Chapter 3

3.0: MATERIALS AND METHODS

3.1 GENERAL INTRODUCTION

This study was carried out to diagnose pregnancy in goats using images of ultrasound scanner at two farms. The does underwent AI or/and natural mating after natural oestrus or oestrus synchronization. The study was performed from May 2007 until December 2008.

3.2 LOCATION OF STUDY

The experiments were carried out at the Institute of Biological Sciences (ISB) Mini Farm (Livestock), University of Malaya (Figure 3.1) and Kambing Bakabaik Kepala Batas Farm (KBKB), Kepala Batas, Penang (Figure 3.2).

3.3 EXPERIMENTAL ANIMALS

Jermasia does from ISB Mini Farm (Livestock), University of Malaya and Boer crossbred female goats from KBKB Farm, Kepala Batas, Penang, aged 1 to 7 years old were used in this study. The pregnancy detection was conducted with the does using the ultrasound scanner in a standing position. Goats were fed with Napier grass and commercial pellets twice daily, with access to water *ad libitum*. A total of 425 scanning procedures were carried out on 243 goats. Oestrus synchronization was carried out on some females before AI or natural mating while the rest were naturally mated.

Figure 3.1: Institute of Biological Sciences (ISB) Mini Farm (Livestock), University of Malaya.

Figure 3.2: Kambing Bakabaik Kepala Batas Farm (KBKB), Kepala Batas, Penang.

3.4 MATERIALS

3.4.1 Equipment and Instruments

Pregnancy detection was conducted using real-time ultrasound scanner, ALOKA SSD500 (Figure 3.3) equipped with a convex 5.0 MHz transabdominal scanner (4.0 cm length) (Figure 3.4) and linear array 7.5 MHz transrectal scanner (shaft length: 35 cm, shaft diameter: 1.4 cm) (Figure 3.5). Desired images were printed by thermic printer Sony UP-860 (Figure 3.6) to obtain hard copies in high density Type II., paper Sony UPP-110HD.

3.4.2 Disposables/Miscellaneous

Contact fluid (carboxymethylcellulose gel) that reacts as a coupling agent for ultrasound transmission was used to the test site of the does in transabdominal approach. In transrectal, the transducer was fastened to a plastic rod lubricated with the gel. Electric shaver was needed to shave pregnancy detection site before ultrasound examination using transabdominal probe took place. Goat feed, probe cover, gloves and masks were also used in the experiments.

3.5 METHODS

3.5.1: General Overview

In this study, five experiments were carried out. Females that were synchronized for oestrus or naturally oestrus were restrained in a standing position before pregnancy detection. Ultrasound machine and probes were properly set up. Data such as tag number of goat and date were keyed in. Ultrasound scanning examinations were executed using two probes; transrectal and transabdominal probes. Transrectal probe was preferable in early stages whereas transabdominal probe gave effective result in later stages. Table 3.1 shows

Figure 3.3: Ultrasound monitor (ALOKA SSD500).

Figure 3.4: Transabdominal probe (5.0 MHz) with 4.0 cm length.

Figure 3.5: Transrectal probe (7.5 MHz) (shaft length: 35 cm, shaft diameter: 1.4 cm).

Figure 3.6: Thermal printer Sony UP-860.

stages of gestation classification and probable probe suitability for pregnancy detection.

Table 3.1: Classification stages of gestation and probable probe suitability for pregnancy detection

Stages of gestation	Days	Probe / transducer
Initial	1-21	Transrectal
Early	22-49	Transrectal
Middle	50-83	Transrectal & transabdominal
Late	84-119	Transabdominal
Final	120-147	Transabdominal

3.5.2: Transrectal Scanner Procedure

With transrectal approach, faeces were cleared from the rectum. The 7.5 MHz transducer was attached to the tip of a rigid extension rod. The tip of the transducer was lubricated with carboxymethylcellulose contact gel (Figure 3.7). The transducer was inserted gently until the urinary bladder was identifiable. Probe was moved gently forwards and backwards and rotated it 90 degrees clockwise and counter-clockwise (Figure 3.8). Images of embryonic vesicles, placentomes, heart, heartbeat and foetus were positive indicators of pregnancy in early stages.

3.5.3 Transabdominal Scanner Procedure

Transabdominal ultrasonography was performed at middle, late and final stages of gestation using a 5.0 MHz transducer. First, the test site area of 150 to 200 cm^2 was shaved. The contact fluid, carboxymethylcellulose gel was later applied to the test site and the transducer was placed at the right side of the goat, 5 cm in front of the rear leg and 2.5 cm above the teat. Then, surface probe was covered with gel (Figure 3.9) and probe was

Figure 3.7: The tip of the transducer was lubricated with carboxymethylcellulose contact gel.

Figure 3.8: Probe was moved gently forwards and backwards and rotated 90 degrees clockwise and counter-clockwise.

orientated perpendicularly towards the ventral abdominal wall (Figure 3.10). The observed images of placentomes, heart, skeletal structures, heartbeat, spinal cord, head, limbs and foetus which were positive indicators of pregnancy in later stages were printed. The images were analyzed and compared for the different stages of pregnancy. Detection of pregnancy on does differ with stages of gestation.

3.6 EXPERIMENTAL DESIGN

A total of 245 scanning procedures were carried out on naturally oestrus and mated does. In addition, 75 does were synchronized for oestrus and underwent natural mating or artificial insemination (AI) (Figure 3.11) to obtain pregnancy. A total of five experiments using ultrasound scanner with 7.5 MHz transrectal and/or 5.0 MHz transabdominal probes were carried out in this study (Figure 3.12). Experiments 1 and 2 were carried out to observe images of foetus and related images in pregnancy diagnosis using both probes specifically i.e., transrectal probe (Experiment 1) and transabdominal probe (Experiment 2). Specific image pregnancy indicators obtained in Experiments 1 and 2 were specifically identified and sequentially arranged throughout the goat pregnancy period. Experiments 3 and 4 were carried out to optimize the combination of structural images obtained from the two probes for pregnancy diagnosis in goat. Images showing specific structures were used as indicators to optimize the prediction for different stages of pregnancy. The introduction of bucks to females and date of mating were unknown to the researcher. The does that were naturally mated and/or AI were scanned using both probes. Percentage of correct diagnosis was evaluated based on the predetermined optimal combination of structural images.

Figure 3.9: Surface probe was covered with gel.

Figure 3.10: Probe was moved orientated perpendicularly towards the ventral abdominal wall.

Figure 3.11: Female underwent artificial insemination (AI).

3.6.1 Observation on Foetal Structures and Related Images in Reproductive System of Does using Transrectal Probe (Experiment 1)

A total of 9 naturally-mated and pregnant does at KBKB Farm were used in the experiment. The pregnant does were diagnosed using 7.5 MHz transrectal probe throughout the gestation period. The foetal structural images and other related images were recorded. The age of foetus involved in this experiment was determined retrospectively after obtaining date of kidding (approximately 147 days). Three does were designated for each specific period of pregnancy decided for this experiment. Images from the non-pregnant does were obtained as control. The foetal structural images and other related images obtained were sequentially arranged and correlated with the specific period of pregnancy. Parameters on early pregnancy such as sac, non-echogenic (NE) area, placentomes, uterine wall, amniotic fluid and foetus with heartbeat were observed and recorded during pregnancy detection. The objective of the experiment was to observe foetal structures and related images in

reproductive system using 7.5 MHz transrectal probe. Analysis obtained from the experiment was fact finding for Experiment 3.

3.6.2 Observation on Foetal Development and Related Images in Reproductive System of Does using Transabdominal Probe (Experiment 2)

In this experiment, a total of 13 naturally mated does at KBKB Farm were used. The objective of the experiment was to observe foetal structures and related images in reproductive system using 5.0 MHz transabdominal probe throughout the gestation period. Three does were designated for each specific period of pregnancy decided for this experiment. Gestational age at the time of scanning of does was calculated retrospectively by subtracting the number of days elapsing between scanning date and kidding date, that is from the average duration of gestation (approximately 147 days). Images from the non-pregnant does were obtained as control. The foetal structural images and other related images, such as presence of placentomes, foetus with discernible heartbeat, ribs, spinal cord, limbs, head and other skeletal structures were recorded. Images obtained during pregnancy detection were indicators found during later stages of pregnancy. From our preliminary survey, no indicators were expected during early stages of pregnancy with transabdominal probe. Images were analyzed, sequentially arranged and compiled to form as bases for Experiment 4.

3.6.3 Optimization of Pregnancy Diagnosis of Does with Transrectal Probe (Experiment 3)

A total of 95 naturally mated does at KBKB Farm and ISB Farm were used in this experiment. It was designed to optimize pregnancy diagnosis in does using ultrasound

scanner with 7.5 MHz transrectal probe. Chronologically arranged images during pregnancy obtained from Experiment 1 formed the bases as a reference for images acquired from Experiment 3. Briefly, structural and related images were categorized based on related stages of pregnancy as indicated in Experiment 1. Specific does of different pregnancy stages were selected and were diagnosed using 7.5 MHz transrectal probe. The foetal structural and other related images, such as presence of embryonic vesicles, placentomes, uterine wall, amniotic fluid and foetus with heartbeat were recorded. The age of foetus involved in this experiment was also confirmed retrospectively after obtaining date of kidding (calculated based on duration of pregnancy of 147 days). Various combinations of images for specific designated stages of pregnancy were analyzed and formed the percent predicted accuracy for different stages of pregnancy. The reliability for the combination of structures with highest predictive value was diagnosed and confirmed in Experiment 5.

3.6.4 Optimization of Pregnancy Diagnosis of Does with Transabdominal Probe (Experiment 4)

A total of 150 naturally mated does at KBKB Farm and ISB Farm were used in this study. The experimental design was followed as described in Experiment 3 (Section 3.6.3) using 5.0 MHz transabdominal probe throughout the gestation period. The foetal structural images and other related images such presence of placentomes, foetus with discernible heartbeat, ribs, spinal cord, head, limbs and other skeletal structures were recorded. The age of foetus involved in this experiment was determined retrospectively after obtaining date of kidding (approximately 147 days). The objective of this experiment was to optimize pregnancy diagnosis in does using ultrasound scanner with 5.0 MHz transabdominal probe. Chronologically arranged images during pregnancy obtained from Experiment 2 formed the

bases as a reference for images acquired from Experiment 4. Various combinations of images for specific designated stages of pregnancy were analyzed and formed the percent predicted accuracy for different stages of pregnancy. The reliability for the combination of structures with highest predictive value was designed and confirmed in Experiment 5.

3.6.5 Pregnancy Diagnosis in Does Undergo Natural Mating and AI with Transrectal and Transabdominal Probes (Experiment 5)

The objective of this experiment was to determine the efficacy of ultrasound scanning in detecting different stages of pregnancy in goats using the optimal combination of structural images obtained from Experiments 3 and 4 using transrectal and transabdominal probes. A total of 75 does, synchronized for oestrus with CIDR, Estrumate, PMSG and hCG were used in this experiment which included 53 does grouped with two fertile bucks for natural mating and 22 does in oestrus were inseminated twice by experienced operators. For the AI group, four fertile bucks were introduced to the flock after two weeks of AI to ensure maximum pregnancy. The experimental does were scanned using both probes at designated pregnancy period for various foetal and related structures as described in previous experiments. The dates of introduction of buck to the female and artificial insemination were unknown to the scanning operator. Images were recorded and gestational age at the time of scanning of these does was calculated retrospectively by subtracting the number of days elapsing between scanning and kidding from the average duration of gestation (approximately 147 days). Percentages of pregnancy were calculated for each stage of pregnancy and pregnancy due to AI or natural mating was differentiated and determined.

3.7 STATISTICAL ANALYSIS

Table 3.2: Independent and dependant variables

Independent variables	Dependent variables
Transrectal or/and transabdominal probes	Placentomes, sac and foetus
Days of pregnancy	Indicators/criteria of pregnancy
Stages of gestation	% pregnancy
AI or natural mating	% kidding
	% correct negative (specificity)
	% correct positive (sensitivity)
	% accuracy

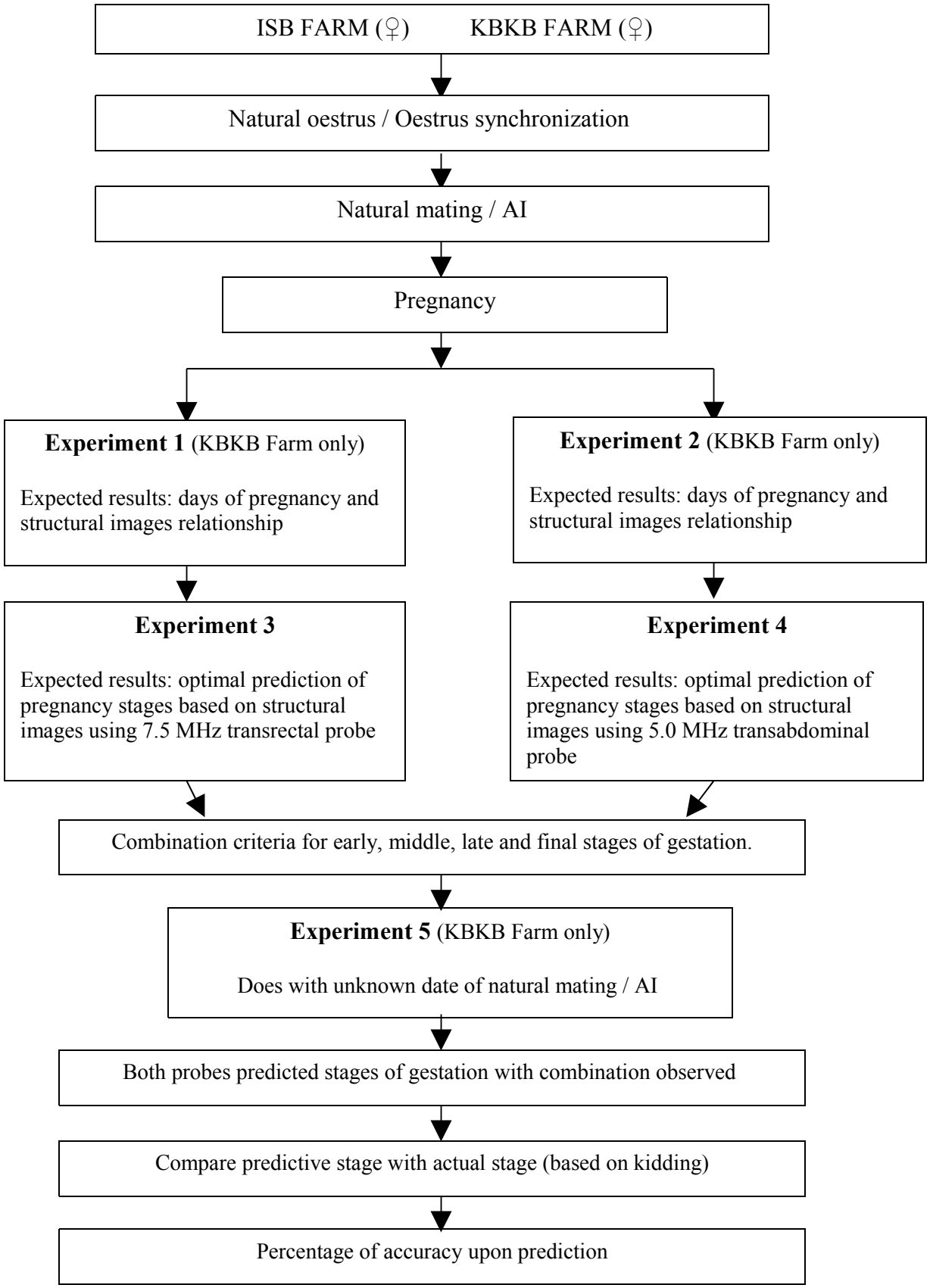

Figure 3.12a: A schematic overview of the experimental design for diagnosis of pregnant does using both probes.

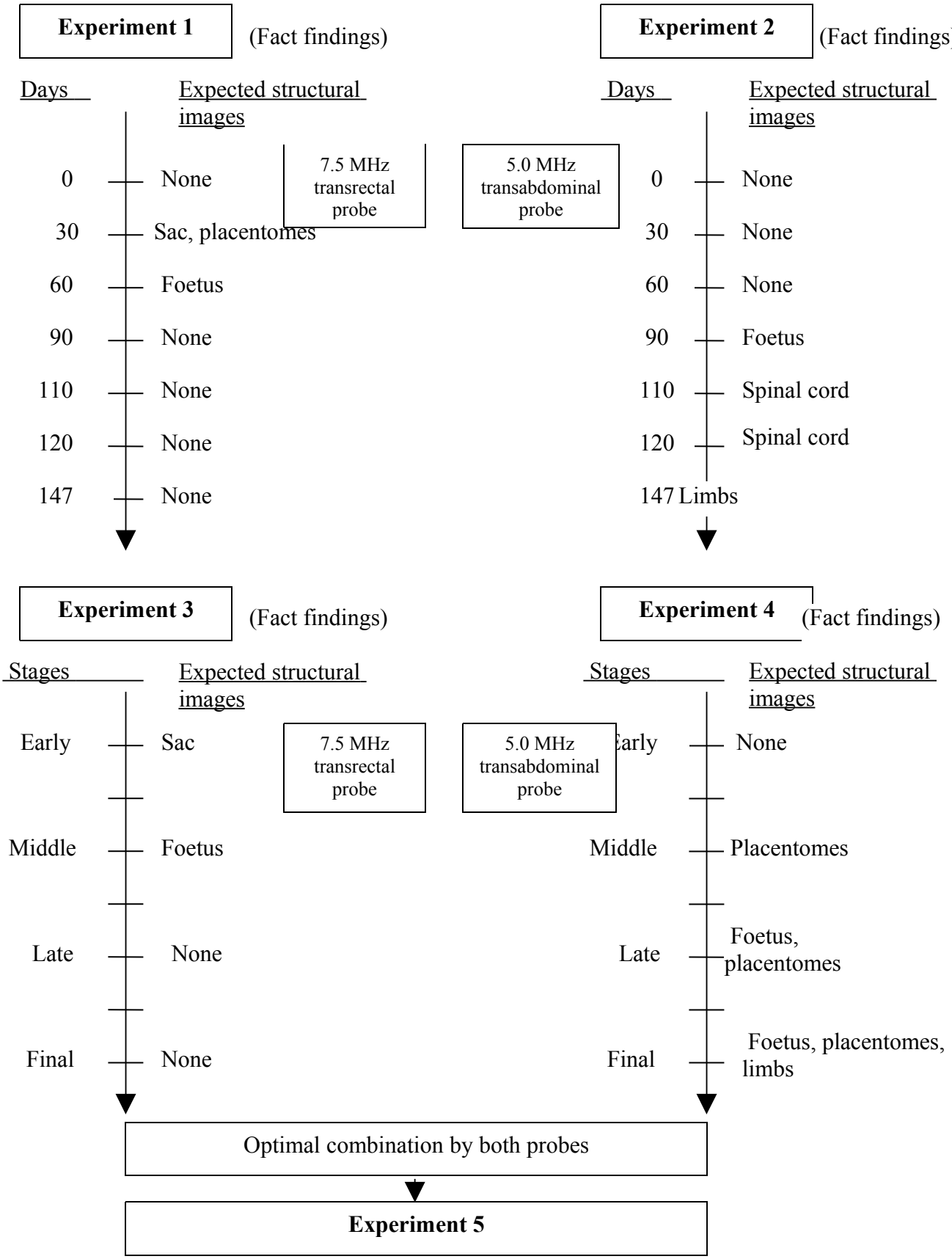

Figure 3.12b: A schematic overview of the experimental design for diagnosis of pregnant does using both probes.

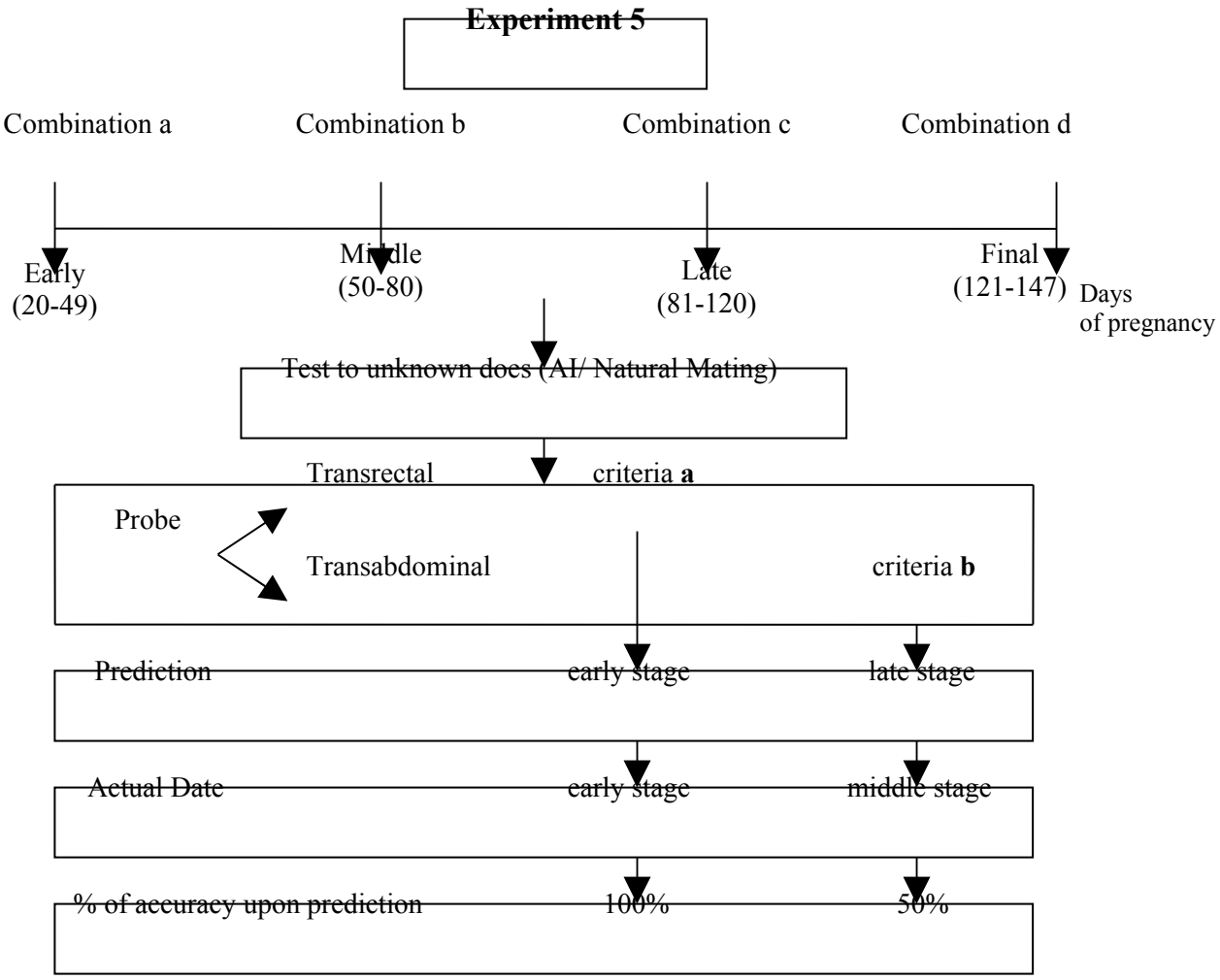

Figure 3.12c: A schematic overview of the experimental design for diagnosis of pregnant does using both probes

Figure 3.12: Summary of experimental design.