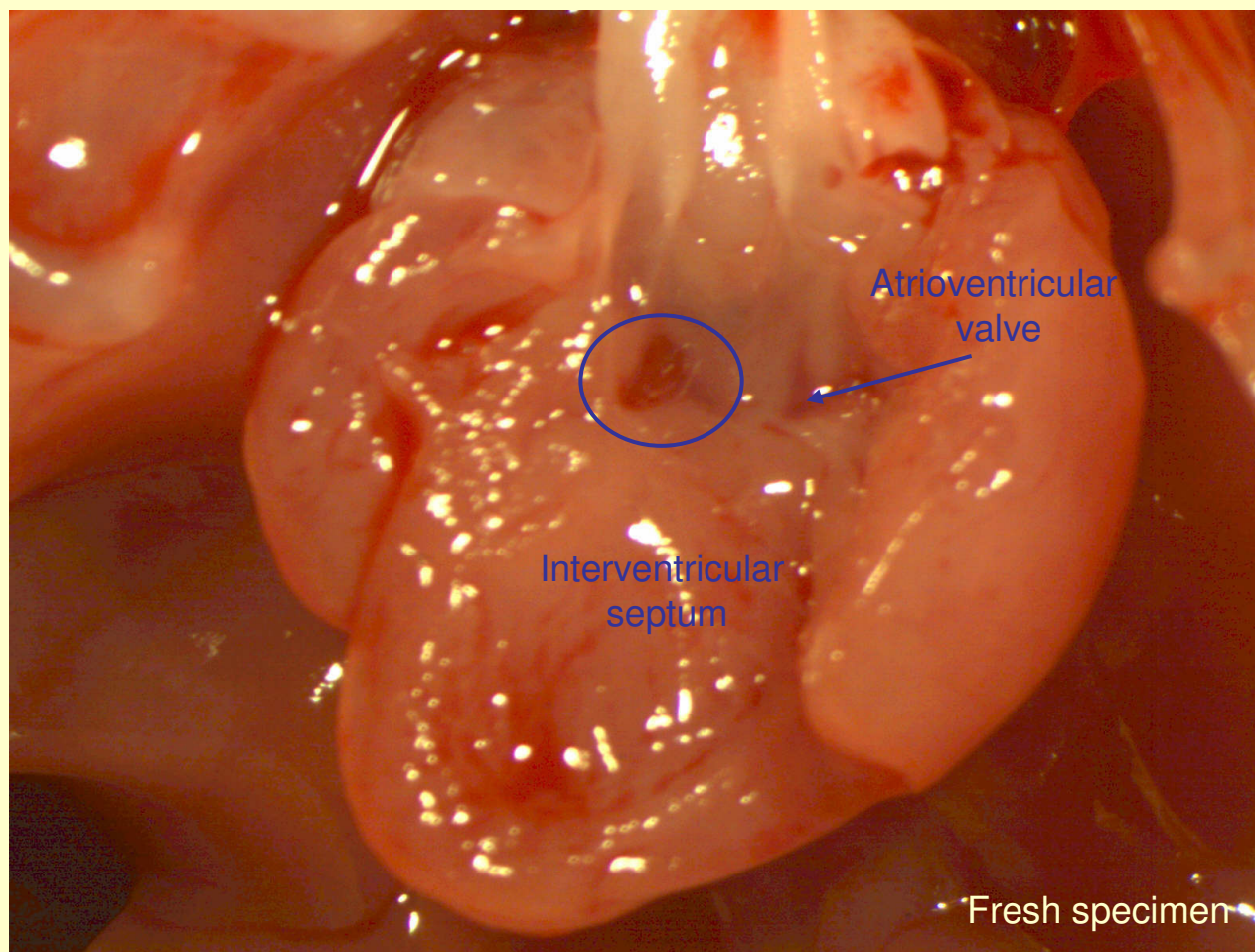


○ = perimembranous region

Rabbit, Day 28/29 of pregnancy

Interventricular septal “defect”

– viewed from inside left ventricle

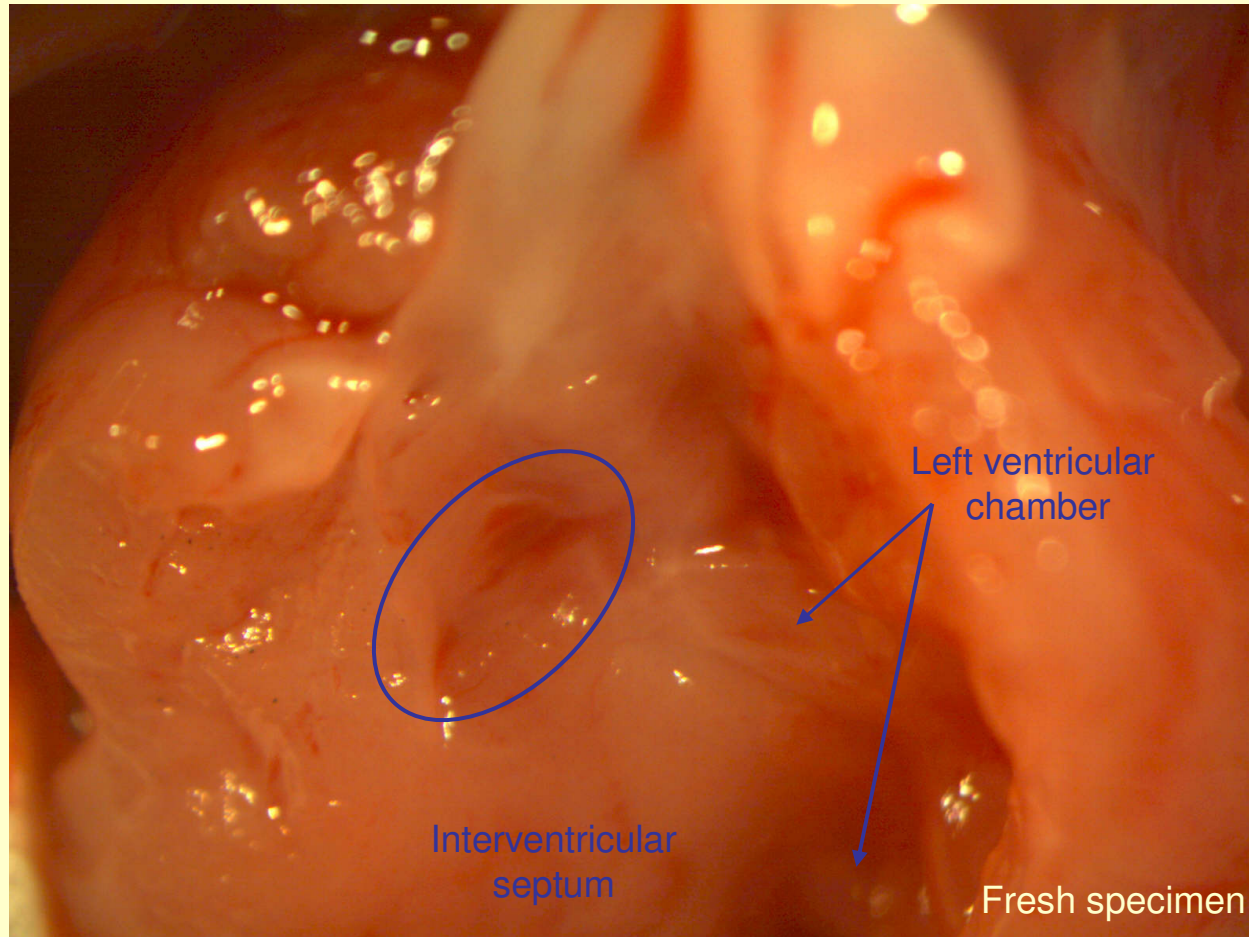


○ = perimembranous region

Rabbit, Day 28/29 of pregnancy

Interventricular septal “defect”

– viewed from inside left ventricle



○ = perimembranous region

Rabbit, Day 28/29 of pregnancy

Classification

Interventricular septal defect

= malformation

Classification

Interventricular septal defect

= malformation

But :

- the numbers in controls increased so classification as a malformation became less comfortable

Considerations

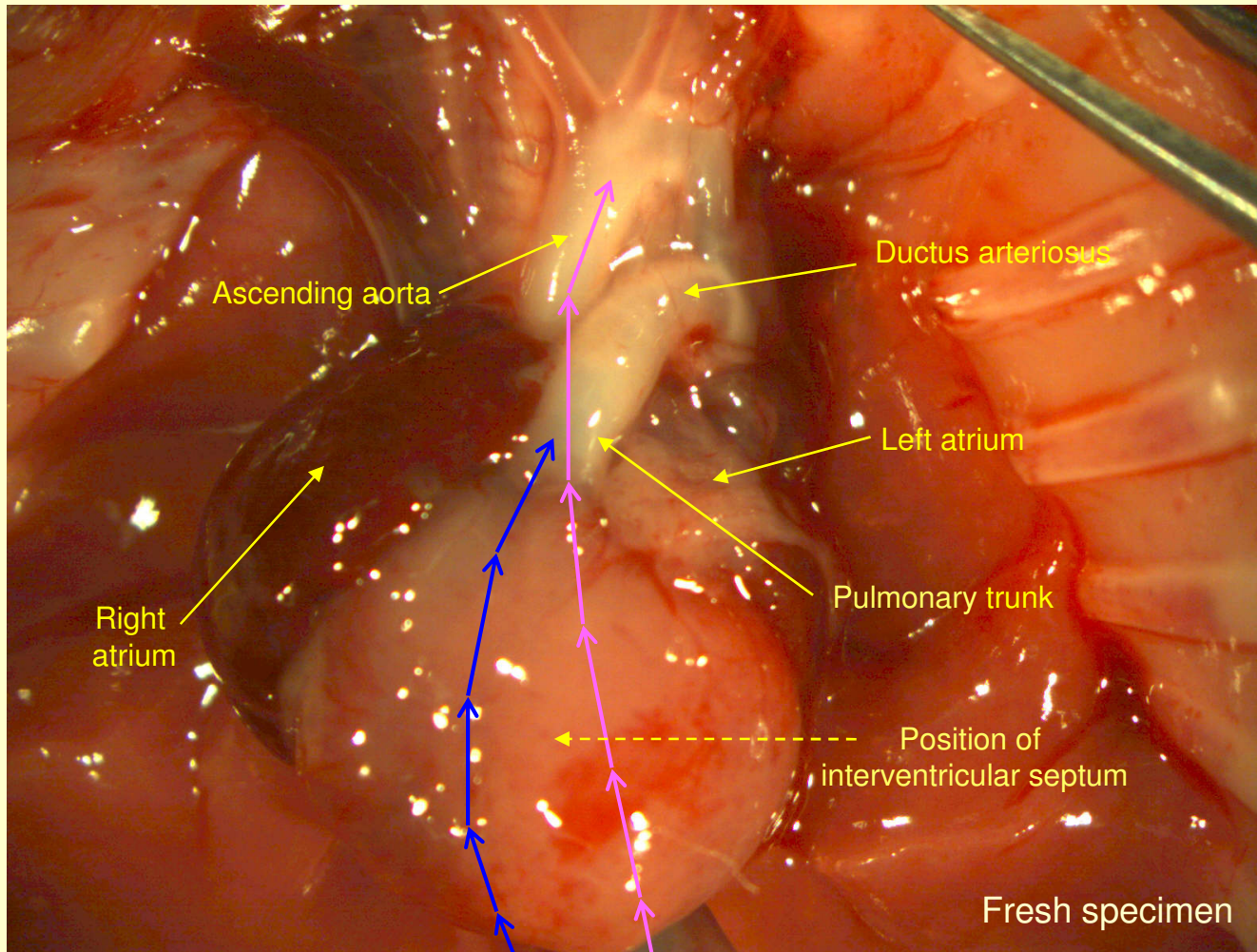
- Were we damaging the hearts with the scissors ?
- Were we seeing something that we thought was abnormal but was actually normal ?
- Were there really so many interventricular septal defects ?

What to do ?

- Try to improve the technique
 - change the heart opening method (open the left chamber first)
 - less chance of damaging the left chamber
 - better visibility
 - clean the perimembranous area of the interventricular septum more carefully before examination

Routine heart sections

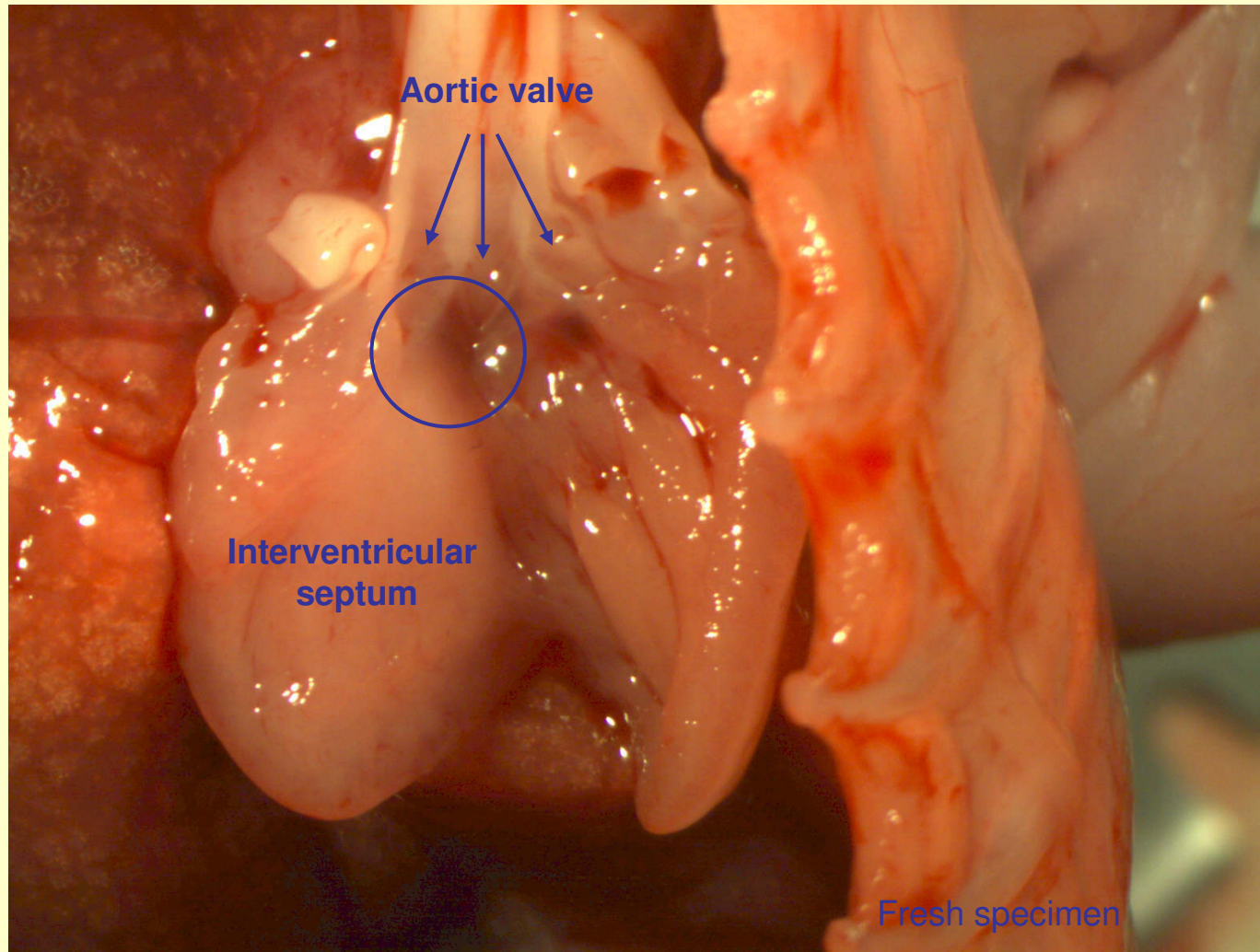
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- Left ventricle to ascending aorta
- Right ventricle to pulmonary trunk

Rabbit, Day 28/29 of pregnancy

Normal left ventricle - viewed at fresh dissection

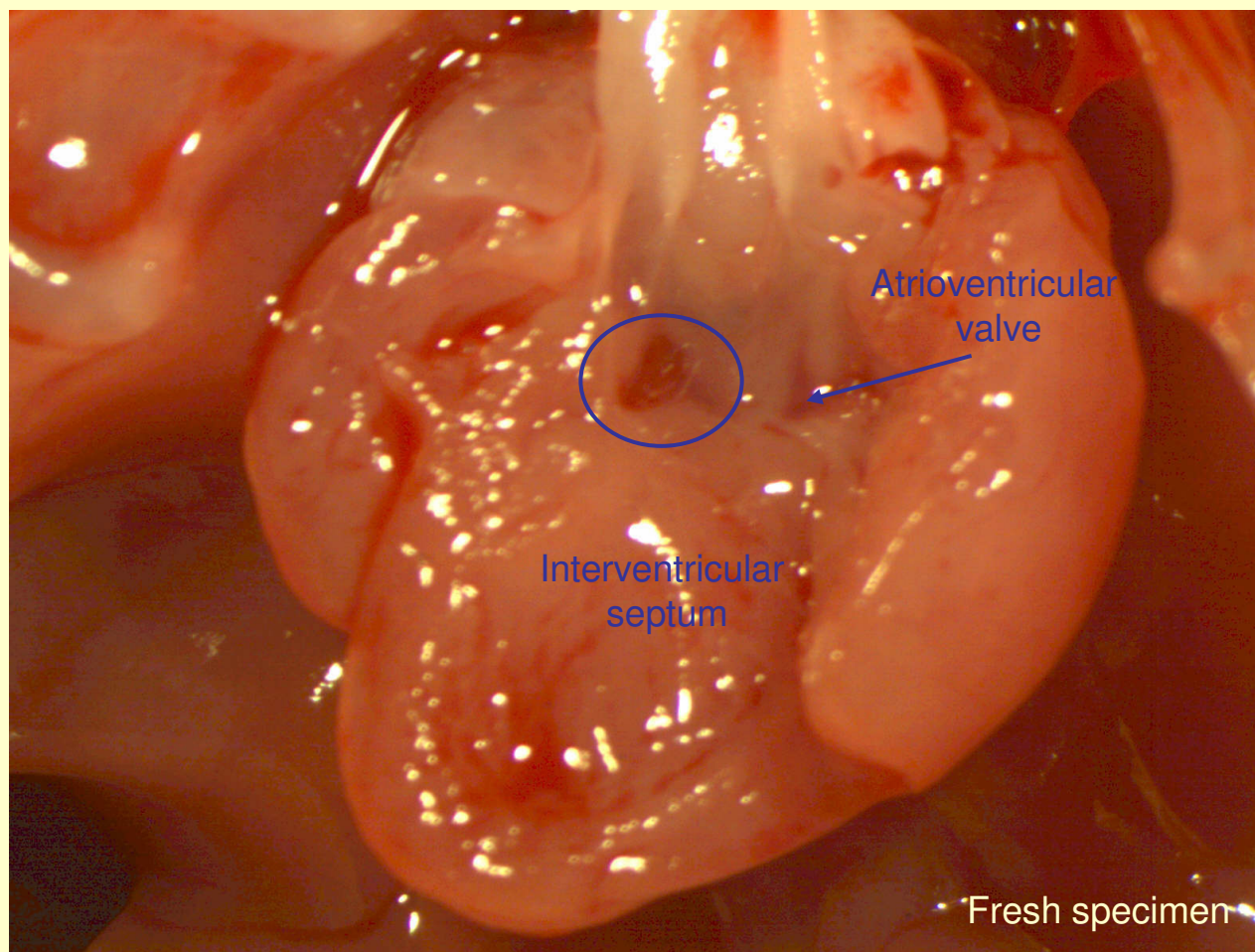


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Rabbit, Day 28/29 of pregnancy

Interventricular septal “defect”

– viewed from inside left ventricle

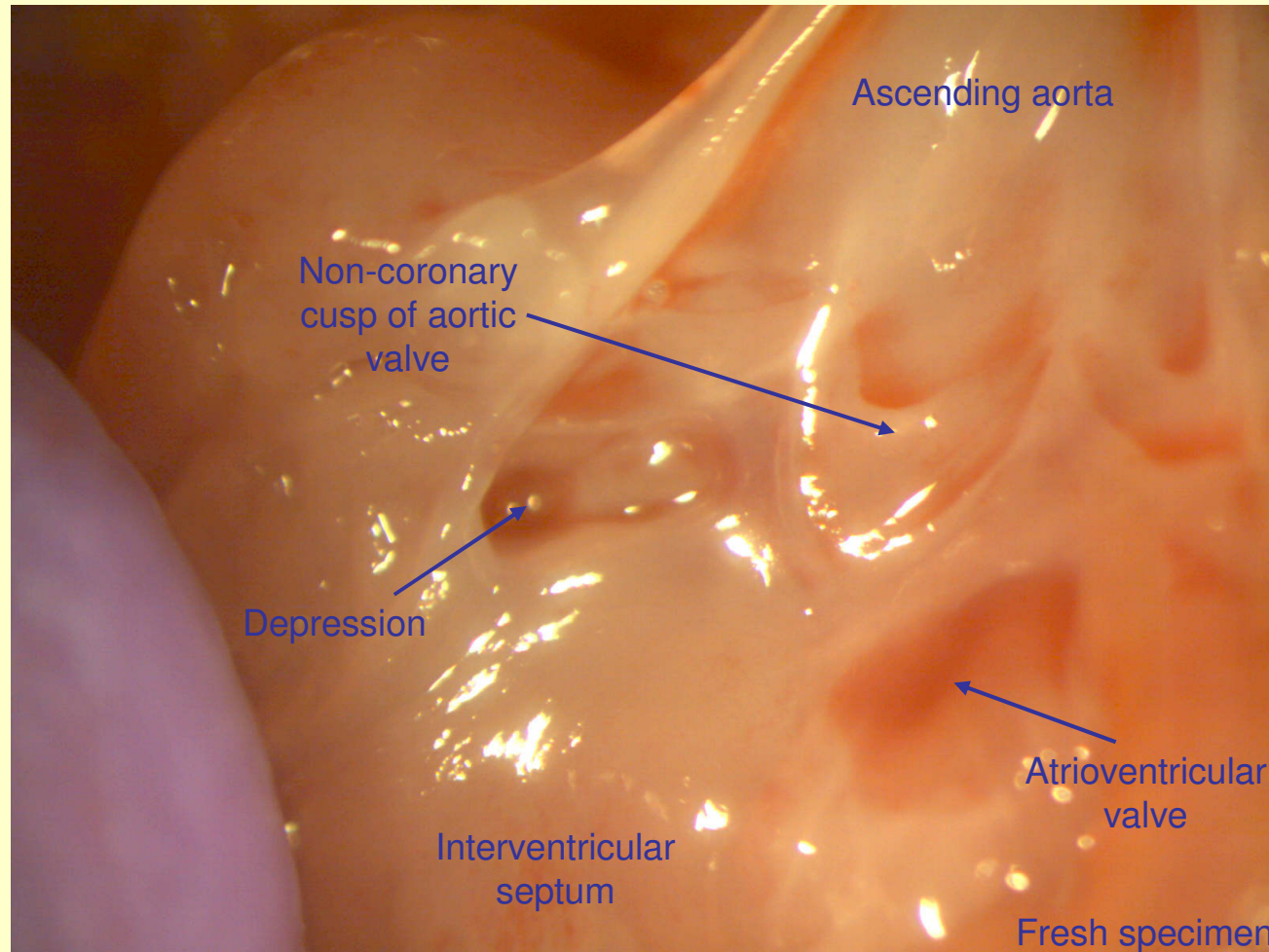


○ = perimembranous region

Rabbit, Day 28/29 of pregnancy

Interventricular septal “defect”

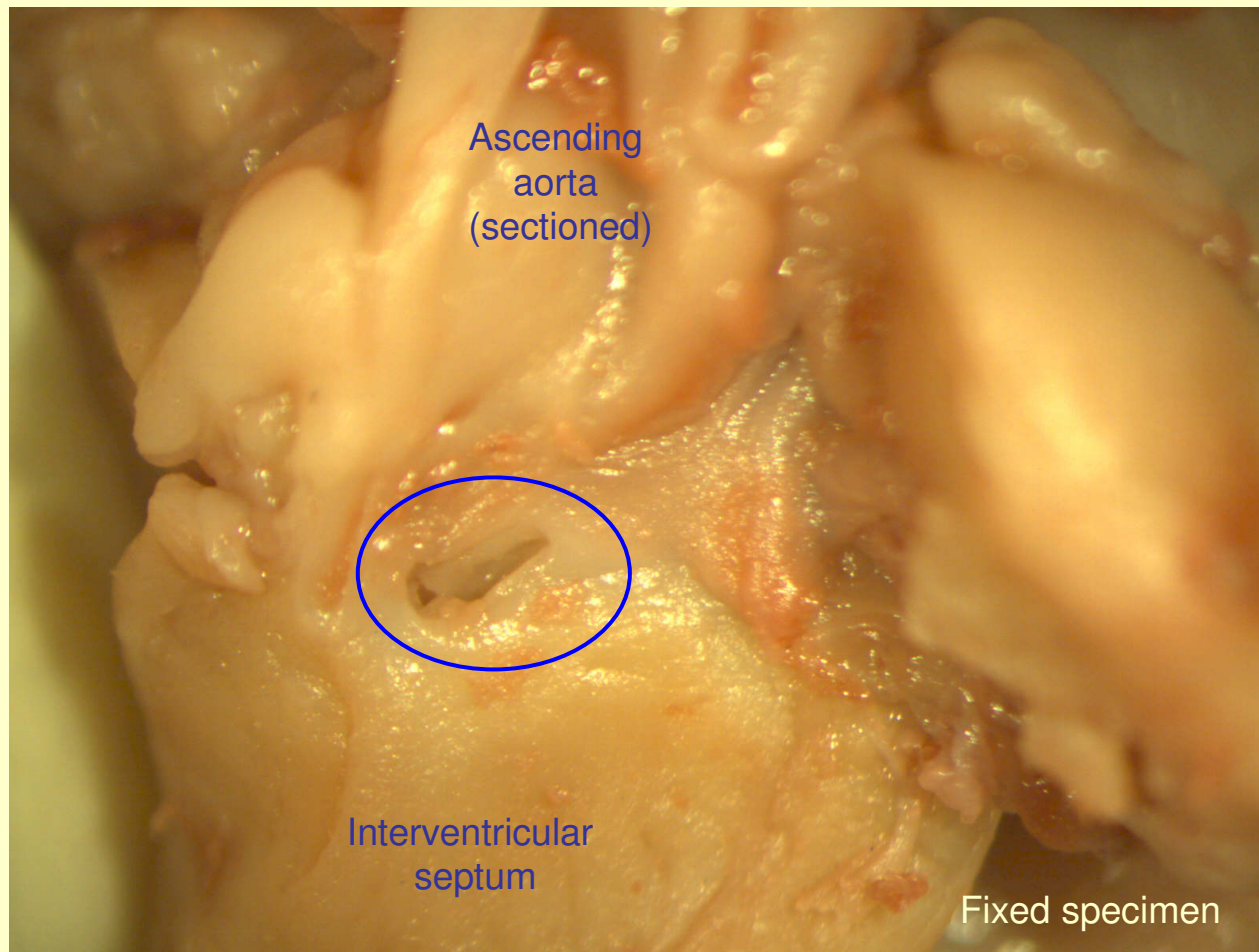
– viewed from inside left ventricle



Rabbit, Day 28/29 of pregnancy

Interventricular septal 'defect'

– viewed from inside left ventricle

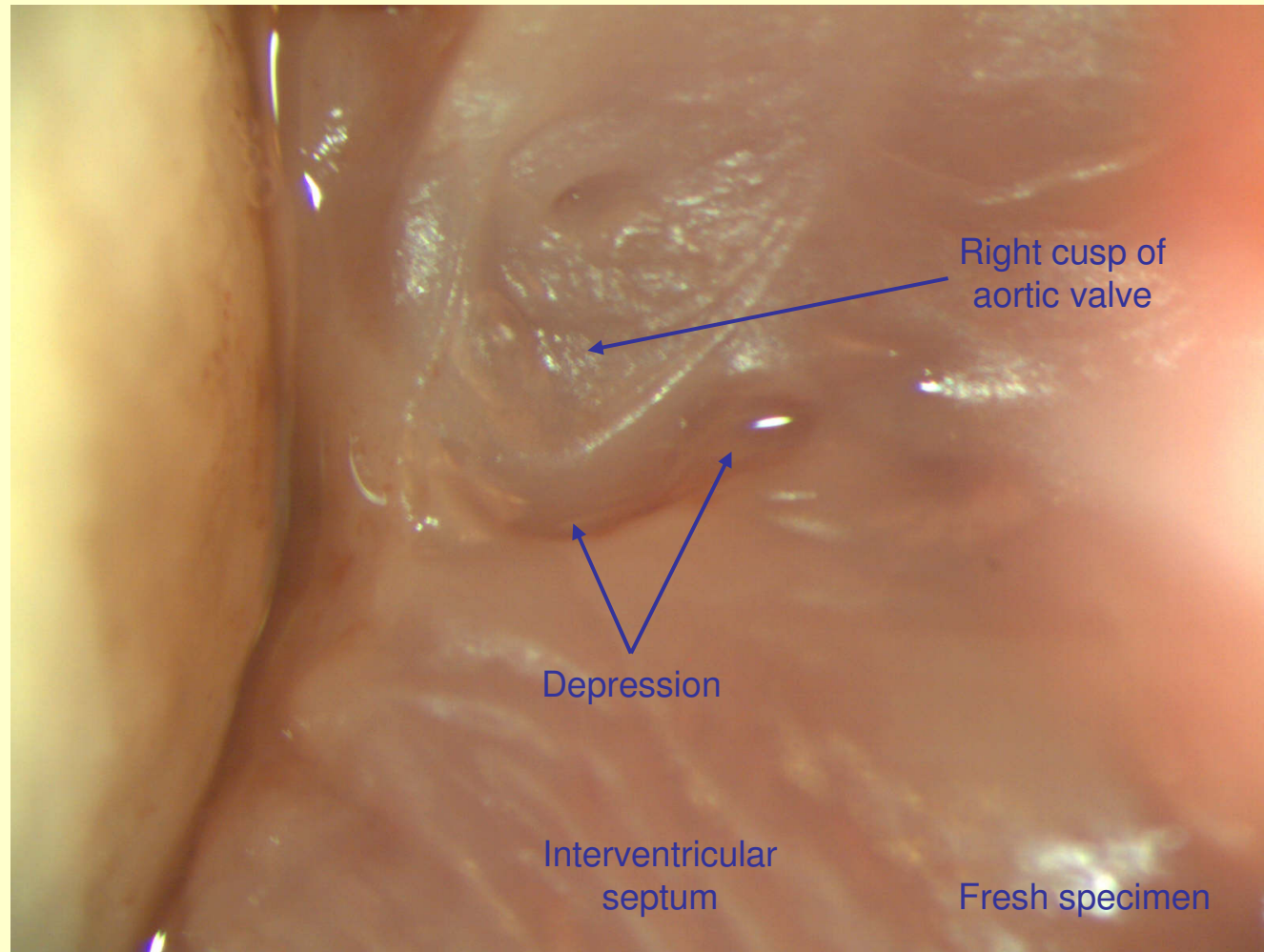


○ = perimembranous region

Rabbit, Day 28/29 of pregnancy

Interventricular septal “defect”

– viewed from inside left ventricle



Rabbit, Day 28/29 of pregnancy

The numbers increased even more
and they clearly were not damaged

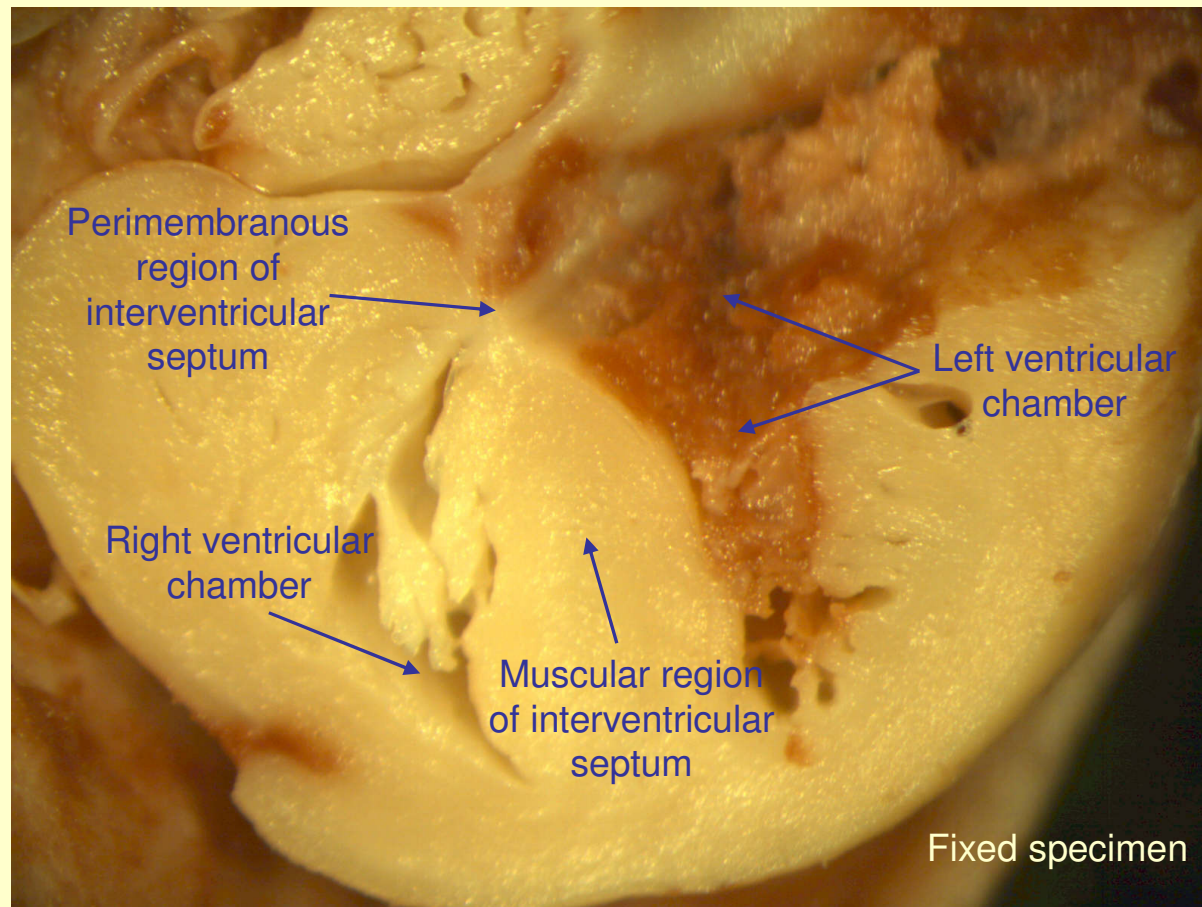
We were in the Grey-Zone

What to do next ?

- Try to improve the technique further
 - fix some hearts after fresh examination ('normal', and with abnormal findings) and section the interventricular septum at the point of the lesion

Normal septum

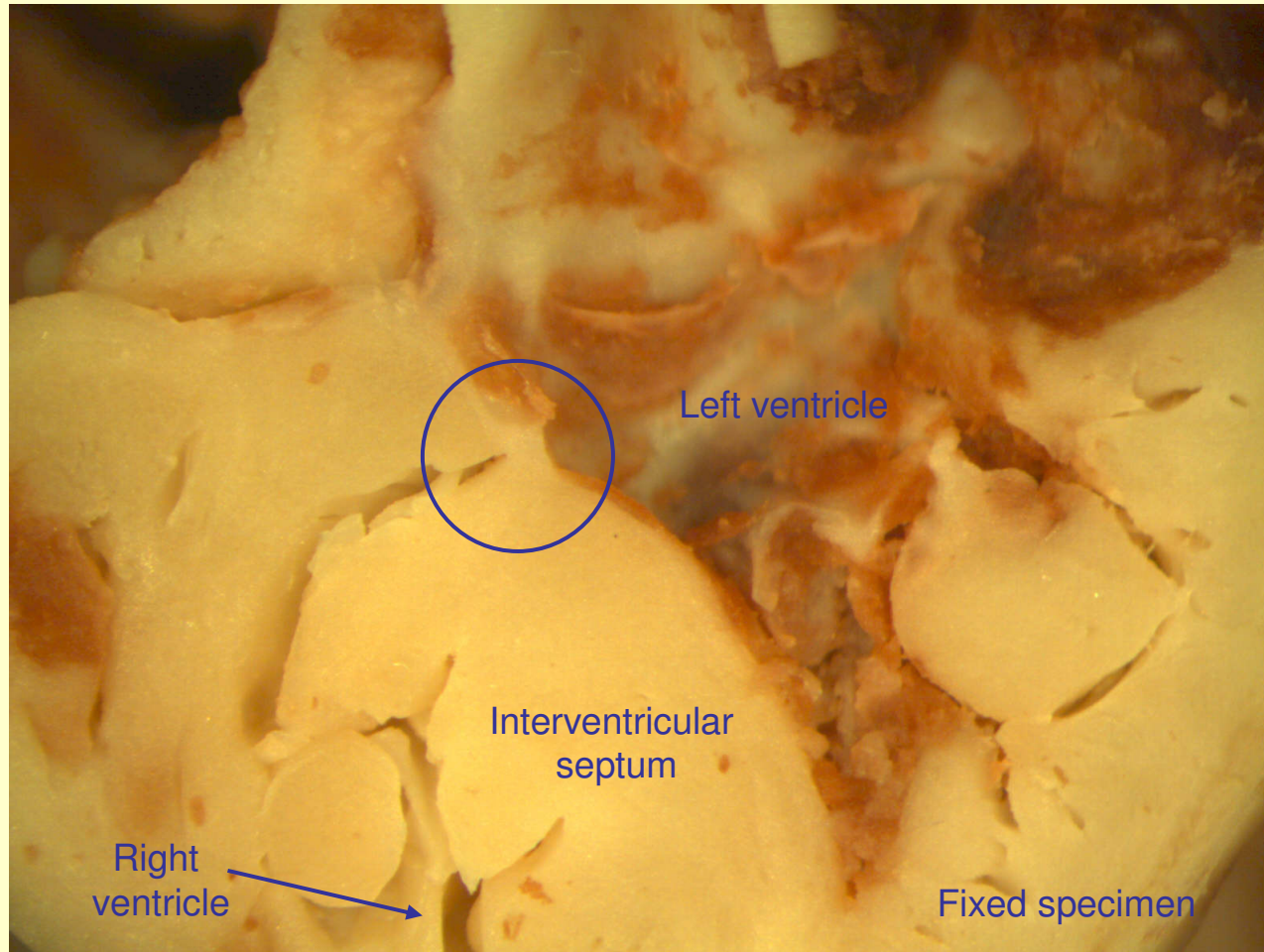
- viewed from frontal section through opened heart



Rabbit, Day 28/29 of pregnancy

Normal perimembranous septum

- viewed from frontal section through opened heart

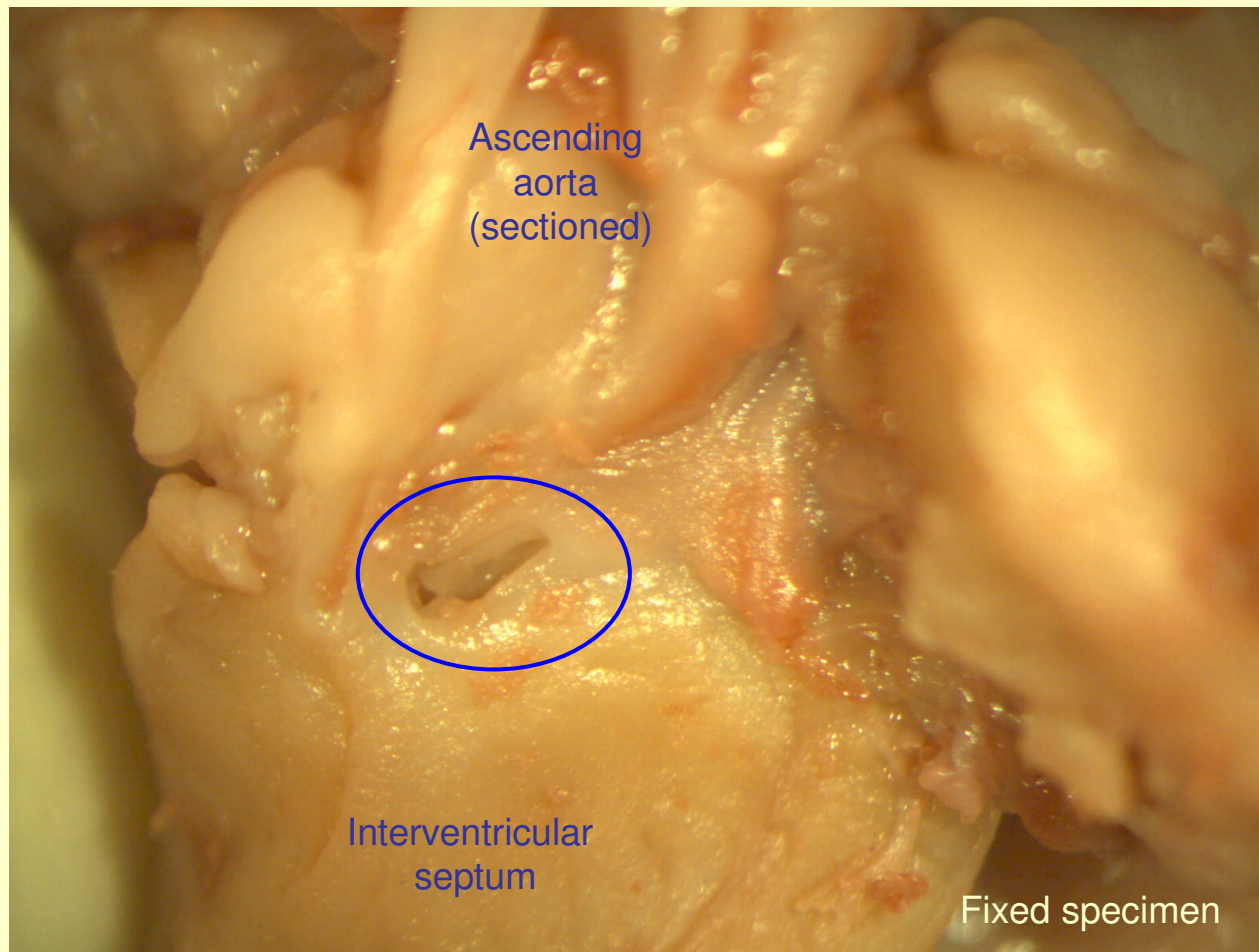


○ = perimembranous septum

Rabbit, Day 28/29 of pregnancy

Interventricular septal 'defect'

– viewed from inside left ventricle

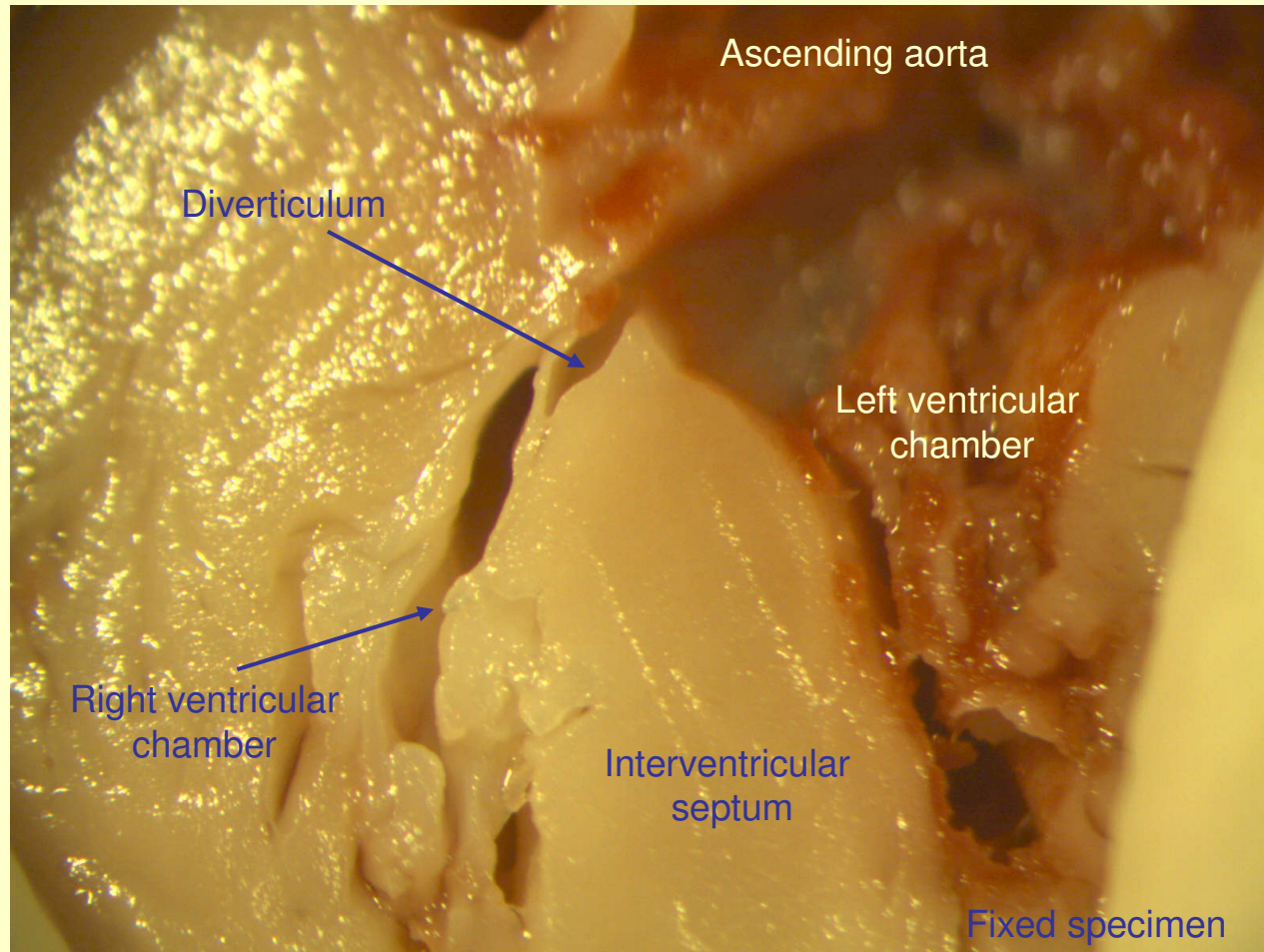


○ = perimembranous region

Rabbit, Day 28/29 of pregnancy

Diverticulum

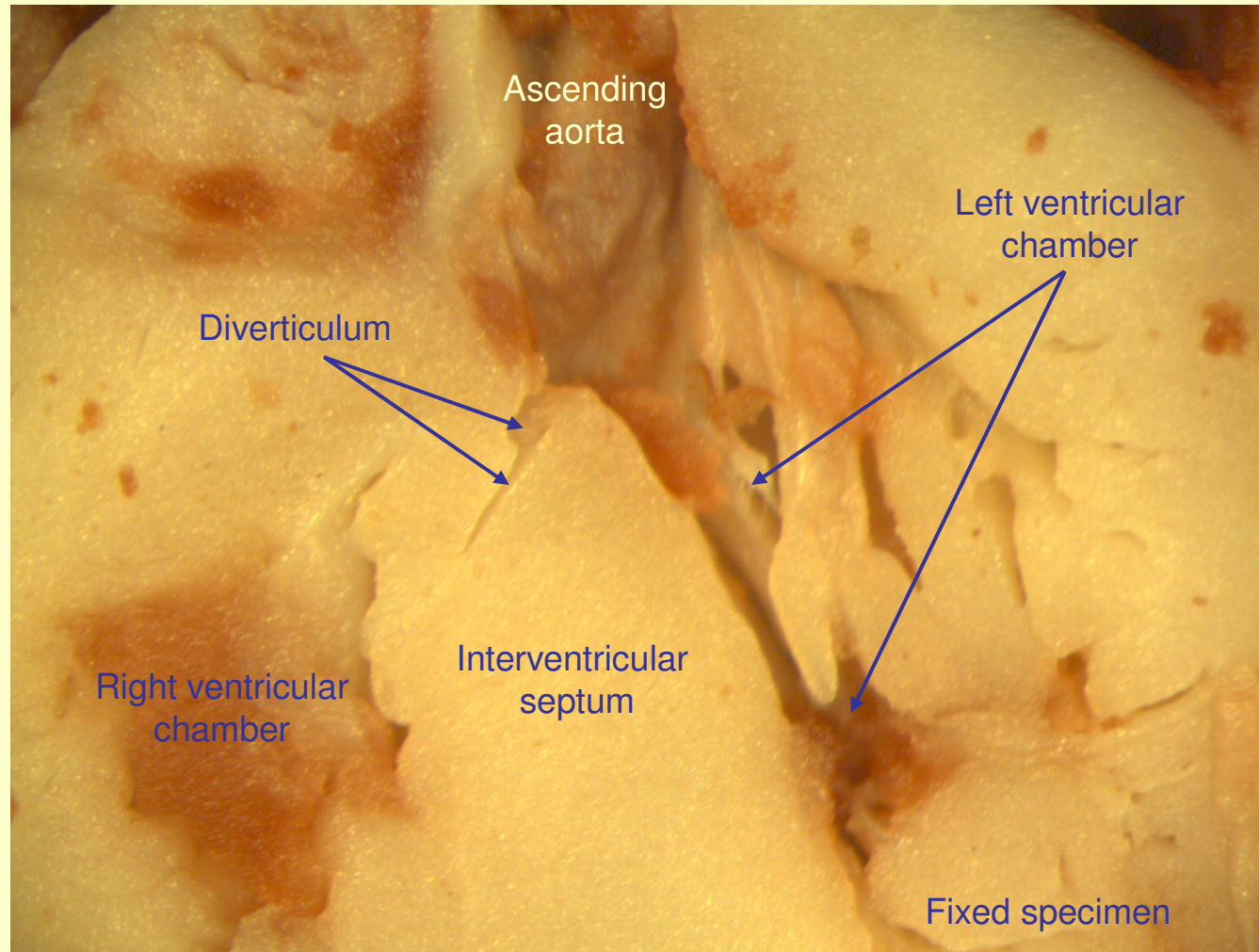
– viewed from frontal section through opened heart



Rabbit, Day 28/29 of pregnancy

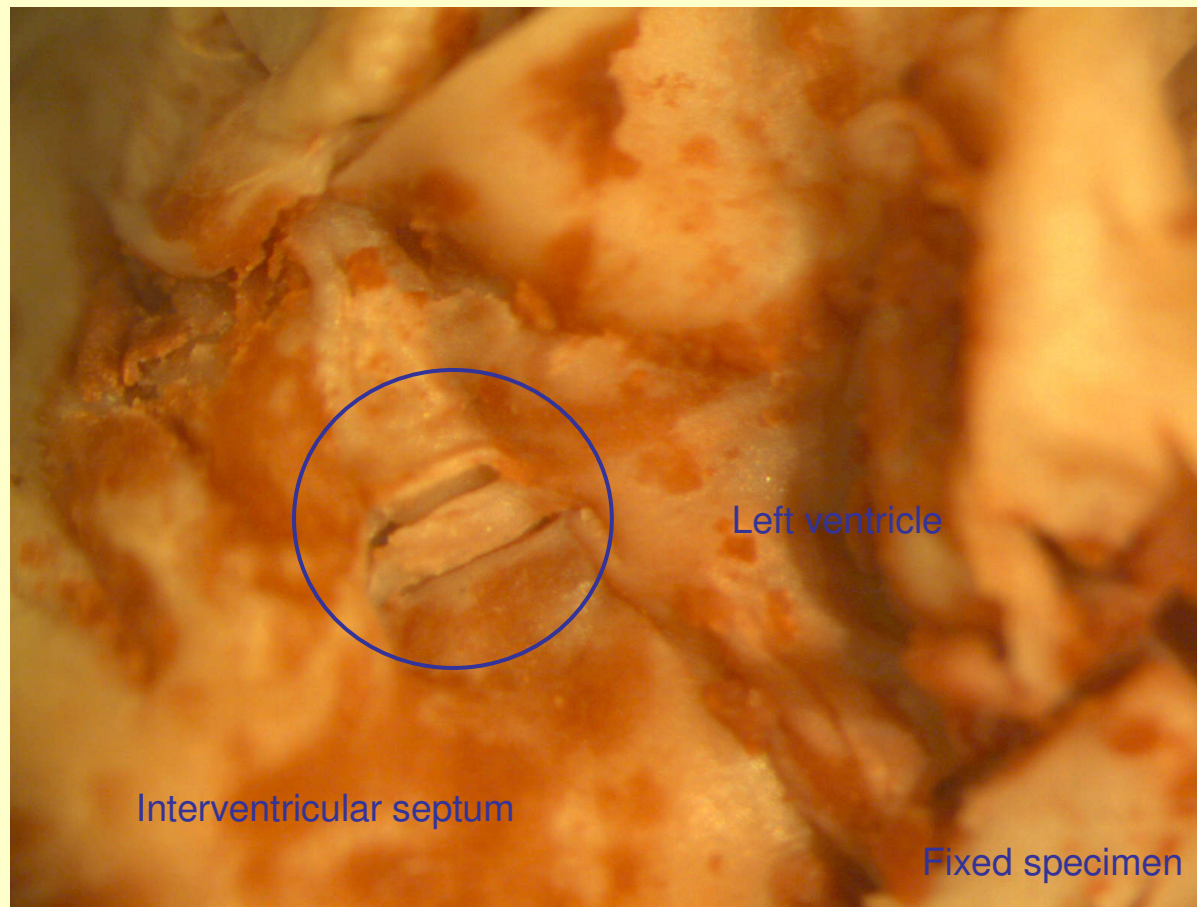
Diverticulum

– viewed from frontal section through opened heart



Interventricular septal “defects”

– viewed from inside left ventricle

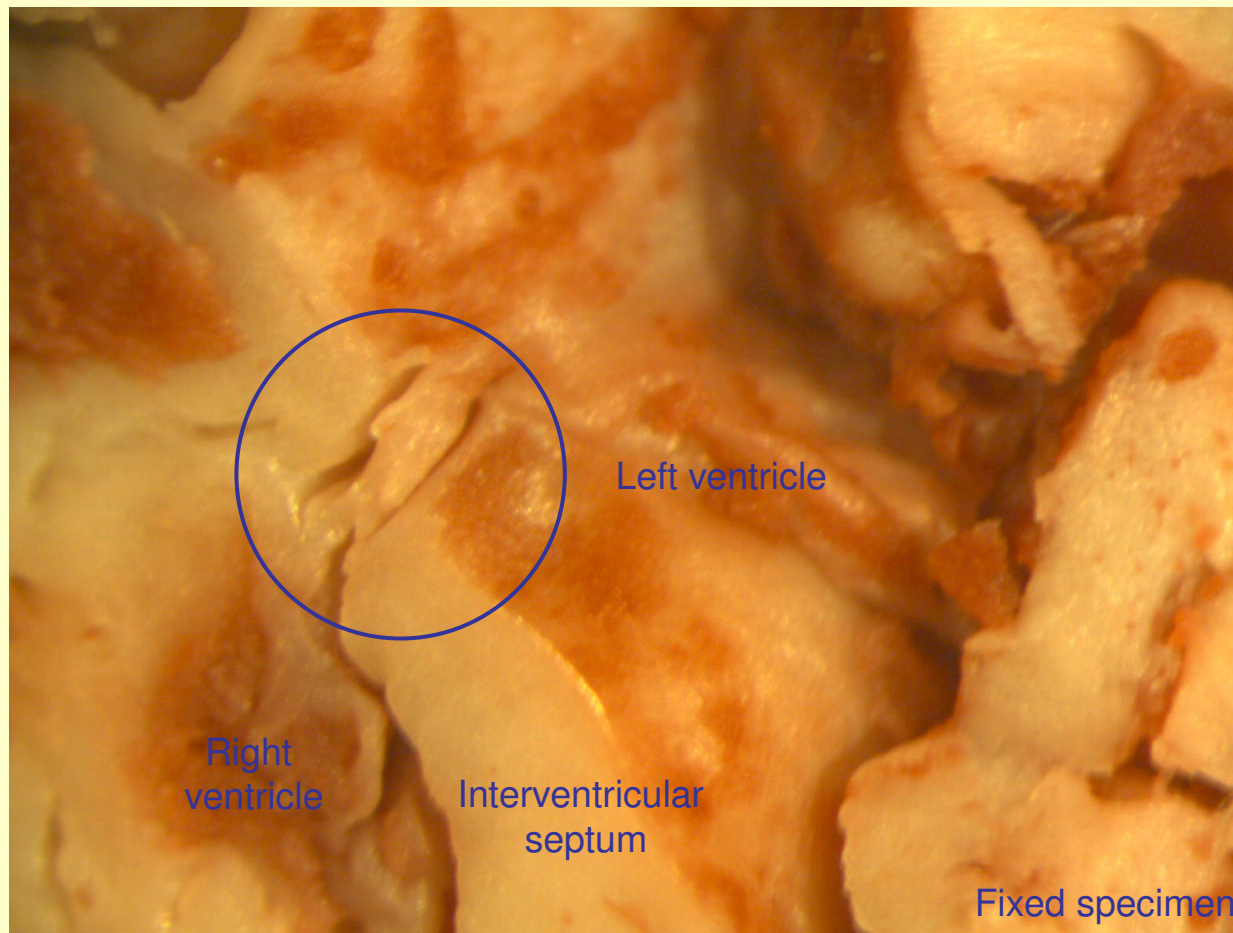


○ = perimembranous region

Rabbit, Day 28/29 of pregnancy

Diverticula

– viewed from frontal section through opened heart

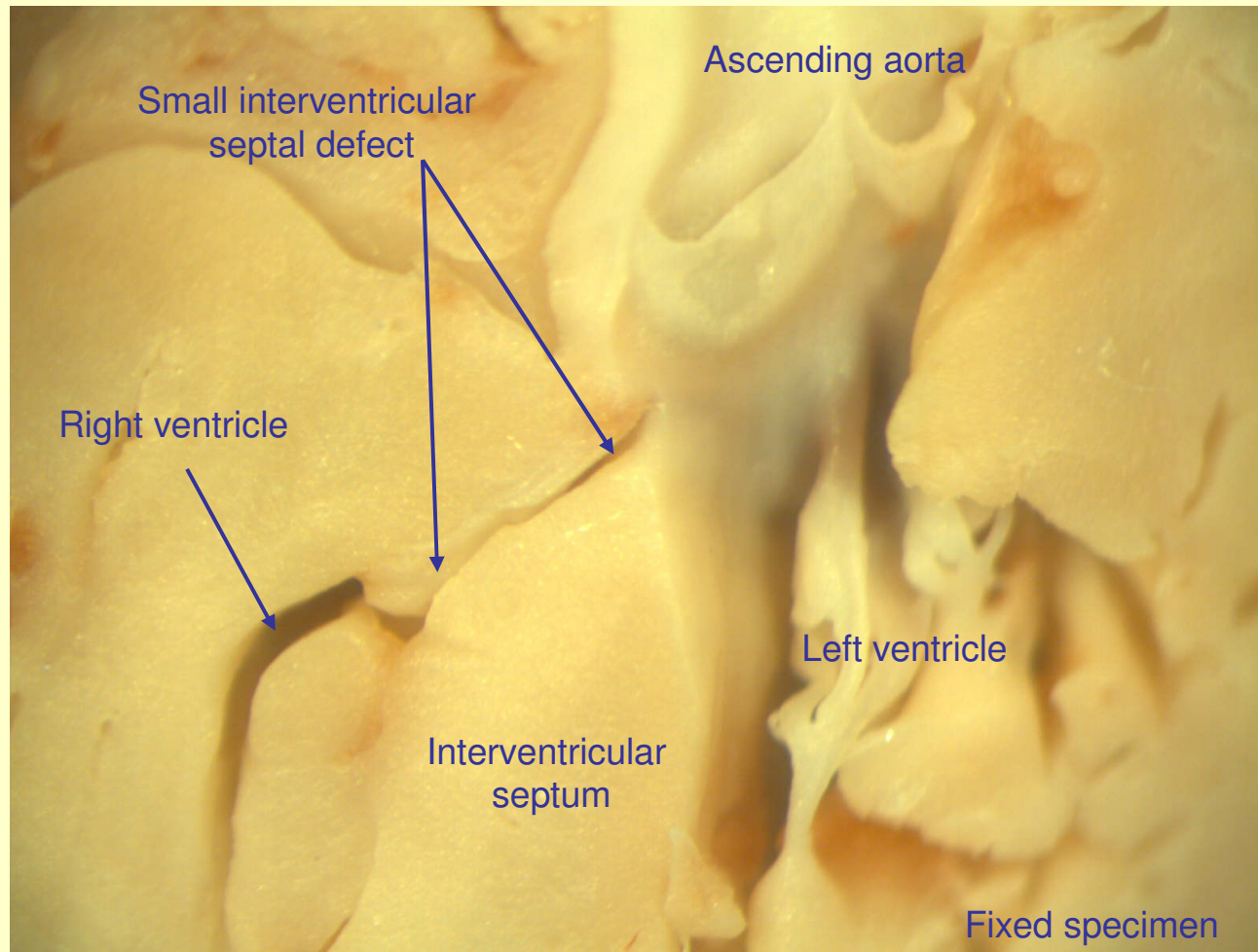


○ = Diverticula

Rabbit, Day 28/29 of pregnancy

Small interventricular septal defect

– viewed from frontal section through opened heart

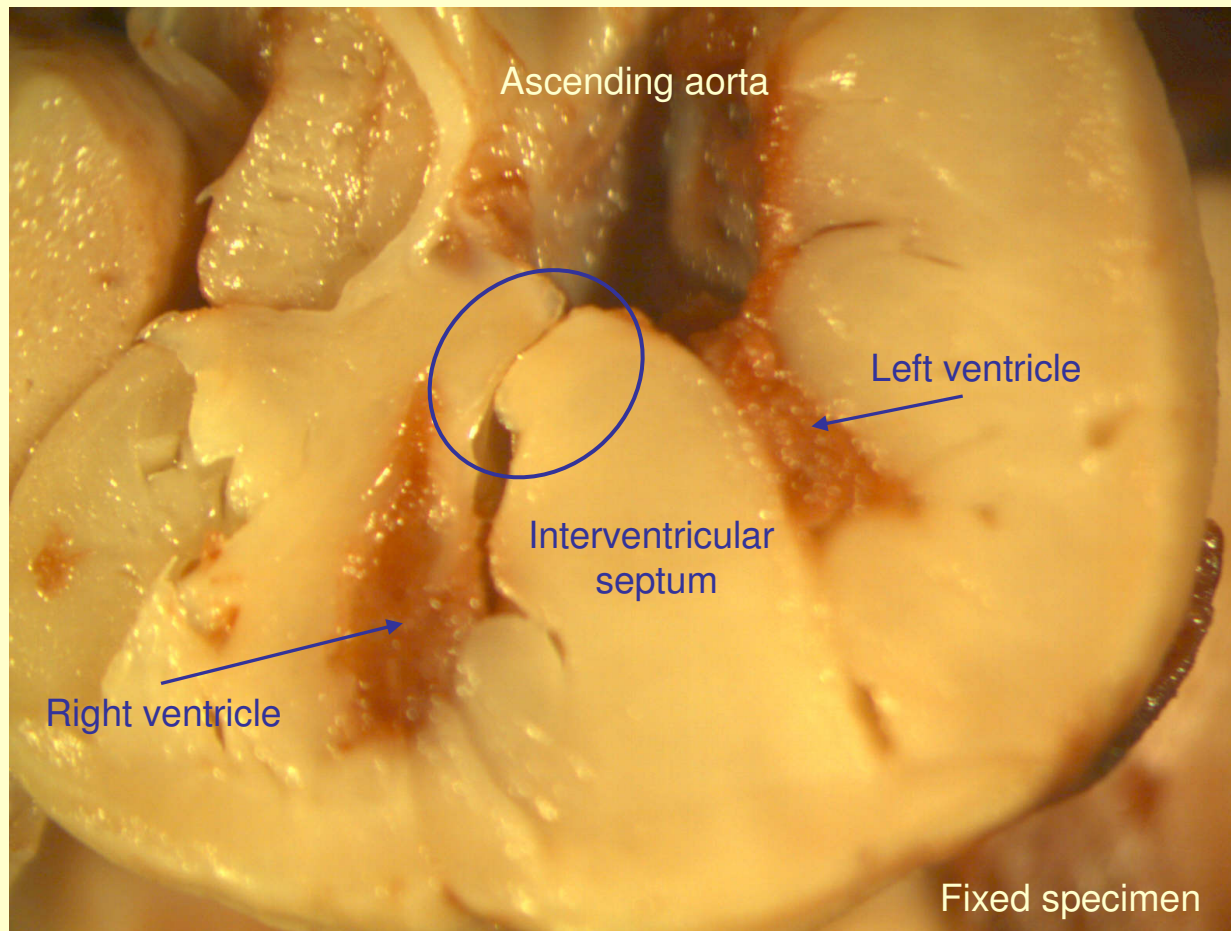


○ = perimembranous region

Rabbit, Day 28/29 of pregnancy

Small interventricular septal defect

– viewed from frontal section through opened heart



○ = perimembranous region

Rabbit, Day 28/29 of pregnancy

Classification

Diverticulum

perimembranous region

and

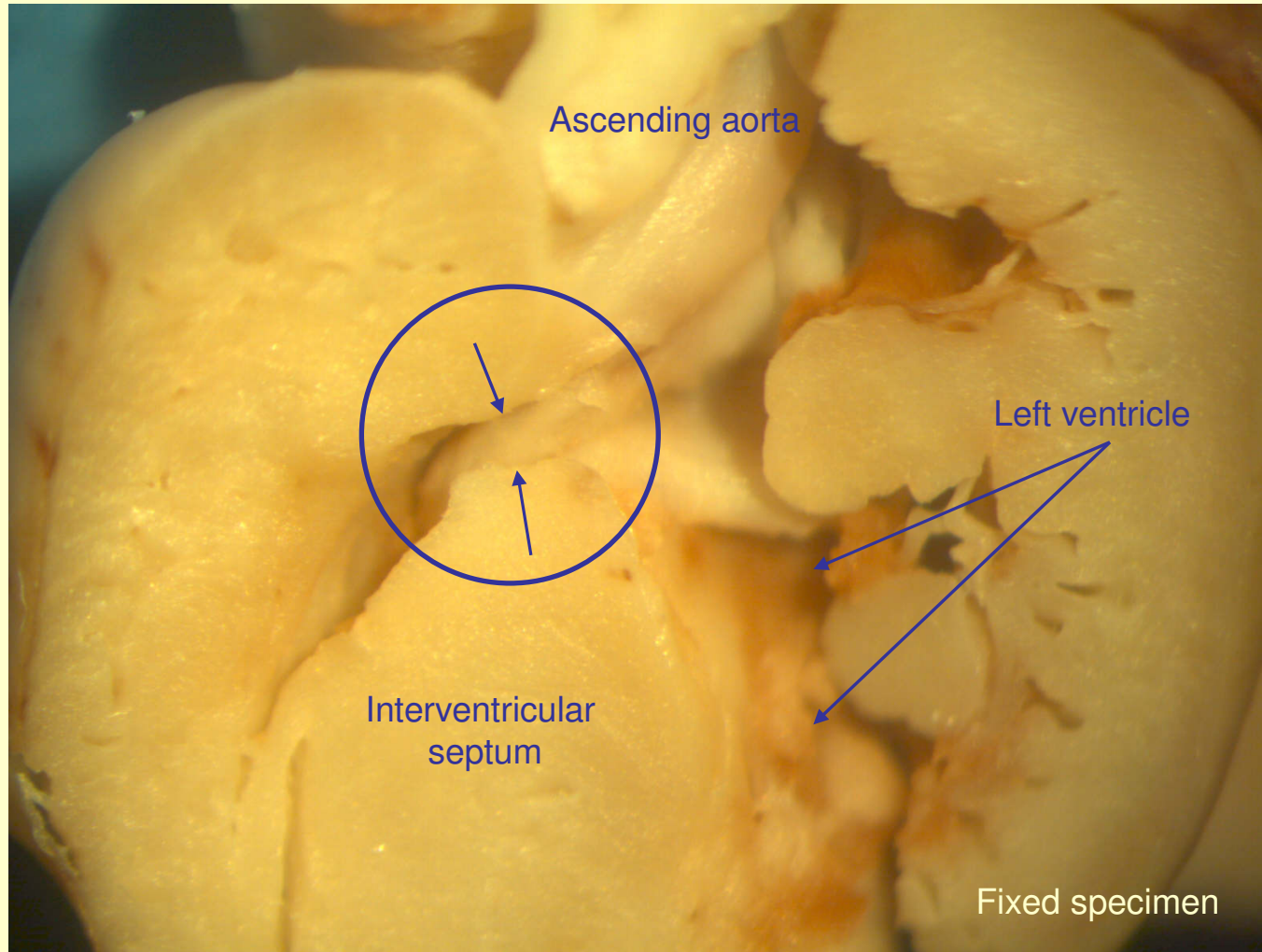
Small interventricular septal defect

perimembranous region

= Variation

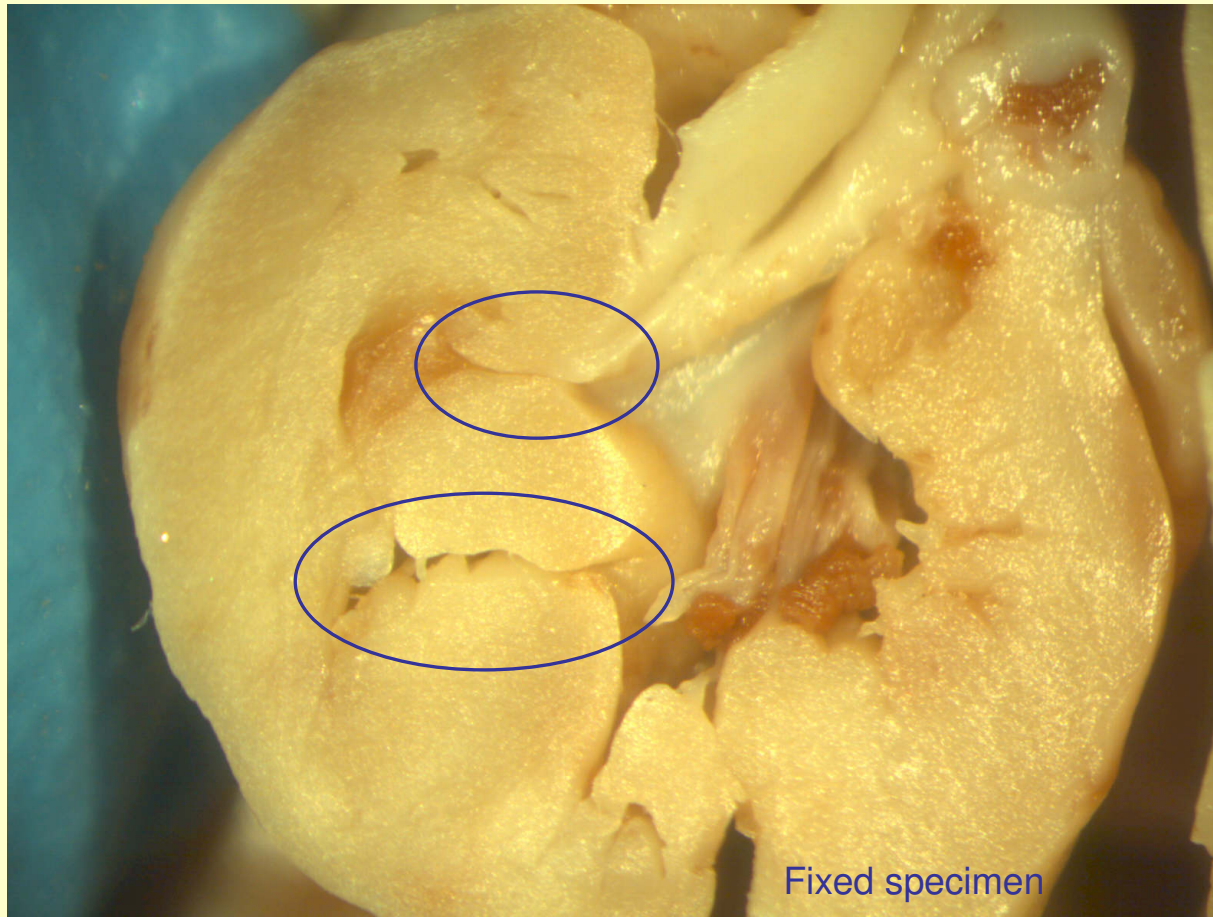
Interventricular septal defect

– viewed from frontal section through opened heart




Interventricular septal defects, perimembranous and muscular

– viewed from frontal section through opened heart



Rabbit, Day 28/29 of pregnancy

 = Septal defect

Classification

Interventricular septal defect
muscular region

= Malformation

By developing our lab method, we have gained more information on potential adversity and thus avoided the Grey-Zone

Classifications

Fresh examination:

Depression perimembranous region = variation

Fixed examination:

Diverticulum perimembranous region = variation

Small interventricular septal defect perimembranous region = variation

Interventricular septal defect muscular region = malformation

Solomon HM et al (1997)

Spontaneous and Induced Alterations in
the Cardiac Membranous Ventricular
Septum of Fetal, Weanling and Adult Rats

Teratology 55:185 to 194

Acknowledgement

Harlan Laboratories, Switzerland

Discussion of external and
visceral Grey-Zone anomalies:

Reclassification due to new
knowledge

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