

Investigation on Implantation /Attachment Process in Small Ruminants

Arjuma Khatun

(2005-V-59-M)



Division of Animal Reproduction, Gynaecology and Obstetrics

Faculty of Post-graduate Studies

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Arjuma Khatun

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2007

Dedicated to
My mother,
Mrs. Aziza Begum

Sher - e - Kashmir
University of Agricultural Sciences and Technology of Kashmir
Division of Animal Reproduction, Gynaecology and Obstetrics,
Shuhama Campus, Alusteng, Srinagar-190006

Certificate – I

This is to certify that the thesis entitled, **“Investigation on Implantation / Attachment Process in Small Ruminants”** submitted in partial fulfillment of the requirements for the award of the degree of **Master of Veterinary Science (Animal Reproduction, Gynaecology and Obstetrics)**, to the **Faculty of Post-graduate Studies, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir** is a record of bonafide research work carried out by **Dr. Arjuma Khatun (Regd. No. 2005-V-59-M)** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

It is further certified that such help or information received during the course of investigation has duly been acknowledged.

(Prof. G. M. Wani)
Chairman
Advisory Committee

Endorsed

Head,
Division of Animal Reproduction,
Gynaecology and Obstetrics.

Sher-e-Kashmir
University of Agricultural Sciences and Technology of Kashmir
Division of Animal Reproduction, Gynaecology and Obstetrics,
Shuhama Campus, Alusteng, Srinagar-190006

Certificate – II

We, the members of the Advisory Committee of **Dr. Arjuma Khatun (Regd. No. 2005-V-59-M)**, a candidate for the degree of **Master of Veterinary Science (Animal Reproduction, Gynaecology and Obstetrics)** have gone through the manuscript of the thesis entitled, **“Investigation on Implantation/Attachment Process in Small Ruminants”** and recommended that it may be submitted by the student in partial fulfillment of the requirements for the award of the degree.

Advisory Committee

Chairman

Prof. G.M. Wani,
Director, Extension Education

Members

1. Prof. M. Z. Khan,
Head, Animal Reproduction,
Gynaecology and Obstetrics

2. Prof. S. A. Wani,
Head, Division of Veterinary
Microbiology & Immunology

3. Mr. A. H. Lone,
Assistant Professor, Division of
Agri-statistics and Economics

Dean P.G. Nominee

4. Dr. M. A. Kirmani,
Dean, F.V.Sc. & A.H., Shuhama,

Sher - e – Kashmir
University of Agricultural Sciences and Technology of Kashmir
Shalimar Campus, Srinagar-191121

Certificate – III

This is to certify that the thesis entitled, **“Investigation on Implantation/Attachment Process in Small Ruminants”** submitted by **Dr. Arjuma Khatun (Regd. No. 2005-V-59-M)** to the **Faculty of Post-graduate Studies, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir** in partial fulfillment of the requirements for the award of the degree of **Master of Veterinary Science (Animal Reproduction, Gynaecology and Obstetrics)** was examined and approved by the Advisory Committee and External Examiner on

Chairman
Advisory Committee

External Examiner

Professor & Head,
Division of Animal Reproduction,
Gynaecology and Obstetrics.

Director Resident Instruction-cum-Dean
Post-graduate Studies, SKUAST (K)

Sher - e – Kashmir
University of Agricultural Sciences and Technology of Kashmir
Division of Animal Reproduction, Gynaecology and Obstetrics,
Shuhama, Alusteng, Srinagar-190006

Name of the student	Dr. Arjuma Khatun
Registration No.	2005-V-59-M
Major Subject	Animal Reproduction, Gynaecology and Obstetrics
Minor Subject	1. Veterinary Microbiology & Immunology and 2. Veterinary Medicine
Major Advisor	Prof. G.M. Wani, Director, Extension Education
Title of the Thesis	Investigation on Implantation / Attachment Process in Small Ruminants

ABSTRACT

The whole study consisted of collecting eighty six samples of gravid and non-gravid uteri from the different local slaughter houses. The blood samples were collected from each of the animals. The age of the foetus during the gravidity ranged from 14 -140 days. As per the objectives of our study, all the samples were divided into two main groups which have been marked as Group-1 and Group-2. Group-1 was subdivided into five groups viz. Group A (GpA), Group B (GpB), Group C (GpC), Group D (GpD) and Group E (GpE). Group-2 was also subdivided into five groups viz. Group A₁ (GpA₁), Group A₂ (GpA₂), Group A₃ (GpA₃), Group A₄ (GpA₄) and Group A₅ (GpA₅). The Group-2 was curtailed and restricted to the samples whose foetal age varied from 14 -57 days only. Foetal age determination in between 15 to 39 days was difficult; no formula could be used directly due to some drawback. Therefore, we have innovated a new graph/scale wherein we have incorporated embryonic length given by Gordon (2004) i.e. 0.05 cm (12 days old embryo) and 0.07 cm (14 days old embryo) as lower values and by using the crown anus length from 2.1 cm (40 days of gestation onwards) as higher values

given by Richardson (1980). This graph has given practical age determination and also different changes in different age groups also supported by literature (Hafez, 1987, Hafez and Hafez, 2000 and Roberts, 1971). Our graph is also supported by Noden and Lahunta (1985). This new graph is known as **“Khatun-Wani graph, 2007”**.

Biometrical increase of different parts of female genitalia was observed during different stages of pregnancy. The numbers of attached caruncles increased and unattached caruncles decreased with progressive gestational age. As the gestation advanced the length of uterine horns increased four folds to adjust the growing foetus. Volume of allantoic fluid increased with higher gestation period however, the amniotic fluid decreased during the terminal stage of gestation. There was change of colour and consistency of foetal fluids from transparent white to yellow, granular and viscous with higher gestational age.

The pH of amniotic fluid and maternal serum was alkaline but in allantoic fluid became acidic towards term. Glucose in maternal serum gradually increased but decreased slightly in foetal fluids as the pregnancy progressed. Total protein continuously increased both in maternal serum and foetal fluids. Cholesterol decreased in maternal serum and remained constant almost in all the groups in foetal fluids as the gestation period advanced. Creatinine, urea and urea nitrogen gradually increased with higher gestational periods. Sodium increased in maternal serum but decreased in foetal fluids and the concentration of sodium was higher in amniotic fluid than that of allantoic fluid in all the groups. Whereas, the potassium showed reverse changes as observed in sodium. Copper decreased in maternal serum but remained almost constant in foetal fluids. The level of zinc gradually increased in foetal fluids while decreased in maternal serum. Concentration of manganese was frequently changing both in maternal serum and foetal fluids throughout pregnancy. Iron level in maternal serum gradually decreased but increased in foetal fluids.

The levels of Glutamate Oxalo Transaminase (GOT) and Glutamate Pyruvate Transaminase (GPT) remained almost constant in foetal fluids and showed gradual decrease in maternal serum. Whereas, the alkaline phosphatase increased gradually in foetal fluids and maternal serum.

The concentrations of progesterone and estrogen increased in maternal serum as the gestation period advanced. There was slight increased level of progesterone receptor with higher gestational period with low value during the time of attachment whereas; the estrogen receptor remained almost constant in all the groups.

Microscopically, on 18 days of gestation, a transitory attachment was observed with projections of trophoblastic villi into the uterine gland. Binucleate cells appeared around 18 days of gestation. At 18 days intercaruncular region was involved with attachment process. From 18-33 days of pregnancy, there was partial loss of epithelium in the intercaruncular region only but the caruncular epithelium remained regular and smooth. From 36 days of gestation onwards the caruncular epithelium also became loosen. At 39 days of pregnancy, cotyledons appeared for the first time as single layered irregular red granules.

Around 40 days of gestation there were projections or dentations in the caruncular epithelium and at this very stage, gross caruncular–cotyledonary attachment was observed. Syncytial formation was observed from 42 days of gestation onwards.

Initially, the caruncular-cotyledonary attachment was very fragile and could be separated by holding the uterine horn without imposing any pressure. But from 48 days onwards, it was not separating just by holding the uterine horn and gradually it became strong. So, it can be said that during early stage, the attachment might be non invasive and superficial type but as the gestation period advanced it became invasive and invading type.

From 80 days onwards, cotyledones were completely attached by the caruncles which were observed both grossly and histologically. Both intercaruncular and caruncular attachments were supported by histological, gross and progesterone receptor changes.

Epithelial lining of the cervix of non-pregnant and early pregnant animals was simple low columnar with goblet cells containing shorter mucosal folds and the lamina propria-submucosa possessed numerous smaller glands. As the gestation period advanced the height and loosening of the lamina epithelialis mucosa occurred with longer mucosal folds. Glands became larger gradually and their proliferation also occurred with higher gestational age. Vascular proliferation occurred from lamina propria upto the tunica serosa with higher gestation periods.

Key words: Caruncle, Elongated blastocyst, Estrogen receptor, Foetal development, Foeto-maternal union, Progesterone receptor.

Signature of Student
Dated :

Signature of Major Advisor
Dated :

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Dated :

(Arjuma Khatun)

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CONTENTS

Chapter	Particulars	Page No.
1.	INTRODUCTION	1 - 4
2.	REVIEW OF LITERATURE	5 – 21
	2.1. Biometric studies of female genital organs	6 – 8
	2.2. Developmental changes of the conceptus	8 - 15
	2.3. Hormonal and Receptor status	15 - 19
	2.4. Histological changes in the female genital organ	19 - 21
3.	MATERIALS & METHODS	22 - 33
	3.1. Experimental animals	22
	3.2. Age determination of conceptus	22
	3.3. Grouping of experimental animals	23
	3.4. Grouping of Group A	24

CONTENTS

Chapter	Particulars	Page No.
	3.5. Collection of blood samples	24
	3.6. Collection of female genitalia	24 - 25
	3.7. Observations recorded after opening the genitalia	25
	3.8. Collection of foetal fluids	25 - 26
	3.9. Various biochemical constituents and hormones	26
	3.10. Collection of tissue	26
	3.11. Biochemical Analytical Procedures	26 – 30
	3.12. Analysis of macro and micro minerals	30
	3.13. Hormonal estimation	30 - 31
	3.14. Receptor estimation	32 - 33
	3.15. Microscopic observations	33

CONTENTS

Chapter	Particulars	Page No.
4.	EXPERIMENTAL FINDINGS	34 - 109
	4A. Group - 1	34 - 47
	4B. Group - 2	70 - 83
	4C. The gross changes of genital organ observed in non pregnant and different stages of pregnancy	102 - 105
	4D. Histological changes of genital organ in non pregnant and in different stages of pregnancy	106 - 108
	4E. Histology of cervix in non-pregnant and different stages of pregnancy	109
5.	DISCUSSION	133 - 164
6	SUMMARY & CONCLUSION	165 - 173
7.	LITERATURE CITED	174 - 184

LIST OF TABLES

Table No.	Particulars	Page No.
3.3.	Grouping of experimental animals	23
3.4.	Grouping of Group A	24
4A.1.	Biometry of parts of female genital organ during different stages of pregnancy in sheep	48 - 49
4A.1.1.	Statistical Analysis of Table - 4A.1. (ANOVA + t-test)	50 - 51
4A.2.	Number of caruncles during different stages of pregnancy in sheep	52
4A.2.1.	Statistical Analysis of Table - 4A.2. (ANOVA + t-test)	53
4A.3.	Changes in fetal fluid volume indifferent stages of pregnancy in sheep	54
4A.3.1.	Statistical Analysis of Table - 4A.3. (ANOVA + t-test)	54
4A.3a.	Changes in fetal fluid colour and consistency indifferent stages of pregnancy in sheep	55

LIST OF TABLES

Table No.	Particulars	Page No.
4A.4.	Values of biochemical constituents of fetal fluid and maternal serum during different stages of gestation in sheep	56 – 57
4A.4.1.	Statistical Analysis of Table – 4A.4. (ANOVA + t-test)	58 – 59
4A.5.	Various macro and micro minerals of fetal fluids and maternal serum during different stages of gestation in sheep	60 – 61
4A.5.1.	Statistical Analysis of Table – 4.5. (ANOVA + t-test)	62 - 63
4A.6.	Enzymes of foetal fluid and maternal serum during different stages of gestation in sheep	64
4A.6.1.	Statistical Analysis of Table – 4A.6. (ANOVA + t-test)	65
4A.7.	Hormones and receptor estimation from maternal serum and tissue during different stages of gestation in sheep	66
4A.7.1.	Statistical Analysis of Table – 4A.7. (ANOVA + t-test)	67

LIST OF TABLES

Table No.	Particulars	Page No.
4A.8.	Prediction of age in days in relation to whole embryo/Crown-anus (CA) length	68 – 69
4B.1.	Biometry of parts of female genital organ during early pregnancy in sheep	84
4B.1.1.	Statistical Analysis of Table - 4B.1. (ANOVA + t-test)	85
4B.2.	Biometry of amniotic and allantoic cavities and number of caruncles during early pregnancy in sheep	86
4B.2.1.	Statistical Analysis of Table - 4B.2. (ANOVA + t-test)	87
4B.3.	Changes in fetal fluid volume during early pregnancy in sheep	88
4B.3.1.	Statistical Analysis of Table - 4B.3. (ANOVA + t-test)	88
4B.3a.	Changes in fetal fluid colour and consistency during early pregnancy in sheep	89
4B.4.	Values of biochemical constituents of foetal fluid and maternal serum during early pregnancy in sheep	90 – 91

LIST OF TABLES

Table No.	Particulars	Page No.
4B.4.1.	Statistical Analysis of Table -4B.4. (ANOVA + t-test)	92 - 93
4B.5.	Various macro and micro minerals of fetal fluids and maternal serum during early pregnancy in sheep	94 – 95
4B.5.1.	Statistical Analysis of Table - 4B.5. (ANOVA + t-test)	96 – 97
4B.6.	Enzymes of foetal fluid and maternal serum during early pregnancy in sheep	98
4B.6.1.	Statistical Analysis of Table - 4B.6. (ANOVA + t-test)	99
4B.7.	Hormones and receptor estimation from maternal serum and tissue during early pregnancy	100
4B.7.1.	Statistical Analysis of Table - 4B.7 (ANOVA + t-test)	101

LIST OF FIGURES

Chapter	Particulars	Page No.
Figure 1.	Age (Range : 12 to 140 Days of gestation) and Whole Embryo/Crown-Anus Length (0.05 to 50 cm) Relationship Graph i.e. Khatun-Wani Graph, 2007.	131
Figure 2.	Age (Range : 12 to 40 Days of gestation) and Whole Embryo/Crown-Anus Length (0.05 to 2.1 cm) Relationship Graph i.e. Khatun-Wani Graph, 2007.	132

LIST OF PLATES

Chapter	Particulars	Page No.
Plate 1.	Showing non pregnant genital organ	110
Plate 2.	Showing caruncle in the non pregnant genital organ	110
Plate 3.	Showing Genital organ at 15 days of pregnancy	110
Plate 4.	Showing elongated blastocyst in the uterine horns at 15 days of pregnancy	110
Plate 5.	Showing elongated blastocyst with embryo at 15 days of pregnancy	110
Plate 6.	Showing swollen caruncles and corpus luteum at 15 days of pregnancy	110
Plate 7.	Showing genital organ of 18 days of pregnancy	111
Plate 8.	Showing corpus luteum at 18 days of pregnancy	111
Plate 9.	Showing comma shaped embryo in the elongated blastocyst (18 days of pregnancy)	111
Plate 10.	Showing gross photograph of conceptus (18 days of pregnancy)	111

LIST OF PLATES

Chapter	Particulars	Page No.
Plate 11.	Showing microscopic view of 18 days embryo (2x magnification)	112
Plate 12.	Showing genital organ of 20 days gestation	112
Plate 13.	Blastocyst is coming out by dorsal incision to the right horn (20 days of pregnancy)	112
Plate 14.	Elongated blastocyst containing embryo (20 days of pregnancy)	112
Plate 15.	Showing caruncular and intercaruncular regions (20 days of pregnancy)	112
Plate 16.	Showing 23 days pregnant genital organ	112
Plate 17.	Showing corpus luteum in the left ovary (23 days of pregnancy)	113
Plate 18.	Elongated blastocyst containing embryo (23 days of pregnancy)	113
Plate 19.	Showing 25 days pregnant genital organ	113
Plate 20.	Elongated blastocyst containing embryo (25 days of pregnancy)	113

LIST OF PLATES

Chapter	Particulars	Page No.
Plate 21.	Elongated blastocyst containing double embryos (29 days of pregnancy)	113
Plate 22.	Showing microscopic view of the 29 days embryo and emerging blood vessels from the hind gut	113
Plate 23.	Pregnant genital organ (33 days), showing separation of blastocyst	114
Plate 24.	Showing elongated blastocyst containing embryo (33 days of pregnancy)	114
Plate 25.	Showing 36 days pregnant genital organ	114
Plate 26.	Showing corpus luteum in the right ovary (36 days of pregnancy)	114
Plate 27.	Elongated blastocyst completely filled with fluid and ramified with blood vessels (36 days of pregnancy)	114
Plate 28.	Showing foetus within the amniotic cavity (36 days of pregnancy)	114
Plate 29.	Showing 39 days pregnant genital organ	115
Plate 30.	Showing presence of conceptus in the horn and changes in the caruncular and intercaruncular regions (39 days of pregnancy)	115

LIST OF PLATES

Chapter	Particulars	Page No.
Plate 31.	Showing appearance of cotyledones (39 days of pregnancy)	115
Plate 32.	Showing 40 days pregnant genital organ	115
Plate 33.	Showing separated cotyledone from the caruncle (40 days of pregnancy)	115
Plate 34.	Blastocyst is separating on holding the uterine horn (40 days of pregnancy)	115
Plate 35.	Cotyledones separating from caruncles (40 days of pregnancy)	116
Plate 36.	Cotyledones separating from caruncles at 40 days of pregnancy (Close view)	116
Plate 37.	Elongated blastocyst containing cotyledones nearby to the fetus at 40 days of pregnancy	116
Plate 38.	Showing foetus within the amniotic cavity (40 days of pregnancy)	116
Plate 39.	Showing foetus within the amniotic cavity and spread of Blood vessels at 40 days of pregnancy	116
Plate 40.	Showing flattened caruncles at 40 days of pregnancy	116

LIST OF PLATES

Chapter	Particulars	Page No.
Plate 41.	Showing 42 days pregnant genital organ	117
Plate 42.	Showing separation of cotyledone from the caruncle at 42 days of pregnancy	117
Plate 43.	Showing emerging blood vessels from the hind gut of foetus at 42 days of gestation	117
Plate 44.	Showing 44 days pregnant genital organ	117
Plate 45.	Showing corpus luteum at 44 days of pregnancy	117
Plate 46.	Showing prominent cotyledons nearby foetus at 44 days of gestation.	117
Plate 47.	Showing amniotic cavity and umbilical chord at 44 days of pregnancy	118
Plate 48.	Showing 45 days pregnant genital organ	118
Plate 49.	Showing arrangement of cotyledons to the blastocyst at 45 days of pregnancy	118
Plate 50.	Showing 46 days pregnant genital organ	118

LIST OF PLATES

Chapter	Particulars	Page No.
Plate 51.	Showing elongated blastocyst at 46 days of pregnancy	118
Plate 52.	Showing 47 days pregnant genital organ	118
Plate 53.	Showing depressed caruncles at 47 days of gestation	119
Plate 54.	Showing visible placentomes from outside horn at 48 days of gestation	119
Plate 55.	Showing strong cotyledonary – caruncular attachment at 48 days of pregnancy	119
Plate 56.	Showing cavity formation in the placentome at 48 days of pregnancy	119
Plate 57.	Showing rounded and raised type Cotyledones at 48 days of pregnancy	119
Plate 58.	Showing distinct placentomes from outside at 52 days of pregnancy	119
Plate 59.	Showing ball like cotuyledones and cup shaped placentomes at 52 days of pregnancy	120
Plate 60.	Showing elongated blastocyst at 53 days of pregnancy	120

LIST OF PLATES

Chapter	Particulars	Page No.
Plate 61.	Showing 57 days pregnant genital organ	120
Plate 62.	Showing strong placentome formation at 57 days of pregnancy	120
Plate 63.	Showing arrangement of blood vessels with the placentomes at 68 days of pregnancy	121
Plate 64.	Showing 87 days pregnant genital organ	121
Plate 65.	Cotyledons are completely attached with caruncles at 87 days of pregnancy	121
Plate 66.	Showing 134 days pregnant genital organ	121
Plate 67.	Showing full grown foetus at 134 days of gestation	121
Plate 68.	Showing corpus luteum at 134 days of gestation	121
Plate 69.	Showing transparent white amniotic fluid and creamy white allantoic fluid	122
Plate 70.	Showing transparent white amniotic fluid and pale yellow coloured allantoic fluid	122

LIST OF PLATES

Chapter	Particulars	Page No.
Plate 71.	Showing pale yellow coloured amniotic fluid and intense yellow coloured allantoic fluid	122
Plate 72.	Showing yellow coloured amniotic fluid and intense yellow allantoic fluid	122
Plate 73.	Showing off white amniotic fluid and intense yellow allantoic fluid	122
Plate 74.	Showing caruncle of non pregnant genital organ (4x)	123
Plate 75.	Showing intercaruncular region of non pregnant genital organ	123
Plate 76.	Showing caruncle of pregnant genital organ at 4x (15 days of pregnancy)	123
Plate 77.	Intercaruncular region showing endometrial gland proliferation (15 days of pregnancy)	123
Plate 78.	Showing endometrial gland proliferation (18 days of pregnancy)	123
Plate 79.	Intercaruncular region showing glandular opening (18 days of pregnancy)	123
Plate 80.	Showing simple cuboidal epithelium in the endometrial gland (18 days of pregnancy)	124

LIST OF PLATES

Chapter	Particulars	Page No.
Plate 81.	Showing trophoblastic villi with binucleate cells (18 days of pregnancy)	124
Plate 82.	Showing binucleate & elongated nucleate cells in the FM (18 days pregnancy)	124
Plate 83.	Showing caruncle and vascular proliferation (20 days pregnancy)	124
Plate 84.	Vascular proliferation at the base of the caruncle (20 days of pregnancy)	124
Plate 85.	Showing opened endometrial gland in the intercaruncle region (20 days of pregnancy)	124
Plate 86.	Showing binucleate cells in the FM (20 days of pregnancy)	125
Plate 87.	Binucleate cells in the fetal membrane under 100x (20 days of pregnancy)	125
Plate 88.	Showing caruncle and vascular proliferation at 23 days of pregnancy	125
Plate 89.	Showing caruncular and intercaruncular regions with glandular opening at 23 days of pregnancy	125
Plate 90.	Binucleate cells in the FM under 100x (23 days of pregnancy)	125

LIST OF PLATES

Chapter	Particulars	Page No.
Plate 91.	Showing endometrial glands and vascular proliferation at 25 days of pregnancy	125
Plate 92.	Showing vascular stock at the base of the caruncle (33 days of pregnancy)	126
Plate 93.	Showing trophoblastic villi (33 days of pregnancy)	126
Plate 94.	Showing vascular proliferation in the fetal membrane at 33 days of pregnancy (Direct tissue fixation)	126
Plate 95.	Showing trophoblastic villi and vascular proliferation in the FM (36 days pregnancy)	126
Plate 96.	Showing vascular proliferation in the fetal membrane at 36 days of pregnancy (Direct tissue fixation)	126
Plate 97.	Fetal membrane showing presence of large nucleate , elongated nucleate and free binucleate cells at 36 days of pregnancy	126
Plate 98.	Caruncle showing loose epithelium and more vascular stock at 39 days of pregnancy	127
Plate 99.	Fetal membrane showing free binucleate cell (39 days of pregnancy)	127
Plate 100.	Showing dentations in the caruncular epithelium, 4x (40 days of pregnancy)	127

LIST OF PLATES

Chapter	Particulars	Page No.
Plate 101.	Showing dentations in the caruncular epithelium under 40x (40 days of pregnancy)	127
Plate 102.	Showing binucleate cells and vascular proliferation in the fetal membrane (40 days of pregnancy)	128
Plate 103.	Showing projections in the caruncular epithelium and syncytial formation at 42 days of pregnancy	128
Plate 104.	Showing short trophoblastic villi in the fetal membrane (42 days of pregnancy)	128
Plate 105.	Placentome showing fetal parts entered into the caruncular projections (45 days of pregnancy)	128
Plate 106.	Showing syncytial formation in the placentome under 40x (45 days of pregnancy)	128
Plate 107.	Showing syncytial formation in the placentome under 100x (45 days of pregnancy)	128
Plate 108.	Placentome showing fetal parts into the caruncle (48 days of gestation)	129

LIST OF PLATES

Chapter	Particulars	Page No.
Plate 109.	Showing syncytial formation in the placentome under 40x (48 days of gestation)	129
Plate 110.	Placentome showing intermixing of foetal and maternal parts (52 days of pregnancy)	129
Plate 111.	Placentome showing intermixing of foetal and maternal parts (56 days of pregnancy)	129
Plate 112.	Showing foetal parts with blood vessels at the centre of the placentome (87 days of pregnancy)	129
Plate 113.	Placentome showing complete mixing of foetal and maternal parts (87 days of pregnancy)	129
Plate 114.	Short mucosal folds containing simple columnar epithelium and numerous tiny mucous glands present in the lamina propria-submucosa in non pregnant cervix.	130
Plate 115.	Gland which is mucous in type during mid pregnancy	130
Plate 116.	Large number of mucosal folds showing mucous secretion in the stellate shaped lumen	130
Plate 117.	Epithelium showing simple columnar with goblet cells	130

Chapter – 1

INTRODUCTION

1. INTRODUCTION

Deficiency of Meat and Meat Products in Jammu and Kashmir has aroused many eye brows. On the national level too we are having deficiency in meat products. Against requirements of 25 gms of meat per day per person the availability in India is half of it. However, meat and meat products deficiency at national level is not felt because of more vegetarian population in the country. For self sufficiency in meat products in India and for export in future emphasis on meat and meat products is needed. Due to the religious sentiments of our population, beef and pork do not have much available market in India. Therefore, sheep and goat meat needs more emphasis both in research and development. Fertility and multiple births in sheep and goats are desirable for meat production. However, a high mortality rate during the embryonic and foetal life deters fertility. In order to achieve significant improvement in meat production we must have sheep and goats giving at least three lambs/kids crop in two years. The rate of twins and triplets has to be increased. The embryonic mortality has to be minimized.

Embryonic losses in sheep and goats are very high. About (22-44%) embryos are lost during early pregnancy, maximum losses occur during pre-implantation period (Wani, 2006). Various biochemical, nutritional and genetic causes have been identified with embryonic mortality. (Aminoor Rehman *et al.*, 2004; Lee and De Mayo, 2004; Rowe *et al.*, 2003 and Wani, 2005).

Pregnancy is an intimate association between mother and foetus. Foetus is regarded as a **“Homograph”** in spite of being foreign to mother’s immunological status and gene mapping. The protective cover is hidden in the formation of placenta which consists of foetal and maternal parts. The common questions asked, is this foetal portion akin to mother? If so why then it is rejected and expelled soon after delivery as foetal

placenta or after birth. The mechanism of its formation is hidden in caruncles, the upright projections of the non-pregnant uterus or in the foetal membranes developed before attachment or implantation. The exact mechanism is obscure. Ruminant uteri have caruncles as the proposed sites of implantation (Gray *et al.*, 2002).

Ovine pre-implantation **blastocyst** undergoes elongation for upto 30 days and services on uterine milk through histotrophic nutritional process (Wani *et al.*, 2006). Uterine epithelium undergoes various changes. The morphological nature of these changes is not recorded but has been postulated. The changes in caruncles and endometrium under the influence of functional corpus luteum are the beginning of immunological protective cover which prevents rejection of conceptus (Wani, 1996).

Placental production of progesterone is known to increase markedly between 70th and 100th days of gestation in the ewe after the placenta has reached its maximum size (Gordon, 2004). The corpus luteum of pregnancy is essential throughout pregnancy almost in all farm animals except, ewe and mare as placenta produces sufficient progesterone to maintain pregnancy. The blood progesterone level remains constant in the ewe and cow and attains a high level early in pregnancy in the sow (Hafez, 1987).

The uterine endometrium of pregnant females is dominated by progesterone which stimulates development of glandular epithelium that becomes highly secretory and produces histotrophe which is essential for conceptus development. Secretory vesicles accumulate in the epithelium of endometrial glands (Bazer and Roberts, 1983). Protein released from the secretory vesicles into the uterine lumen serve as transport proteins, enzymes and regulatory proteins (Hafez, 1987).

Serum progesterone rapidly rise following formation of corpus luteum . Timing of implantation may be regulated by length of time the uterine endometrium is exposed to progesterone stimulation. The conceptus can not attach to the uterine epithelial surface until the large glycoprotein called MUC-1 has been removed. Over 8 -10 days of exposure to progesterone, nuclear progesterone receptors in the uterine epithelium down-regulate leading to a loss of the direct effect of progesterone on this cell type. Since epithelial synthesis of MUC-1 (glycoprotein) is stimulated by progesterone, loss of progesterone receptor from the uterine epithelium would reduce MUC-1 production and open a receptive state for conceptus attachment (Hafez and Hafez, 2000).

IFN-tau is believed to act by down-regulating oestrogen receptor thus preventing the oxytocin receptor and regulate the progesterone secretion (Gordon, 2004).

Placentation in case of ruminant can be regarded as superficial and relatively non-invasive. In this type of placentation the chorionic epithelium contains both mononucleate and binucleate cells. Binucleate cells in ruminant placenta differentiated from foetal mononucleate trophoblast cells and are known to secrete glycoproteins. As attachment is initiated, the trophoblast binucleate cells migrate to fuse with maternal endometrial cells and formed syncytium. It is at this stage the contents of binucleate cell secretory granules are released into maternal tissues; among these secretory products are many of the pregnancy associated glycoproteins. These binucleate cells facilitate the transfer of complex molecules across the embryo-maternal junction into the maternal circulation (Gordon, 2004).

Endometrial receptivity is stimulated by ovarian steroids. Proliferation of uterine cells is influenced by E2 and P4 and they have their cognate nuclear receptors in ovine or caprine uteri called E2 (ER) and P4 (PR) receptors (Lee and De Mayo, 2004). The model to identify gene expression in endometrial epithelium has been described too (Gray *et al.*, 2002). The changes of endometrium or foetal membranes have not been correlated with biochemical markers as described. Therefore, the present study will be conducted to investigate morphological and histological changes in blastocyst and endometrium during pre-implantation period in ovine.

Scanty literature is available in implantation, it's morphological, histological and biochemical aspects. Still very meager information is available on the morphology of attachment of the conceptus with maternal tissues in small ruminants. As such, present investigation on implantation/attachment has been planned with the following objectives :-

1. To study the biometry of different parts of the female genitalia.
2. To correlate external and internal uterine morphology of the female genital organs during early pregnancy and non-pregnancy.
3. To study the histological changes occurring during attachment.
4. To investigate the conceptus attachments and its mechanization.

5. To study the biochemical constituents of fetal fluids and their correlation with fertility.
6. To study the changes of estrogen, progesterone ratio during gestation and early pregnancy.
7. To study the changes of estrogen receptor, progesterone receptor ratio during gestation, early pregnancy and attachment.

Chapter - 2

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

In the era of consciousness for health, food quality, animal welfare, environmental protection, balanced food is need of the hour. We need hygienic and nutrient of the food at low cost. International zoo-sanitation has put more pressure on food distribution and production in the world. More food animal protein is thus demanded. More food from animals means more lambs/calves or offsprings per dam.

The modern techniques of Artificial Insemination (AI), Embryo Transfer (ET) and Multiple Ovulation Embryo Transfer (MOET) have been in uses since last few decades. Now it is time to use them under field conditions for more efficient quality of the livestock.

India has 124 million goats and 61 million sheep (Ramana Rao, 2006). In industrialized countries, sheep farming is more important (40%), than goat (4.6%). They are important both in terms of meat, milk, and fibers. The European countries have a challenge market nowadays for the lamb meat (Gordon, 2004). Asian countries especially in India, China and Pakistan have highest ranks in goat population. In India sheep contributes 5% of total meat and goat contribute 10% (Gordon, 2004). Now-a- days even USA demands more sheep and goat. The demand for lamb meat or lean meat in general is increasing due to health consciousness and fatty deposition of whole American and prosperous Indians too.

The lean meat production needs more studies on development of conceptus. We know that prenatal development undergoes in three stages. During early pregnancy skeletal and nervous tissues grow, on priority. During mid-gestation the hind and forelimbs develop. During late gestation muscular development along with peripheral extremities including hair develops. In order to increase lean meat production, research is needed to investigate relationship between hard and soft tissues. A maternal and foetal interaction needs a probe. Some basic studies (Mufti, 1995) indicated prospects of manipulating lean meat production (Wani, 2002) by bio-stimulators during late gestation,

which may help, the production of muscular lambs / kids at birth, besides, increasing birth weights.

Lean meat is a global demand increasing over years. The Wazwan, very peculiar to Kashmiris too needs tender lamb's meat for preparation (Mufti, 1995). Sheep and goat farming in Jammu and Kashmir state is an important task to improve household nutrition with very less investment as the small ruminants are more acclimatized with the geographic and climatic conditions of the state. Due to religious sentiments of our population, sheep and goat meats are more popular amongst people. Therefore, sheep and goat needs more emphasis both in research and development. Knowledge on foetal biology is important to increase the production.

The embryonic and fetal development completed within the uterus and the period is known as gestation period. Many scientists reviewed on prenatal development (Green and Winters, 1945, Hafez and Jainudeen, 1993, Wani and buchoo, 1990, Wallace, 1948, Joubert, 1956 and Surgeev *et al.*, 1990). Conceptus attachment is a very critical periods for prenatal life, as most of the embryonic losses and teratological defects occurs at this stage (Roberts, 1971). Attachment of conceptus with the uterine epithelium is the theme of our research. Failure of attachment leads to infertility. The actual time sequence of attachment in sheep is not very clearly defined. Many scientists reported various findings related directly or indirectly to conceptus attachment in farm animals cited below under different headings. The broad review on the topic is summarized as:

2.1. Biometrical studies of female genital organs:

Biometry of the vagina was reported by many workers. Singh *et al.* (1974) measured the averages of length of the vagina as 8.0 cm in goat, Pareek *et al.* (1981) as 7.6, 11.2 and 34.8 cm for a 3 day old, a 1 month old and an adult female camel respectively, Hafez (1987) as 10-14 cm in non pregnant adult sheep and Khatri *et al.*, (2004) recorded as 23.93 cm in buffalo.

Biometry of the cervix was reported by many workers. Singh *et al.* (1974) measured the averages of length of the cervix as 3.42 cm in female goat. Pareek *et al.* (1981) observed the cervical length 3.2, 4.7 and 7.6 cm for a 3 day old, a 1 month old and an adult female camel respectively, Hafez (1987) as 4 -10 cm in non pregnant adult sheep

and Khatri *et al.* (2004) recorded the average length as 8.50 cm in buffalo. Hafez (1987) also recorded the presence of several cervical rings in non pregnant adult sheep.

Biometry of the uterus was reported by many workers. Singh *et al.* (1974) measured the averages of length of left and right horns and corpus uterus as 16.06 cm, 15.42 cm and 1.30 cm respectively in goat. Pareek *et al.* (1981) observed the uterine body length as 0.5, 0.8 and 8.0 cm; left uterine horn length 4.3, 5.0 and 15.4 and right uterine horn length 3.4, 4.5 and 11.7 cm for a 3 day old, a 1 month old and an adult female camel respectively. Hafez (1987) mentioned the length of uterine horns and body as 10-12 cm and 1-2 cm respectively and the uterine horn presented 88-96 number of caruncles in non pregnant adult sheep. Shalini and Sharma (2004) recorded the corpus uteri as 1.60 cm in gaddi goats; Khatri *et al.* (2004) measured the average length of the left and right uterine horns as 17.17 cm and 15.97 cm. and the average length of the uterine body as 2.63 cm respectively in buffalo. Hafez (1987) further postulated that the dimensions cited above in non pregnant adult sheep varied with age, breed, parity and plane of nutrition.

Biometry of the ovary was reviewed by many scientists. Singh *et al.* (1974) reported the length, width and thickness as 1.58, 0.74 and 0.97 for the left ovary and 1.53, 0.80 and 1.03 cm for the right ovary; Mufti *et al.* (2000) studied in different stages of pregnancy i.e. Stage-I (30-60 days); Stage-II (61-90 days); Stage-III (91-120 days); and Stage-IV (120 days to term) and recorded the length as 1.4, 1.7, 1.6 and 2.0 cm, breadth as 1.0, 1.2, 1.2 and 1.5 cm and thickness as 0.7, 0.8, 0.8 and 0.9 cm, respectively. The length, width and thickness of the ovary were also measured by Shalini and Sharma (2004) in gaddi goats as 1.57, 1.11 and 0.83 cm respectively.

2.2. Developmental changes of the conceptus:

2.2.1. Prenatal development:

Ovine embryonic attachment begins at about 17 to 20 days and is completed by about 28 to 30 days (Roberts, 1971). Prenatal development on farm animals has been reviewed by many workers (Hafez and Jainudeen, 1993 and Hafez and Hafez, 2000). They have divided the prenatal life into three periods. The ovum period culminated with the initial attachment of blastocyst and before the establishment of intra-embryonic circulation which lasted upto 12 days in sheep and Mare and 15 days in Cattle. The embryonic period extended from day 12 to 34 in sheep, 15 to 45 in cow and day 12 to 60

in mare. During this period rapid growth and differentiation occurred and major parts of body become recognizable. The foetal period extended from about day 34 of gestation in sheep, day 45 in cattle and from day 60 in mare until birth.

2.2.2. Changes in the foetal fluid:

2.2.2a. Foetal Fluid Volume:

Malan and Curson (1937) and Cloete (1939) reported that the total volume of foetal fluids increased with advancing age of the foetus in sheep. They found that during first three months of gestation, the allantoic fluid accumulated slowly whereas the amniotic fluid accumulated rapidly. But in the fourth months of gestation the volume of allantoic fluid increased rapidly with slight increase in the amniotic fluid. During last month (5th) of gestation the allantoic fluid became almost doubled of its volume but the volume of amniotic fluid diminishes than that of previous stage.

Arthur (1956) observed that the volume of foetal fluids during twin pregnancy was approximately doubled.

Bongso and Basrur (1976) measured the volume of allantoic fluid that exceeded the amniotic fluid during 0-76 days of gestation whereas during 76-164 days the volume of the amniotic fluid was greater thereafter again allantoic fluid exceeded in cattle. Bongso *et al.* (1979) again studied the volume of foetal fluid in goat and reported that the allantoic fluid was higher compared to amniotic fluid until 5.5 cm CRL but amniotic fluid predominated at 31cm CRL thereafter allantoic fluid increased again.

Goldstein *et al.* (1980) found that the volume of allantoic fluid of porcine conceptus increased on day 20 (3.7 ± 0.3 ml), peak on day 30 (189.0 ± 5.6 ml) and second peak on day 58 (451.3 ± 53.5 ml), while decreased on day 40 (69.5 ± 4.6 ml) and then steadily decreased on day 112 (23.8 ± 5.5 ml).

Hafez (1987) reported that foetus can reduce amniotic fluid by swallowing or by drawing fluid into the fetal lungs during respiratory movement during final stages of gestation.

Amle *et al.* (1992) recorded that the relative volumes of foetal fluids showed much fluctuations during the entire gestation. Further they suggested that variations might be due to the contributions of the foetal and maternal compartments and the control of fluid compartments regulated by foetal endocrine system and foetal kidneys.

Mufti *et al.* (2000) recorded the volume of fetal fluids as 64 ± 15 , 256 ± 34 , 287 ± 25 and 502 ± 29 ml in allantoic fluid and 22 ± 6 , 128 ± 50 , 304 ± 22 and 353 ± 51 ml in amniotic fluid, respectively in different stages of pregnancy in sheep i.e. Stage-I (30-60 days); Stage-II (61-90 days); Stage-III (91-120 days); and Stage-IV (120 days to term).

2.2.2b. Foetal Fluid Colour and Consistency:

Bongso *et al.* (1979) observed the colour and consistency of the foetal fluid in goat. During their observation they noted that the allantoic fluid was colourless in the initial stages of gestation (< 6 cm CRL), amber through the major part of gestation (6- 24 cm CRL) and turning dark amber in the terminal stages of gestation. Amniotic fluid followed similar shifts in colour except that it turned colourless during the final stages of gestation. The consistency of allantoic fluid was watery throughout gestation whereas that of amniotic fluid was watery only until the final stages of gestation (>24 cm CRL), subsequently became mucoid and gelatinous. Arthur (1957) found that the allantoic fluid was watery or urine like in colour throughout gestation while the amniotic fluid was similar in the first two thirds of pregnancy but mucoid for the remaining gestation period. Raj Sundrum *et al.* (1990) reported that foetal fluids of buffaloes contain metabolic constituents, electrolytes, enzymes, hormones, cells and other structures. The concentration of different constituents of these fluids was influenced by metabolic products of foetus, flow of foetal urine through urachus and foetal secretions from lungs and salivary glands.

Amle *et al.* (1992) reported that foetal fluids were important in the efficient handling of foetal waste products during the entire gestation in buffalo.

2.2.3. Foetal Nutrition and Metabolism :

Alexander *et al.* (1955 and 1958) have reported that the sheep foetus is capable of producing considerable amounts of urine. The introduction of this urine, low in sodium and chloride, into the foetal fluids resulted in progressive reduction in the sodium and chloride concentration of foetal fluids as gestation advanced. No consistent trend was discernible in the sodium concentration in either of the foetal fluids but that in the amniotic fluid sodium was higher than that in the allantoic fluid throughout gestation. In general the potassium concentration of the amniotic fluid declined as term approached, while the converse was suggested by the values encountered in the allantoic fluid.

Dukes (1955) reported that gravid uterine muscles are richer in creatinine, this being due mainly to an increase in protein catabolism, because the

liberation of creatine is more rapid than its conversion to creatinine. Creatinine is a normal excretory product of creatine metabolism.

High levels of creatinine in the allantoic fluid have been reported by Hafez and Kamal (1955), Alexander *et al.* (1955) and Lupke *et al.* (1967).

Hafez *et al.* (1958) reported that the Cholesterol was found to be the precursor of progesterone and its level increased during pregnancy. The blood cholesterol level was reported to show a declining trend when animals were exposed to direct sun-light.

Alexander *et al.* (1958) reported that as the gestational age advanced the concentration of urea also increased in the foetal fluid and also changes in the concentration of urea nitrogen with the increase in gestation period have been reported in sheep foetal fluids and foetal urine (Alexander *et al.*, 1958 and Wales and Murdoch, 1973), foetal urine and relationship with foetal fluids and maternal plasma in sheep (Mellor and Slater, 1971 and 1972), buffalo (Soliman, 1975) and in caprines (Chauhan, *et al.*, 1992)

Stainer (1965) reported that lack of exchange of solutes from the allantoic fluid may be cause for the increase of creatinine level in the allantoic fluid.

Changes in the concentration of plasma proteins occurred during pregnancy as protein metabolism was put under an extreme stress during foetal development (Kancko and Corneleous, 1970).

Mellor and Slater (1971 and 1972) reported that the entry of foetal urine into the fluid sacs tends to decrease the osmolarity of Na⁺ and K⁺. Further they reported that the chorio allantois contained a pumping mechanism operating between allantoic fluid and foetal blood which act to decrease the sodium and increase potassium of allantoic fluid also reported that the entry of foetal urine into the fluid sacs tended to increase the urea concentration both in amniotic and allantoic fluid in ewes.

In between 31 and 44 days of pregnancy in sheep the allantoic fluid showed an increase level of Protein, Calcium (Ca) and Magnesium (Mg) and decrease in Potassium

(K) while amniotic fluid had less Protein, Calcium (Ca), Magnesium (Mg) and more Sodium (Na) and Potassium (K) (Wales and Murdoch, 1973)

Baetz *et al.* (1976) reported that as the crown rump length increased creatinine and uric acid concentration increased in serum and in allantoic fluid and total iron binding capacity in amniotic fluid, while the glucose concentrations decreased in allantoic and amniotic fluid and potassium in amniotic fluid in bovine.

Hafez and Kamal (1955) reported that the urea concentration was similar in both amniotic and allantoic fluids except that its levels increased with foetal maturity.

McDonald (1980) reported that an increase protein concentration in maternal circulation indicated a positive association with pregnancy establishment. The fluctuations revealed its demand and utilization for the physiological events that took place during fertilization.

Progesterone has been reported to reduce the excretion of potassium in rats (Garland, 1983).

Lathura *et al.* (1987) reported that urea and urea nitrogen increased both in allantoic fluid and maternal serum as the pregnancy advanced and also reported that total protein concentration in maternal plasma was much higher than either of the foetal fluids at all the stages of gestation. .

The concentrations of potassium and sodium ions in the amniotic fluid ranged from 3.6 to 27.4 mmol / lit and 14.0 to 97.0 mmol / lit (12 to 195 mm fetal body length and 22 to 77 days old). On the other hand, at the same fetal body length and age same ions in the allantoic fluid ranged from 1.2 to 47.6 and 8.0 to 113.0 mmol/ lit, reviewed by Kleflin *et al.* (1988) in case of pig fetus.

Raj Sundrum *et al.* (1990) studied the changes in biochemical constituents of foetal fluids of buffaloes and reported that sodium concentration in allantoic fluid was 80.25 ± 5.80 mmol / l and in amniotic fluid 117.5 ± 2.70 mmol / l. As the stage of gestation increased the sodium concentration declined and the potassium level increased in the allantoic fluid while in the amniotic fluid, it showed non significant variation and also reported that the concentration of urea nitrogen increased (from 40.00 ± 2.43 to 62.5 ± 9.16 mg/dl) in allantoic fluid due to entering of foetal urine into the allantoic cavity through the urachus and allantoic duct.

Bazar and Sharp (1991) reported that a protein produced by sheep conceptus between 12-21 days of pregnancy called ovine trophoblastic protein-1 (OTP-1) that enhanced secretions of proteins by uterine endometrium.

McCrabb *et al.* (1991) reported that factors like maternal under nutrition during mid-pregnancy in sheep resulted in retarded growth of placenta but there was no significant effect on the whole body glucose metabolism during late pregnancy.

Chauhan *et al.* (1992) reported that the potassium concentration increased progressively on foetal fluids with advancing pregnancy.

Mufti (1995) reported that as the gestational age advanced total protein concentration increased in allantoic fluid and maternal serum, glucose concentration increased in serum but decreased in allantoic fluid, urea increased both in allantoic as well as in serum, cholesterol remain almost constant in allantoic fluid and decreased in serum, creatinine increased both in serum and allantoic fluid, Sodium showed declining phase both in serum and in allantoic fluid whereas, potassium increased in allantoic fluid and decreased in serum in ewe.

Activity of enzymes like Aspartate Amino Transferase (ASAT), Alanine Amino Transferase (ALAT) and Alkaline Phosphatase (AKP) studied by Chauhan and Tiwari (1996) in the amniotic fluids of goats. ASAT and ALAT activity was maximum during stage I of gestation (crown-rump length 4 cm or less) AKP was higher during the stage II of gestation (crown-rump length 4 -20 cm).

Interferon tau, a member of type-1 interferon, is the first protein secreted by the trophoblast of the ruminant conceptus between days 15 -24 for cows, days 12-22 for sheep and days 15-21 for foals is essential for pregnancy recognition, IFN-tau induces several protein involved in embryo implantation, reviewed by (Fakowska-Podstawka *et al.*, 2002).

Dhami *et al.* (2003) evaluated the micromineral profile in blood plasma of female Holstein Friesian (HF) cattle of different age groups and reproductive status. They found that Zinc level was significantly low in lactating and anoestrus animals compared to young growing calves, cycling and pregnant heifers. Iron was lowest in repeat breeders

and highest in pubertal and pregnant heifers. Copper content was low in parturient and lactating cows compared to young calves, pregnant heifers and repeat breeding cows. Copper was also low in lactating cows and heifers around one year of age.

Ismail *et al.* (2003) suggested about mean concentrations of alkaline phosphatase as 57.0 ± 16.5 IU/ litre in the serum of female camels between their second and third trimester of pregnancy.

Xu-HuiZhong *et al.* (2004) reported about various microminerals in blood of beef cattle of different ages and sexes. The contents of copper (Cu), Zinc (Zn), Iron (Fe), Magnesium (Mg) and Potassium (K) were 0.402 ± 0.18 , 2.530 ± 0.75 , 412.526 ± 67.52 , 26.580 ± 3.04 and 371.422 ± 172.704 micro g/ml, respectively. They also found that altitude, sex, feed level and pregnancy had no significant effect on the contents of the above mentioned five minerals.

Manish *et al.* (2004) reported about various enzymes along with their activity viz alkaline phosphatase (AKP) (11.75 ± 0.42 King Armstrong's Units/dl), glutamate-oxaloacetate transaminase (GOT) (51.62 ± 0.70 Karmen's Units/ml), and glutamate-pyruvate transaminase (GPT) (16.84 ± 0.50 Karmen's Units/ml) in the serum samples of Jamunapuri and Marwari bucks and they also noticed that the activity of AKP was higher in the Jamunapuri breed (13.91 ± 0.63 King Armstrong's Units/dl) than that in the Marwari breed (9.58 ± 0.45 King Armstrong's Units/dl).

Antunovic and Steiner (2004) investigated the changes of biochemical constituents in the serum of pregnant, non-pregnant and lactating ewes. He found that there were low concentrations of sodium in the serum of pregnant and non-pregnant ewes and high concentration of potassium in non pregnant and lactating ewes, cholesterol and Glutamate Pyruvate Transaminase (GPT) in pregnant, total protein in non-pregnant ewes.

Zogno *et al.* (2004) studied certain biochemical constituents of fetal fluids of female moco (*K. rupestris*) and he found that the concentrations (mg/ml) of glucose, creatinine, urea and total protein as 45.87 ± 22.38 , 0.45 ± 0.12 , 34.03 ± 5.96 and 31.24 ± 16.67 respectively in the amniotic fluid whereas, the concentrations for the similar parameters in the allantoic fluid were 59.17 ± 10.85 , 0.38 ± 0.38 , 31.49 ± 2.28 and 30.70 ± 18.39 respectively.

Pourouchottamane *et al.* (2005) reported certain blood biochemical constituents in female yaks at different physiological statuses and recorded that the young animals had lower blood protein values compared to lactating and non-lactating adult females. The values of alkaline phosphatase, serum glutamate oxaloacetate transaminase (SGOT) and glutamate pyruvate transaminase (SGPT) ranged from 59.7 to 385.2, 18.4 to 82.06 and 5.71 to 37.82 IU/litre, respectively.

2.3. Hormonal and Receptor status :

Estrogen receptor (ER) concentration was inversely related to the level of plasma progesterone (P4) in cycling heifers. Endometrial oestrogen and plasma estrogen levels were also inversely correlated with plasma P4 levels. Thus, if P4

levels influences ER levels, the hormone may make the tissue less responsive to ovarian oestrogen at such times as the luteal phase of the cycle and during pregnancy (Henricks and Harris, 1978).

The uterine endometrium of pregnant females is dominated by progesterone which stimulates development of glandular epithelium that becomes highly secretory and produces histotroph which is essential for conceptus development. Secretory vesicles accumulate in the epithelium of endometrial glands (Bazer and Roberts, 1983). Protein released from the secretory vesicles into the uterine lumen serve as transport proteins, enzymes and regulatory proteins (Hafez, 1987).

The levels of oestradiol (E) and progesterone (P) receptors in cytosolic and nuclear fractions of cells in the porcine myometrium reported by Thailender *et al.* (1990). The concentration of oestradiol cytosolic receptors (ERc) and oestradiol nuclear receptors (ERn) in non placental and placental myometrium was low in early and mid pregnancy, increasing in late pregnancy and was highest at parturition. The levels of progesterone cytosolic receptor (PRc) in non placental regions were low in early pregnancy, increased in late pregnancy but declined during parturition whereas progesterone cytosolic receptor (PRc) in placental regions and progesterone nuclear receptor (PRn) in placental and non placental regions showed reverse character.

Thomas and Fuller (1995) suggested that uterine estrogen receptor (ER) and progesterone receptor (PR) gene expression is regulated in a tissue and cell type specific manner during estrous cycle and early pregnancy and also reported that in the ovine uterus, estrogen increases both estrogen receptor (ER) and progesterone receptor (PR) expression, whereas progesterone (P) inhibits estrogen receptor (ER) and the expression of its own receptor. Further they have recorded that alterations in tissue and cell specific expression of uterine estrogen receptor (ER) and progesterone receptor (PR) not only depend on circulating steroid hormone concentrations but also depend on locally produced, paracrine acting factors.

Rodrigues *et al.* (1998) reported that serum levels of progesterone increased during gestation and showed an abrupt decrease by two days preterm in ewes.

Progesterone attenuates the proliferative effect of estrogen in the uterus, whereas estrogen also induces the progesterone receptor (PRmRNA and PRprotein) (Hewitt and Korach, 2000).

Serum progesterone rapidly rises following formation of corpus luteum. Timing of implantation may be regulated by length of time the uterine endometrium is exposed to progesterone stimulation. The conceptus can not attach to the uterine epithelial surface until the large glycoprotein called MUC-1 has been removed. Over 8-10 days of exposure to progesterone, nuclear progesterone receptors in the uterine epithelium down-regulate leading to a loss of the direct effect of progesterone on this cell type. Since epithelial synthesis of MUC-1 is stimulated by progesterone, loss of progesterone receptor from the uterine epithelium would reduce MUC-1 production and open a receptive state for conceptus attachment (Hafez and Hafez, 2000).

Hafez and Hafez (2000) reported that estrogen and progesterone are placental hormones in ewe.

Cengiz *et al.* (2000) studied serum samples of cows at the last pregnancy period for estradiol 17β (E₂) and progesterone. They found estradiol values as 87.83, 117.33 and 592.50 in Holstein cows whereas in Brown swiss cows 85.57, 105.57 and 783.14

pg/ml during 8th, 9th and just before parturition respectively. They also recorded the Progesterone values 10.78, 8.21 and 3.61 ng/ml in Holstein cows and 10.24, 9.78 and 4.38 in Brown Swiss cows respectively during the same period.

Upregulation of progesterone receptors (PR) in endometrial stroma was mediated by estrogen signal which induces stromal cells to differentiate into decidual cells (Kurita *et al.*, 2001).

Hewitt *et al.* (2002) illustrated that estrogen was essential for epithelial proliferation and embryo implantation and was dispensable for stromal desidualization. Schuler (2002) suggested that placental estrogen and progesterone are important factors controlling caruncular growth, differentiation and function.

Endometrial receptivity is stimulated by ovarian steroids. The role of ovine and caprine ovarian steroids in initiating endometrial receptivity has been reviewed by various workers (Huct-Hudson *et al.*, 1989, Wani, 1995, Wani 1996, Wani, 2003, Wani 2005 and Wani, 2006). Proliferation of uterine cells is influenced by estrogen (E2) and progesterone (P4). These hormones have their cognate nuclear receptors in ovine/ caprine uteri called E2 receptor (ER) and P4 receptor (PR). (Lee and De Mayo, 2004). This intrinsic ability of uterine epithelium has been reviewed (Dey, 2004).

IFN-tau is believed to act by down-regulating oestrogen receptor thus preventing the oxytocin receptor and regulate the progesterone secretion (Gordon, 2004).

Gene expressions under the stimuli from progesterone (P4) are essential for implantation and maintenance of pregnancy. Various other factors having significance for inducing implantation are calcitonin, Indian hedge Log (IHA) Ambhiregulin etc (Lee and De Mayo, 2004).

Serum progesterone concentration remained at luteal levels 1.5 ± 0.5 ng/ml for the first two months of pregnancy and then gradually increased in pregnant Sumatran rhinoceros. However, progesterone decreased almost to luteal levels during the 5th months after that it increased again and eventually reached peak concentration (13.3 ± 1.9 ng/ml) shortly before parturition (Roth *et al.*, 2004).

2.4. Histological changes in the female genital organ:

Extensive erosion of pseudostratified columnar epithelium of the uterus occurred at the time of invasion of binucleate cells of the trophoblast (Bhavsar *et al.*, 1974).

Joshi *et al.* (1976) observed cervical glands of goats aged 3 months to 5 years with regard to age, oestrous cycle, pregnancy and mode of secretion and observed simple, tubular, branched, mucous type gland.

Eulenberger *et al.* (1977) studied endometrium and chorioepithelium of sows after implantation and reported that the cytoplasm of the epithelial cells was vacuolar on 28th day and granular later, reflecting their secretory activity.

King *et al.* (1981) observed that on day 18th the presence of blastocyst reduced the height of the uterine epithelium, on day 20th the trophoblast was starting to contact with maternal epithelium; while on day 21st and 30th attachment became more complex. Also reported that attachment occurred simultaneously intercaruncular and caruncular regions near the embryo and subsequently spread towards each end of the blastocyst in case of bovine.

A glycoprotein substance binds the trophoblast and the uterine epithelium visualized by the phosphotungstic acid staining. This review suggests that biochemical changes occur in the composition or distribution of the trophoblastic cell coat during the process of blastocyst attachment in the ewe (Guillomot *et al.*, 1982).

Michel and Stief (1985) demonstrated the conversion of maternal epithelium in caruncular crypts to syncytial tissue.

Banks (1986) reported that the cervix of the cow was highly glandular. A clear mucous was secreted during estrus and a thick cervical seal was produced during pregnancy. The lamina epithelialis mucosa was lined by simple columnar with goblet like cells which may be ciliated. The lamina propria-submucosa contains loose connective tissues. Tunica muscularis was well developed and rich in elastic fibres.

Hafez (1987) postulated that the lining epithelium of the cervix was simple columnar which may be ciliated or non-ciliated. The cervical crypts were more prominent in case cow compared to the other farm animals. The wall of the cervix was composed of fibrous, elastic and collagenous tissues and a small amount of muscle fibres. Because of these connective tissue fibres the wall of the cervix has high tensile strengths.

Bacha and Wood (1990) observed that the lining cells of the cervix of cow were glandular. Their secretory activity varies with the stages of estrus cycle and pregnancy. A clear mucous was secreted during estrus and a thick cervical seal was produced during pregnancy.

Uterus of four pregnant camels of 14, 25, 35 and 65 days after ovulation had studied by Skidmore *et al.* (1997). As per their observation at 14 days, majority of trophoblast had become closely apposed to uterine lumen and formed epitheliochorial placenta, at 25 day a well developed microvillar junction formed between fetal and maternal tissue and at 35 and 65 days fetus was in the middle uterine horn and large multinuclear giant trophoblast cells of a syncytial nature had developed.

Placental attachment in ruminants is characterized by the appearance of binucleate cells arising from uninucleate cells of the trophoblast, first appears on day 17 and is present throughout the gestation. These cells migrate and fuse with the underlying uterine surface epithelium to form multinucleate cells or syncytium, may be involved in immunologic protection of the conceptus and the transfer of placental lactogen. Implantation in large domestic farm species is superficial and noninvasive and involves phases of trophoblast - uterine epithelial cell apposition and adhesion, reviewed by Hafez and Hafez (2000).

Conceptus attachment in Ruminant species involves both caruncular and intercaruncular areas of the endometrium. A transitory attachment first occurs as the bovine and ovine conceptus develop finger like villi (Papillae) that project into the lumen of the uterine glands. These papillae provide a temporary anchor and absorptive structure for the conceptus as more complete attachment progress. The loss of MUC -1 from the epithelial surface permits the contact of integrins with their receptors bringing the conceptus in close contact with the uterine surface and forming epitheliochorial type of placentation, reviewed by (Hafez and Hafez, 2000).

Paul *et al.* (2003) studied the gross anatomical and histological changes during pregnancy in non-descript cow. As per observation diameter of the gravid horn increased as pregnancy progressed and the thickness of pregnant uterine wall decreased with the progress of gestation up to 71- 80 days. After that, thinning of the pregnant wall ceased. A number of binucleate giant cells (diplokaryocytes) appeared within epithelial lining.

They also observed the extensive elongation of caruncular processes and formation of branching patterns and uterine gland became more prominent close to the caruncular area and within the lamina propria.

The uterine epithelium remain unmodified in adjacent areas of the chorionic necrotic zones towards the ovarian extremities of the uterine horns and in the openings of the glandular ducts, since the trophoblast had no contact with the uterus in those areas and first changes in early implantation are contact between the trophoblast and the uterine epithelium (Rodriguez *et al.*, 2005).

Shameha *et al.* (2005) cited about the infiltration of lymphocytes and macrophages in the uterine mucosa which were diffusely scattered in pregnant rather than non pregnant rats.

Chapter – 3

MATERIALS AND METHODS

3. Materials and Methods

3.1. Experimental animals: Eighty six (86) numbers of female genitalia (both pregnant and non-pregnant) from healthy adult ewes, average age in between one to four years (1-4 years) were utilized for this experiment. The age was detected on the basis of dentition (Sastry *et al.*, 1982 and Ramadhar Singh, 1988). The organs were collected from local abattoirs present in and around Srinagar city soon after slaughter. The samples collected immediately after slaughter and carried to the laboratory in thermos flask for investigation.

3.2. Age determination of conceptus: Age of the conceptus was determined by the application of embryonic length (.05 cm = 12 days , .07 cm = 14 days) (Gordon, 2004) and Richardson's formula (Richardson, 1980) i.e. $X=2.1(Y+17)$ where, X denoted the developmental age in days and Y denoted the crown-anus length in centimeter .Age determination from fifteen (15) to thirty nine (39) days was difficult because the day of conception was unknown and also crown anus length were not distinct, so, approximate ages were determined by plotting a graph (Figure 1 and Figure 2) considering the length of embryo proper as a lower value (Ian Gordon, 2004) and crown-anus length (from 40 days onwards) as a higher value in a graph. This graph is our innovation for determining foetal age and is named as “**Khatun - Wani graph, 2007**” for foetal age determination.

3.3. Grouping of experimental animals: The animals were grouped on the basis of whole - embryo length (WEL for embryos) and crown - anus length (CAL for foetus) as given below and will be known as Group - 1:-

Sl. No.	Groups	No. of	Range	W.E / C.A.	Range	Age group
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		genitalia studied	(W.E / C.A. length in cm)	length in cm (Mean $\pm$ S.E.)	(Age group in days)	in days (Mean $\pm$ S.E.)
1	A	30	0.1-10.0	4.50 $\pm$ 0.59	14 - 57	41.40 $\pm$ 2.17
2	B	08	10.1-20.0	15.37 $\pm$ 0.69	58 -74	67.87 $\pm$ 1.38
3	C	05	20.1-30.0	25.60 $\pm$ 1.15	75 - 93	89.20 $\pm$ 2.33
4	D	05	30.1-40.0	35.74 $\pm$ 1.46	94 - 120	110.60 $\pm$ 3.14
5	E	04	40.1-50.0	45.17 $\pm$ 1.33	121 - 140	130.0 $\pm$ 2.79

Abbreviations :

W.E.L. = Whole Embryo Length

C.A.L. = Crown-anus length

S.E. = Standard Error

3.4. Group A was divided into Group A₁ (GpA₁), Group A₂ (GpA₂), Group A₃ (GpA₃), Group A₄ (GpA₄) and Group A₅ (GpA₅) because our aim was to investigate the attachment process during early pregnancy. The group A₁ - A₅ details are given below and will be known as Group - 2: -

Sl. No.	Groups	No. of genitalia studied	Range (W.E / C.A. Length in cm)	Range (W.E / C.A. length in cm) (Mean $\pm$ S.E.)	Range (Age group in days)	Range (Age group in days) (Mean $\pm$ S.E.)
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1	A ₁	10	0.1–2.0	1.16 ± 0.21	14 - 39	27.5 ± 2.70
2	A ₂	06	2.1-4.0	3.20 ± 0.34	40-44	42.16± 0.75
3	A ₃	05	4.1-6.0	5.32 ± 0.32	45-48	46.8 ± 0.58
4	A ₄	05	6.1-8.0	7.48 ± 0.22	49-52	51.4 ± 0.51
5	A ₅	04	8.1-10.0	9.55 ± 0.26	53-57	55.75 ± 0.63

3.5. Collection of blood samples: Venous blood samples were collected by jugular venupuncture from dams without knowing any history (Whether it was pregnant or non pregnant) in sterile glass tube. The blood was allowed to clot by keeping the glass tube in a slanting position for over night at room temperature. The serum was decanted and collected in centrifuge tube. It was centrifuged at 2500 rpm for 15 minutes to make clear the serum. The centrifuged serum was transferred to 5 ml screw cap plastic vials and stored at deep freeze (at -70° C) till analysis.

3.6. Collection of female genitalia: Female genitalia from slaughtered animals (Whose blood were collected) were removed from vulva up to ovaries without any tear and brought to the laboratory in thermos flask for details study.

The genitalia were spread in natural position in a tray. Various measurements of different genital organs before opening the genitalia were undertaken as follows–

1. Length of vagina, cervix, left and right uterine horns were recorded by using thread and measuring scale.
2. Length, breadth and thickness of ovary containing follicle and corpus luteum were recorded by using vernier calipers and measurements recorded upto two decimal.

3.7. Observations recorded after opening the genitalia:

1. The developmental status of the conceptus (Whether the blastocyst is spherical or elongated , the condition of the elongated blastocyst, development of the blood vessels and fluid accumulation to the blastocyst, developmental status of the cotyledons and embryo or fetus), caruncles (Whether they are sharp, flat or caviated, degree of blood circulation to the caruncle)
2. Implantation/Attachment process (Whether the conceptus implanted or just attached to the endometrium and site of implantation / attachment and stage of implantation/attachment)

3. Length of body of uterus
4. Number of attached and unattached caruncles
5. The length of embryo proper (For embryo) and crown-anus length (For fetus) were recorded.

Photographic evidences of each and every step before and after opening of genitalia were also recorded and are presented in Plate 1 to Plate 73.

3.8. Collection of foetal fluids:

The gravid uteri were cut open through the dorsal curvature without damaging the foetal sacs. The foetal cotyledons were separated gently from maternal caruncles in advance pregnancy. The blastocyst sac as a whole comes out as intact entirely in early pregnancy. Sample of allantoic and amniotic fluid were collected from respective sacs. A sterile syringe and needle was used for collection of this fluid. The fluid 15ml in volume was stored in screw cap glass tube. All glassware used in this study was sterilized at 160°C temperature for one hour in hot air oven and other surgical pack sterilized at 121°C under 15 pound pressure for 15 minutes.

The volumes of the fetal fluid by using measuring cylinder and colour of foetal fluids at different stages of pregnancy were also recorded.

3.9. Various biochemical constituents and hormones viz. pH, Glutamate Pyruvate Transaminase (GPT), Glutamate Oxaloacetate Transaminase (GOT), Alkaline phosphatase, Glucose, Total protein, Urea, Cholesterol, Creatinine, Blood urea nitrogen (BUN), Sodium (Na), Potassium (K), Zinc (Zn), Copper (Cu), Manganese (Mn), Iron (Fe), Estrogen (E) and Progesterone (P) were estimated as described under 3.10 and 3.11.

3.10. Collection of tissue:

A piece of uterine tissue both from left and right uterine horn were kept in the sterile plastic vial containing phosphate buffer saline (PBS) and stored at deep freeze (-70°C) for estrogen and progesterone receptor estimation and remaining parts of the genital organ kept at 10 % formalin for histological examination.

3.11. Biochemical Analytical Procedures:

All the following biochemical analysis was done in the laboratory of veterinary Biochemistry, Faculty of veterinary Sciences and animal husbandry, Shuhama.

3.11.1. pH : p^H of serum and foetal fluids were recorded by using digital pH meter.

3.11.2. Glutamate Pyruvate Transaminase (GPT)/Alanin Amino Transferase (ALAT) : Serum and fluid GPT/ ALAT was estimated by the method of Reitman and Frankels (1957), using SGPT(ALAT) kit and the values were expressed in Reitman and Frankel unit per ml of serum and fluid. The procedure followed as per kit instruction both for serum and fluid. The optical density (O.D) was read on a colorimeter at 505nm. Calculations were done by plotting a standard graph. Enzyme activity was plotted on X-axis and O.D on Y- axis.

3.11.3. Glutamate Oxalo Transaminase (GOT) / Aspartate Amino Transferase (ASAT) : Serum and fluid GOT/ASAT was estimated by the method of Reitman and Frankels (1957), using SGOT (ASAT) kit and the values were expressed in Reitman and Frankel unit per ml of serum and fluid. The procedure followed as per kit instruction both for serum and fluid. The optical density (O.D) was read, using colorimeter at 505nm. Calculations was done by plotting a standard graph, where enzyme activity was on X-axis and O.D on Y- axis

3.11.4. Alkaline Phosphatase :

Alkaline phosphatase was estimated by the method of Kind and Kings (1954), using Alkaline Phosphatase kit and the values were expressed in KA units. The procedure followed as per kit instruction both for serum and fluid. The optical density (O.D) was read on a colorimeter at 510nm. Calculations were undertaken using the following formula,

$$\text{Alkaline phosphatase activity in KA units} = \frac{\text{O.D. Test} - \text{O.D. control}}{\text{O.D. Standard} - \text{O.D. Blank}} \times 10$$

3.11.5. Glucose estimation :

Glucose was estimated by orthotoluidine method (Hugh *et al.*, 1971) and the values were expressed in mg %. An amount of 0.5ml and 1.0 ml serum and fluid were taken analysed as per procedures. Optical density (O.D) was read on a colorimeter at 630 nm. Values were calculated by the using following formula,

T

$$\text{Foetal fluids} = \frac{\text{-----}}{\text{S}} \times 10 \text{ (Where, T = O.D. of test and S = O.D. of standard)}$$

$$\text{T}$$

$$\text{Serum} = \frac{\text{-----}}{\text{S}} \times 10 \times 4 \text{ (Where, T = O.D. of test and S = O.D. of standard)}$$

3.11.6. Total protein :

Estimated by Biuret method (Reinhold, 1953) and the values were expressed in g/dl. Amount of serum and fluid were 0.5 ml and 1.0 ml respectively and analysis done as per method described (Reinhold, 1953). The optical density (O.D) was read on a colorimeter at 540 nm. Calculations were done by the following formula,

$$\text{T}$$

$$\text{Foetal fluids} = \frac{\text{-----}}{\text{S}} \times 200 \text{ (Where, T = O.D. of test and S = O.D. of standard)}$$

$$\text{T}$$

$$\text{Serum} = \frac{\text{-----}}{\text{S}} \times 200 \times 10 \text{ (Where, T = O.D. of test and S = O.D. of standard)}$$

3.11.7. Urea:

Estimated by Diacetyl monoxime (DAM) method (Natelson *et al.*, 1951) and the values were expressed in mg %. An amount of 0.5 ml serum and 1.0 ml foetal fluid were processed as per DAM methods described (Natelson *et al.*, 1951). The estimation of actual values was calibrated by reading the optical density (O.D) on a colorimeter at 540 nm. For calculations the formula used was as follows,

$$\text{T}$$

$$\text{Foetal fluids} = \frac{\text{-----}}{\text{S}} \times 1 \text{ (Where, T = O.D. of test and S = O.D. of standard)}$$

$$\text{T}$$

$$\text{Serum} = \frac{\text{-----}}{\text{S}} \times 10 \text{ (Where, T = O.D. of test and S = O.D. of standard)}$$

S

3.11.8. Cholesterol :

Estimated by Zlatkis *et al.*, method (1953) and the values were expressed in mg %. Amount of serum and fluid were 0.5 ml and 1.0 ml respectively and analysis done as per method described (Zlatkis *et al.*, 1953) .The optical density (O.D) was read on a colorimeter at 560 nm. Calculations were done by using the following formula,

T

Foetal fluids = ----- X 100 (Where, T = O.D. of test and S = O.D. of standard)

S

T

Serum = ----- X 100 X 25 (Where, T = O.D. of test and S = O.D. of standard)

S

3.11.9. Creatinine :

Estimated by Jaffe's method (Owen *et al.*, 1954) and the values were expressed in mg %. An amount of 0.5 ml serum and 1.0 ml foetal fluid were processed as per Jaffe's method (Owen *et al.*, 1954). The optical density (O.D) was read on a colorimeter at 520 nm. Calculations were done by using the following formula,

T

Foetal fluids = ----- X 100 (Where, T = O.D. of test and S = O.D. of standard)

S

T

Serum = ----- X 100 X 4 (Where, T = O.D. of test and S = O.D. of standard)

S

3.11.10. Urea nitrogen:

Estimated by Diacetyl Monoxime (DAM) method (Natelson *et al.*, 1951) and the values were expressed in mg %. Calculations were done by using the following formula,

Fetal fluids = Urea of fluids X 0.467

Serum = Urea of serum X 0.467

3.12. Analysis of macro and micro minerals :

Values of sodium (Na) and Potassium (K) were estimated by using Flame Photometer whereas, Zinc (Zn), Copper (Cu), Manganese (Mn) and Iron (Fe) by using Atomic Absorption Spectrophotometer (AAS) after digestion of the sample.

Digestion of Samples: Samples were digested as per procedure described by Kolmer *et al.*, 1951. One ml of sample (Both fetal fluid and serum) was mix with 10 ml of concentrated nitric acid (conc.HNO₃) heated upto 200° c for forty five minutes in the microwave and after cooling final volume made upto 50 ml. During the time of calculation the results were multiplied by dilution factor.

Analysis was done at the division of Soil Science, Sher- e- Kashmir University of Agricultural Sciences and Technology, Shalimar.

3.13. Hormonal estimation:

Both hormonal and receptor estimation were undertaken at the Department of Immunology and molecular medicine, Sher – I – Kashmir University of Medical Science (Deemed University), Soura, Kashmir.

3.13.1. Progesterone :

Values of Progesterone were estimated by using Enzyme Immunoassay for quantitative determination of serum progesterone levels. The procedure was followed as per kit instructions, kit Catalog, 4210- 96, Syntrope Bioresearch, Inc.

The details of ELISA reader was as:

Measurment filter: 450nm, Reference filter: 620nm, Measurment Range: Normal, Shaking: yes, Shaking duration: 2 sec, Shaking intensity: low

Calculations:

1. calculated the average absorbance value (A₄₅₀) for each reference standard, control and test sample.
2. Divided the average A₄₅₀ value for each standard, control and test sample by the average A₄₅₀ of standard 0 ng/ml and multiplied by 100 to obtain % A/A₀ for each sample.

3. Prepared a standard curve by plotting the % A/Ao value for each standard on y- axis verses the corresponding concentration of each standard on x- axis in a graph paper. Using the % A/Ao value for each control and test sample, determined the corresponding concentration of progesterone in ng/ml from the standard curve.

3.13.2. Estrogen:

Values of Estrogen were estimated by using Microwell Estradiol (E₂) Enzyme Immunoassay for quantitative determination of serum, Estradiol kit, Catalog, 4310A- 96, Syntrone Bioresearch, Inc. Procedure were followed as per kit instructions.

Details of ELISA reader: Same as with progesterone

Calculations: Same as with Progesterone, but the corresponding concentration of estrogen expressed in pg/ml from the standard curve.

3.14. Receptor estimation:

3.14.1. Progesterone receptor (PR):

Values of Progesterone receptor protein were estimated by using Nuclear Receptor Sandwich progesterone Receptor Enzyme Linked Immuno Sorbent assay (NR Sandwich PR ELISA kit) for quantitative determination of uterine tissue progesterone receptor levels ,Catalog , 49396 & 49896, Active Motif . Procedure followed as per kit instructions.

Details of ELISA reader: Same as used to progesterone

Calculations: Plotted the absorbance value/ optical density (O. D) of the standards on Y-axis verses the corresponding concentration (ng) of each standards on X-axis and a standard curve was drawn. The sample quantitated by plotting O.D value of the sample on the Y- axis and extends a horizontal line to the standard curve. At the intersection point extended a vertical line to the X-axis and read the corresponding standard value. The values expressed in ng / mg.

3.14.2. Estrogen receptor (ER):

Values of Estrogen receptor protein were estimated by using Nuclear Receptor Sandwich Estrogen Receptor Enzyme Linked Immuno Sorbent assay (NR Sandwich ER α

ELISA kit) for quantitative determination of uterine tissue Estrogen receptor levels, Catalog No.s, 49296 & 49796, Active Motif. Procedures here followed as per kit instructions.

Details of ELISA reader: As described under progesterone estimation above.

Calculations: Same as with progesterone receptor.

Details of Estrogen receptor (ER) and Progesterone receptor (PR) Nuclear Extract:

ER α and PR belongs to the nuclear receptor super family present in the uterine tissue. To quantitate the level of ER α and PR from the uterine tissue the nucleus was extracted out by using Nuclear Extract Kit, Catalog Nos. 40010 & 40410, and Active Motif. Frozen uterine tissue was used to extract the nucleus. The procedure followed was as per kit instruction.

The data thus generated were subjected to statistical analysis (Snedecor and Cochran, 1980).

3.15. Microscopic observations:

Uterine tissues of gravid and non gravid genitalia were fixed in 10 % formalin. The tissues were processed by paraffin embedding technique and were sectioned at 5 μ m thickness. The sections were studied by routine histomorphology by using Heamatoxylin and Eosin stain (Luna, 1968). The facility available at the Division of Vety. Anatomy & Histology and Vety. Pathology of this university was used.

Chapter – 4

EXPERIMENTAL FINDINGS

4A. EXPERIMENTAL FINDINGS (Group - 1)

In the present study the gravid uteri of sheep ranging from 14 to 140 days was divided into five groups e.g. GpA, Range: 14 – 57 days and Mean $\pm$ S.E : 41.40 ± 2.17 , GpB, Range: 58 -74 and Mean $\pm$ S.E : 67.87 ± 1.38 , GpC, Range:75 – 93 and Mean $\pm$ S.E : 89.20 ± 2.33 , GpD, Range: 94 – 120 and Mean $\pm$ S.E : 110.60 ± 3.14 and GpE, Range:121 – 140 and Mean $\pm$ S.E : 130.0 ± 2.79 (Table: 3.3).

Gross appearance of genitalia :

The mean vaginal lengths in five different groups in the present study from GpA to GpE were 7.31 ± 0.18 , 7.30 ± 0.39 , 7.32 ± 0.54 , 7.68 ± 0.54 and 7.59 ± 0.11 respectively (Table: 4A.1). The lengths of vagina increased upto GpD and then slightly decreased in GpE. But all the changes were non-significant (Table: 4A.1.1).

The mean cervical lengths from GpA to GpE were 5.96 ± 0.24 , 6.75 ± 0.43 , 6.72 ± 0.66 , 7.63 ± 0.61 and 7.0 ± 0.37 respectively (Table: 4A.1). The mean cervical lengths showed similar changes as there were in vagina. But in between GpA and Gp B, GpA and GpD and GpA and GpE the lengths of cervix increased significantly ($p = 0.05$) (Table: 4A.1.1). The average number of cervical rings both in pregnant and non pregnant animal was 5.33 ± 0.41 .

The mean lengths from cervix to left utero-tubal junction from GpA to GpE were 27.10 ± 1.11 , 47.25 ± 3.82 , 47.22 ± 5.69 , 58.8 ± 5.44 and 66.08 ± 3.86 respectively (Table: 4A.1). The lengths increased as the gestation period advanced. The difference in between Gp A and GpB, GpA and GpC, GpA and GpD, GpA and GpE, GpB and GpE, GpC and GpE were significant ($p = 0.05$) (Table: 4A.1.1).

The mean lengths from cervix to right utero-tubal junction in five groups of gestation period were 27.67 ± 1.30 , 47.03 ± 3.77 , 52.5 ± 4.73 , 54.9 ± 13.38 and 78.73 ± 12.92 respectively (Table: 4A.1). The mean lengths, from cervix to right utero-tubal junction from GpA to GpE showed similar changes as with the mean lengths from cervix

to left utero-tubal junction. The difference in between GpA and GpB, GpA and GpC, GpA and GpE, GpB and GpE were significant ($p = 0.05$). Another changes were observed from GpC to GpE that the mean lengths, from cervix to right utero-tubal junction were higher in comparison with mean lengths, from cervix to left utero-tubal junction (Table: 4A.1.1).

The mean lengths, from cervix to inter-cornual ligament were 16.41 ± 0.70 , 26.75 ± 2.24 , 35.58 ± 2.63 , 42.4 ± 0.99 and 44.65 ± 0.70 respectively, from GpA to GpE (Table: 4A.1). Here, all the groups increased significantly ($p = 0.05$) (Table: 4A.1.1).

The mean lengths of body of uterus were 1.47 ± 0.05 , 1.68 ± 0.18 , 2.1 ± 0.04 , 2.38 ± 0.13 and 2.58 ± 0.05 (Table: 4A.1). Body of uterus was increasing as the gestation period advanced. The difference in between GpA and GpC, GpA and GpD, GpA and GpE, GpB and GpC, GpB and GpD, GpB and GpE, GpC and GpD and GpC and GpE were significant ($p = 0.05$) (Table: 4A.1.1).

The details of statistical data analysis for the biometry of different parts of female genitalia are given in the Table: 4A.1.1 by employing “t” test and ANOVA.

Size of ovaries :

The mean lengths of left ovary from GpA to GpE were 1.45 ± 0.05 , 1.68 ± 0.15 , 1.66 ± 0.07 , 1.60 ± 0.06 and 1.40 ± 0.04 respectively (Table: 4A.1). The lengths of left ovary increased upto GpC but slightly decreased in GpD and GpE. The lengths of left ovary increased significantly ($p=0.05$) in between GpA and GpC, GpA and GpD and decreased significantly ($p=0.05$) in between GpC and GpE, GpD and GpE. The mean lengths of right ovary from GpA to GpE were 1.42 ± 0.05 , 1.64 ± 0.145 , 1.74 ± 0.17 , 1.55 ± 0.03 and 1.50 ± 0.04 respectively (Table: 4A.1). From the above mentioned data we can say that both left as well as right ovary showed similar changes in length as gestation period advanced. The difference in between Gp A and Gp C, GpA and Gp D were significant ($p=0.05$) in the right ovary (Table: 4A.1.1).

The mean breadth of left ovary from GpA to GpE were 1.01 ± 0.03 , 1.11 ± 0.09 , 1.16 ± 0.07 , 1.27 ± 0.03 and 1.10 ± 0.07 respectively (Table: 4A.1). The mean values increased upto GpD and slightly decreased in GpE. The breadth increased significantly ($p=0.05$) in between GpA and GpC, GpA and GpD, GpB and GpD, GpC and GpD and decreased significantly ($p=0.05$) in between GpD and GpE (Table: 4A.1.1). The mean

breadth of right ovary from GpA to GpE were 1.11 ± 0.04 , 1.15 ± 0.05 , 1.16 ± 0.04 , 1.20 ± 0.04 and 1.00 ± 0.04 respectively (Table: 4A.1). Similar parameters for the right ovary showed similar changes as there was in left ovary. In the right ovary, in between GpA and GpE, GpB and GpE, GpC and GpE, GpD and GpE showed significant ($p=0.05$) decreasing trend (Table: 4A.1.1).

The mean thickness of left ovary from GpA to GpE were 0.62 ± 0.02 , 0.63 ± 0.03 , 0.64 ± 0.02 , 0.65 ± 0.03 and 0.60 ± 0.12 respectively (Table: 4A.1). The thickness of left ovary increased upto GpD but slightly decreased in GpE. All the groups differ non-significantly (Table: 4A.1.1). The mean thickness of right ovary from GpA to GpE were 0.73 ± 0.02 , 0.74 ± 0.07 , 0.74 ± 0.05 , 0.78 ± 0.05 and 0.75 ± 0.03 respectively (Table: 4A.1). The changes of mean in thickness in different groups of pregnancy were same as in the left ovary. But the mean values were more in right ovary in all the groups of gestation period compared to the left and the values differed non-significantly in all the groups of gestation periods (Table: 4A.1.1).

Details of statistical data analysis for the biometry of ovary are given in Table: 4A.1.1 by employing “t” test and ANOVA.

Changes in the caruncle:

The average numbers of attached caruncles in the left and right horn from GpA to GpE were 32.00 ± 1.67 , 34.00 ± 1.71 , 34.00 ± 1.28 , 38.00 ± 0.71 , 39.00 ± 0.85 and 35.00 ± 0.64 , 38.00 ± 2.70 , 39.00 ± 3.29 , 39.00 ± 1.90 , 40.00 ± 2.06 respectively (Table: 4A.2). The average numbers of attached caruncles increased both in left and right horns as the gestation progressed but the average numbers of caruncles in the right horn were more in all the groups of gestation compared to the left. In case of left horn GpA and GpD, GpA and GpE, GpB and GpD, GpB and GpE, GpC and GpD, GpC and GpE differ significantly ($p=0.05$) and in case of right horn GpA and GpD, GpA and GpE differed significantly ($p=0.05$) (Table: 4A.2.1) .

The average numbers of unattached caruncles in the left and right horn from GpA to GpE were 18.00 ± 2.65 , 8.00 ± 0.53 , 7.00 ± 0.66 , 7.00 ± 0.51 , 7.00 ± 0.48 and 17.00 ± 2.82 , 8.00 ± 0.70 , 7.00 ± 0.66 , 7.00 ± 0.51 , 7.00 ± 0.48 respectively (Table: 4A.2). The average numbers of unattached caruncles decreased both in left and right horns as the

gestation period advanced. Both in the left and right horns GpA and GpB, GpA and GpC, GpA and GpD, GpA and GpE differ significantly ($p=0.05$) (Table: 4A.2.1) .

Statistical data analysis of caruncles in details is presented in Table: 4A.2.1. by employing “t” test and ANOVA.

Changes in the foetal fluid volume:

The mean foetal fluid volume in amniotic and allantoic fluid from GpA to GpE were 62.77 ± 13.13 , 259.75 ± 14.16 , 290.00 ± 14.72 , 374.00 ± 10.80 , 200.00 ± 17.91 and 54.62 ± 8.78 , 133.75 ± 16.76 , 191.25 ± 5.56 , 314.25 ± 1.93 , 523.75 ± 41.99 respectively (Table: 4A.3). Amniotic fluid increased significantly ($p=0.05$) upto GpD and then decreased significantly ($p=0.05$) in GpE. But the allantoic fluid progressively increased from GpA to GpE and all the groups showed significant differences ($p=0.05$) (Table: 4A.3.1).

Statistical data analysis for foetal fluids in details is depicted in Table: 4A.3.1. by employing “t” test and ANOVA.

Changes in the foetal fluid colour and consistency :

Colour and consistency of amniotic and allantoic fluid in different groups of pregnancy from Gp A to Gp E were, in amniotic fluid it varied from watery and transparent to very mild yellow and granular in GpA, in GpB it was light yellow and viscous, in GpC was moderate yellow and viscous, in GpD was yellow and highly viscous and in GpE was off - white and highly viscous. On the other hand in allantoic fluid in GpA it was creamy - white to yellow, opaque and slightly viscous with granules, in GpB fluid was yellow, opaque and slightly viscous with granules, in GpC was dark yellow and viscous, in GpD and GpE allantoic fluid showed similar changes i.e. intense yellow and viscous (Table: 4A.3a) (Plate 69 to Plate 73).

Changes of pH in the foetal fluid and maternal serum :

The mean values from GpA to GpE in amniotic and allantoic fluids were 7.58 ± 0.18 , 7.37 ± 0.29 , 6.88 ± 0.23 , 7.03 ± 0.34 , 6.53 ± 0.09 and 7.46 ± 0.17 , 6.89 ± 0.21 , 6.72 ± 0.43 , 6.9 ± 0.15 , 6.34 ± 0.12 respectively (Table: 4A.4). pH in the amniotic fluid was

almost alkaline in all the groups but in allantoic fluid it became acidic in GpE, otherwise it was alkaline in remaining groups. In amniotic fluid GpA and GpC, GpA and GpE, GpB and GpE differed significantly ($p=0.05$). In case of allantoic fluid GpA and GpB, GpA and GpC, GpA and GpD, GpA and GpE, GpB and GpE, GpD and GpE differed significantly ($p=0.05$) (Table: 4A.4.1).

The mean values of pH in maternal serum were alkaline in all the groups of gestation period. The mean values from GpA to GpE were 7.53 ± 0.09 , 7.60 ± 0.12 , 7.62 ± 0.16 , 7.6 ± 0.20 and 7.38 ± 0.17 respectively (Table: 4A.4). The differences in between GpA and GpE were significant ($p=0.05$) (Table: 4A.4.1).

Glucose :

The mean glucose levels in amniotic and allantoic fluids were 4.33 ± 0.42 , 4.24 ± 0.62 , 3.88 ± 0.14 , 3.41 ± 0.21 , 2.92 ± 0.15 and 3.57 ± 0.34 , 3.49 ± 0.78 , 3.0 ± 0.85 , 3.09 ± 0.93 , 2.3 ± 0.43 (Table: 4A.4) at the five gestational groups from GpA to GpE were recorded. Mean values of glucose both in amniotic and allantoic fluid showed similar shift i.e. slightly declining trend as the gestation advanced but the changes were very less amongst groups. In case of amniotic fluid GpA and GpD, GpA and GpE, GpB and GpE, GpC and GpD, GpC and GpE, GpD and GpE had significant differences ($p=0.05$), however in case of allantoic fluid only GpA and GpE differed significantly ($p=0.05$) (Table: 4A.4.1).

The mean maternal serum glucose concentrations were 56.85 ± 2.22 , 54.31 ± 1.33 , 59.8 ± 0.88 , 63.42 ± 1.75 and 65.22 ± 1.90 respectively (Table: 4A.4) at the five gestational groups. The glucose levels in maternal serum increased as the gestational period advanced and they increased significantly almost in all the groups except, in GpA and GpB, GpA and GpC, GpD and GpE (Table: 4A.4.1).

Total protein :

The mean total protein values in amniotic and allantoic fluids in five groups of gestation were 0.40 ± 0.06 , 0.42 ± 0.12 , 0.43 ± 0.17 , 0.55 ± 0.12 , 0.84 ± 0.26 and 0.48 ± 0.06 , 0.47 ± 0.03 , 0.56 ± 0.087 , 0.93 ± 0.09 , 1.42 ± 0.20 and also in serum were 6.94

± 0.82 , $11.1.0 \pm 0.62$, 13.0 ± 0.75 , $13.11 \pm .97$, 16.92 ± 0.69 (Table: 4A.4.). The concentration in serum was much higher in comparison to foetal fluids. The mean protein concentrations both in foetal fluids and maternal serum increased as the gestation period progressed. Almost all the groups in maternal serum and allantoic fluid differed significantly ($p=0.05$) except, GpB and GpD, GpC and GpD in serum and GpA and GpB, GpA and GpC, GpB and GpC in allantoic fluid. In amniotic fluid in between GpA and GpE differed significantly ($p=0.05$) (Table: 4A.4.1).

Cholesterol :

The mean cholesterol values in amniotic and allantoic fluids observed from GpA to GpE were 4.26 ± 0.15 , 4.25 ± 0.09 , 4.2 ± 0.09 , 4.36 ± 0.16 , 4.15 ± 0.10 and 3.13 ± 0.19 , 3.13 ± 0.08 , 3.16 ± 0.08 , 3.18 ± 0.09 , 3.15 ± 0.10 (Table: 4A.4) with no significant differences between the groups except, GpA and GpB in allantoic fluid which differed significantly ($p=0.05$) (Table: 4A.4.1).

The maternal serum cholesterol values showed a decreasing trend from 107.62 ± 3.44 in GpA to 57.75 ± 1.31 in GpE (Table: 4A.4). The observed values from GpA to GpE in serum were 107.62 ± 3.44 , 82 ± 5.20 , 65.8 ± 2.40 , 63.8 ± 1.91 and 57.75 ± 1.31 respectively (Table: 4A.4). Here, all the groups differed significantly ($p=0.05$) except, GpC and GpD (Table: 4A.4.1).

Creatinine:

The mean creatinine values in amniotic and allantoic fluids recorded in five groups from GpA to GpE were 1.52 ± 0.26 , 5.34 ± 0.35 , 7.34 ± 1.19 , 9.30 ± 0.65 , 13.67 ± 0.75 and 2.21 ± 0.37 , 6.09 ± 0.43 , 11.36 ± 0.88 , 15.01 ± 0.10 , 16.77 ± 0.31 and in serum were 2.23 ± 0.36 , 6.25 ± 0.26 , 11.64 ± 1.26 , 16.54 ± 0.58 , 23.88 ± 1.14 (Table: 4A.4). Creatinine concentrations in foetal fluids and maternal serum increased significantly ($p=0.05$) almost in all the groups except in between GpC and GpD which differed non-significantly in amniotic fluid as the gestation period advanced (Table: 4A.4.1).

Urea:

The mean urea concentrations in amniotic and allantoic fluids recorded in five groups from GpA to GpE were 14.01 ± 0.72 , 20.16 ± 0.66 , 23.32 ± 1.20 , 34.3 ± 1.41 , 38.1 ± 0.47 and 16.60 ± 1.02 , 28.93 ± 1.26 , 37.63 ± 3.48 , 52.08 ± 2.96 , 72.36 ± 1.08 and in serum were 61.93 ± 2.46 , 88.19 ± 3.42 , 96.95 ± 0.39 , 107.70 ± 4.96 , 114.71 ± 1.68 (Table: 4A.4).. The values in serum in all the groups of gestation period were much higher in comparison to foetal fluids. Urea concentrations in foetal fluids and maternal serum increased significantly ($p=0.05$) in all the groups except, in between GpC and GpD, GpD and GpE which differed non-significantly in serum as the gestation period advanced (Table: 4A.4.1).

Urea Nitrogen (UN):

The mean urea nitrogen concentration in amniotic and allantoic fluids observed from GpA to GpE were 6.54 ± 0.34 , 9.41 ± 0.31 , 10.89 ± 0.56 , 16.02 ± 0.66 , 17.79 ± 0.22 and 7.75 ± 0.48 , 13.51 ± 0.59 , 17.57 ± 1.62 , 24.32 ± 1.38 , 33.79 ± 0.50 and in serum were 28.92 ± 1.15 , 41.19 ± 1.60 , 45.28 ± 0.18 , 50.30 ± 2.32 , 53.57 ± 0.79 (Table: 4A.4). The concentration in serum was much higher as compared to foetal fluids. Urea nitrogen concentrations in foetal fluids and maternal serum increased significantly ($p=0.05$) in all the groups except, in between GpD and GpE in serum which differed non-significantly as the gestation period advanced (Table: 4A.4.1).

The details of statistical data analysis for biochemical constituents are given in Table: 4A.4.1 by employing “t” test and ANOVA.

Sodium:

The sodium concentration was more in amniotic fluid in comparison to allantoic fluid. The mean sodium concentrations in amniotic and allantoic fluids observed from GpA to GpE were 96.06 ± 3.14 , 96.34 ± 8.31 , 95.96 ± 6.96 , 92.78 ± 0.81 , 90.48 ± 0.86 and 88.07 ± 1.19 , 84.62 ± 3.10 , 78.8 ± 3.15 , 75.20 ± 1.46 , 72.0 ± 1.58 (Table: 4A.5). Sodium concentration in amniotic and allantoic fluids showed similar shift i.e. the values were decreasing as the gestation advanced. In amniotic fluid GpA and GpE, GpD and GpE differed significantly ($p=0.05$). However, in allantoic fluid in all the groups’ values

decreased significantly ($p=0.05$) except, GpA and GpB, GpB and GpC and GpC and GpD (Table: 4A.5.1).

The sodium concentrations in maternal serum in five groups of gestation period were 120.36 ± 3.36 , 119.21 ± 2.84 , 125.84 ± 12.59 , 136.97 ± 4.40 , 138.70 ± 3.45 (Table: 4.5). Sodium concentrations in maternal serum were more in all the stages of gestation period than foetal fluids. The differences in between GpA and GpD, GpA and GpE, GpB and GpD, GpB and GpE were significant ($p=0.05$) (Table: 4A.5.1).

Potassium:

The concentrations of sodium and potassium in foetal fluids and maternal serum showed completely reverse changes in all the groups of gestation. In amniotic and allantoic fluids mean potassium concentration from GpA to GpE were 5.23 ± 0.14 , 5.38 ± 0.39 , 6.92 ± 0.70 , 8.13 ± 0.44 , 7.69 ± 0.37 and 8.97 ± 0.36 , 12.25 ± 0.31 , 14.39 ± 0.47 , 16.80 ± 0.73 , 20.86 ± 1.33 and in serum were 7.33 ± 0.14 , 6.77 ± 0.12 , 6.40 ± 0.11 , 6.22 ± 0.12 , 6.18 ± 0.11 respectively (Table: 4A.5). In amniotic fluid the potassium concentrations increased significantly ($p=0.05$) almost in all the groups except, in GpA and GpB, GpC and GpE, GpD and GpE. But in allantoic fluid potassium concentrations increased significantly ($p=0.05$) in all the groups and in case of maternal serum decreased significantly ($p=0.05$) in all the groups except, in GpC and GpD, GpC and GpE, GpD and GpE (Table: 4A.5.1).

Copper:

The copper concentrations in amniotic fluid and maternal serum in five groups of gestation from GpA to GpE were 2.54 ± 0.22 , 2.03 ± 0.03 , 2.05 ± 0.10 , 1.98 ± 0.01 , 1.88 ± 0.05 and 3.18 ± 0.24 , 2.54 ± 0.24 , 2.34 ± 0.07 , 2.09 ± 0.09 , 2.20 ± 0.08 (Table: 4A.5). Both amniotic fluid and serum showed a decrease trend from GpA to GpE but the differences were less. In amniotic fluid and serum almost all the groups differed significantly ($p=0.05$) except, in between GpB and GpC, GpB and GpD, GpC and GpD in amniotic fluid and GpB and GpC, GpB and GpE, GpC and GpE, GpD and GpE in serum (Table: 4A.5.1).

In allantoic fluid the concentrations from GpA to GpC were 2.44 ± 0.13 , 2.83 ± 0.23 , 3.12 ± 0.46 and then slightly decreased in GpD and GpE were 2.20 ± 0.10 , 2.31 ± 0.07 (Table: 4A.5). In allantoic fluid GpB and GpD, GpB and GpE, GpC and GpD, GpC and GpE differed significantly ($p=0.05$) (Table: 4A.5.1).

Zinc:

The mean concentrations of zinc in amniotic and allantoic fluids in five groups of gestation from GpA to GpE were 5.84 ± 0.39 , 6.86 ± 0.59 , 6.95 ± 1.29 , 8.35 ± 0.30 , 8.18 ± 0.23 and 5.83 ± 0.33 , 8.88 ± 0.94 , 6.00 ± 1.10 , 10.90 ± 1.05 , 12.13 ± 0.13 respectively (Table: 4A.5). Zinc concentration in foetal fluids increased significantly ($p=0.05$) as the gestation period advanced except, in between GpA and GpC, GpD and GpE in amniotic fluid and in between GpA and GpD, GpA and GpE, GpB and GpD, GpB and GpE in allantoic fluid in which, values differed non-significantly (Table: 4A.5.1).

The zinc concentrations in maternal serum in GpA and GpB were 6.42 ± 0.49 , 7.88 ± 0.63 and then slightly decreased in GpC and GpD were 6.82 ± 0.32 , 4.88 ± 0.32 and again showed an increase trend in GpE, 4.93 ± 0.15 (Table: 4A.5). All the groups differed significantly ($p=0.05$) except, in GpA and GpC, GpD and GpE (Table: 4A.5.1).

Manganese :

The mean concentration of manganese in amniotic and allantoic fluid were fluctuating in different groups of gestation and the concentration from GpA to GpE were 3.37 ± 0.15 , 2.75 ± 0.09 , 3.06 ± 0.04 , 3.14 ± 0.17 , 2.90 ± 0.07 and 3.17 ± 0.10 , 2.81 ± 0.10 , 3.15 ± 0.17 , 3.47 ± 0.40 , 2.85 ± 0.25 (Table: 4A.5). In amniotic fluid GpA and GpB, GpA and GpC, GpA and GpE, GpB and GpC, GpB and GpD, GpC and GpE differed significantly ($p=0.05$) and in case of allantoic fluid GpA and GpB, GpB and GpC, GpB and GpD, GpC and GpD differed significantly ($p=0.05$) (Table: 4A.5.1).

The manganese concentrations in maternal serum were 3.58 ± 0.17 , 3.18 ± 0.06 , 3.20 ± 0.08 , 2.61 ± 0.16 , 2.50 ± 0.03 (Table: 4A.5). In serum the values decreased

significantly ($p=0.05$) in almost all the groups except, in between GpB and GpC, GpD and GpE which differed non-significantly (Table: 4A.5.1).

Iron:

The mean concentrations of iron in amniotic and allantoic fluids observed in the present study from GpA to GpE were 8.61 ± 0.34 , 9.50 ± 0.37 , 9.43 ± 0.27 , 10.17 ± 0.48 , 10.16 ± 0.44 and 5.82 ± 0.15 , 6.89 ± 0.28 , 7.76 ± 0.23 , 8.22 ± 0.21 , 9.43 ± 0.54 and in serum were 13.05 ± 0.28 , 12.43 ± 0.35 , 12.34 ± 0.39 , 10.00 ± 0.45 , 8.62 ± 0.49 respectively (Table: 4A.5). The concentration of iron in foetal fluids increased while in maternal serum decreased as the pregnancy progressed. Iron in allantoic fluid increased significantly ($p=0.05$) in all the groups but in maternal serum decreased significantly ($p=0.05$) almost in all the groups except, in GpA and GpB, GpB and GpC, where they differed non-significantly. In amniotic fluid in between GpA and GpB, GpA and GpC, GpA and GpD, GpA and GpE increased significantly ($p=0.05$) (Table: 4A.5.1).

The details of statistical data analysis for all the minerals are given in Table: 4A.5.1 by employing “t” test and ANOVA.

Glutamate Oxalo Transaminase (GOT)/Aspartate Amino Transferase (ASAT):

The mean concentrations of GOT/ASAT showed similar changes both in amniotic and allantoic fluids. In amniotic and allantoic fluids the concentrations decreased from GpA to GpC were 15.26 ± 0.45 , 13.18 ± 1.07 , 13.40 ± 0.4 and 13.48 ± 0.42 , 12.05 ± 1.13 , 12.43 ± 0.50 and again increased in GpD and GpE were 14.80 ± 0.86 , 15.00 ± 1.08 and 13.4 ± 0.93 , 13.75 ± 0.95 (Table: 4A.6). In maternal serum concentrations decreased as gestation period advanced and were 104.10 ± 4.03 , 94.38 ± 3.05 , 86.6 ± 3.79 , 83.6 ± 3.83 , 78.5 ± 1.32 (Table: 4A.6). In amniotic fluid GpA and GpB, GpA and GpC decreased significantly ($p=0.05$) and in allantoic fluid GpA and GpC decreased significantly ($p=0.05$). In serum in all the groups the concentration decreased significantly except, GpC and GpD, GpD and GpE (Table: 4A.6.1).

Glutamate Pyruvate Transaminase (GPT)/Alanin Amino Transferase (ALAT):

The mean values of GPT/ALAT in amniotic fluid decreased from GpA to GpC were 7.56 ± 0.24 , 6.36 ± 0.39 , 6.98 ± 0.76 and increased in GpD and GpE were 7.04 ± 0.50 , 7.00 ± 0.33 (Table: 4A.6). The differences in between GpA and GpB, GpA and GpE differed significantly ($p=0.05$). The mean values of GPT/ALAT in allantoic fluid decreased from GpA to GpD were 6.34 ± 0.24 , 5.49 ± 0.37 , 5.62 ± 0.82 , 5.82 ± 0.51 and increased in GpE, 5.90 ± 0.19 (Table: 4A.6). In allantoic fluid in between GpA and GpB differed significantly ($p=0.05$) (Table: 4A.6.1).

In maternal serum concentrations decreased from GpA to GpE were 34.63 ± 1.19 , 28.20 ± 0.80 , 29.75 ± 0.97 , 26.00 ± 1.30 , 24.00 ± 2.04 (Table: 4A.6). Here, almost in all the groups it decreased significantly ($p=0.05$) except in GpB and GpC, GpC and GpD, GpD and GpE (Table: 4A.6.1).

Alkaline Phosphatase:

The mean concentrations of alkaline phosphatase both in foetal fluids and maternal serum increased as the gestation period advanced. In amniotic and allantoic fluids the concentration observed from GpA to GpE were 30.73 ± 1.51 , 35.75 ± 1.63 , 40.2 ± 1.50 , 41.2 ± 1.59 , 43.75 ± 1.31 and 27.33 ± 1.42 , 31.88 ± 1.44 , 33.60 ± 1.60 , 32.40 ± 2.40 , 36.50 ± 1.26 and in serum were 60.70 ± 3.43 , 71.88 ± 2.96 , 76.80 ± 4.99 , 78.80 ± 2.75 , 92.50 ± 4.99 respectively (Table: 4A.6). Both in foetal fluids and maternal serum almost in all the groups the values increased significantly ($p=0.05$) except, Gpc and GpD, GpD and GpE in amniotic fluid GpB and GpC, GpB and GpD, GpC and GpD, GpC and GpE in allantoic fluid GpB and GpC, GpC and GpD in serum (Table: 4A.6.1).

The details of statistical data analysis for the enzymes are given in Table: 4A.6.1 by employing “t” test and ANOVA.

Progesterone:

The mean concentrations of progesterone in maternal serum observed from GpA to GpE were 5.32 ± 0.33 , 8.26 ± 0.11 , 7.70 ± 0.23 , 7.08 ± 0.35 , 7.00 ± 0.32 respectively (Table: 4A.7). The progesterone concentrations increased significantly ($p=0.05$) from GpA to GpB and decreased significantly ($p=0.05$) in GpC but decreased non-significantly from GpD to GpE. The progesterone concentration increased significantly ($p=0.05$) in

between GpA and GpB, GpA and GpC, GpA and GpD, GpA and GpE, GpB and GpC, GpB and GpD, GpB and GpE and decreased significantly ($p=0.05$) in between GpC and GpE (Table: 4A.7.1)

Estrogen:

The mean concentrations of estrogen followed almost similar shift from GpA to GpE as in progesterone. The concentrations of estrogen recorded from maternal serum in the present study from GpA to GpE were 138.50 ± 11.42 , 250.0 ± 0.0 , 230.0 ± 12.25 , 210.0 ± 18.71 , 210.0 ± 10.0 respectively (Table: 4A.7). The estrogen concentration increased significantly ($p=0.05$) in between GpA and GpB, GpA and GpC, GpA and GpD, GpA and GpE and decreased significantly ($p=0.05$) in between GpB and GpC, GpB and GpD, GpB and GpE (Table: 4A.7.1).

Progesterone receptor:

The mean concentrations of progesterone receptor in uterine tissue from GpA to GpE were 3.81 ± 0.04 , 4.33 ± 0.06 , 4.38 ± 0.04 , 4.09 ± 0.09 , 3.86 ± 0.12 respectively (Table: 4A.7). The mean concentration of progesterone receptor followed similar shift as in progesterone from GpA to GpE. The progesterone receptor concentration increased significantly ($p=0.05$) in between GpA and GpB, GpA and GpC, GpA and GpD and decreased significantly ($p=0.05$) in between GpB and GpD, GpB and GpE, GpC and GpE, GpD and GpE (Table: 4A.7.1)

Estrogen receptor:

The mean concentrations of estrogen receptor in uterine tissue from GpA to GpE were 0.37 ± 0.01 , 0.41 ± 0.01 , 0.41 ± 0.01 , 0.37 ± 0.02 , 0.36 ± 0.03 respectively (Table: 4A.7). The estrogen receptor concentrations increased significantly ($p=0.05$) in between GpA and GpB, GpA and GpC and decreased significantly ($p=0.05$) in between GpB and GpD, GpB and GpE, GpC and GpD (Table: 4A.7.1).

The details of statistical data analysis for the hormones and receptors are given in Table: 4A.7.1 by employing “t” test and ANOVA.

TABLES

(Group-1)

Table: 4A.1. Biometry of parts of female genital organ during different stages of pregnancy in sheep

Parameters (cm)	Groups (Length of embryo and CA length of fetus in cm / Age in days)				
	A (0.1 – 10 / 14 - 57)	B (10.1 – 20 / 58 -74)	C (20.1 - 30 / 75 - 93)	D (30.1 – 40 / 94 - 120)	E (40.1 – 50 / 121 - 140)
Length Of Vagina	7.31 ± 0.18 ^a	7.28 ± 0.39 ^a	7.32 ± 0.54 ^a	7.68 ± 0.54 ^a	7.59 ± 0.11 ^a
Length Of Cervix	5.96 ± 0.24 ^a	6.75 ± 0.43 ^{bf}	6.72 ± 0.66 ^{af}	7.63 ± 0.61 ^{df}	7.0 ± 0.37 ^{ef}
From Cervix to Lt UTJ (Length)	27.10 ± 1.11 ^a	47.25 ± 3.82 ^{bf}	47.22 ± 5.69 ^{cf}	58.8 ± 5.44 ^{dfg}	66.08 ± 3.86 ^{eg}
From Cervix to Rt UTJ (Length)	27.67 ± 1.30 ^a	47.03 ± 3.77 ^b	52.5 ± 4.73 ^{cb}	54.9 ± 13.38 ^{abf}	78.73 ± 12.92 ^{ecf}
From Cervix to ICL (Length)	16.41 ± 0.70 ^a	26.75 ± 2.24 ^b	35.58 ± 2.63 ^c	42.4 ± 0.99 ^d	44.65 ± 0.70 ^e
Length of Body of uterus	1.47 ± 0.05 ^a	1.68 ± 0.18 ^a	2.1 ± 0.04 ^c	2.38 ± 0.13 ^{df}	2.58 ± 0.05 ^{ef}

Parameters (cm)		Groups (Length of embryo and CA length of fetus in cm / Age in days)				
		A (0.1 – 10 / 14 - 57)	B (10.1 – 20 / 58 -74)	C (20.1 - 30 / 75 - 93)	D (30.1 – 40 / 94 - 120)	E (40.1 – 50 / 121 - 140)
Left Ovary	Length	1.45 ± 0.05 ^a	1.68 ± 0.15 ^{ae}	1.66 ± 0.07 ^{ce}	1.60 ± 0.06 ^{de}	1.40 ± 0.04 ^a
	Breadth	1.01 ± 0.03 ^a	1.11 ± 0.09 ^{ae}	1.16 ± 0.07 ^{ce}	1.27 ± 0.03 ^d	1.10 ± 0.07 ^{ae}
	Thickness	0.62 ± 0.02 ^a	0.63 ± 0.03 ^a	0.64 ± 0.02 ^a	0.65 ± 0.03 ^a	0.60 ± 0.12 ^a
Right Ovary	Length	1.42 ± 0.05 ^a	1.64 ± 0.145 ^{ae}	1.74 ± 0.17 ^{be}	1.55 ± 0.03 ^{de}	1.50 ± 0.04 ^{ae}
	Breadth	1.11 ± 0.04 ^a	1.15 ± 0.05 ^a	1.16 ± 0.04 ^a	1.20 ± 0.04 ^a	1.00 ± 0.04 ^e
	Thickness	0.73 ± 0.02 ^a	0.74 ± 0.07 ^a	0.74 ± 0.05 ^a	0.78 ± 0.05 ^a	0.75 ± 0.03 ^a

Values with different superscript are significant and having any one of the common superscript are non significant, p = 0.05.

Comparison was made by applying students “t” test.

Abbreviations : Lt UTJ = Left Utero Tubal Junction, Rt UTJ = Right Utero Tubal Junction, ICL = Inter Cornual Ligament

Table: 4A.1.1. Statistical Analysis of Table - 4A.1. (ANOVA + t-test)

Parameters	ANOVA	t-test									
		AB	AC	AD	AE	BC	BD	BE	CD	CE	DE
Length Of Vagina	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Length Of Cervix	NS	S	NS	S	S	NS	NS	NS	NS	NS	NS
From Cervix to Lt UTJ (Length)	HS	HS	S	S	HS	NS	NS	S	NS	S	NS
From Cervix to Rt UTJ (Length)	HS	S	HS	NS	S	NS	NS	S	NS	NS	NS
From Cervix to ICL (Length)	HS	S	HS	HS	HS	S	HS	HS	S	S	S

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Abbreviations : S = Significant, NS = Non Significant, HS = Highly Significant

Table: 4A.2. Number of caruncles during different stages of pregnancy in sheep

Parameters (No.s)		Groups (Length of embryo and CA length of fetus in cm / Age in days)				
		A (0.1 – 10 / 14 - 57)	B (10.1 – 20 / 58 -74)	C (20.1 - 30 / 75 - 93)	D (30.1 – 40 / 94 - 120)	E (40.1 – 50 / 121 - 140)
Caruncles (Left horn)	Attached	32.00 ± 1.67 ^a	34.00 ± 1.71 ^a	34.00 ± 1.28 ^a	38.00 ± 0.71 ^{de}	39.00 ± 0.85 ^e
	Unattached	18.00 ± 2.65 ^a	8.00 ± 0.53 ^{bf}	7.00 ± 0.66 ^{cf}	7.00 ± 0.51 ^{df}	7.00 ± 0.4 8 ^{ef}
Caruncles	Attached	35.00 ± 0.64 ^a	38.00 ± 2.70 ^{af}	39.00 ± 3.29 ^{af}	39.00 ± 1.90 ^{df}	40.00 ± 2.06 ^{ef}

(Right horn)	Unattached	a 17.00 ± 2.82	bf 8.00 ± 0.70	cf 7.00 ± 0.66	df 7.00 ± 0.51	ef 7.00 ± 0.48
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Values with different superscript are significant and having any one of the common superscript are non significant, $p = 0.05$.

Comparison was made by applying students “t” test.

Table: 4A.2.1. Statistical Analysis of Table - 4A.2. (ANOVA + t-test)

Parameters	ANOVA	t-test									
		AB	AC	AD	AE	BC	BD	BE	CD	CE	DE
Caruncles (Left horn)											

Attached	NS	NS	NS	S	S	NS	S	S	S	S	NS
Unattached	S	S	S	S	S	NS	NS	NS	NS	NS	NS
Caruncles (Right horn)											
Attached	NS	NS	NS	S	S	NS	NS	NS	NS	NS	NS
Unattached	NS	S	S	S	S	NS	NS	NS	NS	NS	NS

Abbreviations : S = Significant, NS = Non Significant, HS = Highly Significant

Table: 4A.3. Changes in fetal fluid volume indifferent stages of pregnancy in sheep

Fetal fluid (ml)	Groups (Length of embryo and CA length of fetus in cm / Age in days)				
	A (0.1 – 10 /	B (10.1 – 20 /	C (20.1 - 30 /	D (30.1 – 40 /	E (40.1 – 50 /

	14 - 57)	58 -74)	75 - 93)	94 - 120)	121 - 140)
Amniotic fluid	62.77 ± 13.13 ^a	259.75 ± 14.16 ^{bf}	290.00 ± 14.72 ^{cf}	374.00 ± 10.80 ^d	200.00 ± 17.91 ^e
Allantoic fluid	54.62 ± 8.78 ^a	133.75 ± 16.76 ^b	191.25 ± 5.56 ^c	314.25 ± 1.93 ^d	523.75 ± 41.99 ^e

Values with different superscript are significant and having any one of the common superscript are non significant, p = 0.05.
Comparison was made by applying students “t” test.

Table: 4A.3.1. Statistical Analysis of Table – 4A.3. (ANOVA + t-test)

Parameters	ANOVA	t-test									
		AB	AC	AD	AE	BC	BD	BE	CD	CE	DE
FOETAL FLUID											
Amniotic	HS	HS	HS	HS	HS	NS	HS	S	S	S	HS
Allantoic	HS	S	HS	HS	HS	S	HS	HS	HS	HS	S

Abbreviations : S = Significant, NS = Non Significant, HS = Highly Significant

Table: 4A.3a. Changes in fetal fluid colour and consistency indifferent stages of pregnancy in sheep

Foetal fluid (ml)	Groups (Length of embryo and CA length of fetus in cm / Age in days)				
	A (0.1 – 10 / 14 - 57)	B (10.1 – 20 / 58 -74)	C (20.1 - 30 / 75 - 93)	D (30.1 – 40 / 94 - 120)	E (40.1 – 50 / 121 - 140)
Amniotic fluid	Watery and transparent without granules to very mild yellow with granules	Mild yellow and viscous	Moderate yellow and viscous	Yellow and highly viscous	Off white and highly viscous
Allantoic fluid	Creamy white to Yellow, opaque and slightly viscous with granules	Yellow, opaque and slightly viscous with granules	Dark yellow and viscous	Intense yellow and viscous	Intense yellow and viscous

Table: 4A.4. Values of biochemical constituents of fetal fluid and maternal serum during different stages of gestation in sheep

Parameters	Types of fluid	Groups (Length of embryo and CA length of fetus in cm / Age in days)				
		A (0.1 – 10 / 14 - 57)	B (10.1 – 20 / 58 -74)	C (20.1 - 30 / 75 - 93)	D (30.1 – 40 / 94 - 120)	E (40.1 – 50 / 121 - 140)
pH (Mean ± SE)	Amniotic	7.58 ± 0.18 ^a	7.37 ± 0.29 ^{af}	6.88 ± 0.23 ^{cfg}	7.03 ± 0.34 ^{afg}	6.53 ± 0.09 ^{eg}
	Allantoic	7.46 ± 0.17 ^a	6.89 ± 0.21 ^{bf}	6.72 ± 0.43 ^{cfg}	6.9 ± 0.15 ^{df}	6.34 ± 0.12 ^{eg}
	Serum	7.53 ± 0.09 ^a	7.60 ± 0.12 ^{af}	7.62 ± 0.16 ^{ag}	7.6 ± 0.20 ^{ag}	7.38± 0.17 ^{efg}
Glucose (Mean ± SE)	Amniotic fluid (mg%)	4.33 ± 0.42 ^a	4.24 ± 0.62 ^{afg}	3.88 ± 0.14 ^{af}	3.41 ± 0.21 ^{dg}	2.92 ± 0.15 ^e
	Allantoic fluid (mg%)	3.57 ± 0.34 ^a	3.49 ± 0.78 ^{af}	2.8 ± 0.85 ^{af}	3.09 ± 0.93 ^{af}	2.3 ± 0.43 ^{ef}
	Serum (mg%)	56.85 ± 2.22 ^{ac}	54.31 ± 1.33 ^a	59.8 ± 0.88 ^c	63.42 ± 1.75 ^{df}	65.22 ± 1.90 ^{ef}
Total protein (Mean ± SE)	Amniotic fluid (g/dl)	0.40 ± 0.06 ^a	0.42 ± 0.12 ^{af}	0.43 ± 0.17 ^{af}	0.55 ± 0.12 ^{af}	0.84 ± 0.26 ^{ef}
	Allantoic fluid (g/dl)	0.48 ± 0.06 ^a	0.47 ± 0.03 ^a	0.56 ± 0.087 ^a	0.93 ± 0.09 ^d	1.42 ± 0.20 ^e
	Serum (g/dl)	6.94 ± 0.82 ^a	11.1.0 ± 0.62 ^{bf}	13.0 ± 0.75 ^{cg}	13.11 ± .97 ^{dfg}	16.92 ± 0.69 ^e

Cholesterol (Mean ± SE)	Amniotic fluid (mg%)	4.26 ± 0.15 a	4.25 ± 0.09 a	4.2 ± 0.09 a	4.36 ± 0.16 a	4.15 ± 0.10 a
	Allantoic fluid (mg%)	3.13 ± 0.19 a	3.13 ± 0.08 bf	3.16 ± 0.08 af	3.18 ± 0.09 af	3.15 ± 0.10 af
	Serum (mg%)	107.62 ± 3.44 a	82 ± 5.20 b	65.8 ± 2.40 cf	63.8 ± 1.91 df	57.75 ± 1.31 e
Parameters	Types of fluid	Groups (Length of embryo and CA length of fetus in cm / Age in days)				
		A (0.1 – 10 / 14 - 57)	B (10.1 – 20 / 58 -74)	C (20.1 - 30 / 75 - 93)	D (30.1 – 40 / 94 - 120)	E (40.1 – 50 / 121 - 140)
Creatinine (Mean ± SE)	Amniotic fluid (mg%)	1.52 ± 0.26 a	5.34 ± 0.35 b	7.34 ± 1.19 cf	9.30 ± 0.65 df	13.67 ± 0.75 e
	Allantoic fluid (mg%)	2.21 ± 0.37 a	6.09 ± 0.43 b	11.36 ± 0.88 c	15.01 ± 0.10 d	16.77 ± 0.31 e
	Serum (mg%)	2.23 ± 0.36 a	6.25 ± 0.26 b	11.64 ± 1.26 c	16.54 ± 0.58 d	23.88 ± 1.14 e
Urea (Mean ± SE)	Amniotic fluid (mg%)	14.01 ± 0.72 a	20.16 ± 0.66 b	23.32 ± 1.20 c	34.3 ± 1.41 d	38.1 ± 0.47 e
	Allantoic fluid (mg%)	16.60 ± 1.02 a	28.93 ± 1.26 b	37.63 ± 3.48 c	52.08 ± 2.96 d	72.36 ± 1.08 e
	Serum (mg%)	61.93 ± 2.46 a	88.19 ± 3.42 b	96.95 ± 0.39 cf	107.70 ± 4.96 dfg	114.71 ± 1.68 eg
Urea Nitrogen	Amniotic fluid (mg%)	6.54 ± 0.34 a	9.41 ± 0.31 b	10.89 ± 0.56 c	16.02 ± 0.66 d	17.79 ± 0.22 e

(Mean ± SE)	Allantoic fluid (mg%)	7.75 ± 0.48 ^a	13.51 ± 0.59 ^b	17.57 ± 1.62 ^c	24.32 ± 1.38 ^d	33.79 ± 0.50 ^e
	Serum(mg%)	28.92 ± 1.15 ^a	41.19 ± 1.60 ^b	45.28 ± 0.18 ^c	50.30 ± 2.32 ^{df}	53.57 ± 0.79 ^{ef}

Values with different superscript are significant and having any one of the common superscript are non significant, p = 0.05.
Comparison was made by applying students “t” test.

Table: 4A.4.1. Statistical Analysis of Table – 4A.4. (ANOVA + t-test)

Parameters	ANOVA	t-test									
		AB	AC	AD	AE	BC	BD	BE	CD	CE	DE
pH											
Amniotic	NS	NS	S	NS	HS	NS	NS	S	NS	NS	NS
Allantoic	S	S	S	S	HS	NS	NS	S	NS	NS	S
Serum	NS	NS	NS	NS	S	NS	NS	NS	NS	NS	NS
GLUCOSE											
Amniotic	NS	NS	NS	S	S	NS	NS	S	S	S	S
Allantoic	NS	NS	NS	NS	S	NS	NS	NS	NS	NS	NS
Serum	NS	NS	NS	S	S	S	S	S	S	S	NS

TOTAL PROTEIN											
Amniotic	NS	NS	NS	NS	S	NS	NS	NS	NS	NS	NS
Allantoic	HS	NS	NS	S	S	NS	S	S	S	S	S
Serum	HS	S	HS	S	HS	S	NS	HS	NS	S	S
CHOLESTEROL											
Amniotic	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Allantoic	NS	S	NS	NS	NS	NS	NS	NS	NS	NS	NS
Serum	HS	S	HS	HS	HS	S	S	S	NS	S	S

Parameters	ANOVA	t-test									
		AB	AC	AD	AE	BC	BD	BE	CD	CE	DE
CREATININE											
Amniotic	HS	HS	S	HS	HS	S	HS	HS	NS	S	S
Allantoic	HS	HS	HS	HS	HS	HS	HS	HS	S	HS	S
Serum	HS	HS	HS	HS	HS	S	HS	HS	S	HS	S
UREA											

Amniotic	HS	HS	HS	HS	HS	S	HS	HS	HS	HS	S
Allantoic	HS	HS	HS	HS	HS	S	HS	HS	S	HS	HS
Serum	HS	HS	HS	HS	HS	S	S	HS	NS	HS	NS
UREA NITROGEN (UN)											
Amniotic	HS	HS	HS	HS	HS	S	HS	HS	HS	HS	S
Allantoic	HS	HS	HS	HS	HS	S	HS	HS	S	HS	HS
Serum	HS	HS	HS	HS	HS	S	S	HS	S	HS	NS

Abbreviations : S = Significant, NS = Non Significant, HS = Highly Significant

Table: 4A.5. Various macro and micro minerals of fetal fluids and maternal serum during different stages of gestation in sheep

Parameters	Types of fluid	Groups (Length of embryo and CA length of fetus in cm / Age in days)				
		A (0.1 – 10 / 14 - 57)	B (10.1 – 20 / 58 -74)	C (20.1 - 30 / 75 - 93)	D (30.1 – 40 / 94 - 120)	E (40.1 – 50 / 121 - 140)

Sodium (Mean ± SE)	Amniotic (mmol/L)	96.06 ± 3.14 a	96.34 ± 8.31 af	95.96 ± 6.96 ag	92.78 ± 0.81 a	90.48 ± 0.86 efg
	Allantoic (mmol/L)	88.07± 1.19 a	84.62 ± 3.10 af	78.8 ± 3.15 cfg	75.20± 1.46 dg	72.0 ± 1.58 e
	Serum (mmol/L)	120.36 ± 3.36 a	119.21 ± 2.84 a	125.84 ± 12.59 af	136.97 ± 4.40 df	138.70 ± 3.45 ef
Potassium (Mean ± SE)	Amniotic fluid (mmol/L)	5.23 ± 0.14 a	5.38 ± 0.39 a	6.92 ± 0.70 cg	8.13 ± 0.44 df	7.69 ± 0.37 egf
	Allantoic fluid (mmol/L)	8.97 ± 0.36 a	12.25 ± 0.31 b	14.39 ± 0.47 c	16.80 ± 0.73 d	20.86 ± 1.33 e
	Serum (mmol/L)	7.33 ± 0.14 a	6.77 ± 0.12 b	6.40 ± 0.11 cf	6.22 ± 0.12 df	6.18 ± 0.11 ef
Copper (Mean ± SE)	Amniotic fluid (ppm)	2.54 ± 0.22 a	2.03 ± 0.03 bf	2.05 ± 0.10 cf	1.98 ± 0.01 df	1.88 ± 0.05 e
	Allantoic fluid (ppm)	2.44 ± 0.13 afh	2.83 ± 0.23 af	3.12 ± 0.4 6 af	2.20 ± 0.10 dgh	2.31 ± 0.07 gh
	Serum (ppm)	3.18 ± 0.24 a	2.54 ± 0.24 bf	2.34 ± 0.07 cf	2.09 ± 0.09 dhg	2.20 ± 0.08 efhg

Parameters	Types of fluid	Groups (Length of embryo and CA length of fetus in cm / Age in days)				
		A (0.1 – 10 / 14 - 57)	B (10.1 – 20 / 58 -74)	C (20.1 - 30 / 75 - 93)	D (30.1 – 40 / 94 - 120)	E (40.1 – 50 / 121 - 140)

Zinc (Mean ± SE)	Amniotic fluid (ppm)	5.84 ± 0.39 a	6.86 ± 0.59 a	6.95 ± 1.29 af	8.35 ± 0.30 df	8.18 ± 0.23 ef
	Allantoic fluid (ppm)	5.83 ± 0.33 a	8.88 ± 0.94 b	6.00 ± 1.10 a	10.90 ± 1.05 df	12.13 ± 0.13 ef
	Serum (ppm)	6.42 ± 0.49 a	7.88 ± 0.63 a	6.82 ± 0.32 a	4.88 ± 0.32 a	4.93 ± 0.15 e
Manganese (Mean ± SE)	Amniotic fluid (ppm)	3.37 ± 0.15 a	2.75 ± 0.09 bf	3.06 ± 0.04 ch	3.14 ± 0.17 agh	2.90 ± 0.07 egf
	Allantoic fluid (ppm)	3.17 ± 0.10 ah	2.81 ± 0.10 bf	3.47 ± 0.40 a	3.15 ± 0.17 dhg	2.85 ± 0.25 afg
	Serum (ppm)	3.58 ± 0.17 a	3.18 ± 0.06 bf	3.20 ± 0.08 cf	2.61 ± 0.16 dg	2.50 ± 0.03 eg
Iron (Mean ± SE)	Amniotic fluid (ppm)	8.61 ± 0.34 a	9.50 ± 0.37 bf	9.43 ± 0.27 cf	10.17 ± 0.48 df	10.16 ± 0.44 ef
	Allantoic fluid (ppm)	5.82 ± 0.15 a	6.89 ± 0.28 b	7.76 ± 0.23 c	8.22 ± 0.21 d	9.43 ± 0.54 e
	Serum (ppm)	13.05 ± 0.28 af	12.43 ± 0.35 bfg	12.34 ± 0.39 cg	10.00 ± 0.45 d	8.62 ± 0.49 e

Values with different superscript are significant and having any one of the common superscript are non significant, p= 0.05.
Comparison was made by applying students “t” test.

Table: 4A.5.1. Statistical Analysis of Table – 4.5. (ANOVA + t-test)

Parameters	ANOVA	t-test									
		AB	AC	AD	AE	BC	BD	BE	CD	CE	DE
SODIUM (Na)											
Amniotic	NS	NS	NS	NS	S	NS	NS	NS	NS	NS	S
Allantoic	HS	NS	S	HS	HS	NS	S	S	NS	S	S
Serum	NS	NS	NS	S	S	NS	S	S	NS	NS	NS
POTASSIUM (K)											
Amniotic	HS	NS	S	HS	HS	S	S	S	S	NS	NS
Allantoic	HS	HS	HS	HS	HS	S	HS	HS	S	S	S
Serum	HS	S	S	HS	HS	S	HS	HS	NS	NS	NS
COPPER (Cu)											
Amniotic	NS	S	S	S	S	NS	NS	S	NS	S	S
Allantoic	NS	NS	NS	NS	NS	NS	S	S	S	S	NS
Serum	NS	S	S	S	S	NS	S	NS	S	NS	NS

Parameters	ANOVA	t-test									
		AB	AC	AD	AE	BC	BD	BE	CD	CE	DE
ZINC (Zn)											
Amniotic	S	NS	NS	S	S	NS	S	S	NS	NS	NS
Allantoic	HS	S	NS	S	HS	S	S	S	S	S	NS
Serum	NS	S	NS	S	S	S	S	S	S	S	NS
MANGANESE (Mn)											
Amniotic	NS	S	S	NS	S	S	S	NS	NS	S	NS
Allantoic	NS	S	NS	NS	NS	S	S	NS	S	NS	NS
Serum	S	S	S	S	HS	NS	S	HS	S	HS	NS
IRON (Fe)											
Amniotic	NS	S	S	S	S	NS	NS	NS	NS	NS	NS
Allantoic	HS	S	HS	HS	S	S	S	S	S	S	S
Serum	HS	NS	S	S	HS	NS	S	HS	S	HS	S

Abbreviations : S = Significant, NS = Non Significant, HS = Highly Significant

Table: 4A.6. Enzymes of foetal fluid and maternal serum during different stages of gestation in sheep

Parameters	Types of fluid	Groups (Length of embryo and CA length of fetus in cm / Age in days)				
		A (0.1 – 10 / 14 - 57)	B (10.1 – 20 / 58 -74)	C (20.1 - 30 / 75 - 93)	D (30.1 – 40 / 94 - 120)	E (40.1 – 50 / 121 - 140)
GOT/AST (Mean ± SE)	Amniotic (U/ L)	15.26 ± 0.45 ^a	13.18 ± 1.07 ^{bf}	13.40 ± 0.4 ^{cf}	14.80 ± 0.86 ^{af}	15.00 ± 1.08 ^{af}
	Allantoic (U/ L)	13.48 ± 0.42 ^a	12.05 ± 1.13 ^{af}	12.43 ± 0.50 ^{cf}	13.4 ± 0.93 ^{af}	13.75 ± 0.95 ^{af}
	Serum (U/ L)	104.10 ± 4.03 ^a	94.38 ± 3.05 ^b	86.6 ± 3.79 ^{cf}	83.6 ± 3.83 ^{dfg}	78.5 ± 1.32 ^{eg}
GPT/ALAT (Mean ± SE)	Amniotic fluid (U/ L)	7.56 ± 0.24 ^a	6.36 ± 0.39 ^{bf}	6.98 ± 0.76 ^{af}	7.04 ± 0.50 ^{af}	6.83 ± 0.33 ^{ef}
	Allantoic fluid (U/ L)	6.34 ± 0.24 ^a	5.49 ± 0.37 ^{bf}	5.62 ± 0.82 ^{af}	5.82 ± 0.51 ^{af}	5.90 ± 0.19 ^{af}

	Serum (U/ L)	34.63 ± 1.19 ^a	29.75 ± 0.97 ^{bf}	28.20 ± 0.80 ^{cfg}	26.00 ± 1.30 ^{dgh}	24.00 ± 2.04 ^{eh}
Alkaline Phosphatase (Mean ± SE)	Amniotic fluid (KA Units)	30.73 ± 1.51 ^a	35.75 ± 1.63 ^b	40.2 ± 1.50 ^{cf}	41.2 ± 1.59 ^{dfg}	43.75 ± 1.31 ^{eg}
	Allantoic fluid (KA Units)	27.33 ± 1.42 ^a	31.88 ± 1.44 ^{bf}	33.60 ± 1.60 ^{cfg}	32.40 ± 2.40 ^{df}	36.50 ± 1.26 ^{eg}
	Serum (KA Units)	60.70 ± 3.43 ^a	71.88 ± 2.96 ^{bf}	76.80 ± 4.99 ^{cfg}	78.80 ± 2.75 ^{dg}	92.50 ± 4.99 ^e

Values with different superscript are significant and having any one of the common superscript are non significant, p = 0.05.

Comparison was made by applying students “t” test.

Table: 4A.6.1. Statistical Analysis of Table – 4A.6. (ANOVA + t-test)

Parameters	ANOVA	t-test									
		AB	AC	AD	AE	BC	BD	BE	CD	CE	DE
GOT / AST											
Amniotic	NS	S	S	NS	NS	NS	NS	NS	NS	NS	NS
Allantoic	NS	NS	S	NS	NS	NS	NS	NS	NS	NS	NS
Serum	S	S	S	S	HS	S	S	S	NS	S	NS
GPT / ALAT											
Amniotic	NS	S	NS	NS	S	NS	NS	NS	NS	NS	NS
Allantoic	NS	S	NS	NS	NS	NS	NS	NS	NS	NS	NS
Serum	HS	S	S	S	S	NS	S	S	NS	S	NS

ALKALINE PHOSPHATASE											
Amniotic	S	S	S	S	HS	S	S	S	NS	S	NS
Allantoic	S	S	S	S	S	NS	NS	S	NS	NS	S
Serum	S	S	S	S	S	NS	S	S	NS	S	S

Abbreviations : S = Significant, NS = Non Significant, HS = Highly Significant

Table: 4A.7. Hormones and receptor estimation from maternal serum and tissue during different stages of gestation in sheep

Parameters	Types of material	Groups (Length of embryo and CA length of fetus in cm / Age in days)				
		A (0.1 – 10 / 14 - 57)	B (10.1 – 20 / 58 -74)	C (20.1 - 30 / 75 - 93)	D (30.1 – 40 / 94 - 120)	E (40.1 – 50 / 121 - 140)
Progesterone (Mean ± SE)	Serum (ng/ml)	5.32 ± 0.33 ^a	8.26 ± 0.11 ^b	7.70 ± 0.23 ^c	7.08 ± 0.35 ^{dcf}	7.00 ± 0.32 ^{ef}
Estrogen (Mean ± SE)	Serum (pg/ml)	138.50 ± 11.42 ^a	250.0 ± 0.0 ^b	230.0 ± 12.25 ^{cf}	210.0 ± 18.71 ^{df}	210.0 ± 10.0 ^{ef}

ESTROGEN											
Serum	HS	HS	S	S	S	S	S	S	NS	NS	NS
PROGESTERONE RECEPTOR											
Tissue	HS	HS	HS	S	NS	NS	S	S	S	S	S
ESTROGEN RECEPTOR											
Tissue	S	S	S	NS	NS	NS	S	S	S	NS	NS

Abbreviations : S = Significant, NS = Non Significant, HS = Highly Significant

Table: 4A.8. Prediction of age in days in relation to whole embryo/Crown-anus (CA) length

Sl. No.	Whole/CA Length in cm	Age in days
1	0.05	12
2.	0.07	14
3.	0.2	15.4
4.	0.4	18.0
5.	0.6	20.2
6.	0.8	23
7.	1.0	25.5
8.	1.3	29.5
9.	1.6	33.5
10.	1.8	36
11.	1.9	37.5
12.	2.0	39
13.	2.1	40.11
14.	2.2	40.03
15.	3.2	42.42
16.	3.7	43.47
17.	3.9	43.89
18.	4.0	44.1
19.	4.3	44.73
20.	4.9	45.99
21.	5.5	47.25
22.	5.9	48.09
23.	6	48.30
24.	6.9	50.19
25.	7.1	50.61
26.	7.5	51.54
27.	7.9	52.29
28.	8.0	52.5

Sl. No.	Whole/CA Length in cm	Age in days
29.	8.8	54.18
30.	9.6	55.86
31.	9.8	56.28
32.	10	57
33.	12.5	61.95
34.	12.9	62.79
35.	14.8	66.78
36.	15.5	68.25
37.	16.0	69.3
38.	16.4	70.14
39.	16.5	70.35
40.	18.4	74.34
41.	22.5	82.95
42.	24.6	87.36
43.	27.0	92.4
44.	27.4	93.24
45.	27.5	93.45
46.	31.8	102.48
47.	33.9	106.89
48.	35.0	109.2
49.	38.0	115.5
50.	40.0	119.7
51.	42.0	123.9
52.	44.0	128.1
53.	47.0	134.4
54.	47.5	135.45
55.	50.0	140.0

4B. EXPERIMENTAL FINDINGS (Group - 2)

Here, GpA, Range: 14 – 57 days and Mean $\pm$ S.E : 41.40 ± 2.17 is sub divided into five groups viz. GpA₁, Range:14 – 39 days and Mean $\pm$ S.E : 27.5 ± 2.70 , GpA₂, Range: 40-44 days and Mean $\pm$ S.E : 42.16 ± 0.75 , GpA₃, Range: 45-48 days and Mean $\pm$ S.E : 46.8 ± 0.58 , GpA₄, Range: 49-52 days and Mean $\pm$ S.E : 51.4 ± 0.51 and GpA₅, Range: 53-57 days and Mean $\pm$ S.E : 55.75 ± 0.63 as our main aim was to investigate the attachment process during early pregnancy (Table: 3.4).

Gross appearance of genitalia :

The mean vaginal lengths observed in present study from GpA₁ to GpA₅ were 7.08 ± 0.32 , 7.32 ± 0.56 , 7.48 ± 0.52 , 7.54 ± 0.44 and 7.40 ± 0.20 respectively (Table: 4B.1). In all the groups mean vaginal lengths did not vary significantly (Table: 4B.1.1).

The mean cervical lengths from GpA₁ to GpA₅ were 5.54 ± 0.44 , 5.57 ± 0.22 , 6.42 ± 0.57 , 6.48 ± 0.43 , 6.43 ± 0.96 respectively (Table: 4B.1). The mean cervical lengths increased as the gestation period advanced. The Cervical lengths increased ($p=0.05$) in between GpA₁ and Gp A₄, GpA₂ and Gp A₄ (Table: 4B.1.1).

The mean lengths from cervix to left utero-tubal junction from GpA₁ to GpA₅ were 22.36 ± 1.10 , 23.68 ± 1.00 , 27.92 ± 1.21 , 34.22 ± 1.60 and 34.20 ± 2.88 respectively (Table: 4B.1). The lengths from cervix to left utero-tubal junction increased significantly ($p=0.05$) almost in all the groups except in between GpA₁ and Gp A₂, GpA₃ and Gp A₅, A₄ and Gp A₅ (Table: 4B.1.1).

The mean lengths from cervix to right utero-tubal junction from GpA₁ to GpA₅ were 21.39 ± 0.95 , 26.03 ± 1.89 , 29.0 ± 1.86 , 30.48 ± 1.93 and 40.65 ± 0.77 respectively (Table: 4B.1). The lengths increased significantly ($p=0.05$) almost in all the groups except in GpA₂ and GpA₃, GpA₂ and Gp A₄, GpA₃ and GpA₄ (Table: 4B.1.1).

The mean lengths from cervix to inter-cornual ligament were 13.48 ± 0.63 , 14.42 ± 0.78 , 18.24 ± 1.33 , 18.42 ± 1.64 and 21.90 ± 1.20 respectively (Table: 4B.1). Here, also all the groups increased significantly ($p=0.05$) except, GpA₁ and GpA₂, GpA₃ and GpA₄, GpA₄ and Gp A₅ (Table: 4B.1.1).

The mean lengths of body of uterus recorded from GpA₁ to GpA₅ were 1.21 ± 0.05 , 1.45 ± 0.03 , 1.50 ± 0.03 , 1.66 ± 0.08 and 1.85 ± 0.06 respectively (Table: 4B.1). Body of uterus was increasing significantly ($p=0.05$) as the gestational age advanced almost in all the groups except, GpA₂ and GpA₃, GpA₄ and GpA₅ (Table: 4B.1.1).

The details of statistical data analysis for the biometry of different parts of female genitalia are given in Table: 4B.1.1 by employing “t” test and ANOVA.

Size of ovaries :

The mean lengths and breadths of left ovary recorded in present study from GpA₁ to GpA₅ were 1.33 ± 0.08 , 1.47 ± 0.14 , 1.42 ± 0.12 , 1.48 ± 0.13 , 1.75 ± 0.09 and 0.97 ± 0.03 , 0.95 ± 0.12 , 1.04 ± 0.11 , 1.16 ± 0.05 , 1.00 ± 0.09 and also thickness were 0.53 ± 0.04 , 0.67 ± 0.02 , 0.64 ± 0.02 , 0.66 ± 0.02 , 0.70 ± 0.04 respectively (Table: 4B.1). Length, Breadth and thickness increased as the gestational age advanced. In case of length GpA₁ and GpA₅, GpA₃ and GpA₅ differed significantly ($p=0.05$), in case of breadth GpA₁ and GpA₄, GpA₂ and GpA₄, GpA₄ and GpA₅ differed significantly ($p=0.05$) and in thickness GpA₁ and GpA₂, GpA₁ and GpA₃, GpA₁ and GpA₄, GpA₁ and GpA₅ differed significantly ($p=0.05$) (Table: 4B.1.1).

The mean lengths and breadths of right ovary recorded in present study from GpA₁ to GpA₅ were 1.35 ± 0.09 , 1.38 ± 0.09 , 1.32 ± 0.07 , 1.54 ± 0.02 , 1.65 ± 0.12 and 1.00 ± 0.04 , 1.12 ± 0.03 , 1.18 ± 0.04 , 1.20 ± 0.03 , 1.20 ± 0.04 and also thickness were 0.67 ± 0.03 , 0.73 ± 0.05 , 0.74 ± 0.02 , 0.78 ± 0.04 , 0.78 ± 0.03 respectively (Table: 4B.1). Length, Breadth and thickness increased as the gestation period advanced. In case of length GpA₁ and GpA₄, GpA₁ and GpA₅, GpA₃ and GpA₄, GpA₃ and GpA₅ differed significantly ($p=0.05$), in case of breadth GpA₁ and GpA₂, GpA₁ and GpA₃, GpA₁ and GpA₄, GpA₁ and GpA₅, GpA₂ and GpA₄, GpA₄ and GpA₅ differ significantly ($p=0.05$) and in thickness GpA₁ and GpA₃, GpA₁ and GpA₄, GpA₁ and GpA₅ differ significantly ($p=0.05$) (Table: 4B.1.1).

Details of statistical analysis for the biometry of ovary can be seen in Table: 4B.1.1 by employing “t” test and ANOVA.

Changes in the length of allantoic and amniotic cavity and diameter of amniotic cavity:

The mean lengths of allantoic and amniotic cavity from GpA₁ to GpA₅ were 46.28 ± 2.60, 57.52 ± 1.50, 68.86 ± 1.47, 78.62 ± 2.24, 92.58 ± 1.86 and 158 ± 0.28, 4.43 ± 0.67, 7.66 ± 0.57, 13.22 ± 1.10, 18.20 ± 0.62 and diameter of amniotic cavity were 1.46 ± 0.27, 3.37 ± 0.40, 5.50 ± 0.42, 8.32 ± 0.58, 12.65 ± 1.14 respectively (Table: 4B.2). The lengths of allantoic and amniotic cavity and diameter of amniotic cavity increased significantly in all the groups as gestation advanced (Table: 4B.2.1).

Statistical data analysis for the length of allantoic and amniotic cavity and diameter of amniotic cavity in details is presented in Table: 4B.2.1.

Changes in the caruncle:

The average numbers of attached caruncles in the left and right horn from GpA₂ to GpA₅ (As in GpA₁ all the caruncles were unattached) were 29.00 ± 3.89, 33.00 ± 2.84, 33.00 ± 2.60, 34.00 ± 2.97 and 34.00 ± 0.68, 34.00 ± 0.24, 34.00 ± 1.98, 36.00 ± 1.78 respectively (Table: 4B.2). The numbers of attached caruncles increased non-significantly in all the groups as gestational age advanced but the average numbers of caruncles in the right horn were more compared to the left (Table: 4B.2.1).

The average numbers of unattached caruncles in the left and right horn from GpA₁ to GpA₅ were 37.00 ± 0.64, 12.00 ± 5.05, 8.00 ± 0.40, 6.00 ± 0.86, 7.00 ± 0.71 and 37.00 ± 1.06, 11.00 ± 5.65, 6.00 ± 0.37, 6.00 ± 0.97, 6.00 ± 0.75 respectively (Table: 4B.2). Average numbers of unattached caruncles decreased as the pregnancy advanced. In the left horn, GpA₁ and GpA₂, GpA₁ and GpA₃, GpA₁ and GpA₄, GpA₁ and GpA₅, GpA₃ and GpA₄, GpA₃ and GpA₅ differed significantly (p=0.05) and in the right horn GpA₁ and GpA₂, GpA₁ and GpA₃, GpA₁ and GpA₄, GpA₁ and GpA₅ differed significantly (p=0.05) (Table: 4B.2.1).

Statistical data analysis of caruncles in detail is presented in Table: 4B.2.1.

Changes in the foetal fluid volume:

The mean of foetal fluid volumes for amniotic and allantoic fluids from GpA₁ to GpA₅ were 1.37 ± 0.23, 11.88 ± 3.11, 61.80 ± 11.11, 133.50 ± 6.13, 148.70 ± 0.51 and

12.87 $\pm$ 3.25, 37.82 $\pm$ 3.60, 53.23 $\pm$ 1.94, 109.50 $\pm$ 10.17, 126.50 $\pm$ 1.55 respectively (Table: 4B.3). Amniotic fluid increased significantly ($p=0.05$) in all the groups and the allantoic fluid also increased significantly ($p=0.05$) almost in all the groups except GpA₄ and GpA₅ (Table: 4B.3.1).

Statistical data analysis for foetal fluids in detail is depicted in Table: 4B.3.1.

Changes in the foetal fluid colour and consistency:

The colour and consistency of foetal fluids recorded in the present study from GpA₁ to GpA₅ were in GpA₁. It was watery and transparent in amniotic fluid and in GpA₁ and GpA₂ in allantoic fluid the changes were creamy white with granules in GpA₂, GpA₃ and GpA₄, in amniotic fluid the changes were watery and transparent with granules whereas in GpA₃, the allantoic fluid was light yellow and transparent with granules, in GpA₄ the allantoic fluid was yellow, translucent and slightly viscous with granules and in GpA₅ the amniotic fluid was very mild yellow with granules and allantoic fluid was yellow, opaque and slightly viscous with granules (Table: 4B.3a) (Plate 69 to Plate 70).

Changes of pH in the foetal fluids and maternal serum :

The mean values of pH in foetal fluids and maternal serum were almost similar i.e. alkaline in all the groups of gestation (Table: 4B.4). The mean values from GpA₁ to GpA₅, in amniotic and allantoic fluids were 7.33 $\pm$ 0.09, 7.70 $\pm$ 0.27, 7.00 $\pm$ 0.64, 8.34 $\pm$ 0.41, 7.54 $\pm$ 0.39 and 7.50 $\pm$ 0.35, 7.28 $\pm$ 0.17, 8.07 $\pm$ 0.21, 7.08 $\pm$ 0.17, 7.28 $\pm$ 0.76 and in serum were 7.73 $\pm$ 0.17, 7.72 $\pm$ 0.16, 7.26 $\pm$ 0.27, 7.26 $\pm$ 0.07, 7.45 $\pm$ 0.16 respectively (Table: 4B.4). In case of amniotic fluid the pH increased significantly ($p=0.05$) in between GpA₁ and GpA₄, GpA₃ and GpA₄, in allantoic fluid the pH increased significantly ($p=0.05$) in between GpA₂ and GpA₃ and decreased significantly ($p=0.05$) in between GpA₃ and GpA₄ and in serum the pH decreased significantly ($p=0.05$) in between GpA₁ and GpA₄, GpA₂ and GpA₄ (Table: 4B.4.1).

Glucose :

The mean glucose levels in amniotic and allantoic fluids were 6.87 ± 0.43 , 3.52 ± 0.22 , 3.82 ± 0.90 , 3.11 ± 0.64 , 3.09 ± 1.20 and 3.14 ± 0.60 , 3.13 ± 0.70 , 3.36 ± 1.17 , 4.14 ± 0.38 , 4.60 ± 0.97 respectively (Table: 4B.4) at the five gestational groups from GpA₁ to GpA₅. Glucose in amniotic fluid showed a decreasing and allantoic fluid showed an increasing trend as the pregnancy advanced. In case of amniotic fluid glucose concentration showed significant ($p=0.05$) decreasing trend in between GpA₁ and GpA₂, GpA₁ and GpA₃, GpA₁ and GpA₄, GpA₁ and GpA₅ and in case of allantoic fluid all the groups increased non-significantly (Table: 4B.4.1).

The mean maternal serum glucose concentrations showed a significant ($p=0.05$) declining phase from GpA₁ to GpA₃ were 68.53 ± 2.46 , 52.51 ± 2.66 , 41.92 ± 0.91 , and also showed a significant ($p=0.05$) increasing trend from GpA₃ to GpA₅ and the values were 41.92 ± 0.91 , 54.04 ± 2.15 , 66.94 ± 2.18 (Table: 4B.4). The glucose levels in maternal serum showed significant ($p=0.05$) differences almost in all the groups except, GpA₁ and GpA₅, GpA₂ and GpA₄ (Table: 4B.4.1).

Total Protein:

The mean total protein values in amniotic and allantoic fluids at five groups of gestation from GpA₁ to GpA₅ were 0.20 ± 0.03 , 0.39 ± 0.11 , 0.46 ± 0.20 , 0.51 ± 0.14 , 0.60 ± 0.15 and 0.25 ± 0.11 , 0.45 ± 0.12 , 0.50 ± 0.10 , 0.68 ± 0.12 , 0.51 ± 0.12 and also in serum were 2.96 ± 0.15 , 3.56 ± 0.81 , 10.24 ± 0.43 , 11.85 ± 0.55 , 12.51 ± 0.69 respectively (Table: 4B.4). The concentration in serum was much higher in comparison to foetal fluids. The mean protein concentrations both in foetal fluids and maternal serum increased as the gestation period progressed. In maternal serum total protein increased significantly ($p=0.05$) almost in all the groups except, GpA₁ and GpA₂, GpA₄ and GpA₅. In amniotic fluid only GpA₁ and GpA₅ differed significantly ($p=0.05$) but in allantoic fluid GpA₁ and GpA₃, GpA₁ and GpA₄, GpA₁ and GpA₅ differed significantly ($p=0.05$) (Table: 4B.4.1).

Cholesterol:

The mean cholesterol values in amniotic and allantoic fluids observed in present study showed similar shift. Both in amniotic and allantoic fluids the cholesterol

concentrations from GpA₁ to GpA₃ increased and decreased in GpA₄ and GpA₅ and the values from GpA₁ to GpA₅ were 3.96 ± 0.17 , 3.85 ± 0.19 , 5.68 ± 0.37 , 4.18 ± 0.13 , 3.98 ± 0.06 and 2.45 ± 0.15 , 2.73 ± 0.35 , 5.06 ± 0.22 , 3.18 ± 0.13 , 2.98 ± 0.06 respectively (Table: 4B.4). In amniotic fluid in between GpA₁ and GpA₃, GpA₂ and GpA₃ the concentration increased significantly ($p=0.05$) and in between GpA₃ and GpA₄, GpA₃ and GpA₅ the concentration decreased significantly ($p=0.05$). In allantoic fluids almost all the groups differed significantly ($p=0.05$) except GpA₁ and GpA₂, GpA₂ and GpA₄, GpA₂ and GpA₅, GpA₄ and GpA₅ (Table: 4B.4.1).

The maternal serum cholesterol values showed a decreasing trend from 121.06 ± 8.41 in GpA₁ to 96.25 ± 5.48 in GpA₅ (Table: 4B.4). The observed values from GpA₁ to GpA₅ in serum were 121.06 ± 8.41 , 107.67 ± 1.58 , 99.18 ± 3.18 , 98.20 ± 3.02 , 96.25 ± 5.48 (Table: 4B.4). In maternal serum almost all the groups differed significantly ($p=0.05$) except GpA₃ and GpA₄, GpA₃ and GpA₅, GpA₄ and GpA₅ (Table: 4B.4.1).

Creatinine:

The mean creatinine values in amniotic and allantoic fluids recorded in five groups from GpA₁ to GpA₅ were 0.16 ± 0.04 , 0.96 ± 0.21 , 1.61 ± 0.47 , 2.28 ± 0.57 , 3.69 ± 0.11 and 0.33 ± 0.08 , 1.01 ± 0.21 , 2.12 ± 0.29 , 3.85 ± 0.38 , 5.39 ± 0.23 and in serum were 0.54 ± 0.07 , 0.86 ± 0.05 , 2.51 ± 0.32 , 4.08 ± 0.34 , 5.50 ± 0.22 respectively (Table: 4B.4). Creatinine concentrations in foetal fluids and maternal serum increased significantly ($p=0.05$) in all the groups except, in between GpA₂ and GpA₃, GpA₃ and GpA₄ which differed non-significantly in amniotic fluid as the gestational age advanced (Table: 4B.4.1).

Urea:

The mean urea concentrations in amniotic and allantoic fluids recorded in five groups from GpA₁ to GpA₅ were 9.92 ± 0.84 , 12.90 ± 0.35 , 14.91 ± 0.86 , 16.92 ± 0.74 , 18.56 ± 1.10 and 11.15 ± 0.91 , 14.46 ± 0.58 , 17.22 ± 0.47 , 18.07 ± 0.82 , 26.22 ± 0.53 and in serum were 49.20 ± 3.00 , 61.06 ± 0.10 , 67.21 ± 2.44 , 73.49 ± 1.39 , 78.00 ± 1.34 (Table: 4B.4). The values in serum in all the groups of gestation were much higher in comparison to foetal fluids. Urea concentration in maternal serum increased

significantly ($p=0.05$) in all the groups and in foetal fluids increased significantly ($p=0.05$) almost in all the groups except, in between GpA₄ and GpA₅ in amniotic fluid and GpA₃ and GpA₄ in allantoic fluid which differed non-significantly (Table: 4B.4.1).

Urea Nitrogen (UN):

The mean blood urea nitrogen concentrations in amniotic and allantoic fluids observed from GpA₁ to GpA₅ were 4.63 ± 0.39 , 6.02 ± 0.16 , 6.96 ± 0.40 , 7.90 ± 0.35 , 8.67 ± 0.51 and 5.20 ± 0.43 , 6.75 ± 0.27 , 8.05 ± 0.22 , 8.44 ± 0.39 , 12.24 ± 12.24 and in serum were 22.98 ± 1.40 , 28.51 ± 0.46 , 31.39 ± 1.14 , 34.32 ± 0.65 , 36.43 ± 0.63 (Table: 4B.4). The concentrations in serum were much higher in comparison to the foetal fluids. Urea nitrogen concentrations in maternal serum increased significantly ($p=0.05$) in all the groups and in foetal fluids increased significantly ($p=0.05$) almost in all the groups except, in between GpA₄ and GpA₅ in amniotic fluid and GpA₃ and GpA₄ in allantoic which differed non-significantly (Table: 4B.4.1).

The details of statistical data analysis for all the data of biochemical constituents are given in Table: 4B.4.1 by employing “t” test and ANOVA.

Sodium:

The sodium concentrations were more in amniotic fluid in comparison to allantoic fluid in all the groups of gestation. The mean sodium concentrations in amniotic and allantoic fluids observed from GpA₁ to GpA₅ were 97.58 ± 5.20 , 97.38 ± 8.37 , 95.09 ± 10.89 , 95.09 ± 8.17 , 92.72 ± 2.43 and 94.50 ± 1.22 , 88.50 ± 0.76 , 87.20 ± 1.50 , 81.6 ± 2.00 , 80.50 ± 2.87 respectively (Table: 4B.5). Sodium concentrations in amniotic and allantoic fluids showed similar shift, the values were decreasing as the gestation advanced. In amniotic fluid the concentration decreased non-significantly in all the groups. However, in allantoic fluid almost all the groups decreased significantly ($p=0.05$) except, GpA₂ and GpA₃, GpA₄ and GpA₅ (Table: 4B.5.1).

The sodium concentrations in maternal serum in five groups of gestation were 113.79 ± 4.48 , 122.12 ± 9.10 , 123.20 ± 8.94 , 124.60 ± 12.63 , 124.98 ± 1.81 (Table: 4B.5). Sodium concentrations in between group GpA₁ and GpA₅ increased significantly ($p=0.05$) in serum (Table: 4B.5.1).

Potassium:

The concentrations of sodium and potassium in foetal fluids and maternal serum showed completely reverse phases in different groups of gestation. In amniotic and allantoic fluids mean potassium concentrations from GpA₁ to GpA₅ were 4.99 ± 0.22 , 5.15 ± 0.27 , 5.39 ± 0.53 , 5.54 ± 0.35 , 5.38 ± 0.10 and 6.73 ± 0.26 , 8.96 ± 0.24 , 9.21 ± 0.39 , 11.09 ± 0.22 , 11.60 ± 0.07 and in serum were 7.83 ± 0.15 , 7.72 ± 0.15 , 7.55 ± 0.25 , 6.65 ± 0.16 , 6.11 ± 0.36 (Table: 4B.5). In amniotic fluid the potassium concentration increased significantly ($p=0.05$) in between GpA₁ and GpA₅. On the other hand potassium concentrations increased significantly ($p=0.05$) almost in all the groups except, GpA₂ and GpA₃ in allantoic fluid and in case of maternal serum decreased significantly ($p=0.05$) in all the groups except, in GpA₁ and GpA₂, GpA₁ and GpA₃, GpA₂ and GpA₃, GpA₄ and GpA₅ (Table: 4B.5.1).

Copper:

The copper concentrations in amniotic and allantoic fluids in five groups of gestation from GpA₁ to GpA₅ were 2.74 ± 0.83 , 3.20 ± 0.36 , 2.14 ± 0.14 , 2.18 ± 0.11 , 2.21 ± 0.14 and 2.13 ± 0.16 , 2.78 ± 0.38 , 2.75 ± 0.32 , 2.45 ± 0.26 , 1.98 ± 0.01 and in serum were 2.95 ± 0.48 , 3.09 ± 0.38 , 2.47 ± 0.14 , 4.61 ± 0.60 , 2.95 ± 0.55 respectively (Table: 4B.5). In amniotic fluid GpA₂ and GpA₃, GpA₂ and GpA₄, GpA₂ and GpA₅ decreased significantly ($p=0.05$). In allantoic fluid GpA₁ and GpA₂, GpA₁ and GpA₃ increased significantly ($p=0.05$) and GpA₂ and GpA₅, GpA₃ and GpA₅, GpA₄ and GpA₅ decreased significantly ($p=0.05$). In serum GpA₁ and GpA₄, GpA₂ and GpA₄, GpA₃ and GpA₄ increased significantly ($p=0.05$) on the other hand, GpA₂ and GpA₃, GpA₄ and GpA₅ decreased significantly ($p=0.05$) (Table: 4B.5.1).

Zinc:

The mean concentrations of zinc in amniotic and allantoic fluids in five groups of gestation from GpA₁ to GpA₅ were 5.12 ± 1.13 , 6.78 ± 1.00 , 4.70 ± 0.57 , 5.90 ± 0.49 , 6.90 ± 0.07 and 4.89 ± 0.63 , 6.15 ± 0.63 , 4.88 ± 0.51 , 5.73 ± 0.60 , 8.10 ± 0.06 and in serum were 6.52 ± 1.16 , 6.51 ± 0.10 , 8.00 ± 0.95 , 6.41 ± 0.49 , 4.10 ± 0.33 respectively

(Table: 4B.5). In amniotic fluid GpA₁ and GpA₅, GpA₃ and GpA₄, GpA₃ and GpA₅, GpA₄ and GpA₅ increased significantly ($p=0.05$) and GpA₂ and GpA₃ decreased significantly ($p=0.05$). In allantoic fluid GpA₁ and GpA₅, GpA₂ and GpA₅, GpA₃ and GpA₅, GpA₄ and GpA₅ increased significantly ($p=0.05$), GpA₂ and GpA₃ decreased significantly ($p=0.05$). In serum GpA₁ and GpA₅, GpA₂ and GpA₅, GpA₃ and GpA₅, GpA₄ and GpA₅ decreased significantly. Zinc concentration in amniotic and allantoic fluids increased from GpA₁ to GpA₂ decreased in GpA₃ and again increased in GpA₄ and GpA₅, in serum zinc concentrations increased from GpA₁ to GpA₃ decreased in GpA₄ and GpA₅ (Table: 4B.5.1).

Manganese :

The mean concentrations of manganese in amniotic and allantoic fluid from GpA₁ to GpA₅ were 4.19 ± 0.51 , 3.15 ± 0.15 , 3.26 ± 0.20 , 3.05 ± 0.09 , 3.00 ± 0.00 and 3.47 ± 0.29 , 3.23 ± 0.17 , 3.01 ± 0.14 , 3.44 ± 0.04 , 2.5 ± 0.14 and in serum were 4.34 ± 0.41 , 3.34 ± 0.16 , 3.45 ± 0.11 , 3.19 ± 0.18 , 2.86 ± 0.21 respectively (Table: 4B.5). Manganese concentrations showed declining trend both in foetal fluids and maternal serum as the gestation period advanced. Manganese concentrations in amniotic fluid showed a significant ($p=0.05$) decrease in between GpA₁ and GpA₂, GpA₁ and GpA₃, GpA₁ and GpA₄, GpA₁ and GpA₅. In allantoic fluid GpA₁ and GpA₅, GpA₂ and GpA₅, GpA₃ and GpA₅, GpA₄ and GpA₅ showed a significant ($p=0.05$) decrease and in between GpA₃ and GpA₄ showed a significant ($p=0.05$) increase. In serum in between GpA₁ and GpA₂, GpA₁ and GpA₃, GpA₁ and GpA₄, GpA₁ and GpA₅, GpA₂ and GpA₅, GpA₃ and GpA₅ showed a significant ($p=0.05$) decreasing trend (Table: 4B.5.1).

Iron:

The mean concentrations of iron in amniotic and allantoic fluids observed in present study from GpA₁ to GpA₅ were 6.68 ± 0.11 , 8.00 ± 0.52 , 9.66 ± 0.42 , 10.31 ± 0.08 , 10.94 ± 0.71 and 5.31 ± 0.25 , 5.58 ± 0.36 , 6.04 ± 0.12 , 6.18 ± 0.19 , 6.73 ± 0.28 and in serum were 14.37 ± 0.53 , 12.50 ± 0.56 , 12.58 ± 0.24 , 12.40 ± 0.40 , 12.00 ± 0.41 respectively (Table: 4B.5). The iron concentrations in foetal fluids were increasing and in maternal serum was decreasing as the pregnancy advanced. Iron in amniotic fluid

increased significantly ($p=0.05$) almost in all the groups except, in between GpA₁ and GpA₅. On the other hand in allantoic fluid in between GpA₁ and GpA₃, GpA₁ and GpA₄, GpA₁ and GpA₅, GpA₂ and GpA₄, GpA₂ and GpA₅, GpA₃ and GpA₅, GpA₄ and GpA₅ increased significantly ($p=0.05$) and in serum in between GpA₁ and GpA₂, GpA₁ and GpA₃, GpA₁ and GpA₄, GpA₁ and GpA₅ decreased significantly ($p=0.05$) (Table: 4B.5.1).

The details of statistical data analysis for all the minerals are given in Table: 4B.5.1 by employing “t” test and ANOVA.

Glutamate Oxalo Transaminase (GOT) / Aspartate Amino Transferase (ASAT):

In amniotic and allantoic fluids the mean concentrations of GOT from GpA₁ to GpA₅ were 15.22 ± 0.95 , 14.69 ± 0.91 , 16.10 ± 1.25 , 16.13 ± 0.87 , 14.03 ± 0.65 and 13.40 ± 0.65 , 12.82 ± 1.22 , 14.91 ± 1.21 , 13.70 ± 0.89 , 12.63 ± 0.85 and in maternal serum were 122.90 ± 8.76 , 88.83 ± 2.39 , 92.80 ± 4.10 , 106.0 ± 4.74 , 91.75 ± 2.63 respectively (Table: 4B.6).

In amniotic fluid in between GpA₃ and GpA₅, GpA₄ and GpA₅ decreased significantly ($p=0.05$) and in allantoic fluid in between GpA₃ and GpA₅ decreased significantly ($p=0.05$). In serum in between GpA₁ and GpA₂, GpA₁ and GpA₃, GpA₁ and GpA₄, GpA₁ and GpA₅, GpA₄ and GpA₅ decreased significantly and in between GpA₂ and GpA₄, GpA₃ and GpA₄ increased significantly ($p=0.05$) (Table: 4B.6. 1).

Glutamate Pyruvate Transaminase (GPT) / Alanin Amino Transferase (ALAT):

The mean concentrations of GPT in amniotic and allantoic fluids from GpA₁ to GpA₅ were 7.45 ± 0.24 , 6.38 ± 0.41 , 8.56 ± 0.70 , 8.02 ± 0.58 , 7.75 ± 0.79 and 6.65 ± 0.24 , 6.67 ± 0.97 , 5.57 ± 0.30 , 7.18 ± 0.65 , 5.28 ± 0.45 and in serum were 37.00 ± 3.19 , 31.50 ± 1.78 , 32.20 ± 0.66 , 34.80 ± 1.46 , 36.25 ± 1.38 respectively (Table: 4B.6). In amniotic fluid in between GpA₁ and GpA₂ decreased significantly ($p=0.05$) and in between GpA₁ and GpA₃, GpA₂ and GpA₃, GpA₂ and GpA₄, GpA₂ and GpA₅ increased significantly ($p=0.05$). In allantoic fluid in between GpA₂ and GpA₄ increased significantly ($p=0.05$) and GpA₁ and GpA₂, GpA₁ and GpA₅, GpA₄ and GpA₅ decreased significantly ($p=0.05$). In serum in between GpA₁ and GpA₂, GpA₁ and GpA₃ decreased

significantly and in between GpA₂ and GpA₅, GpA₃ and GpA₄, GpA₃ and GpA₅ increased significantly (p=0.05) (Table: 4B.6. 1).

Alkaline Phosphatase:

The mean concentrations of alkaline phosphatase both in foetal fluids and maternal serum increased as the gestation advanced. In amniotic and allantoic fluids the concentrations observed from GpA₁ to GpA₅ were 20.90 ± 1.85 , 33.50 ± 1.15 , 35.40 ± 1.72 , 37.60 ± 0.51 , 36.75 ± 2.36 and 19.10 ± 1.86 , 28.67 ± 1.69 , 32.0 ± 2.61 , 34.0 ± 2.10 , 31.75 ± 1.55 and in serum were 37.50 ± 2.62 , 66.67 ± 2.40 , 70.80 ± 3.65 , 81.6 ± 2.46 , 71.0 ± 5.26 respectively (Table: 4B.6). Both in amniotic and allantoic fluids in between GpA₁ and GpA₂, GpA₁ and GpA₃, GpA₁ and GpA₄, GpA₁ and GpA₅, GpA₂ and GpA₄ increased significantly (p=0.05). In serum in between GpA₁ and GpA₂, GpA₁ and GpA₃, GpA₁ and GpA₄, GpA₁ and GpA₅, GpA₂ and GpA₄, GpA₃ and GpA₄ increased significantly (p=0.05) but in between GpA₄ and GpA₅ decreased significantly (p=0.05) (Table: 4B.6. 1).

The details of statistical data analysis for the enzymes are given in Table: 4B.6.1 by employing “t” test and ANOVA.

Progesterone:

The mean concentrations of progesterone in maternal serum observed from GpA₁ to GpA₅ were 3.26 ± 0.32 , 5.19 ± 0.08 , 6.00 ± 0.14 , 7.00 ± 0.21 , 7.89 ± 0.08 respectively (Table: 4B.7). The progesterone concentrations increased significantly (p=0.05) in all the groups as the gestation advanced (Table: 4B.7.1).

Estrogen:

The mean concentrations of estrogen followed almost similar shift from GpA₁ to GpA₅ as there were in progesterone. The mean concentrations of estrogen in maternal serum observed from GpA₁ to GpA₅ were 74.0 ± 8.59 , 113.33 ± 6.67 , 160.0 ± 8.37 , 198.0 ± 5.0 and 236.25 ± 2.39 respectively (Table: 4B.7). The estrogen concentration increased significantly (p=0.05) in all the groups as the gestational age advanced (Table: 4B.7.1).

Progesterone receptor:

The mean concentrations of progesterone receptor in uterine tissue from GpA₁ to GpA₅ were 3.74 ± 0.05 , 3.62 ± 0.09 , 3.82 ± 0.04 , 3.98 ± 0.02 , 4.06 ± 0.02 respectively (Table: 4B.7). The mean concentrations of progesterone receptor followed similar shift as in progesterone from GpA₁ to GpA₅, which showed increasing phase as the gestation advanced. The progesterone receptor concentrations increased significantly ($p=0.05$) almost in all the groups except, in between GpA₁ and GpA₂, GpA₁ and GpA₃ (Table: 4B.7.1).

Estrogen receptor:

The mean concentrations of estrogen receptor in uterine tissue from GpA₁ to GpA₅ were 0.37 ± 0.01 , 0.36 ± 0.01 , 0.36 ± 0.02 , 0.39 ± 0.01 , 0.37 ± 0.01 respectively (Table: 4B.7). Estrogen receptor in all the groups was almost constant. Estrogen receptor increased significantly ($p=0.05$) in between GpA₂ and GpA₄, GpA₂ and GpA₅ (Table: 4B.7.1).

The details of statistical data analysis for the hormones and receptors are given in Table: 4B.7.1 by employing “t” test and ANOVA.

TABLES

(Group-2)

Table: 4B.1. Biometry of parts of female genital organ during early pregnancy in sheep

Parameters (cm)		Groups (Length of embryo and CA length of fetus in cm / Age in days)				
		A ₁ (0.1 - 2.0 / 14 - 39)	A ₂ (2.1 - 4.0 / 40-44)	A ₃ (4.1 - 6.0 / 45-48)	A ₄ (6.1 - 8.0 / 49-52)	A ₅ (8.1 - 10.0 / 53-57)
Length Of Vagina		7.08 ± 0.32 ^a	7.32 ± 0.56 ^a	7.48 ± 0.52 ^a	7.54 ± 0.44 ^a	7.40 ± 0.20 ^a
Length Of Cervix		5.54 ± 0.44 ^a	5.57 ± 0.22 ^a	6.42 ± 0.57 ^{af}	6.48 ± 0.43 ^{dfg}	6.43 ± 0.96 ^{ag}
From Cervix to Lt UTJ (Length)		22.36 ± 1.10 ^a	23.68 ± 1.00 ^a	27.92 ± 1.21 ^{cf}	34.22 ± 1.60 ^{dg}	34.18 ± 2.88 ^{efg}
From Cervix to Rt UTJ (Length)		21.39 ± 0.95 ^a	26.03 ± 1.89 ^{bf}	29.0 ± 1.86 ^{cf}	30.48 ± 1.93 ^{df}	40.65 ± 0.77 ^e
From Cervix to ICL (Length)		13.48 ± 0.63 ^a	14.42 ± 0.78 ^a	18.24 ± 1.33 ^{cf}	18.42 ± 1.64 ^{dfg}	21.90 ± 1.20 ^{eg}
Length of Body of uterus		1.21 ± 0.05 ^a	1.45 ± 0.03 ^{bf}	1.50 ± 0.03 ^{cf}	1.66 ± 0.08 ^{dg}	1.85 ± 0.06 ^{eg}
Left Ovary	Length	1.33 ± 0.08 ^a	1.47 ± 0.14 ^{af}	1.42 ± 0.12 ^a	1.48 ± 0.13 ^{ag}	1.75 ± 0.09 ^{efg}
	Breadth	0.97 ± 0.03 ^a	0.95 ± 0.12 ^a	1.04 ± 0.11 ^{af}	1.16 ± 0.05 ^{df}	1.00 ± 0.09 ^a
	Thickness	0.53 ± 0.04 ^a	0.67 ± 0.02 ^{bf}	0.66 ± 0.02 ^{cf}	0.64 ± 0.02 ^{df}	0.70 ± 0.04 ^{ef}
Right Ovary	Length	1.35 ± 0.09 ^a	1.38 ± 0.09 ^{af}	1.32 ± 0.07 ^a	1.54 ± 0.02 ^{df}	1.65 ± 0.12 ^{ef}
	Breadth	1.00 ± 0.04 ^a	1.12 ± 0.03 ^{bf}	1.18 ± 0.04 ^{cfg}	1.20 ± 0.03 ^{dg}	1.20 ± 0.04 ^{ef}

	Thickness	a 0.67 ± 0.03	af 0.73 ± 0.05	cf 0.74 ± 0.02	df 0.78 ± 0.04	ef 0.78 ± 0.03
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Values with different superscript are significant and having any one of the common superscript are non significant, p = 0.05.

Comparison was made by applying students “t” test.

Abbreviations : Lt UTJ = Left Utero Tubal Junction, Rt UTJ = Right Utero Tubal Junction, ICL = Inter Cornual Ligament

Table: 4B.1.1. Statistical Analysis of Table - 4B.1. (ANOVA + t-test)

Parameters	ANOVA	t-test									
		A ₁ A ₂	A ₁ A ₃	A ₁ A ₄	A ₁ A ₅	A ₂ A ₃	A ₂ A ₄	A ₂ A ₅	A ₃ A ₄	A ₃ A ₅	A ₄ A ₅
Length Of Vagina	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Length Of Cervix	NS	NS	NS	S	NS	NS	S	NS	NS	NS	NS
From Cervix to Lt UTJ (Length)	HS	NS	S	HS	S	S	HS	S	S	NS	NS
From Cervix to Rt UTJ (Length)	HS	S	S	S	HS	NS	NS	HS	NS	HS	S
From Cervix to ICL (Length)	HS	NS	S	S	HS	S	S	S	NS	S	NS
Length of Body of uterus	HS	S	S	S	HS	NS	S	S	S	S	NS
LEFT OVARY											
Length	NS	NS	NS	NS	S	NS	NS	NS	NS	S	NS
Breadth	NS	NS	NS	S	NS	NS	S	NS	NS	NS	S

Thickness	S	S	S	S	S	NS	NS	NS	NS	NS	NS
RIGHT OVARY											
Length	NS	NS	NS	S	S	NS	NS	NS	S	S	NS
Breadth	S	S	S	S	S	NS	S	NS	NS	NS	S
Thickness	NS	NS	S	S	S	NS	NS	NS	NS	NS	NS

Abbreviations : S = Significant, NS = Non Significant, HS = Highly Significant

Table: 4B.2. Biometry of amniotic and allantoic cavities and number of caruncles during early pregnancy in sheep

Parameters		Groups (Length of embryo and CA length of fetus in cm / Age in days)				
		A ₁ (0.1 - 2.0 / 14 - 39)	A ₂ (2.1 - 4.0 / 40-44)	A ₃ (4.1 - 6.0 / 45-48)	A ₄ (6.1 - 8.0 / 49-52)	A ₅ (8.1 - 10.0 / 53-57)
Amniotic cavity (cm)	Diameter	1.46 ± 0.27 ^a	3.37 ± 0.40 ^b	5.5 ± 0.42 ^c	8.32 ± 0.58 ^d	12.65 ± 1.14 ^e
	Length	1.58 ± 0.28 ^a	4.43 ± 0.67 ^b	7.66 ± 0.57 ^c	13.22 ± 1.10 ^d	18.20 ± 0.62 ^e
Length of Allantoic cavity (cm)		46.28 ± 2.60 ^a	57.52 ± 1.50 ^b	68.86 ± 1.47 ^c	78.62 ± 2.24 ^d	92.58 ± 1.86 ^e

Caruncles (Left horn) (No.s)	Attached	Nil	29.00 ± 3.89 ^a	33.00 ± 2.84 ^a	33.00 ± 2.60 ^a	34.00 ± 2.97 ^a
	Unattached	37.00 ± 0.64 ^a	12.00 ± 5.05 ^{bfg}	8.00 ± 0.40 ^{cg}	6.00 ± 0.86 ^{df}	7.00 ± 0.71 ^{ef}
Caruncles (Right horn) (No.s)	Attached	Nil	34.00 ± 0.68 ^a	34.00 ± 0.24 ^a	34.00 ± 1.98 ^a	36.00 ± 1.78 ^a
	Unattached	37.00 ± 1.06 ^a	11.00 ± 5.65 ^{bf}	6.00 ± 0.37 ^{cf}	6.00 ± 0.97 ^{df}	6.00 ± 0.75 ^{ef}

Values with different superscript are significant and having any one of the common superscript are non significant, p = 0.05.
Comparison was made by applying students “t” test.

Table: 4B.2.1. Statistical Analysis of Table - 4B.2. (ANOVA + t-test)

Parameters	ANOVA	t-test									
		A₁ A₂	A₁ A₃	A₁ A₄	A₁ A₅	A₂ A₃	A₂ A₄	A₂ A₅	A₃ A₄	A₃ A₅	A₄ A₅
Length (All Cav)	HS	S	HS	HS	HS	HS	HS	HS	S	HS	S
AMNIOTIC CAVITY											
Length	HS	S	HS	HS	HS	S	HS	HS	S	HS	S
Diameter	HS	S	HS	HS	HS	S	HS	HS	S	HS	S

CAR-LT HORN (NO.)											
Attached	NS	Nil	Nil	Nil	Nil	NS	NS	NS	NS	NS	NS
Unattached	HS	S	HS	HS	HS	NS	NS	NS	S	S	NS
CAR-RT HORN (NO.)											
Attached	NS	Nil	Nil	Nil	Nil	NS	NS	NS	NS	NS	NS
Unattached	HS	S	HS	HS	HS	NS	NS	NS	NS	NS	NS

Abbreviations : S = Significant, NS = Non Significant, HS = Highly Significant

Table: 4B.3. Changes in fetal fluid volume during early pregnancy in sheep

Fetal fluid (ml)	Groups (Length of embryo and CA length of fetus in cm / Age in days)
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	A₁ (0.1 - 2.0 / 14 - 39)	A₂ (2.1 - 4.0 / 40-44)	A₃ (4.1 - 6.0 / 45-48)	A₄ (6.1 - 8.0 / 49-52)	A₅ (8.1 - 10.0 / 53-57)
Amniotic fluid	1.37 ± 0.23 ^{a}	11.88 ± 3.11 ^{b}	61.80 ± 11.11 ^{c}	133.50 ± 6.13 ^{d}	148.70 ± 0.51 ^{e}
Allantoic fluid	12.87 ± 3.25 ^{a}	37.82 ± 3.60 ^{b}	53.23 ± 1.94 ^{c}	109.50 ± 10.17 ^{df}	126.50 ± 1.55 ^{ef}

Values with different superscript are significant and having any one of the common superscript are non significant, p = 0.05.
Comparison was made by applying students “t” test.

Table: 4B.3.1. Statistical Analysis of Table - 4B.3. (ANOVA + t-test)

Parameters	ANOVA	t-test									
		A ₁ A ₂	A ₁ A ₃	A ₁ A ₄	A ₁ A ₅	A ₂ A ₃	A ₂ A ₄	A ₂ A ₅	A ₃ A ₄	A ₃ A ₅	A ₄ A ₅
FOETAL FLUID											
Amniotic	HS	S	S	HS	HS	S	HS	HS	HS	HS	S
Allantoic	HS	HS	HS	HS	HS	S	HS	HS	HS	HS	NS

Abbreviations : S = Significant, NS = Non Significant, HS = Highly Significant

Table: 4B.3a. Changes in fetal fluid colour and consistency during early pregnancy in sheep

Fetal fluid (ml)	Groups (Length of embryo and CA length of fetus in cm / Age in days)				
	A₁ (0.1 - 2.0 / 14 - 39)	A₂ (2.1 - 4.0 / 40-44)	A₃ (4.1 - 6.0 / 45-48)	A₄ (6.1 - 8.0 / 49-52)	A₅ (8.1 - 10.0 / 53-57)
Amniotic fluid	Watery and transparent	Watery and transparent with granules	Watery and transparent with granules	Watery and transparent with granules	very mild yellow with granules
Allantoic fluid	Creamy white with granules	Creamy white with granules	Transparent, light yellow with granules	Yellow, translucent and slightly viscous with granules	Yellow, opaque and slightly viscous with granules

Table: 4B.4. Values of biochemical constituents of foetal fluid and maternal serum during early pregnancy in sheep

Parameters	Types of fluid	Groups (Length of embryo and CA length of fetus in cm / Age in days)				
		A ₁ (0.1 - 2.0 / 14 - 39)	A ₂ (2.1 - 4.0 / 40-44)	A ₃ (4.1 - 6.0 / 45-48)	A ₄ (6.1 - 8.0 / 49-52)	A ₅ (8.1 - 10.0 / 53-57)
pH (Mean ± SE)	Amniotic	7.33 ± 0.09 ^a	7.70 ± 0.27 ^{af}	6.90 ± 0.64 ^a	8.34 ± 0.41 ^{dfg}	7.54 ± 0.39 ^{ag}
	Allantoic	7.50 ± 0.35 ^{ag}	7.28 ± 0.17 ^a	8.07 ± 0.21 ^{fgh}	7.08 ± 0.17 ^a	7.28 ± 0.76 ^{ah}
	Serum	7.73 ± 0.17 ^a	7.72 ± 0.16 ^a	7.26 ± 0.27 ^{af}	7.26 ± 0.07 ^{df}	7.45 ± 0.16 ^{af}
Glucose (Mean ± SE)	Amniotic fluid (mg%)	6.87 ± 0.43 ^a	3.52 ± 0.22 ^{bf}	3.82 ± 0.90 ^{cf}	3.11 ± 0.64 ^{df}	3.09 ± 1.20 ^{ef}
	Allantoic fluid (mg%)	3.14 ± 0.60 ^a	3.13 ± 0.70 ^a	3.36 ± 1.17 ^a	4.14 ± 0.38 ^a	4.60 ± 0.97 ^a
	Serum (mg%)	68.53 ± 2.46 ^a	52.51 ± 2.66 ^{bf}	41.92 ± 0.91 ^c	54.04 ± 2.15 ^{df}	66.94 ± 2.18 ^a
Total protein (Mean ± SE)	Amniotic fluid (g/dl)	0.20 ± 0.03 ^a	0.39 ± 0.11 ^{af}	0.46 ± 0.20 ^{ag}	0.51 ± 0.14 ^{af}	0.60 ± 0.15 ^{efg}
	Allantoic fluid (g/dl)	0.25 ± 0.11 ^a	0.45 ± 0.12 ^{af}	0.50 ± 0.10 ^{cf}	0.68 ± 0.12 ^{df}	0.51 ± 0.12 ^{ef}
	Serum (g/dl)	2.96 ± 0.15 ^a	3.56 ± 0.81 ^a	10.24 ± 0.43 ^c	11.85 ± 0.55 ^{df}	12.51 ± 0.69 ^{ef}

Parameters	Types of fluid	Groups (Length of embryo and CA length of fetus in cm / Age in days)				
		A ₁ (0.1 - 2.0 / 14 - 39)	A ₂ (2.1 - 4.0 / 40-44)	A ₃ (4.1 - 6.0 / 45-48)	A ₄ (6.1 - 8.0 / 49-52)	A ₅ (8.1 - 10.0 / 53-57)
Cholesterol (Mean ± SE)	Amniotic fluid (mg%)	3.96 ± 0.17 ^a	3.85 ± 0.19 ^a	5.68 ± 0.37 ^c	4.18 ± 0.13 ^a	3.98 ± 0.06 ^a
	Allantoic fluid (mg%)	2.45 ± 0.15 ^a	2.73 ± 0.35 ^{af}	5.06 ± 0.22 ^c	3.18 ± 0.13 ^{df}	2.98 ± 0.06 ^{ef}
	Serum (mg%)	121.06 ± 8.41 ^a	107.67 ± 1.58 ^b	99.18 ± 3.18 ^{cf}	98.20 ± 3.02 ^{df}	96.25 ± 5.48 ^{ef}
Creatinine (Mean ± SE)	Amniotic fluid (mg%)	0.16 ± 0.04 ^a	0.96 ± 0.21 ^{bf}	1.61 ± 0.47 ^{cfg}	2.28 ± 0.57 ^{dg}	3.69 ± 0.11 ^e
	Allantoic fluid (mg%)	0.33 ± 0.08 ^a	1.01 ± 0.21 ^b	2.12 ± 0.29 ^c	3.85 ± 0.38 ^d	5.39 ± 0.23 ^e
	Serum (mg%)	0.54 ± 0.07 ^a	0.86 ± 0.05 ^b	2.51 ± 0.32 ^c	4.08 ± 0.34 ^d	5.50 ± 0.22 ^e
Urea (Mean ± SE)	Amniotic fluid (mg%)	9.92 ± 0.84 ^a	12.90 ± 0.35 ^b	14.91 ± 0.86 ^c	16.92 ± 0.74 ^{df}	18.56 ± 1.10 ^{ef}
	Allantoic fluid (mg%)	11.15 ± 0.91 ^a	14.46 ± 0.58 ^b	17.22 ± 0.47 ^{cf}	18.07 ± 0.82 ^{df}	26.22 ± 0.53 ^e
	Serum (mg%)	49.20 ± 3.00 ^a	61.06 ± 0.10 ^b	67.21 ± 2.44 ^c	73.49 ± 1.39 ^d	78.00 ± 1.34 ^e

Urea Nitrogen (Mean ± SE)	Amniotic fluid (mg%)	a 4.63 ± 0.39	b 6.02 ± 0.16	c 6.96 ± 0.40	df 7.90 ± 0.35	ef 8.67 ± 0.51
	Allantoic fluid (mg%)	a 5.20 ± 0.43	b 6.75 ± 0.27	cf 8.05 ± 0.22	df 8.44 ± 0.39	e 12.24 ± 12.24
	Serum(mg%)	a 22.98 ± 1.40	b 28.51 ± 0.46	c 31.39 ± 1.14	d 34.32 ± 0.65	e 36.43 ± 0.63

Values with different superscript are significant and having any one of the common superscript are non significant, p = 0.05.

Comparison was made by applying students “t” test.

Table: 4B.4.1. Statistical Analysis of Table -4B.4. (ANOVA + t-test)

Parameters	ANOVA	t-test									
		A ₁ A ₂	A ₁ A ₃	A ₁ A ₄	A ₁ A ₅	A ₂ A ₃	A ₂ A ₄	A ₂ A ₅	A ₃ A ₄	A ₃ A ₅	A ₄ A ₅
pH											
Amniotic	NS	NS	NS	S	NS	NS	NS	NS	S	NS	NS
Allantoic	NS	NS	NS	NS	NS	S	NS	NS	S	NS	NS
Serum	NS	NS	NS	S	NS	NS	S	NS	NS	NS	NS
GLUCOSE											
Amniotic	HS	HS	S	S	S	NS	NS	NS	NS	NS	NS
Allantoic	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Serum	HS	S	HS	S	NS	S	NS	S	S	HS	S
TOTAL PROTEIN											
Amniotic	NS	NS	NS	NS	S	NS	NS	NS	NS	NS	NS

Allantoic	NS	NS	S	S	S	NS	NS	NS	NS	NS	NS
Serum	HS	NS	HS	HS	HS	HS	HS	HS	S	S	NS
CHOLESTEROL											
Amniotic	HS	NS	S	NS	NS	S	NS	NS	S	S	NS
Allantoic	HS	NS	HS	S	S	S	NS	NS	HS	HS	NS
Serum	NS	S	S	S	S	S	S	S	NS	NS	NS

Parameters	ANOVA	t-test									
		A ₁ A ₂	A ₁ A ₃	A ₁ A ₄	A ₁ A ₅	A ₂ A ₃	A ₂ A ₄	A ₂ A ₅	A ₃ A ₄	A ₃ A ₅	A ₄ A ₅
CREATININE											
Amniotic	HS	S	S	S	HS	NS	S	HS	NS	S	S
Allantoic	HS	S	HS	HS	HS	S	HS	HS	S	HS	S
Serum	HS	S	S	HS	HS	S	HS	HS	S	HS	S
UREA											
Amniotic	HS	S	S	HS	HS	S	S	S	S	S	NS
Allantoic	HS	S	HS	HS	HS	S	S	HS	NS	HS	HS
Serum	HS	S	S	HS	HS	S	HS	HS	S	S	S

UREA NITROGEN (UN)											
Amniotic	HS	S	S	HS	HS	S	S	S	S	S	NS
Allantoic	HS	S	HS	S	HS	S	S	HS	NS	HS	HS
Serum	HS	S	S	HS	HS	S	HS	HS	S	S	S

Abbreviations : S = Significant, NS = Non Significant, HS = Highly Significant

Table: 4B.5. Various macro and micro minerals of fetal fluids and maternal serum during early pregnancy in sheep

Parameters	Types of fluid	Groups (Length of embryo and CA length of fetus in cm / Age in days)				
		A ₁ (0.1 - 2.0 / 14 - 39)	A ₂ (2.1 - 4.0 / 40-44)	A ₃ (4.1 - 6.0 / 45-48)	A ₄ (6.1 - 8.0 / 49-52)	A ₅ (8.1 - 10.0 / 53-57)
Sodium (Mean ± SE)	Amniotic (mmol/L)	97.58 ± 5.20 ^a	97.38 ± 8.37 ^a	95.09 ± 10.89 ^a	95.09 ± 8.17 ^a	92.72 ± 2.43 ^a

	Allantoic (mmol/L)	94.50 ± 1.22 ^a	88.50± 0.76 ^{bf}	87.20 ± 1.50 ^{cf}	81.6 ± 2.00 ^{dg}	80.50 ± 2.87 ^{eg}
	Serum (mmol/L)	113.79 ± 4.48 ^a	122.12 ± 9.10 ^{af}	123.20 ± 8.94 ^{af}	124.60 ± 12.63 ^{af}	124.98 ± 1.81 ^{ef}
Potassium (Mean ± SE)	Amniotic fluid (mmol/L)	4.99 ± 0.22 ^a	5.15 ± 0.27 ^{af}	5.39 ± 0.53 ^{af}	5.54 ± 0.35 ^{af}	5.38 ± 0.10 ^{ef}
	Allantoic fluid (mmol/L)	6.73 ± 0.26 ^a	8.96 ± 0.24 ^{bf}	9.21 ± 0.39 ^{cf}	11.09 ± 0.22 ^d	11.60 ± 0.07 ^e
	Serum (mmol/L)	7.83 ± 0.15 ^a	7.72 ± 0.15 ^a	7.55 ± 0.25 ^a	6.65 ± 0.16 ^{df}	6.11 ± 0.36 ^{ef}
Copper (Mean ± SE)	Amniotic fluid (g/dl)	2.74 ± 0.83 ^{af}	3.20 ± 0.36 ^{bf}	2.14 ± 0.14 ^a	2.18 ± 0.11 ^a	2.21 ± 0.14 ^a
	Allantoic fluid (ppm)	2.13 ± 0.16 ^{ag}	2.78 ± 0.38 ^{bf}	2.75 ± 0.32 ^{cf}	2.45 ± 0.26 ^{fg}	1.98 ± 0.01 ^a
	Serum (ppm)	2.95 ± 0.48 ^{ag}	3.09 ± 0.38 ^{af}	2.47 ± 0.14 ^{cgh}	4.61 ± 0.60 ^d	2.95 ± 0.55 ^{efh}

Parameters	Types of fluid	Groups (Length of embryo and CA length of fetus in cm / Age in days)				
		A ₁ (0.1 - 2.0 / 14 - 39)	A ₂ (2.1 - 4.0 / 40-44)	A ₃ (4.1 - 6.0 / 45-48)	A ₄ (6.1 - 8.0 / 49-52)	A ₅ (8.1 - 10.0 / 53-57)
Zinc (Mean ± SE)	Amniotic fluid (ppm)	5.12 ± 1.13 ^{ag}	6.78 ± 1.00 ^{bfg}	4.70 ± 0.57 ^a	5.90 ± 0.49 ^{gh}	6.90 ± 0.07 ^{ef}

	Allantoic fluid (ppm)	4.89 ± 0.63 ^{af}	6.15 ± 0.63 ^a	4.88 ± 0.51 ^{cf}	5.73 ± 0.60 ^{af}	8.10 ± 0.06 ^e
	Serum (mg%)	6.52 ± 1.16 ^a	6.51 ± 0.10 ^a	8.00 ± 0.95 ^a	6.41 ± 0.49 ^a	4.10 ± 0.33 ^e
Manganese (Mean ± SE)	Amniotic fluid (ppm)	4.19 ± 0.51 ^a	3.15 ± 0.15 ^{bf}	3.26 ± 0.20 ^{cf}	3.05 ± 0.09 ^{df}	3.00 ± 0.00 ^{ef}
	Allantoic fluid (ppm)	3.47 ± 0.29 ^{af}	3.23 ± 0.17 ^{af}	3.01 ± 0.14 ^{cf}	3.44 ± 0.04 ^a	2.5 ± 0.14 ^e
	Serum (ppm)	4.34 ± 0.41 ^a	3.34 ± 0.16 ^{bf}	3.45 ± 0.11 ^{cf}	3.19 ± 0.18 ^{dfg}	2.86 ± 0.21 ^{eg}
Iron (Mean ± SE)	Amniotic fluid (ppm)	6.68 ± 0.11 ^a	8.00 ± 0.52 ^b	9.66 ± 0.42 ^c	10.31 ± 0.08 ^{df}	10.94 ± 0.71 ^{ef}
	Allantoic fluid (ppm)	5.31 ± 0.25 ^{af}	5.58 ± 0.36 ^{bfg}	6.04 ± 0.12 ^{cgh}	6.18 ± 0.19 ^{dh}	6.73 ± 0.28 ^e
	Serum (ppm)	14.37 ± 0.53 ^a	12.50 ± 0.56 ^{bf}	12.58 ± 0.24 ^{cf}	12.40 ± 0.40 ^{df}	12.00 ± 0.41 ^{ef}

Values with different superscript are significant and having any one of the common superscript are non significant, p = 0.05.

Comparison was made by applying students “t” test.

Table: 4B.5.1. Statistical Analysis of Table - 4B.5. (ANOVA + t-test)

Parameters	ANOVA	t-test									
		A ₁ A ₂	A ₁ A ₃	A ₁ A ₄	A ₁ A ₅	A ₂ A ₃	A ₂ A ₄	A ₂ A ₅	A ₃ A ₄	A ₃ A ₅	A ₄ A ₅
SODIUM (Na)											
Amniotic	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Allantoic	HS	S	S	S	S	NS	S	S	S	S	NS
Serum	NS	NS	NS	NS	S	NS	NS	NS	NS	NS	NS
POTASSIUM (K)											
Amniotic	NS	NS	NS	NS	S	NS	NS	NS	NS	NS	NS
Allantoic	HS	HS	S	HS	HS	NS	HS	HS	S	HS	S
Serum	HS	NS	NS	S	S	NS	S	S	S	S	NS
COPPER (Cu)											
Amniotic	NS	NS	NS	NS	NS	S	S	S	NS	NS	NS
Allantoic	NS	S	S	NS	NS	NS	NS	S	NS	S	S
Serum	S	NS	NS	S	HS	S	S	NS	S	NS	S

Parameters	ANOVA	t-test									
		A ₁ A ₂	A ₁ A ₃	A ₁ A ₄	A ₁ A ₅	A ₂ A ₃	A ₂ A ₄	A ₂ A ₅	A ₃ A ₄	A ₃ A ₅	A ₄ A ₅
ZINC (Zn)											
Amniotic	NS	NS	NS	NS	S	S	NS	NS	S	S	S
Allantoic	S	NS	NS	NS	S	S	NS	S	NS	HS	S
Serum	NS	NS	NS	NS	S	NS	NS	S	NS	S	S
MANGANESE (Mn)											
Amniotic	S	S	S	S	S	NS	NS	NS	NS	NS	NS
Allantoic	S	NS	NS	NS	S	NS	NS	S	S	S	HS
Serum	S	S	S	S	S	NS	NS	S	NS	S	NS
IRON (Fe)											
Amniotic	S	S	S	S	S	S	S	S	S	S	NS
Allantoic	S	NS	S	S	S	NS	S	S	NS	S	S
Serum	S	S	S	S	S	NS	NS	NS	NS	NS	NS

Abbreviations : S = Significant, NS = Non Significant, HS = Highly Significant

Table: 4B.6. Enzymes of foetal fluid and maternal serum during early pregnancy in sheep

Parameters	Types of fluid	Groups (Length of embryo and CA length of fetus in cm / Age in days)				
		A ₁ (0.1 - 2.0 / 14 - 39)	A ₂ (2.1 - 4.0 / 40-44)	A ₃ (4.1 - 6.0 / 45-48)	A ₄ (6.1 - 8.0 / 49-52)	A ₅ (8.1 - 10.0 / 53-57)
GOT/AST (Mean ± SE)	Amniotic (U/ L)	15.22 ± 0.95 ^{af}	14.69 ± 0.91 ^{af}	16.10 ± 1.25 ^a	16.13 ± 0.87 ^a	14.03 ± 0.65 ^{ef}
	Allantoic (U/ L)	13.40 ± 0.65 ^{af}	12.82 ± 1.22 ^{af}	14.91 ± 1.21 ^a	13.70 ± 0.89 ^{af}	12.63 ± 0.85 ^{ef}
	Serum (U/ L)	122.90 ± 8.76 ^a	88.83 ± 2.39 ^{bf}	92.80 ± 4.10 ^{cf}	106.0 ± 4.74 ^d	91.75 ± 2.63 ^{ef}
GPT/ALAT (Mean ± SE)	Amniotic fluid (U/ L)	7.45 ± 0.24 ^a	6.38 ± 0.41 ^b	8.56 ± 0.70 ^{cf}	8.02 ± 0.58 ^{af}	7.75 ± 0.79 ^{af}
	Allantoic fluid (U/ L)	6.65 ± 0.24 ^a	5.57 ± 0.30 ^{bf}	6.67 ± 0.97 ^{af}	7.18 ± 0.65 ^{ag}	5.28 ± 0.45 ^{ef}

	Serum (U/ L)	37.00 ± 3.19 ^a	31.50 ± 1.78 ^{bfg}	32.20 ± 0.66 ^{cf}	34.80 ± 1.46 ^{ag}	36.25 ± 1.38 ^a
Alkaline Phosphatase (Mean ± SE)	Amniotic fluid (KA Units)	20.90 ± 1.85 ^a	33.50 ± 1.15 ^{bf}	35.40 ± 1.72 ^{cfg}	37.60 ± 0.51 ^{dg}	36.75 ± 2.36 ^{efg}
	Allantoic fluid (KA Units)	19.10 ± 1.86 ^a	28.67 ± 1.69 ^{bf}	32.0 ± 2.61 ^{cfg}	34.0 ± 2.10 ^{dg}	31.75 ± 1.55 ^{efg}
	Serum (KA Units)	37.50 ± 2.62 ^a	66.67 ± 2.40 ^{bf}	70.80 ± 3.65 ^{cf}	81.6 ± 2.46 ^d	71.0 ± 5.26 ^{ef}

Values with different superscript are significant and having any one of the common superscript are non significant, p = 0.05.
Comparison was made by applying students “t” test.

Table: 4B.6.1. Statistical Analysis of Table - 4B.6. (ANOVA + t-test)

Parameters	ANOVA	t-test									
		A ₁ A ₂	A ₁ A ₃	A ₁ A ₄	A ₁ A ₅	A ₂ A ₃	A ₂ A ₄	A ₂ A ₅	A ₃ A ₄	A ₃ A ₅	A ₄ A ₅
GOT / AST											
Amniotic	NS	NS	NS	NS	NS	NS	NS	NS	NS	S	S
Allantoic	NS	NS	NS	NS	NS	NS	NS	NS	NS	S	NS
Serum	HS	S	S	S	S	NS	S	NS	S	NS	S
GPT / ALAT											
Amniotic	S	S	S	NS	NS	S	S	S	NS	NS	NS
Allantoic	NS	S	NS	NS	S	NS	S	NS	NS	NS	S

Serum	NS	S	S	NS	NS	NS	NS	S	S	S	NS
ALKALINE PHOSPHATASE											
Amniotic	HS	HS	HS	HS	HS	NS	S	NS	NS	NS	NS
Allantoic	HS	S	S	S	S	NS	S	NS	NS	NS	NS
Serum	HS	HS	HS	HS	S	NS	S	NS	S	NS	S

Abbreviations : S = Significant, NS = Non Significant, HS = Highly Significant

Table: 4B.7. Hormones and receptor estimation from maternal serum and tissue during early pregnancy in sheep

Parameters	Types of material	Groups (Length of embryo and CA length of fetus in cm / Age in days)				
		A ₁ (0.1 - 2.0 / 14 - 39)	A ₂ (2.1 - 4.0 / 40-44)	A ₃ (4.1 - 6.0 / 45-48)	A ₄ (6.1 - 8.0 / 49-52)	A ₅ (8.1 - 10.0 / 53-57)
Progesterone e (Mean ± SE)	Serum (ng/ml)	a 3.26 ± 0.32	b 5.19 ± 0.08	c 6.00 ± 0.14	d 7.00 ± 0.21	e 7.89 ± 0.08

Estrogen (Mean ± SE)	Serum (pg/ml)	74.0 ± 8.59 ^a	113.33 ± 6.67 ^b	160.0± 8.37 ^c	198.0 ± 5.0 ^d	236.25 ± 2.39 ^e
Progesteron e receptor (Mean ± SE)	Tissue (ng/mg)	3.74 ± 0.05 ^{af}	3.62 ± 0.09 ^a	3.82 ± 0.04 ^{cf}	3.98 ± 0.02 ^d	4.06 ± 0.02 ^e
Estrogen receptor (Mean ± SE)	Tissue (ng/mg)	0.37± 0.01 ^{afg}	0.36 ± 0.01 ^{bf}	0.36 ± 0.02 ^{af}	0.39 ± 0.01 ^{ag}	0.37 ± 0.01 ^a

Values with different superscript are significant and having any one of the common superscript are non significant, $p = 0.05$. Comparison was made by applying students “t” test.

Table: 4B.7.1. Statistical Analysis of Table - 4B.7 (ANOVA + t-test)

Parameters	ANOVA	t-test									
		$A_1 A_2$	$A_1 A_3$	$A_1 A_4$	$A_1 A_5$	$A_2 A_3$	$A_2 A_4$	$A_2 A_5$	$A_3 A_4$	$A_3 A_5$	$A_4 A_5$
PROGESTERONE											

Serum	HS	S	HS	HS	HS	S	HS	HS	S	HS	S
ESTROGEN											
Serum	HS	S	HS	HS	HS	S	HS	HS	S	HS	HS
PROGESTERONE RECEPTOR											
Tissue	HS	NS	NS	S	HS	S	S	S	S	S	S
ESTROGEN RECEPTOR											
Tissue	NS	NS	NS	NS	NS	NS	S	S	NS	NS	NS

Abbreviations : S = Significant, NS = Non Significant, HS = Highly Significant

4C. EXPERIMENTAL FINDINGS (The gross changes of genital organ observed in non pregnant and in different stages of pregnancy):

In non pregnant genital organ both the horns were symmetrical and the caruncles were sharp, smaller and rounded type (Plate 1 and Plate 2).

At 15 days of pregnancy, the horns were slightly asymmetrical but only the body of uterus was bulged and the right ovary had the corpus luteum. After giving incision to the dorsal part of the horns, an elongated blastocyst was observed and the embryo which was present in it, appeared like a blood clot that could be recognized only after microscopic observation. Caruncles were swollen and rounded that might be the effect of progesterone. Some caruncles possessed gelatinous material on the centre. As the gestation period advanced the size of the caruncles were increasing progressively (Plate 3 to Plate 6).

At 18 days of gestation, the right horn was larger than the left and right ovary contained corpus luteum. An elongated blastocyst was present with comma shaped embryo. The changes in the caruncles were same with previous sample (Plate 7 to Plate 11).

At 20 days of gestation, the right horn was larger than the left and right ovary having the corpus luteum. An elongated blastocyst was present with embryo. Both caruncular and intercaruncular area gave live appearance. The centre of the caruncles was more reddish compared to other parts of the horns. Caruncles were swollen, slightly more larger than earlier and rounded (Plate 12 to Plate 15).

At 23 days of gestation, the horns were asymmetrical and body of the uterus bulged dorsally. The left ovary had corpus luteum. An elongated blastocyst was present with embryo. The changes in the caruncle and intercaruncular areas were same with 20 days of pregnancy (Plate 16 to Plate 18).

At 25 days of gestation, the right horn was slightly larger than the left. An elongated blastocyst was present with embryo. Both caruncular and intercaruncular area gave lively appearance. The centre of the caruncles had slight circular depression (Plate 19 to Plate 20).

At 29 days of gestation right ovary had double corpus luteum and comma shaped embryos were present one in each of the horns. Changes in the caruncles were as like 25

days gestation (Plate 21 to Plate 22). Stereomicroscopic view gave the distinct comma shaped feature of the embryo (Plate 22). Under microscope some other structures like somites, head, eye bud, tail bud and emerging blood vessels from hind gut could also be observed in the elongated blastocyst. Two vessels emerged from each of the embryos and entered into the either of the horns along with its ramification could also be observed grossly. From this stage onwards emerging of blood vessels from the hind gut of foetus became distinct grossly (Plate 21).

At 33 days of gestation right horn was more enlarged than the left. A curved embryo was present in the right horn, emerging of blood vessels from the hind gut of foetus became distinct grossly (Plate 23 to Plate 24). Caruncles were flattened type.

At 36 days of gestation left horn was more enlarged than the right and the corpus luteum was present in the right ovary. Foetus was present in the left horn. At this stage the blastocyst was completely filled with fluid and whole blastocyst ramified with blood vessels and it was almost reddish in colour. From this stage head, eye bud and tail could be recognized grossly, this might be the beginning of foetal stage (Jainndeen and Hafez, 1984 and Hafez and Hafez, 2000). Circulation increased to the caruncle and intercaruncular areas. Caruncles were flattened; reddish colouration was present at the centre (Plate 25 to Plate 28)

At 39 days of gestation left horn was more enlarged than the right and the corpus luteum was present in the left ovary. Foetus was present in the left horn. At this stage single layered reddish granules were present mostly in the membrane of the left side were nothing but the cotyledons, appeared for the first time. Cotyledones were arranged in single layered which was reddish in colour and granular. Ramification of blood vessels was present both the horns but was more prominent in the left side. Head, eye bud, limb and tail bud in the foetus could be observed grossly. Caruncles were flattened (Plate 29 to Plate 31).

At 40 days of gestation left horn was larger than that of the right and the corpus luteum was present in the left ovary. At this stage blastocyst separated out from right horn by giving dorsal incision. But, as soon as incision given to the left horn on the dorsal curvature immediately caruncular-cotyledonary attachment could be observed but it was so fragile and temporary that without imposing any pressure it got separated (Plate 33 to

Plate 36). This is the stage, where for the first time caruncular-cotyledonary attachment could be observed grossly. Foetus was present in the left horn which was located nearer to the body of the uterus. Grossly, the caruncles became flattened with very slight depression at the centre (Plate 40).

At 42 and 43 days of gestation right horn was larger than the left and the corpus luteum was present in the right ovary. Other changes were same as there was in 40 days of gestation except their size and shape of the foetus which were increasing (Plate 41 to Plate 43).

At 44 days of gestation right horn was larger than the left and the corpus luteum was present on the right ovary and at around 45 days of gestation left horn was larger than the right and the corpus luteum was present in the left ovary. Other changes were almost same as was in 40 days gestation except, the size of the foetus which was larger and the umbilical chord was very distinct and showed total four numbers of blood vessels, two of them entered on either side of the horns. Cotyledons were multilayered and reddish in colour at this stage (Plate 44 to Plate 47).

At 46 and 47 days of gestation right horn was larger than the left and the corpus luteum was present on the right ovary. From this stage onwards the cotyledons were raised type and the depression at the centre of the caruncles was increasing. The size of the cotyledons and caruncles increased with higher gestational age. The external body parts of the foetus became distinct from this stage onwards. Cotyledons could easily be separated from the caruncles by holding the uterine horn or by imposing slight pressure (Plate 49 to Plate 53).

At 48 days of gestation, right horn was larger than the left and the corpus luteum was present on the right ovary. At this stage demarcation of placentomes was visible from outside of the uterine horns. Caruncular-cotyledonary attachment was strong that could not be separated easily and needed pressure for separation. The placentomes were cup shaped and cotyledons were raised type. From this stage onwards all the external body parts of the foetus developed properly and their size and shape increased as the age advanced (Plate 54 to Plate 57).

From 49 to 57 days of gestation all the changes were almost same with 48 days of gestation, except the distinctness of placentomes from outside of the horns. Placentomes

became rounded with having cavity at the centre and the cavity was gradually filling up with cotyledons. Length, size and shape of the foetus were increased as the age advanced (Plate 58 to Plate 62).

From 58 days to 79 days all the changes were same as 57 days of gestation except increasing length, size and shape of the foetus.

From 80 days to 135 days the cotyledons were almost engulfed by the placentomes leaving no space at the centre of the caruncles. At this stage separation of cotyledones from the caruncles were very difficult but could be separated by imposing strong pressure (Plate 64 to Plate 68).

The umbilical chords were consisting of four numbers of blood vessels out of which two vessels entered on either of the horns with elongated blastocyst. The sub branches developed from main two vessels and entered each of the cotyledons and ultimately connection developed between the cotyledons and the caruncles. Arrangement of blood vessels with the cotyledons were just like a full blooming tree, where blood vessels were just like branches of the tree and cotyledons were like blooming flowers at the terminal ends of the branches. As the time advanced the vessels were becoming thick and prominent (Plate 63).

4D. EXPERIMENTAL FINDINGS (Histological changes of genital organ in non pregnant and in different stages of pregnancy) :

In non pregnant genitalia the size and shape of the caruncles and the glands in the intercaruncular region were small. Caruncular epithelium was smooth and regular. Few blood vessels were present beneath the caruncle (Plate 74 to Plate 75).

At 15 days of pregnancy the caruncles were larger compared to non pregnant stage. Proliferation of blood vessels was observed at the base of the caruncle. Endometrial glands proliferated in the intercaruncular region (Plate 77). Foetal membrane did not show any specific changes except, the presence of some basophilic cells. As the gestation progressed the size of the caruncles were increasing progressively (Plate 76 to Plate 77).

At 18 days of pregnancy changes in the caruncle was almost similar with 15 days of pregnancy. In the intercaruncular region glands were proliferating and glandular

openings were present in the epithelial lining of the intercaruncular region and the glandular lining was simple cuboidal, this indicated the secretory activity of the glands (Plate 78 to Plate 80). The fetal membrane possessed some binucleate cells and presented some villi (Papillae) like projections (Plate 81 to Plate 82). A transitory attachment first occurs as the ovine conceptus develop finger like villi (Papillae) that project into the lumen of the uterine glands. These papillae provide a temporary anchor and absorptive structure for the conceptus as more complete or actual cotyledonary-caruncular attachment developed (Hafez and Hafez, 2000). The secretory products of the lining and glandular epithelia include mucus, lipids, glycogen and proteins (Banks, 1986). Endometrial glands secrete histotrophe (Dellmann and Eurell, 1998). Binucleate cells secrete glycoprotein. As attachment is initiated, the trophoblast binucleate cells migrate from the trophoblast to fuse with maternal endometrial epithelial cells (Gordon, 2004).

At 20 days of pregnancy changes in the caruncles were larger with increased microvascularisation and the blood vessels started to infiltrate from the base of the caruncle towards mid. Endometrial glands were proliferating and glandular openings were emerging towards the epithelial lining of the intercaruncular region and the lining of the endometrial glands was simple cuboidal type. The fetal membrane presented binucleate cells, villi like projections and increased vascularization (Plate 83 to Plate 87).

At 23, 25, 29, 33, 36 days of pregnancy changes in the caruncle, intercaruncular region and fetal membrane were almost same with 20 days of pregnancy except the proliferation of blood vessels in the caruncle as well as in the fetal membrane and size of the caruncles increased with higher gestational periods (Plate 88 to Plate 96). The lining epithelium of caruncle was regular upto 33 days of gestation while it became loosen from 36th day of gestation and free binucleate cells were also observed in the foetal membrane at this stage (Plate 97).

At 39 days of pregnancy the caruncles were larger with increased vascularisation and the vessels infiltrated the caruncles compared to earlier stages. Caruncular epithelial lining was irregular and more loosening than that of previous stage. Other changes were same as there were in the previous stage (Plate 98 to Plate 99).

At 40 days of pregnancy the caruncular epithelium showed some projections/dentations. This is the stage where we observed caruncle-cotyledonary

attachment grossly for the first time. Other changes in caruncle and fetal membrane were almost same with 39 days of pregnancy. But from this stage the height of the villi like projections in the fetal membrane became shorter compared to earlier stage. The lining epithelium of caruncle was lost totally from this stage onwards (Plate 100 to Plate 102).

At 42 days of pregnancy the epithelium of placentome showed some projections which were longer than that of 40 days of pregnancy. Microscopically, syncytial formation was observed from this stage onwards. Other changes in the caruncle and fetal membrane were almost same with 40 days of pregnancy (Plate 103 to Plate 104).

Around 45 days of pregnancy the epithelium of placentome showed finger like projection which were longer than the 42 days and also infiltration of fetal membrane into the caruncle was more compared to than that of 44 days of pregnancy and also syncytial formation was very clear (Plate 105 to Plate 107).

At 48 days of gestation projections of fetal membrane entered more deeply in to the placentome with increased number of syncytia (Plate 108 to Plate 109). As the gestation period advanced the degree of intrusion of the fetal membrane towards the deeper part of the placentome was increased (Plate 110 to Plate 111). But from 80 days of gestation onwards the placentome was invaded by the fetal membrane in such a way that the parts of foetal and maternal were intermixed with each other (Plate 112 to Plate 113).

4E. EXPERIMENTAL FINDINGS (Histology of cervix in non-pregnant and in different stages of pregnancy):

In case of non-pregnant animals' cervix contained short mucosal folds (Plate 114). The lining epithelium was simple low columnar type. Numerous tiny mucous glands were present in the lamina propria-submucosa as the muscularis mucosae remained absent in between the lamina propria and tunica submucosa of the cervix.

In early pregnancy the tunica mucosa became more folded with primary, secondary and tertiary folds. As the gestational period progressed the folds became elongated with more branching that occupied most part of the lumen (Plate 116).

The lamina epithelialis mucosa was lined by simple low columnar with goblet cells during non-pregnant and early pregnancy period (Plate 117). The height of the lining epithelial cells increased with higher gestational periods.

The lamina propria was directly connected with tunica submucosa as the cervix is devoid of lamina muscularis mucosae. The lamina propria-submucosa consisted of loose connective tissue with dense irregular arrangement. It also contained numerous glands and blood vessels in early gestation period. As the pregnancy advanced the glandular and vascular proliferation occurred. The glands were mucous in type and became larger with higher gestational age (Plate 115).

The muscle layers of tunica muscularis were arranged as inner circular and outer longitudinal pattern. Loosening of connective tissue fibres occurred with higher gestation.

Tunica serosa was rich in loose connective tissue fibres and blood vessels. Vacularization increased as the gestation period advanced. Tunica serosa was lined by simple squamous epithelium in non pregnant as well as in pregnant stages.

PLATES



Plate 1. Showing non pregnant genital organ



Plate 2. Showing caruncle in the non pregnant genital organ



Plate 3. Showing Genital organ at 15 days of pregnancy

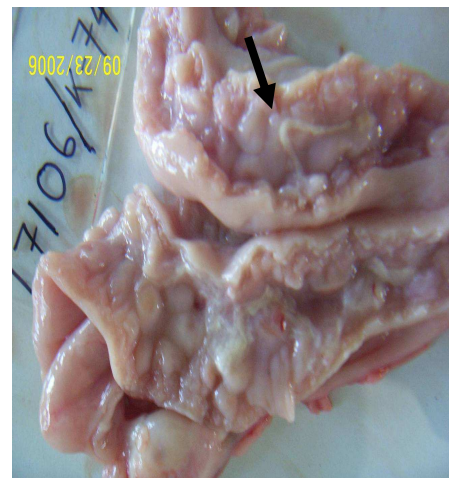


Plate 4. Showing elongated blastocyst in the uterine horns at 15 days of pregnancy

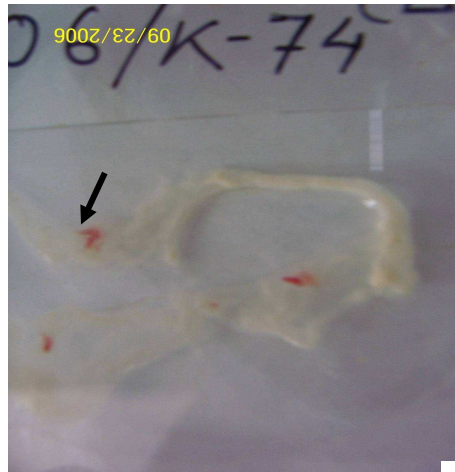


Plate 5. Showing elongated blastocyst with embryo at 15 days of pregnancy

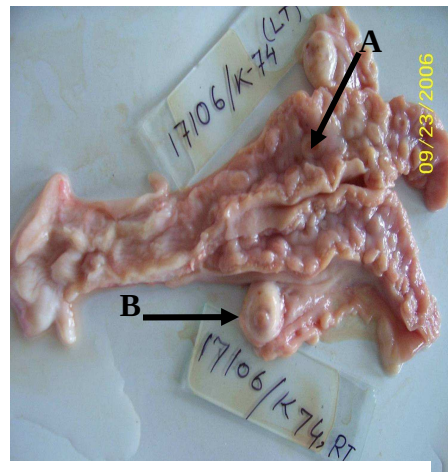


Plate 6. Showing swollen caruncles (A) and corpus luteum (B) at 15 days of pregnancy

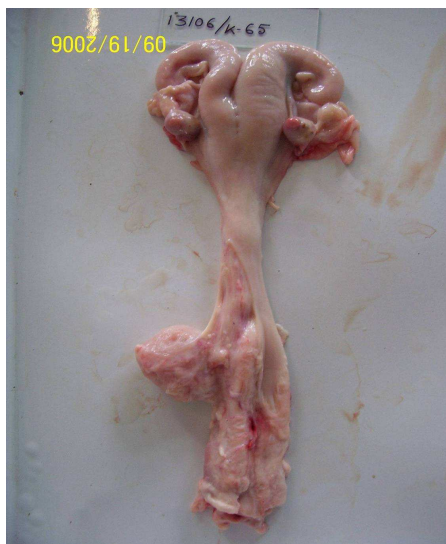


Plate 7. Showing genital organ of 18 days of pregnancy



Plate 8. Showing corpus luteum at 18 days of pregnancy



Plate 9. Showing comma shaped embryo in the elongated blastocyst (18 days of pregnancy)

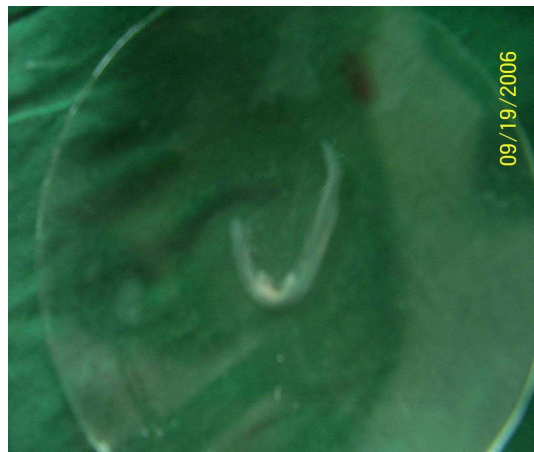


Plate 10. Showing gross photograph of conceptus at 18 days of pregnancy

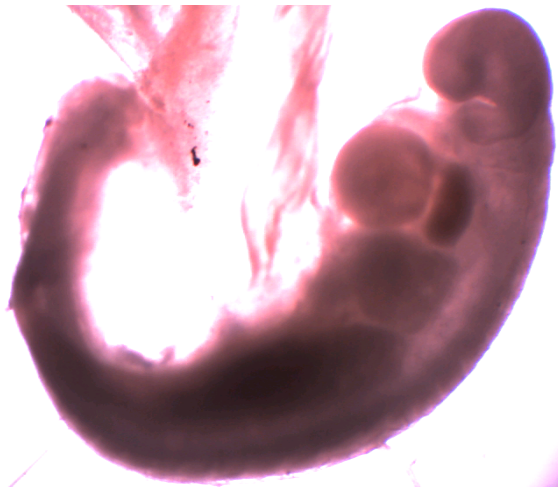


Plate 11. Showing microscopic view of 18 days embryo (2x magnification)

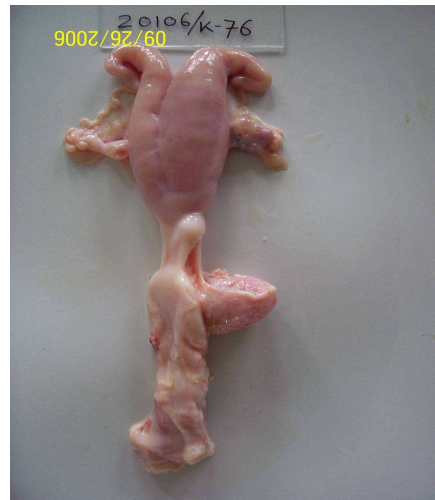


Plate 12. Showing genital organ of 20 days of gestation

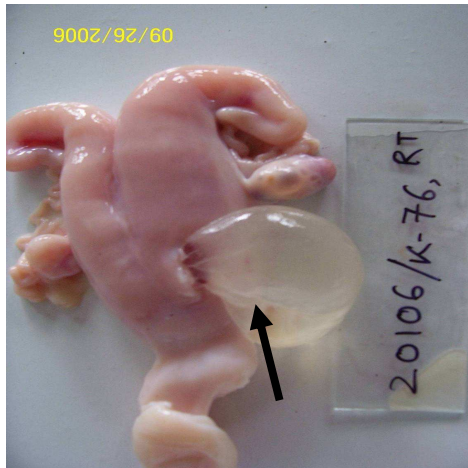


Plate 13. Blastocyst is coming out by dorsal incision to the right horn (20 days of pregnancy)



Plate 14. Elongated blastocyst containing embryo (20 days of pregnancy)

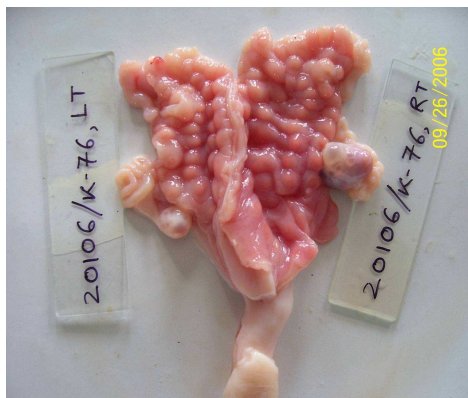


Plate 15. Showing caruncular and intercaruncular regions (20 days of pregnancy)

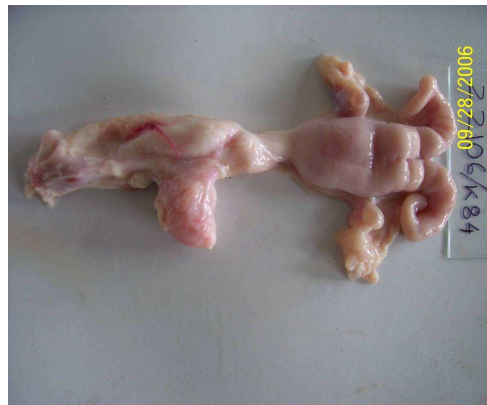


Plate 16. Showing 23 days pregnant genital organ

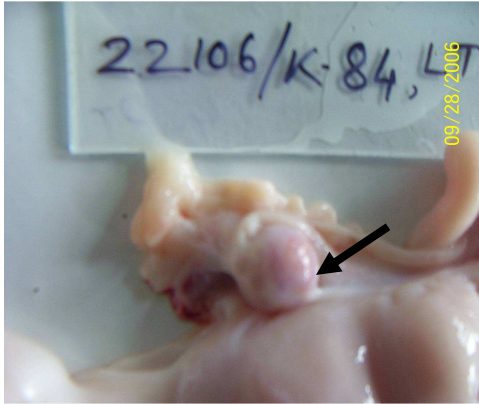


**Plate 17. Showing corpus luteum
in the left ovary (23 days of
pregnancy)**

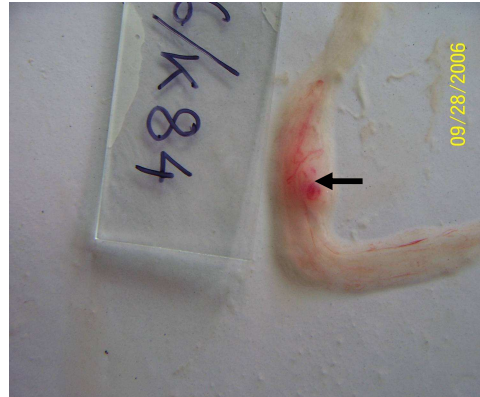


**Plate 18. Elongated blastocyst
containing embryo (23 days of
pregnancy)**

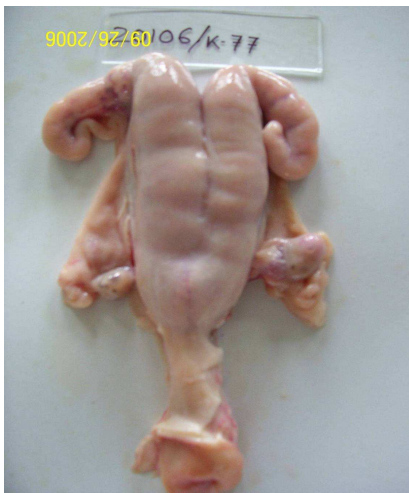


**Plate 19. Showing 25 days pregnant
genital organ**

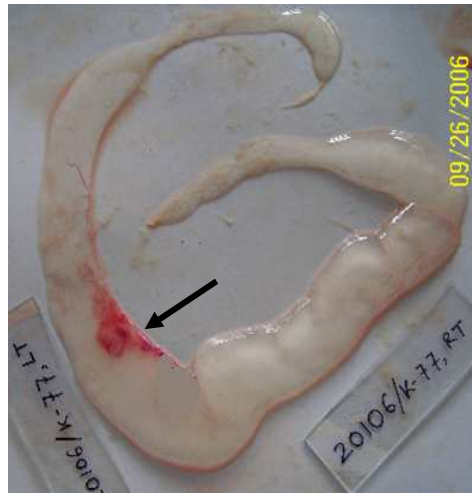


**Plate 20. Elongated blastocyst containing
embryo (25 days of pregnancy)**

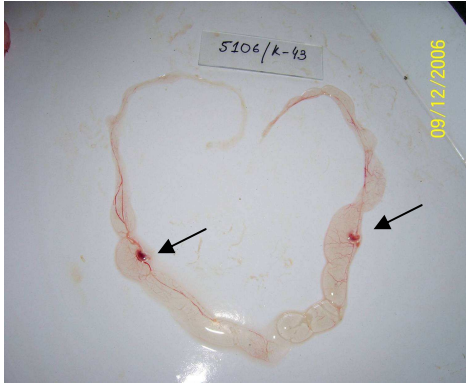


Plate 21. Elongated blastocyst containing double embryos (29 days of pregnancy)



Plate 22. Showing microscopic view of the 29 days embryo and emerging blood vessels

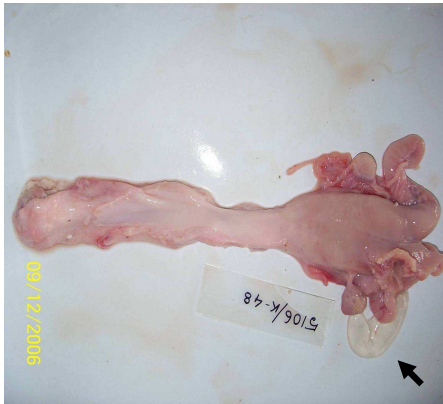


Plate 23. Pregnant genital organ (33 days), showing separation of blastocyst

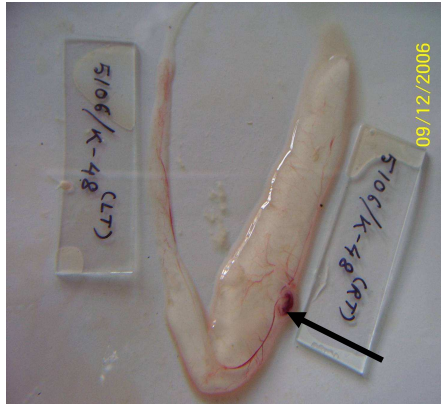


Plate 24. Showing elongated blastocyst containing embryo (33 days of pregnancy)



Plate 25. Showing 36 days pregnant genital organ

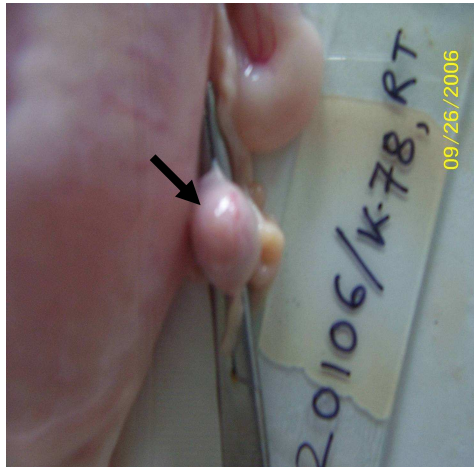


Plate 26. Showing corpus luteum in the right ovary (36 days of pregnancy)



Plate 27. Elongated blastocyst completely filled with fluid and ramified with blood vessels (36 days of pregnancy)



Plate 28. Showing foetus within the amniotic cavity (36 days of pregnancy)

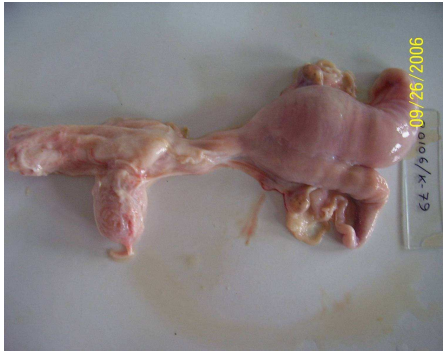


Plate 29. Showing 39 days pregnant genital organ

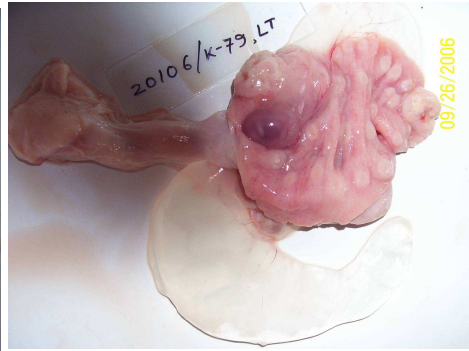


Plate 30. Showing presence of conceptus in the horn and changes in the caruncular and intercaruncular regions (39 days of pregnancy)



Plate 31. Showing appearance of cotyledones (39 days of pregnancy)

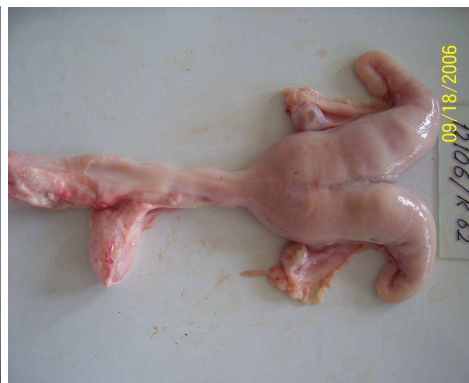


Plate 32. Showing 40 days pregnant genital organ



Plate 33. Showing separated cotyledone from the caruncle (40 days of pregnancy)



Plate 34. Blastocyst is separating on holding the uterine horn (40 days of pregnancy)



Plate 35. Cotyledones separating from caruncles (40 days of pregnancy)

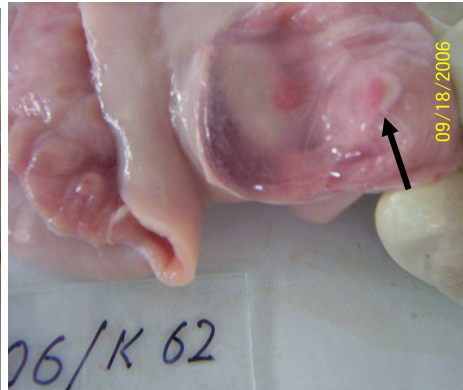


Plate 36. Cotyledones separating from caruncles at 40 days of pregnancy (Close view)



Plate 37. Elongated blastocyst containing cotyledones nearby to the fetus at 40 days of pregnancy



Plate 38. Showing foetus within the amniotic cavity and spread of blood vessels (40 days of pregnancy)

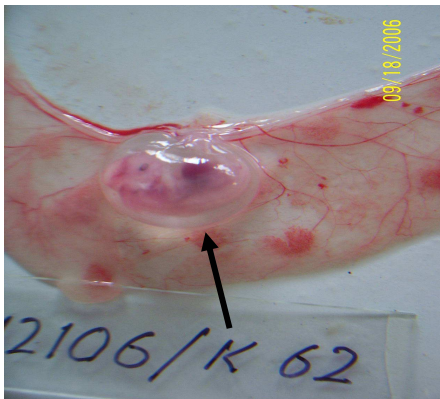


Plate 39. Showing foetus within the amniotic cavity at 40 days of pregnancy

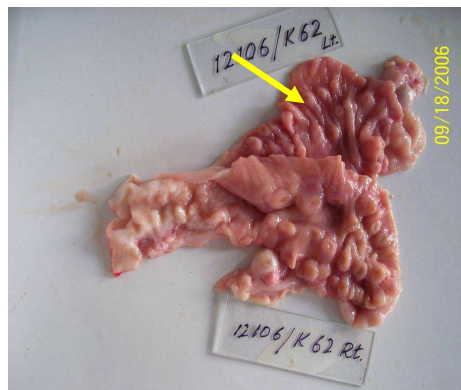


Plate 40. Showing flattened caruncles at 40 days of pregnancy

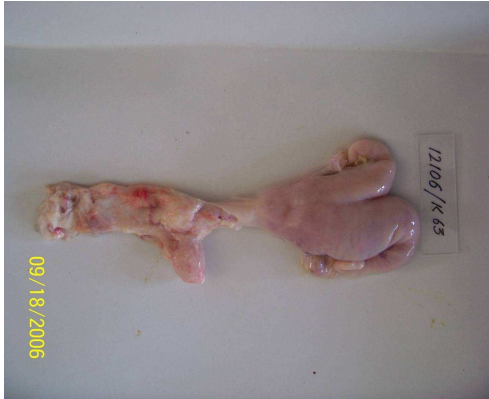


Plate 41. Showing 42 days pregnant genital organ



Plate 42. Showing separation of cotyledone from the caruncle at 42 days of pregnancy

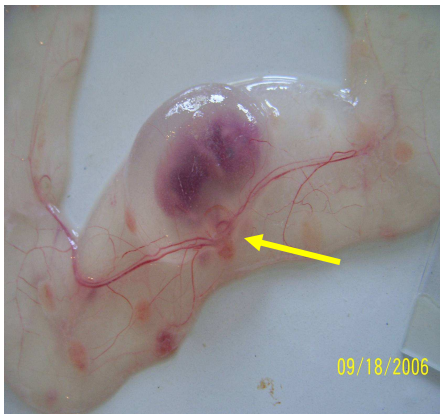


Plate 43. Showing emerging blood vessels from the hind gut of foetus at 42 days of gestation



Plate 44. Showing 44 days pregnant genital organ

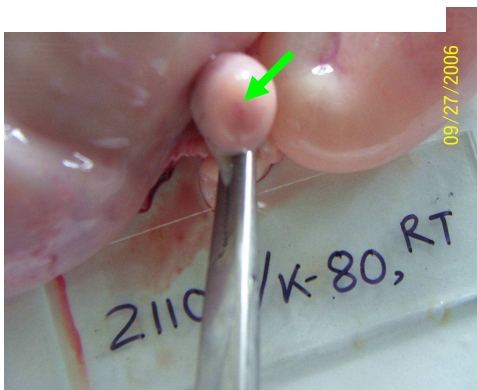


Plate 45. Showing corpus luteum at 44 days of pregnancy



Plate 46. Showing prominent cotyledons nearby foetus at 44 days of gestation.

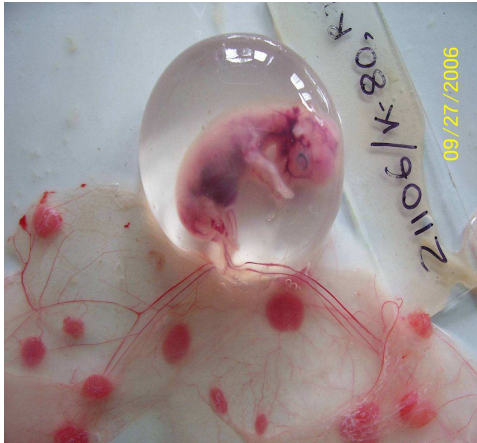


Plate 47. Showing amniotic cavity and umbilical chord at 44 days of pregnancy

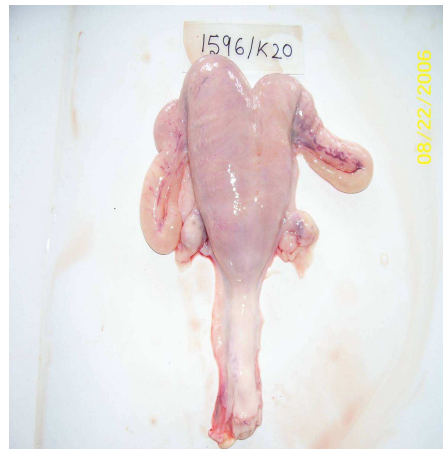


Plate 48. Showing 45 days pregnant genital organ



Plate 49. Showing arrangement of cotyledons to the blastocyst at 45 days of pregnancy

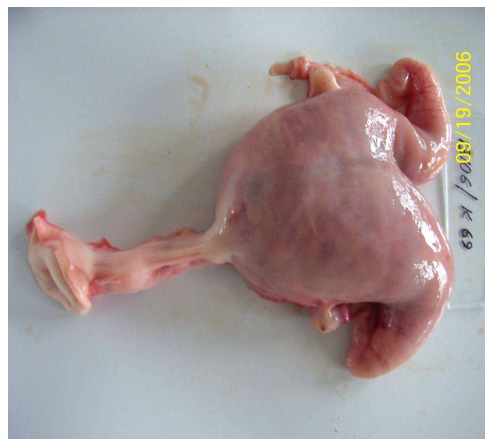


Plate 50. Showing 46 days pregnant genital organ



Plate 51. Showing elongated blastocyst at 46 days of pregnancy



Plate 52. Showing 47 days pregnant genital organ

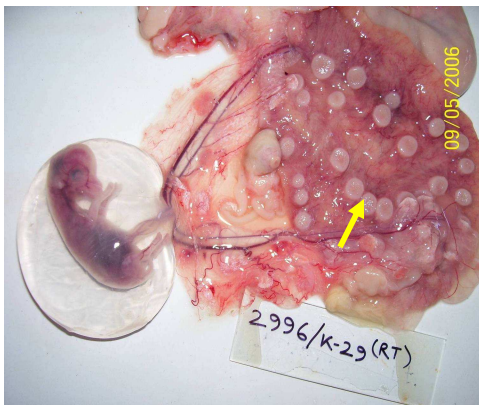


Plate 53. Showing depressed caruncles at 47 days of gestation



Plate 54. Showing visible placentomes from outside horn at 48 days of gestation



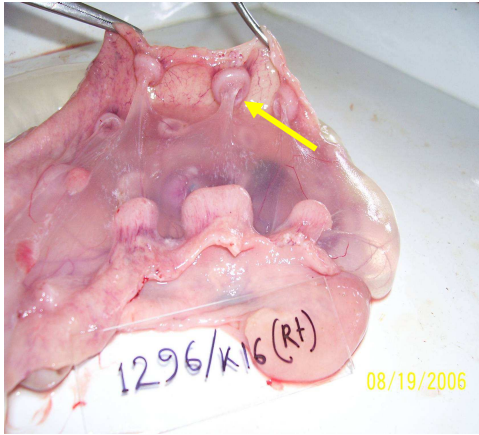


Plate 55. Showing strong cotyledonary – caruncular attachment at 48 days of pregnancy

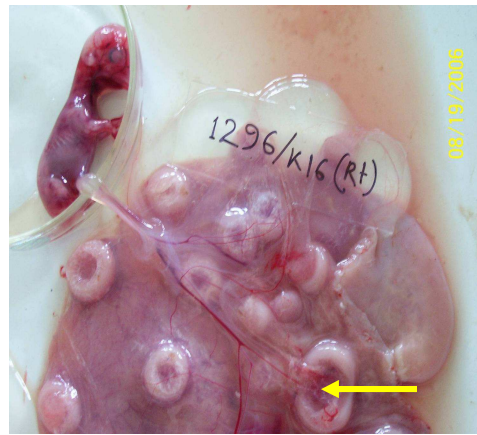


Plate 56. Showing cavity formation in the placentome at 48 days of pregnancy

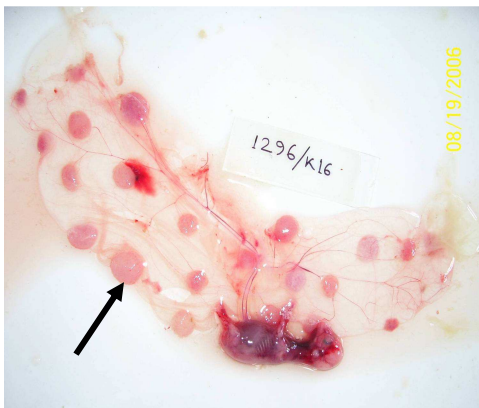


Plate 57. Showing rounded and raised type Cotyledones at 48 days of pregnancy

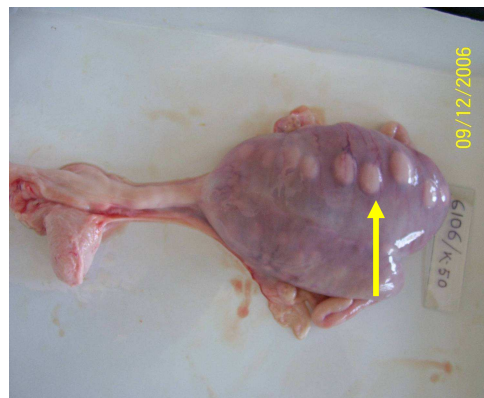


Plate 58. Showing distinct placentomes from outside at 52 days of pregnancy



Plate 59. Showing ball like cotyledones (A) and cup shaped placentomes (B) at 52 days of pregnancy



Plate 60. Showing elongated blastocyst at 53 days of pregnancy



Plate 61. Showing 57 days pregnant genital organ

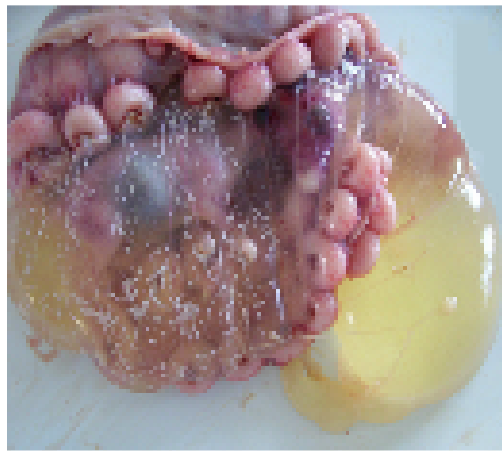


Plate 62. Showing strong placentome formation at 57 days of pregnancy

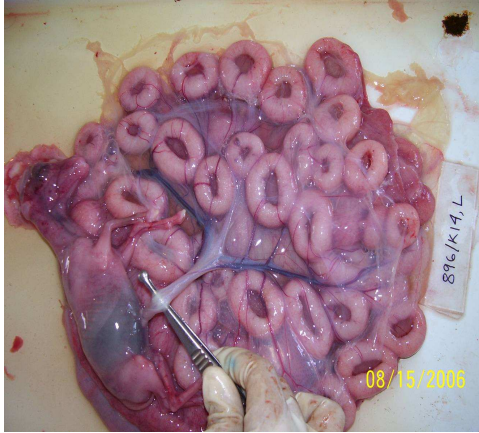


Plate 63. Showing arrangement of blood vessels with the placentomes at 68 days of pregnancy



Plate 64. Showing 87 days pregnant genital organ

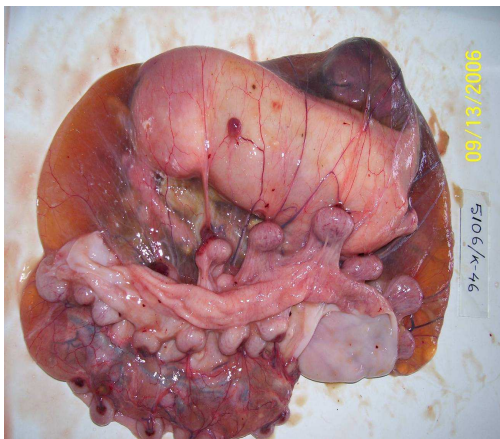


Plate 65. Cotyledons are completely attached with caruncles at 87 days of pregnancy

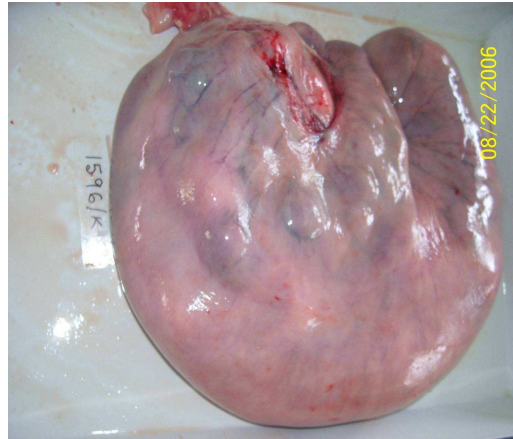


Plate 66. Showing 134 days pregnant genital organ

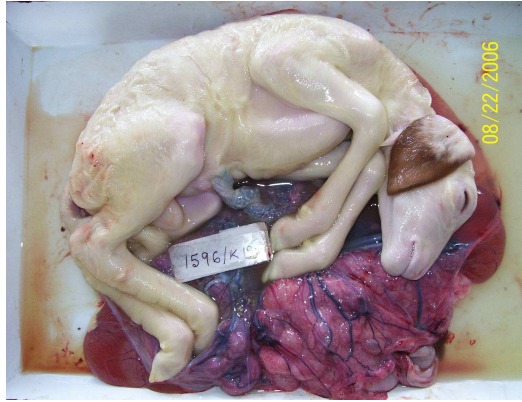


Plate 67. Showing full grown foetus at 134 days of gestation

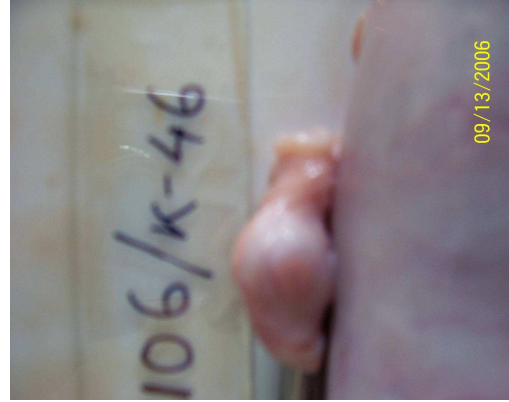


Plate 68. Showing corpus luteum at 134 days of gestation



Plate 69. Showing transparent white amniotic (AM) and creamy white allantoic (AL) fluids

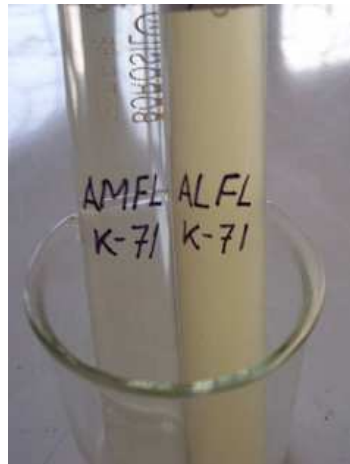


Plate 70. Showing transparent white amniotic (AM) and pale yellow coloured allantoic (AL) fluids



Plate 71. Showing pale yellow coloured amniotic fluid and intense yellow coloured



Plate 72. Showing yellow coloured amniotic (AM) and intense yellow allantoic (AL) fluids



Plate 73. Showing off white amniotic (AM) and intense yellow allantoic (AL) fluids



Plate 74. Showing caruncle of non pregnant genital organ (4x)

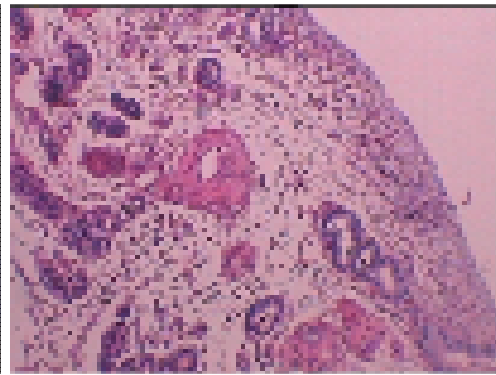


Plate 75. Showing intercaruncular region of non pregnant genital organ



Plate 76. Showing caruncle of pregnant genital organ at 4x (15 days of pregnancy)

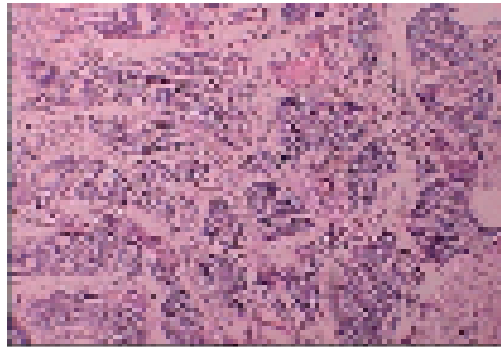


Plate 77. Intercaruncular region showing endometrial gland proliferation (15 days of pregnancy)

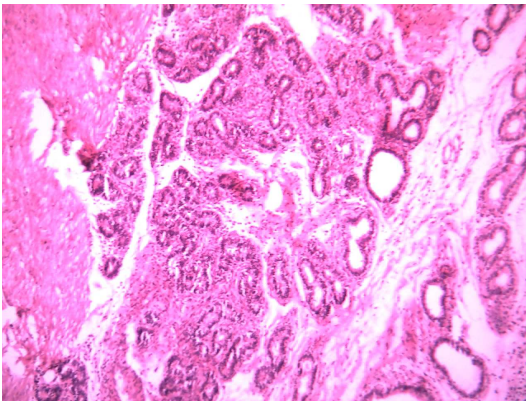


Plate 78. Showing endometrial gland proliferation (18 days of pregnancy)

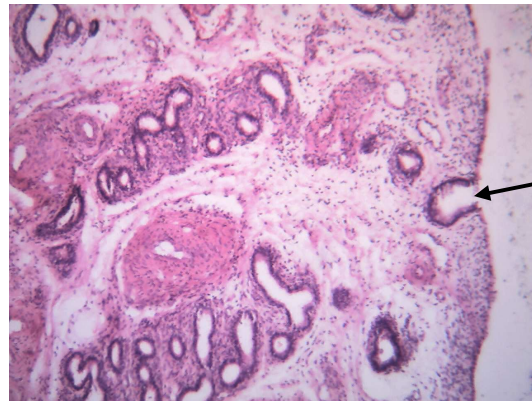


Plate 79. Intercaruncular region showing glandular opening (18 days of pregnancy)

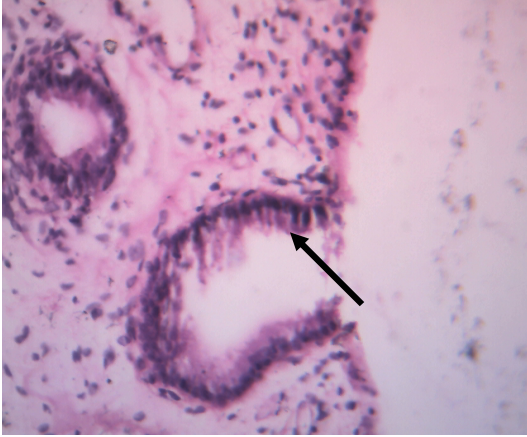


Plate 80. Showing simple cuboidal epithelium in the endometrial gland (18 days of pregnancy)



Plate 81. Showing trophoblastic villi with binucleate cells (18 days of pregnancy)

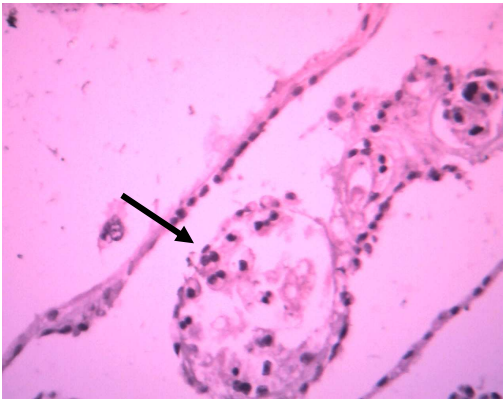


Plate 82. Showing binucleate cells in the fetal membrane (18 days of pregnancy)

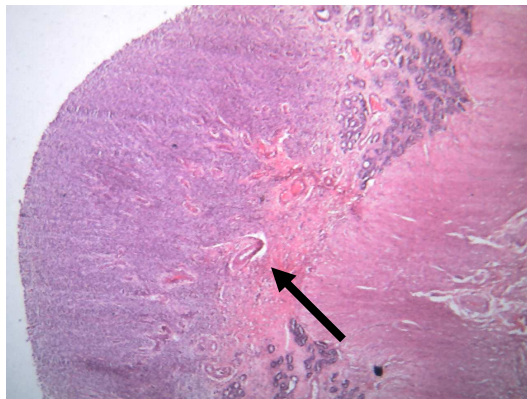


Plate 83. Showing caruncle and vascular proliferation (20 days of pregnancy)

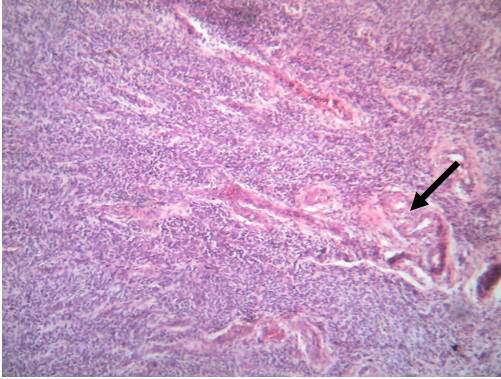


Plate 84. Vascular proliferation at the base of the caruncle (20 days of pregnancy)

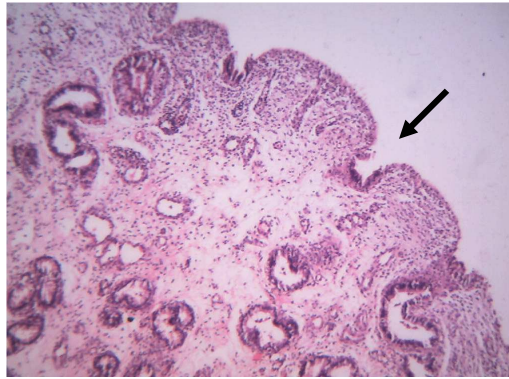


Plate 85. Showing opened endometrial gland in the intercaruncle region (20 days of pregnancy)

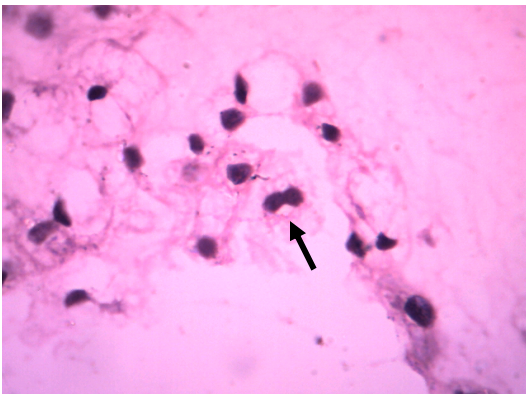


Plate 86. Showing binucleate cells in the fetal membrane (20 days of pregnancy)

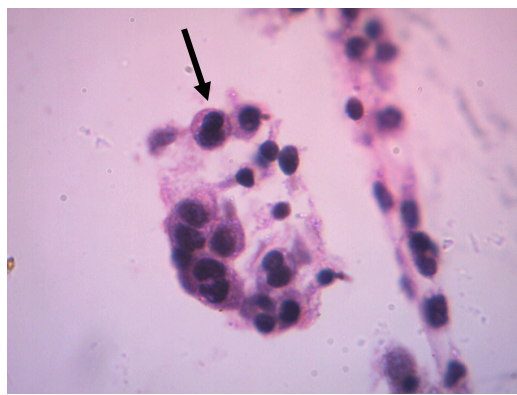


Plate 87. Binucleate cells in the fetal membrane under 100x (20 days of pregnancy)

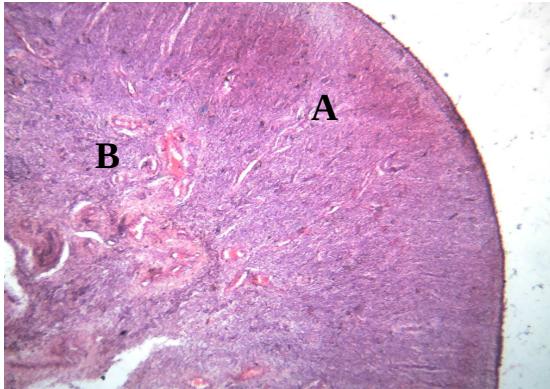


Plate 88. Showing caruncle (A) and vascular proliferation (B) at 23 days of pregnancy

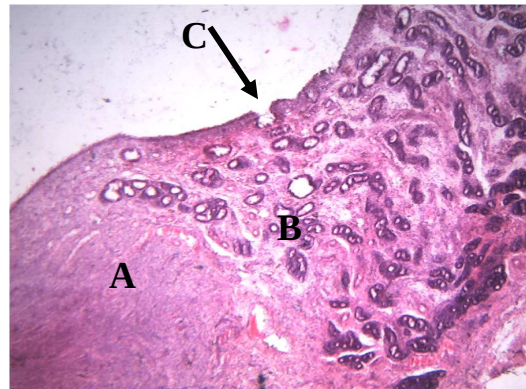


Plate 89. Showing caruncular (A) and intercaruncular regions (B) with glandular opening(C) at 23 days of pregnancy

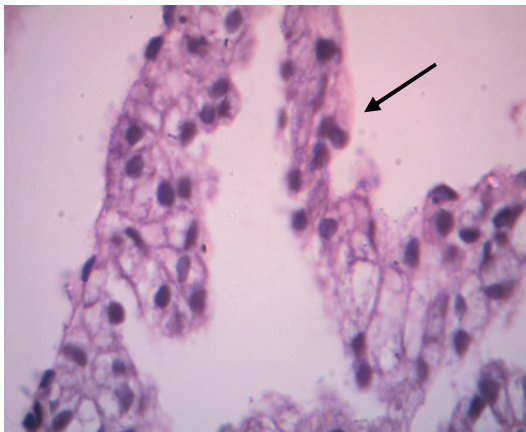


Plate 90. Binucleate cells in the fetal membrane under 100x (23 days of pregnancy)

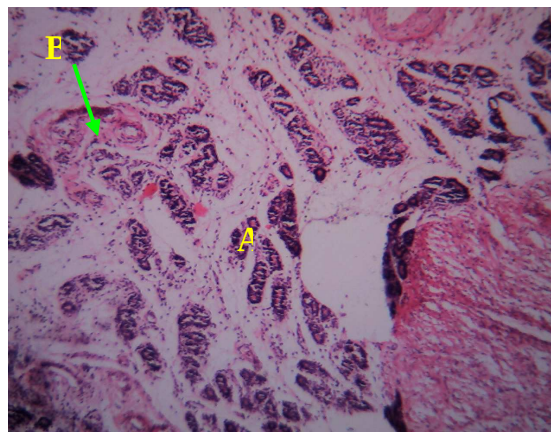


Plate 91. Showing endometrial glands (A) and vascular proliferation (B) at 25 days of pregnancy

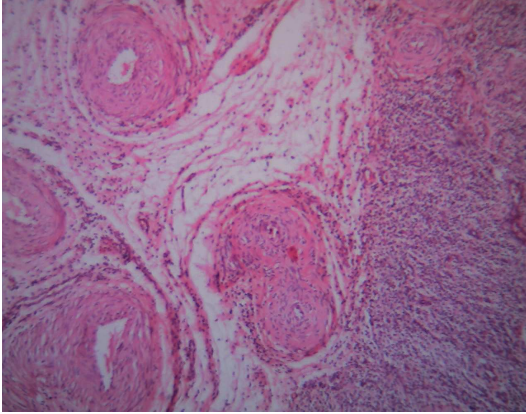


Plate 92. Showing vascular stock at the base of the caruncle (33 days of pregnancy)

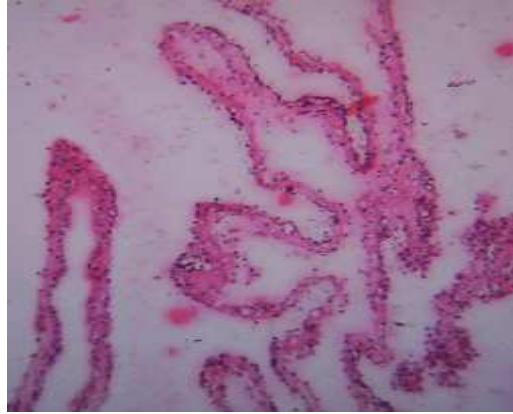


Plate 93. Showing trophoblastic villi (33 days of pregnancy)

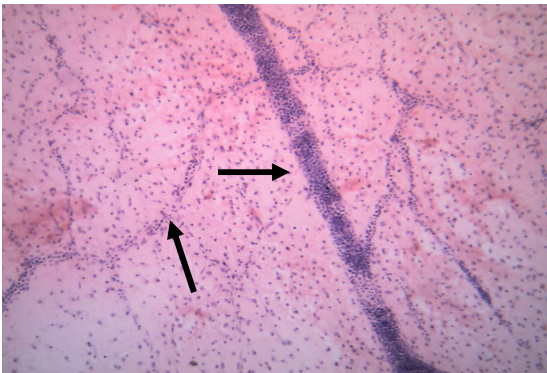


Plate 94. Showing vascular proliferation in the fetal membrane at 33 days of pregnancy (Direct tissue fixation)

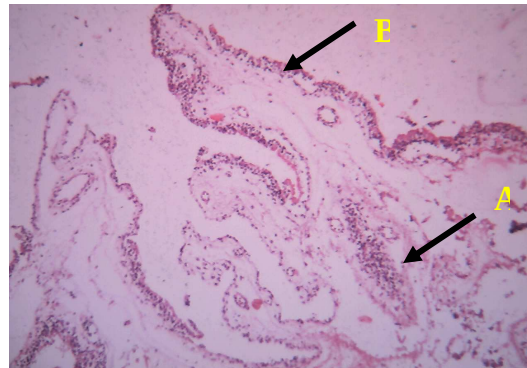


Plate 95. Showing trophoblastic villi (B) and vascular proliferation (A) in the fetal membrane (36 days of pregnancy)

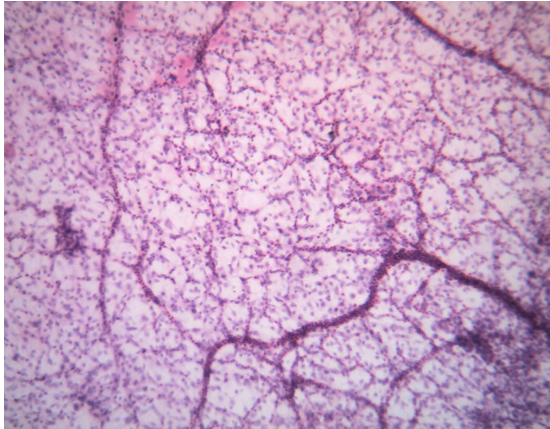


Plate 96. Showing vascular proliferation in the fetal membrane at 36 days of pregnancy (Direct tissue fixation)

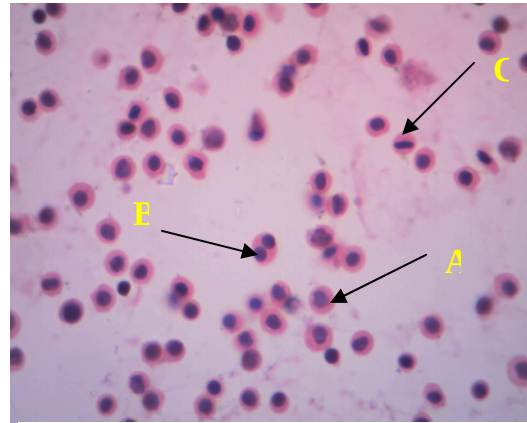


Plate 97. Fetal membrane showing presence of large nucleate (A), elongated nucleate (C) and free binucleate cells (B) at 36 days of pregnancy

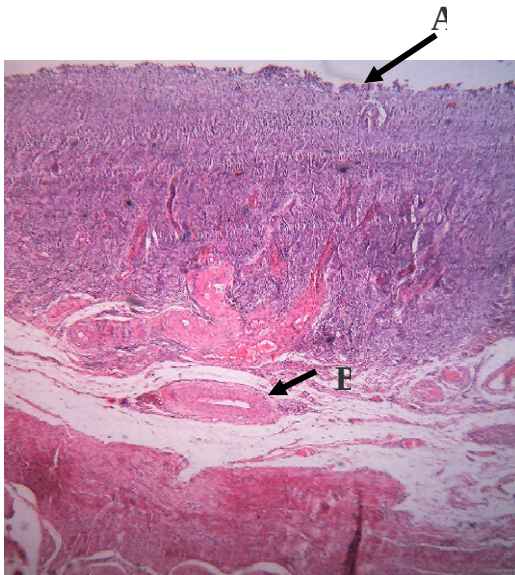


Plate 98. Caruncle showing loose epithelium (A) and more vascular stock

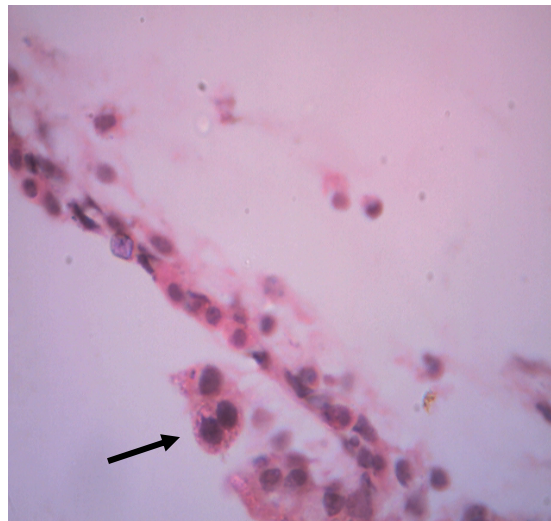


Plate 99. Fetal membrane showing free binucleate cell (39 days of pregnancy)

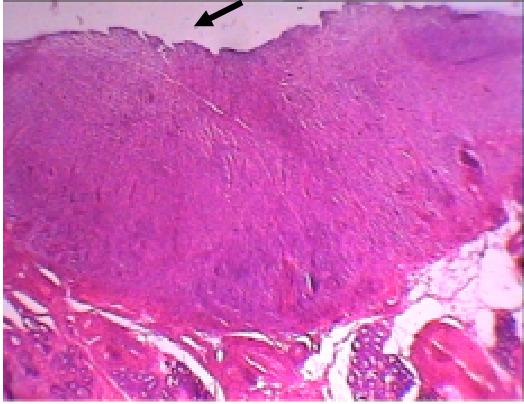


Plate 100. Showing dentations in the caruncular epithelium, 4x (40 days of pregnancy)

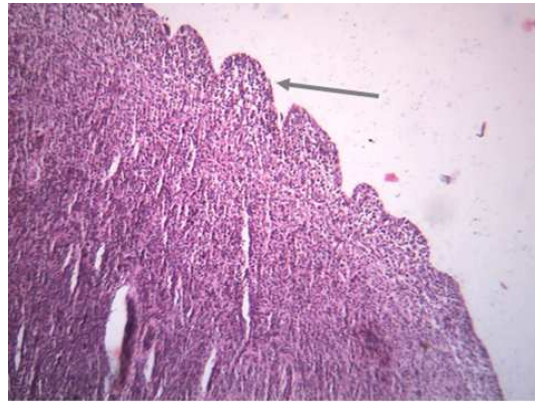


Plate 101. Showing dentations in the caruncular epithelium under 40x (40 days of pregnancy)

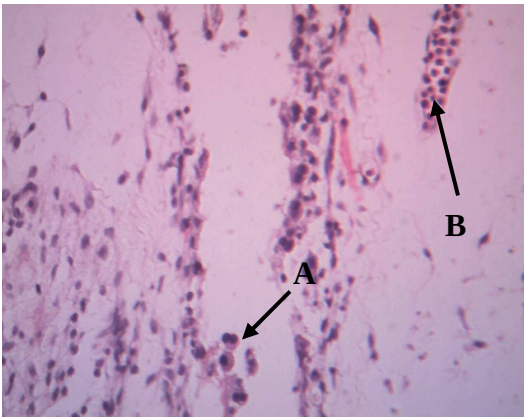


Plate 102. Showing binucleate cells (A) and vascular proliferation (B) in the fetal membrane (40 days of pregnancy)

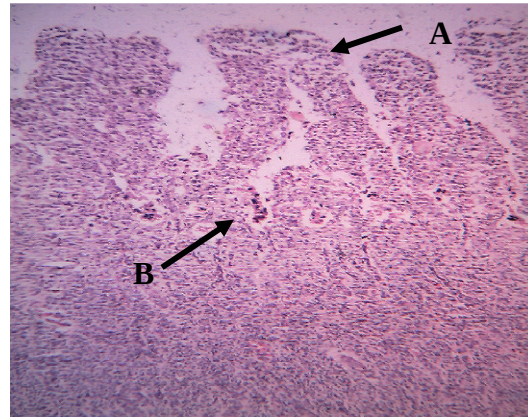


Plate 103. Showing projections in the caruncular epithelium (A) and syncytial formation (B) at 42 days of pregnancy

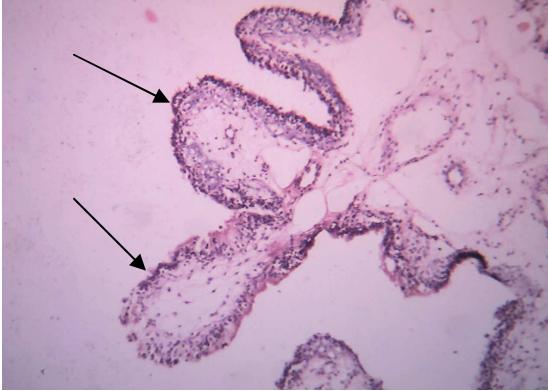


Plate 104. Showing short trophoblastic villi in the fetal membrane (42 days of pregnancy)

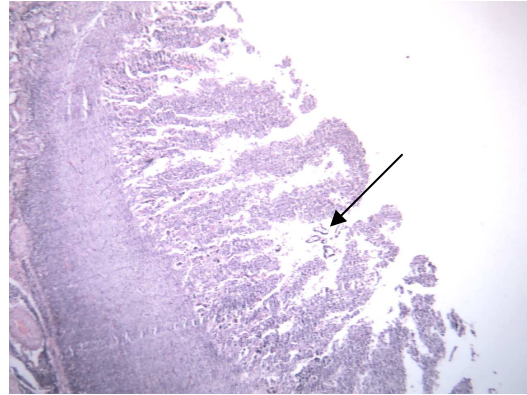


Plate 105. Placentome showing fetal parts entered into the caruncular projections (45 days of pregnancy)

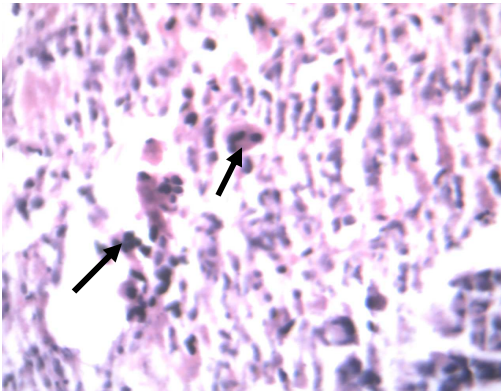


Plate 106. Showing syncytial formation in the placentome under 40x (45 days of pregnancy)

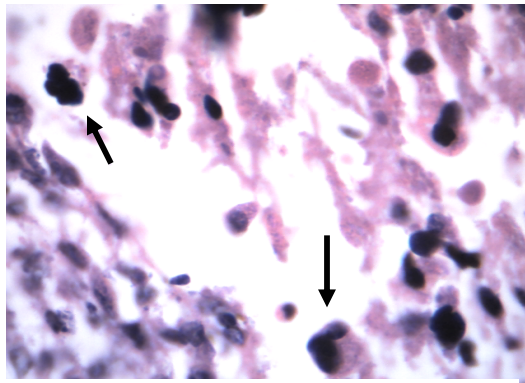


Plate 107. Showing syncytial formation in the placentome under 100x (45 days of pregnancy)

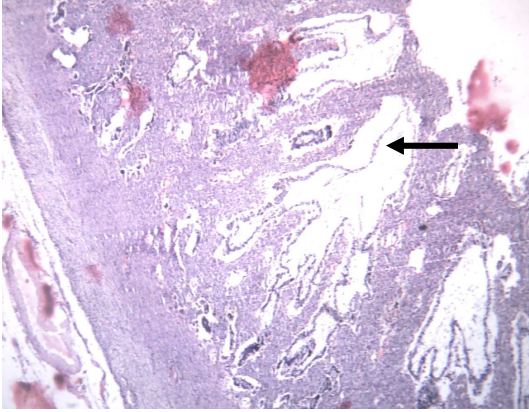


Plate 108. Placentome showing fetal parts into the caruncle (48 days of gestation)

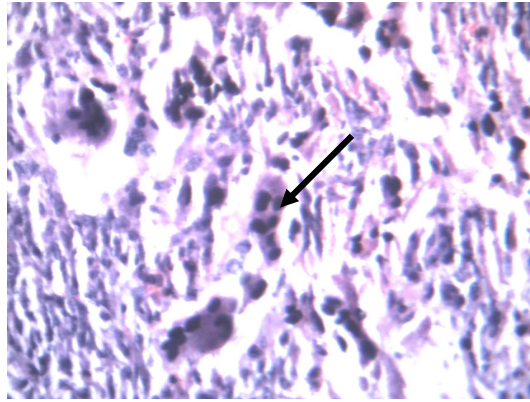


Plate 109. Showing syncytial formation in the placentome under 40x (48 days of gestation)

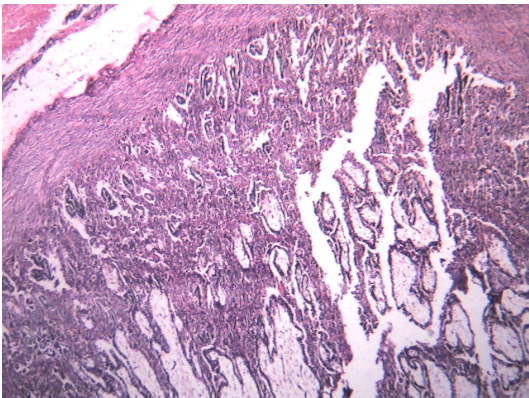


Plate 110. Placentome showing intermixing of foetal and maternal parts (52 days of pregnancy)

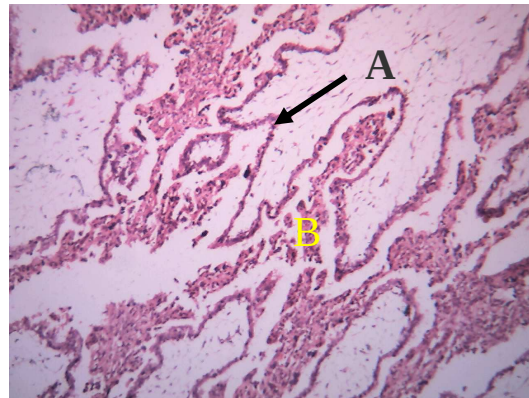


Plate 111. Placentome showing intermixing of foetal (A) and maternal parts (B) (56 days of pregnancy)

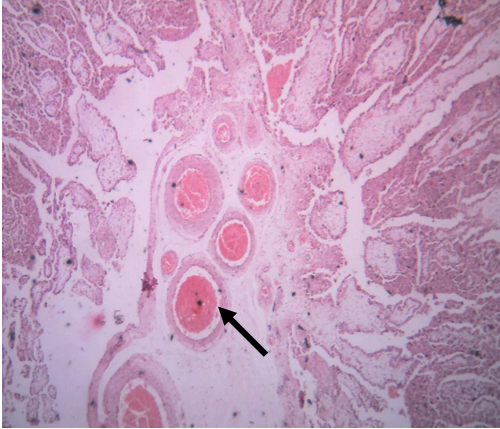


Plate 112. Showing foetal parts with blood vessels at the centre of the placentome (87 days of pregnancy)

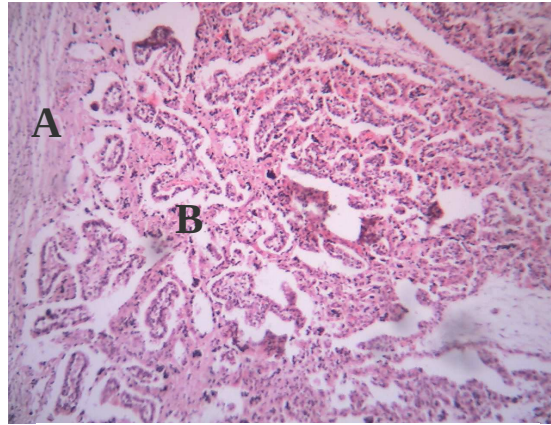


Plate 113. Placentome showing capsule (A) and complete inter mixing of foetal and maternal parts (B) (87 days of pregnancy)

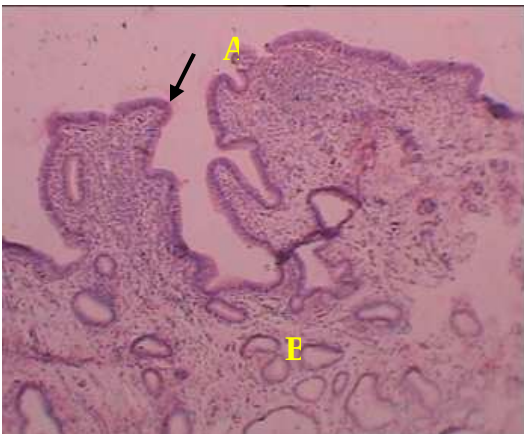


Plate 114. Short mucosal folds containing simple columnar epithelium (A) and numerous tiny mucous glands (B) present in the lamina propria-submucosa in non pregnant cervix.

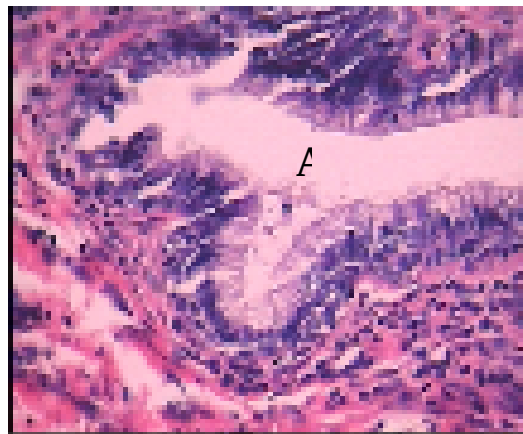


Plate 115. Gland which is mucous in type (A) during mid pregnancy

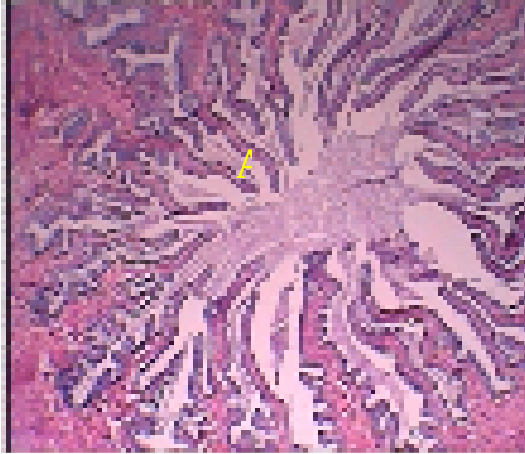


Plate 116. Large number of mucosal folds showing mucous secretion in the stellate shaped lumen (A)



Plate 117. Epithelium showing simple columnar (A) with goblet cells (B)

FIGURES

Figure 1. Age (Range : 12 to 140 Days of gestation) and Whole Embryo/Crown-Anus Length (Range : 0.05 to 50 cm) Relationship Graph i.e. Khatun-Wani Graph, 2007.

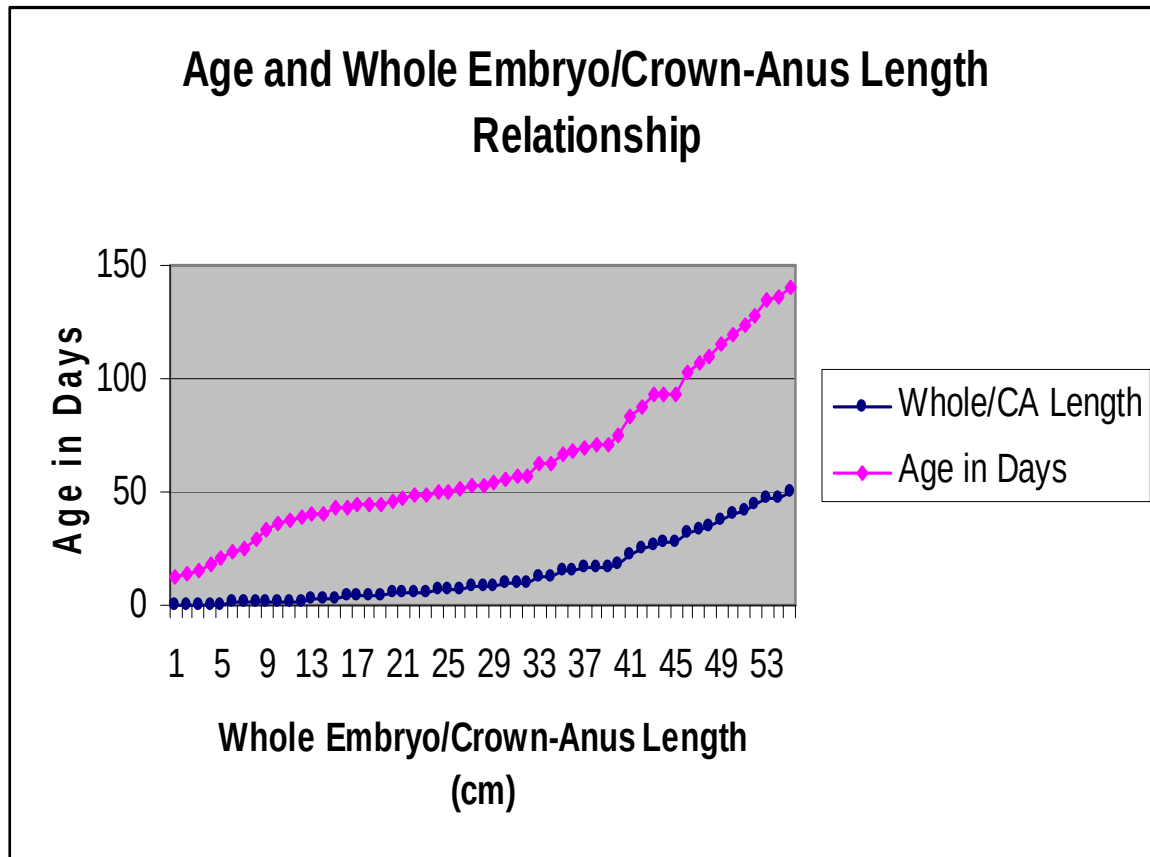
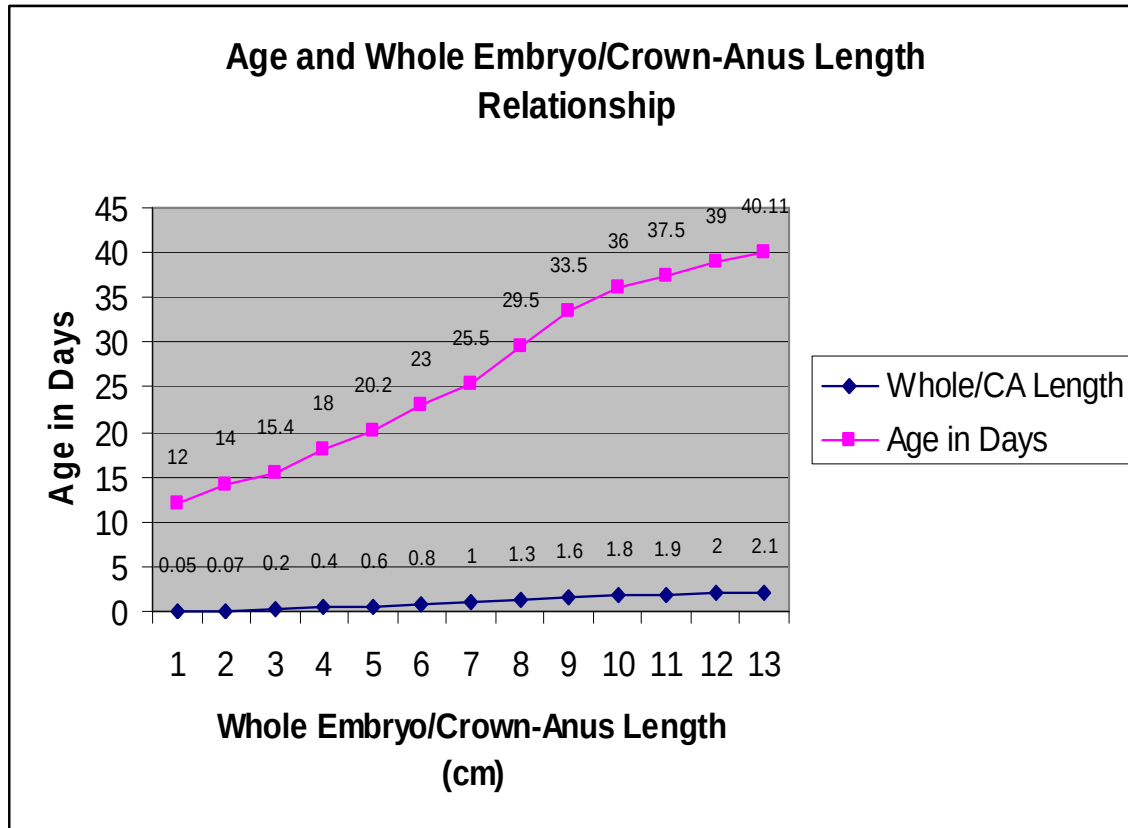


Figure 2. Age (Range : 12 to 40 Days of gestation) and Whole Embryo/Crown-Anus Length (Range : 0.05 to 2.1 cm) Relationship Graph i.e. Khatun-Wani Graph, 2007.



Chapter – 5

DISCUSSION

5C. Discussion on gross and histological changes in non pregnant and in different stages of pregnancy:

Gross and histological changes of ewe genitalia in non pregnant and in different stages of pregnancy narrated in details under results 4C and 4D along with photographic evidences (Plate1 to Plate 68 and Plate 74 to Plate 113). Gross changes in non pregnant genitalia were externally symmetrical horns and internally the caruncles were pointed and histologically, the size and shape of the caruncle and the endometrial glands in the intercaruncular region were smaller and caruncular epithelium was smooth and regular. Few blood vessels were present beneath the caruncle (Plate 74 to Plate 75). These changes were supported by Wani *et al.*, 2006. As per pregnancy concerned we have studied from 15 days upto 135 days of gestation (Range: 14 to 140 days). Caruncular size and vascular proliferation were increasing continuously as the gestation advanced. At 15 days the horns were slightly asymmetrical but the base of the horn (Part of the horn nearer to the body of the uterus) bulged dorsally, the reason might be due to the presence of conceptus at the base of the uterine horn. The blastocyst was not completely elongated upto the tip of the horns and also the cavities were not completely filled up with foetal fluid. At 15 days, histologically, the size of the caruncles were larger in comparison with non pregnant genitalia. Glandular proliferation started in the inter-caruncular region but caruncular epithelial lining was regular and smooth just like non pregnant sample. Vascular proliferation observed at the base of the caruncle. The foetal membrane did not show any specific changes except, the presence of some basophilic cells. Asymmetry of the horns was observed from 18 days onwards and was increasing as the gestation period advanced. At 18 days of gestation the changes in the caruncle (histologically) were almost same with 15 days of gestation except, the increasing size of caruncle and vascular proliferation. Important observation by histologically at 18 days of gestation was appearance of binucleate cells (binucleate cells observed constantly in the fetal membrane from 18 days onwards) and villi (papillae) like extension in the fetal membrane and endometrial glandular opening in the inter-carucarular region, which was an indication of transitory attachment supported by earlier author, Hafez and Hafez

(2000). Dellmann and Eurell (1998) postulated that histotrophe, secreted by uterine glands nourished the embryo before implantation. The postulation of Dellmann and Eurell (1998) also supported our observation. This attachment was also supported by progesterone receptor, where progesterone receptor decreased slightly (Hafez and Hafez, 2000). Upto 20 day's corpus luteum possessed neck and was bulged out from the ovarian surface but from 23 days onwards it started embedding within the ovary and was present upto the term. From 15 days upto 23 days of gestation grossly, the caruncles were rounded and swollen type that might be the effect of progesterone (Schular, 2002) but at 25 days of gestation a circular depression was observed at the centre of the caruncle and gradually it became flattened. Continuously presence of depression at the centre of the caruncle was observed from 40 days and it was increasing as the gestation advanced and gradually they became cup shaped. Important point to be noted that as the age advanced the size of the caruncles were continuously increasing both gross and histologically (Schular, 2002). At 15 days the blastocyst was not elongated upto the tip of the horns and embryo was just like a red clot, it could only be recognized after microscopic observation. At this very stage the amniotic cavity was not distinct and fluid accumulation to the allantoic cavity was very less. But from 18 days onwards there was continuous accumulation of foetal fluids. From 20 days onwards the blastocyst was completely elongated upto the tip of the horns but was not completely filled with fluids. Emerging blood vessels from the hind gut of foetus was grossly recognized from 20 days onwards. Two vessels emerged from the hind gut of foetus but actually it was not two, were total of four vessels recognized after 25 days of gestation and two of each entered either of the horns. As the gestation period advanced the vessels were becoming thick and prominent and also the ramifications from the main vessels were increasing. From 25 days onwards the amniotic cavity could be recognized grossly. At 36 days of gestation, blastocyst was completely filled with fluid and whole blastocyst ramified with blood vessels and it was almost reddish in colour (Plate 27). From this stage head, eye bud and tail could be recognized grossly (Plate 28) and this might be the beginning of foetal stage (Hafez and Jainndeen, 1993 and Hafez and Hafez, 2000). At 39 days single layered reddish granules were present mostly in the membrane of the left side were nothing but the cotyledons that appeared for the first time (Plate 31). Cotyledones were arranged in

single layered which was reddish in colour and granular. Very important changes were observed from 40 days of gestation onwards. At 40 days we have observed gross caruncular-cotyledonary attachment (Plate 33 to Plate 36) that was supported by histological changes and progesterone receptor concentration (Hafez and Hafez, 2000), which was the main motto of our research. At this stage histologically, a typical change was observed in the caruncular epithelial lining. There were some projections rather to say dentations on the caruncular epithelial lining. Gradually, from 40 days of gestation onwards the lengths of these projections were increasing. Within these projections the trophoblastic villi were invading or infiltrating gradually and developed syncytium (Hafez and Hafez, 2000 and Gordon, 2004). In our studies we have observed the formation of syncytia from 42 days onwards. Hafez and Hafez, 2000 and Gordon, 2004 mentioned about syncytial formation but they did not cite the actual time of syncytial formation which might be cleared from our observation. From 18th days of gestation upto 40th day's changes in the fetal membrane were presence of binucleate cells, villi like extension and increasing vascular proliferation, but from 40 days onwards other changes were continuing except villi like extension i.e. the height of villi like extension reduced. From 40 days upto 47 days of gestation the caruncular-cotyledonary attachment was very fragile and they got separated by holding the uterine horn without imposing any pressure or by putting very slight pressure. But from 48 days onwards the attachment could not be separated by holding the uterine horn (Plate 55), it was becoming strong gradually. Depression at the centre of the caruncles was observed from 40 days onwards and gradually it was becoming deep and rounded. The cotyledonary invasion increased gradually as if the placentomes were engulfing the cotyledons. From 80 days onwards the placentomes completely attached the cotyledons, leaving no space at the centre of the placentome. The placentome became completely rounded, this gross change was also supported by histological observation of our study. Histologically, the foetal and maternal parts were completely intermixed and a capsule was present around the placentome (Plate 113). From this stage onwards it was difficult to separate the fetal and maternal parts and needed very strong pressure. Our observation partly supported the postulation of Gordon (2004). He postulated that the attachment process in ruminant was superficial and non invasive, actually as per our observation the attachment was non invasive at the early

stage only but it was becoming strong from 48 days onwards and cotyledons were completely attached by the placentomes from 80 days onwards. As per our observation attachment started microscopically from 18 days of gestation (embryonic stage), it might be started from 17 days as we did not have 17 days of pregnant sample (Roberts, 1971), and completed grossly by establishing caruncular-cotyledonary attachment at around 40 days (Foetal stage) of gestation. Our observation also supported the report of Roberts (1971). He reported that ovine embryonic attachment begins at about 17 to 20 days and is completed by about 28 to 30 days (Roberts, 1971). Our observation also supported by Bazer and Roberts (1983), Hafez (1987), Wani (1996), Gray *et al.* (2002) and Wani *et al.* (2006) directly or indirectly.

5D. Discussion for histology of cervix:

We have studied histology of cervix in non-pregnant and different stages of pregnancy i.e. early, mid and late pregnancy. In case of non-pregnant animals' cervix contained short mucosal folds. The lining epithelium was simple low columnar type. Numerous tiny mucous glands were present in the lamina propria-submucosa. This study was supported by Banks (1986) and Hafez (1987).

In early pregnancy, the tunica mucosa became more folded with primary, secondary and tertiary folds. As the gestational period progressed the folds became elongated with more branching that occupied most part of the lumen.

The lamina epithelialis mucosa was lined by simple columnar with goblet cells during early pregnancy. As the gestation period advanced height and loosening of the lining epithelial cells increased. The secretory activity of the mucosa increased with higher gestational age and formed cervical seal in the cervical lumen (Banks, 1986).

The lamina propria-submucosa consisted of loose connective tissue with dense irregular arrangement (Banks, 1986). It also contained numerous glands and blood vessels in early gestation period. As the pregnancy advanced the glandular and vascular proliferation occurred. The glands were mucous in type and became larger with higher gestational age due to increase secretory nature.

The muscle layers of tunica muscularis were arranged as inner circular and outer longitudinal pattern. Loosening of connective tissue fibres occurred with higher gestation. The tunica muscularis was rich in collagen and elastic fibres (Banks, 1986 and Hafez, 1987) as these fibres have a high tensile strength.

Tunica serosa was rich in loose connective tissue fibres and blood vessels. Vascularization increased with higher gestational ages.

5E. Discussion on attachment/implantation process, placentation, caruncular changes, appearance of binucleate cells and syncytial foramation :

Ovine embryonic attachments are reported to begin at about 17-20 days of post conception and have been reported to be completed by about 28-30th day of gestation (Roberts, 1971). In our study the actual attachment has been reported microscopically when examined it could detect villi like projection in the foetal membrane (Plate 81). The actual morphological union macroscopically could not be established before day 40th of gestation in the present study. As the above authors announcing or describing the attachment around day 17-20 in sheep did not specify whether they have observed it macroscopically or microscopically. The narration of the above authors pointed to microscopic observation which could not be corroborated in their studies. However, on the basis of microvilli projections between foetal and maternal tissues one could safely confirm the transitory attachment begins between 18-20 days and was visible macroscopically around day 40th of gestation. To that extend our observations have given a new concept of foeto-maternal attachment around day 40th. However, transitory microscopic connection has been established at 18th days of gestation. We have seen hatched elongated blastocyst with a red clot was nothing but embryo (recognized under microscope) at 15 days of gestation and initial development of the placenta. As the time advanced there were development of amniotic and allantoic cavity and was increasing accumulation of fluids. Gradually there were increasing vascular proliferation in the fetal membrane and whole fetal membrane ramified with blood vessels was also completely

filled with foetal fluids and head, eye bud and tail could be recognized grossly, this might be the beginning of foetal stage. At 39 days of gestation some single layered reddish granules were present in the fetal membrane were nothing but the cotyledons, appeared for the first time. Cotyledones were arranged in single layered which was reddish in colour and granular. Both this process i.e. formation of placenta and organogenesis of the fetal parts occurred within unattached (before morphological attachment) elongated blastocyst (Plate 3 to Plate 31). On the basis of our observation, one could safely deduce that the placentation begins with the thickening of the blood drop. The embryonic mass is enveloped within a small cavity which appeared like a round mass and gradually converted to a fluid filled cavity and ramification and networking of blood vessels occurred around the conceptus. The conceptus was fully formed within the elongated blastocyst. We have recorded sequential images of beginning of placentation and formation of cotyledons (Plate 3 to Plate 31). The whole process begins when the conceptus still wrapped up within the elongated blastocyst. The placental membranes differentiated within elongated blastocyst before physical or gross attachment. The vascularisation of conceptus begins during the earliest stage. Caruncles also undergone tremendous changes and the size were increasing as the gestation advanced (Schular, 2002). It consisted of angiogenic (increased blood supply); mucification (formation of a mucous coat) and finally solid projected caruncles were differentiated into cup shaped structures. The morphological differentiation of caruncles during different stages of pregnancy is shown (Plate 3 to Plate 65).

In brief, our studies have revealed that during non pregnant condition the caruncles were projected type (Plate 2). Changes in the caruncles started from the beginning of pregnancy and caruncles were swollen and rounded type during the early pregnancy that might be the early effect of progesterone, gradually developed depression at the centre and ultimately became cup shaped. Histologically, proliferated endometrial glands were observed in the intercaruncular region from 15 days of gestation onwards and from 18 days onwards glandular openings were present in the intercaruncular epithelial lining. The lining cells of the gland were simple cuboidal, indicated secreting something and might be histotrophe (Dellmann and Eurell, 1998). On this vary stage a specific phenomenon observed through histological observation were binucleate cells and

villi (papillae) like extension in the fetal membrane, indicated the development of transitory attachment by projecting villi (papillae) like extension to the opening of endometrial gland presented in the intercaruncular region.

Details changes of caruncles, intercaruncles, fetal membranes, conceptus both grossly and histologically described under result (4C and 4D), and the changes can be seen from Plate 3 to Plate 67 and Plate 76 to Plate 113. There are very little informations available on caruncular development, morphological status of conceptus in literature. However, our earlier observations have been confirmed by the details of present study (Wani, 2006).

In our preliminary studies we had not incorporated histological, endocrinological and other morphological details, which have been under taken in these studies. Although all the observations reported by the above authors have been partially confirmed, like nobody reported regarding the actual day of suncytial appearance in ovine and many others. The present study have come up with more elaborate evidences which clearly showed that the process of attachment starts almost during the 3rd week of pregnancy, where as the fully formed attachment consisting union of cotyledons and caruncles observed at 40th day of gestation.

In our observations we have found that the elongated blastocyst contains the fully differentiated conceptus, wrapped up in a finely coated membrane which gradually gave rise to amniotic sac. The only connection between the rest of the foetal membrane and growing conceptus was through a tiny blood vessel which eventually transformed into umbilical chord. This blood vessels coming out of the conceptus spread into a network throughout the blastocyst. Subsequently, with the development of the pregnancy the interviewing blood vessels meeting at various ends developed into a ball of blood mass. As per our observations each blood vessel terminated into a small blood drop. Thus, initially the cotyledonary mass looks like scattered red dots which eventually joint together and formed a loose mass of blood vessels. With compaction they became solid red round masses which were later on shaped themselves as cotyledons. These cotyledons during their formation may ramify and send projections across the blastocyst borders. As per our histological evidences we have noticed such projections even during early stages (From 18 days of gestation). Macroscopically, we were unable to find such projections or

indication of such attachment until day 40th of gestation. Morphologically, from 40 days we had evidences of caruncular-cotyledonary attachments which have been described under result 4C and can be seen in (Plate 33 to Plate 36). We claimed these results to be reported for the first time. Literature reviewed did not indicate any such reports till date. However, the reports of King *et al.* (1981), Wani (1996), Hafez (2000), Gray *et al.* (2002), Gordon (2004) and Wani *et al.* (2006) indicated certain biochemical markers which supported histological and morphological observations found in this study. The observations on the appearances of changes in epithelium, appearance of binucleate cells and the glandular development as observed in this study was in confirmatory with the findings of Bhavsar *et al.* (1974), King *et al.* (1981), Michel and Stief (1985), Skidmore *et al.* (1997), Hafez (2000), Paul *et al.* (2003) and Rodriguez *et al.* (2005). We do differ with the observations with some of the above authors; we have based their conclusion on scanty observation. Our attachment process as detailed above has been hinted by Roberts (1971) and Hafez (2000) in their observation, where in Roberts (1971) stated that ovine embryonic attachment begins at about 17 to 20 days and is completed by about 28th to 30th days of gestation and Hafez and Hafez (2000) stated that placental attachment in ruminants was characterized by the appearance of binucleate cells arising from uninucleate cells of the trophoblast, first appeared on day 17 and are present throughout the gestation. These cells migrated and fused with the underlying uterine surface epithelium to form multinucleate cells or syncytium and conceptus attachment in ruminant species involves both caruncular and inter-caruncular areas of the endometrium. A transitory attachment first occurred as the bovine and ovine conceptus developed finger like villi (Papillae) that projected into the lumen of the uterine glands. These papillae provided a temporary anchor and absorptive structure for the conceptus as more complete attachment progressed. Our studies were in confirmation of this earlier speculation. To that extend the evidences put forth in this study fortified the theoretical model proposed by Roberts (1971) and Hafez and Hafez (2000).

Placentation observed by earlier authors was described as superficial and non-invasive type, Gordon (2004). Our observation in this study did not fully support the views of the above author. He postulated that the attachment process in ruminant was superficial and non invasive, actually as per our observation the attachment was non

invasive at the early stage only but it was becoming strong from 48 days onwards and cotyledone completely invaded the caruncle from 80 days onwards and after 80 days of gestation it was very difficult to separate the foetal cotyledon and maternal caruncle, it needed strong pressure to separate and also sometime the foetal and maternal parts torned out. However, the presence of mononucleate cells and binucleate cells in the foetal membrane observed at 15th day and 18th day of gestation, gross/morphologic fusion of foeto-maternal cells observed at 40th days of gestation and formation of syncytium was observed in 42 days of gestation respectively in the present study. In present study binucleate cells were observed from 18 days of gestation but syncytial formation occurred only after gross or morphological attachment of foetal and maternal tissue. At the beginning the binucleate cells were within the foetal membrane but from 36 days onwards we have observed free binucleate cells in the foetal membrane that indicated the migration of binucleate cells to the maternal tissue and formed syncytium from 42 days and observed continuously as pregnancy progressed. Our studies were in complete agreement with Gordon (2004) so far as the release of binucleate cells through secretory granules is concerned. Gordon has postulated that the binucleate cells secrete pregnancy associated glycoproteins and facilitates the transfer of complex molecules across the placental barrier into the maternal circulation. He has also postulated that as attachment is initiated, the trophoblast binucleate cells migrate and fuse with maternal endometrial cells and formed syncytium. Hafez and Hafez (2000) postulated that placental attachment in ruminants is characterized by the appearance of binucleate cells arising from uninucleate cells of the trophoblast, first appears on day 17 and are present throughout the gestation. These cells migrate and fuse with the underlying uterine surface epithelium to form multinucleate cells or syncytium, may be involved in immunologic protection of the ceonceptus and the transfer of placental lactogen. The reports of Gordon (2004) and Hafez and Hafez (2000) in case of binucleate and syncytial formation were complete agreement with our study.

Chapter – 6

SUMMARY AND CONCLUSION

6. SUMMARY AND CONCLUSION

The whole study consisted of collecting eighty six samples of gravid and non-gravid uteri from the different local slaughter houses. The blood samples were also collected from each of the animals. In the present study the gravid uteri of sheep ranging from 14 to 140 days was divided into five groups e.g. Group A (GpA), Range: 14 – 57 days and Mean $\pm$ S.E : 41.40 ± 2.17 , Group B (GpB), Range: 58 -74 and Mean $\pm$ S.E : 67.87 ± 1.38 , Group C (GpC), Range: 75 – 93 and Mean $\pm$ S.E : 89.20 ± 2.33 , Group D (GpD), Range: 94 – 120 and Mean $\pm$ S.E : 110.60 ± 3.14 and Group E (GpE), Range:121 – 140 and Mean $\pm$ S.E : 130.0 ± 2.79 . Again, GpA is subdivided into five groups viz. Group A₁ (GpA₁), Range: 14 – 39 days and Mean $\pm$ S.E: 27.5 ± 2.70 , Group A₂ (GpA₂), Range: 40-44 days and Mean $\pm$ S.E: 42.16 ± 0.75 , Group A₃ (GpA₃), Range: 45-48 days and Mean $\pm$ S.E: 46.8 ± 0.58 , Group A₄ (GpA₄), Range: 49-52 days and Mean $\pm$ S.E: 51.4 ± 0.51 and Group A₅ (GpA₅), Range: 53-57 days and Mean $\pm$ S.E: 55.75 ± 0.63 . Foetal age determination in between 15 to 39 days was difficult; no formula could be used directly due to some drawback. Therefore, we have innovated a new graph/scale known as “**Khatun–Wani graph, 2007**”.

The blood samples were collected by jugular venupuncture, decanted serum and were analysed for various biochemical constituents, minerals, enzymes and hormones. The foetal fluids were also collected to estimate above all the constituents except, hormones to ascertain systemic changes with increasing gestational age. The tissue samples were collected from uterine horn kept in the sterile plastic vial containing phosphate buffer saline (PBS) and stored at deep freeze (-70°C) for estrogen and progesterone receptors estimation and remaining parts of the genital organ kept at 10 % formalin for histological examination.

Pregnancy is an intimate association between mother and foetus. Foetus is given a special status of “Homograph” in spite of its foreign or heterographic structure. This protective immunological cover to foetus is hidden in the formation and exact attachment

of the foeto-maternal placenta viz. cotyledons and caruncles union. This main objective was the preamble of this investigation, with following objectives:-

1. Comparisons of female genitalia, biometric changes during pregnancy and non pregnancy.
2. Correlate external and internal morphology of the uterine caruncles during pregnancy with non pregnancy.
3. The histological changes during the attachment of cotyledons with caruncles and their mode of union.
4. To study conceptus attachment and changes occurring in dams oestrogen and progesterone, estrogen and progesterone receptor and other biochemical constituents ranging from glucose, protein and enzymes to trace elements. The chronological observations concomitant with the above objectives can be briefly presented as :

At 15 days of gestation, caruncles were swollen and mucinous. Around day 18th, an elongated worm like blastocyst with small comma or curved embryonic mass was recorded. Around day 29th microscopically, somites, head, eyebud, tailbud and heart with emerging blood vessels were observed. Around day 36th of gestation elongated blastocyst with ramification and networking of the blood vessels was seen. At day 39th of pregnancy, cotyledons were seen as small reddish granules as if blood vessels ends joining together gave rise to these round red balls i.e. cotyledones. The first attachment or formation of placentome was observed around day 40th of the gestation. Since this time line an intimate association between foetal cotyledons and uterine caruncles gave rise to prominent placentomes, in uterine tissue which could easily seen grossly.

Various changes in the uterine caruncles and intercaruncular area observed have been described. Trophoblastic villi were observed around day 18th and projections or dentations in the caruncles were discerned around day 40th of gestation. Syncytial formation was observed around day 42nd of post conception. From day 80th onward complete intermixing of cotyledons and caruncles was observed.

The changes in allantoic and amniotic cavities along with other parts of foetal and maternal components have been recorded and analyzed. The data revealed significant

changes between pregnancy stages. The main characteristics of foetal fluids were analyzed. The pH of amniotic fluid and maternal serum was alkaline but in allantoic fluid became acidic towards term. The concentration of glucose in whole gestation period differed in GpA (4.33 ± 0.42), GpD (3.41 ± 0.21), GpE (2.92 ± 0.15) in amniotic fluid, In GpA (3.57 ± 0.34), GpE (2.3 ± 0.43) in allantoic fluid and GpA (56.85 ± 2.22), GpC (59.8 ± 0.88), GpD (63.42 ± 1.75), GpE (65.22 ± 1.90) in serum (Whole gestation period), again in case of early pregnancy differed in GpA₁ (6.87 ± 0.43), GpA₂ (3.52 ± 0.22), GpA₃ (3.82 ± 0.90), GpA₄ (3.11 ± 0.64), GpA₅ (3.09 ± 1.20) in amniotic fluid, GpA₁ (68.53 ± 2.46), GpA₂ (52.51 ± 2.66), GpA₃ (41.92 ± 0.91), GpA₄ (54.04 ± 2.15) in serum.

The concentrations of total protein in whole gestation period differed in GpA (0.40 ± 0.06) and GpE (0.84 ± 0.26) in amniotic fluid), in GpA (0.48 ± 0.06), GpD (0.93 ± 0.09), GpE (1.42 ± 0.20) in allantoic fluid, in GpA (6.94 ± 0.82), GpB (11.10 ± 0.62), GpC (13.0 ± 0.75), GpD (13.11 ± 0.97) and GpE (16.92 ± 0.69) in serum whereas, the similar parameter for the early pregnancy differed in GpA₁ (0.20 ± 0.03) and GpA₅ (0.60 ± 0.15) in amniotic fluid, GpA₁ (0.25 ± 0.11), GpA₃ (0.50 ± 0.10), GpA₄ (0.68 ± 0.12), GpA₅ (0.51 ± 0.12) in allantoic fluid; GpA₁ (2.96 ± 0.15), GpA₃ (10.24 ± 0.43), GpA₄ (11.85 ± 0.55), GpA₅ (12.51 ± 0.69) in serum.

The concentrations of cholesterol in whole gestation period differed in GpA (3.13 ± 0.19) and GpB (3.13 ± 0.08) in allantoic fluid, GpA (107.62 ± 3.44), GpB (82.0 ± 5.2), GpC (65.80 ± 2.40), GpD (63.80 ± 1.91), GpE (57.75 ± 1.31) in serum. Similar parameter for the early pregnancy differed in GpA₁ (3.96 ± 0.17) and GpA₃ (5.68 ± 0.37) in amniotic fluid, GpA₁ (2.45 ± 0.15), GpA₃ (5.06 ± 0.22), GpA₄ (3.18 ± 0.13), GpA₅ (2.98 ± 0.06) in allantoic fluid and GpA₁ (121.06 ± 8.41), GpA₂ (107.67 ± 1.58), GpA₃ (99.18 ± 3.18), GpA₄ (98.20 ± 3.02), GpA₅ (96.25 ± 5.48) in serum.

The concentrations of creatinine in whole gestation period differed in GpA (1.52 ± 0.26), GpB (5.34 ± 0.35), GpC (7.34 ± 1.19), GpD (9.30 ± 0.65) and GpE (13.67 ± 0.75) in amniotic fluid; GpA (2.21 ± 0.37), GpB (6.09 ± 0.43), GpC (11.36 ± 0.88), GpD (15.01 ± 0.10) and GpE (16.77 ± 0.31) in allantoic fluid and GpA (2.23 ± 0.36), GpB (6.25 ± 0.26), GpC (11.64 ± 1.26), GpD (16.54 ± 0.58) and GpE (23.88 ± 1.14) in serum. Similar parameter for the early pregnancy differed in GpA₁ (0.16 ± 0.04),

GpA₂ (0.96 ± 0.21), GpA₃ (1.61 ± 0.47), GpA₄ (2.28 ± 0.57) and GpA₅ (3.69 ± 0.11) in amniotic fluid, GpA₁ (0.33 ± 0.08), GpA₂ (1.01 ± 0.21), GpA₃ (2.12 ± 0.29), GpA₄ (3.85 ± 0.38) and GpA₅ (5.39 ± 0.23) in allantoic fluid and GpA₁ (0.54 ± 0.07), GpA₂ (0.86 ± 0.05), GpA₃ (2.51 ± 0.32), GpA₄ (4.08 ± 0.34) and GpA₅ (5.5 ± 0.22) in serum.

The concentrations of urea in whole gestation period differed in GpA (14.01 ± 0.72), GpB (20.16 ± 0.66), GpC (23.32 ± 1.20), GpD (34.30 ± 1.41) and GpE (38.10 ± 0.47) in amniotic fluid; GpA (16.60 ± 1.02), GpB (28.93 ± 1.26), GpC (37.63 ± 3.48), GpD (52.08 ± 2.96) and GpE (72.36 ± 1.08) in allantoic fluid and GpA (61.93 ± 2.46), GpB (88.19 ± 3.42), GpC (96.95 ± 0.39), GpD (107.70 ± 4.96) and GpE (114.71 ± 1.68) in serum. Similar parameter for the early pregnancy differed in GpA₁ (9.92 ± 0.84), GpA₂ (12.90 ± 0.35), GpA₃ (14.91 ± 0.86), GpA₄ (16.92 ± 0.74) and GpA₅ (18.56 ± 1.10) in amniotic fluid, GpA₁ (11.15 ± 0.91), GpA₂ (14.46 ± 0.58), GpA₃ (17.22 ± 0.47), GpA₄ (18.07 ± 0.82) and GpA₅ (26.22 ± 0.53) in allantoic fluid and GpA₁ (49.20 ± 3.00), GpA₂ (61.06 ± 0.10), GpA₃ (67.21 ± 2.44), GpA₄ (73.49 ± 1.39) and GpA₅ (78.00 ± 1.34) in serum.

The concentrations of urea nitrogen in whole gestation period differed in GpA (6.54 ± 0.34), GpB (9.41 ± 0.31), GpC (10.89 ± 0.56), GpD (16.02 ± 0.66) and GpE (17.79 ± 0.22) in amniotic fluid; GpA (7.75 ± 0.48), GpB (13.51 ± 0.59), GpC (17.57 ± 1.62), GpD (24.32 ± 1.38) and GpE (33.79 ± 0.50) in allantoic fluid and GpA (28.92 ± 1.15), GpB (41.19 ± 1.60), GpC (45.28 ± 0.18), GpD (50.30 ± 2.32) and GpE (53.57 ± 0.79) in serum. Similar parameter for the early pregnancy differed in GpA₁ (4.63 ± 0.39), GpA₂ (6.02 ± 0.16), GpA₃ (6.96 ± 0.40), GpA₄ (7.90 ± 0.35) and GpA₅ (8.67 ± 0.51) in amniotic fluid, GpA₁ (5.20 ± 0.43), GpA₂ (6.75 ± 0.27), GpA₃ (8.05 ± 0.22), GpA₄ (8.44 ± 0.39) and GpA₅ (12.24 ± 12.24) in allantoic fluid and GpA₁ (22.98 ± 1.40), GpA₂ (28.51 ± 0.46), GpA₃ (31.39 ± 1.14), GpA₄ (34.32 ± 0.65) and GpA₅ (36.43 ± 0.63) in serum.

The concentrations of sodium in whole gestation period differed in GpA (96.06 ± 3.14) and GpE (90.48 ± 0.86) in amniotic fluid; GpA (88.07 ± 1.19), GpC (78.8 ± 3.15), GpD (75.20 ± 1.46) and GpE (72.0 ± 1.58) in allantoic fluid and GpA (120.36 ± 3.36), GpD (136.97 ± 4.40) and GpE (138.70 ± 3.45) in serum. Similar parameter

for the early pregnancy differed in GpA₁ (94.50 ± 1.22), GpA₂ (88.50 ± 0.76), GpA₃ (87.20 ± 1.50), GpA₄ (81.60 ± 2.00) and GpA₅ (80.50 ± 2.87) in allantoic fluid, GpA₁ (113.79 ± 4.48), and GpA₅ (124.98 ± 1.81) in serum.

The concentrations of potassium in whole gestation period differed in GpA (5.23 ± 0.14), GpC (6.92 ± 0.70), GpD (8.13 ± 0.44) and GpE (7.69 ± 0.37) in amniotic fluid; GpA (8.97 ± 0.36), GpB (12.25 ± 0.31), GpC (14.39 ± 0.47), GpD (16.80 ± 0.73) and GpE (20.86 ± 1.33) in allantoic fluid and GpA (7.33 ± 0.14), GpB (6.77 ± 0.12), GpC (6.40 ± 0.11), GpD (6.22 ± 0.12) and GpE (6.18 ± 0.11) in serum. Similar parameter for the early pregnancy differed in GpA₁ (4.99 ± 0.22), and GpA₅ (5.38 ± 0.10) in amniotic fluid, GpA₁ (6.73 ± 0.26), GpA₂ (8.96 ± 0.24), GpA₃ (9.21 ± 0.39), GpA₄ (11.09 ± 0.22) and GpA₅ (11.60 ± 0.07) in allantoic fluid and GpA₁ (7.83 ± 0.15), GpA₄ (6.65 ± 0.16) and GpA₅ (6.11 ± 0.36) in serum.

The concentrations of copper in whole gestation period differed in GpA (2.54 ± 0.22), GpB (2.03 ± 0.03), GpC (2.05 ± 0.10), GpD (1.98 ± 0.01) and GpE (1.88 ± 0.05) in amniotic fluid; GpC (3.12 ± 0.46), GpD (2.20 ± 0.10) and GpE (2.31 ± 0.07) in allantoic fluid and GpA (3.18 ± 0.24), GpB (2.54 ± 0.24), GpC (2.34 ± 0.07), GpD (2.09 ± 0.09) and GpE (2.20 ± 0.08) in serum. Similar parameter for the early pregnancy differed in GpA₁ (2.74 ± 0.83), GpA₂ (3.20 ± 0.36) in amniotic fluid, GpA₁ (2.13 ± 0.16), GpA₂ (2.78 ± 0.38), GpA₃ (2.75 ± 0.32) and GpA₄ (2.45 ± 0.26) in allantoic fluid and GpA₁ (2.95 ± 0.48), GpA₃ (2.47 ± 0.14), GpA₄ (4.61 ± 0.60) and GpA₅ (2.95 ± 0.55) in serum.

The concentrations of zinc in whole gestation period differed in GpA (5.84 ± 0.39), GpD (8.35 ± 0.30) and GpE (8.18 ± 0.23) in amniotic fluid; GpA (5.83 ± 0.33), GpB (8.88 ± 0.94), GpD (10.90 ± 1.05) and GpE (12.13 ± 0.13) in allantoic fluid and GpA (6.42 ± 0.49) and GpE (4.93 ± 0.15) in serum. Similar parameter for the early pregnancy differed in GpA₂ (6.78 ± 1.00) and GpA₃ (4.70 ± 0.57) in amniotic fluid, GpA₁ (4.89 ± 0.63) and GpA₅ (8.10 ± 0.06) in allantoic fluid and GpA₁ (6.52 ± 1.16) and GpA₅ (4.10 ± 0.33) in serum.

The concentrations of manganese in whole gestation period differed in GpA (3.37 ± 0.15), GpB (2.75 ± 0.09), GpC (3.06 ± 0.04) and GpE (2.90 ± 0.07) in amniotic fluid; GpA (3.17 ± 0.10) and GpB (2.81 ± 0.10) in allantoic fluid and GpA ($3.58 \pm$

0.17), GpB (3.18 ± 0.06), GpC (3.20 ± 0.08), GpD (2.61 ± 0.16) and GpE (2.50 ± 0.03) in serum. Similar parameter for the early pregnancy differed in GpA₁ (4.19 ± 0.51), GpA₂ (3.15 ± 0.15), GpA₃ (3.26 ± 0.20), GpA₄ (3.05 ± 0.09) and GpA₅ (3.00 ± 0.00) in amniotic fluid, GpA₁ (3.47 ± 0.29) and GpA₅ (2.50 ± 0.14) in allantoic fluid and GpA₁ (4.34 ± 0.41), GpA₂ (3.34 ± 0.16), GpA₃ (3.45 ± 0.11), GpA₄ (3.19 ± 0.18) and GpA₅ (2.86 ± 0.21) in serum.

The concentrations of iron in whole gestation period differed in GpA (8.61 ± 0.34), GpB (9.50 ± 0.37), GpC (9.43 ± 0.27), GpD (10.17 ± 0.48) and GpE (10.16 ± 0.44) in amniotic fluid; GpA (5.82 ± 0.15), GpB (6.89 ± 0.28), GpC (7.76 ± 0.23), GpD (8.22 ± 0.21) and GpE (9.43 ± 0.54) in allantoic fluid and GpA (13.05 ± 0.28), GpB (12.43 ± 0.35), GpC (12.34 ± 0.39), GpD (10.00 ± 0.45) and GpE (8.62 ± 0.49) in serum. Similar parameter for the early pregnancy differed in GpA₁ (6.68 ± 0.11), GpA₂ (8.00 ± 0.52), GpA₃ (9.66 ± 0.42), GpA₄ (10.31 ± 0.08) and GpA₅ (10.94 ± 0.71) in amniotic fluid, GpA₁ (5.31 ± 0.25), GpA₂ (5.58 ± 0.36), GpA₃ (6.04 ± 0.12), GpA₄ (6.18 ± 0.19) and GpA₅ (6.73 ± 0.28) in allantoic fluid and GpA₁ (14.37 ± 0.53), GpA₂ (12.50 ± 0.56), GpA₃ (12.58 ± 0.24), GpA₄ (12.40 ± 0.40) and GpA₅ (12.00 ± 0.41) in serum.

The concentrations of Glutamate Oxalo Transaminase (GOT) in whole gestation period differed in GpA (15.26 ± 0.45), GpB (13.18 ± 1.07) and GpC (13.40 ± 0.40) in amniotic fluid; GpA (13.48 ± 0.42) and GpC (12.43 ± 0.50) in allantoic fluid and GpA (104.10 ± 4.03), GpB (94.38 ± 3.05), GpC (86.60 ± 3.79), GpD (83.60 ± 3.83) and GpE (78.50 ± 1.32) in serum. Similar parameter for the early pregnancy differed in GpA₁ (122.90 ± 8.76), GpA₂ (88.83 ± 2.39), GpA₃ (92.80 ± 4.10), GpA₄ (106.00 ± 4.74) and GpA₅ (91.75 ± 2.63) in serum.

The concentrations of Glutamate Pyruvate Transaminase (GPT) in whole gestation period differed in GpA (7.56 ± 0.24), GpB (6.36 ± 0.39) and GpE (6.83 ± 0.33) in amniotic fluid; GpA (6.34 ± 0.24) and GpB (5.49 ± 0.37) in allantoic fluid and GpA (34.63 ± 1.19), GpB (29.75 ± 0.97), GpC (28.20 ± 0.80), GpD (26.00 ± 1.30) and GpE (24.00 ± 2.04) in serum. Similar parameter for the early pregnancy differed in GpA₁ (7.45 ± 0.24), GpA₂ (6.38 ± 0.41) and GpA₃ (8.56 ± 0.70) in amniotic fluid, GpA₁ (6.65 ± 0.24), GpA₂ (5.57 ± 0.30) and GpA₅ (5.28 ± 0.45) in

allantoic fluid and GpA₁ (37.00 ± 3.19), GpA₂ (31.50 ± 1.78) and GpA₃ (32.20 ± 0.66) in serum.

The concentrations of alkaline phosphatase in whole gestation period differed in GpA (30.73 ± 1.51), GpB (35.75 ± 1.63), GpC (40.20 ± 1.50), GpD (41.20 ± 1.59) and GpE (43.75 ± 1.31) in amniotic fluid; GpA (27.33 ± 1.42), GpB (31.88 ± 1.44), GpC (33.60 ± 1.60), GpD (32.40 ± 2.40) and GpE (36.50 ± 1.26) in allantoic fluid and GpA (60.70 ± 3.43), GpB (71.88 ± 2.96), GpC (76.80 ± 4.99), GpD (78.80 ± 2.75) and GpE (92.50 ± 4.99) in serum. Similar parameter for the early pregnancy differed in GpA₁ (20.90 ± 1.85), GpA₂ (33.50 ± 1.15), GpA₃ (35.40 ± 1.72), GpA₄ (37.60 ± 0.51) and GpA₅ (36.75 ± 2.36) in amniotic fluid, GpA₁ (19.10 ± 1.86), GpA₂ (28.67 ± 1.69), GpA₃ (32.00 ± 2.61), GpA₄ (34.00 ± 2.10) and GpA₅ (31.75 ± 1.55) in allantoic fluid and GpA₁ (37.50 ± 2.62), GpA₂ (66.67 ± 2.40), GpA₃ (70.80 ± 3.65), GpA₄ (81.60 ± 2.46) and GpA₅ (71.00 ± 5.26) in serum.

The concentrations of progesterone and estrogen in whole gestation period differed in GpA (5.32 ± 0.33), GpB (8.26 ± 0.11), GpC (7.70 ± 0.23), GpD (7.08 ± 0.35), GpE (7.00 ± 0.32) and in GpA (138.50 ± 11.42), GpB (250.00 ± 0.00), GpC (230.00 ± 12.25), GpD (210.00 ± 18.71), GpE (210.00 ± 10.00) respectively in serum.

The concentrations of progesterone and estrogen receptors in whole gestation period differed in GpA (3.81 ± 0.04), GpB (4.33 ± 0.06), GpC (4.38 ± 0.04), GpD (4.09 ± 0.09) and in GpA (0.37 ± 0.01), GpB (0.41 ± 0.01), GpC (0.41 ± 0.01) respectively in uterine tissue.

The concentrations of progesterone and estrogen during early pregnancy differed in GpA₁ (3.26 ± 0.32), GpA₂ (5.19 ± 0.08), GpA₃ (6.00 ± 0.14), GpA₄ (7.00 ± 0.21), GpA₅ (7.89 ± 0.08) and in GpA₁ (74.00 ± 8.59), GpA₂ (113.33 ± 6.67), GpA₃ (160.00 ± 8.37), GpA₄ (198.00 ± 5.00), GpA₅ (236.25 ± 2.39) respectively in serum.

The concentrations of progesterone and estrogen receptors during early pregnancy differed in GpA₁ (3.74 ± 0.05), GpA₃ (3.82 ± 0.04), GpA₄ (3.98 ± 0.02), GpA₅ (4.06 ± 0.02) and in GpA₂ (0.36 ± 0.01), GpA₄ (0.39 ± 0.01), GpA₅ (0.37 ± 0.01) respectively in uterine tissue.

Status of progesterone (5.19 ± 0.08) and estrogen (113.33 ± 6.67) in dam serum and progesterone receptor (3.62 ± 0.09) and estrogen receptor (0.36 ± 0.01) in uterine tissue were there during attachment.

On the basis of above results we deduce that the caruncular-cotyledonary attachment occurred at 40 days of gestation but microscopically transitory attachment occurred at 18 days of gestation by the trophoblastic villi. The morphological changes leading to intimate association viz. formation of cotyledons, changes in caruncles, microvilli projections and final union as placentomes is completed with 18-40th day of post conception. As a result of our above study we dare to claim first inventions as follows:

1. Preparation of a graph for predicting the age of conceptus on the basis of whole length, crown-anus length which we name as **“Khatun-Wani graph, 2007”**.
2. We describe the possible mechanism of formation and union of cotyledons and caruncles and fostering a new idea that elongated blastocyst upto day 36th could recovered from uterus and cultured invitro, thereby preparing the first step for invitro foetus investigation in sheep.

Chapter – 7

LITERATURE

CITED

7. LITERATURE CITED

- Alexander, D. P., Nixon, D. A., Widdas, W. F. and Wohlzogen, F. X. 1955. Gestational variation in the composition of foetal fluids and foetal urine in sheep. *Journal of Physiology*, **140**: 1.
- Alexander, D. P., Nixon, D. A., Widdas, W. F. and Wohlzogen, F. X. 1958. Renal function in sheep foetus. *Journal of Physiology*, **140**: 14-22.
- Aminoor-Rahman, A. N. M., Snibson, K. J., Lee-Chee-Seong and Meeusen, E. N. T. 2004. Effects of implantation and early pregnancy on the expression of cytokines and vascular surface molecules in the Sheep endometrium. *Journal of Reproductive Immunology*, **64** (1/2): 45-58.
- Amle, M. B., Chinckar, S. R., Hukari, V. B. and Deopurkar, V. L. 1992. Studies on Foetal Fluids of Buffaloes. *Indian Journal of Animal Reproduction*, **13** (2): 165- 167.
- Antunovic, Z., Speranda and M., Steiner, Z. 2004. The influence of age and reproductive status on the blood indicators of ewes. *Archiv-fur-Tierzucht*, **47**(3): 265-273.
- Arthur, G. H. (1956). **In:** Vety. Reproduction and Obstetrics, 5th edition, p. 43.
- Arthur, G.H. (1957). Some notes on quantities of foetal fluids in ruminants. *British Vety. Journal*, **113**: 17.
- Bacha J. William, and Jr.Linda M. Wood, 1990. **In:** Colour Atlas of Vety. Histology. Lea & Febiger, Philadelphia, London. pp 207.
- Baetz, A. L., Hubbert, W. T. and Graham, C. K. 1976. Changes of biochemical constituents in bovine foetal fluids with gestational age. *American Journal of Veterinary Research*, **37**(9): 1047- 1052.
- Banks, W.J. 1986. Applied Veterinary Histology, Hoesy Year book, Philadelphia, p. 503.
- Bazer, F. W. and Roberts, R. M. 1983. Biochemical aspects of conceptus – endometrial interections. *J. Exp. Zool.*, **228**: 373.

- Bazer, F.W. and Sharp, D.C. 1991. **In:** Reproduction in farm animals by Hafez, 6th edition, p. 83.
- Bhavsar, B. K., Pattabiraman, S. R. and Venkataswami, V. 1974. Endometrial changes during early pregnancy of *Bubalus bubalis*. *Indian veterinary journal*, **51**(3): 175-176.
- Bongso, T. A., Edirisinghe, R. and Athuraliya, D. 1979. Studies on foetal fluids in goat. *Indian veterinary journal*, **56**(7): 562- 569.
- Bongso, T.A and Basrur, P.K.1976. Foetal fluids in cattle. *Canadian Journal*, **17**(2): 38-41.
- Cengiz, F., Yaman, K. and Galip, N. 2000. Studies on progesterone, estrogen and calcium values in cows before and after calving. *Veteriner-Fakultesi-Dergisi-Uludag-Universitesi*, **19**(3): 37-43.
- Chauhan, R. A. S. and Tiwari, S. 1996. Variation in the transaminase and alkaline phosphatase activity of caprine foetal fluids with gestational age. *Indian Veterinary Journal*, **73**(10): 1045- 1047.
- Chauhan, R.A.S., Tiwari, S. and Datta, I. C. 1992. Changes in the volume and biochemical constituents of caprine foetal fluids with gestational age. *Indian Journal of Animal Reproduction*, **13**(1): 72-74.
- Cloete, J. H. L. 1939. Onderstepoort. *J.Vet.Sci.Anim.Indust.*, **13**: 418.
- Dellmann, H. Dieter and Eurell Joann. 1998. **In:** Textbook of Veterinary Histology, Fifth Edition, Lippincott Williams & Wilkins
- Dey, S. K. 2004. Focus on implantation reproduction, **128**: 655-656.
- Dhami, A. J., Patel, P. M., Lakum, P. D., Ramani, V. P. and Pande, M. B. 2003. Micro-nutrient profile of blood plasma in relation to age reproductive status of Holstein Friesian cattle. *Indian-Journal-of-Animal-Nutrition*, **20**(2): 206-211.
- Dukes, H. H. 1955. Protein Metabolism. *Physiology of Domestic Animals*. 7th Edition, pp 562.
- Eulenberger, K. H., Neubauer, K. and Elze, K. 1977. Histological studies of the endometrium and chorioepithelium of sows during early pregnancy. *Monatshefte- fur-Veterinarmedizin*, **32**(9):333-336.

- Fakowska - Podstawka, M., Wrona, Z. and Krakowski, L. 2002. Biological role of interferontau. *Medycyna-Weterynaryjna*, **58**(8): 568-571.
- Garland, H. O. 1983. Cited in "The effects of pregnancy and sodium intake on water and electrolyte balance in sheep. *British Veterinary Journal*, **144**: 147.
- Goldstein, M. H., Bazer, F. W. and Barron, D. H. 1980. Characterisation of changes in volume, osmolarity and electrolyte composition of porcine foetal fluids during gestation. *Biology of Reproduction*, **22**(5): 1168 – 1180.
- Gordon Ian, 2004. **In**: Reproductive technologies in farm animals. CABI publishing.
- Gray, G. A., Barghardt, R. C., Johnson, G. A., Bazer, F. W. and Spencer, T. E. 2002. Evidence that absence of endometrial gland secretions in uterine gland knockout ewes compromises conceptus survival and elongation. *Reproduction*, **124**: 289-300.
- Green, W.W. and Winters, L. M. 1945. Tech. Bull. Minn. Agric. Exp., Ta No. 169.
- Guillomot, M., Flechon, J. E. and Wintenberger Torres, S. 1982. Cytochemical studies of uterine and trophoblastic surface coats during blastocyst attachment in the ewe. *Journal of Reproduction and Fertility*. **65**(1): 1-8.
- Hafez, E. S. E. 1987. **In**: Reproduction in farm animals, 5th edition, Lea & Febiger, Verghese Company
- Hafez, E. S. E. and Hafez, B. 2000. Reproduction in farm Animals. Seventh Edition, Lippincott Williams & Wilkins, A Wolters Kluwer Company, pp. 126-139.
- Hafez, E. S. E. and Jainudeen, M. R. 1993. Gestation, Prenatal physiology and parturition. **In**: Reproduction in farm animals. 6th edition, pp. 225-226.
- Hafez, E. S. E. and Kamal, M. A. M. 1955. Physiochemical investigation on placental fluids of buffaloes. *Indian Journal of Vety. Science*, **25**(3): 39-45.
- Hafez, E. S. E., Magharabi, M. M. and Anwar, A. 1958. Some factors effecting blood cholesterol level in buffaloes. *Indian Journal of Vety. Science*, **28**(3): 133-138.
- Henricks, D. M and Harris, R. B. Jr. 1978. Cytoplasmic estrogen receptors and estrogen concentration in bovine uterine endometrium. *Endocrinology*, **103**(1):176-185.
- Hewitt, S. C. and Korach, K. S. 2000. Progesterone action and responses in the alpha ERKO mouse. *Steroids*, **65**(10/ 11): 551- 557.

- Hewitt, S. C., Goulding, E. H., Eddy, E. M. and Korach, K. S. 2002. Studies using the estrogen receptor alpha knockout uterus demonstrate that implantation but not decidualisation associated signaling is estrogen dependent. *Biology of reproduction*, **67**(4): 1268-1277.
- Huct-Hudson, M. Y., Andres, G. K. and Dey, S. K. 1989. Cell type specific localization of C-MYC-Protein in mouse uterus-modupition by steroid hormones and analysis of the pre implantation period. *Endocrionology*, **125**: 1683-1690.
- Hugget, A. St. G. and Widdas, W. F. 1951. The relationship between mammalian foetal weight and conception age. *Journal of physiology*, **114**: 306- 317.
- Hugh Y. Yee, Edward S. Jenest, and Fred R. Bowels, 1971. Modified manual or automated o- toluidin system for determining glucose in serum, with an improved aquous reagent. *Clin chem.*, **17**(2):103-107.
- Ismail, Z. B., Qureshi, T., Levy, M., Khamas, W. and Nour, A. 2003. Preliminary report on some physiological parameters in pregnant female camels (*Camelus dromedarius*) in North America. *Journal-of-Camel-Practice-and-Research*, **10**(2): 125-129.
- Joshi, C. L., Nanda, B. S. and Saigal, R. P. 1976. Histomorphological and histochemical studies on the female genitalia of ageing goat, glands in cervix uteri. *Anatomischer-Anzeiger*, **139**(1/2): 193-199.
- Joubert, D. M. 1956. A study of prenatal growth and development of components of caprine conceptus. *Journal of Agricultural Science*, **47**: 382- 428.
- Kancko, J. J. and Corneleous, C. E. 1970. Clinical biochemistry of domestic animals. Volume I, 2nd edition, pp. 313-394.
- Khatri Pershotam, Hamayun Khan and Rind, M. M. 2004. Biometrical studies of reproductive organs of non pregnant buffaloes slaughtered in Hyderabad. *Journal of Animal and Veterinary Advances*, **3**(8): 500-502.
- Kind, P. R. .M. and Kings, E. J. 1954. *J. Clin. Pathol.*, **7**: 322.
- King, G. J., Atkinson, B. A and Robertson, H. A. 1981. Development of the intercaruncular areas during early gestation and establishment of the placenta. *Journal of reproduction and fertility*, **61**(2): 469-474.

- Kleflin, A., Zobundzija, M., Martincic, T. and Sladic, Z. 1988. Concentration changes of potassium and sodium ions in the pigs foetal fluids during the first half of gravidity. *Veterinarski – Archiv*, **58** (5): 225- 232.
- Kolmer, J. A., Spanbling, E. H. and Robinson, H. W. 1951. Approved Laboratory Techniques. Appleton Century Crofts, New york.
- Kurita, T., Lee - Kijun, Saunders, P. T. K., Cooke, P. S., Taylor, J. A., Dahiya, R. and Cunha, G. R. 2001. Regulation of progesterone receptors and decidualisation in uterine stroma of the estrogen receptor- alpha knockout mouse. *Biology of reproduction*, **64**(1): 272-283.
- Lathura, R. A., Gupta, R. C., Khar, S. K. and Baru, P. 1987. Variation in metabolic constituents of caprine foetal fluids during different stages of gestation. *Indian Journal of Animal Reproduction*, **8**(2): 133-137.
- Lee, K. Y. and De Mayo, F. J. 2004. Animal models of implantation. *Reproduction*. **128**: 679-695.
- Luna, L. G. 1968. Manual of Histological Staining methods of the Armed Forces Institute of pathology, 3rd Edn. The Blakistan Division, McGraw-Hill Book company, New Work
- Lupke, H., Mulling, M. and Sohn, E. 1967. Maternal foetal relationship in cattle, content to total nitrogen, urea, uric acid and creatinine in foetal fluids. *Vety. Bulletin*, **38**: 2047.
- Malan, A. P. and Curson, H. H. 1937. Onderstepoort. *J. Vet. Sci. Anim. Indust.*, **8**: 417.
- Manish - Mahawar, Jain, A. K. and Puneet – Kumar, 2004. Clinically important enzymes in Jamunapari and Marwari goats. *Indian-Journal-of-Animal-Sciences*, **74**(9): 924-925.
- McCrabb, G. J., Egan, A. R. and Hosking, B. J. 1991. Maternal under nutrition during mid pregnancy in sheep. *British Journal of Nutrition*, **65**(2): 157-168.
- McDonald, L.E. 1980. *Veterinary endocrinology and reproduction*, 3rd edition, p. 61, pp. 388-389.
- Mellor, D. J. and Slatter, J. S. 1971. Daily changes in foetal fluids during last three months of pregnancy in ewes. *Journal of Physiology*, **217**: 573-604.

- Mellor, D. J. and Slatter, J. S. 1972. Daily changes in foetal urine and relationship with foetal fluids and maternal plasma during last three months of pregnancy in ewes. *Journal of Physiology*, **227**: 503-525.
- Michel, G. and Stief, E. 1985. Placentation and foetal development in sheep. *Monatshefte-fur-veterinarmedizin*, **40**(10): 344- 347.
- Mufti, A. M. 1995. Thesis on Morphological characters and some metabolic constituents at different stages of development of ovine foetus. SKUAST-K, Shalimar.
- Mufti, A. M., Wani, G. M., Wani, N. A., Buchoo, B. A. and Khan, M. Z. 2000. Prenatal development of ovine fetus. *Small-Ruminant-Research*, **38**(1): 87-89.
- Natelson, Scatt, M. L. and Beffa, C. 1951. *Americ. J. Chem, Path.* **21**: 275.
- Noden M. Drew and Lahunta De Alexander, 1985. **In**: Embryology of Domestic Animals. Williams & Wilkins.
- Owen, J. A., Iggo, B., Scand rett F. J., Stewart, C. P. 1954. *Biochem. J*, **58**: 426.
- Pareek, P. K., Joshi, C. K. and Vyas, K. K. 1981. Note on the biometry of genital organs of female camel. *Indian Journal of Animal Sciences*. **51**(10): 1010.
- Paul, S., Ghosh, R. K., Das, P., Mahata, T. K. and Thakur, A. M. 2003. Studies on gross anatomy and histology of the non pregnant and pregnant (upto 90 days) uterus in the non descript cow. *Indian journal of animal health*, **42**(2): 178-181.
- Pourouchottamane, R., Chatterjee, A., Sheikh, I. U., Sarkar, M. and Gogoi, D. 2005. Blood biochemical constituents of female yaks in different physiological status. *Indian-Veterinary-Journal*, **82**(10): 1108-1109.
- Raj Sundrum, R. C., Kathireson, D. and Pattabiraman, S. R. 1990. Changes in biochemical constituents of foetal fluids with increase in gestation period in buffaloes. *Indian Journal of Animal Reproduction*, **11**(1): 24-28.
- Ramadhar Singh, 1988. Ageing and age. Essential of Animal Production and Management. First Edition. Kalyani publisher, pp. 210- 227.

- Ramana Rao, K.V. 2006. **In:** General studies for Agricultural Research services and other Competitive Examinations. 4th Revised and Enlarged Edition, Jain Brothers, New Delhi, pp. 35-36.
- Reinhold, J.G. 1953. **In:** standard methods of clinical chemistry (Reiner M.ed), New York and London, Academic press, **1**: 88.
- Reitman, S. and Frankel, S. 1957. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. *Am. J. Clin.Pathol.*, **28**: 56- 63.
- Richardson, C. 1980. **In:** Vety. Reproduction and Obstetrics (Editor, Noakes David E, Parkinson Timothy J, England Gary C W) Eighth Edition, p. 68.
- Roberts J. Stephen, 1971. Veterinary Obstetrics and Genital Diseases, Second Edition (Indian Edition), CBS Publishers and Distributors, pp. 36-47.
- Rodrigues, P. A., Vicenta, W. R. R., Cipolla-Neto, J. and Oliveira, C. A. 1998. Evaluation of the progesterone hormone profile in ewes at early pregnancy, pre-term and early lactation. *Arquivo-Brasileiro-de-Medicina-Veterinaria-e-Zootecnia*, **50**(2): 133-139.
- Rodriguez-Marquez, J. M., Hernandez-Vasquez, A., Hindalgo-Delgado, G. 2005. Ovine endometrial lining modification during the implantation in natural and induced multiple pregnancies. *Revista-cientifica, Facultad –de- Ciencias-Veterinarians, Universidad-del-Zulia*, **15**(4): 326-333.
- Roth, T. L., Bateman, H. L., Kroll, J. L., Steinetz, B. G. and Reinhart, P. R. 2004. Endocrine and ultrasonographic characterization of a successful pregnancy in a sumatran rhinoceros (*Dicerorhinus sumatrensis*) supplemented with a synthetic progestin. *Zoo- Biology*, **23**(3): 219-238.
- Rowe, A. J., Wulff, C. and Fraser, H. M. 2003. Localization of MRNA for vascular endothelial growth factor (VEGF), angiopoitins and their receptors during peri-implantation period and early pregnancy in marmosets (*Callitrix jacchus*). *Reproduction*, **126**: 227-238.
- Sastry, N. S. R., Thomas, C. K. and Singh, R. A. 1982. Dentition and aging of animals. Farm Management and Poultry Production. Second revised Edition. Vikas Publishing House Pvt. Ltd., pp. 43-50.

- Schular, 2002. **In:** Reproductive Technology in Farm Animals. CABI publishing, pp. 237- 254.
- Shalini and Sharma, D. N. 2004. Biometrical study of internal genital organs of gaddi goats. *Indian Journal of Animal Reproduction*, **25**(2): 148-150.
- Shameha-Intan, A. R., Zuki, A. B., Nornajiban, K. and Zamri-Saad, M. 2005. Microscopic evaluation of uterine mucosa in pregnant and non pregnant rats. *Journal of Animal and veterinary advances*, **4**(6): 590- 594.
- Singh Yashwant, Sharma, D. N. and Dhingral, L. D. 1979. Morphogenesis of the testis in goat. *Indian J. Anim. Sci.*, **11**: 925-931.
- Singh, S. K, Bhattacharya, A. R and Luktuke, S. N. 1974. Studies on biometry of genital organs of female goat. *Indian Veterinary Journal*, **51**(2): 81- 85.
- Skidmore, J. A., Wooding, F. B. P. and Allen, W. R. 1997. Placentation during the first 60 days of gestation in the dromedary camel (*camelus dromedarius*). *Journal of camel practice and research*, **4**(2): 199-202.
- Snedecor, G. W. and Cochran, W. G. 1980. Statistical Methods. 6th Indian Edition. Oxford and IBH Publishing Company, New Delhi
- Soliman, M. K. 1975. Studies on physiological chemistry of allantoic and amniotic fluids of buffaloes at various stages of gestation. *Indian Vety. Journal*, **52**: 106-112.
- Stainer, M. W. 1965. Transfer of radioactive water, urea and glycine between maternal and foetal body fluids in rabbit. *Journal of Physiology*, **178**: 127-149.
- Surgeev, L., Kleeman and Walker, 1990. Prediction of ovine foetal age using real time ultrasound imaging. Australian Society, *Animal production*, **18**: 547.
- Thailender, G., Eriksson, H., Edquist, L. E., Rodriguez-Martinez, H. 1990. Variations in oestradiol and progesterone receptor levels in the porcine myometrium and cervix during pregnancy and parturition. *Journal of veterinary medicine, Series- A*, **37**(5): 329 -338.
- Thomas E. Spencer and Fuller W. Bazer, 1995. Temporal and spatial alterations in uterine estrogen receptor and progesterone receptor gene expression during

- the estrous cycle and early pregnancy in ewe. *Biology of reproduction*. **53**: 1527-1543.
- Wales, R. G. and Murdoch, R. N. 1973. Changes in the composition of sheep foetal fluids during early pregnancy. *Journal of Reproduction and Fertility*. **33**(2): 197- 205.
- Wallace, L. R. 1948. *Journal of Agricultural Science*, **38**: 93, 243.
- Wani, G. M and Buchoo, B. A. 1990. Laparoscopic view of ovine conceptus. *Indian Journal of Animal Science*, **60**(5): 564 – 568.
- Wani, G. M. 1995. Early diagnosis of pregnancy in surrogate mothers (Sheep and Goat). **In** National Symposium on Embryo Transfer and Genetic Engineering Technologies in Small Ruminants, May 7-8, CIRG, Makhdoom, Farah, India.
- Wani, G. M. 1996. Embryo Biotechnology in Sheep and Goats. Publisher Valley Book House, University Road, Hazratbal, Srinagar, Kashmir.
- Wani, G. M. 2000. Effect of oocyte harvesting techniques on invitro maturation and invitro fertilization in sheep. *Small Ruminant Research*, **36**(1): 63-67.
- Wani, G. M. 2002. Multipurpose Management/ Goat Husbandry. Encyclopedia, Dairy sciences, Editor Roginski, H, Academic Press, An imprint of Elsevier Science, London, Boston, Amsterdam, New York, Oxford paris, San Diego, San Francisco, Singapore, Sydney, Tokyo, **II**: 1259-1270.
- Wani, G. M. 2003. Biotechnological advances in goats production. **In** *National Work-Shope on “Recent Advances in Goat Rearing”*. MPKKV, Agric.Univ. Rahuri, 29-30 December.
- Wani, G. M. 2005. Advances in ovine and Caprine Reproduction, www.word-mart.com. Research Paper, **9**: 1-25.
- Wani, G. M. 2006. Biotechnological Advances in Goat Production. www.word-mart.com. Research paper, p. 10.
- Wani, G. M., Khatun, A., Wani, G., Goswami, P. and Khan, M.Z. 2006. Preimplatation endometrial and foetal structural changes in sheep. Paper presented, **In** *International Symposium on “Emerging Trends in Genomic and proteomic sciences”* Mumbai, India, 15-18 october.

- Wani, G.M.1984. Investigation on ovarian activity, ovulation and pregnancy diagnosis in sheep and goats. Ph.D.Dissertation. Rohilakhand University, Bareilly, Izatnagar.
- Xu-HuiZhong, Liu-Dan, Zhong-YuanQiang and Yang-FengXing, 2004. Mineral element contents in the blood of cattle and their variations. *Journal-of-Southwest-Agricultural-University*, **26**(3): 236-239.
- Zietzschmann, O., Krolling, Oh. and Vitmus, 1955. **In:** Vety. Obstetrics and Genital Diseases (S.J. Roberts eds), 2nd Indian Edition, p. 31.
- Zlatkis, A., Zak, B. and Boyle, G. J. 1953. *J. lab. Clin. Med.*, **41**: 486.
- Zogno, M. A., Miglino, M. A. and Oliveira, M. F. 2004. Biochemical analysis of fetal fluids and cytology of the amniotic fluid of female moco (*Kerodon upestris*). *Brazilian-Journal-of-Veterinary-Research-and-Animal-Science*, **41**(4): 228-235.

Sher - e – Kashmir
University of Agricultural Sciences and Technology of Kashmir
Division of Animal Reproduction, Gynaecology and Obstetrics,
Shuhama, Alusteng, Srinagar-190006

Certificate

This is to certify that all the corrections and modifications suggested by the external examiner in the thesis manuscript of **Dr. Arjuma Khatun (Regd. No. 2005-V-59-M)** entitled, **“Investigation on Implantation/Attachment Process in Small Ruminants”** have been taken care of before final binding of the same.

(Prof. G. M. Wani)
Major Advisor