APPENDICES

APPENDICES A

ESTRUS SYNCHRONIZATION AND TRANSCERVICAL AI

The procedure of first and re-use CIDR for estrus synchronization in Thai-native goat

Figure 1 Controlled internal drug release (CIDR) device

Figure 2 Thai-native goats

Figure 3 Preparation of CIDR device for estrus synchronization

Figure 2 CIDR device insertion

Figure 5 CIDR device removal on day 14

Figure 6 Cleaning CIDR device with warm water and solution disinfectant

Figure 7 CIDR device after first use

Figure 8 Re-use CIDR device

Transcervical artificial insemination (TCAI) in Thai-native goat

Figure 9 Estrus synchronization

Figure 10 Preparation of goat semen for artificial insemination

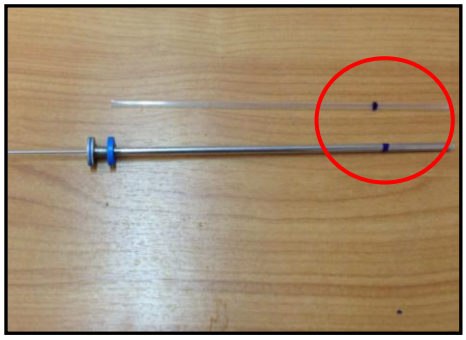

Figure 11 Marking AI catheter and AI sheath (5 cm) position for deposited of the semen into the cervix from external cervical os

Figure 12 Artificial insemination at 54 h after hormone removal

APPENDICES B

PROGESTERONE ANALYSIS

Progesterone ELISA assay

1. Plate down 100 µL secondary goat anti mouse IgG with coating buffer at 4 °C overnight.

2. Wash plate using microplate washer 4 times with wash buffer to remove excess secondary goat anti mouse off plate.

3. Add 100 µL primary antibody (monoclonal progesterone) to each well except NSB wells. Cover with plate sealer and shake at room temperature for 1.5 hours minimum.

4. Prepare samples and progesterone (P4) standard curve diluted in assay buffer. Extracted samples; add desired amount of assay buffer to extracted samples. Cap and vortex for 15 seconds, incubate at 37 °C for 30 minutes and vortex again.

5. Transfer 130 µl of each sample P4 and standard curve dilution into a disposable 96 well plate.

6. Wash plate (that has antibody on it) 4 times to remove excess primary antibody.

7. Transfer 100 µl samples and standard curve from disposable plate to plate with antibody. Incubate at room temperature for 1.5 hours.

8. Add 50 µl P3-P4-HRP conjugate to each well on top samples and stand curve.

9. Incubate at room temperature for 1.5 hours.

10. Wash plate 4 times.

11. Add 125 µl of substrate solution to each well. Covers with plate sealer incubate on shaker at 37 °C for 15 minutes. Plate will turn blue.

12. Add 50 µl stop solution (0.5 M H_2SO_4). Solution will turn yellow.

13. Read plate on mode 4 (OD = 450 & 600 nm) of plate reader.

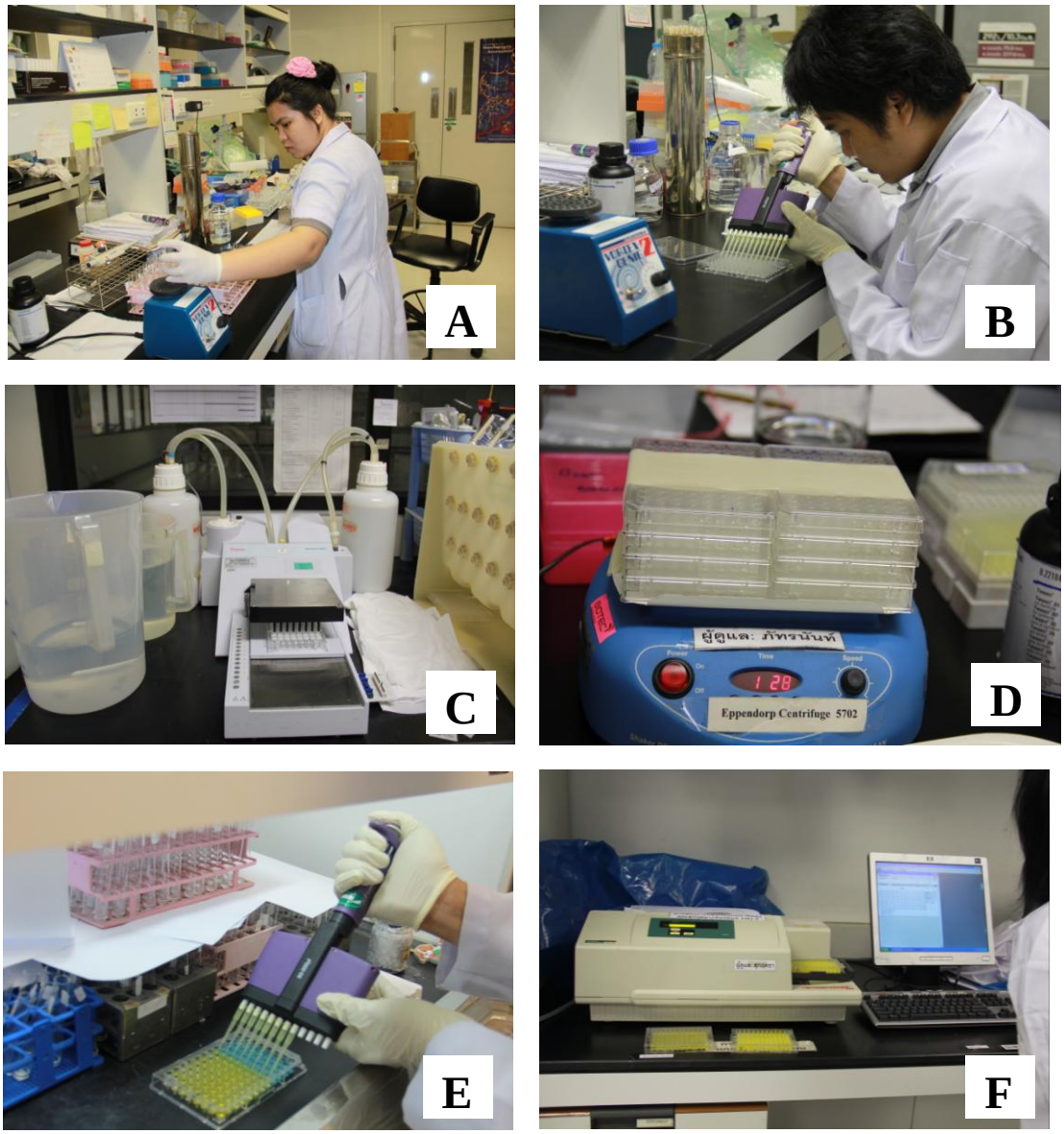

Figure 13 Progesterone ELISA Assay

A Extraction samples with Petroleum ether

B Coating goat anti-mouse IgG in 96 wells plate

C Microplate washer for washing 96 wells plate with PBST

D Incubation and shaking unknown sample and standard concentration

E 0.5 M H_2SO_4 (stop solution)

F Analyse data with software and spectrophotometry

	1	2	3	4	5	6	7	8	9	10	11	12
A	Bo	10.00	5.00	2.50	1.25	0.63	0.31	0.16	0.78			NSB
B	Bo	10.00	5.00	2.50	1.25	0.63	0.31	0.16	0.78			NSB
C	1	2	3	4	5	6	7	8	9	10	11	12
D	1	2	3	4	5	6	7	8	9	10	11	12
E	13	14	15	16	17	18	19	20	21	22	23	24
F	13	14	15	16	17	18	19	20	21	22	23	24
G												
H												

Figure 14 Disposable 96 well plate example, number 1-24 indicate sample numbers, rows 10-11 are for the stand curve and NSB wells should contain assay buffer.

KHON KAEN UNIVERSITY

This is certify that

The Project Entitled : Efficiency of goat semen diluents using transcervical deep artificial insemination technique

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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 : 25 November 2010

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