

APPENDICES

1. Factor VIII staining protocol

The staining protocol was applied from Cell Biology Center, North Dakota State University (NDSU), Hultz Hall 109.

Staining purpose:	Fluorescent
Fixative used:	Carnoy's
Tissue section:	Paraffin section at 6 μ m
IgG:	Rabbit
Dilution preference:	1:100
Antibody source:	Sigma, rabbit anti-Factor VIII, #F-3520
Procedure:	

Solution	Time	Comments
1. Clearing agent	2 x 3 min/each	-
2. Graded ethanol	5 min/each	-
3. dH ₂ O	5 min	Rinsing
4. 3% H ₂ O ₂	5 min	-
5. dH ₂ O	5 min	Rinsing
6. 10 mM PBS with 0.3% Triton X-100	10 min	-
7. 10 mM PBS	10 min	Rinsing
8. Blocking buffer with 2% goat serum	20 min	-
9. Rabbit anti-Factor VIII IgG	1 h	-
10. Rinsed 10 mM PBS	2 x 10 min/each	-
11. Goat anti-rabbit IgG with Alexa Fluor Grn [®] 488 at 1:250	45 min	Under dark condition
12. dH ₂ O	5 min	Rinsing
13. Coverslip with Vectashield hardset with DAPI	-	Use #1.5 coverglass

2. eNOS staining protocol

The staining protocol was applied from Cell Biology Center, North Dakota State University (NDSU), Hultz Hall 109.

Staining purpose:	Fluorescent
Fixative used:	Carnoy's
Tissue section:	Paraffin section at 6 μ m
IgG:	Mouse
Dilution preference:	1:500
Antibody source:	Transduction Laboratories, mouse anti-eNOS, #N30020-050
Procedure:	

Solution	Time	Comments
1. Clearing agent	2 x 10 min/each	-
2. Graded ethanol	5 min/each	-
3. dH ₂ O	5 min	Rinsing
4. 3% H ₂ O ₂	5 min	-
5. dH ₂ O	5 min	Rinsing
6. 10 mM PBS with 0.3% Triton X-100	10 min	-
7. 10 mM PBS	10 min	Rinsing
8. Blocking buffer with 2% goat serum	20 min	-
9. Mouse anti-eNOS IgG	Overnight	In 4 °C refrigerator
10. 10 mM PBS	2 x 5 min/each	Rinsing
11. Goat anti-mouse IgG with Alexa Fluor Grn [®] 488 at 1:250	45 min	Under dark condition
12. dH ₂ O	5 min	Rinsing
13. Coverslip with Vectashield hardset with DAPI	-	Use #1.5 coverglass

3. Ovarian tissue preparation

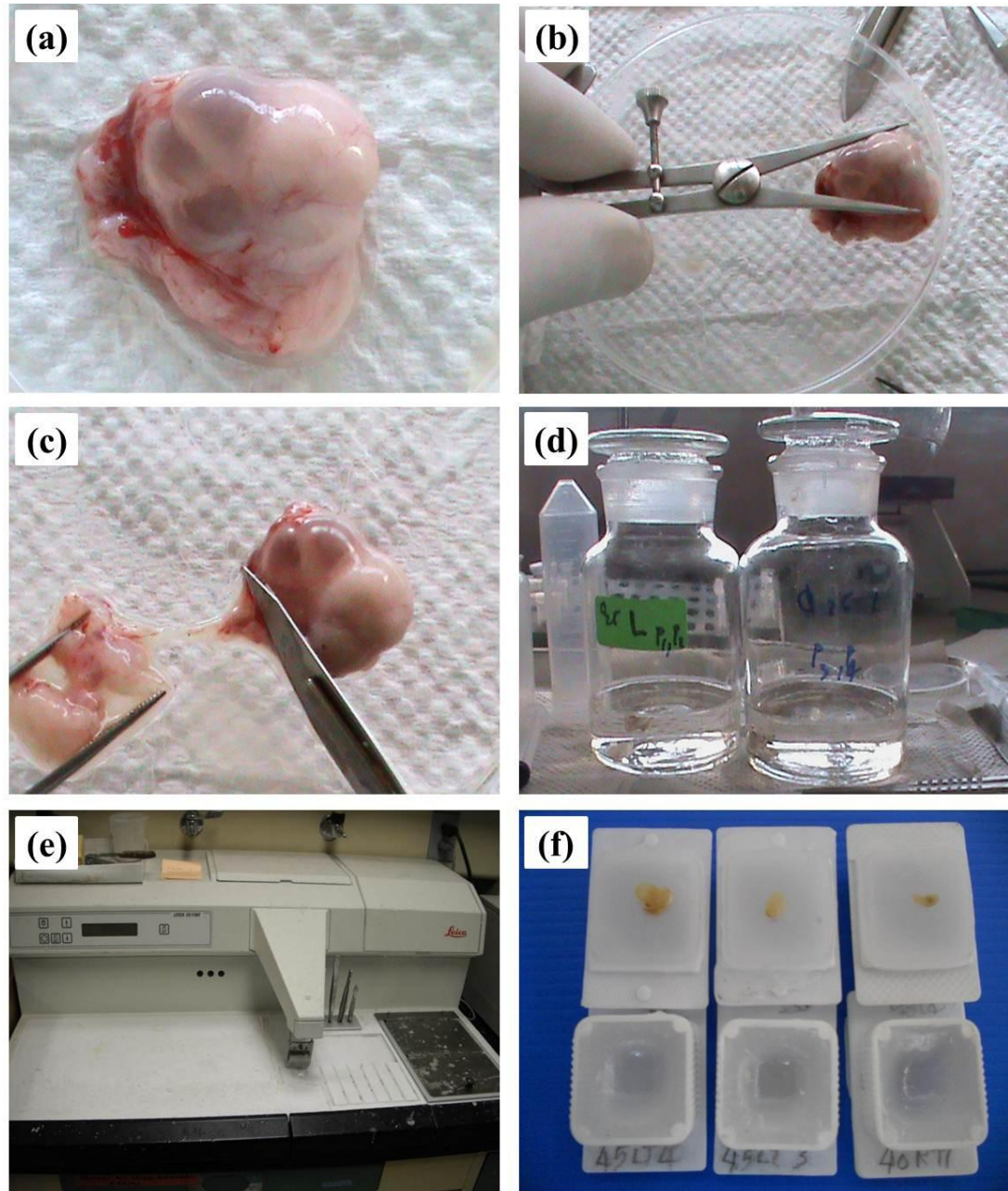


Figure 6.1 Ovary collection and preparation was used for histological method and staining; (a), ovarian tissue; (b), classification of follicles; (c), ovaries were sectioned into several pieces; (d), tissue sections were fixed in Carnoy's solution; (e), paraffin embedded; (f), paraffin blocks

4. Immunofluorescence localization of Factor VIII and eNOS

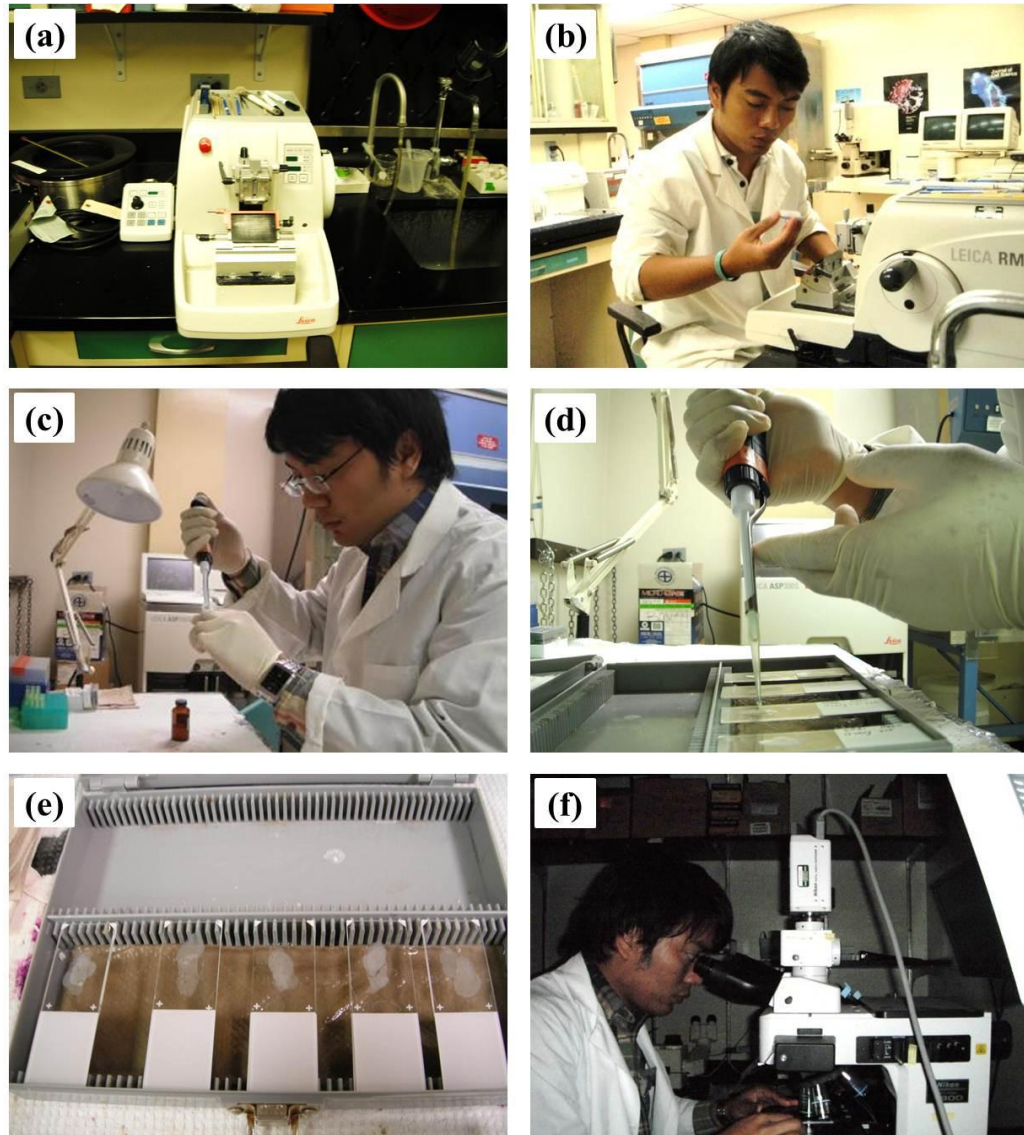


Figure 6.2 Immunofluorescence detection of Factor VIII and eNOS; (a and b), preparation tissue sections by microtome; (c and d), tissue sections were covered with primary antibody for Factor VIII or eNOS; (e), slides were incubated with the fluorophores-labeled goat anti-rabbit IgG antibody for Factor VIII or the fluorophores-labeled goat anti-mouse IgG antibody for eNOS; (f), tissue sections were conducted to test quality staining for Factor VIII and eNOS using a fluorescent microscope under dark condition

5. Image analysis

The basic steps involved in taking measurements are:

1. The photographic files of ovarian tissue were opened using Image-Pro Plus, version 4.5.1 software (Figure 6.1a and b).
2. The images were magnified in the software program (Figure 1c).
3. The area tool of software program was used to create a polygon feature on the current image and the specific area was selected for automatic analysis (Figure 6.1d).
4. Within the unit area, the positive staining for Factor VIII was analyzed by software program (red areas; Figure 6.1e).
5. When we take a measurement, the measurement is highlighted and numbered in the image, and the result is placed in the measurements data sheet (Figure 6.1f).
6. The measurements data sheet included the total area (tissue area and positive staining area), % area (tissue and positive staining areas), the perimeter of positive staining areas (range), the number of samples, and the standard deviation (Figure 6.1f).
7. After finishing, we can export the analyzed data capillary area into MS Office Excel to calculate data (capillary area density, capillary area density, and area per capillary) in the next step as follow.
 - 7.1 Capillary area density (CAD, %) is the capillary area per tissue area.
 - 7.2 Capillary number density (CND, number/ μm^2) is the capillary number per tissue area.
 - 7.3 Area per capillary (APC, $\mu\text{m}^2/\text{number}$) is the capillary area per capillary number.

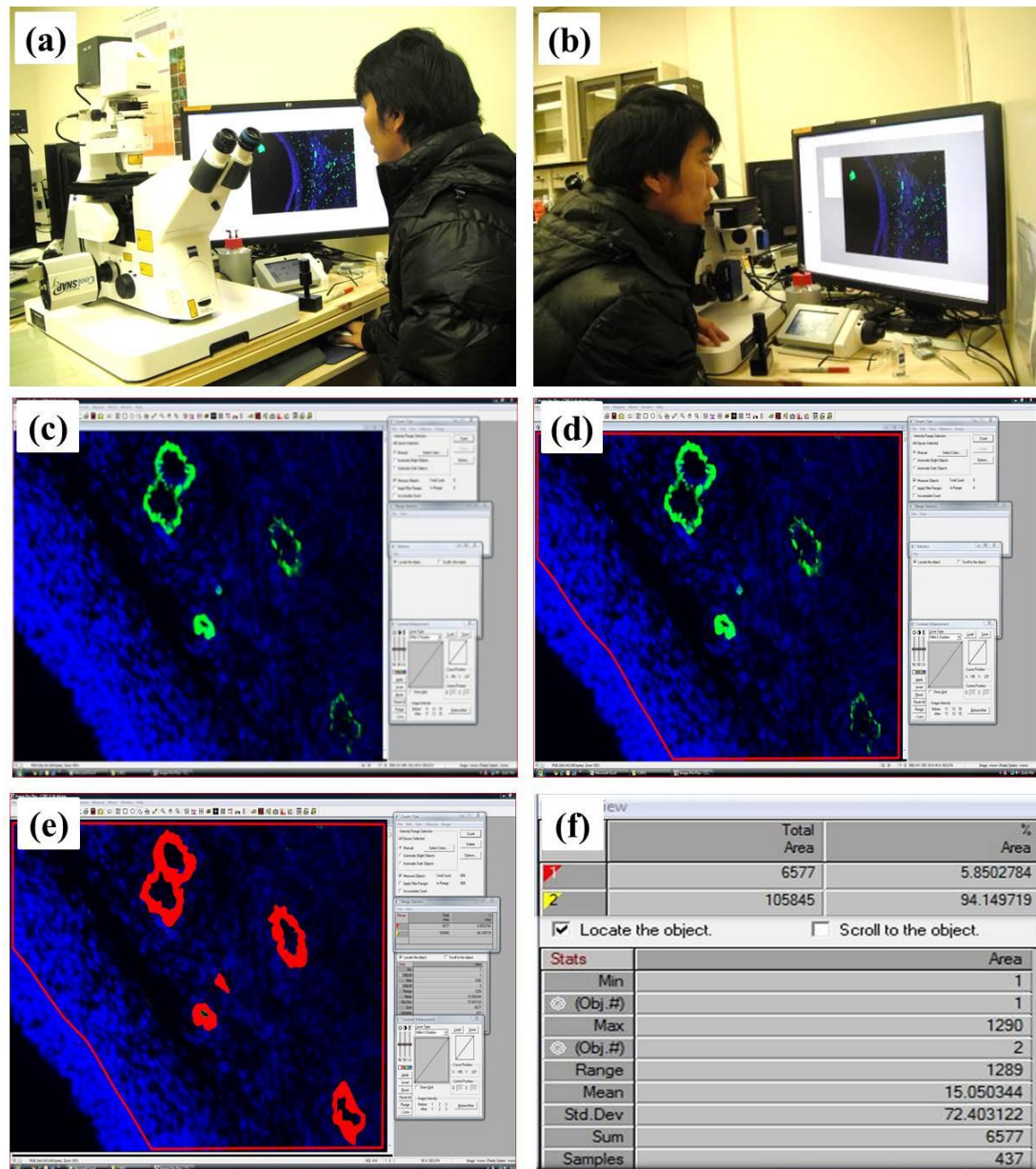


Figure 6.3 The basic steps involved in the image analysis of ovarian tissue.

5. Laparotomy preparation

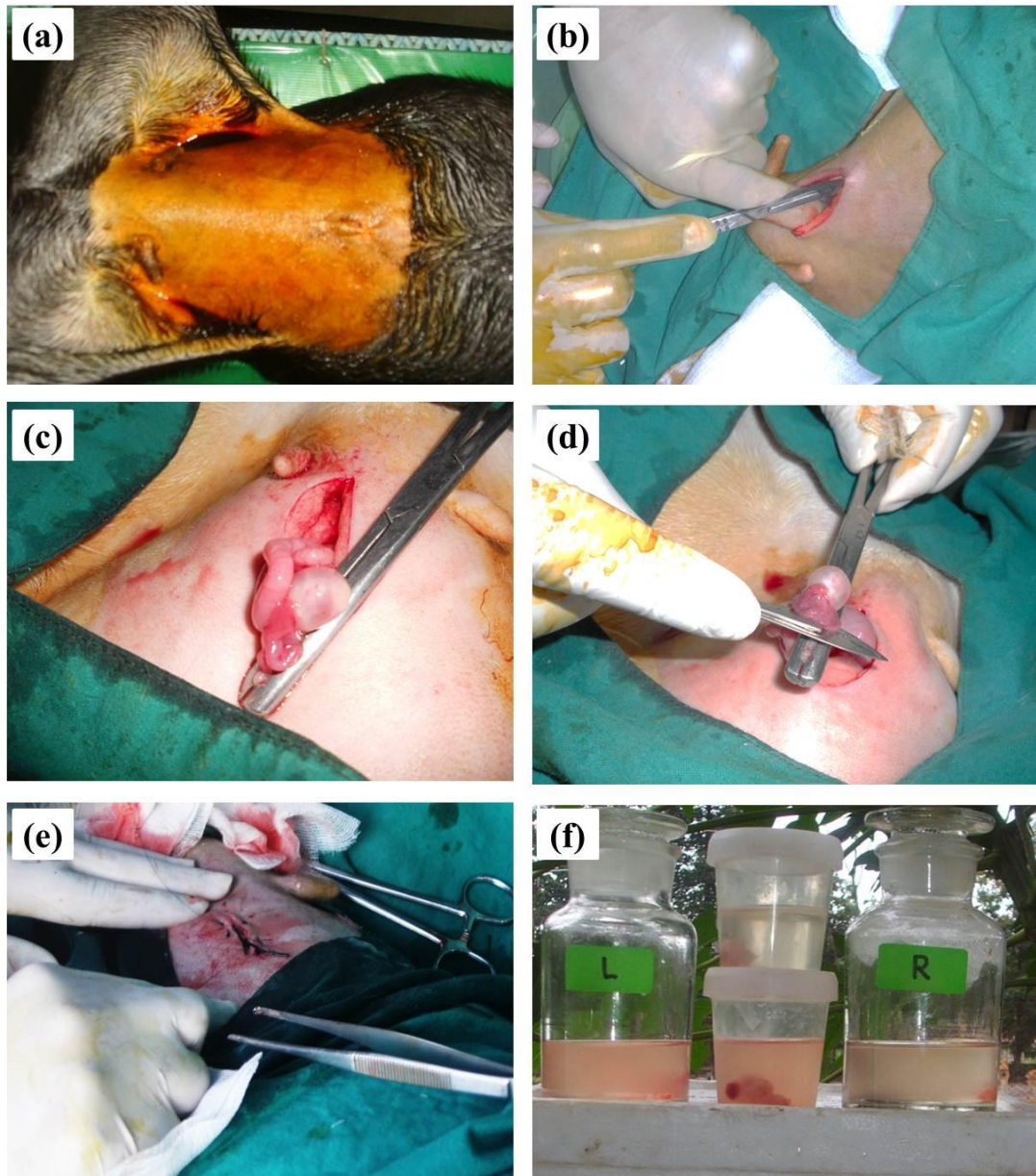


Figure 6.4 Laparotomy preparations in goat; (a), the arrangement for goat on the cradle; (b), the slitting cuts to abdomen; (c), the ovary of goat; (d), ovariectomy; (e), peritoneal cavity was closed with absorbable sutures using catgut and skin was closed with non-absorbable suture using braided silk; (f), ovaries were removed and placed in PBS for transport to the laboratory

6. Oocyte collection and grading

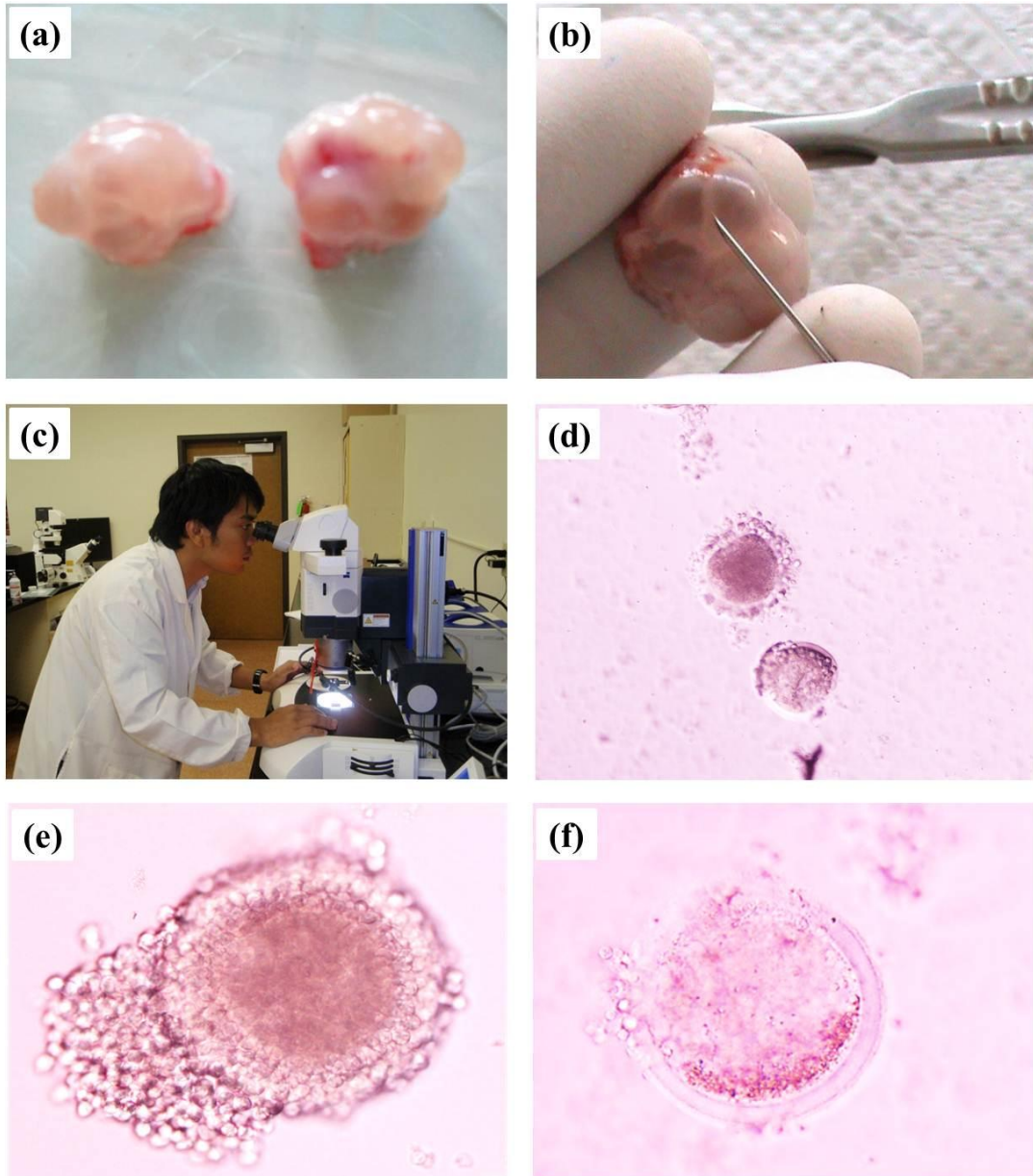


Figure 6.5 Oocyte collection and grading in goat; (a), the ovaries; (b), oocyte aspiration; (c and d), oocyte grading; (e), healthy oocyte; (f), nonhealthy oocyte



KHON KAEN UNIVERSITY

This is certify that

The Project Entitled : Association among follicular growth, cell proliferation, vascularity and IGFBP-4 expression on day 6 of the bovine follicular wave

Researcher : Mr. Tossapol Moonmanee/Assoc.Prof.Chainarong navanukraw

Name of Department : Department of Animal Science, Faculty of Agriculture, Khon Kaen University

has been reviewed and approved by the Animal Ethics Committee of Khon Kaen University, based on the Ethic of Animal Experimentation of National Research Council of Thailand.

Date of Approval : 19 July 2012

Associate Professor Paiboon Sithithaworn
Chairman,
Animal Ethics Committee of Khon Kaen University

Professor Suthipun Jitpimolmard
Vice President,
Research and Technology Transfer Affairs
Khon Kaen University

Record No. AEKKU 54/2555

Reference No. 0514.1.12.2/55



KHON KAEN UNIVERSITY

This is certify that

The Project Entitled : A study on model of follicular growth and atresia induction in Thai-native goat

Researcher : Mr. Tossapol Moonmanee/Assoc.Prof.Chainarong navanukraw

Name of Department : Department of Animal Science, Faculty of Agriculture, Khon Kaen University

has been reviewed and approved by the Animal Ethics Committee of Khon Kaen University, based on the Ethic of Animal Experimentation of National Research Council of Thailand.

Date of Approval : 19 July 2012

Associate Professor Paiboon Sithithaworn
Chairman,
Animal Ethics Committee of Khon Kaen University

Professor Suthipun Jitpimolmard
Vice President,
Research and Technology Transfer Affairs
Khon Kaen University

Record No. AEKKU 53/255

Reference No. 0514.1.12.2/54

Research and Technology transfer Affairs, Khon Kaen University, THAILAND

Tel.-66-43-202011 Fax-66-43-202015

RESEARCH PUBLICATIONS

1. **Moonmanee, T.**, C. Navanukraw, S. Uriyapongson, A. Kraison, S. Aiumlamai, S. Guntaprom, T. Rittirod, P. P. Borowicz, and D. A. Redmer. 2013. Relationships among vasculature, mitotic activity, and endothelial nitric oxide synthase (eNOS) in bovine antral follicles of the first follicular wave. *Domestic Animal Endocrinology* (Article in Press).
2. **Moonmanee, T.**, C. Navanukraw, S. Aiumlamai, K. Jarukamjorn, J. Thammasiri, and D. A. Redmer. 2012. Quantitative vascularity of antral follicle in *Bos indicus* using Factor VIII immunolocalization. *Livestock Science*. 150: 128-134.
3. **Moonmanee, T.**, C. Navanukraw, J. Thammasiri, S. Aiumlami, and C. Kamollirt. 2012. Evaluation of ovarian follicular health with the markers of endothelial and granulosa cells. *Chiang Mai University Journal of Natural Sciences*. 11: 461-467.
4. **Moonmanee, T.**, C. Navanukraw, J. Thammasiri, S. Aiumlmai, and C. Kamollirt. 2011. Quantitative vascularity analysis of the bovine preovulatory follicle using Factor VIII immunolocalization. In *International Conference on Global Issues Influencing Human and Animal Health for ASEAN One Health Concept*, Khon Kaen, Thailand. 256 p. (abstract).
5. **Moonmanee, T.**, C. Navanukraw, and S. Guntaprom. 2010. Associations of the bovine follicular health, mitotic activity and intrafollicular amounts of IGFBP-4 on d 5 or 6 of the first follicular wave. In *the 8th Ruminant Reproduction*, Anchorage, Alaska, USA. 554 p. (abstract).
6. **ทศพล มุลมณี**, ไชยณรงค์ นาวานุกเราะห์, สุวิทย์ อุปสัย, ศรุติวงศ์ บุญคง, จิรัฐติ ธรรมศิริ, วิไลวรรณ ชันธุแสง และ อารีย์ ไกรสุรย์. 2555. การทดสอบแบบจำลองการเหนี่ยวนำการเจริญเติบโตและการเสื่อมสลายของฟอลลิเคิลในแพะ. การประชุมวิชาการสุขภาพสัตว์ภาคเหนือ ประจำปี 2555 วันที่ 13-14 ธันวาคม พ.ศ. 2555 คณะสัตวแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่. 56-57.