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ORIGINAL LITERARY WORK DECLARATION

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Field of Study : Reproductive Biotechnology

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ABSTRACT

Pregnancy diagnosis plays important role in modern goat management, especially when technologies such as artificial insemination and embryo transfer are employed in goat. Accurate pregnancy diagnosis may provide essential information for effective herd management in farm animals (Doize et al., 1997). This study was carried out with the following objectives: a) to determine accuracy of pregnancy prediction by using ultrasound scanner, b) to determine the criteria for single and twin pregnancies using ultrasound scanner, c) to predict the gestation age based on ultrasound scanning using constructed regression equation, d) to compare the efficacy of two different probes on pregnancy diagnosis and e) to determine the effect of breeds and unknown date of mating on pregnancy diagnosis, using ultrasound scanning. Pregnancy diagnosis was conducted using real-time B-mode ultrasound scanner with transrectal (7.5 MHz) and transabdominal (5.0 MHz) probes. Experiments 1 and 2 were conducted as preliminary studies on detection of pregnancy-related structures and their echogenicity. Experiments 3 and 4 were designed for echogenicity and size measurement of pregnancy-related structures, using both probes as well as to derive equations for gestational age estimation. Flock test was conducted in Experiment 5 to test the accuracy of gestational age estimation equations derived from Experiments 3 and 4. Embryonic vesicles with foetus and foetal heart were detected on week 4 and week 5 of gestation, respectively. Single and twin foetuses were differentiated between weeks 4 to 7 and weeks 5 to 10 of gestation, respectively. Both probes firstly detected placentome on week 7 of gestation and measurable up to the delivery day (week 21 of gestation). Foetal heart measurement was possible starting from week 8 of gestation, owing to the changes in echogenicity of the foetal heart. Transrectal probe visualised foetal heart up to week 10 of gestation, while transabdominal probe until week 21 of gestation.
Skeletal structures, such as foetal head, were first viewed on week 6 and week 7 of gestation using transrectal and transabdominal probes, respectively. From Experiment 5, polynomial relationship between foetal heart area and gestational age (single pregnancy: \( y = 0.033x^2 - 0.129x - 0.842; \) twin pregnancy: \( y = 0.088x^2 - 1.501x + 7.274; \) where \( y: \) heart area (cm\(^2\)), \( x: \) gestational age (week)) gave highest accuracy with 58% accuracy for 1 week delivery difference and maximum accuracy of 94%, of which does delivered within 3 weeks difference from estimated date. Equation for gestational age estimation from placentome diameter derived from current research data (\( y = -0.016x^2 + 0.605x - 1.759; \) where \( y: \) placentome diameter (cm), \( x: \) gestational age (week)) only gave 8% and 38% accuracy, respectively. In summary, transrectal probe is reliable to detect pregnancy and estimate gestational age from weeks 4 to 10 of gestation, while transabdominal probe from weeks 5 to 21 of gestation. Foetal heart is proved to be a reliable indicator for determination of foetal number, viability of foetus and gestational age of goats. It is hoped that, in the near future, the outcomes of ultrasound scanning studies on pregnancy diagnosis will complement efficiently with other farm management practices for goat industry.
ABSTRAK

Diagnosis kebuntingan memainkan peranan penting dalam pengurusan ternakan kambing terutamanya apabila teknologi seperti peramanian beradas dan pemindahan embrio diaplikasikan ke atas ternakan. Kajian ini dijalankan dengan objektif a) untuk menentukan ketepatan pengesan kebuntingan dengan menggunakan pengesan ultrabunyi b) untuk menentukan kriteria kandungan tunggal dan kembar menggunakan pengesan ultrabunyi c) untuk menentukan usia kandungan melalui persamaan regresi d) untuk membandingkan keberkesanan kedua-dua jenis prob ke atas diagnosis kebuntingan e) untuk menentukan kesan baka dan ketiadaan maklumat pembiakan kambing ke atas diagnosis kebuntingan dengan pengesan ultrabunyi. Diagnosis kebuntingan dijalankan dengan menggunakan pengesan bunyi real-time B-mode yang dilengkapi dengan prob transrektal (7.5 MHz) dan transabdominal (5.0 MHz). Eksperimen 1 dan 2 merupakan kajian awal untuk pengesan struktur berkait kebuntingan dan ekogenisitinya. Eksperimen 3 dan 4 dirangka untuk kajian ekogenisiti dan pengukuran saiz struktur berkait kebuntingan serta menerbitkan persamaan regresi. Ujian flok dijalankan dalam Eksperimen 5 untuk mengkaji ketepatan persamaan regresi dari Eksperimen 3 dan 4. Vesikel embrionik dengan fetus dan jantung fetus dikesan pada minggu 4 dan minggu 5 kebuntingan dengan prob transrektal dan transabdominal. Bilangan fetus boleh ditentukan antara minggu 4 hingga 7 dan minggu 5 hingga 10 kebuntingan, masing-masing. Kedua-dua prob pertama kali mengesan plasentom pada minggu 7 kebuntingan dan boleh diukur hingga hari kelahiran (minggu 21 kebuntingan). Pengukuran jantung fetus hanya boleh dijalankan mulai minggu 8 kebuntingan, dengan perubahan ekogenisiti jantung. Bagaimanapun, prob transrektal cuma boleh mengesan jantung sehingga minggu 10 kebuntingan, manakala prob transabdominal
hingga minggu 21 kebuntingan. Struktur bertulang, seperti kepala fetus, pertama kali dikestan pada hari minggu 6 dan minggu 7 kebuntingan menggunakan prob transrektal dan transabdominal, masing-masing. Dari Eksperimen 5, perhubungan polinomial antara jantung fetus dan usia kebuntingan (Kebuntingan tunggal: \( y = 0.033x^2 - 0.129x - 0.842 \); Kembar: \( y = 0.088x^2 - 1.501x + 7.274 \); di mana \( y \): saiz jantung (cm\(^2\)), \( x \): usia kebuntingan (minggu)) memberikan ketepatan tertinggi untuk perbezaan tarikh kelahiran seminggu iaitu 58% dan ketepatan maksimum 94% untuk kelahiran dalam tempoh masa 3 minggu dari tarikh jangkaan. Persamaan dari diameter placentum (\( y = -0.016x^2 + 0.605x - 1.759 \); di mana \( y \): diameter placentum (cm), \( x \): usia kebuntingan (minggu)) memberikan ketepatan 8% dan 38%, masing-masing. Kesimpulannya, prob transrektal boleh sesuai digunakan untuk mengesan dan menentukan usia kebuntingan dari minggu 4 hingga 10 kebuntingan, manakala prob transabdominal dari minggu 5 hingga 21 kebuntingan. Jantung fetus terbukti sebagai petunjuk yang berkesan untuk penentuan bilangan fetus, mati-hidup fetus dan usia kebuntingan kambing. Adalah diharapkan supaya pada masa depan, penemuan dari kajian diagnosis kebuntingan menggunakan pengesan ultrabunyi ini akan membantu dalam pengurusan ladang yang efektif bagi industri kambing.
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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>DECLARATION</td>
<td>i</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>ii</td>
</tr>
<tr>
<td>ABSTRAK</td>
<td>iv</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>vi</td>
</tr>
<tr>
<td>LIST OF PUBLICATIONS, PRESENTATIONS AND AWARDS</td>
<td>viii</td>
</tr>
<tr>
<td>TABLE OF CONTENTS</td>
<td>ix</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xvi</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xix</td>
</tr>
<tr>
<td>LIST OF APPENDICES</td>
<td>xxvi</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>xxvii</td>
</tr>
</tbody>
</table>

Chapter 1

1.0 INTRODUCTION  
1.1 INTRODUCTION  
1.2 BACKGROUND  
1.3 STATEMENT OF PROBLEMS  
1.4 JUSTIFICATION  
1.5 OBJECTIVES

Chapter 2

2.0 LITERATURE REVIEW  
2.1 TIMELINE OF SIGNIFICANT FINDINGS IN SMALL RUMINANTS ULTRASOUND SCANNING  

...
2.2 PREGNANCY DIAGNOSIS IN FARM ANIMALS 19

2.2.1 Pregnancy Diagnosis: Clinical Methods 21

2.2.2 Pregnancy Diagnosis: Immunologic Diagnosis 26

2.2.3 Pregnancy Diagnosis: Return of Oestrus 28

2.2.4 Overall Comparison of Efficiency between Currently Available Pregnancy Diagnosis 30

2.3 DIFFERENT TYPES OF ULTRASOUND SCANNERS 31

2.4 SUITABILITY OF SCANNING METHOD FOR DIFFERENT STRUCTURES ALONG PREGNANCY PERIOD 36

2.5 PREGNANCY-RELATED STRUCTURES 38

2.5.1 Embryonic Vesicles 38

2.5.2 Crown-rump Length 40

2.5.3 Foetal Heart 42

2.5.4 Placentome 44

2.6 TIMEFRAME FOR ULTRASOUND SCANNING PERFORMED FOR PREGNANCY DIAGNOSIS IN SMALL RUMINANT 50

2.7 EFFECT OF GOAT BREED ON PREGNANCY DIAGNOSIS ACCURACY 51
Chapter 3

3.0 MATERIALS AND METHODS 53

3.1 GENERAL INTRODUCTION 53

3.2 LOCATION OF STUDY 53

3.3 EXPERIMENTAL ANIMALS 53

3.4 MATERIALS 56

3.4.1 Equipments and Instruments 56

3.4.2 Disposable/Miscellaneous 56

3.5 METHODS 58

3.5.1 General Overview 58

3.5.2 Transrectal Scanning Procedure 58

3.5.3 Transabdominal Scanning Procedure 58

3.6 EXPERIMENTAL DESIGN 59

3.6.1 Observation of Foetal Structures and Related Images in Reproductive System of Does Using Transrectal and Transabdominal probes (Experiment 1) 61

3.6.2 Gestational Age Estimation based on Analysis of Pregnancy-related Structures in Different Breeds of Goats based on Characteristics Derived from Experiment 1 (Experiment 2) 61

3.6.3 Optimisation of Pregnancy Diagnosis of Does with Transrectal Probe (Expriment 3) 63

3.6.4 Optimisation of Pregnancy Diagnosis of Does with Transabdominal Probe (Experiment 4) 63
3.6.5 Prediction of Gestational Age based on Equations on Placentome Diameter and Heart Size Derived from Experiments 3 and 4 Using Transrectal and Transabdominal Probe

Chapter 4

4.0 RESULTS

4.1 OBSERVATION ON FOETAL STRUCTURES AND RELATED IMAGES IN REPRODUCTIVE SYSTEM OF DOES USING TRANSRECTAL AND TRANSABDOMINAL PROBES (EXPERIMENT 1)

4.2 GESTATIONAL AGE ESTIMATION BASED ON ANALYSIS OF PREGNANCY-RELATED STRUCTURES IN DIFFERENT BREEDS OF GOATS BASED ON CHARACTERISTICS DERIVED FROM EXPERIMENT 1 (EXPERIMENT 2)

4.3 OPTIMISATION OF PREGNANCY DIAGNOSIS OF DOES WITH TRANSRECTAL PROBE (EXPERIMENT 3)

4.4 OPTIMISATION OF PREGNANCY DIAGNOSIS OF DOES WITH TRANSABDOMINAL PROBE (EXPERIMENT 4)
4.5 PREDICTION OF GESTATIONAL AGE BASED ON EQUATIONS ON PLACENTOME DIAMETER AND HEART SIZE DERIVED FROM EXPERIMENTS 3 AND 4 USING TRANSRECTAL AND TRANSABDOMINAL PROBES (EXPERIMENT 5)

4.5.1 Correlation between foetal heart development and gestational age 141

4.5.2 Flock test for gestational age estimation from placentome diameter 148

4.5.3 Flock test for gestational age estimation from foetal heartbeat rate 150

4.5.4 Flock test for gestational age estimation from foetal heart area 151

4.5.5 Summary of accuracy of gestational age estimation using different pregnancy-related structures 156

Chapter 5

5.0 DISCUSSION 160

5.1 INTRODUCTION 160
5.2 OBSERVATION ON FOETAL STRUCTURES AND RELATED IMAGES IN REPRODUCTIVE SYSTEM OF DOES USING TRANSRECTAL AND TRANSABDOMINAL PROBES

5.3 GESTATIONAL AGE ESTIMATION BASED ON ANALYSIS OF PREGNANCY-RELATED STRUCTURES IN DIFFERENT BREEDS OF GOATS BASED ON CHARACTERISTICS DERIVED FROM EXPERIMENT 1 (EXPERIMENT 2)

5.4 OPTIMISATION OF PREGNANCY DIAGNOSIS OF DOES WITH TRANSRECTAL PROBE (EXPERIMENT 3)

5.5 OPTIMISATION OF PREGNANCY DIAGNOSIS OF DOES WITH TRANSABDOMINAL PROBE (EXPERIMENT 4)

5.6 PREDICTION OF GESTATIONAL AGE BASED ON EQUATIONS ON HEART SIZE AND PLACENTOME DIAMETER DERIVED FROM EXPERIMENT 3 AND 4 USING TRANSRECTAL AND TRANSABDOMINAL PROBE (EXPERIMENT 5)

5.7 GENERAL DISCUSSION
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 2.1</td>
<td>Timeline of significant findings by various authors of ultrasound scanning in small ruminant reproduction</td>
<td>10</td>
</tr>
<tr>
<td>Table 2.2</td>
<td>Interpretation of the “On-farm milk progesterone” test for pregnancy diagnosis in cattle (Adapted from: Jainudeen and Hafez, 2000)</td>
<td>27</td>
</tr>
<tr>
<td>Table 2.3</td>
<td>Oestrous cycle, oestrous, and ovulation in farm animals (Adapted from: Jainudeen and Hafez, 2000)</td>
<td>28</td>
</tr>
<tr>
<td>Table 2.4</td>
<td>Overall comparison between ultrasonography, hormonal assay and return to oestrus responds for pregnancy diagnosis (Modified from: Jainudeen and Hafez, 2000)</td>
<td>30</td>
</tr>
<tr>
<td>Table 2.5</td>
<td>The presence or absence and measurability of foetus and foetal-related structures during different stages of pregnancy</td>
<td>36</td>
</tr>
<tr>
<td>Table 2.6</td>
<td>Timeframe for ultrasonography as previously conducted in small ruminants</td>
<td>50</td>
</tr>
<tr>
<td>Table 2.7</td>
<td>Comparison of diagnosis on selected pregnancy-related structures in different breed of pregnant does</td>
<td>51</td>
</tr>
<tr>
<td>Table 4.1</td>
<td>Characteristic of ultrasonographic images of pregnancy diagnosis in pregnant does via both transrectal and transabdominal probes based on gestation age classification</td>
<td>71</td>
</tr>
<tr>
<td>Table 4.2</td>
<td>Comparison of detectability for transrectal and transabdominal probes throughout gestation</td>
<td>83</td>
</tr>
<tr>
<td>Table 4.3</td>
<td>Range of pregnancy-related structures detection throughout pregnancy using both transrectal and transabdominal probes</td>
<td>86</td>
</tr>
<tr>
<td>Table 4.4</td>
<td>Analysis of images obtained from confirmed pregnant does</td>
<td>88</td>
</tr>
<tr>
<td>Table 4.5</td>
<td>Summary of pregnancy detection and gestational age accuracy estimation based on criteria derived from Experiment 1</td>
<td>97</td>
</tr>
<tr>
<td>Table 4.6</td>
<td>Details of pregnancy-related structures detected throughout pregnancy using transrectal probe</td>
<td>100</td>
</tr>
<tr>
<td>Table 4.7</td>
<td>Measurability of pregnancy-related structures throughout pregnancy via transrectal procedure</td>
<td>105</td>
</tr>
</tbody>
</table>
Table 4.8 Details of pregnancy-related structures detected throughout pregnancy using transabdominal probe

Table 4.9 Measurability of pregnancy-related structures throughout pregnancy via transabdominal procedure

Table 4.10 Changes in foetal heart echogenicity and area throughout both single and twin pregnancy

Table 4.11 Accuracy of gestational age estimation based on Karen et al. (2009) equation using placentome diameter and gestational age relationship

Table 4.12 Accuracy of gestational age estimation based on Suguna et al. (2008) equation using placentome diameter and gestational age relationship

Table 4.13 Accuracy of gestational age estimation based on current research equation (polynomial regression) using placentome diameter and gestational age relationship

Table 4.14 Accuracy of gestational age estimation based on current research equation (linear regression) using placentome diameter and gestational age relationship

Table 4.15 Accuracy of gestational age estimation based on Karen et al. (2009) equation using foetal heartbeat rate and gestational age relationship

Table 4.16 Accuracy of gestational age estimation based on current research equation (polynomial regression) using heart area and gestational age relationship from Jermasia does

Table 4.17 Accuracy of gestational age estimation based on current research equation (linear regression) using heart area and gestational age relationship from Jermasia does

Table 4.18 Accuracy of gestational age estimation based on current research equation (polynomial regression) using heart area and gestational age relationship from Jermasia and Boer-crossbred does

Table 4.19 Accuracy of gestational age estimation based on current research equation (linear regression) using heart area and gestational age relationship from Jermasia and Boer-crossbred does

Table 4.20 Accuracy of gestational age estimation based on current research equation (polynomial) using heart area and gestational age relationship from Jermasia does, when conducted during different gestational age
Table 4.21  Accuracy of gestational age estimation based on current research equation (linear) using heart area and gestational age relationship from Jermasia does, when conducted during different gestational age

Table 4.22  Accuracy of gestational age estimation based on current research equation (polynomial) using heart area and gestational age relationship from Jermasia and Boer-crossbred does, when conducted during different gestational age

Table 4.23  Accuracy of gestational age estimation based on current research equation (linear) using heart area and gestational age relationship from Jermasia and Boer-crossbred does, when conducted during different gestational age

Table 4.24  Summary of accuracy for gestational age estimation using equations from previous researchers and current findings

Table 5.1  Details of equations used for gestational age relationship estimation from placentome diameter
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 2.1</td>
<td>Pregnancy diagnosis techniques in farm animal (Adapted from Jainudeen and Hafez, 2000).</td>
<td>20</td>
</tr>
<tr>
<td>Figure 2.2</td>
<td>Rectal palpation is being performed (Adapted from <a href="http://cal.vet.upenn.edu">http://cal.vet.upenn.edu</a>).</td>
<td>21</td>
</tr>
<tr>
<td>Figure 2.3</td>
<td>Transrectal probe and rectangular image generated. a) Transrectal probe (Adapted from <a href="http://www.aloka-europe.com">www.aloka-europe.com</a>) and b) rectangular image generated from transrectal ultrasound scanning (Adapted from Ali and Hayder, 2007).</td>
<td>23</td>
</tr>
<tr>
<td>Figure 2.4</td>
<td>Transabdominal probe and sector image generated. a) Transabdominal probe (Adapted from: <a href="http://www.aloka-europe.com">www.aloka-europe.com</a>) and b) sector image generated from transabdominal ultrasound scanning (Adapted from Medan and Abd El-Aty, 2010).</td>
<td>23</td>
</tr>
<tr>
<td>Figure 2.5</td>
<td>Images of single and twin foetuses using transrectal ultrasonography. a) Single foetus (arrow) detected as a distinct echodense structure on day-35 of gestation. b) Twin foetuses (arrows) are apparent as echodense structures within the uterine lumen of a 28-day pregnant doe (Adapted from Suguna et al., 2008).</td>
<td>25</td>
</tr>
<tr>
<td>Figure 2.6</td>
<td>Embryonic vesicle (arrow) in the uterine lumen of a doe 21 days pregnant (Adapted from Suguna et al., 2008).</td>
<td>39</td>
</tr>
<tr>
<td>Figure 2.7</td>
<td>Foetus (arrow) at the age of 32 days. Black area enclosed the foetus is the amniotic fluid (Adapted from Ali and Hayder, 2007).</td>
<td>40</td>
</tr>
<tr>
<td>Figure 2.8</td>
<td>Relationship between CRL and gestational age in Egyptian goat (Adapted from Karen et al., 2009).</td>
<td>41</td>
</tr>
<tr>
<td>Figure 2.9</td>
<td>Heart (arrow) and chest as observed on day-83 of pregnancy (Adapted from Ali and Hayder, 2007).</td>
<td>42</td>
</tr>
<tr>
<td>Figure 2.10</td>
<td>Relationship between foetal heartbeat rate (FHB) with gestational age (Adapted from Karen et al., 2009).</td>
<td>43</td>
</tr>
<tr>
<td>Figure 2.11</td>
<td>Relationship between foetal heartbeat rate (FHB) with gestational age (Adapted from Suguna et al., 2008).</td>
<td>44</td>
</tr>
</tbody>
</table>
Figure 2.12 Cross-section of caruncles, placentomes and chorioallantois in sheep. a) caruncles in an incised non-pregnant sheep uterus. b) cross-section through placentomes from a mid-gestation sheep pregnancy. c) The image to the right shows an incised uterus from a pregnant sheep, roughly day 50 of gestation. The numerous button-shaped structures are placentomes, and the surfaces in view are actually cotyledons - the foetal side of the placentome. The slightly milky-looking membrane covering and between placentomes is the chorioallantois. The foetus is clearly visible inside the amnion (Adapted from Bowen, 2000).

Figure 2.13 Comparison of the exact image of C-shaped placentome (Adapted from: Bowen, 2000) a) and b) placentome as observed on day 84 of gestation ultrasonically (Adapted from Ali and Hayder, 2007).

Figure 2.14 Collapsed placentome as observed on day 102 of gestation (Adapted from Ali and Hayder, 2007).

Figure 2.15 Relationship between placentome diameter and gestational age (Adapted from Suguna et al., 2008).

Figure 2.16 Relationship between placentome diameter and gestational age (Adapted from Karen et al., 2009).

Figure 3.1 Jermasia breed does.

Figure 3.2 Boer-crossbreed does.

Figure 3.3 Katjang breed does.

Figure 3.4 Dentition to determine age of goat (Adapted from http://members.psyber.com/macgoats.htm).

Figure 3.5 Ultrasound machine ALOKA SSD500. This ultrasound machine comprises of three major parts; monitor, probes and printer. ALOKA SSD500 can be used for B-mode and M-mode ultrasound diagnosis (Adapted from http://www.medwrench.com).

Figure 3.6 Artificial insemination (AI) is being performed on oestrus synchronized does.

Figure 3.7 Close up: AI is being performed on oestrus synchronized does.

Figure 3.8 A schematic overview of the experimental design for diagnosis of pregnant does using transrectal and transabdominal probes (Experiment 1) and flock test (Experiment 2).
Figure 3.9  A schematic overview of the experimental design for optimisation of pregnancy diagnosis in does using transrectal (Experiment 3) and transabdominal (Experiment 4) probes.

Figure 3.10  A schematic overview of gestational age estimation based on analysis of pregnancy-related structures in does (Experiment 5).

Figure 4.1  A) Original image of an open doe via transrectal ultrasonography. No pregnancy related structure detected. Ovary with follicle could be detected easily. B) Labeled image of ovary (arrow: →) and follicles (arrows: ←) as detected in non-pregnant does.

Figure 4.2  A) Original image of pregnant doe as visualized by via transrectal ultrasonography (presumed day 21 of pregnancy). Embryonic sacs were detected at 3 to 5 o’clock direction to the urinary bladder. B) Labeled image of detected embryonic sacs (arrows).

Figure 4.3  A) Original image of pregnant doe as visualized by via transrectal ultrasonography (presumed day 30 of pregnancy). B) Labeled image of detected foetus (arrow: →), foetal heart (arrow: ←) and umbilical cord (arrow: ↓).

Figure 4.4  A) Original image twin foetuses as visualised by via transrectal ultrasonography (presumed day 30 of pregnancy). B) Labeled image of detected foetuses (arrows).

Figure 4.5  A) Image of an open doe via transabdominal probe. No pregnancy-related structure detected. B) Labeled image.

Figure 4.6  A) Original image of foetus as detected using transabdominal probe (presumed day 29 of gestation). B) Labeled image of foetus detected (arrow).

Figure 4.7  A) Original image of foetus as detected using transabdominal transducer (presumed day 44 of gestation). Placentomes were detected around the embryonic fluid enclosing foetus. B) Labeled image of foetus (arrow: →) and placentomes (arrow: ↑).

Figure 4.8  A) Original image of foetus as detected using transabdominal probe (presumed day 75 of gestation). Placentomes were detected around the embryonic fluid enclosing foetus and heart detected as grey coloured structure with fast rhythmic beating enclosed within thoracic area. B) Labeled image of foetus (arrow: →) and placentomes (arrow: ↑).
Figure 4.9  Image of foetus as detected using transabdominal probe (presumed day 119 of pregnancy). Heart detected (arrow) as grey-black coloured structure with slower rhythmic beating as compared to earlier stage of pregnancy. A) Original image, B) Labeled image.

Figure 4.10  A) Original image of placentomes as detected using transabdominal probe (presumed day 147 of pregnancy). B) Labeled image of C-shaped placentomes (arrows).

Figure 4.11  A) Foetal heart (arrow: ←) and V-shaped thoracic area (arrows: →) at gestational age of approximately 60 days. A) Original image. B) Labeled image. Gestational age was confirmed to be accurate from the actual mating date.

Figure 4.12  Foetal heart (arrow: →) V-shaped thoracic area (arrows: ←) at gestational age of approximately 120 days. A) Original image. B) Labeled image. Gestational age was confirmed to be accurate from the delivery date.

Figure 4.13  Placentome (arrow) at gestational age of approximately 60 days appear as echogenic structure. A) Original image. B) Labeled image. Gestational age was confirmed to be accurate from the actual mating date.

Figure 4.14  Placentome (arrow) at gestational age of approximately 120 days appears as less echogenic structure (grey colour instead of white). A) Original image. B) Labeled image. Gestational age was confirmed to be accurate from the delivery date.

Figure 4.15  Single foetus at day 23 (week 4) of pregnancy. A) Original image detected foetus (arrow). B) Labeled image of the detected foetus (arrow).

Figure 4.16  Twin foetuses as observed on day-23 (week 4) of pregnancy. A) Original image of the detected foetuses. B) Labeled image of the detected foetuses (arrows).

Figure 4.17  Foetus as observed on day-38 (week 6) of pregnancy. A) Original image. B) Labeled image: foetal head (arrow: ↓), umbilical cord (arrow: →), leg (arrow: ←) and heart (arrow: ↑) can be detected.

Figure 4.18  Foetus as observed on day 46 (week 7) of gestation. Foetal head, umbilical cord, leg and heart can be detected easily. Foetal head was well differentiated from foetal trunk at this age. A) Original image. B) Labeled image: head (arrow: →) and leg (arrow: ←).
Figure 4.19  Foetus as observed on day 53 (week 8) of gestation. Foetal heart shape, thoracic area and foetal trunk can be accessed. Foetal heart colour changed from white to grey, results in definable shape. A) Original image. B) Labeled image: heart (arrow: →) and ribs (arrow: ←).

Figure 4.20  Image recorded on day 65 (week 10) of gestation. Foetus and foetal head appeared to be too big visualised as a whole on screen. Only placentome can be visualised as a whole at this age. A) Original image. B) Labeled image: placentome (arrow).

Figure 4.21  Image recorded on day 72 (week 11) of gestation. Foetus and other pregnancy-related structures were difficult to be visualised on screen. Only placentome can be visualised as a whole at this age. A) Original image. B) Labeled image: placentome (arrow: →).

Figure 4.22  Image of pregnant doe at day 33 (week 5) of gestation. A) Original image. B) Labeled image: foetus (arrow: →), allantoic fluid (arrows: ↑).

Figure 4.23  Image of pregnant doe at day 36 (week 6) of gestation. Foetus and heart were detected. A) Original image. B) Labeled image: embryonic sac (arrow: →), foetus (arrow: ↑) and foetal heart (arrow: ↓).

Figure 4.24  Image of pregnant doe at day 36 (week 6) of gestation. C-shaped placentome was detected. A) Original image. B) Labeled image: placentome (arrow: →).

Figure 4.25  Image of pregnant doe at day 46 (week 7) of gestation. Foetal head, body (arrow: →) and placentome (arrow: ↓) were detected. A) Original image. B) Labeled image.

Figure 4.26  Image recorded at day 53 (week 8) of gestation. Foetal head (arrow: →) increased in size and foetal neck (arrow↓) can be detected easily. Foetal heart and thoracic area can be detected as well. A) Original image. B) Labeled image.

Figure 4.27  Image recorded at day 53 (week 8) of gestation. Placentome (arrow) can be detected and measured easily. A) Original image. B) Labeled image.

Figure 4.28  Image recorded at day 60 (week 9) of gestation. Bony structure becomes more echodense. Foetal head (arrow: →) can be detected easily. A) Original image. B) Labeled image.
Figure 4.29 Image recorded at day 65 (week 10) of gestation. Legs (arrows: ↓) and scrotum (arrow: ←) can be observed. A) Original image. B) Labeled image.

Figure 4.30 Image recorded at day 72 (week 11) of gestation. Foetal head (arrow: ←) and neck (arrow: ↓) became more ossified. A) Original image. B) Labeled image.

Figure 4.31 Image recorded at day 72 (week 11) of gestation. Foetal heart (arrow: →) and ribs (arrows: ↓) could be detected easily. A) Original image. B) Labeled image.

Figure 4.32 Image recorded at day 84 (week 12) of gestation. Foetal heart (arrow: ←) and ribs (arrows: ↓) could be detected easily. A) Original image. B) Labeled image.

Figure 4.33 Image recorded at day 91 (week 13) of gestation. Scrotum could be detected (arrow). A) Original image. B) Labeled image.

Figure 4.34 Image recorded at day 98 (week 14) of gestation. High increase in foetal heart size (arrow) as compared to two weeks earlier could be observed. A) Original image. B) Labeled image.

Figure 4.35 Image recorded at day 105 (week 15) of gestation. Foetal heart (arrow) started to change its echogenicity and became black in colour. A) Original image. B) Labeled image.

Figure 4.36 Image recorded at day 112 (week 16) of gestation. Foetal heart (arrow) increased in size (4.66 cm²) as compared to one week earlier (Figure 4.35: 3.75 cm²).

Figure 4.37 Image recorded at day 119 (week 17) of gestation. Foetal heart (arrow) increased in size and appeared as less echogenic structure (black in colour).

Figure 4.38 Image recorded at day 135 (week 20) of gestation. Placentome (arrow) have degraded and change in colour. A) Original image. B) Labeled image.

Figure 4.39 Image recorded at day 142 (week 21) of gestation. Heart achieved maximum size and appeared as slow beating non-echogenic structure.

Figure 4.40 Fixed foetal position for heart measurement to be made. Foetal heart (H) appeared as non-echogenic structure between the white dots (arrows) which represent ribs. A) Original image. B) Labeled image.
<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.41</td>
<td>Polynomial regression between placentome diameter and gestational age in Jermasia and Boer-crossbred does.</td>
</tr>
<tr>
<td>4.42</td>
<td>Linear regression between placentome diameter and gestational age in Jermasia and Boer-crossbred does.</td>
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<tr>
<td>4.43</td>
<td>Polynomial regression between gestational age and heart area in Jermasia does (single pregnancy).</td>
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<tr>
<td>4.44</td>
<td>Linear regression between gestational age and heart area in Jermasia does (single pregnancy).</td>
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<tr>
<td>4.45</td>
<td>Polynomial regression between heart area and gestational age in Jermasia and Boer-crossbred does (single pregnancy).</td>
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</tr>
<tr>
<td>4.47</td>
<td>Polynomial regression between heart area and gestational age in Jermasia does (twin pregnancy).</td>
</tr>
<tr>
<td>4.48</td>
<td>Linear regression between heart area and gestational age in Jermasia does (twin pregnancy).</td>
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<tr>
<td>Appendix</td>
<td>Title</td>
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<tr>
<td>---------------------</td>
<td>-----------------------------------------------------------------------</td>
</tr>
<tr>
<td>Appendix Table 1</td>
<td>Detailed findings from selected authors of ultrasound scanning in goat</td>
</tr>
<tr>
<td>Appendix Figure 1</td>
<td>Image of fluid-filled vesicle (arrow) in the uterine lumen of pregnant doe at day 22 of gestation, observed by transrectal real-time ultrasonography with a 7.5 MHz linear-array transducer (Modified from: Padilla-Rivas et al., 2005). A) Original image. B) Labelled image.</td>
</tr>
<tr>
<td>Appendix Figure 2</td>
<td>Image of small foetus (arrow) immersed in embryonic fluid of a doe at day 28 of gestation, observed by transrectal real-time ultrasonography with a 7.5 MHz linear-array transducer (Modified from: Padilla-Rivas et al., 2005). A) Original image. B) Labelled image.</td>
</tr>
<tr>
<td>Appendix Figure 3</td>
<td>Image of larger foetus (big arrow) and umbilical cord (arrow: ←) immersed in embryonic fluid of a doe at day 34 of gestation, observed by transrectal real-time ultrasonography with a 7.5 MHz linear-array transducer (Modified from: Padilla-Rivas et al., 2005). A) Original image. B) Labelled image.</td>
</tr>
<tr>
<td>Appendix Figure 4</td>
<td>Image of heart (arrow: ←) and thorax in goat foetus at 2 months of gestation using 5.0 MHz transabdominal transducer (note that the heart (H) appears as an anechoic structure between the white dots which represents ribs (arrow: ↑) (Modified from: Medan et al., 2004). A) Original image. B) Labelled image.</td>
</tr>
<tr>
<td>Appendix Figure 5</td>
<td>Foetal head (arrow) detected in Saanen does using 3.5 MHz transabdominal convex transducer (Modified from: Abdelghafar et al., 2007). A) Original image. B) Labelled image.</td>
</tr>
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</table>
# LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABEL</td>
<td>Animal Biotechnology-Embryo Laboratory</td>
</tr>
<tr>
<td>CL</td>
<td>Corpus luteum</td>
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<td>Heartbeat</td>
<td>Detection of heart in foetus</td>
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<td>KBKB</td>
<td>Kambing Bakabaik Kepala Batas</td>
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<td>NE</td>
<td>Non-echogenic</td>
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<td>RASB</td>
<td>Rumpun Asia Sdn. Bhd.</td>
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