SERUM AND LUTEAL PROGESTERONE PROFILE AND HISTOMORPHOLOGY OF CORPUS LUTEUM DURING PREGNANCY IN BUFFALO

BY

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A1

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ABSTRACT

The present study on Nili-Ravi buffalo indicates that 58.11% of the foetuses were in right and 41.89% in the left uterine horn. The frequency of male and female foetuses observed in Nili-Ravi buffalo was 54.2% and 45.8% respectively. No case of twinning or intrauterine migration of ova was observed during this collection.

The mean length (2.78 ± 0.04 cm; $P<0.01$), width (2.16± 0.02 cm; $P<0.001$) and weight (5.40±0.10g; $P<0.001$) of the luteal ovary during 31st to 315th day of pregnancy were significantly higher than that of the non-luteal ovary (Table 1). The mean length (1.72 ± 0.02 cm), width (1.75 ± 0.02 cm), thickness (1.32 ± 0.01 cm), weight, (2.15 ± 0.04 g) and volume (2.18 ± 0.04 cm^3) of corpus luteum were observed during 31st to 315th day of pregnancy. Significant decrease in thickness ($P<0.05$), weight ($P<0.001$) and volume ($P<0.01$) of corpus luteum was

observed in gestation group II (8th to 10.5th month) as compared to group I (1.5th to 7th month), however, the length ($P>0.05$) and width ($P>0.05$) did not differ significantly in the two gestation groups.

Mean serum progesterone concentration observed during 1.5th to 9.5th month of pregnancy was 2.7 ± 0.15 ng/ml. Mean serum progesterone concentration in gestation group I (3.11 ± 0.16 ng/ml) was significantly greater ($P<0.001$) than in group II (1.76 ± 0.16 ng/ml), (Table 6).

Mean luteal progesterone concentration during 1.5th to 10.5th month of pregnancy was 27.36 ± 1.52 μg/g. Mean luteal progesterone concentration in gestation group I (31.99 ± 1.88 μg/g) was significantly higher ($P<0.001$) than in group II (22.00 ± 1.82 μg/g), (Table 7).

Mean serum cholesterol concentration reported from 1.5th to 9.5th month of pregnancy was 54.67±1.67mg/100ml. Mean serum cholesterol concentration in gestation group I (58.68 ± 1.94 mg/100 ml) was significantly higher (P<0.001) than in group II (47.77 ± 1.90 mg/100 ml), (Table 9).

Mean luteal cholesterol concentration obtained during the period of pregnancy (1.5th to 10.5th month) was 10.74 ± 0.31 mg/g. Mean luteal cholesterol concentration in gestation group I and group II is 10.73 ± 2.08 mg/g and 10.80 ± 0.44 mg/g respectively. Mean luteal cholesterol concentration observed in gestation group II is non-significantly greater (P>0.9) than in group I (Table 10).

Histomorphological study of pregnant Nili-Ravi buffalo corpus luteum shows morphologically two different types of luteal cells. Large luteal cells appear mostly spherical in shape (Fig.6a & 5b). These range from

23.12 μm to 33.88 μm in diameter and have moderate (Fig.6b) to highly convoluted cell membrane (Fig.5c). Large luteal cells have spherical, darkly stained nuclei with distinct nuclear membrane (Fig.9a & 5c). These range from 5.99 μm to 9.24 μm in diameter during 1.5th to 10th month of pregnancy. Cytoplasm of the large luteal cells is brightly stained and contains conspicuous membrane-bound secretory granules (Fig.8c) and vacuoles(Fig.5c).

Small luteal cells observed in Nili-Ravi buffalo corpus luteum are spindle-shaped and range from 10.66 μm to 15.7 μm in length and 3.40 μm to 4.90 μm in width during 1.5th to 10th month of pregnancy.

INTRODUCTION

The domesticated water buffalo (Bubalus bubalis) is used as a dairy, beef and draught animal in many countries of the world. About 97% of the world's domesticated buffalo population is maintained in Asia (Kamonpatana et.al.,1979). Pakistan is gifted with a live stock wealth which includes a large number of different species of farm animals. Pakistan is the home tract of the world's finest dairy buffalo, Nili-Ravi, which are ranked among the highest milk producing breeds of domesticated water buffalo (Cockrill., 1974) . There is estimated to be 17 million heads of buffalo (Pirzada and Jalali, 1990) in our country. Pakistani buffaloes contribute 7.46 million metric tones of milk and 0.241 million metric tones of beef to the annual national milk and meat production (Shah., 1988) and in addition, 56% of hides are also contributed by buffaloes (Government of Pakistan 1976).

Inspite of the fact that the buffalo has provided a significant contribution to our overall national economy, it is unfortunate, that it remains the least understood dairy animal as regards its physiology in general and reproductive physiology in particular. Although with the recent advent of sensitive assay procedures, like the radioimmunoassay (RIA) and competitive protein binding (CPB) techniques, some scattered information on various aspects of its reproductive physiology is becoming available. However, such estimations of the endocrine status of the Pakistani buffalo are yet to be initiated. The main endocrine approach to study the reproductive pattern in the bovine females is the evaluation of the progesterone levels in the blood (Shemsh et al., 1971) which almost correlate with the development, growth and regression of the corpus luteum (Karg et al., 1976).

The reason thus necessitates the establishment of normal hormone levels of Pakistani buffaloes during its

various physiological states i.e., pre-puberty, oestrous cycle, pregnancy, parturition and lactation. Hormonal factors regulating oestrous cycle and early pregnancy in Murrah buffaloes have been studied (Sanwal et al., 1980; Batra et al., 1982; Prakash and Madan., 1984b) but such information about Nili-Ravi buffalo in Pakistan is meagre and is limited only to oestrous cycle and early pregnancy (Chohan et al., 1989; Chohan et al., 1992). Thus an attempt to elucidate the histomorphology of corpus luteum and serum and luteal progesterone profiles throughout pregnancy can lead to meaningful conclusions.

In Brazilian buffaloes Vale et al. (1982) observed that 54.3% of the foetuses are in the right and 40% in the left uterine horn.

In Murrah Buffaloes 51.1% of the calves at birth are male (Rao and Murari., 1956) whereas in Egyptian buffaloes 53.44% calves at birth are male (Shalash and El-Mikkawi.,1956). Twinning frequency in Egyptian

buffaloes is 0.63% (Tantawy and Ahmed., 1957) and in Italian buffaloes is 0.294% (Ferrara., 1960), however very low incidence of twin births (0.0578%) is estimated by Goswami and Nair (1968) during their observation on 10363 calvings from 3096 Indian buffaloes.

The luteal ovary compared to non-luteal ovary is significantly greater in length (24.6 mm and 20.4 mm), weight (4.5 g and 3.1 g) and volume (5.00 cm^3 and 2.9cm^3) respectively. However, width (16.2 mm and 13.3 mm) and thickness (12.5 and 10.5 mm) of luteal and non-luteal ovary respectively do not differ significantly from each other during oestrous cycle (Usmani et al., 1985).

The corpus luteum of Indian buffalo ranges from 1.19 cm to 1.52 cm in length, 1.08 cm to 1.34 cm in width and in weight from 0.72 g to 1.54 g at its maximum stage of development during oestrous cycle (Luktuke and Rao., 1962). Roy and Mullick (1964) reported that the average weight of corpus luteum at 15th day of oestrous cycle is

2.285 ± 0.17 g. The average weight and size of the corpus luteum in Nili-Ravi buffaloes at various stages of oestrous cycle is 1.00 ± 0.2g (range, 0.1 - 3.00 g) and 6.6 ± 0.7 mm (range, 3 - 12 mm) respectively (Usmani et al., 1985).

In Murrah buffaloes Rao et al (1983) reported 4.4 ng/ml serum progesterone level during the first 25 days of pregnancy, but Sanwal et al. (1980) observed lower levels of serum progesterone which range from 1.41 to 2.53 ng/ml during the first 36 days of pregnancy in the same species. Chohan et al. (1992) mentioned that mean serum progesterone concentration ranges from 3.53 ± 1.04 ng/ml to 4.05 ± 1.07 ng/ml during the first 13 to 42 days of pregnancy in Nili-Ravi buffaloes. Nanda et al., (1984) mentioned that mean serum progesterone concentration during the period of 3 to 4 months of pregnancy is 2.40 ± 0.7 ng/ml (range, 1.7 - 4.0 ng/ml) in Indian buffaloes. However, during the later stages of pregnancy in Murrah buffaloes, a gradual decline in the

mean plasma progesterone levels occurs from 30 days prepartum (1.82 ng/ml) to 2 days before term (1.21 ng/ml). Thereafter, the mean progesterone level falls sharply ($P< .05$) to a concentration of 0.32 ng/ml at partum (Prakash and Madan., 1984 b).

El-Belely et al. (1988) reported that the Plasma progesterone concentration in Egyptian buffaloes declined from 3.00±0.2 ng/ml during days 21-17 before calving to 2.1±0.1 ng/ml at 3 days before calving, followed by a fall during the last 3 days of gestation to 1.2±0.3 ng/ml at calving.

Gao et al. (1988) reported that the plasma progesterone concentration observed during first and second trimesters of pregnancy are usually greater than those in the third trimester in Friesian cows. In Zebu cows the overall progesterone concentration (average) is 2.64 ± 0.16 ng/ml (rang, 0.34 - 6.7 ng/ml) of blood serum and it remains fairly constant throughout pregnancy

(Agarwal et al., 1980). The concentration of progesterone in maternal blood plasma decreases gradually during the last 20 days of pregnancy and then falls more rapidly in the 2 to 3 days before parturition in cows (Donaldson et al., 1970). These progesterone concentrations correlate with the low levels of uterine activity upto 2-4 days prepartum (Gillette 1966) and support the applicability of the progesterone block hypothesis to the Cow (Csapo., 1956).

Frankel (1903) reported for the first time that corpus luteum is required to maintain pregnancy in rabbit. Allen and Corner (1929) showed that a component of the lipoidal extract of corpus luteum, which was subsequently called progesterone, is responsible for the maintenance of pregnancy.

The maximum development of the corpus luteum of oestrous cycle (corpus luteum spurum) is observed at 12 to 15 days of oestrous cycle, followed by regression as

the next oestrous cycle approaches in Murrah buffaloes (Roy and Mullick., 1964) and in Nili-Ravi buffaloes (Abbas et al., 1981).

Betteridge et al. (1980) stated that an essential manifestation of pregnancy in large domestic species is the extended corpus luteum function. The corpus luteum or an exogenous source of progesterone is essential throughout pregnancy in the rabbit, rat, and goat but for less than full term in the guinea pig, mare and primate (Gomes., and Erb., 1965). In the cow corpus luteum is the major site of progesterone production, which remains functional throughout pregnancy (Erb. et al., 1967) and its regression occurs before the onset of parturition. The average weight and progesterone concentration of corpus luteum in cows from 11 to 284 days of pregnancy is 6.5 $\pm$ 0.5 g and 31.1 $\pm$ 1.14 μg/g respectively, however, the weight and progesterone concentration of corpus luteum declines after 200 days of pregnancy (Erb et al. 1967).

Removal of the corpus luteum before day 200 of pregnancy always causes abortion with delivery of a dead foetus in cows. (McDonald et al., 1953; Estergreen et al., 1967; Edquist et al., 1973). Erb et al., (1967) reported that most of the cows maintain pregnancy following ovariectomy after 200 to 237 days of pregnancy, however, corpus luteum is necessary during the last month (from 240 days till term) of pregnancy for normal gestation period and normal process of parturition. They suggested that maintenance of pregnancy during 200 to 237 days of pregnancy in cows may be due to extra-ovarian sources of progesterone. The extra-ovarian sources of progesterone may be considerable because Jugular venus plasma progesterone concentration remains high after third month, even though the concentration in ovarian venus plasma is surprisingly low from 199 - 237 days of pregnancy. But progesterone from extra-ovarian sources during the last month of pregnancy appears inadequate to maintain pregnancy in ovariectomized cows. Furthermore the secretory activity of corpus luteum (indicated by a

greater concentration of progesterone in ovarian venous plasma) also increases during the last month of pregnancy.

Blood cholesterol plays important role in reproduction. It is fairly well established that cholesterol is an important precursor to many steroid hormones secreted by the gonads and adrenals (Kellie., 1966). Cholesterol represents a key metabolite in the pathway leading to progesterone synthesis and luteal cells can obtain cholesterol for steroidogenesis through de novo synthesis and/or from circulatory plasma lipoprotein (Anderson and Dietschy., 1978; Grummer and Carrel., 1988). But the primary source of cholesterol for luteal cell progesterone synthesis among mammalian species is that present in blood (Armstrong et al., 1970; Bolte et.al., 1974, Anderson and Dietschy, 1978., Grummer and Carrel., 1988). Thus changes in the quantity of cholesterol present in blood could play a significant role in regulating steroid hormone synthesis by the ovary

(Henderson et al., 1981). There are reports indicating a positive correlation between high blood cholesterol concentration and good reproductive performance in cattle (Aminudden et al., 1984; Shahukar et al., 1985; Staish and Sharma., 1991).

Cholesterol level in blood plasma is influenced by both genetic and non-genetic factors. Among non-genetic factors, age, sex, gestation status, and lactation are of considerable importance. Arave et al.(1975) reported that serum cholesterol level in cattle increases with advancing age. Sex affects the blood cholesterol concentration differently in various species of animals. In cattle and humans, the females have the highest values (Schaefer et al., 1958; Maza and Dizon., 1968) in contrast to chicken and mice where males have the highest level (Hardy et al., 1962). Cholesterol concentration is also influenced by the stage of gestation and lactation. The concentration in cattle increases during gestation and decreases rapidly in connection with parturition.

(Lennon and Mixner., 1957; Memon and Mullick., 1961). The level of cholesterol increases during the lactation period and reaches its maximum about the third to fifth lactation month (Memon and Mullick., 1961). Prakash and Tandon (1979) studied total serum cholesterol concentration 5 weeks prepartum to 6 weeks post partum in cows and reported 155 mg/dl of total serum cholesterol concentration during 1 to 5 weeks prepartum, 111 mg/dl on the day of parturition, which subsequently increases (123 - 258 mg/dl) during the first 6 weeks postpartum.

Shashikumar and Dubay (1986) reported that mean total cholesterole concentration in uterine secretion is 125.80±4.96 , 54.40±6.15 , 207.03 ± 10.04 and 156.10 ± 11.71 mg/100 ml during pro-oestrus, oestrus, dioestrus and early pregnancy (25-30 days) respectively. Raju et al. (1991) observed that the mean total cholesterol concentration on the day of oestrus is 152.48 ± 2.57 mg/100 ml and day 10 of oestrous cycle is 132.73 ± 6.5 mg/100 ml in Murrah buffaloes.

In Holstein heifers the highest mean concentration of serum cholesterol (96.3 ± 8.2 mg/dl) occurred between the day of oestrus and the first 2 days following oestrus. A transient decline ($P<.05$) in serum total cholesterol is observed during the luteal phase beginning on day 2 and reached a nadir 6 days after oestrus (76.3 ± 10.3 mg/dl). The concentration of total cholesterol are negatively correlated ($r = -0.40$; $P< 0.01$) with serum progesterone between day 2 to 9 but not throughout cycle (Talavera et al., 1985).

Corpus luteum in cows (Fields et al., 1985; Alila et al., 1988) and sheep (Fitz et al., 1982) consists of at least two morphologically and biochemically distinct cells types known as small and large luteal cells. In addition to luteal cells, the corpus luteum also contains vascular elements, connective tissues and fibroblasts (Nett et al., 1976., Rodgers et al., 1984).

Fields et al., (1985) reported that large luteal cells are always close to capillaries, appear as polyhedral and have a diameter of 20-50 μm in cows during the period of 45 to 280 days of pregnancy. The outer membrane of the large luteal cells is highly convulated, providing the cell with large surface area. The nuclei are large, round and approximately 10 μm in diameter (Parry et al., 1980). Large luteal cells have lightly stained cytoplasm and contained an abundance of small dense membrane-bound granules (150-300 nm in diameter) in their cytoplasm during mid-luteal phase of oestrous cycle (Parry et al., 1980) and pregnancy (Fields et al., 1985). The granules are sphericle, however some pleomorphism is also observed e.g. "dumb-bell" configuration. These granules are distributed in clusters throughout the cytoplasm, and are also often found near the edges of the cell (Parry et al., 1980). However, Fields et al., (1985) observed cytoplasmic polarity of these granules in large luteal cells during pregnancy (45 to 280 days) which is not found in small luteal cells. Numerous secretory granules

are seen in the paranuclear area of the cell. In other large luteal cells these granules are seen grouped in the centre or at the periphery, where the granule membrane is incorporated in to the cell membrane and the dense material exocytosed. Parry et al., (1980) also observed these granules in the extra-cellular space during mid-luteal phase of the oestrous cycle in cows, which suggest that they had been secreted.

Fields et al., (1985) observed secretory granules in the cytoplasm of large luteal cells as early as day 45 of pregnancy in cows. Although these secretory granules are present during the first and last quarter of pregnancy, their number are less than during the second and third quarter. They further reported that after the peak in granules number (200 days of pregnancy), there is an accumulation of cytoplasmic lipids inclusions in the large luteal cells. Parry et al., (1980) also reported that lipid droplets are also present in large luteal cells during oestrous cycle in cows and their

concentration varied inversly to the number of electron dense membrane-bound secretory granules present. In cells containing large number of lipid droplets, evidence for exocytosis of granules are rare. Thus lipid droplets increased by day 20 when progesterone secretion had ceased.

Fields et al. (1985) reported that small luteal cells appear spindle-shaped, have darkly stained cytoplasm and an irregular shaped nucleus in cows. The cross sectional diameter of small luteal cells during the period of 45 to 280 days of pregnancy ranges from 10 to 15 μm and their cytoplasm also contain small number of mature and immature secretory granules.

It is well documented that progesterone is responsible for the maintenance of pregnancy in domestic animals. Corpus luteum is the major source of progesterone for most, if not all of the gestation period in cows. Evaluation of serum progesterone concentration

is used as an index for pregnancy diagnosis in domestic animals. Information regarding the functional activity and histomorphology of corpus luteum is not available in Nili-Ravi buffaloes. Serum progesterone concentration has been determined in Nili-Ravi buffaloes, but such information are limited to early pregnancy.

The present investigation on Nili-Ravi buffalo is thus designed to study progesterone , cholesterol and concentration and histomorphology of corpus luteum throughout pregnancy. This study also includes serum progesterone , cholesterol and concentration throughout pregnancy.

MATERIALS AND METHODS

Blood and corpora lutea of pregnant Nili-Ravi buffaloes from 31st to 315th day of pregnancy were collected for this study from Sihala Abbatoir, Islamabad, Pakistan.

Blood was collected at the time of slaughtering in clean centrifuge glass tubes. The blood samples were chilled in ice, transported to the laboratory and centrifuged at 3000 rpm for 10 minutes. Serum was separated and stored at - 20^{o}C until analyzed for progesterone and cholesterol. After slaughtering the cornual implantation and sex of the foetuses were noted. Pregnancy stage was determined by measuring foetal crown to rump length as described by Pirzada and Jalali [1990] in Nili-Ravi buffaloes. Both the luteal and non-luteal ovaries were dissected out and transported to the laboratory in an ice container. These ovaries were weighed separately on Metler's balance, measured for their length and width with vernier caliper. The corpora

lutea were enucleated, weighed and measured for their length, width and thickness. Volume of corpora lutea was determined by fluid displacement method and stored at $-20^{o}C$ until used for progesterone and cholesterol determination.

The variation in size (length, width, thickness) weight and volume of corpus luteum was studied at an interval of 30 days from 31st to 315th day of pregnancy. The gestation period was divided into two groups. Group I consists of first and second trimester (1.5th to 7th month) and group II comp rises of third trimester (8th to 10.5th month) of pregnancy. This grouping was carried out to find if the size, weight and volume of corpus luteum shows significant changes in group II as compared to group I. This grouping of the gestation period was also utilized to study the changes in luteal progesterone and cholesterol concentration. For the comparison of mean serum progesterone and cholesterol concentration between two gestation groups, the gestation group II includes

8th to 9.5th month of pregnancy due to the unavailability of serum samples at 10th and 10.5th month of pregnancy.

PROGESTERONE RADIOIMMUNOASSAY

For the determination of progesterone, serum and corpus luteum was taken from the same animal at selected stages of pregnancy (Table 5).

1. Serum Progesterone Radioimmunoassay:

Serum progesterone radioimmunoassay was carried out at Nuclear Medicine, Oncology and Radiotherpy Institute (NORI), Islamabad, Pakistan by using Amerlex-M progesterone radioimmunoassay kit (Kodak diagnostics Ltd., Amersham, UK). The procedure was adopted as provided by Kodak Clinical diagnostics, Amersham, U.K.

2. Luteal Tissues Progesterone Radioimmunoassay:

For luteal tissues progesterone radioimmunoassay the tissues were first extracted in anaesthetic ether through the following procedure.

25 mg of corpus luteum was homogenized in 5 ml normal saline (0.9% NaCl). To 100 μl of this homogenate 2.5ml x 2 anaesthetic ether was added and vortexed. The supernatant anaesthetic ether was transfered into clean scintillation vials and dried under air in water bath at 60^{o}C. The anaesthetic ether extracts wre reconstituted with appropriate volume (4 ml) of steroid phosphate buffer (0.1M containing NaCl 0.9%, gelatin 0.1%, sodium azide 0.1%, pH 7.2). The samples were then assayed by using Amerlex-M progesterone radioimmunoassay kit (Kodak Clinical diagnostics, Amersham, U.K.)

CHOLESTEROL DETERMINATION

Serum and luteal tissue total cholesterol concentration at selected stages of pregnancy (Table 8) was determined as described by Arslan and Jalali [1973]. Cholesterol concentration was expressed as mg of cholesterol per 100 ml of serum and mg of cholesterol per 100 mg of luteal tissue.

HISTOLOGY

For histomorphological observation the corpora lutea at 1.5th and 3rd month (First trimester), 6th month (second trimester) and 10th month (third trimester) of pregnancy were enucleated from ovaries just after slaughtering and cut into small pieces to allow prompt fixation. The tissues were fixed in sera (60 ml absolute alcohol, 30 ml formaldehyde, 10 ml glacial acetic acid) for 4-5 hours. Then the tissues were dehydrated as

follows in ascending grades of propanol:

Propanol	70% for 4-5 hours
	80% overnight
	90% for two hours
	95% for two hours
	100% for 5 hours.

The tissues were transfered to cedar wood oil and left in it until they became transparent. The tissues were then embedded in paraplast by the following procedure:

Benzol I	10 minutes
Benzol II	10 minutes
Benzol + Paraplast	20 minutes (at 60^{o}C)
Paraplast I	Morning (60^{o}C)
Paraplast II	Evening (60^{o}C)
Paraplast III	Morning (60^{o}C).

In the evening the tissues were ready to make blocks. The sections were cut out of paraffin blocks at a thickness of 5-6 μm by using Reichert microtome and stretched at 60^{o}C on Fisher slide warmer.

After deparaffinizing, the slides were transferred to xylol for 15 minutes to remove any remaining wax. The tissues were then hydrated in the descending grades of alcohol, washed in tap water and stained in Harris Haematoxylin. After that the tissues were dehydrated in the ascending grades of alcohol and then counter-stained with Eosin (McMannus and Mowry., 1964). Microphotographs of the sections were then prepared later on.

CYTOMETRY

The cytometric studies of large and small luteal cells were carried out with the help of an ocular micrometer. The size of large and small luteal cells and the diameter of their nuclei were calculated by taking

average of 10 measurements. Size of the luteal cells and diameter of their nuclei were expressed in μm.

Statistical Analysis:

Mean, standard error and student's t-tests were calculated for various comparisons.

RESULTS

CORNUAL IMPLANTATION AND SEX RATIO

The present investigation is based on 148 pregnant Nili-Ravi buffaloes slaughtered at Sihala Abbatoir, Islamabad.

This investigation reveals that 58.11% of foetuses are in the right and 41.89% in the left uterine horn. 54.2% of the foetuses are male while 45.8% are female. Twinning or intrauterine migration of ova has not been observed during this study.

GROSS OVARIAN AND CORPUS LUTEUM MORPHOLOGY

Ovarian Morphology:

The mean length, width and weight of luteal ovary is 2.78 ± 0.04 cm, 2.16 ± 0.02 cm and 5.40 ± 0.10 g while that of non-luteal ovary is 2.59 ± 0.04 cm, 1.72 ± 0.02 cm and 3.43 ± 0.09 g respectively from 31st to 315th day of

pregnancy in Nili-Ravi buffaloes (Table 1). The mean values for the length ($P < 0.01$), width ($P < 0.001$) and weight ($P < 0.001$) of luteal ovary is significantly higher than that of non-luteal ovary (Table 1).

Corpus Luteum Morphology:

The mean length, width, thickness, weight and volume of the corpus luteum is 1.76 ± 0.02 cm, 1.75 ± 0.02 cm, 1.32 ± 0.01 cm, 2.15 ± 0.04 g and 2.18 ± 0.04 cm^3 respectively from 31st to 315th day of pregnancy (Table 2).

The mean values with respective ranges for the length, width, thickness, weight and volume of corpus luteum at various stages of pregnancy are shown in Table 3, (Fig. 1 & 2). The corpus luteum does not show much variation in length from 31st to 210th day of pregnancy (Table 3, Fig.1) but from this stage onward a slight, non-significant ($P>0.05$; Table 4) decrease occurs. The

width of the corpus luteum remains fairly constant from 31st to 300th day of pregnancy (Table 3) and show a non-significant change ($P > .05$; Table 4) between the two gestation groups. There is no appreciable change in the thickness, weight and volume of corpus luteum from 31st to 210th day of pregnancy (Table 3), but from this stage onward a significant decrease in thickness ($P < 0.05$), weight ($P<0.001$) and volume ($P<0.01$) occurs (Table 4). However, the lowest values for all these characteristics of corpus luteum are observed at 301st - 315th day of pregnancy (Table 3).

Table 1: Effect of presence of a corpus luteum on characteristics of Nili-Ravi buffalo ovaries during pregnancy.

Ovarian Characteristics	Luteal Ovary		Non-luteal Ovary				
	Mean ± S.E.	Range	Mean ± S.E.	Range	t	d.f	P
Length(cm)	2.78±0.04	1.77-4.15	2.59±0.04	1.2-3.64	3.15	260	<0.01
Width(cm)	2.16±0.02	1.5 -3.50	1.72±0.03	1.22-2.75	11.94	259	<0.001
Weight(g)	5.4±0.10	3.13-9.31	3.4±0.09	1.42-6.12	15.25	280	<0.001

Table 2: Mean length, width, thickness, weight and volume of corpus luteum during pregnancy in Nili-Ravi buffalo.

Size (cm)			Weight (g)	Volume (cm^3)
Length	Width	Thickness		
1.72±0.02	1.76±0.02	1.32±0.01	2.15±0.04	2.18±0.04
(1.54-1.89)	(1.69-1.84)	(0.98-1.43)	(1.27-2.31)	(1.5-2.41)

Mean ± S.E.

Table 3: Mean length, width, thickness, weight and volume of corpus luteum at various stages of pregnancy in Nili-Ravi buffalo.

Pregnancy stage (days)	Size (cm)			Weight (g)	Volume (cm^3)
	Length	Width	Thickness		
31-60	1.79±0.06 (1.40-2.67)	1.84±0.08 (1.55-2.50)	1.42±0.04 (1.17-1.87)	2.30±0.12 (1.61-3.44)	2.41±0.13 (1.7-3.50)
61-90	1.78±0.04 (1.40-2.16)	1.82±0.02 (1.43-2.22)	1.33±0.02 [1.00-1.60)	2.31±0.07 (1.60-3.31)	2.38±0.08 (1.60-3.30)
91-120	1.73±0.12 (1.20-2.16)	1.71±0.04 (1.40-2.02)	1.39±0.04 (0.93-1.70)	2.21±0.11 (1.28-3.13)	2.14±0.10 (1.40-3.10)
121-150	1.70±0.05 (1.40-2.10)	1.75±0.05 (1.20-2.10)	1.30±0.04 (1.06-1.46)	2.03±0.10 (1.20-2.84)	2.11±0.13 (1.40-3.98)
151-180	1.81±0.07 (1.24-2.25)	1.77±0.06 (1.40-2.24)	1.29±0.05 (0.90-1.67)	2.18±0.11 (1.37-3.06)	2.13±0.10 (1.40-3.00)
181-210	1.81±0.07 (1.24-2.25)	1.77±0.06 (1.40-2.24)	1.29±0.05 (0.90-1.67)	2.18±0.11 (1.37-3.06)	2.13±0.10 (1.40-3.00)
211-240	1.67±0.04 (1.22-1.90)	1.64±0.08 (1.34-1.92)	1.19±0.05 (1.00-1.37)	1.73±0.12 (1.22-2.10)	1.89±0.15 (1.30-2.48)
241-270	1.67±0.18 (1.4-2.07)	1.84±0.18 (1.48-2.10)	1.43±0.05 (1.33-1.50)	2.02±0.28 (1.47-2.43)	1.97±0.30 (1.40-2.40)
271-300	1.66±0.05 (1.54-1.80)	1.80±0.15 (1.50-1.96)	1.22±0.01 (1.20-1.25)	1.74±0.08 (1.59-1.87)	1.90±0.06 (1.80-2.00)
301-315	1.54±0.18 (1.22-1.86)	1.62±0.08 (1.48-1.70)	0.99±0.13 (0.75-1.22)	1.27±0.22 (0.89-2.77)	1.50±0.29 (1.00-2.00)

Table 4: Comparison of various characteristics of corpus luteum between two gestation groups in pregnant Nili-Ravi buffalo.

Corpus luteum characteristics	Group I	Group II	t	d.f.	P
Length	1.778±0.028	1.752±0.25	1.949	142	>0.05
Width	1.764±0.021	1.702±0.06	1.033	141	>0.05
Thickness	1.337±0.016	1.219±0.048	2.314	125	<0.05
Weight	2.195±0.042	1.732±0.099	4.287	141	<0.001
Volume	2.223±0.045	1.855±0.106	3.2	141	<0.01

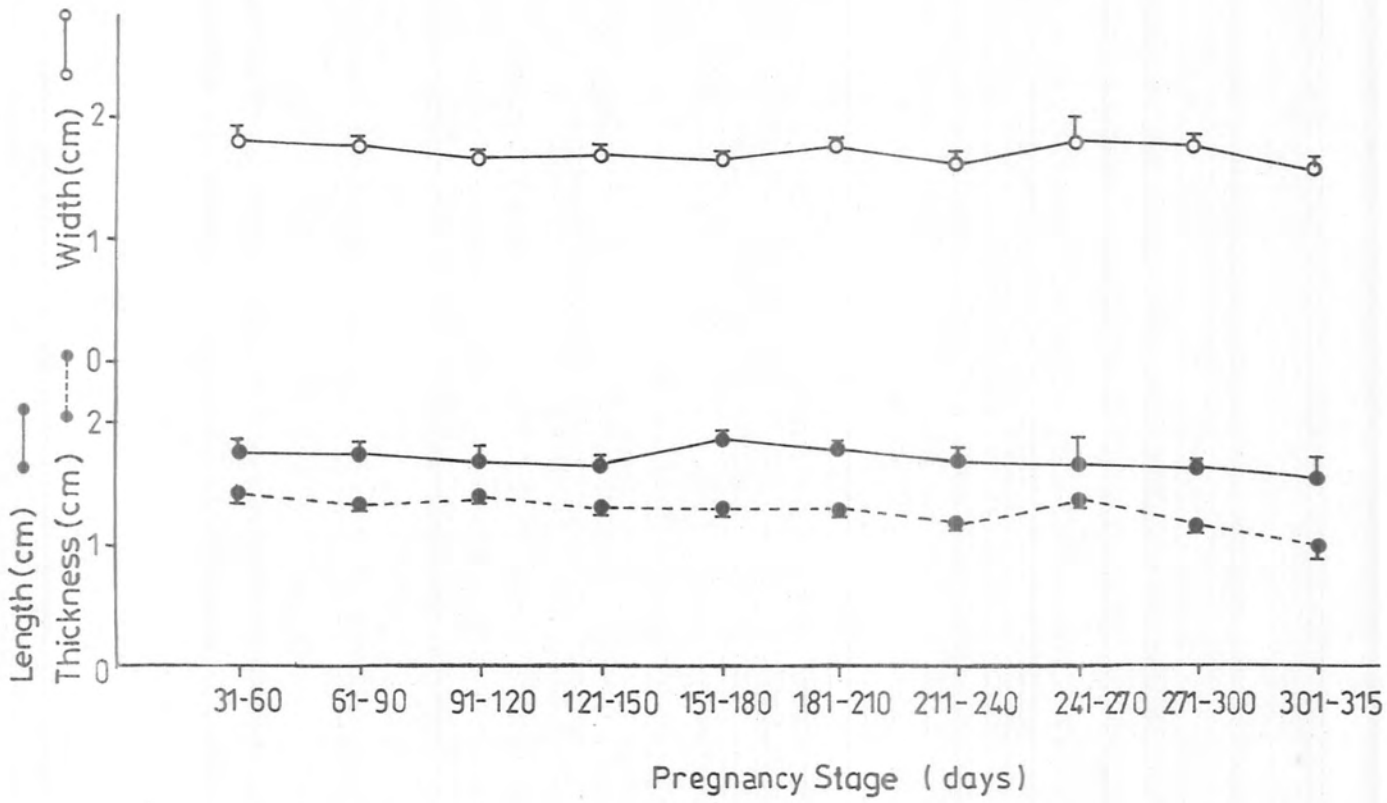

Fig.1: Mean length, width and thickness of corpus luteum at various stages of pregnancy in Nili-Ravi buffalo.

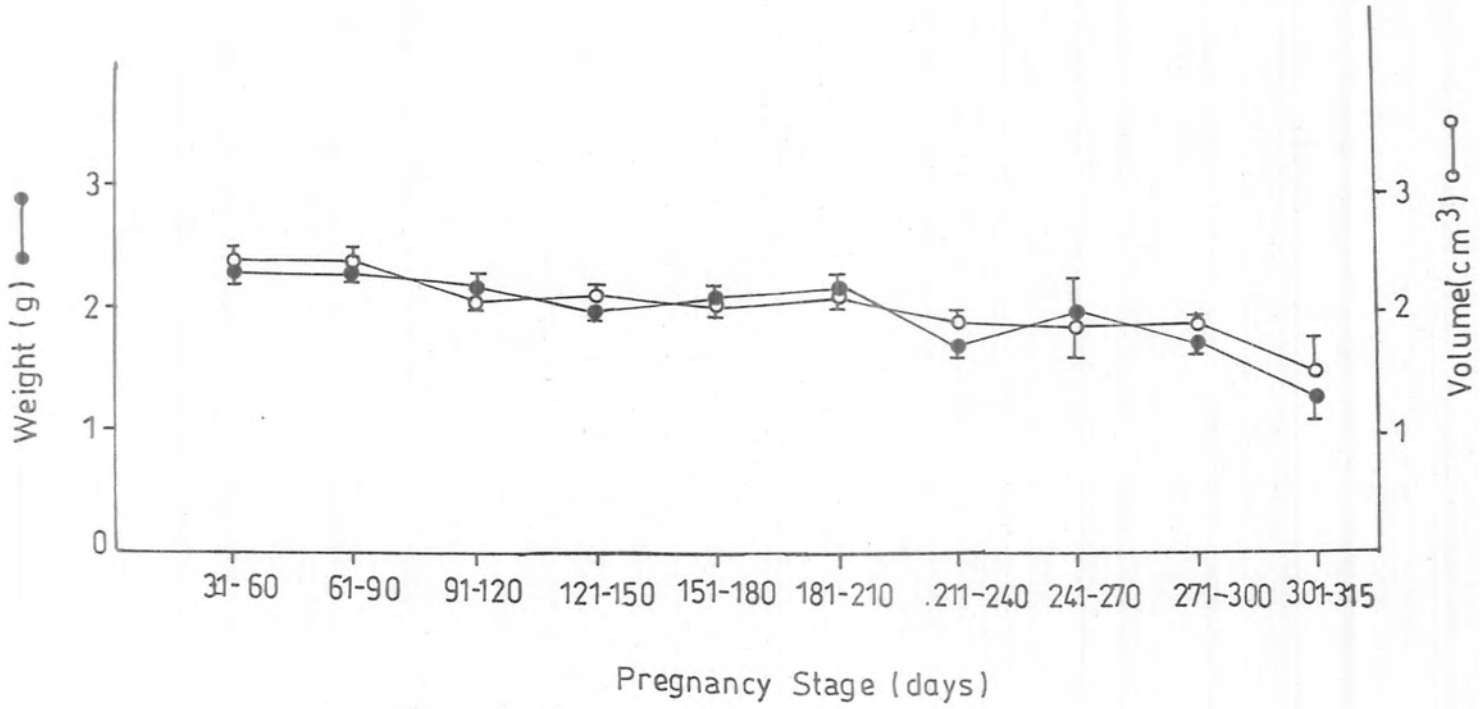

Fig.2: Weight and volume of corpus luteum at various stages of pregnancy in Nili-Ravi buffalo.

SERUM PROGESTERONE

Gestation status (1.5th - 9.5th month) and mean serum progesterone concentration is shown in Table 5 (Fig.3). Mean serum progesterone concentration is 2.7 ± 0.15 ng/ml (range, 1.46 - 3.94 ng/ml) during the investigated period of pregnancy. Serum progesterone concentration from 1.5th to 7th month has not shown much variation but gestation status 8th to 9.5th month indicates an abrupt decrease in serum progesterone concentration.

Mean serum progesterone concentration in gestation group I and group II is 3.11 ± 0.16 ng/ml and 1.76 ± 0.16 ng/ml respectively (Table 6). The comparison of mean serum progesterone concentration in the two gestation groups shows highly significant decrease in group II (Table 6).

LUTEAL PROGESTERONE

Mean luteal progesterone concentration at various stages of pregnancy (1.5th - 10.5th month) are presented in Table 5, (Fig.3). Mean luteal progesterone concentration is 27.36 ± 1.52 μg/g (range, 9.45 - 35.76 μg/g) during the investigated period of pregnancy. No appreciable difference is observed in luteal progesterone concentration from 1.5th to 7th month of pregnancy. A sharp decline in luteal progesterone concentration occurs during 8th to 9th month which is followed by a progressive increase from 9.5th to 10th month of pregnancy. Progesterone concentration markedly dropped at 10.5 month of pregnancy (9.45 ± 2.02 μg/g). Mean luteal progesterone concentration in gestation group I and II are 31.99 ± 1.88 and 22.00 ± 1.82 μg/g respectively (Table 7). Comparison of mean luteal progesterone concentration reveals a highly significant decrease in gestation group II compared to group I.

Table 5

Serum and luteal progesterone concentration at various stages of pregnancy in Nili-Ravi Buffaloes

Gestation Status (Months)	Serum progesterone (ng/ml)	Luteal progesterone (μg/g)
1.5	3.94 ± 0.56	34.64 ± 6.23
2	2.91 ± 0.18	31.35 ± 2.81
3	2.99 ± 0.40	29.52 ± 2.07
4	3.31 ± 0.44	34.91 ± 9.07
5	3.54 ± 0.59	29.39 ± 6.00
6	2.85 ± 0.59	29.34 ± 3.26
7	2.28 ± 0.66	35.76 ± 7.44
8	1.46 ± 0.26	20.84 ± 2.08
9	1.85 ± 0.22	25.40 ± 2.55
9.5	1.93 ± 0.32	26.9 ± 1.19
10	-	28.65 ± 0.63
10.5	-	9.45 ± 2.02

Mean ± S.E.

Table 6

Comparison of mean serum progesterone concentration in two gestation groups.

Gestation group	Serum progesterone (ng/ml)	t	d.f	P
Group I	3.11 ± 0.16	6.14	44	<0.001
Group II	1.76 ± 0.16			

Mean ± S.E.

Table 7

Comparison of mean luteal progesterone concentration in two gestation groups.

Gestation group	Luteal Progesteron (μg/g)	t	d.f	P
Group I	31.99 ± 1.88	3.82	39	<0.001
Group II	22.00 ± 1.82			

Mean ± S.E.

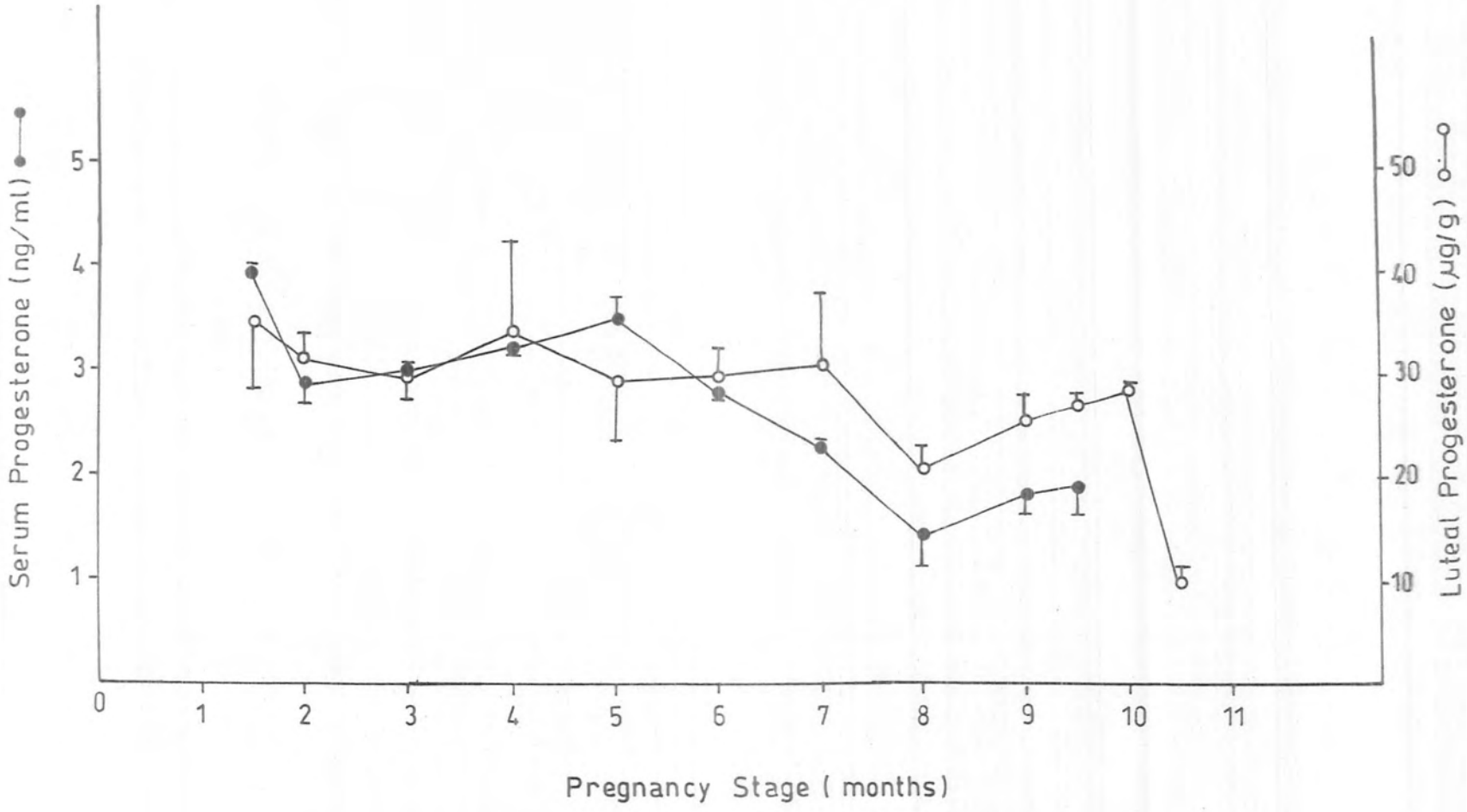

Fig.3: Serum and luteal progesterone concentration at various stages of pregnancy in Nili-Ravi buffalo.

SERUM CHOLESTEROL

Mean serum cholesterol concentration at various stages of pregnancy are summurized in Table 8, (Fig.4). Mean serum cholesterol concentration is 54.67 ± 1.67 mg/100 ml (range, 42.76 - 63.97 mg/100 ml). during the investigated period of pregnancy (1.5th to 9.5th month). Serum cholesterol concentration shows not much variation from 1.5th to 6th month of pregnancy except at fifth month (53.81 ± 3.73 mg/100 ml). A sharp fall is observed from 6th month to 9th month with a slight increase at 9.5th month of pregnancy.

Mean serum cholesterol concentration is 58.62 ± 1.94 and 47.77 ± 1.90 mg/100 ml in gestation group I and II respectively (Table 9). The comparison of mean serum cholesterol concentratin in the two gestation groups reveals a highly significant decrease in group II as compared to group I.

LUTEAL CHOLESTEROL

Mean luteal cholesterol concentration at various stages of pregnancy are shown in Table 8, (Fig.4). Mean luteal cholesterol concentration is 10.74 ± 0.31 mg/g (range, 8.99 - 13.16 mg/g) during the investigated period of pregnancy. Luteal cholesterol concentration is fairly constant during pregnancy but a slight increase is observed at 10.5th month (13.16 ± 0.60 mg/g) of pregnancy. Mean luteal cholesterol concentration is 10.73±2.08 mg/g in gestation group I and 10.80±0.52 mg/g in group II. The comparison of mean luteal cholesterol concentration in two gestation groups indicates a slight increase in group II which is statistically non-significant (Table 10).

Table 8

Mean serum and luteal cholesterol concentration at various stages of pregnancy in Nili-Ravi buffaloes.

Gestation status (Months)	Serum cholesterol mg/100 ml	Luteal cholesterol mg/g
1.5	63.97 ± 6.65	10.14 ± 1.22
2	62.30 ± 5.51	11.75 ± 1.98
3	63.48 ± 1.20	12.13 ± 0.61
4	59.41 ± 7.02	11.09 ± 1.01
5	53.81 ± 3.73	10.59 ± 1.19
6	60.58 ± 1.63	9.44 ± 1.17
7	46.76 ± 2.30	10.45± 1.18
8	42.18 ± 0.80	8.99 ± 0.36
9	48.00 ± 3.81	11.82 ± 0.72
9.5	53.12 ± 2.20	10.53 ± 0.60
10	-	9.25 ± 0.49
10.5	-	13.16 ± 0.60

Mean ± S.E.

Table 9

Comparison of mean serum cholesterol concentration in two gestation groups

Gestation group	Serum cholesterol mg/100 ml	t	d.f	P
Group I	58.62 ± 1.94	3.99	31	<0.001
Group II	47.77 ± 1.90			

Mean ± S.E.

Table 10

Comparison of mean luteal cholesterol concentration in two gestation groups.

Gestation group	Luteal cholesterol (mg/g)	t	d.f	P
Group I	10.73 ± 2.08	0.014	40	>0.9
Group II	10.80 ± 0.44			

Mean ± S.E.

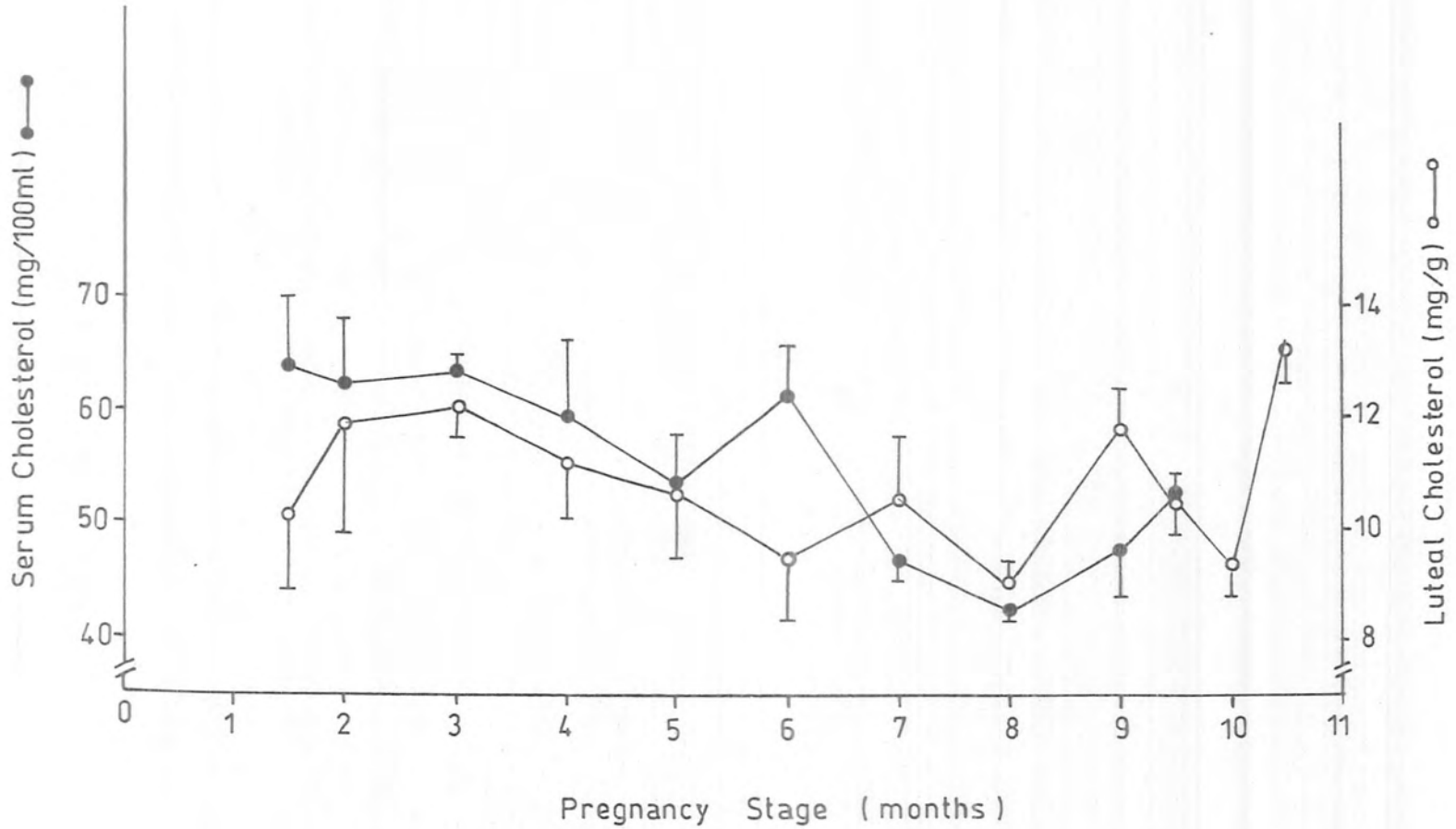

Fig.4: Serum and luteal cholesterol concentration at various stages of pregnancy in Nili-Ravi buffalo.

HISTOMORPHOLOGY OF CORPUS LUTEUM

Histomorphological observation of the pregnant Nili-Ravi buffalo corpus luteum were carried out at first (1.5th and 3rd month), second (6th month) and third trimester (10th month) of pregnancy.

The study of cross section of corpus luteum during pregnancy indicates that corpus luteum is surrounded by relatively thick capsule (Fig.5a). Corpus luteum consists of morphologically two different types of cells namely large and small luteal cells (Fig.5b). Large luteal cells are always in close association with blood vessels (Fig.8a), appear sphericle in shape (Fig.5b & 6a) and have an average diameter of 27.20 $\pm$ 0.36 μm (range, 23.12 μm - 33.88 μm) during the period of 1.5th to 10th month of pregnancy.

Large luteal cells have sphericle darkly stained nuclei which have an average diameter of 7.21 $\pm$ 0.14 μm

(range, 5.99 μm - 9.24 μm) and brightly stained cytoplasm (Fig.5c) which contains secretory granules and vacules (Fig.5c & 8c).

Small luteal cells in Nili-Ravi buffalo corpus luteum are spindle-shaped and range from 10.66 to 15.78 μm (average, 12.93±1.13 μm) in length and 3.40 μm to 4.90 μm (average, 4.21 ± 0.35 μm) in width during the period of 1.5th to 10th month of pregnancy. They have lightly stained cytoplasm and darkly stained nuclei (Fig.9b).

1.5th Month of Pregnancy:

At this stage of pregnancy the corpus luteum is surrounded by a thick capsule which also contains blood vessels (Fig. 5a). Large luteal cells are in close association with blood vessels and are of variable shapes. Most of them appear sphericle in shape, however, few elongated large luteal cells are also observed (Fig.5b). Large luteal cells have an average diameter of

26.87 μm (range 24.48 μm - 27.88 μm) and posses highly convulated cell membrane (Fig.5c). These cells have sphericle and darkly stained nuclei which are eccentric in position. The nuclei of large luteal cells at this stage of pregnancy ranges from 6.12 μm to 8.16 μm (7.06±0.2 μm) in diameter. These cells have brightly stained cytoplasm which contains few, inconspicuous secretory granules located in the peripheral region of the cell. The most prominent feature of the large luteal cells cytoplasm at this stage is peripheral vaculation (Fig.5c). Small luteal cells are spindle- shaped in appearance, and have an average length and width of 10.66 ± 0.13 μm and 3.4 ± 0.02 μm respectively. They posses lightly stained cytoplasm and darkly stained nuclei (Fig.5b).

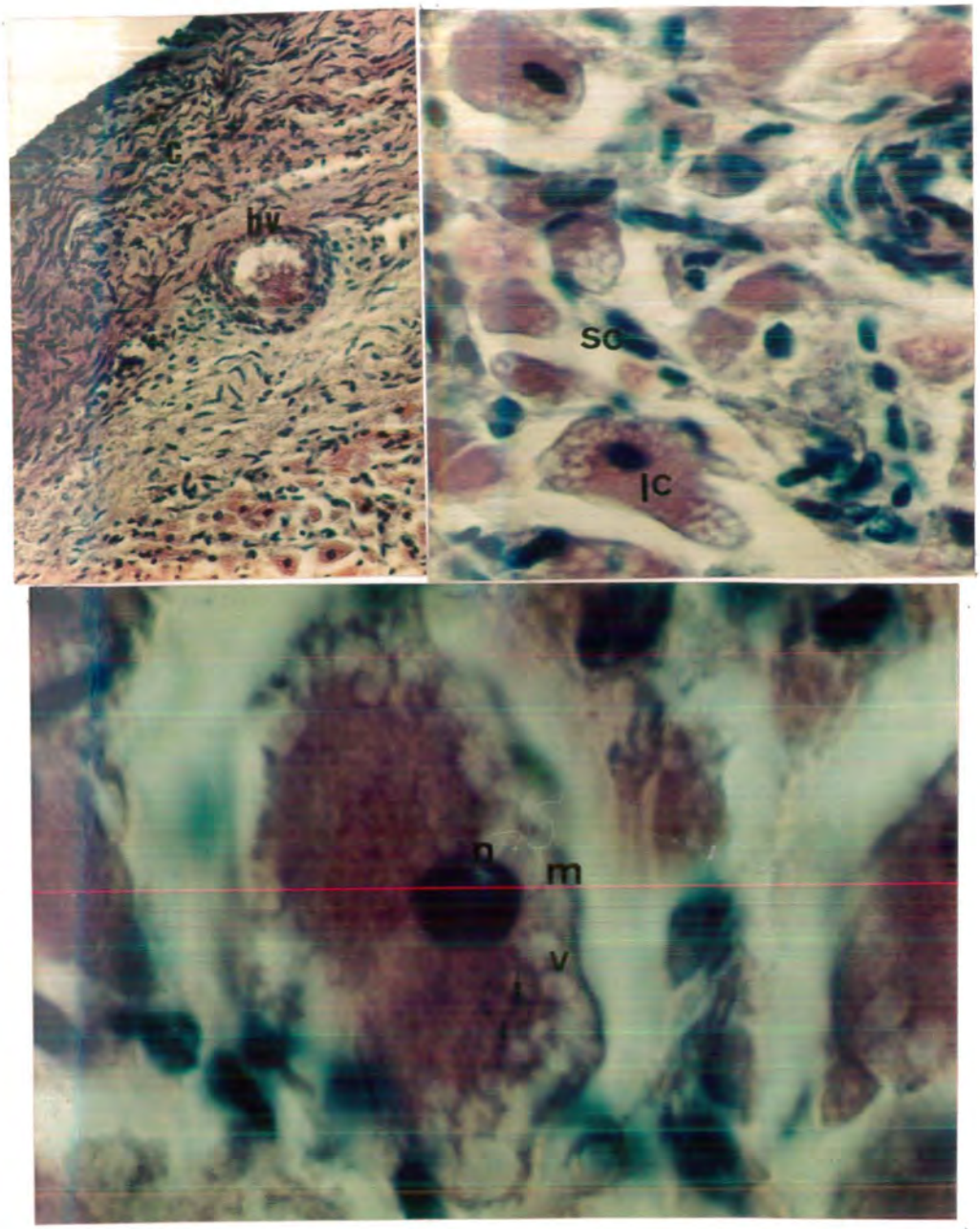

Fig.5: Photomicrograph of cross-section of Nili-Ravi buffalo corpus luteum at 1.5th month of pregnancy showing a) thick capsule (c) and blood vessel (bv). x56- b) higher magnification of (a) illustrating large (lc) and small luteal cells (sc). x -224c) magnified large luteal cell (lc) possesses highly convoluted cell membrane (m) and sphericle darkly stained nucleus (n). Note cytoplasmic granules (arrow) and peripheral vacuoles (v) along the inner side of cell membrane (m). H.E. x560

3rd Month of Pregnancy:

Capsule of corpus luteum at this stage of pregnancy is of similar thickness and consistency as that of the previous stage. Large luteal cells are in close association with blood vessels and have an average diameter of 25.16 μm $\pm$ 0.43 μm (range, 23.12 - 26.66 μm). At this stage sphericle large luteal cells are abundant as compared to the previous stage, however, few slightly elongated large luteal cells are also observed (Fig.6c). Cell membrane of the large luteal cells at this stage are moderately convulated compared to the previous stage (Fig.6b). Nuclei of the large luteal cells are sphericle, and central in position with intact nuclear membrane. They have an average diameter of 6.62 $\pm$ 0.15 μm (range, 5.94 μm - 7.48 μm). These nuclei contain sphericle, darkly stained peripheral nucleolus and slightly granulated chromatin (Fig.6b). Cytoplasmic secretory granules at this stage are more conspicuous and abundant than previous stage. Prominent cytoplasmic vacules are

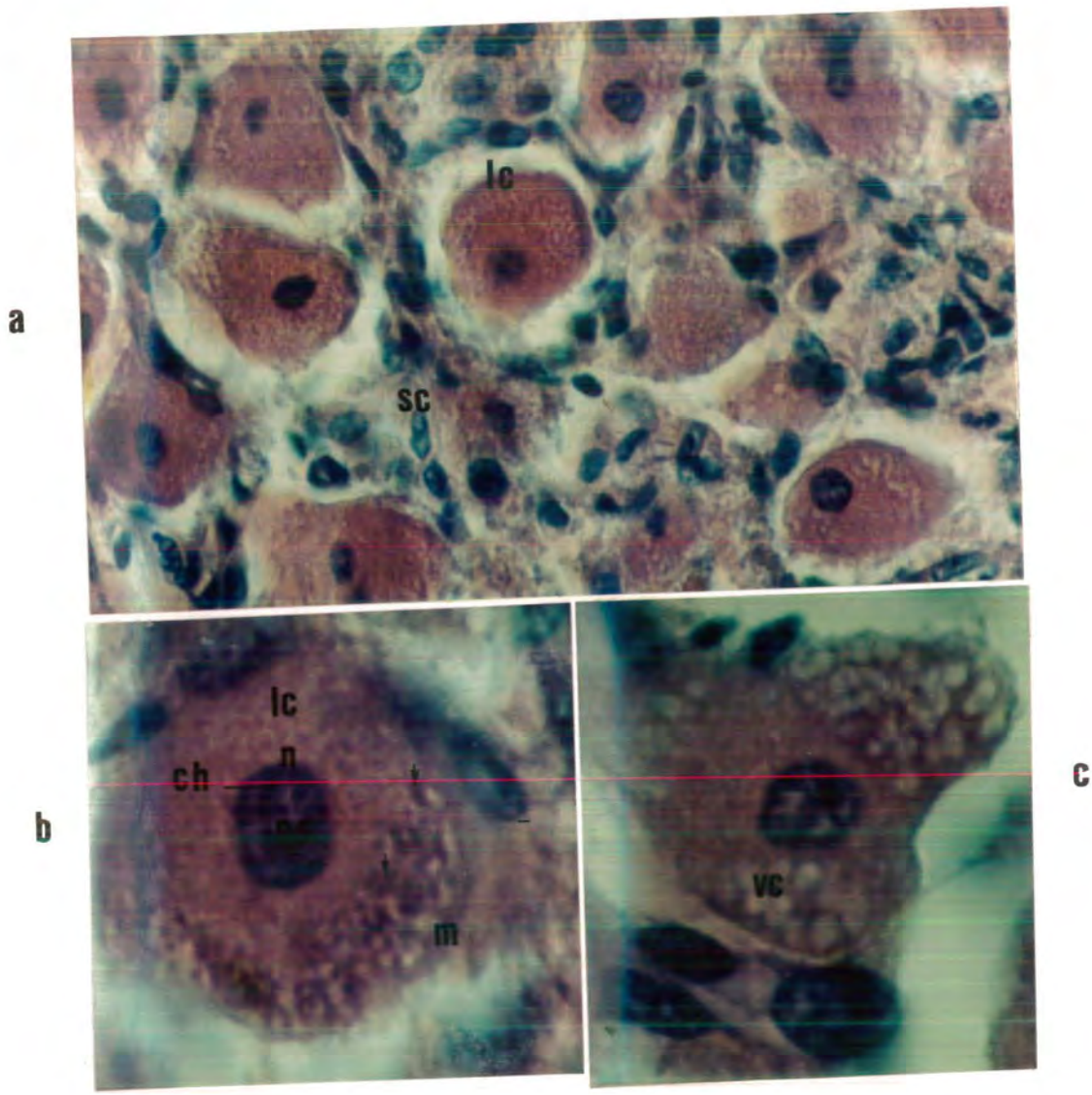

Fig.6: Photomicrograph of cross section of corpus luteum at 3rd month of pregnancy showing a) large (lc) and small luteal cells (sc). x224 b) Magnified large luteal cell possesses moderately convoluted cell membrane (m) conspicuous secretory granules (arrow) and darkly stained sphericle nucleus (n) situated in the centre the of cell. Note dark, sphericle eccentrically located nucleolus (ns) and granulated chromatin (ch) in nucleus. x c) higher magnification of large luteal cell representing prominent cytoplsmic vacuoles (vc) arranged in groups. H.E. x560

also seen in large luteal cells, but they are arranged more or less in groups (Fig.6c) in contrast to that of the previous stage.

Small luteal cells are similar to that observed in the previous stage in shape and arrangement, however, they are slightly longer (11.68 ± 0.32 μm) and wider (3.86 ± 0.08 μm) as compared to small luteal cells at 1.5th month of pregnancy.

6th Month of Pregnancy:

Capsule of the corpus luteum is thick and compact, however, connective tissues underneath it are quite loosely arranged as compared to the previous stages (Fig.7a).

Large luteal cells are in close association with blood vessels like that of the previous stages. These cells appear sphericle or polyhedral in shape and have an average diameter of 28.12 ± 0.55 μm (range, 24.98 μm - 29.93μm). The cell membrane of these cells is moderately convulated (Fig.7c). Large luteal cells have sphericle, darkly stained nuclei which range from 6.46 μm - 8.33 μm (average, 7.4 ± 0.27 μm) in diameter and are eccentric in position like previous stages (Fig.7c). These nuclei posses sphericle, darkly stained nucleolus which most of the cases are central in position (Fig.7c). The major differences observed in large luteal cells at this stage are the inconspicuous secretory granules and vacules compared to the previous stages. There is no change in the shape and arrangement of small luteal cells at this stage as compared to the previous satages. The average length and width of small luteal cells at this stage is 13.6 ± 0.43 μm and 4.7 ± 0.13 μm respectively.

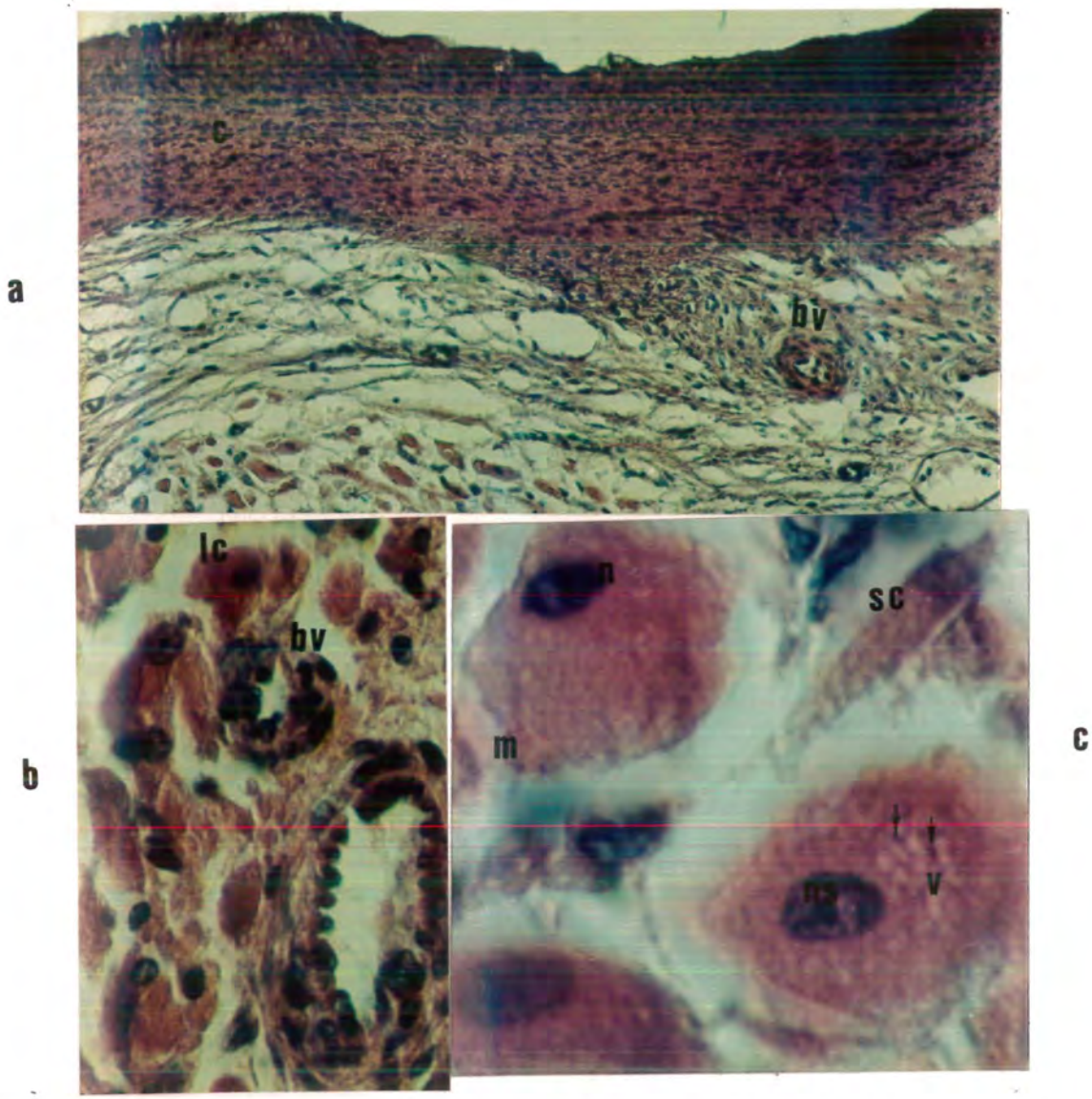

Fig.7: Photomicrograph of cross-section of corpus luteum at 6th month of pregnancy illustrating a) thick capsule c) and blood vessel (bv). x 56 b) large luteal cells (lc) in close association with blood vessel (bv.). x 224 c) higher magnification of large luteal cells with moderately convoluted cell membrane (m), sphericle darkly stained nuclei (n) which posses sphericle darkly stained nucleolus (ns). Note inconspicuous secretory granules (arrow) and vacuoles (v) in cytoplasm of large luteal cells. Small luteal cells (sc) are spindle-shape with lightly stained cytoplasm and darkly stained nuclei. H.E.x 560

10th Month of Pregnancy:

Capsule of the corpus luteum is thin and not as compact as observed at earlier stages of pregnancy. Connective tissues underneath the capsule is loosely arranged as compared to the previous stages. Blood vessels are also present at the junction of the capsule and connective tissues (Fig.8a).

Large luteal cells are almost sphericle in shape (Fig.8c) and lie close to blood vessels (Fig.8a). They have an average diameter of 28.64 ± 0.95 μm (range, 24.64 μm - 33.88 μm) and posses moderately convulated cell membrane (Fig.8c). Nuclei of the large luteal cells are sphericle in shape with distinct nuclear membrane, and are eccentric in position (Fig.8b & 8c). These nuclei range from 6.16 μm to 9.24 μm (average, 7.78 ± 0.35 μm) in diameter. Nuclei of the large luteal cells have sphericle, darkly stained nucleolus which are mostly situated in the centre of the nucleus. The chromatin is granulated and arranged on the inner side of nuclear

membrane (Fig.9a).

Cytoplasm of the large luteal cells at this stage is lightly stained and contain sphericle membrane-bound secretory granules. These granules are abundent in number and more conspicuous as compared to the previous stages. Furthermore, these granules are found in the paranuclear area or centre of the cell either in scattered (Fig.8c), are group form (Fig.9a). Many secretory granules are also seen adjuscent to the inner border of cell membrane (Fig.8c) and some are seen in the extra-cellular spaces (Fig.8c). Large luteal cells also posses prominent peripheral vacules in their cytoplasm (Fig.8b).

Small luteal cells are similar in shape and arrangement to that observed in the previous stages. The average length (15.78 $\pm$ 0.69 μm) and width (4.90$\pm$0.33 μm) observed at this stage are greater than any of the other stages studied. Nuclei of small luteal cells at this

stage are lightly stained and posses granular chromatin present on the inner side of nuclear membrane (Fig.9b). Cytoplasm is lightly stained and inconspicuous at this stage.

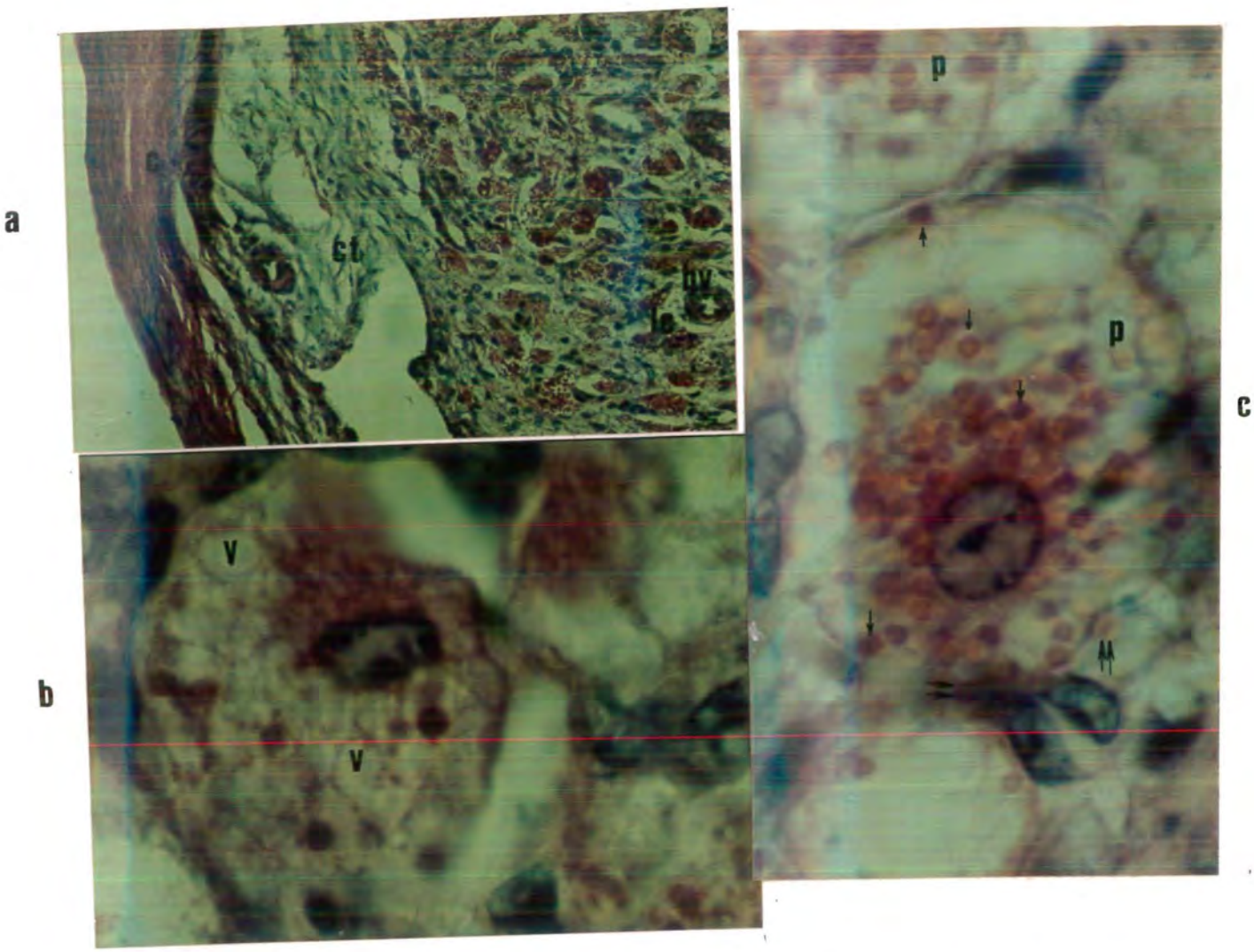

Fig.8: Photomicrograph of cross-section of corpus luteum at 10th month of pregnancy illustrating a) thin capsule (c) and loosely arranged connective (c.t.) below it. Large luteal cells (lc) are in close association with blood vessels (b.v). x56 b) Magnified large luteal cell containing prominent cytoplasmic vacuoles (v). x560 c) Higher magnification of large luteal cell showing moderately convoluted cell membrane. Prominent secretory granules (arrow) are visible in paranuclear area, adjacent to the inner side of cell membrane and in extra-cellular space (double arrow). Note light pale coloured granules (p) scattered in the cytoplasm and adjacent to the inner side of cell membrane.

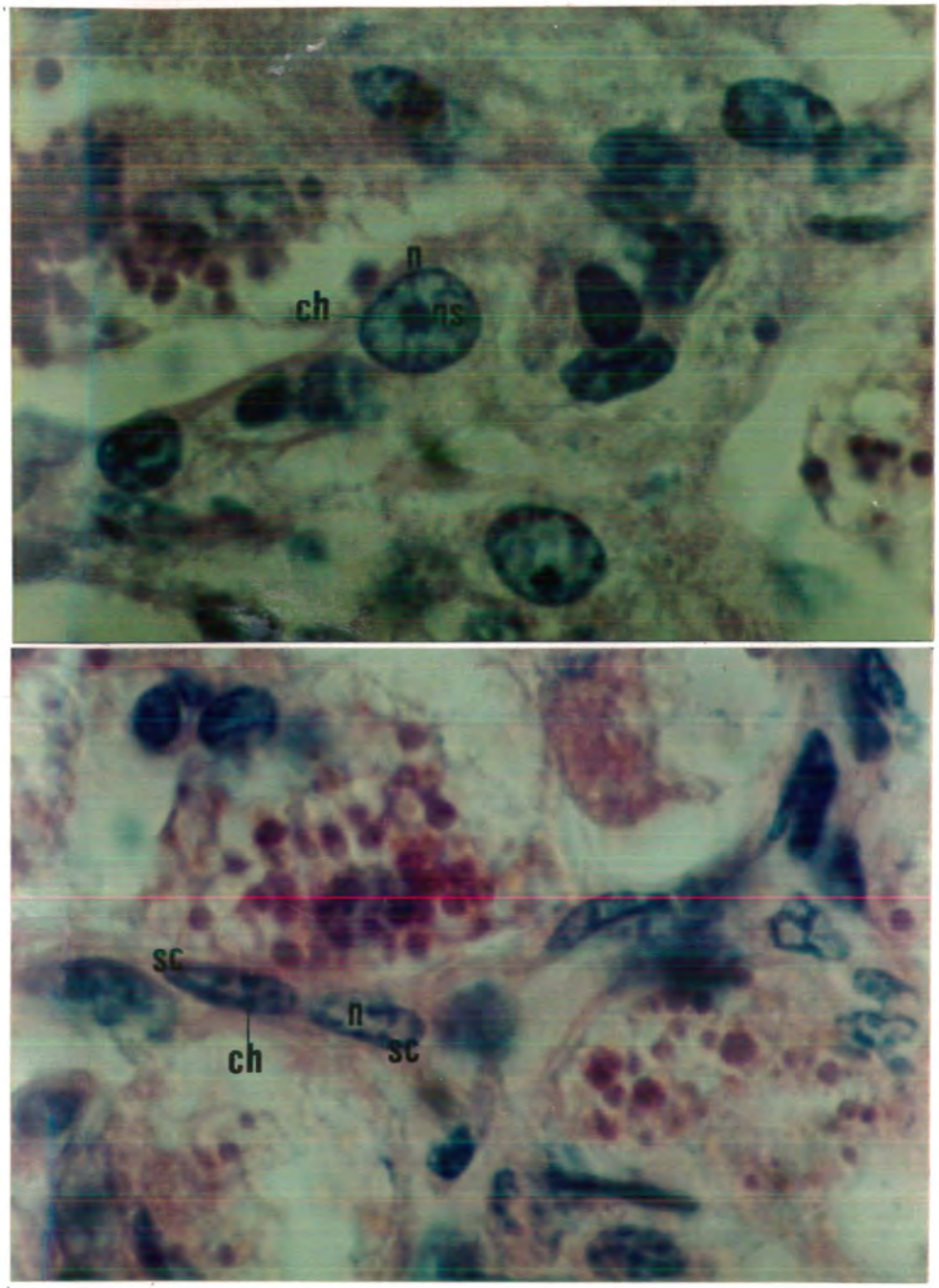

Fig.9: Photomicrograph of cross-section of corpus luteum at 10th month of pregnancy showing a) sphericle, lightly stained nucleus (n) of large luteal cell (lc) with distinct sphericle darkly stained nucleolus (ns). Note granulated chromatin (ch) on the inner side of distinct nuclear membrane (nm). b) spindle-shaped small luteal cell (sc) with lightly stained nucleus (n). Note granulated chromatin (ch) on the inner side of nuclear membrane in small luteal cell. H.E. x560

DISCUSSION

This study is carried out on 148 pregnant Nili-Ravi buffaloes of unknown age and reproductive history. The frequency of right cornual pregnancy (58.11%) in Nili-Ravi buffalo is slightly higher than reported by Vale et al. (1982), in Brazil (54.3%). In Nili-Ravi buffalo left cornual pregnancy (41.89%) is comparable to Brazilian buffaloes, 40%, (Vale et al., 1982). This indicates that right ovary is more functional than left ovary in Nili-Ravi buffalo.

The higher frequency of male foetuses (54.2%) than female foetuses in Nili-Ravi buffalo in this study is in agreement with the results reported for Murrah buffaloes (51.1%), (Rao and Murari., 1956) and Egyptian buffaloes (53.44%), (El-Mikkawi., 1956).

No case of twinning or intrauterine migration of ova is observed in Nili-Ravi buffaloes in this collection. A

very low percentage of twinning (0.057%) in Indian buffalo (Goswami and Nair., 1968) is reported compared to Egyptian buffalo, 0.63%, (Tantawy and Ahmed., 1957) and in Italian buffalo, 0.244%, (Ferrara., 1960).

Ovarian and corpus luteum characteristics like length, width, thickness, weight and volume in Nili-Ravi buffalo during pregnancy are not reported in literature. However, these characteristics are reported by Usmani et al., (1985) during oestrous cycle in Nili-Ravi buffalo. The values for various characteristics of luteal and non-luteal ovaries recorded in this study are higher than reported by Usmani et al., (1985) (Table 11).

In this study it is observed that the length ($P<0.01$), width ($P<0.001$) and weight ($P<0.001$) of luteal ovaries are significantly higher than that of non-luteal ovaries during pregnancy. However, during oestrous cycle Usmani et al., (1985) showed a significant difference in length ($P<0.01$) and weight ($P<0.01$) of luteal and non-

luteal ovaries but non-significant difference in width in them (Table 11).

The ranges for characteristics of corpus luteum (length, width, thickness, weight and volume) during oestrous cycle (Indian buffalo, Luktuke and Rao., 1962; Nili-Ravi buffalo, Usmani et al., 1985) and pregnancy (this study) are shown in Table 12. All these characteristics for corpus luteum show a higher range during pregnancy compared to oestrous cycle. This difference in corpus luteum characteristics could be due to two different reproductive states studied in Indian buffalo and Nili-Ravi buffalo.

TABLE 11

Characteristics of luteal and non-luteal ovaries during oestrous cycle and pregnancy

Ovarian Characteristics	Oestrous Cycle*		Pregnancy**	
	Ovary		Ovary	
	Luteal	Non-Luteal	Luteal	Non-Luteal
Length (cm)	2.46±0.08	2.09±0.10	2.78±0.04	2.59±0.04
Width (cm)	1.62±0.06	1.33±0.09	2.16±0.02	1.72±0.02
Weight (g)	4.5±0.03	3.10±0.03	5.40±0.10	3.43±0.04

Mean ± S.E.

* Usmani et al., (1985)

** Present study.

Table 12: Characteristics of corpus luteum during oestrous cycle and pregnancy

Corpus luteum characteristics	Oestrous Cycle			Pregnancy	
	Indian buffalo*	Nili-Ravi buffalo**		Nili-Ravi buffalo***	
	Range	Mean ± S.E.	Range	Mean ± S.E.	Range
Length (cm)	1.19 - 1.52	-	-	1.72±0.02	1.54-1.89
Width (cm)	1.08 - 1.34	-	-	1.76±0.02	1.69-1.84
Thickness (cm)	-	-	-	1.32±0.01	0.98-1.43
Weight (g)	0.72 - 1.54	1.00±0.20	0.1-3.00	2.15±0.04	1.27-2.31
Volume (cm^3)	-	-		2.18±0.04	1.50-2.41

* Luk tuke and Rao (1962)

** Usmani et al. (1985)

*** Present study

The average serum progesterone concentration recorded in this study from 1.5th to 9.5th month of pregnancy is 2.7 ± 0.15 ng/ml (range, 1.46 - 3.94 ng/ml). Progesterone concentration remains relatively constant during first (1.5th to 3rd month; 3.17 ± 0.21 ng/ml) and second trimester (4th to 7th month; 3.07 ± 1.00 ng/ml) of pregnancy, however, a significant decrease ($P<0.001$) is observed during third trimester (1.76 ± 0.16 ng/ml; $P<0.001$) compared to first and second trimesters of pregnancy. A similar pattern of plasma progesterone concentration is also reported by Gao et al., (1988) in Friesian cows in which the plasma progesterone concentration during first and second trimester of pregnancy is usually greater than those in the third trimester.

Mean serum progesterone concentration at 1.5th month (45 days) of pregnancy in Nili-Ravi buffalo is 3.94 ± 0.56 ng/ml (range, 3.28 - 5.06 ng/ml), (Table 5). This is in agreement to mean serum progesterone concen-

tration (3.71 ng/ml) reported by Chohan et al. (1992) during first 13 to 42 days of pregnancy in Nili-Ravi buffalo.Mean serum progesterone concentration during the period of 3-4 months of pregnancy (2.40 ± 0.7 ng/ml) in Indian buffalo reported by Nanda et al. (1984) is lower than investigated in this study during the same period (3.17±0.29 ng/ml).

In the late stages of pregnancy (9 to 9.5th month) serum progesterone concentration (1.88±0.17 ng/ml) recorded in this study is in agreement with Prakash and Madan (1984b) for Murrah buffalo (1.82 ng/ml) in the same pregnancy period. However, in Egyptian buffalo serum progesterone concentration at the same period of pregnancy (3.00 ± 0.2 ng/ml) is higher than in Nili-Ravi buffalo and Murrah buffalo (El-Beleley et al., 1988).

Agarwal et al. (1980) stated that overall average serum progesterone concentration in Zebu cows is 2.64±0.16 ng/ml (range, 0.34 - 6.7 ng/ml) and it remains

fairly constant throughout pregnancy. The overall average serum progesterone concentration obtained in this investi- gation is 2.7 ± 0.15 ng/ml which is in agreement with Agarwal et al.(1980) among Zebu cows. In Nili-Ravi buffalo, however, serum progesterone concentration does not remain constant throughout pregnancy but a significant decrease ($P<0.001$) occurs from 8th month onward compared to first seven months of pregnancy (Table 5).

No data are available about luteal progesterone concentration during oestrous cycle or pregnancy in buffaloes. However, some valuable information regarding the functional activity and the effects of removal of corpus luteum on pregnancy in cows is presented by Erb et al. (1967). According to Erb et al. (1967) the average weight and progesterone concentration of corpus luteum is 6.5 ± 0.5 g and 31.1 ± 1.14 μg/g respectively during the period of 11 to 284 days of pregnancy. However both of them (weight and progesterone concentration)

decline after 200 days of pregnancy in cows. A similar pattern of decline in weight (Table 3, Fig. 2) and progesterone concentration (Table 5, Fig. 3) of corpus luteum after 7th month (210 days) of pregnancy is also observed in Nili-Ravi buffalo. However, the average weight (2.15 ± 0.04 g) and progesterone concentration (27.36 ± 1.52 μg/g) observed in Nili-Ravi buffalo during the period of 1.5th to 10.5th month of pregnancy are lower than mentioned by Erb et al. (1967) in cows.

Corpus luteum is necessary for the maintenance of pregnancy during first 200 days (McDonald et al., 1953; Estergreen et al., 1967; Edquist et al., 1973) and last 30 to 40 days (Erb et al., 1967) of pregnancy in cows. However, most of the cows maintain pregnancy during 200 to 237 days of pregnancy due to adequate amount of progesterone from extra-ovarian sources in cows (Erb et al., 1967). Functional activity of corpus luteum based on luteal progesterone concentration in Nili-Ravi buffalo (this study) remains fairly constant during the

first 7 months (210 days) of pregnancy (Table 5). Luteal progesterone concentration in Nili-Ravi buffalo declines during the period of 8th to 9th month (240-270 days) followed by a progressive increase during 9.5th to 10th month of pregnancy (Table 5). This is similar to that stated by Erb et al. (1967) that during the period of 200 to 237 days of pregnancy the secretory activity of corpus luteum, indicated by a low ovarian venous plasma progesterone concentration, decreases and again increases during the last month of pregnancy in cows.

Information about serum cholesterol concentration is meagre in buffalo and mostly limited to oestrous cycle (Dhaliwal and Sharma., 1990). These estimations in Nili-Ravi buffalo have not been reported. This study shows mean serum cholesterol concen- tration as 54.67 $\pm$ 1.67 mg/100 ml during the period of 1.5th to 9.5th month of pregnancy in Nili-Ravi buffalo. Serum cholesterol concentration shows not much variation during the first 6 months of pregnancy (Table 8). However, a significant

decline ($P<0.001$) is observed in serum cholesterol concentration from 7th month onward compared to first six months of pregnancy. Serum cholesterol concentration noted during different stages of pregnancy in Nili-Ravi buffalo are less than those reported by Prakash and Tandon. (1979) in cows 5 weeks pre-partum (155 mg/dl) and during first 6 weeks post-partum (123-258 mg/dl).

Shashikumar and Dubay (1986) reported that constant utilization of cholesterol by concepts and release of high amount of cholesterol into uterine secretion (156.10 ± 11.72 mg/100 ml) under the influence of persistent high progesterone level during pregnancy may lead to low blood cholesterol level during pregnancy. These factors may also be responsible for low blood cholesterol level noted in Nili-Rivi buffalo in this study.

In the present investigation the mean luteal cholesterol concentration observed during pregnancy (1.5th to 10.5th month) in Nili-Ravi buffalo is

10.74 ± 0.31 mg/g. Luteal cholesterol concentration in Nili-Ravi buffalo remains fairly constant upto 10th month of pregnancy. A slight increase is observed at 10.5th month of pregnancy (Table 8, Fig.4) whereas luteal progesterone concentration at the same stage of pregnancy is markedly low (Table 5). A negative relationship is found between luteal progesterone and luteal cholesterol concentration ($r = -0.36$, $P > 0.1$) during the period of 1.5th to 10.5th month of pregnancy.

Histomorphological study of corpus luteum in Nili-Ravi buffalo at various stages of pregnancy indicates that it consists of morphologically two different types of cells. Large luteal cells are in close association with blood vessels (Fig.8a), sphericle (Fig.5b & 6a) or slightly elongated in shape (Fig.6c) and possess moderate to highly convoluted cell membrane (Fig.6b & 5c). These findings are similar to that observed by Fields et al. (1985) in cows during the period of 45 to 280 days of pregnancy. Large luteal cells

range in diameter from 23.12 μm to 33.88 μm (average, 27.20 ± 0.36 μm) in Nili-Ravi buffalo corpus luteum during the pregnancy period of 1.5th to 10th month. Fields et al. (1985) reported higher range for large luteal cells diameter (20-50 μm) in cows during the period of 45 to 280 days of pregnancy.

Large luteal cells in Nili-Ravi buffalo corpus luteum possess large, sphericle darkly stained nuclei which range from 5.94 μm to 9.24 μm (average, 7.21±0.14 μm) in diameter (Fig.5c). Similar findings were also reported by Parry et al. (1980) in cows during oestrous cycle, however, the average diameter of nuclei (10 μm) reported in cows by Parry et al. (1980) are greater than observed in this study. Cytoplasm of large luteal cells in Nili-Ravi buffalo corpus luteum is brightly stained and contains secretory granules. Numerous secretory granules are seen in paranuclear area of the cell (Fig.8c), or in the form of groups in the centre or periphery of the cell (Fig.9a & 9b). Many secretory

granules are also observed adjacent to the inner side of cell membrane (Fig.8c). These observations obtained in Nili-Ravi buffalo corpus luteum are identical to that studied by Fields et al. (1985) in cows during pregnancy. Parry et al. (1980) observed secretory granules in the extra-cellular space during the active phase of progesterone synthesis and release (mid-luteal phase) of oestrous cycle and suggested that they had been secreted. Secretory granules in the extra-cellular space are also observed in this study which indicates that the secretion of secretory granules also occurs in Nili-Ravi buffalo (Fig.8c). Secretory granules in the cytoplasm of large luteal cells are observed by Fields et al. (1985) as early as day 45 of pregnancy. In Nili-Ravi buffalo secretory granules are seen at 1.5th month (day 45) of pregnancy, the earliest stage studied during this investigation (Fig.5c).

Fields et al. (1985) reported that the organization of the secretory granules around nucleus, their appearent

(after 200 days of pregnancy) and Parry et al. (1980) on day 20 of oestrous cycle in cows.

Donaldson and Hansel. (1965) reported cytoplasmic vacules in large luteal cells at the active phase of progesterone synthesis and release (mid-luteal phase) during oestrous cycle in cows. In Nili-Ravi buffalo corpus luteum, similar findings regarding cytoplasmic vacuoles are observed at all stages of pregnancy studied during this investigation (Fig.5c, 6c, 7c & 8b).

Fields et al. (1985) reported spindle-shaped small luteal cells with darkly stained cytoplasm and irregularly shaped nuclei in cows during pregnancy. In Nili-Ravi buffalo corpus luteum, small luteal cells are spindle-shaped during pregnancy, however, they have darkly stained nuclei and lightly stained cytoplasm (Fig.7c) compared to that of cows. The cross sectional diameter of small luteal cells during the period of 45 to 280 days of pregnancy range from 10 to 15 μm in cows

(Fields et al., 1985). In Nili-Ravi buffalo corpus luteum (this study) the small luteal cells are more elongated and range in length and width from 10.66 to 15.78 μm (average, 12.93 $\pm$ 1.13 μm) and 3.40 to 4.90 μm (average, 4.21 $\pm$ 0.35 μm) respectively during the investigated period of pregnancy.

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