EVALUATION OF EFFECTIVENESS OF DIFFERENT EARLY PREGNANCY DIAGNOSIS TECHNIQUES IN SOWS AND MONITORING OF FETOMETRY BY USING ULTRASOUND SCANNING

By
Dr. A. Priyanka
B.V.Sc & A.H
RVM/14-35

THESIS SUBMITTED TO
P.V.NARSIMHA RAO TELANGANA VETERINARY UNIVERSITY
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF

MASTER OF VETERINARY SCIENCE
(VETERINARY GYNAECOLGY AND OBSTETRICS)

DEPARTMENT OF VETERINARY GYNAECOLOGY AND OBSTETRICS
COLLEGE OF VETERINARY SCIENCE
RAJENDRANAGAR, HYDERABAD-500 030
P.V.NARSIMHA RAO TELANGANA VETERINARY UNIVERSITY
HYDERABAD
JANUARY 2017
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Chapter No</th>
<th>Title</th>
<th>Page No</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1. INTRODUCTION</td>
<td>1-3</td>
</tr>
<tr>
<td>II</td>
<td>2. REVIEW OF LITERATURE</td>
<td>4-22</td>
</tr>
<tr>
<td></td>
<td>2.1 Historical background</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>2.2 Non-return rates</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>2.3 Salivary Crystallization</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>2.4 Punyakoti test</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>2.4.1 Seed germination inhibition</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>2.4.2 Shoot length</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>2.5 Real-Time B mode ultrasonography</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>2.5.1 Ultrasonography on days 19-24 post insemination</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>2.5.2 Ultrasonography on days 25-29 post insemination</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>2.5.3 Ultrasonography from day 30 post insemination</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>2.6 Doppler Echo (A-mode)</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>2.7 Visual Inspection</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>2.7.1 Postural behaviour of the sows during gestation</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>2.7.2 Abdominal distension</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>2.7.3 Mammary gland development</td>
<td>22</td>
</tr>
<tr>
<td>III</td>
<td>3. MATERIALS AND METHODS</td>
<td>23-35</td>
</tr>
<tr>
<td></td>
<td>3.1 Experimental sows</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>3.2 Experimental design and methods</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>3.3 Non-return to estrus</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>3.3.1 Test procedure</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>3.4 Salivary crystallization</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>3.4.1 Collection of saliva samples</td>
<td>27</td>
</tr>
</tbody>
</table>
3.4.2 Test procedure
3.5 Punyakoti test
3.5.1 Collection of urine
3.5.2 Test procedure
3.5.3 Germination inhibition per cent
3.5.4 Shoot length per cent
3.6 Transabdominal ultrasonography
3.6.1 Technique of trans-abdominal ultrasonography for pregnancy diagnosis
3.6.2 Criteria for pregnancy diagnosis
3.6.2.1 Ultrasonographic findings on day 20 post insemination
3.6.2.2 Ultrasonographic findings on day 25 post insemination
3.6.2.3 Ultrasonographic findings on day 30 post insemination
3.7 Doppler Echo
3.7.1 Technique of Doppler Echo
3.8 Visual inspection
3.9 Statistical analysis
3.9.1 Accuracy
3.9.2 Correct and incorrect diagnosis
3.9.3 Calculation of accuracy of different early pregnancy diagnostic methods
3.9.4 Comparison of different methods of early pregnancy diagnosis

IV RESULTS

4.1 Non-Return to estrus
4.1.1 Accuracy of non-return to estrus
4.2 Salivary crystallization
4.2.1 Overall accuracy of Salivary crystallization
4.3 Punyakoti test
<table>
<thead>
<tr>
<th>Section</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.3.1</td>
<td>Germination inhibition per cent</td>
<td>41</td>
</tr>
<tr>
<td>4.3.2</td>
<td>Shoot length</td>
<td>41</td>
</tr>
<tr>
<td>4.3.3</td>
<td>Accuracy of punyakoti test</td>
<td>42</td>
</tr>
<tr>
<td>4.4</td>
<td>Real-time B mode ultrasonography for pregnancy diagnosis</td>
<td>46</td>
</tr>
<tr>
<td>4.4.1</td>
<td>Ultrasonography on day 20 post insemination</td>
<td>46</td>
</tr>
<tr>
<td>4.4.2</td>
<td>Ultrasonography on day 25 post insemination</td>
<td>46</td>
</tr>
<tr>
<td>4.4.3</td>
<td>Ultrasonography on day 30 post insemination</td>
<td>47</td>
</tr>
<tr>
<td>4.4.4</td>
<td>Description of embryonic vesicle and embryo proper</td>
<td>47</td>
</tr>
<tr>
<td>4.5</td>
<td>Doppler echo (A-mode)</td>
<td>57</td>
</tr>
<tr>
<td>4.5.1</td>
<td>Overall accuracy of Doppler echo</td>
<td>57</td>
</tr>
<tr>
<td>4.6</td>
<td>Visual inspection of physical changes</td>
<td>60</td>
</tr>
<tr>
<td>4.6.1</td>
<td>Postural behaviour of sow during gestation</td>
<td>60</td>
</tr>
<tr>
<td>4.6.2</td>
<td>Abdominal distension</td>
<td>60</td>
</tr>
<tr>
<td>4.6.3</td>
<td>Mammary gland development</td>
<td>62</td>
</tr>
<tr>
<td>4.7</td>
<td>Comparative accuracy of different pregnancy diagnostic techniques</td>
<td>67</td>
</tr>
<tr>
<td>V</td>
<td>DISCUSSION</td>
<td>68-81</td>
</tr>
<tr>
<td>VI</td>
<td>SUMMARY</td>
<td>82-84</td>
</tr>
<tr>
<td></td>
<td>LITERATURE CITED</td>
<td>85-94</td>
</tr>
<tr>
<td>TABLE NO</td>
<td>TITLE</td>
<td>PAGE NO</td>
</tr>
<tr>
<td>----------</td>
<td>----------------------------------------------------------------------</td>
<td>---------</td>
</tr>
<tr>
<td>1.</td>
<td>Methods of early pregnancy diagnosis in LWY sows</td>
<td>25</td>
</tr>
<tr>
<td>2.</td>
<td>Confirmation of early pregnancy diagnosis using non-return to estrus in comparison with real-time B mode ultrasonography</td>
<td>36</td>
</tr>
<tr>
<td>3.</td>
<td>Accuracy of early pregnancy diagnosis using non-return rate in comparison with real-time B mode ultrasonography</td>
<td>36</td>
</tr>
<tr>
<td>4.</td>
<td>Confirmation of early pregnancy diagnosis using salivary crystallization in comparison with the real-time B mode ultrasonography</td>
<td>38</td>
</tr>
<tr>
<td>5.</td>
<td>Accuracy of pregnancy diagnosis using salivary crystallization</td>
<td>38</td>
</tr>
<tr>
<td>6.</td>
<td>Showing Germination Inhibition per cent and shoot length of wheat seeds in different groups</td>
<td>44</td>
</tr>
<tr>
<td>7.</td>
<td>Confirmation of early pregnancy diagnosis using salivary crystallization in comparison with the real-time B mode ultrasonography</td>
<td>44</td>
</tr>
<tr>
<td>8.</td>
<td>Accuracy of early pregnancy diagnosis using punyakoti test</td>
<td>44</td>
</tr>
<tr>
<td>9.</td>
<td>Measurement of Embryonic vesicle and embryo at different stages of gestation</td>
<td>48</td>
</tr>
<tr>
<td>10.</td>
<td>Measurements of amniotic vesicle and CRL of embryo in different days of gestation</td>
<td>48</td>
</tr>
<tr>
<td>11.</td>
<td>Accuracy of ultrasonography on day 20 post insemination in comparison with real-time B ultrasonography on day 45-60 post insemination</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>Accuracy of ultrasonography on day 25 post insemination in comparison with real-time B mode ultrasonography on day 45-60 post insemination</td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>-----------------------------------------------------------------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>12.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Accuracy of ultrasonography on day 30 post insemination in comparison with real-time B ultrasonography on day 45 post insemination</td>
<td></td>
</tr>
<tr>
<td>13.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Overall accuracy of early pregnancy diagnosis using ultrasonography in comparison with the real-time B mode ultrasonography on day 45-60 post insemination</td>
<td></td>
</tr>
<tr>
<td>14.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Confirmation of pregnancy diagnosis using doppler echo in comparison with the real-time B mode ultrasonography</td>
<td></td>
</tr>
<tr>
<td>15.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Accuracy of pregnancy diagnosis using doppler echo (A-mode) in comparison with real-time B ultrasonography on day 45-60 post insemination</td>
<td></td>
</tr>
<tr>
<td>16.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Accuracy of pregnancy diagnosis by fetal Doppler during different gestation period in all experimental LWY sows</td>
<td></td>
</tr>
<tr>
<td>17.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Comparison of accuracy of early pregnancy diagnosis among different early pregnancy diagnostic techniques</td>
<td></td>
</tr>
<tr>
<td>18.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
## LIST OF PLATES

<table>
<thead>
<tr>
<th>PLATE No.</th>
<th>TITLE</th>
<th>PAGE No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Equipment and materials used for early pregnancy diagnosis</td>
<td>26</td>
</tr>
<tr>
<td>2.</td>
<td>Showing different crystallization patterns of sow’s saliva</td>
<td>39</td>
</tr>
<tr>
<td>3.</td>
<td>Per cent of salivary crystallization</td>
<td>40</td>
</tr>
<tr>
<td>4.</td>
<td>Showing shoot length (cm) of wheat seeds</td>
<td>43</td>
</tr>
<tr>
<td>5.</td>
<td>Showing germination inhibition per cent of test groups in punyakoti test</td>
<td>45</td>
</tr>
<tr>
<td>6.</td>
<td>Showing mean values of shoot length (cm) of test groups in punyakoti test.</td>
<td>45</td>
</tr>
<tr>
<td>7.</td>
<td>Anechogenic smaller amniotic vesicle on day 20 of gestation</td>
<td>53</td>
</tr>
<tr>
<td>8.</td>
<td>Anechogenic amniotic vesicle on day 25 of gestation</td>
<td>53</td>
</tr>
<tr>
<td>9.</td>
<td>Real-time B mode ultrasonography image on day 30 with amniotic vesicle and echogenic embryo</td>
<td>54</td>
</tr>
<tr>
<td>10.</td>
<td>Real-time B mode ultrasonography image on day 45 of gestation with fetus in anechogenic vesicle</td>
<td>54</td>
</tr>
<tr>
<td>11.</td>
<td>Real-time B mode ultrasonography image on day 55 of gestation</td>
<td>55</td>
</tr>
<tr>
<td>12.</td>
<td>Real-time B mode ultrasonography image on day 65 of gestation</td>
<td>55</td>
</tr>
<tr>
<td>13.</td>
<td>Real-time B mode ultrasonography image on day 78 of gestation</td>
<td>56</td>
</tr>
<tr>
<td>14.</td>
<td>Real-time B mode ultrasonography shown length of BPD</td>
<td>56</td>
</tr>
<tr>
<td>15.</td>
<td>Performing Doppler echo (A-mode) in pregnant sow</td>
<td>58</td>
</tr>
<tr>
<td>16.</td>
<td>Showing various postures adopted by LWY pregnant sows</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>Description</td>
<td>Page</td>
</tr>
<tr>
<td>---</td>
<td>-----------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>17.</td>
<td>Showing various abdominal distension of pregnant sows during gestation</td>
<td>63</td>
</tr>
<tr>
<td>18.</td>
<td>Showing development of mammary gland of sow during gestation</td>
<td>64</td>
</tr>
<tr>
<td>19.</td>
<td>Showing development of teats of sow during 3½ month of gestation period</td>
<td>65</td>
</tr>
</tbody>
</table>
# LIST OF SYMBOLS AND ABBREVIATIONS

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>LWY</td>
<td>Large White Yorkshire</td>
</tr>
<tr>
<td>FAOSTAT</td>
<td>The State Of Food and Agriculture</td>
</tr>
<tr>
<td>AI</td>
<td>Artificial insemination</td>
</tr>
<tr>
<td>MHz</td>
<td>Mega hertz</td>
</tr>
<tr>
<td>mm</td>
<td>millimeter</td>
</tr>
<tr>
<td>ABA</td>
<td>Abscissic acid</td>
</tr>
<tr>
<td>%</td>
<td>Per cent</td>
</tr>
<tr>
<td>cm</td>
<td>Centimeter</td>
</tr>
<tr>
<td>mg</td>
<td>Milligram</td>
</tr>
<tr>
<td>ml</td>
<td>Milli liter</td>
</tr>
<tr>
<td><em>et. al.</em>,</td>
<td>Associates / co-workers / others</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>SE</td>
<td>Standard Error</td>
</tr>
<tr>
<td>h</td>
<td>hours</td>
</tr>
<tr>
<td>CRL</td>
<td>Crown-rump Length</td>
</tr>
</tbody>
</table>
ACKNOWLEDGEMENT

Words are not enough to express the sense of respect and gratitude that I feel for my major advisor and Chairman of my Advisory Committee, Dr.K. VENKATARAMANA, Associate Professor and Principal, Department of Veterinary Gynaecology and Obstetrics, Animal Husbandry Polytechnic College, Mahabubnagar. As a major advisor, he has been extremely patient with me, especially during those occasions when I was not able to meet his expectations. Even though he was busy with his academic and personal works, he allocated his valuable time to ameliorate this dissertation with all his commitment and wholeheartedness. He has not only provided me knowledge that are related to my subject, but also about life itself.

I am deeply grateful to the esteemed members of my Advisory committee, Dr. K. Ramchandra Reddy, Associate Professor, Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science, Korutla for his whole hearted support, sustained encouragement, care, unretiring help, interesting ideas, active cooperation with simplicity, advice in all aspects during the course of my research and I am also thankful to him for encouraging the use of correct grammar and consistent notation in my writings and for carefully reading and commenting on countless revisions of this manuscript.

It gives me great pleasure to owe my deepest gratitude to Dr.D.Naga Lakshmi, Professor and Univ. Head, Department of Animal Nutrition, College of Veterinary Science, Rajendranagar, member of Advisory committee for her help in writing dissertation, encouragement, constructive critics, valuable suggestions and friendly approach that were profoundly led to fruitful evaluation of manuscript.

I express my profound thanks to Dr.K.Chandrashekar Reddy, Professor and Univ.Head, Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science, R.Nagar for his help during my research.

I would like to show my greatest appreciation to Dr.K.Solman Raju, Professor, Teaching Veterinary Clinical Complex, College of Veterinary Science, Rajendranagar for his wise counsel, cordial support throughout the post graduation period and for delivering valuable ideas and suggestions during my research work.

I express my special thanks to Dr. T. Raghunandan, Associate Dean, C.V.Sc, Korutla, and Dr. Sushma, Assistant professor, ILFC, C.V.Sc, Rajendranagar, Hyderabad, for permitting me to carry out my research work at ILFC and for their kind help during period of study.

I heartly respect my sincere thanks to Dr.Ramsingh, and Dr.SunilAnand, Assistant Professors, Department of Veterinary Gynaecology and Obstetrics, College
of Veterinary Science, Rajendranagar for their moral support, generous help and encouragement rendered in completing this piece of work.

I am highly grateful to Dr. T. Madhava Rao, Professor and Head, TVCC, College of Veterinary Science, Hyderabad, Dr. N. Nalini Kumari, Associate professor and Head, Department of Animal Nutrition, C.V.sc, Korutla.

Special thanks to my senior Dr. B. Srilatha, Ph.D for teaching patiently the basics of the subject to me in the wards throughout the 1st year, her friendly approach, her valuable suggestions about life and for helping technically in bringing out the manuscript in an effective way.

I am extremely delighted to extend thanks to Dr. P. Vishal Kumar, Ph.D, Dr. M. Rajashri Ph.D and Mr. B. Sandeep for their time sparing, kind cooperation during my research work who have been with me till the end of my work.

I would like to convey my immense love and gratitude to my friends Dr. D. Neelima Prabha, Dr. M. Sindhu Priya Reddy, and my seniors Dr. Vidya, Asst. Professor, Dr. Razia Sultana (Ph.D), Dr. Vinaya Sheela, Asst. Professor without whose motivation I would have not joined M.V.Sc. I am thankful to them for their assistance and encouragement throughout my research work and for cheering me up in my bad times. They are my well-wisher, teacher and the one who always guided me to take correct decisions and renovated my life in a better way. They know me better than I know myself and I am very fortunate for this. I am very grateful to god for embracing them in my life.

I owe my deepest gratitude to my M.V.Sc colleagues, Dr. B. Priyanka, Dr. N. Vamsi Krishna Reddy, Dr. M. Mahesh, Dr. B. Gopi, Dr. Srisailam and Dr. Lingaswamy who have been with me in my happiness and sorrows. Being fresh PG and in-service PG scholars, they educated me about different gynaecological techniques in the wards. Helped me a lot in completion of my work with their moral and technical support. I, from the deep of my heart thank you all for tolerating me throughout these two years.

I am very much grateful to junior M.V.Sc students Dr. Yashaswini, Dr. Anil Kumar, Dr. Ashok, Dr. Raju, Dr. Pranay and Dr. Prashanth for their candid cooperation and help during my research work.

I would like to offer my special thanks to and Mrs. L. Achamamba Madam and Library Staff of PVNR, College of Veterinary Science, Rajendranagar for their help in literature collection.

It is the time to surface out my genuine love and deep sense of honour to my mother and father Smt. A. Mariyamma and Sri. A. Bayyanna who constantly educated,
guided and moulded me into the present position and whose boundless love, unparalleled affection, encouragement and moral support is a constant source of motivation for me. And also I wish to acknowledge my brother Srikanth for his everlasting love on me without whose affection I would not come up to this level, which I shall remember throughout my life.

I would like to thank my friends and all my M.V.Sc colleagues of all departments Dr.B.Kavya, Dr.Soma.Shekhar goud, Dr.Ch.Vinod kumar, Dr.BH.Alekhya, Dr.V.Gopinath, Dr.D.Tagore, Dr.Y.Ajay, Dr.E.Vivek, Dr.N.Ramesh, Dr.T.Sukanya, Dr.Sailaja and Dr.Radhika for their encouragement and support showed on me during my research period and cheering me up in my bad times. Thank god for introducing these gifted people in my life.

I am also happy to express my sincere gratitude to Mahesh, Mahendar, Rambabu, staff of ILFC, Moghulamma, Swaroopa, Anjaneyulu, Sriramulu and Raju staff of Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Science, Rajendranagar, HYD for their co-operation and help rendered throughout the research period.

I express my deep sense of gratitude to the farmers Mr. Krishnamraju and Mr. Anjaneyulu, Mahabubnagar for allowing me to continue the research work at their private farm, kind cooperation, assistance and encouragement throughout my research work.

I am thankful to P. V. Narsimha Rao Telangana Veterinary University, Hyderabad for providing financial assistance in the form of stipend during my post graduate study.

Finally I fill my heart with praise, bow my heads with reverence and fold my hands with thanks giving towards my Lord Jesus and Mother Maria for their abundant grace without which I could not have taken my P.G. seat here and accomplished my studies.

Place: Rajendranagar

Date:

(Allapuri.Priyanka)
DECLARATION

I, A.PRIYANKA, RVM/14-35 hereby declare that the thesis entitled

“EVALUATION OF EFFECTIVENESS OF DIFFERENT EARLY PREGNANCY Diagnosis
techniques in Sows and Monitoring of Fetometry by Using Ultrasound
Scanning” submitted to P.V. Narasimha Rao Telangana Veterinary University,
Hyderabad for the degree of Master of Veterinary Science is the
result of original research work done by me. It is further declared that the thesis
or any part thereof has not been published earlier in any manner.

Date: A.PRIYANKA
Name of the author : PRIYANKA ALLAPURI

Title of the thesis : EVALUATION OF EFFECTIVENESS OF DIFFERENT EARLY PREGNANCY DIAGNOSIS TECHNIQUES IN SOWS AND MONITORING OF FETOMETRY BY USING ULTRASOUND SCANNING

Degree to which it is submitted : MASTER OF VETERINARY SCIENCE

Faculty : VETERINARY SCIENCE

Discipline : VETERINARY GYNAECOLOGY AND OBSTETRICS

Major Advisor : Dr. K. VENKATARAMANA, ASSOCIATE PROFESSOR, VETERINARY GYNAECOLOGY AND OBSTETRICS

University : P.V. NARASIMHA RAO TELANGANA VETERINARY UNIVERSITY, HYDERABAD 500030.

Year of submission : 2017

ABSTRACT

The aim of the present investigation was to evaluate the different methods of early pregnancy diagnosis viz., Nonreturn rates, Salivary crystallization, Punyakoti test, Real-time B ultrasonography and Doppler echo+ and to compare between and among different methods. The present study was conducted on Large White Yorkshire aged between 2 to 3 years showing estrus signs and were bred. These were randomly divided and pregnancy diagnosis was carried out at an early stage from day 20 post insemination. Pregnancy diagnosis in Nonreturn rates was carried out using visual observations on day 21 post insemination with presence of boar in pregnant sows. Sows in Salivary crystallization were analyzed for early pregnancy with estimation of saliva progesterone concentration by different types of crystallization on day 18 post insemination using 400x microscope and were recorded in pregnant and nonpregnant sows, respectively. Pregnancy diagnosis in Punyakoti test was undertaken using seed
germination inhibition test at day 26 post insemination and the sows diagnosed pregnant showed significant inhibition of seed germination and shoot length than the nonpregnant sows with a mean germination inhibition percentage (%) and shoot length (cm) of 74.66 ± 4.42 ; 3.33 ± 0.061 and 52.44 ± 2.33 ; 5.44 ± 0.92 in pregnant and nonpregnant sows, respectively. Sows in Real-time B mode ultrasonography were analyzed for early pregnancy using ultrasound at day 20, 25 and 30 post insemination. Pregnant sows showed anechoic embryonic vesicle within the echogenic uterus on day 20, expansion of embryonic vesicle and embryo proper in some sows on day 25 and clear visualization of anechoic embryonic vesicle and echogenic embryo proper in all pregnant sows on day 30 post insemination. Pregnancy diagnosis by Doppler echo+ were analysed using transabdominal transducer at day 30, 35 post insemination. Confirmation of pregnancy in all the sows that were selected in the five test groups was done by real-time B ultrasonography at day 45-60 post insemination.

The overall diagnostic accuracy of Nonreturn rates, Salivary crystallization, Punyakoti test, Real-time ultrasonography and Doppler echo+ was 80.0, 75.00, 75.00, 100 and 85 per cent on days 21, 30, 26, 30 and 30-35 post insemination. The study concluded that Real-time transabdominal ultrasonography was 100 per cent accurate in diagnosing nonpregnancy. Among all the diagnostic techniques in the present study, transabdominal ultrasonography was more accurate in diagnosing pregnancy and nonpregnancy from day 25 post insemination.
CHAPTER I

INTRODUCTION

Swine husbandry has gained importance in India during the recent years due to increased demand for pork and pork products. Pig farming has become a profitable enterprise since pig possesses many important economic traits such as high prolificacy, faster growth rate, better feed conversion efficiency and shorter generation interval.

The pig farming has emerged as an enterprise in Telangana as it provides a substantial income to the poor rural and urban unemployed youth. For the emancipation of economic status of weaker sections of the society Government has started several research schemes on pigs. Of late, crossbreeding programmes are being undertaken to bring about the genetic improvement of the indigenous pig population by crossing these pigs with the exotic boars, to evolve a new breed suited to agro-climatic conditions of Telangana state. In India, 70% of the pig population is reared under traditional small holder, low-input demand driven production system, except for limited number of semi-commercial pig farms in Kerala, Punjab and Goa.

As per the XIX Livestock census (2012), the swine population of India is 10.29 million, with a decrease in population by 7.54% over the previous census. Porcine population of Telangana state is 2.51 lakhs ranking 17th position in the country (Census,2012). Average meat yield of pigs in India is 39kg/animal which is about 55 per cent less than the corresponding value of world average 79kg/animal, the pig population of India constitute, 1.47 per cent of world pig population (FAOSTAT 2009).

Information on the early pregnancy diagnosis is vital to enable the farmer to maintain the pig herd in a more economic way and thus earn more profits by prompt culling of non-productive sows, reducing the piglet losses, reducing farrowing interval.

Successful integration of reproductive management system also depends on ideal early pregnancy test for sows which should be sensitive, specific, inexpensive, simple to conduct under field conditions and able to determine pregnancy on farm itself (Williams 2008). Lack of reliable sow side early pregnancy diagnostic methods has been drawn the attention of several researchers to develop a novel way to reduce farrowing to conception interval by diagnosis of early pregnancy.
Early pregnancy diagnosis is a key to shorten the farrowing interval through early identification of open animals and their timely treatment and rebreeding so as to maintain a postpartum barren interval of 60 days. Early identification of non-pregnant sows can improve reproductive efficiency and pregnancy rate. Thus, latest technologies to identify non-pregnant early after breeding may play a key role in managerial strategies to improve reproductive efficiency and profitability of commercial pig farms (Kruger and Bilkei, 2002).

Establishing detection of Estrus (Heat) observation of physiological and behavioral signs of estrus after breeding is the most common method used for pregnancy diagnosis on 18 to 24 days with an average of 21 days (Almond and Dial, 1987). Estrus detection with a mature boar daily after 17 days of breeding is an accurate means of predicting farrowing rate with an accuracy 98% (Flowers et al., 2000). Failure to return to estrus provides circumstantial evidence that the previous service resulted in pregnancy (Cowart, 2008). Saliva sampling is a non-invasive, simple and low cost procedure (Skalova et al., 2013). The aim of presented research was the confirmation of the presence and changes of saliva crystallization in sows during early pregnancy and verification of differences in crystallization between pregnant and non-pregnant.

Even though the techniques such as rectal palpation, radiography, ultrasonography, progesterone assay and rosette inhibition assay are available (Jainudeen and Hafez, 1993 and Wani et al., 2003) due to practical constraints in their application, there is a consistent effort in search of a simple, economical and non-invasive technique. Punyakoti test a simple non-invasive test has been developed to diagnose pregnancy (Veena and Narendranath, 1993). This seed germination inhibition test is recognized as a door step technology to the farmers that can be done by the farmers since it require inexpensive materials and does not need special skills.

Ultrasound pregnancy diagnosis is a reliable method of determining the presence of a conceptus and viability of an embryo, which is essential to increase the profitability of the animal (Tiwari et al., 2002). Additional sensitivity can be achieved by using trans-abdominal ultrasound for early pregnancy detection. Trans-abdominal ultrasound can be used as early as 20 days after breeding but is more typically applied after day 30 (Fricke, 2002). In the 1980’s, real time ultrasonography was developed for use in domestic animals. Ultrasound is a less invasive technique for early pregnancy diagnosis. In sows, trans-
abdominal ultrasonography is most commonly used to determine early pregnancy.

Doppler echo is the one of the method to confirm early pregnancy in sows and gilts. The accuracy of pregnancy diagnosis with Doppler echo is greater than 95 per cent when conducted after day 29 of gestation (Flowers et al., 2003).

Many sows display external physical signs of pregnancy between 60 and 90 days of pregnancy. Signs may include abdominal distension, udder enlargement, and enlargement of the vulva (Meredith, 1980).

On perusal of the available literature, information on the early pregnancy diagnosis of Large White Yorkshire (LWY) pigs under Indian conditions was found to be scanty and these can hardly be construed as authentic. Since the available information on the various aspects of early pregnancy diagnosis in LWY appeared to be vary among the methods of pregnancy diagnosis, hence a study was undertaken to investigate early pregnancy on scientific lines in LWY breed to facilitate better package of management practices for a successful and profitable pig enterprises with the following objectives:

• To diagnose the early pregnancy using Non return rates, Salivary crystallization, Punyakoti test, Doppler (A-mode), Visual inspection (Physical changes) during pregnancy and Ultrasonography.
• To study the accuracy of each method of the pregnancy diagnosis.
• To reconfirm the early pregnancy by real-time B ultrasonography on day 45-60.
• To evaluate the sensitivity and specificity of each method and to draw a comparison between and among the different methods of pregnancy diagnosis.
CHAPTER II

REVIEW OF LITERATURE

Early pregnancy diagnosis has become a key to economic success in sow production and confirming pregnancy is essential for improving fertility herd rate. Early identification of nonpregnant sows post breeding can improve reproductive efficiency and pregnancy rate. Accurate early detection of pregnant and non-pregnant sows is an essential factor for optimizing reproductive performance in swine farms (Miller, 2003). In sow, pregnancy diagnosis is an important tool to measure the success of a reproductive management. The assessment of pregnancy diagnosis can be conducted at varying intervals after service. The methods used can have varying degrees of accuracy. However, pregnancy diagnosis only gives an assessment at a particular point of time, after which undetected loss of pregnancy may occur and give a false result.

Methods of pregnancy diagnosis in sows can be classified as visual methods i.e. non-return to estrus, and some of the laboratory methods which are indirect include hormonal assays such as progesterone, estrone sulphate etc., assays of early pregnancy factor, pregnancy associated glycoprotein (PAG) and the most practical and direct methods such as rectal palpation, radiography and transrectal, transabdominal ultrasonography are currently used for an accurate and early pregnancy diagnosis.

Since there is paucity of information in sows, the literature cited on these aspects for sows was also reviewed. In the present study, five methods of early pregnancy diagnosis were reviewed.
2.1 HISTORICAL BACKGROUND

The assessment of an early and reliable pregnancy diagnosis in sows is very important for
limiting the number of non-productive days in sow herds (Flowers, 2000). A simple
pregnancy diagnostic technique was developed on the basis of an ancient practice
deciphered from medical papyri, dating back to 400 years, recovered from tombs of Egypt.
According to papyri, in ancient Egypt, human doctors diagnosed pregnancy in women on
the basis of a seed germination method. On the basis of this ancient clue, a simple
technique was developed that involves the germination of wheat seeds in the diluted urine
of cow whose pregnancy is to be diagnosed, which were followed same technique in swine.
Inhibited germination suggests pregnancy and uninhibited growth suggests non-pregnancy.
The technique has been christened the Punyakoti test and can be easily be carried out by
field veterinarians and dairy owners and farmers in rural areas on their farms (Veena and
Narendranath, 1993).

Lindahl (1966) was probably the first to describe the use of ultrasound for
pregnancy diagnosis in ewes and he found the amplitude-depth presentation (A-mode)
more useful than real-time.

Non-return to estrus for pregnancy diagnosis has been a routine procedure for more
than 100 years (Almond and Dial, 1986).

Saliva crystallization was mainly studied in women (Kullander and Sonesson 1965;
Berardono et.al., 1993) and we noticed only four studies in animals – in beagle bitches
(Pardo-Carmona et.al., 2010), camels (Camelus bactrianus) (Haberova, 2010), cattle
(Skalova et.al., 2013) and White sheep (Sangeetha and Ramesh kumar, 2015).
Real time ultrasound for pregnancy diagnosis in swine became popular in the late 1970’s and specialized transducers were developed (Flowers et.al., 1999).

Fraser and Robertson (1967) was probably the first to apply the use of ultrasounic Doppler method for pregnancy diagnosis in ewes and sows and he found the amplitude-depth presentation (A-mode) more useful than real-time.

**2.2 NON-RETURN TO ESTRUS:**

Almond and Dial (1986) analyzed nonreturn to estrus in postpartum and pregnant as a monitor of reproductive status in LWY sows. The most common and time – horned means of diagnosing pregnancy in swine is observing signs of estrus. Sexually mature, non-pregnant sows and gilts normally complete an estrous cycle every 18 to 24 days with an average of 21 days.

In presence of boar, Atkinson et.al. (1986) observed most widely used method of pregnancy diagnosis in sows is nonreturn to estrus around 20 d after service. Sows that return to estrus are classified as nonpregnant, and pregnancy was assumed if a sow did not display signs of estrus. Commonly referred as the “boar test,” which was not accurate, and its accuracy decreased markedly in the presence of some reproductive disorders.

Glossop and Foulkes (1998) observed the occurrence of two phases of return to estrus in sows on commercial units using a boar for heat detection must have an accuracy of 98 per cent.

Flowers et.al. (1999) reported that the detection of estrus via daily boar exposure from day 17 to 23 after breeding resulted accuracy of more than 85 per cent.
Knox and Althouse (1999) visualized the reproductive tract of the female pig and conformed the pregnancy has traditionally been confirmed based on the absence of signs of estrus at 18-24 days after mating showed the accuracy of 80 per cent.

Flowers and Knox (2003) observed daily estrus detection with a mature boar was an accurate means of predicting farrowing rate. Studies in commercial farms reported an accuracy of 98 percentage.

Cowart (2008) reported daily exposure to mature, sexually aggressive boars and observation for signs of estrus was 98 per cent accurate in predicting farrowing.

Gaggini (2012) reported the most widely used management for identifying empty female daily was the detection of return to estrus through stimulation by the presence of boars. Accuracy was high and requires a lot of time invested, trained employees and can even result in false diagnoses due to low specificity to identify empty sows.

Merck (2016) Pregnancy was most commonly diagnosed by noting that the female does not return to estrus in 18–25 days with 75–85 per cent accuracy.

2.3 SALIVARY CRYSTALLIZATION:

Salivary ferning may be used as a new parameter to aid women to detect their fertile period. Later there was increase in the usage of salivary ferning test in different clinical settings such as to predict the ovulatory period (Guida et. al., 1993), to detect the resumption of ovarian function in the post-partum period (Tommaselli et. al., 2000).

Noonan et.al. (1975) observed that only two types of crystalline patterns during insemination period, namely mixed branch like.
Guida et. al. (1993) reported that ferning days were found throughout the cycle, clear ferning was found only on 1 or 2 days and there was also no discernible beginning or end to the fertility cycle as determined by the salivary ferning test.

Haberova, (2010) showed that branch like crystallization belonged among the most frequent types with overall incidence of 36.27 per cent in camels.

Pardo-Carmona et.al. (2010) determined the optimal mating time were highly variable, largely in bitches because saliva crystallization appears during heat and also reported that the positive crystallization patterns type 1 and 2 i.e. partial and total crystallization appeared same days before and after the optimal time for breeding and saliva crystallization persisted for several days before and after the expected date of ovulation.

Skalova et. al. (2013) confirmed the presence and changes of saliva crystallization in domestic cattle during pregnancy and also in non-pregnant animals and found significant differences in crystalline pattern between 20 and 29 days after artificial insemination.

Gnanamuthu and Ramesh kumar (2015) conducted the studies to determine the day of ovulation in cattle by the salivary fern test and observed different types of salivary fern patterns like none, dotted, branch like, fir like, mixed branch+fern like; mixed fern +fir like; and mixed branch+fir+fern like.

Sangeetha and Ramesh kumar (2015) in Ramnad white sheep and suggested that during estrus, changes in saliva crystallization patterns occurred due to variations in electrolyte concentrations associated with hormonal changes.
2.4 PUNYAKOTI TEST:

Veena and Narendranath (1993) studied the ancient Egyptian pregnancy test extended to cattle. Based on clues provided in papyri from ancient Egypt (2100-2200 BC), they attempted to device a simple test to diagnose pregnancy in cow, similar test was reported in swine. The germination and shoot length of wheat seeds were suppressed by the urine of pregnant sows and persists for 2-3 months after farrowing. However, the urine of non-pregnant sows did not cause such inhibition. Such a differential response was not due to pH. They suggested that these results would be important in developing a simple test to diagnose pregnancy in sow.

2.4.1 SEED GERMINATION INHIBITION:

Nirmala et.al. (2008) studied the effect of estrogen and progesterone at different concentration on seed germination. Stock solutions of estrogen and progesterone were prepared in alcohol (1mg/ml) and serial dilutions made using distilled water. The average seed germination and shoot length did not significantly (p>0.05) when compared with that of control groups. They concluded that estrogen and progesterone in their natural forms did not affect either the seed germination or shoot length.

Dilrukshi and Perera (2009) reported that the urine of pregnant animals has inhibitory effect on seed germination and shoot length which were significant (p<0.05) than non pregnant cows. The urine of the pregnant cows suppressed the seed germination (57.93 per cent) significantly than the non-pregnant (79.2 per cent).

Swamy et.al. (2010) applied the seed germination inhibition technique to diagnose pregnancy in Malnad Gidda cattle using samples collected at 2 months of post insemination
and were diluted with distilled water in the ratio of 1:4. It was resulted that mean germination inhibition percentage was 73.65 ± 2.81, 27.90 ± 2.56 and 21.48 ± 2.69, respectively in positive, negative and control groups.

Perumal (2014) assessed the seed germination inhibition test on pregnancy diagnosis in Mithun cows using urine samples and were diluted with distilled water at the ratio of 1:4. The mean seed germination inhibition percentage was 19.57 ± 1.54, 24.02 ± 1.80 and 78.91 ± 2.09 in control, negative and positive group, respectively.

Rine et.al. (2014) evaluated seed germination inhibition test for early pregnancy detection in cross bred dairy cattle using urine samples on day 14, 21, 28,35 and 45 post insemination. A significant difference of seed germination inhibition percentage was noticed between pregnant and nonpregnants with mean values of 38.13 ± 1.25, 40.51 ± 1.63, 54.12 ± 2.29, 56.53 ± 3.31 and 65.31 ± 4.76 per cent in pregnant and 37.60 ± 1.58, 38.40 ± 1.96, 39.73 ± 2.15, 40.87 ± 1.52 and 40.60 ± 1.79 per cent in nonpregnant animals on day 14, 21, 28, 35 and 45, respectively.

2.4.2 SHOOT LENGTH:

Dilrukshi and Perera (2009) recorded shoot length was significantly less when treated with the urine samples collected from pregnant cows (mean shoot length 3.89 ± 3.16) when compared to that of non-pregnant cows (mean shoot length 6.1 ± 3.24) and water which was used as control treatment (mean shoot length 13.6 ± 3.41). Urine of pregnant cows dramatically inhibited the germination and shoot growth of mung beans than the nonpregnant cows.

Rao and Veena (2009) conducted seed germination test for pregnancy diagnosis in cows using urine samples collected from 40 crossbred cows / heifers early in the morning
on day 14, 21, 28, 35 and 45 after artificial insemination (AI) and were subjected to seed germination test using wheat seeds and green gram. The mean values of shoot lengths (cm) in wheat seeds and green gram on 14, 21, 28, 35 and 45 days were 4.63 ± 0.14, 2.69 ± 0.08; 4.49 ± 0.19, 2.49 ± 0.08; 2.72 ± 0.33, 1.58 ± 0.09; 1.43± 0.06, 0.77 ± 0.09; and 1.38 ± 0.06, 0.65 ± 0.07 in pregnant animals and 4.92 ± 0.19, 2.40 ± 0.16; 4.42 ± 0.21, 2.46 ± 0.13; 4.80 ± 0.23, 2.58 ± 0.16; 4.77 ± 0.28, 2.39 ± 0.14; and 4.76 ± 0.20, 2.64 ± 0.15 in non-pregnant animals, respectively. The results indicated that significance inhibition of seed germination after 48hrs and shoot growth after 4 days in wheat and green gram seeds, discoloration of either seeds or both after 48 hrs could be noted for first time in 68 per cent of test group, especially 28 days post insemination. But the efficacies for true pregnancy detection on days 35 and 45 were 100 per cent.

Swamy et al. (2010) recorded the shoot length of wheat seeds in the seed germination inhibition technique to diagnose pregnancy in Malnad Gidda cattle using samples collected at 2months of post insemination and were diluted with distilled water in the ratio of 1:4. The mean shoot length of the germinated wheat seeds on fifth day was 0.95 ± 0.47, 3.62 ± 0.51 and 5.54 ± 0.68 cm in positive, negative and control groups, respectively and concluded that the seed germination inhibition technique was useful to detect pregnancy as a simple, non-invasive and economical method.

Hussain (2012) compared seed germination test at 1:4 and 1:8 dilutions for pregnancy diagnosis in cow with Punyakoti test and rectal palpation test. There was no level of significance observed for the first 0-14 days at both dilutions. The dilutions 1:4 showed best results for pregnancy diagnosis in cows as compared to 1:8 dilutions. The germination percentage and shoot length on 14th day for 1:4 dilutions was 75 per cent and 5.98 cm in non-pregnant animals, 77.33 per cent and 5.90 cm in pregnant animals
respectively. Similarly for 1:8 dilution 81.66 per cent and 6.22 cm in non-pregnant animals, 82.33 per cent and 6.17 cm in pregnant animals, respectively.

Perumal (2014) measured the shoot length of paddy seeds which were treated with diluted urine samples in the ratio 1:4 in the seed germination inhibition test of pregnancy diagnosis. The mean shoot length of paddy seeds was 5.91 ± 0.86, 3.67 ± 0.73 and 0.53 ± 0.52 cm in control, negative and positive group, respectively. Further concluded that the seed germination inhibition technique was useful to detect pregnancy in Mithun cows as a simple, non-invasive and economical method.

Rine et.al. (2014) measured the shoot lengths of wheat seeds treated with diluted urine of 1:4 ratio as a method of early pregnancy diagnosis and reported as 3.37 ± 0.8, 3.44 ± 0.05, 2.84 ± 0.09, 2.88 ± 0.14 and 2.39 ± 0.16 cm in pregnant and 3.38 ± 0.06, 3.38 ± 0.06, 3.40 ± 0.05, 3.37 ± 0.06 and 3.43 ± 0.06 cm in nonpregnants on day 14, 21, 28, 35 and 45, respectively. A significant difference was noticed between pregnant and nonpregnant animals on days 28, 35 and 45 post insemination.

2.5 REAL-TIME B MODE ULTRASONOGRAPHY:

In swine, the allantoic vesicle undergoes particularly rapid growth in length around day 23 of pregnancy (Almond and Dial 1987). Flowers and Knox (2000) reported that, a 5 MHz or 7.5 MHz transducer tends to provide more reliable results than a 3.0 MHz transducer for early pregnancy diagnosis. The level of accuracy depended on factors like, the type of ultrasound equipment used (sector or linear), transducer frequency (3.5, 5.0 or 7.5 MHz), stage of gestation and the experience of the operator.
2.5.1 ULTRASONOGRAPHY ON DAY 19-24 POST INSEMINATION:

Embryonic vesicles (fluid–filled membrane) can be visualized as early as 14 to 15 days of gestation in the sow with advanced RTU equipment. However, at present, the earliest that acceptable accuracy was achieved with the portable on-farm units was about 22 days postmating (Flowers et al., 1999). If an improved RTU machine or some other ideal pregnancy test could be developed to accurately diagnose pregnancy at 18 to 42 days postmating, just before most sows would return to estrus, it would have substantial economic benefits.

Inaba (1983) reported that, the accuracy approached 100 per cent for diagnosing pregnancy from day 22 of gestation in sows was developed with 3.5 MHz multitransducers.

Almond and Dial et al. (1986) monitored the swine conceptus in sows and gilts by intra abdominal ultrasonic imaging and reported that the heart beat was detected on day 21 confirming viability of the embryo and heart beat was expected to be first seen on day 25.

Curran et al., (1986) monitored the bovine conceptus in heifers by intrarectal ultrasonic imaging and reported that the heart beat can be detected on day 21 confirming viability of the embryo and heart beat is expected to be first seen on days 25 or 26.

Almond and Dial (1987) monitored the swine conceptus in sows and gilts by intrabdominal ultrasonic imaging and reported that the heart beat can be detected on day 22 confirming viability of the embryo and heart beat is expected to be first seen on days 25 or 26.

Glossop and Foulkes (1988) reported that, pregnancy diagnosis at days 10 to 14 was based on detection of the conceptus and observed that detection of the conceptus was not accurate prior to visualization of the embryo proper (mean day 22, range days 20 to 24).
Kastelic et. al. (1989) reported that, pregnancy diagnosis at days 10 to 14 was based on detection of the conceptus and observed that detection of the conceptus was not accurate prior to visualization of the embryo proper (mean day 22, range days 20 to 24).

Bonato et. al. (1990) performed ultrasound scanning in cows for detection of pregnancy between days 17 and 21, 22 and 26, 27 and 30 post insemination. The accuracy of pregnancy diagnosis was 90, 92 and 97 per cent, respectively in 3 groups of animals. The size of embryonic vesicle was 5 × 7mm on day 17.

Boyd et. al. (1990) used real time B-mode ultrasound scanner with a 7.5 MHz rectal linear transducer for pregnancy detection in dairy cows with an accuracy of 33 per cent in cows up to 16 days after estrus, increased markedly after 17 days and was 100 per cent by day 20.

Knox and Althouse, (1999) conducted transrectal and transabdominal ultrasound scanning examinations of uterus to determine the accuracy of detecting pregnancy and nonpregnancy in sows between days 16 and 20 following inseminations. Diagnostic accuracy and sensitivity of the ultrasound examinations between days 16 and 22 were 50.0 and 25.0 per cent & 70.2 and 68.8 per cent, respectively.

Flowers et. al. (1999) discussed the possibilities for early pregnancy diagnosis in sows. Pregnancy diagnosis was possible as early as 25th day after breeding by the detection of fluid in the apex of uterine horn and presence of corpus luteum. However, accurate pregnancy diagnosis was only possible at day 28 by the detection of embryo and its heart beat with 96.0 per cent.

Flowers (2000) noted that the presence of a fetus at 28 to 35 days after insemination was accurately predicted by real-time transrectal and transabdominal ultrasonography,
wherein accumulation of uterine fluid and embryonic membranes were observed. Accuracy of pregnancy diagnosis from days 21 to 23 post insemination were 93 per cent, respectively.

Miller et.al. (2003) performed pregnancy diagnosis in 100 cows between 21 and 70 days post insemination using a linear-array and sector real-time ultrasound scanner with a 3.0 MHz rectal transducer. The total number of correct positive diagnoses (n=97) incorrect positive diagnoses (n = 3 made on days 36, 40 and 44 after A I) made on days 25, 28, 28, 29, 30, 31, and 33 after A I) recorded a sensitivity of 99 per cent, specificity of 45 per cent, accuracy of 95 per cent. From these preliminary results it was concluded that, real-time ultrasound scanning was a useful and reliable technique for early pregnancy diagnosis in sows.

Knox and Flowers (2004) reported that the accuracy of transrectal ultrasound scanning for diagnosis of early pregnancy was more than 90 per cent on days 25 and 29 in sows.

Razdan et.al. (2004) conducted ultrasonographic examinations from day 26 to 58 post insemination in sows. They resulted that under field conditions, accurate results were reached by ultrasound scanning of the uterus from day 29 or 30 after AI, when the presence of (allantoic) fluid was used as the criterion for a positive pregnancy diagnosis.

Cortez et.al. (2006) stated that, the real-time B-mode ultrasonography was the most accurate method for the diagnosis of early pregnancy in sows. Ultrasonographic pregnancy diagnosis performed with 3-5 MHz linear-array or sector transducer between 25 and 70 days after AI in swine showed, sensitivity of 95 and 99 per cent, respectively and the specificity of the test varied greatly, ranging from 76 to 97 per cent, respectively.
92 per cent accuracy in diagnosing pregnancy and 80 per cent accuracy in diagnosing non-pregnancy.

Boma and Bilkei (2008) observed the field experiences with early pregnancy diagnosis carried out in two methods transrectal ultrasonography between days 17-22 postmating by ultrasound. Sensitivity and specificity was 97 and 89 per cent.

Williams et.al. (2008) compared ultrasound scan of uterus with Doppler Echo+ for diagnosing early pregnancy in sows. A considerable difference was noted between the reliability of the scanning performed at a early stage (days 21 to 25) and those performed at a later stage (days 26 to 33). The sensitivity and specificity of the ultrasound examination between days 21 and 25 were 90 and 45 per cent, respectively.

Nakhashi et.al. (2010) compared the efficacy of ultrasound scanning and plasma progesterone assay to diagnose early pregnancy in Mehsana buffaloes and reported the sensitivity and specificity for early pregnancy through ultrasound scanning on day 20 were 75.0 and 66.7 per cent, respectively.

Pawshe et.al. (2011) reported that, the conceptus was first detected on day 20 to day 22. The embryonic vesicle was first observed on mean day 19.2 ± 1 which was initially spherical in shape with a mean height of 7.03 ± 1.30 mm on day 18 and gradually increases to 47.80 ± 2.61 mm on day 50.

Ferreira et.al. (2012) aimed to monitor the early pregnancy development in buffaloes and to establish biometric threshold of different gestational vesicles and embryo or fetal parts from the day of diagnosis to the 70th day of pregnancy. The gestational vesicle was assessed on days 20.55 ± 2.34 of pregnancy.
Ivan Stancic *et.al.* (2012) evaluated the sensitivity and specificity of pregnancy diagnosis using real-time ultrasonography between sows and gilts from days 17 to 24. In sows, sensitivity was 100 per cent at day 29. Specificity was 96.6 per cent on day 26. In heifers, sensitivity was 100 per cent at day 26. The sensitivity for sows and gilts was 89.2 and 96.8 per cent, respectively and the specificity was 93.0 and 93.4 per cent.

Moharrami *et.al.* (2013) evaluated the accuracy rate of early pregnancy diagnosis in Holstein heifers using transrectal ultrasonography during days 24, 26 and 28. They observed that the sensitivity and specificity was 93.05 and 100 per cent, respectively on day 24. Positive and negative predictive values were 100 and 84.86 per cent on day 24, respectively.

### 2.5.2 ULTRASONOGRAPHY ON DAY 25-29 POST INSEMINATION:

Taverne *et.al.* (1985) performed pregnancy diagnosis in 201 cows between 21 and 70 days post insemination using a linear-array real-time ultrasound scanner with a 3.0 MHz rectal transducer. Sensitivity, specificity, positive predictive value and negative predictive value were 94.8, 95.3, 97.7 and 89.8 per cent respectively. From these preliminary results it was concluded that, real-time ultrasound scanning was a useful and reliable technique for early pregnancy diagnosis in cows.

Flowers *et.al.* (1999) worked on ultrasound scanning for diagnosis of pregnancy in sows between 30 and 45 days post insemination and recorded the overall accuracy of pregnancy diagnosis as 98.3 per cent.

Flowers and Knox (2000) studied the accuracy of pregnancy diagnosis by real-time transrectal ultrasonography at different intervals after AI and it was found that
ultrasonography results were 100 per cent reliable for negative diagnosis from 40 days onwards and found to be positive upon ultrasonography examination after 30 days.

Miller *et al.* (2003) compared the efficacy of transabdominal ultrasound scanning and transrectal ultrasonography to diagnose early pregnancy in sows and reported the sensitivity and specificity for early pregnancy through ultrasound scanning on day 30 of gestation as 100 and 100 per cent, visualized the embryonic vesicle and or embryo clearly in every animal that was found pregnant from day 30 post breeding.

Flowers (2000) expressed that, the presence of an embryo within the embryonic vesicle in sows and gilts were confirmed by observing an echogenic area with rhythmic pulsations (heartbeat). The embryonic vesicle gradually increased in length from the day of first observation until day 26 when it extended past the curvature of the horn and began to encroach into the contralateral horn. The embryo was first visible between days 26 and 29 when the mean length was 10 mm.

Gaggini *et al.* (2012) detected both embryonic vesicle and embryo proper in pregnant sows on day 40 post insemination using ultrasound scanning. They reported that the sensitivity, specificity, positive and negative predictive values as 93.75, 100, 100 and 90.91 per cent, respectively.

### 2.5.3 ULTRASONOGRAPHY FROM DAY 30 POST INSEMINATION:

Almond and Dial (1987) worked on ultrasound scanning for diagnosis of pregnancy in sows between 30 and 68 days post insemination, recorded the overall accuracy for pregnancy diagnosis as 98.3 per cent.

Miller *et al.* (2003) expressed that, the presence of an embryo within the embryonic vesicle in sow was confirmed by observing an echogenic area with rhythmic pulsations (heartbeat). In all sows, by day 32 the vesicle extended to the tip of the contralateral horn.
Kauffold and Althouse (2007) performed pregnancy diagnosis in sows at 30 to 35 day but occasionally was done upto 45 days by ultrasonography using B-mode diagnostic ultrasound scanner equipped with a linear array 5 MHz transducer and observed that the embryonic vesicle was seen as bean shaped at early stages of pregnancies. The mean length of the embryonic vesicle increased from 2.3 cm at 30 days to 4.1 cm at 45 days pregnancy and found 97.9 per cent accuracy in diagnosing pregnancy which was confirmed by ultrasonography at 3 months of pregnancy.

Williams (2008) performed pregnancy diagnosis in sows between 21 and 70 days post insemination using a linear-array real-time ultrasound scanner with a 3.0 MHz rectal transducer. Sensitivity, specificity, positive predictive value and negative predictive value were 94.8, 95.3, 97.7 and 89.8 per cent respectively. From these preliminary results it was concluded that, real-time B ultrasound scanning was a useful and reliable technique for early pregnancy diagnosis in sows.

Radu et. al. (2012) conducted studies on reproductive performance of Landrace x Yorkshire crossbreed sows used transabdominal scanning and revealed that the accuracy was 92 per cent on 21-28 days of gestation.

Stancic (2012) studied the pregnancy diagnosis in 132 gilts and sows using a 3 MHz ultrasonic transducer on day 41 post insemination, reported that the accuracy of positive diagnosis (99.17 per cent) was higher than the negative diagnosis (89.7 per cent).

2.6 DOPPLER ECHO (A-mode):

Doppler Echo+ of the uterus for pregnancy diagnosis in sows was first described in the 1974’s (Weiss, 1975) which was the oldest and most widely used method for early pregnancy diagnosis in sows.
Too *et.al.* (1974) observed the pregnancy diagnosis in the pig and fetal heart rate changes during pregnancy between the days 30-39 and after 40 with the accuracy of 81.8 and 100 per cent.

Weiss (1975) discussed the possibilities for early pregnancy diagnosis in sows. Pregnancy diagnosis was possible as early as 26\textsuperscript{th} day after breeding by the detection of rhythmic pulsation from uterine artery. However, accurate pregnancy diagnosis was only possible at day 28 by the detection of embryo and its heart beat.

Almond and Dial (1986) reported that, the conceptus was detected with a high accuracy on day 26 to 45 with fetal heart beats which was important for confirmation of pregnancy in sows by doppler.

Atkinson (1986) aimed to monitor the early pregnancy development in sows and to establish biometric threshold of different gestational vesicle and embryo or fetal parts from the day of diagnosis to the 70\textsuperscript{th} day of pregnancy. The embryo and its heart beats were assessed, respectively, by gestational days of pregnancy.

Almond and Dial (1987) reported that, the conceptus was detected with a high accuracy on day 26 to 30 with fetal heart beats which was important for confirmation of pregnancy in sows by ultrasonography with the accuracy of 86 per cent.

Flowers *et.al.* (1999) worked on ultrasound scanning and doppler for diagnosis of pregnancy in sows between 30 and 40 days post insemination, recorded the overall accuracy for pregnancy diagnosis as 94.5 per cent.
Flowers (2000) expressed that the presence of an embryo within the embryonic vesicle in sows was confirmed by observing an echogenic area with rhythmic pulsations (heartbeat). In all gilts, by day 32, the vesicle extended to the tip of the contralateral horn.

Kauffold and Althouse (2007) observed sows for early pregnancy diagnosis by ultrasonography on 25-30\textsuperscript{th} day post artificial insemination and confirmed pregnancy by Doppler echo+ on 35\textsuperscript{th} day post insemination. In ultrasonography, pregnant animals showed presence of embryo with heart beats, in one of the compartment of the embryonic vesicle which was used as a confirmatory sign of early pregnancy. Overall accuracy of ultrasonography in detecting pregnancy and non-pregnancy was 97.56 per cent.

Cowart (2008) reported that the accuracy of doppler ultrasound scanning for diagnosis of early pregnancy was 85 per cent on days 29 and 35 in gilts and sows.

Gaggini (2012) performed doppler ultrasound to detect and monitor the early conceptus, its growth and its anatomical structures in sows between days 28 and 35 of gestation, reported that the heart beat of embryo proper could be detected on day 29.6 ± 1.57.

2.7 Visual Inspection:

One of the objectives of current study was to standardize and correlate with pregnancy at 45, 60 and 90 days OF gestation. The abdominal distension, udder development was used as a tool for pregnancy diagnosis at 60-90 days post insemination Almond and Dial (1986) and Solmon raju (1991).
2.7.1 Postural behaviour of the sows during gestation.

The literature on the postural behaviour of the sows beginning from the day of conception to farrowing was not available, though very few reports were found regarding behaviour of sows during later part of gestation, which were reviewed hereunder.

A few days prior to the onset of farrowing, most of the sows were either in the lateral recumbency (Jones, 1966) or avoid activities and situations that might cause injury to the fetuses (Sambraus et al., 1978).

2.7.2 Abdominal distension:

The information regarding the visible changes in the abdomen during the gestation period in the sows was not available.

2.7.3 Mammary gland development:

The information on the various visible changes that occur in the mammary gland during the gestation period was also not available in detail. Only stray reports were found which were reviewed.

Jones (1966), Hughes and Varley (1980), Gordon (1983) and Hurnik (1985) reported well developed flaccid mammary gland from several days prior to farrowing, which began to enlarge assuming a cone shape of the individual extremity and becoming turgid a few hours to days prior to farrowing.

Hafez (2000) observed the lobulo-alveolar development of the mammary gland by day 45 of pregnancy, was well developed by day 60.
CHAPTER III
MATERIALS AND METHODS

The present investigation on evaluation of five different methods of early pregnancy diagnosis in sows was carried out in the Department of Veterinary Gynecology and Obstetrics in collaboration with Instructional Livestock Farm Complex, College of Veterinary Science, Rajendranagr, Hyderabad. The study was conducted over a period of six months (from December, 2015 to May, 2016) with an objective to evolve an accurate, simple, and best suitable technique of early pregnancy diagnosis in sows of LWY breed.

3.1 EXPERIMENTAL SOWS

LWY breed of sows aged 6 to 8 months, between second to third farrowing and weighed between 90 to 150kg stationed at ILFC, Rajendranagar and private farm at Mahabubnagar were utilized for the present study. The sows were tagged and housed under uniform conditions of feeding and management and reared in intensive system. Scanning was done by using ultra sound scanner* with 5-7.5 MHz trans-abdominal probe for detecting reproductive abnormalities and pregnancy resulted from previous breeding. The sows without abnormalities were selected and dewormed and vaccinated as per the schedule. After onset of estrus, the sows were bred after 8-12 hours by Artificial Insemination using liquid semen.

3.2 EXPERIMENTAL DESIGN AND METHODS

All selected sows for present study were separated and grouped according to breeding records. All the inseminated LWY Sows did not return to estrus by day 19-21 after breeding were subjected to pregnancy diagnosis using different early pregnancy diagnostic methods. Pregnancy diagnosis was initiated from day 20 post insemination in present study.
Sows which were non-return to estrus by the day 21 post breeding were subjected to the following pregnancy diagnosis (Table. 1).

**A. Non-return rate:** A total of twenty inseminated sows did not return to estrus by day 19-21 were subjected to pregnancy diagnosis. The sows in estrus were detected by boar.

**B. Salivary crystallization:** A total of twenty inseminated sows did not return to estrus by day 19-21 were subjected to pregnancy diagnosis by salivary sampling test on day 30 post insemination.

**C. Punyakoti test:** Early pregnancy diagnosis was carried out on twenty inseminated sows using Punyakoti test i.e., seed germination inhibition test on day 26 post insemination.

**D. Real-time B mode Ultrasonography:** In this group, pregnancy diagnosis was done on thirty inseminated sows using Trans-abdominal ultrasonography on day 20, 25 and 30 post insemination.

**E. Doppler Echo (A-mode):** Early pregnancy diagnosis was carried out on twenty inseminated sows using Doppler echo (A-mode) on days 30-35 post insemination.

1. ILFC farm.
2. Saliva collection on glass slides.
3. Wheat seeds in petri dish.
4. Ultrasonoghraphy- ALOKA Prosound with 5-7.5 MHz trans-abdominal sector probe, Japan.
**Visual inspection**: Pregnancy diagnosis was carried out on all inseminated sows based on Visual Inspection displayed external physical signs of pregnancy between 60 and 90 days of pregnancy.

All the above pregnancy diagnosis methods results were confirmed with the real-time B mode ultrasonography on days 45-60 by considering the definite signs of pregnancy viz., amniotic vesicle and fetometry.

**Table.1: Methods of early pregnancy diagnosis in LWY sows.**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Diagnosis method</th>
<th>Days after insemination</th>
<th>No. of Sows</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Non return to estrus</td>
<td>21</td>
<td>20</td>
</tr>
<tr>
<td>2.</td>
<td>Salivary sampling test</td>
<td>30</td>
<td>20</td>
</tr>
<tr>
<td>3.</td>
<td>Punyakoti test</td>
<td>26</td>
<td>20</td>
</tr>
<tr>
<td>4.</td>
<td>Trans-abdominal ultrasonography</td>
<td>20, 25 and 30</td>
<td>30</td>
</tr>
<tr>
<td>5.</td>
<td>Doppler Echo (A-mode)</td>
<td>30-35</td>
<td>20</td>
</tr>
</tbody>
</table>
PLATE:1 Equipment and materials used for early Pregnancy diagnosis

Saliva sample collection

Petridish containing wheat seeds in punyakoti test

Trans-abdominal ultrasound scanner- Aloka prosound

Doppler echo with Trans-abdominal transducer
3.3 NON-RETURN TO ESTRUS:

This is a rapid detection test that was used for the identification of pregnant and non-pregnant sows on day 21 post insemination. It is a quantitative test indicated by the number of sows that are exposed to boar at the time of testing.

3.3.1 TEST PROCEDURE:

The test was undertaken on day 21 of post insemination using boar exposure for heat detection. The absence of estrus signs indicated the sow as pregnant. The observations were recorded and these sows were tested by real-time B mode ultrasonography on the day 45-60 post insemination.

3.4 SALIVARY CRYSTALLIZATION:

It is known that hormones have a rhythmic variation among the various reproductive phases. The estrogen will be present at significant level present in saliva during early pregnancy, compared to other phases.

3.4.1 COLLECTION OF SALIVA SAMPLES:

The saliva samples from inseminated sows were collected on a glass slide after discarding first few secretions. The saliva samples were air dried thoroughly and tested immediately after collection.

3.4.2 TEST PROCEDURE:

The samples of saliva were obtained daily from day 19 post insemination. Saliva sampling was done using a clean glass rod and these were consequently smeared on glass slide and air dried at room temperature. The samples were microscopically assessed at magnification 400x. Crystallization pattern was classified according to the system adapted by Haberova (2010) as none, dotted, branch-like, fir-like, fern-like, mixed branch-like+fir;
mixed branch–like+fern and mixed fir+fern like. The presence of crystallization in all experimental sows and changes in types of crystallization during early pregnancy were observed.

3.5 PUNYAKOTI TEST:

The present study is the seed germination inhibition test recognized as a door step technology to the farmers. Abscissic acid found in the pregnant sow urine results in decrease germination per cent and shoot growth of seeds.

3.5.1 COLLECTION OF URINE:

The urine samples (n=20) used for detection of pregnancy were collected on day 25 post insemination. The samples were collected during natural micturition as well as by induced micturition, done by continuous stroking of the skin just below the vulva (Rine et al., 2014). The urine samples were collected early in the morning in clean, sterilized and dry plastic containers.

The seed treatment with distilled water (n=10) and diluted urine samples from confirmed non-pregnant sows (n=10) served as control and negative group, respectively.

3.5.2 TEST PROCEDURE:

Seed germination inhibition test was carried out according to the standard procedure (Veena, 2006). About 15 seeds of wheat were placed in a Petri dish containing filter paper added with 15 ml of diluted urine in the ratio of 1:14 (1ml urine + 14 ml distilled water) (Plate. 1). Similarly, a Petri dish containing seeds treated with 15 ml distilled water was kept as control and diluted urine samples from non-pregnant sows were kept as negative group.

The seed germination inhibition and shoot length in centimeters on fifth day were recorded (Rao and Veena, 2009). Inhibition of seed germination and discoloration of seeds
after 48 h and reduced shoot growth after five days were taken as positive criteria for declaring pregnancy. Germination inhibition per cent and shoot length of control, negative and test groups were calculated and compared.

Germination inhibition per cent was calculated as per formula prescribed by Perumal (2014) as follows:

3.5.3 GERMINATION INHIBITION PER CENT (GI): It is defined as the number of seeds not germinated out of the total number of seeds taken in the experiment.

\[
GI\% = \frac{\text{No. of seeds not germinated in Petridish}}{\text{Total no. of seeds taken in Petri dish}} \times 100
\]

3.5.4 SHOOT LENGTH: Shoot length was measured in centimeters and observed for significant difference.

Seed germination inhibition per cent and shoot length were recorded in each treatment group.

3.6 TRANS-ABDOMINAL REAL-TIME B ULTRASONOGRAPHY:

ALOKA Prosound scanner with 5-7.5 MHz transabdominal sector array transducer (Plate.1) was used for scanning the diagnosis of pregnancy in sows.

3.6.1 TECHNIQUE OF TRANS-ABDOMINAL ULTRASONOGRAPHY FOR PREGNANCY DIAGNOSIS:

Transabdominal ultrasonography was done according to the procedure described earlier. For the diagnosis of pregnancy, the probe transabdominal was positioned in direction toward the reproductive tract of sow externally on the abdomen (Almond and Dial, 1987 and Flowers, 2000). The probe was placed in front of the hind leg and lateral to the nipple line. The probe was aimed toward the spine and be angled slightly forward and back to initially visualize the bladder (Knox and Althouse, 1999).
At each examination, an attempt was made to record the first detection of embryonic vesicle and embryo. Positive diagnosis of pregnancy was based on the presence of an anechoic round area of varying size in the lumen of an echogenic uterine lumen representing the fluid filled allantoic cavity termed as the embryonic vesicle (Flowers and Knox, 2000). The presence of an embryo within the embryonic vesicle was confirmed by observing an echogenic (white) area with rhythmic pulsation representing heartbeats as per the observation of Almond and Dial, (1987).

3.6.2 CRITERIA FOR PREGNANCY DIAGNOSIS:

Based on the presence of embryonic vesicle and or embryo proper, diagnosis of pregnancy was made. By ultrasound scanning, different structures found on various days of post insemination were as follows:

3.6.2.1 Ultrasonographic findings on day 20 post insemination

The presence of amniotic vesicle as the anechoic (black) round area in the lumen of echogenic uterine horn was noticed.

3.6.2.2 Ultrasonographic findings on day 25 post insemination

Elongation of embryonic vesicle and visualization of embryo proper as the hypoechoic structure floating within the non-echogenic fluid was observed.

3.6.2.3 Ultrasonographic findings on day 30 and above

Echogenic embryo proper was observed within the anechoic embryonic vesicle. All the different structures were scanned and sonogram was recorded.
3.7 DOPPLER ECHO:

Delta Prosound ultrasonic detector with transabdominal transducer probe was used to detect the sound waves of the audible frequency in the present study.

3.7.1 Technique of Doppler Echo

Doppler devices were designed for pregnancy diagnosis of sows which had transducer probes to be placed on the abdomen. The abdominal probe is positioned on the flank of the sow lateral to the nipple and aimed at the pelvis area. The ultrasound waves are emitted and received by transducer and converted into audible signals. Movements detected include the pulsations of the middle uterine artery and umbilical vessels which were suggestive of pregnancy. Pulsations associated with uterine circulation was described as gushing and swishing sounds at an equal rate to that of maternal heart as per the studies (Cowart, 2008) and friction between the transducer and the blood flow through the umbilical arteries i.e., 150-200 beats per minute.

3.8 VISUAL INSPECTION:

Many sows displayed external physical signs of pregnancy between 60 and 90 days. The signs included were abdominal distension, udder enlargement and enlargement of vulva. These signs were used in confirming a presumed pregnancy.

In the present study, real-time ultrasonography was taken as the gold standard test. Therefore, all the sows that were subjected to different early pregnancy diagnostic tests were scanned transabdominally during 45 to 60 days of early pregnancy for confirmation of pregnancy by observing the definite signs of pregnancy viz., amniotic vesicle and embryo development. Based on the ultrasonography results, the accuracy of these tests were evaluated and compared.
3.9 STATISTICAL ANALYSIS:

The data obtained from all these methods of early pregnancy diagnosis were analyzed statistically as per the methods described by Snedecor and Cochran (1994) and compared for their efficacy in sows under study. Different terms related to the statistical analysis were as follows:

3.9.1 ACCURACY:

The accuracy of the diagnosis was expressed by sensitivity, specificity, positive and negative predictive values. They were described as per the methods of Flowers et al., 1999 and are as follows:

Sensitivity: It is defined as the per cent of sows found pregnant by any of the pregnancy test out of the total number of sows found pregnant by real-time B ultrasonography.

Specificity: It is defined as the per cent of non-pregnant sows diagnosed non-pregnant by any of the pregnancy test and later confirmed non-pregnant by real-time B ultrasonography or returned to estrus at a later date.

Positive predictive value: It is defined as the per cent of actual pregnant sows out of the total number of sows diagnosed pregnant through any of the diagnostic test.

Negative predictive value: It is defined as the per cent of actual non-pregnant sows out of the total number of animals diagnosed non-pregnant through any of the diagnostic test.

Diagnostic accuracy: It is defined as the per cent of the correct diagnosis out of total number of examinations done by any of the test.

3.9.2 CORRECT AND INCORRECT DIAGNOSIS:

As per Flowers et al., (1999), correct and incorrect diagnosis in the present study is described as follows:
A correct diagnosis is described as:

1. **Diagnosis pregnant correct:** A sow diagnosed pregnant with any of the test and subsequently confirmed pregnant during real-time B ultrasonography between 45 to 60 days.

2. **Diagnosis non-pregnant correct:** A sow diagnosed non-pregnant with any of the test and subsequently confirmed non-pregnant during real-time B ultrasonography or returned to estrus at a later date.

An incorrect diagnosis was defined as:

1. **Diagnosis pregnant incorrect:** A sow diagnosed pregnant with any of the test and subsequently confirmed non-pregnant during real-time B ultrasonography or returned to estrus at a later date.

2. **Diagnosis non-pregnant incorrect:** An animal diagnosed non-pregnant with any of the test and subsequently confirmed pregnant during real-time B ultrasonography.

3.9.3 **Calculation of accuracy of different early Pregnancy diagnostic methods:**

Accuracy in the form of sensitivity, specificity, Positive and Negative predictive values for each of the early pregnancy detection technique was calculated as per the formulas given by Flowers *et. al.*, (1999) and were depicted as follows:

<table>
<thead>
<tr>
<th>Pregnant</th>
<th>Non-pregnant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnosis pregnant correct (A)</td>
<td>Diagnosis non-pregnant correct (C)</td>
</tr>
<tr>
<td>Diagnosis pregnant incorrect (B)</td>
<td>Diagnosis non-pregnant incorrect (D)</td>
</tr>
</tbody>
</table>

Number of pregnant sows = A+D

Number of non-pregnant sows = B+C
Sensitivity = \( \frac{A}{A + D} \times 100 \)

Specificity = \( \frac{C}{C + B} \times 100 \)

Positive predictive value = \( \frac{A}{A + B} \times 100 \)

Negative predictive value = \( \frac{C}{C + D} \times 100 \)

Overall diagnostic accuracy = \( \frac{A + C}{A + B + C + D} \times 100 \)

3.9.4 Comparison of different methods of early Pregnancy Diagnosis:

The data regarding sensitivity, specificity, positive and negative predictive values of different early pregnancy detection techniques that were undertaken in the present study were compared.
CHAPTER IV

RESULTS

The investigation on comparative evaluation of five methods on diagnosis of early pregnancy in LWY sows was carried out over a period of six months (from December 2015 to May 2016). All the sows were bred by artificial insemination during estrus and were subjected to five pregnancy diagnostic techniques from day 20 post insemination to evaluate the efficacy of each method.

The methods of early pregnancy diagnosis that were undertaken in the present study were Non-return rate (n=20), Salivary crystallization test (n=20), Punyakoti test (n=20), Trans-abdominal ultrasonography (n=30), Doppler ultrasound (n=20) and Visual Inspection changes from day 45 of gestation. All experimental sows (n=110) subjected to early pregnancy detection were later confirmed by real-time B mode trans-abdominal ultrasonography from day 45-60 by considering the definite signs of pregnancy.

4.1 NON-RETURN TO ESTRUS

Non-return to estrus method was observed on sows from 19-21 day after breeding. Out of 20 sows tested for non-return to estrus, 15 (75 per cent) were not exhibited estrus diagnosed as pregnant, 5 (25 per cent) of sows exhibited estrus were assumed as non-pregnant. As per comparison with the real-time B mode ultrasonography by day 45-60, out of 5 non-pregnant sows diagnosed by this method, 2 (40 per cent) were confirmed non pregnant and 3 (60 per cent) as pregnant. Out of 15 pregnant sows, 14 (93.3 per cent) were confirmed as pregnant and 1 (6.6 per cent) as non-pregnant upon real-time B mode ultrasonography (Table. 2).
4.1.1 Accuracy of Non-return to Estrus

In the present study, non-return rate to estrus was 82.35 per cent accurate in diagnosing pregnancy and 66.66 per cent for non-pregnancy. The evaluation of this method for its sensitivity, specificity, positive and negative predictive values are presented (Table 3).

Table 2: Confirmation of pregnancy diagnosis using non-return to estrus in comparison with real-time B ultrasonography.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Diagnostic results</th>
<th>Non return to estrus (day 21)</th>
<th>Ultrasonography (day 45-60)</th>
<th>Diagnosis incorrect</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Pregnant</td>
<td>15 (75)</td>
<td>14/15 (93.3)</td>
<td>1/15 (6.6)</td>
</tr>
<tr>
<td>2.</td>
<td>Non-pregnant</td>
<td>5 (25)</td>
<td>2/5 (40)</td>
<td>3/5 (60)</td>
</tr>
</tbody>
</table>

*Figures in parentheses are per cent.

Table 3: Accuracy of pregnancy diagnosis using non-return rate in comparison with real-time B ultrasonography.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Particulars</th>
<th>21 days post insemination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of sows tested</td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Diagnosis pregnant correct (a)</td>
<td>14</td>
</tr>
<tr>
<td>2.</td>
<td>Diagnosis pregnant incorrect (b)</td>
<td>1</td>
</tr>
<tr>
<td>3.</td>
<td>Diagnosis non-pregnant correct (c)</td>
<td>2</td>
</tr>
<tr>
<td>4.</td>
<td>Diagnosis non-pregnant incorrect (d)</td>
<td>3</td>
</tr>
<tr>
<td>5.</td>
<td>Sensitivity (Se ; per cent) 100xa/(a+d)</td>
<td>82.35</td>
</tr>
<tr>
<td>6.</td>
<td>Specificity (Sp ; per cent) 100xc/(c+b)</td>
<td>66.66</td>
</tr>
<tr>
<td>7.</td>
<td>Positive predictive value (PPV ; per cent) 100xa/(a+b)</td>
<td>93.33</td>
</tr>
<tr>
<td>8.</td>
<td>Negative predictive value (NPV ; per cent) 100xc/(c+d)</td>
<td>40.0</td>
</tr>
<tr>
<td>9.</td>
<td>Overall diagnostic accuracy 100X(a+c/a+b+c+d)</td>
<td>80.0</td>
</tr>
</tbody>
</table>
4.2 Salivary crystallization:

The saliva crystallization to confirm the new possibility for early pregnancy diagnosis was carried out in 20 sows on day 30 post insemination. Out of 20 sows, the present study diagnosed 7 (35 per cent) sows as pregnant and 13 (65 per cent) as non-pregnant. Out of 7 sows diagnosed pregnant by this test, 1 (14.28 per cent) sow was non-pregnant and out of thirteen sows diagnosed non-pregnant, 3 (23.07 per cent) were pregnant as confirmed later by real-time B mode Ultrasonography between 45-60 days (Table.4). The salivary positive for pregnancy was 66.66 per cent. The different crystallization patterns of saliva of sows during pregnancy were showed in Plate.2.

4.2.1 ACCURACY OF SALIVARY TEST:

The test was 66.66 per cent accurate in diagnosing pregnancy and 90.90 per cent accurate in diagnosing non-pregnancy (Plate.2 & 3). The test sensitivity, specificity, positive and negative predictive values were presented in (Table.5). Salivary crystallization presence of pregnancy were observed and are described as follows.
Table 4: Confirmation of pregnancy diagnosis using salivary crystallization by comparison with the real-time B ultrasonography.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Diagnostic results</th>
<th>Salivary crystallization (day 30)</th>
<th>Ultrasonography (day 45-60)</th>
<th>Diagnosis incorrect</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Pregnant</td>
<td>7 (35)</td>
<td>6/7 (85.72)</td>
<td>1/7 (14.28)</td>
</tr>
<tr>
<td>2.</td>
<td>Nonpregnant</td>
<td>13 (65)</td>
<td>10/13 (76.93)</td>
<td>3/13 (23.07)</td>
</tr>
</tbody>
</table>

*Figures in parentheses are per cent.

Table 5 Accuracy of pregnancy diagnosis using salivary crystallization.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Particular</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of sows tested</td>
<td>20</td>
</tr>
<tr>
<td>1.</td>
<td>Diagnosis pregnant correct</td>
<td>6</td>
</tr>
<tr>
<td>2.</td>
<td>Diagnosis non-pregnant incorrect</td>
<td>1</td>
</tr>
<tr>
<td>3.</td>
<td>Diagnosis non-pregnant correct</td>
<td>10</td>
</tr>
<tr>
<td>4.</td>
<td>Diagnosis non-pregnant incorrect</td>
<td>3</td>
</tr>
<tr>
<td>5.</td>
<td>Sensitivity (Se; per cent) 100xa/(a+d)</td>
<td>66.66</td>
</tr>
<tr>
<td>6.</td>
<td>Specificity (Sp; per cent) 100xc/(c+b)</td>
<td>90.90</td>
</tr>
<tr>
<td>7.</td>
<td>Positive predictive value (PPV; per cent) 100xa/(a+b)</td>
<td>85.71</td>
</tr>
<tr>
<td>8.</td>
<td>Negative predictive value (NPV; per cent) 100xc/(c+d)</td>
<td>76.91</td>
</tr>
<tr>
<td>9.</td>
<td>Overall diagnostic accuracy 100X(a+c/a+b+c+d)</td>
<td>75.0</td>
</tr>
</tbody>
</table>
Plate 2  Showing different crystallization patterns of sow’s saliva

Fir like crystallization pattern.

Combination of fir and fern like crystallization pattern.

Fern like crystallization pattern in estrus on day 21 post insemination.

Combination of Branch and fir like crystallization pattern.

Branch like crystallization pattern.

Combination of Branch and fern like crystallization pattern.
Plate 3: Per cent of salivary crystallization

NONPREGNANT
- BRANCH+FERN 5%
- NONE 10%
- BL+FIL+FERN 20%
- BRANCH+FIR 20%
- FIR 10%
- BRANCH 35%

PREGNANT
- BL+FIL+FEL 10%
- BRANCH+FIR 10%
- NONE 20%
- BRANCH 60%
4.3 PUNYAKOTI TEST:

Seed germination and discoloration after 48 h and shoot lengths after 5 days were observed as follows:

4.3.1 GERMINATION INHIBITION PERCENTAGE:

The germination inhibition per cent of wheat seeds in control, negative and positive groups are presented (Table. 6 & Plate. 4). Germination inhibition per cent as 74.66 ± 4.42 were considered as pregnant in the present study test (Plate.4). Highest germination inhibition was observed in true pregnant sows (Table.6 & Plate.4). The Mean ± SE values of germination inhibition per cent of control, negative and positive sows were 32.66 ± 2.36, 52.44 ± 2.33 and 74.66 ± 4.42 per cent, respectively (Table. 6). A significant difference (p<0.05) regarding germination inhibition per cent among the three groups noticed.

4.3.2 SHOOT LENGTH:

The shoot length of wheat seeds in control, negative and positive groups are presented (Table. 6). The shoot length of less than 3.4 cm was considered as true pregnant in the present study. Shoot length was less in sows that were diagnosed as pregnant (Plate. 6). The shoot length of control, negative and positive sows were 7.46 ± 0.82, 5.44 ± 0.092 and 3.33 ± 0.061 cm, respectively (Table. 6). A significant difference (p<0.05) regarding shoot length among three groups were recorded in the present study.

Out of 20 sows, the present study has diagnosed 5 (25 per cent) as pregnant and 15 (75 per cent) as non-pregnant. Out of 5 sows diagnosed pregnant by the present study, 1 (20.0 per cent) of them was non-pregnant and out of fifteen sows diagnosed non-pregnant, 4 (26.67 per cent) were pregnant with diagnosed by real time B mode trans-abdominal
ultrasonography from day 45-60 (Table. 7). The germination inhibition per cent was 74.66 per cent or more, while shoot length was 3.4 cm or less in sows that were diagnosed as pregnant correct by the present test.

**4.3.3 ACCURACY OF PUNYAKOTI TEST:**

In present study, 57.14 per cent were accurate in diagnosing pregnancy but 91.67 per cent accurate in diagnosing non-pregnancy. The test sensitivity, specificity, positive and negative predictive values are presented in Table-8.
Plate 4 Showing Shoot length (cm) of Wheat seeds

NON-PREGNANT

PREGNANT

CONTROL
Table 6: Showing germination inhibition per cent and shoot length of wheat seeds in different groups.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Particulars</th>
<th>Control</th>
<th>Non-pregnant</th>
<th>Pregnant</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Germination inhibition (per cent)</td>
<td>32.66 ± 2.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>52.44 ± 2.33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>74.66 ± 4.42&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>2.</td>
<td>Shoot length (cm)</td>
<td>7.46 ± 0.82&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5.44 ± 0.092&lt;sup&gt;e&lt;/sup&gt;</td>
<td>3.33 ± 0.061&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means with different superscripts in a row differed significantly. : p<0.05

Table 7: Confirmation of pregnancy diagnosis using Punyakoti test in comparison with real-time B mode ultrasonography.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Diagnostic results</th>
<th>Punyakoti test (day 21)</th>
<th>ultrasonography (day45-60)</th>
<th>Diagnosis incorrect</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Pregnant</td>
<td>5 (25)</td>
<td>4/5 (80.0)</td>
<td>1/5 (20.0)</td>
</tr>
</tbody>
</table>

*Figures in parentheses are per cent.

Table 8 Accuracy of pregnancy diagnosis using Punyakoti test.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Particulars</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of sows tested</td>
<td>20</td>
</tr>
<tr>
<td>1.</td>
<td>Diagnosis pregnant correct</td>
<td>4</td>
</tr>
<tr>
<td>2.</td>
<td>Diagnosis non-pregnant incorrect</td>
<td>1</td>
</tr>
<tr>
<td>3.</td>
<td>Diagnosis non-pregnant correct</td>
<td>11</td>
</tr>
<tr>
<td>4.</td>
<td>Diagnosis non-pregnant incorrect</td>
<td>4</td>
</tr>
<tr>
<td>5.</td>
<td>Sensitivity (Se ; per cent) 100xa/(a+d)</td>
<td>57.14</td>
</tr>
<tr>
<td>6.</td>
<td>Specificity (Sp ; per cent) 100xc/(c+b)</td>
<td>91.67</td>
</tr>
<tr>
<td>7.</td>
<td>Positive predictive value (PPV ; per cent) 100xa/(a+b)</td>
<td>80.00</td>
</tr>
<tr>
<td>8.</td>
<td>Negative predictive value (NPV ; per cent) 100xc/(c+d)</td>
<td>73.31</td>
</tr>
</tbody>
</table>
Overall diagnostic accuracy $100\times \frac{a+c}{a+b+c+d}$

75.0

Plate 5 Showing Germination inhibition per cent of test groups in Punyakoti test

Plate 6 Showing mean values of shoot length (cm) of test groups in Punyakoti test
4.4. REAL-TIME B ULTRASONOGRAPHY FOR PREGNANCY DIAGNOSIS:

A total of 30 sows were scanned using trans-abdominal ultrasound of 5-7.5 MHz sector array transducer for early pregnancy diagnosis on day 20, 25 and 30 post insemination. These sows were scanned in the respective stage of gestation by ultrasonography and pregnancy was confirmed with re-examination by real-time ultrasonography between 45-60 days post insemination. The ultrasound results at different stages of gestation are presented in Plate. 7, 8, 9, 10, 11, 12, 13 and 14.

4.4.1 ULTRASONOGRAPHY ON DAY 20 POST-INSEMINATION:

Out of 30 sows scanned on day 20 post insemination, 4 (13.3 per cent) were diagnosed as pregnant and 26 (86.67 per cent) as non-pregnant. The sows diagnosed pregnant showed anechoic amniotic/embryonic vesicle within the lumen of echogenic uterine horn. Initially the embryonic vesicle was spherical in shape. The measurements of
embryonic vesicle ranged between 2.1 to 2.4 cm. Visualization of echogenic embryo was not detected on day of examination (Table 9).

4.4.2 ULTRASONOGRAPHY ON DAY 25 POST INSEMINATION:

All the 30 sows were again scanned on day 25 post insemination. Out of 30 sows, 6 (20 per cent) were diagnosed as pregnant and 24 (80 per cent) as non-pregnant. The sows that were diagnosed pregnant showed irregular shaped embryonic vesicle with expansion of vesicle and divided into compartments. The measurements of the embryonic vesicle ranged between 2.8 to 3.0 cm and the embryo was 0.8 to 1.2 cm (Table.10). In 4 pregnant sows, embryo proper was detected as echogenic structure with in the anechoic embryonic vesicle (Table.9).

4.4.3 ULTRASONOGRAPHY ON DAY 30 POST INSEMINATION:

Subsequently all the 30 sows were again scanned on day 30 post insemination. Embryo was clearly visualized as an echogenic structure within the anechoic embryonic vesicle. Out of 30 sows, 8 (26.66 per cent) were diagnosed as pregnant and 22 (73.34 per cent) as non-pregnant. All the sows that were diagnosed as pregnant showed presence of embryonic vesicle and embryo proper. The measurements of embryonic vesicle and embryo ranged between 1.5 cm and 1.8 cm, respectively (Table.10).

The correct and incorrect diagnosis of pregnancy, non-pregnancy and their accuracies on day 20, 25 and 30 post insemination, respectively are represented in Table.11-14. Out of 4 sows that were diagnosed non-pregnant on day 20 post insemination, 2 were pregnant on day 25 and 4 were pregnant on day 30 which were confirmed by ultrasonography on day 45-60 post insemination.
4.4.4 DESCRIPTION OF EMBRYONIC VESICLE AND EMBRYO PROPER:

The embryonic vesicle displayed a spherical shape with distinct outline limits on day 20. Later, on day 25 it was visualized as an elongated structure and divided into two compartments which were called as compartmentalization. The embryo proper was clearly visualized as an echogenic structure within the uterine lumen on day 30 of gestation. The average lengths of embryonic vesicle on day 20, 25 and 30 were 2.33 ± 0.06, 2.64 ± 0.06 and 3.07 ± 0.072 cm, respectively (Table.9). The average lengths of embryo on day 25, 30 and 35 were 1.05 ± 0.04, 1.37 ± 0.02 cm, and 1.668 ± 0.05 cm, respectively (Table.9). The mean lengths of embryonic vesicle and embryo proper during different stages of gestation are presented.

Table. 9: Measurements of embryonic vesicle and embryo at different stages of Gestation.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Days of gestation</th>
<th>Embryonic vesicle</th>
<th>Size of embryonic vesicle (cm)</th>
<th>Embryo</th>
<th>Size of embryo (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>(Mean ± S.E)</td>
<td></td>
<td>(Mean± S.E)</td>
</tr>
<tr>
<td>1.</td>
<td>20</td>
<td>4/8 (50)</td>
<td>2.33 ± 0.070</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>25</td>
<td>6/8 (75)</td>
<td>2.64 ± 0.063</td>
<td>5/8 (62.5)</td>
<td>1.05 ± 0.04</td>
</tr>
<tr>
<td>3.</td>
<td>30</td>
<td>8/8 (100)</td>
<td>3.07 ± 0.072</td>
<td>6/8 (75)</td>
<td>1.37 ± 0.02</td>
</tr>
</tbody>
</table>

*Figures on parentheses are per cent.
Table. 10: Measurements of amniotic vesicle and CRL of embryo in different days of gestation.

<table>
<thead>
<tr>
<th>S.NO</th>
<th>DAYS</th>
<th>20</th>
<th>25</th>
<th>30</th>
<th>35</th>
<th>40</th>
<th>45</th>
<th>50</th>
<th>55</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>AMNIOTIC VESICLE diameter</td>
<td>2.2cm</td>
<td>3.0cm</td>
<td>4.5cm</td>
<td>4.9cm</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>EMBRYO SIZE (CRL) diameter</td>
<td>1.1cm</td>
<td>1.2 cm</td>
<td>1.5cm</td>
<td>1.8cm</td>
<td>2.1cm</td>
<td>2.6cm</td>
<td>3.1cm</td>
<td>3.7cm</td>
</tr>
</tbody>
</table>

Table. 11: Accuracy of ultrasonography on day 20 post insemination in comparison with real-time B ultrasonography on day 45-60 post insemination.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Particulars</th>
<th>Day 20 post insemination (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Diagnosis pregnant correct (a)</td>
<td>4</td>
</tr>
<tr>
<td>2.</td>
<td>Diagnosis pregnant incorrect (b)</td>
<td>4</td>
</tr>
<tr>
<td>3.</td>
<td>Diagnosis non-pregnant correct (c)</td>
<td>18</td>
</tr>
<tr>
<td>4.</td>
<td>Diagnosis non-pregnant incorrect (d)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Description</td>
<td>Value</td>
</tr>
<tr>
<td>---</td>
<td>------------------------------------------------------------------------------</td>
<td>--------</td>
</tr>
<tr>
<td>5</td>
<td>Sensitivity (Se; per cent) $100 \times a/(a+d)$</td>
<td>50.0</td>
</tr>
<tr>
<td>6</td>
<td>Specificity (Sp; per cent) $100 \times c/(b+c)$</td>
<td>81.81</td>
</tr>
<tr>
<td>7</td>
<td>Positive predictive value (PPV; per cent) $100 \times a/(a+b)$</td>
<td>50.0</td>
</tr>
<tr>
<td>8</td>
<td>Negative predictive value (NPV; per cent) $100 \times c/(c+d)$</td>
<td>81.81</td>
</tr>
<tr>
<td>9</td>
<td>Overall diagnostic accuracy $100 \times (a+c)/(a+b+c+d)$</td>
<td>73.33</td>
</tr>
</tbody>
</table>

Table 12: Accuracy of ultrasonography on day 25 post insemination in comparison with real-time B mode ultrasonography on day 45-60 post insemination.
<table>
<thead>
<tr>
<th>S.No</th>
<th>Particulars</th>
<th>Day 25 post insemination (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Diagnosis pregnant correct  (a)</td>
<td>6</td>
</tr>
<tr>
<td>2.</td>
<td>Diagnosis pregnant incorrect  (b)</td>
<td>0</td>
</tr>
<tr>
<td>3.</td>
<td>Diagnosis non-pregnant correct (c)</td>
<td>22</td>
</tr>
<tr>
<td>4.</td>
<td>Diagnosis non-pregnant incorrect (d)</td>
<td>2</td>
</tr>
<tr>
<td>5.</td>
<td>Sensitivity (Se ; per cent) 100xa/(a+d)</td>
<td>75.0</td>
</tr>
<tr>
<td>6.</td>
<td>Specificity (Sp ; per cent) 100xc/(b+c)</td>
<td>100</td>
</tr>
<tr>
<td>7.</td>
<td>Positive predictive value (PPV ; per cent) 100xa/(a+b)</td>
<td>100</td>
</tr>
<tr>
<td>8.</td>
<td>Negative predictive value (NPV ; per cent) 100xc/(c+d)</td>
<td>91.66</td>
</tr>
<tr>
<td>9.</td>
<td>Overall diagnostic accuracy100x(a+c)/(a+b+c+d)</td>
<td>93.33</td>
</tr>
</tbody>
</table>
Table. 13: Accuracy of ultrasonography on day 30 post insemination in comparison with real-time B ultrasonography on day 45 post insemination.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Particulars</th>
<th>Day 30 post insemination(n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Diagnosis pregnant correct (a)</td>
<td>8</td>
</tr>
<tr>
<td>2.</td>
<td>Diagnosis pregnant incorrect (b)</td>
<td>0</td>
</tr>
<tr>
<td>3.</td>
<td>Diagnosis non-pregnant correct (c)</td>
<td>22</td>
</tr>
<tr>
<td>4.</td>
<td>Diagnosis non-pregnant incorrect (d)</td>
<td>0</td>
</tr>
<tr>
<td>5.</td>
<td>Sensitivity (Se ; per cent) 100xa/(a+d)</td>
<td>100</td>
</tr>
<tr>
<td>6.</td>
<td>Specificity (Sp ; per cent) 100xc/(b+c)</td>
<td>100</td>
</tr>
<tr>
<td>7.</td>
<td>Positive predictive value (PPV ; per cent) 100xa/(a+b)</td>
<td>100</td>
</tr>
<tr>
<td>8.</td>
<td>Negative predictive value (NPV ; per cent) 100xc/(c+d)</td>
<td>100</td>
</tr>
<tr>
<td>9.</td>
<td>Overall diagnostic accuracy 100x(a+c)/(a+b+c+d)</td>
<td>100</td>
</tr>
</tbody>
</table>

Table. 14: Overall accuracy of early pregnancy diagnosis using ultrasonography in comparison with real-time B ultrasonography on day 45-60 post insemination.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Particulars</th>
<th>Days after insemination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diagnosis pregnant correct (a)</td>
<td>Day 20</td>
</tr>
<tr>
<td>---</td>
<td>--------------------------------</td>
<td>--------</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>Diagnosis pregnant incorrect (b)</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>Diagnosis non-pregnant correct (c)</td>
<td>18</td>
</tr>
<tr>
<td>4</td>
<td>Diagnosis non-pregnant incorrect (d)</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>Sensitivity (Se ; per cent) 100xa/(a+d)</td>
<td>50.0</td>
</tr>
<tr>
<td>6</td>
<td>Specificity (Sp ; per cent) 100xc/(c+b)</td>
<td>81.81</td>
</tr>
<tr>
<td>7</td>
<td>Positive predictive value (PPV ; per cent) 100xa/(a+b)</td>
<td>50.0</td>
</tr>
<tr>
<td>8</td>
<td>Negative predictive value (NPV ; per cent) 100xc/(c+d)</td>
<td>81.81</td>
</tr>
<tr>
<td>9</td>
<td>Overall diagnostic accuracy 100X(a+c)/(a+b+c+d)</td>
<td>73.33</td>
</tr>
</tbody>
</table>

Plate. 7 Anechogenic smaller amniotic vesicles on day 20 of gestation.
Plate. 8 Anechogenic amniotic vesicle on day 25 of gestation.

Plate. 9 Real-time B mode Ultrasonography image of day 30 with amniotic vesicle and echogenic embryo.
Plate 10 Real-time B Ultrasonography image on day 45 of gestation with fetus in anechogenic vesicle.

Plate 11 Real-time B mode ultrasonography image on day 55 of gestation.
Plate.12 Real-time B Ultrasonography image on day 65 of gestation
Plate 13 Real-time B Ultrasonography image on day 78 of gestation.

Plate 14 Real-time B mode ultrasonography shown length of BPD
4.5. Doppler Echo (A-Mode)

Early pregnancy diagnosis was performed in 20 sows using Doppler Echo from day 30 to 35 post insemination (Plate. 15). Out of 20 sows tested for audible signals by Doppler echo, 9 (45 per cent) were diagnosed as pregnant upon observation of audible signal sounds from blood flow of umbilical and uterine arteries and 11 (55 per cent) as non-pregnant upon appearance of absence of audible sounds. Out of 11 non-pregnant sows diagnosed by present study, 9 (81.81 per cent) were confirmed non-pregnant and 2 (18.19 per cent) as pregnant upon real time B ultrasonography. Out of 9 pregnant sows, 8 (88.8 per cent) were confirmed as pregnant and 1 (11.2 per cent) as non-pregnant upon real time B ultrasonography between 45-60 days (Table. 15).

4.5.1 Accuracy of Doppler Echo

In the present study, Doppler ultrasound was 80 per cent accurate in diagnosing pregnancy and 90 per cent for non-pregnancy. The evaluation of this method for its sensitivity, specificity, positive and negative predictive values are presented (Table. 15 & 16).

Table.15: Confirmation of pregnancy diagnosis using Doppler echo in comparison with the real-time B mode ultrasonography.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Diagnosis</th>
<th>Doppler echo (day 30)</th>
<th>Ultrasonography (day 45-60)</th>
<th>Diagnosis incorrect</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Pregnant</td>
<td>9(45)</td>
<td>8/9(88.8)</td>
<td>1/9(11.2)</td>
</tr>
<tr>
<td>2.</td>
<td>Non-pregnant</td>
<td>11(55)</td>
<td>9/11(81.81)</td>
<td>2/11(18.19)</td>
</tr>
</tbody>
</table>

*Figures in parentheses are per cent.
Table. 16: Accuracy of pregnancy diagnosis using Doppler echo (A-mode) in comparison with real-time B ultrasonography on day 45-60 post insemination.

<table>
<thead>
<tr>
<th>S.no</th>
<th>Diagnosis particulars</th>
<th>Diagnosis at day 30 post insemination (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Diagnosis pregnant correct (a)</td>
<td>8</td>
</tr>
<tr>
<td>2.</td>
<td>Diagnosis pregnant incorrect (b)</td>
<td>1</td>
</tr>
<tr>
<td>3.</td>
<td>Diagnosis non-pregnant correct (c)</td>
<td>9</td>
</tr>
<tr>
<td>4.</td>
<td>Diagnosis non-pregnant incorrect (d)</td>
<td>2</td>
</tr>
<tr>
<td>5.</td>
<td>Sensitivity (Se ; per cent) $100x_a/(a+d)$</td>
<td>80.0</td>
</tr>
<tr>
<td>6.</td>
<td>Specificity (Sp ; per cent) $100x_c/(c+b)$</td>
<td>90.0</td>
</tr>
<tr>
<td>7.</td>
<td>Positive predictive value (PPV ; per cent)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$100x_a/(a+b)$</td>
<td>88.88</td>
</tr>
<tr>
<td>8.</td>
<td>Negative predictive value (NPV ; per cent)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$100x_c/(c+d)$</td>
<td>81.81</td>
</tr>
<tr>
<td>9.</td>
<td>Overall diagnostic accuracy $100x(a+c)/(a+b+c+d)$</td>
<td>85.0</td>
</tr>
</tbody>
</table>

Plate.15 Performing Doppler Echo (A-mode) in Pregnant sow
Table 17 ACCURACY OF PREGNANCY DIAGNOSIS BY FETAL DOPPLER DURING DIFFERENT GESTATION PERIOD IN ALL EXPERIMENTAL LWY SOWS:

<table>
<thead>
<tr>
<th>DAY OF GESTATION</th>
<th>NO. OF CASES EXAMINED</th>
<th>CORRECTLY DIAGNOSED (*1)</th>
<th>INCORRECTLY DIAGNOSED *2</th>
<th>ACCURACY RATE PERCENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>22-29</td>
<td>12</td>
<td>7(3)</td>
<td>5</td>
<td>58.3</td>
</tr>
<tr>
<td>30-39</td>
<td>22</td>
<td>18(3)</td>
<td>4</td>
<td>81.8</td>
</tr>
<tr>
<td>40-49</td>
<td>11</td>
<td>11(1)</td>
<td>0</td>
<td>100.0</td>
</tr>
<tr>
<td>50-59</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>100.0</td>
</tr>
<tr>
<td>60-69</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>100.0</td>
</tr>
<tr>
<td>70-79</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>100.0</td>
</tr>
<tr>
<td>80-89</td>
<td>7</td>
<td>7</td>
<td>0</td>
<td>100.0</td>
</tr>
<tr>
<td>90-99</td>
<td>7</td>
<td>7</td>
<td>0</td>
<td>100.0</td>
</tr>
<tr>
<td>100-114</td>
<td>13</td>
<td>13</td>
<td>0</td>
<td>100.0</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>83</strong></td>
<td><strong>74 (7)</strong></td>
<td><strong>9</strong></td>
<td><strong>89.2</strong></td>
</tr>
</tbody>
</table>

*1. Figures in parentheses are non-pregnant
*2. Diagnosed as non-pregnant and confirmed as pregnant from day 45 post insemination.
4.6 Visual inspection of Physiological changes

In Visual inspection group, many sows exhibited external physical signs of pregnancy between 60 and 90 days of pregnancy. Signs included were abdominal distension, udder enlargement.

4.6.1. Postural behaviour of the sow during gestation:

Postures adopted by the sows before and after conception (during pregnancy) varied during different hours/parts of the day i.e., morning, around noon and evening. The various common postures adopted by the sows before and during pregnancy are given below:

Lateral recumbency (Plate. 16) was the commonest posture adopted by the non-pregnant sows during most part of the day.

The postures in pregnant sows varied depending on the month of pregnancy. Ventral recumbency (Plate. 16) was common in the morning hours up to 3rd month, later the sows adopted lateral recumbency (Plate. 16) similarly around noon most of the sows adopted mostly the lateral recumbency.

In the evening, the commonest posture was the lateral recumbency which was noticed both in the pregnancy and non-pregnant sows. Some of the pregnant sows (6-10) adopted a dog-sitting posture (Plate. 16) at times during the day.

4.6.2. Abdominal distension:

There was an increase in the size of the abdomen as the duration of pregnancy advanced. The abdominal distension was negligible by the day 25. Around one and half month of gestation, the distension was noticed by 2 or 2 ½ months. Moderate distension was noticed by 2 or 2 ½ months. (Plate. 17).

The abdominal distension was prominent by 3 and 3 ½ months and well marked and greater as the day of farrowing approached. A distinct demarcation between the abdominal
wall and mammary glands was noticed which became very prominent by 3 and 3½ months of pregnancy but disappeared a day before farrowing (Plate. 17).

4.6.3. Mammary gland development:

The development of mammary gland was progressive as the duration of gestation advanced. The same was not appreciable at the end of first month. By second month of pregnancy the development of mammary gland was slight and by 2½ months it was slight to moderate. Around 3rd month it was moderate and prominent by 3½ months. Marked changes were noticed in the mammary gland one day prior to farrowing. The mammary gland was turgid with distal extremity of each gland assuming cone shape as the hours of farrowing neared, it was much more turgid and the cone shape of the distal extremity was very conspicuous (Plate. 18)

During the early stages of pregnancy not much change was noticed in the size and shape of the teats. But the changes were clear by 2½ month. At 3 and 3½ months the base of the teats was enlarged which was very prominent around a day or a few hours prior to farrowing (Plate. 18 & 19). The teats were very much engorged.
Plate 17 Showing abdominal distension of sows during gestation
Plate. 18 Showing development of mammary gland of sow during gestation
Slight development of mammary gland
Moderate development of mammary gland

Prominent development, showing demarcation between abdominal distension and mammary gland
Showing absence of demarcation between abdominal distension and mammary gland 12 h before farrowing

Plate. 19 Showing observation of teats during 3 ½ month of gestation period
Before one day of farrowing – cone shaped teats

Next day after the farrowing – teats

Feeding of milk to piglets

4.7 COMPARATIVE ACCURACY OF DIFFERENT PREGNANCY DIAGNOSTIC TECHNIQUES:
The results of various methods of early pregnancy diagnosis that were undertaken in the present study were compared and presented (Table.18). By comparing the results, it was concluded that accuracy in diagnosing pregnancy with Ultrasound, Non return estrus, Doppler (A-mode) echo, Salivary sampling test and Punyakoti test was 100, 82.35, 80.0 66.66 per cent and 57.14 per cent respectively while the accuracy in diagnosing non-pregnancy with above methods was 100, 66.66, 90.0, 85.71 and 80.0 per cent, respectively and the overall diagnostic accuracy for the five groups was 100, 80.0, 85.0, 75.0 and 75.0 per cent on days 20, 21, 35, 30 and 26 post insemination, respectively.

Non-return estrus was accurate in detecting pregnancy when compared to Doppler and Salivary sampling test. Doppler ultrasound was accurate in diagnosing pregnancy than Salivary sampling test and Punyakoti test. Salivary sampling test in sows was poor in diagnosing pregnancy but better in diagnosing non-pregnancy with an overall accuracy rate of 75 per cent. Of all the diagnostic techniques that were performed in the present study, Trans-abdominal B mode ultrasonography was more accurate in diagnosing early pregnancy and non-pregnancy from day 25 post insemination.
Table 18 Comparison of accuracy of pregnancy diagnosis among different Early Pregnancy Diagnostic techniques

<table>
<thead>
<tr>
<th>S.n o</th>
<th>Diagnostic test</th>
<th>Days after insemination</th>
<th>Sensitivity (per cent)</th>
<th>Specificity (per cent)</th>
<th>Positive predictive value (per cent)</th>
<th>Negative predictive value (per cent)</th>
<th>Overall accuracy (per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Non return to estrus</td>
<td>19-21</td>
<td>82.35</td>
<td>66.66</td>
<td>93.33</td>
<td>40.0</td>
<td>80.0</td>
</tr>
<tr>
<td>2.</td>
<td>Salivary abortion</td>
<td>30</td>
<td>66.66</td>
<td>90.90</td>
<td>85.71</td>
<td>76.91</td>
<td>75.0</td>
</tr>
<tr>
<td>3.</td>
<td>Punyakoti test</td>
<td>26</td>
<td>57.14</td>
<td>91.67</td>
<td>80.0</td>
<td>73.31</td>
<td>75.0</td>
</tr>
<tr>
<td>4.</td>
<td>Ultrasonography</td>
<td>20</td>
<td>50.0</td>
<td>81.81</td>
<td>50.0</td>
<td>81.81</td>
<td>73.33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25</td>
<td>75.0</td>
<td>100</td>
<td>100</td>
<td>91.66</td>
<td>93.33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>5.</td>
<td>Doppler echo (A-mode)</td>
<td>30-35</td>
<td>80.0</td>
<td>90.0</td>
<td>88.88</td>
<td>81.81</td>
<td>85.0</td>
</tr>
</tbody>
</table>
CHAPTER V

DISCUSSION

In the present study, the accuracy of five different early pregnancy diagnostic methods from day 20 post insemination was evaluated and compared.

Non return to estrus:

One of the objectives of the present study was to detect pregnancy at an early stage i.e 21 days post insemination, by observing non return rate in sows. However, the available literature on non-return rates for pregnancy diagnosis was scanty. Hence discussed with available literature.

A total of 20 sows were used out of which, 15 sows (75 per cent) were diagnosed as pregnant and 5 (25 per cent) as nonpregnant based on observation of non return rates. The pregnancy and nonpregnancy status of these sows was confirmed by real-time B ultrasonography between 45-60 days.

One sow though diagnosed as pregnant using observation on day 21 post insemination was found negative for pregnancy from 45 to 60 days on real-time B ultrasonography scanning. Three sows though diagnosed as nonpregnant by observation of estrus signs on day 21 post insemination was found positive for pregnancy from 45 to 60 days on real-time B ultrasonography scanning.

As per the above mentioned observations in the present study, the sensitivity and specificity of pregnant sows on day 21 post insemination was 82.35 and 66.66 per cent, respectively. The overall diagnostic accuracy in detecting early pregnancy on day 21 post
insemination was 80.0 per cent. Further, the positive and negative predictive values were recorded as 93.33 and 40 per cent, respectively.

Higher per cent accuracy of 85.7 (Williams et. al., 2008), 98.0 (Almond and Dial 1986, Glossop and Foulkes, 1998) and 100 (Viana et. al., 2001) in sows than the present findings and lower per cent of accuracy (78.3) than the present study was reported by Flowers et. al. (1999).

The accuracy of this method in detecting specificity in sows recorded as 66.66 per cent in the present study and was in agreement with the findings of Almond and Dial (1986) and Viana et. al. (2001) in pigs who reported 66 per cent accuracy. The above mentioned finding (66.66) was much higher than the observations of Flowers et. al. (1999) and Williams et. al. (2008) in swine who reported 62.1, 46.1 per cent, respectively.

It was concluded that the Non return rates was 82.5 per cent accurate in diagnosing early pregnancy and less accurate (66.66 per cent) in nonpregnant. Hence, the present method could be used for on farm pregnancy diagnosis for detecting open sows with 66.66 per cent reliability on day 21 post insemination which were in line with the findings of Williams et. al. (2008).

**Salivary sampling assay:**

One of the objectives of the present study was to correlate the salivary crystallization with pregnancy at an early post insemination period. Saliva became popular in recent decades as a medium for the measurement of numerous biomolecules, hence in the present study, selected salivary fern pattern as one of the method for effective early pregnancy diagnosis.
Fern pattern of saliva which showed clear crystallized fern on day 0, which was in estrus, the reason might be the level of estrogen is high and caused the body sodium levels to rise, saliva was affected by the increased salinity, this was noticeable when allowed samples of saliva to dry and a clear crystallized fern was observed, it was due to higher salt content, caused the dried saliva to form crystallization patterns (Sangeetha and Ramesh kumar, 2015). The saliva samples was used as a tool for early pregnancy diagnosis from the day 25 to 30 post insemination in cattle (Skalova et. al., 2013).

In the present study, saliva sampling was undertaken daily from 18 day of post insemination in sows. Basing on crystallization of the saliva sampling, 7 sows out of 20, observed in pregnant, which were found positive on real-time B mode ultrasonography from day 45 to 60 post insemination.

As per the above observations in the present study, the sensitivity, specificity of pregnant and non-pregnant sows on day 29 post insemination was 66.66 and 90.90 per cent, respectively. The overall accuracy of the present study test in the detection of early pregnancy on day 29 was 75.00 per cent.

The recorded salivary crystallization pattern showed significant (p<0.05) difference in pregnant when compared with nonpregnant LWY sows.

The observations recorded in the present study were in alignment with the findings of Haberova (2010) in camels, Pardo-Carmona et. al. (2010) in bitches, Skalova et. al. (2013) in cattle ; Sangeetha and Ramesh kumar (2015) in Ramnad white sheep.

The findings of Haberova (2010) clearly stated that the saliva of the pregnant sows significantly showed the per cent in branch-like crystallization compared to non-pregnant sows.
The present findings were in total agreement with those of Skalova et al. (2013) in cattle; Sangeetha and Ramesh kumar (2015) in Ramnad white sheep and suggested that during estrus and in early pregnancy diagnosis, changes in saliva crystallization patterns occurred due to variations in electrolyte concentrations associated with hormonal changes.

Further it was confirmed that a salivary crystallization pattern was found significantly different in pregnant sows compared to nonpregnant sows by different types of salivary crystallization pattern.

From the present study and comparing the observations of earlier works, it is concluded that salivary crystallization is a simple, non-invasive and do not require any chemicals or sophisticated instruments to diagnose early pregnancy effectively from the day 25 to 30 post insemination.

**Punyakoti test:**

A simple non-invasive technique which was developed on the basis of ancient Egyptian knowledge called as Punyakoti test (Veena, 1997) was used to detect early pregnancy on day 26 post insemination.

In present study, test is based on wheat seed germination inhibition per cent and shoot length in cm in the urine of inseminated sows. In the urine of 5 sows out of 20, the seed germination inhibition was 74.66 ± 4.42 vs. 52.44 ± 2.33 per cent and the shoot length was short as 3.33 ± 0.061 vs. 5.44 ± 0.092 cm in pregnant vs. nonpregnant, respectively which were found positive on real-time B ultrasonography from 45-60 days post insemination.
The observations of the present study with regards to germination inhibition per cent and shoot length in cm 74.66 ± 4.42; 3.33 ± 0.061 vs. 52.44 ± 2.33; 5.44 ± 0.092 in pregnant vs. nonpregnant sows were significantly different (p<0.05). The sows which showed more wheat seed germination inhibition (74.66) and shoot length (5.41) cm later found negative for pregnancy from 45 to 60 days on real-time B ultrasonography. The recorded germination inhibition per cent was higher with reduced shoot length showed significant (p<0.05) difference in pregnant when compared with nonpregnant LWY sows.

The observations recorded in the present study were in close agreement with the findings of Dilrukshi et. al. (2009), Rao and Veena (2009), Swamy et. al. (2010) and Perumal (2014) in cattle.

The findings of Dilrukshi et. al. (2009) clearly stated that the urine of the pregnant cows significantly suppressed the seed germination (57.93 per cent) compared to nonpregnant (79.2 per cent). The recorded shoot length was 3.89 ± 3.16 cm and was significantly less when the seeds were treated with the urine samples collected from the pregnant cows and the same was 6.1 ± 3.24 cm for nonpregnant cows. These findings were similar to those observed in the present study (3.33 and 5.44 cm) for pregnant and nonpregnant buffaloes.

The present findings are in corroboration with those of Swamy et. al. (2010) and Perumal (2014) with regard to seed germination inhibition in pregnant animals (73.65 ± 2.81, 78.91 ± 2.09 per cent). The shoot length observed by these authors were 0.95 ± 0.47, 0.53 ± 0.52 cm.

A significant inhibition of seed germination after 48 hours and shoot length after 4 days with wheat seeds on day 20 and 28 post insemination was observed by Rao and Veena
(2009) indicated the usefulness of this method to detect early pregnancy. However the accuracy of the test was 100 per cent on day 35 and 45 post insemination.

The factors that might be influencing such a differential response in urine treated seeds could be plant growth regulators such as auxins that were excreted in high concentrations in urine during pregnancy which might have caused inhibitory response to seed germination and shoot growth. Further it was stated by Veena and Narendranath (1993) that the presence of increased concentrations of abscissic acid (ABA) which was the inhibitory factor. The most propable factor influencing seed germination and shoot growth excreted in urine must have been a factor associated with the events in the reproductive tract commencing from the time of conception till a few days postpartum because the inhibitory response of urine on seeds has showed to persist for as long as three months postpartum (Veena and Narendranath, 1993).

Further it was confirmed that a high concentration of ABA was found in the urine of pregnant cows (170.62 nmol/ml) as compared to urine of nonpregnant cows (74.46 nmol/ml) (Veena, 2006).

From the present study and comparing the observations of earlier works, it is concluded that Punyakoti test is a simple, non-invasive and do not require any chemicals or sophisticated instruments to diagnose pregnancy from day 25 to 30 post insemination.

**Transabdominal Ultrasonography:**

The literature available on sows was scanty to discuss with the present observations. Use of real time ultrasonography allowed the visualization of the embryonic vesicle and
embryo in the early stages of development with minimal manipulation of the pregnant uterine horn reducing the risk of embryo mortality (Knox and Althouse, 1999).

In the present study, the uterine horns of all the inseminated sows were scanned on day 20, 25 and 30 post insemination. Out of 30 sows scanned on day 20, \( \frac{4}{8} \) (50 per cent) were diagnosed as pregnant based on the presence of anechoic embryonic vesicle which was round in uterine lumen. Sometimes the vesicle appeared as two anechoic areas around the embryo. Similar findings were also reported by Flowers et al. (2000) and Williams et al. (2003). However, Knox and Althouse (2000) and Almond and Dial (1987) reported the visualization of embryonic vesicle by day 30 and 25, respectively. The remaining 26 (86.67 per cent) inseminated sows were considered as nonpregnant as there was no observable presence of embryonic vesicle.

Ultrasound scanning performed on day 25 revealed presence of increased size of embryonic vesicle and compartmentalization of the above four sows and two more sows had also showed the presence of embryonic vesicle, thus lead to a total of 6 (75 per cent) as pregnant and those which did not show embryonic vesicle were treated as nonpregnant (24).

Similarly, ultrasound scanning was performed on day 30, in 8 (8/8 ; 100 per cent) sows the embryonic vesicle visualized with the presence of an echogenic structure within the embryonic vesicle and diagnosed as pregnant while remaining 22 were diagnosed as nonpregnant as these sows did not visualize neither anechoic embryonic vesicle nor echogenic embryo in the uterine lumen.

In the present study, the per cent pregnant sows increased from 4 on day 20 to 8 on day 30, based on the visualization of embryonic vesicle and embryo. The clear visualization
could be due to development of early stage of embryo. The remaining 22 sows did not show any evidence of pregnancy even from 45 to 60 days on real time B ultrasonography. Further it was observed that all 8 were found pregnant on real time B ultrasonography between 45 to 60 days of post insemination. The mean length of embryonic vesicle measured was $2.33 \pm 0.07$, $2.64 \pm 0.063$ and $3.07 \pm 0.072$ cm on day 20, 25 and 30, respectively. The average length of embryo on day 25 and 30 was $1.05 \pm 0.04$ and $1.37 \pm 0.02$ cm, respectively. These findings were in close agreement to the Williams et. al., (2008). On the contrary the present findings were higher than those reported by Knox and Althouse (2000). Based on the above observations, the sensitivity and specificity for early pregnancy diagnosis through ultrasound on day 20, 25 and 30 of gestation were 50.00 and 81.81; 75 and 100; 100 and 100 per cent, respectively. Both negative and positive predictive values improved as the pregnancy advanced from day 20 to 30. From these results, it was concluded that the embryonic vesicle and embryo could be visualized in all the sows that were found pregnant from day 30 post breeding.

The reported detection of embryonic vesicle on day 20 with 50 per cent sensitivity was in agreement with Almond and Dial (1987) and Knox et. al. (1999) who reported that as 44.4 and 40.00 per cent, respectively. However, the present finding was much less to the sensitivity reported by Flowers et. al. (1999) and Viana et. al. (2001) who reported 78.3 and 100 per cent, respectively.

The sensitivity of ultrasonography on day 30 was in line with the observations of many investigators who reported 100 per cent as reported in the present study. Comparing the observations of the present study with those of Viana et. al. (2001), Gaggini et. al. (2012) and Flowers et.al. (2003) it was stated that ultrasound scanning is less sensitive to visualize the embryonic vesicle and embryo by day 30.
75.00 per cent sensitivity in detecting the embryonic vesicle and embryo observed in the study on day 25 was higher than the observations of Miller et. al (2003) and Gaggini et. al. (2012) who reported 66.7 and 70.00 per cent, respectively. The difference could be due to the great difficulty in early identification of the embryo in the present study was due to its position, which might be very close to or in contact with the uterine wall (Knox et.al., 2000) in sows. The same difficulty was also expressed in pigs by Coretz (2006) who considered that signs of pregnancy in bovine ultrasound are positive only when embryonic vesicle, embryo together with its heart beats could be detected.

In sows, the 100 per cent accuracy, sensitivity and specificity of ultrasound detection of pregnancy on day 30 post insemination observed in the present study was in agreement with Cortez et. al. (2006), Williams (2008), Pequeno et. al. (2009) and Gaggini et. al. (2012).

The results observed in the present study for sensitivity, specificity, positive and negative predictive values were in corroboration with the findings of Williams et. al. (2008) who observed the accuracy of pregnancy diagnosis using ultrasound in sows.

Based on the results of the present study, 4 sows pregnant on day 20, 6 on day 25 and 8 on day 30 and the same was confirmed by real time B ultrasonography between 45 to 60 days of post insemination. From these observations, it was inferred that maximum sensitivity and specificity were recorded by an experienced operator, with best time on day 30 post insemination by using transabdominal ultrasound with 5-7.5 MHz sector array transducer in Large White Yokshire pigs.
In conclusion, ultrasonography is a useful tool for the early pregnancy diagnosis. Embryonic vesicle and embryo detection were 100 per cent evident in LWY sows on day 30 post insemination.

**Doppler Echo:**

One of the objectives of the current study was to standardize and correlate doppler echo with pregnancy at an early post insemination period. The doppler echo was used as a tool for early pregnancy diagnosis between 29 to 35 days post insemination in sows (Too, K. *et al.*, 1974 and Almond *et al.*, 1987) using transabdominal transducer.

In the present study using doppler echo, 9 sows (45 per cent) were diagnosed as pregnant and 11 (55 per cent) as nonpregnant based on observation of audible signal sounds from blood flow of uterine and umbilical arteries. The pregnancy and nonpregnancy status of the present study sows was confirmed by real-time B ultrasonography from 45 to 60 days.

Two sows though diagnosed as nonpregnant using doppler echo on day 30 post insemination were found positive for pregnancy at from 45 to 60 days on real-time B ultrasonography examination.

As per the above mentioned observations in the present study, the sensitivity and specificity of pregnant and nonpregnant on day 30 post insemination was 80.0 and 90 per cent, respectively. The overall diagnostic accuracy in detecting early pregnancy using doppler echo on day 30 post insemination was 85.0 per cent. Further, the positive and negative predictive values were recorded as 88.88 and 81.81 per cent, respectively.
The per cent (85 per cent) accuracy in detecting early pregnancy in sows using doppler echo in the current study was comparable with the earlier findings of Kimehiko Too et. al. (1974) in pigs, Almond and Dial (1987) and Cowart (2008) who reported 81.8, 85.6 and 85.00 per cent respectively using doppler echo in sows. The present finding (85 per cent) was much lesser than the observations of Atkinson (1986); Flower et. al. (1999); Flower (2003); Cortez (2006) and Kauffold and Althouse (2007) who recorded 97.7, 94.5, 95, 94.6 and 97.56 per cent, respectively.

The accuracy of doppler echo in detecting nonpregnancy in sows observed in the present study was 90 per cent and these were in agreement with the findings of Almond and Dial (1987). The above mentioned finding (90 per cent) was much higher than the observations of Atkinson (1986) and Cortez (2006), in sows who reported 78 and 70 per cent, respectively.

It was concluded that the doppler echo was 90 per cent accurate in diagnosing nonpregnancy and less accurate (85 per cent) in diagnosing early pregnancy. Hence, this type of doppler echo could be used for on farm pregnancy diagnosis for detecting open sows with 90 per cent reliability on day 30 post insemination which were in accordance with the findings of Flowers (2003).

**Visual Inspection (External physical signs):**

The postures, abdominal distension, mammary gland development was used as a tool for pregnancy diagnosis at 60-90 days post insemination (Almond and Dial., 1986 and Solmon raju., 1991).
In the present study, the use of visual inspection to diagnose pregnancy is advantageous but this method allowed detection of nonpregnant sows only after two months. However, incorrect diagnosis of pregnancy in the present study might be due to reproductive problems like pseudopregnancy, accumulation of body fat which might have consequently similar abdominal distension and can confuse the diagnosis. A major constraint to adopt visual inspection for pregnancy diagnosis could be possible where artificial insemination or breeding dates were known / recorded and not randomly in the hogs.

Variations in the postures adopted by the pregnant and non-pregnant sows was noticed. While most of the non-pregnant sows were in lateral recumbency in the morning, the sows were in early pregnancy were in ventral recumbency. This might be due to changes in the hormonal profiles in the pregnant sows. The maternal recognition of pregnancy in sows occur from day 11 and changes in oestrogen and progestrone level also occur from the day 12 post insemination (Bazer et.al., 1982).

The lateral recumbency during the night and early morning hours in non-pregnant sows might be due to natural or normal resting position during the part of the day. Similarly the lateral recumbency in the pregnant ones during advanced stages might be due to the heaviness of uterine contents with mutiple fetuses as well as being its natural resting position.

The pregnant sows with its heavy uterine contents could not rise immediately and perhaps adopt a dog-sitting posture before rising and standing on all its four legs in order to protect the pregnant uterus/fetuses from external injuries. The changes in the abdomen were
conspicuous as the days of gestation advanced with marked changes 3 days to few hours prior to farrowing.

Gradual abdominal distension with mammary gland demarcation was observed as an external sign of pregnancy in the LWY sows in the study could be attributed to the increase in the size of the fetuses and accumulation of voluminous uterine contents during the pregnancy after implantation of embryo by day 24. The absence of demarcation between the abdomen and the mammary gland prior to a few hours before farrowing was noticed in the present investigation might be due to the action of the hormone “Relaxin” released from the day 105 of pregnancy (Hafez, 2000) as well as due to the droopiness of distended mammary gland just a few hours prior to the farrowing.

The initial development of the mammary gland by the 2nd month of pregnancy in the sows followed by slight to moderate increase in size by 2½ months and becoming prominent by 3½ months as seen in the present investigation in the sows were reported by Hafez (2000) who observed lobulo-alveolar development of the mammary gland by day 45 of gestation and Geyer et.al. (1986) by 4-6 weeks pre-partum. The development of the mammary gland might be due to the combined action of hormones like oestrogens, progesterone, growth hormone and prolactin.

**Comparison of different methods:**

The observations and findings of different early pregnancy diagnosis methods viz., Non-return rate, Salivary crystallization, Punyakoti test, Real-time B mode trans-abdominal ultrasonography, Doppler echo (A-mode) were confirmed with the Real-time B mode ultrasonography on day 45-60 post insemination. By this study, it was observed that Non-return to estrus can be used in diagnosis of early pregnancy with 82.35 per cent reliability.
while compared to Salivary crystallization. Punyakoti test on day 26 post insemination was less accurate in diagnosing pregnancy when compared to other methods. Trans-abdominal ultrasound could be used with higher reliability from day 25 post insemination in comparison with other methods of early pregnancy diagnosis. Further, it is concluded that the diagnostic accuracy was 100 per cent using trans-abdominal ultrasonography against 85 per cent by Doppler echo (A-mode) on day 30 post insemination in sows. By taking all the above mentioned considerations / observations, it was observed that the real time, B-mode ultrasound was found to be a reliable and relatively simple method of diagnosing pregnancy as early as day 25 post insemination in Large White Yorkshire.
CHAPTER VI

SUMMARY

The present investigation was undertaken on LWY sows in the ILFC for a period of six months. LWY sows in second to fourth farrowing (2 to 4 years) reared under controlled conditions were randomly tested by using five methods i.e., non-return rate, Salivary crystallization, Punyakoti test, Transabdominal ultrasonography and Doppler echo. Visual inspection of external physical changes of abdomen and mammary gland development was also observed from day 45 post insemination.

Initially the sows that were inseminated were subjected to pregnancy diagnosis at an early stage i.e 20 days post insemination and later confirmed by using real time B mode diagnostic scanner with 5-7.5 MHz sector array transducer (ALOKA Prosound).

In Nonreturn rate, inseminated sows were subjected to visual inspection on day 21 post insemination for pregnancy, by observing absence of estrus signs, which allows diagnosis of pregnancy between day 17 and 24 post insemination. The ability for early detection of pregnancy associated with high degree of accuracy. It is naturally linked to breeding management and resulted in the shortest interval between the identification of non-pregnant sows and subsequent attempts to rebreed them.

In Salivary sampling group, 20 inseminated sows were subjected to salivary crystallization on day 30 post insemination for early pregnancy diagnosis. Out of 20, 7 (35 per cent) sows were diagnosed as pregnant and 13 (65 per cent) as nonpregnant. In Punyakoti test, 20 inseminated sows were subjected to seed germination inhibition test on day 26 post insemination using wheat seeds and diluted urine samples in 1:14 ratio. Seed germination inhibition per cent after 48 h and shoot length in cms on the fifth day were
recorded. A significant difference (p<0.05) was noticed between pregnant and nonpregnant sows regarding germination inhibition per cent and shoot length (cm), which was recorded as 74.66 ±4.42; 3.33 ± 0.061 and 52.44 ± 2.33; 5.44 ± 0.092, respectively.

In Ultrasonography, 30 inseminated sows were subjected and analysed for pregnancy diagnosis on days 20, 25 and 30 post insemination. In pregnant sows, anechoic area of varying sizes within the lumen of an echogenic uterus representing the fluid filled embryonic vesicle and the embryo within the embryonic vesicle appeared as an echogenic area were observed. On day 20, pregnant sows showed anechoic embryonic vesicle within the echogenic uterine horn. On day 25, pregnant sows showed irregular shaped embryonic vesicle with expansion of vesicle and divided into compartments. Similarly on day 30, embryo was clearly visualized as echogenic structure in all the sows that were diagnosed as pregnant (8/8). The accuracy of ultrasonography was low on day 20 and 25 being 50 and 75 per cent respectively, but as the pregnancy advanced, the technique was 100 per cent accurate in detecting pregnancy from day 30 post insemination.

In Doppler echo+, early pregnancy diagnosis was performed on 20 sows using Doppler echo (A-mode) on day 30-35 post insemination. Out of 20 sows, 9 (45 per cent) were diagnosed as pregnant upon observation of audible signal sounds from blood flow of umbilical and uterine arteries and 11 (55 per cent) as non pregnant.

In Visual inspection, many sows displayed external physical signs of pregnancy observed from day 60 of gestation i.e., postures , abdominal distension and mammary gland development.

The sensitivity, specificity, positive and negative predictive values of Non return estrus on day 21 (82.35, 66.66, 93.33 and 40.0 per cent), Salivary sampling on day 30
(66.66, 90.90, 85.71 and 76.91 per cent), Punyakoti test on day 26 (57.14, 91.67, 80.0 and 73.31 per cent), Transabdominal ultrasonography on day 30 post insemination (100, 100, 100, and 100 per cent), Doppler ultrasound on day 30 (80.0, 90.90, 88.88 and 81.81 per cent) respectively. The overall diagnostic accuracy of above mentioned methods was 80.0, 75.00, 75, 100 and 85.0 per cent, respectively.

From the present investigation, concluded that Transabdominal ultrasonography were 100 per cent accurate in diagnosing pregnancy and nonpregnancy. Doppler echo+ was more accurate in detecting pregnancy than Nonreturn rates, while Nonreturn to estrus was more accurate than Salivary crystallization and Punyakoti test.

Of all the diagnostic techniques that were under taken in the present study, Transabdominal ultrasonography was more accurate in diagnosing pregnancy and nonpregnancy from day 25 post insemination.
LITERATURE CITED


Gaggini, T.S., de Almeida, M.C.S., Bortolozzo, F.P. and Wentz, I. 2012. Diagnóstico
de gestação em fêmeas suínas: uma revisão dos principais métodos. *Current Agricultural Science and Technology, 18*(3).


