ULTRASOUND EVALUATION OF MORPHOMETRY OF BUFFALO OVARY AND CORPUS LUTEUM IN VITRO AND IN VIVO

THESIS SUBMITTED TO THE
NATIONAL DAIRY RESEARCH INSTITUTE, KARNAL
IN PARTIAL FULFILMENT OF THE REQUIREMENT
FOR THE DEGREE OF
MASTER OF SCIENCE
IN
ANIMAL PHYSIOLOGY

BY :
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B. V. Sc. & A.H.

DIVISION OF ANIMAL PHYSIOLOGY
NATIONAL DAIRY RESEARCH INSTITUTE
(I. C. A. R.)
KARNAL-132001 (HARYANA) INDIA

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By

Dr. MIHIR SARKAR

A thesis submitted to the
National Dairy Research Institute
(DEEMED UNIVERSITY)
Karnal (Haryana)
in partial fulfilment of the requirement
for the degree of

MASTER OF SCIENCE
in
ANIMAL PHYSIOLOGY

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Date: June 30th, 1993

R.S. Manik 30/6/93

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MAJOR ADVISOR & CHAIRMAN
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Dedicated to

MY FATHER

for his uncompromising principles that guided his life

MY MOTHER

for leading her children into intellectual pursuits

MY ELDER BROTHER

for his magnificient devotion to our family

SHAMPA

for making every thing worthwhile

SRIPRAKASH MOHANTY

for his magnificient contribution to our profession

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30/6/93

(DR. MIHIR SARKAR)

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CHAPTER 1

INTRODUCTION

1. INTRODUCTION

The role of water buffalo (*Bubalus bubalis*) in the development of our country as well as in the economy of Indian farming as a source of supplementary income to rural folk of India should hardly be over emphasized. An old folk ditty goes like

"Come my beloved,
Let's buy a buffalo,
For then,
We too shall be opulent"

This is true even today, because, it has been reported that the annual growth rate of buffalo population in our country is more than cattle which indicates preference of farmers in rearing this animal. They favour buffalo not only due to it's milk producing capability, but also due to other reasons like its adaptability to Indian climate and better conversion of coarse roughage. The animal has also been used as living tractor as well as for meat, since less religious sentiments are attached to this species.

Inspite of the obvious advantages of this animal, the animal suffers with certain inherit reproductive problems like, late maturity, long calving interval, poor estrous expressivity and distinct seasonal influences. One peculiarity in buffalo ovary often recorded at the time of rectal palpation is the absence of palpable corpus luteum inspite of the fact that there is evidence of animal having ovulated or cycling normally. This suggests the

the possible embedded character of the buffalo corpus luteum. Under these circumstances, it is very difficult to diagnose the stage of estrous cycle and luteal status which is important index of reproductive functioning of the animal.

The results of the modern biotechnologies like embryo transfer and *in vitro* fertilization has met with the limited success in this species when compared with cattle. The success rate is dependent upon the follicle development pattern, follicular reserve, follicle maturation etc. The research data and scientific information available so far on this animal is suggestive of the fact that there are distinct differences in physiological functioning of this species compared to that of cattle. All the time we tried to apply the knowledge available about cattle to buffalo and probably it may be one of the reasons for not being able to exploit this animal's productivity. It is now that thought is being generated to treat this species as a separate "identity". Using the scientific know-how generated and the modern biotechnologies, further attempts are being made to augment reproduction.

For the proper gynaeco-clinical assessment of each individual female in respect to its reproductive ability, it is utmost essential that one should be acquainted with normal dimensions and weight of gonads of mature female.

Current research employing exogenous hormones to modify the tissues or functions of the female reproductive tract, has focussed attention on need for more detailed and precise data on ovarian and luteal dimensions and weights in normal animals in relation to stage of estrous cycle. Further, *in vitro* maturation

of follicular oocytes and their *in vitro* fertilization results in this species needs further detailed investigation on the status of ovaries *in vitro* vs *in vivo*. Knowledge of biometry of the gonads is also necessary in artificial breeding operations and in diagnosis of infertility and in it's treatment.

The size and weight of the ovary and corpus luteum alter during the estrous cycle, according to the development and regression of graffian follicle, and the formation, development and involution of the corpus luteum. Any relationship of the size and weight of the ovary and corpus luteum to the stage of estrous cycle if established, could prove an index of diagnosing the stage of estrous cycle and reproductive functioning of the animal. Earlier attempts were only limited, to study the biometry of ovaries collected from slaughter house without focussing any attention to derive the formulae which could predict the weight of ovary and corpus luteum from it's biometry in living animal. The probable reasons for "no attempt" was that there was no means to measure the biometry of ovary and corpus luteum in living animals.

With the advent of non-invasive technique of ultrasonography now it has become possible to have such dimensions more accurately. The first attempt to visualise the intracranial structure was made by Dussik in 1942 using technique which was based on the principles of echosounding. The credit for introducing the Sonar principle into the area of medical diagnostic goes to Ian Donold, an obstetrician from Glasgow who first used it in 1958. Diagnostic ultrasound instrumentation has been made available to the medical community only since early 1970's. The development of real time or dynamic imaging in late 1970's made this powerful technology adaptable for the study of

internal reproductive tract in large domestic animals via transrectal route. The application of this technology in cattle have been attempted recently (Reeves *et al.*, 1984; Pierson and Ginther, 1984) and no attempt on buffaloes except the reports of Manik *et al.* (1992, 1992,1992).

Ultrasonic assessment of ovarian and corpus luteum biometry *in vitro* (slaughter house specimen) and *in vivo* (living animal) will generate the comparative data of ovarian status. The weight of ovary and corpus luteum in living animal will help to judge the reproductive cyclicity of the animal. Weight of the ovary is correlated to the weight of the corpus luteum, which in turn, correlated to it's secretory activity, is a valuable index of the reproductive functioning of the animal. The profiles of the mean height and width of the corpus luteum are similar to the profiles of peripheral blood progesterone concentrations during the oestrous cycle (Kastelic *et al.*, 1990). Therefore, the biometry of corpus luteum in living animal using ultrasonography will be viable alternative to determine the peripheral progesterone levels for assessment of luteal function. The present investigation, therefore, becomes necessary to investigate the areas about which no information is available, especially using the modern tool of ultrasonography with the following specific objectives.

1. To estimate morphometry of buffalo ovary and corpus luteum (*in vitro*) using ultrasonography.

2. To estimate dimensions of buffalo ovary and corpus luteum using dial calliper measurements.

3. To evaluate the relationship of ultrasonographic (*in vitro*) and dial calliper (*in vitro*) morphometry of buffalo ovary and corpus luteum to derive prediction formulae.

4. To estimate the ovary and corpus luteum dimensions using *in vivo* ultrasonography and prediction of their weight on the basis of prediction formulae derived from *in vitro* study.

CHAPTER 2

REVIEW OF LITERATURE

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2. REVIEW OF LITERATURE

Prior to the introduction of real-time transrectal ultrasonography, the morphology of reproductive organs was evaluated by rectal palpation, at surgery or after excision. Real time ultrasonography has provided a non-invasive technique to directly image, *in situ* the internal and external anatomy of reproductive organs. Morphometry of ovary *in situ*, prediction of weight of corpus luteum and ovary during the various phases of estrous cycle provide an index of reproductive functioning of the animal. Little information is available in literature regarding biometry of buffalo ovary *in vitro* and almost no in the case of *in vivo*. Therefore, with a view to funnel the existing information, the review has been classified as below:

2.1 Biometry of ovary
  2.1.1 *In vitro* study
  2.1.2 *In vivo* study
2.2 Biometry of corpus luteum
  2.2.1 *In vitro* study
  2.2.2 *In vivo* study
2.3 Corpus luteum content and progesterone production
2.4 Ultrasound study of ovary and corpus luteum

2.1 BIOMETRY OF OVARY

2.1.1 *IN VITRO* STUDY

This chapter deals with the review, where ovaries were obtained from the slaughter house and biometrical observations were taken in the laboratory.

Parkale and Hukeri (1989) studied the biometry of 128 pairs of Murrah buffalo ovaries. Biometrical observations of ovaries were taken with the help of Vernier calliper and weight using monopan balance for maximum accuracy. Ovarian measurements were recorded, length from anterior to posterior extremity, width from attached to free border and thickness as diameter between the medial and lateral surface. The length, width, thickness and weight of left ovary as reported by them averaged 24.8 mm, 16.7 mm, 14.6 mm and 3.71 gms, and for right ovary 24.4 mm, 17.4 mm, 14.6 mm and 3.77 gms, respectively. The ovarian weight in buffaloes is comparatively less than in cattle. The dimensions of ovaries from adult cows ranged between 20 to 25 mm in length, 35 to 40 mm in width and 15 to 20 mm in thickness as reported by Zemjanis (1977). There was significant variability in minimum and maximum weight of ovaries in cattle (3.9 vs 6.9 gms) and in buffaloes (2.9 vs 6.1 gms) (Ei-Wishy and Ei-Sayed, 1988).

Bhalla *et al.* (1964) studied the genital organs from 100 adult buffalo cows (*Bubalus bubalis*). They found that length of the left ovaries varies from 1.50 to 3.40 cm with a mean of 2.44 cm, width varies from 0.70 cm to 2.70 cm with a mean of 1.34 cm, height from 0.70 to 2.70 cm with a mean of 1.58 cm and weight 1.00 to 8.00 gms with a mean of 3.32 gms. The length, width, thickness and weight of right ovaries as reported by them were 2.36 cm, 1.37 cm, 1.64 cm and 3.13 gms, respectively.

In the cow, the right ovary is usually reported to be larger and heavier than the left (Runge, 1932; Okliesa, 1934; Polding and Lall, 1945; Hess and Sission, 1953; Sission and Grossman, 1956). However, in the buffalo, no significant difference is seen in the size and weights of the two ovaries when tested statistically (Damodaran, 1958; Luktuke and Rao, 1961; Parkale

and Hukeri, 1989). Damodaran (1958) while studying female genital organs of 15 non-descript nulliparous buffaloes reported that length, width, thickness and weight of left ovary as 2.38 cm, 1.58 cm, 1.23 cm and 2.73 gms and the corresponding values of right ovary were 2.28 cm, 1.52 cm, 1.20 cm and 2.56 gms, respectively

Sane *et al.* (1968) studied the 300 genital organs of adult Jaffri buffalo-cows. They reported that ovaries of Jaffri buffaloes seem to be longer and heavier than those of the other Indian breeds of buffaloes, 3.12 to 3.13 cm in length and 3.87 to 4.01 gms in weight. The length, width and height of left ovaries as reported by them were 3.13 cm, 1.44 cm, 1.15 cm and 3.87 gms and of right ovary 3.12 cm, 1.49 cm, 1.19 cm and 4.01 gms, respectively. In subsequent years, these workers studied over 4000 genital organs of adult Murrah buffalo-cows and reported that significant differences were observed at 1 per cent level in the paired observations on thickness and weight of ovaries. The average length, width, thickness, weight of left ovaries reported by them were 2.97 cm, 1.37 cm, 1.13 cm and 3.66 gms and of right ovary 2.91 cm, 1.39 cm, 1.17 cm and 3.81 gms, respectively (Sane *et al.*, 1970).

Luktuke and Rao (1961), while studying genital organs of 50 adult buffalo-cows, reported that the length, width, thickness and weight of left ovary was 2.60 cm, 1.34 cm, 1.55 cm, 3.93 gms and of right ovary 2.53 cm, 1.32 cm, 1.64 cm and 3.97 gms, respectively.

Mittal (1991) studied the morphometry of ovaries without CL on 100 samples and with CL on 72 samples. She reported that length, width, weight ($\pm$SE) of ovaries without CL was 2.14 $\pm$ 0.03

cm, 2.25 ± 0.03 cm and 3.60 ± 0.08 gms, while these parameters for ovaries with CL were 2.35 ± 0.03 cm, 2.48 ± 0.06 cm and 4.32 ± 0.14 gms, respectively.

Size and weight of the ovary alters during the estrous cycle, according to the development and regression of Graafian follicles and the formation, development and involution of the corpora lutea. A very little information regarding the alteration of size and weight of the ovary during various phases of estrous cycle is available. Luktuke and Bhattacharya (1973) studied the genital organs from 50 adult buffalo-cows. The animals from which the ovaries were obtained were classified into the following groups: (i) in heat, (ii) freshly ovulated (1 to 3 days), (iii) luteal phase (4 to 10 days), (iv) mid-estrous cycle (10 to 15 days) and (v) regressing or follicular phase (16 to 20 days). The weight of ovaries ranged between 0.70 and 5.25 gms, the ovary reaching it's maximum size during 10 to 15 days after the estrous. The mean weights of ovaries during various phases of estrous cycle as reported by them were as follows:

Stage of estrous cycle (days)	Weight of ovary with CL (gms)	Weight of other ovary (gms)
During estrous	(82) 2.35*	2.33
1 - 3	(77) 3.21	2.25
4 - 10	(32) 3.63	2.11
10 - 15	(87) 3.85	2.27
16-20	(35) 3.21	2.19

* ovary containing regressed corpus luteum
Figures in the brackets indicate the number of pairs ovaries studied

2.1.2 *IN VIVO* STUDY

This chapter deals where biometry of the ovaries were measured *in situ* (inside of animal body). The literature on the biometry of the ovaries *in vivo* is extremely scarce due to the fact that it involves use of modern tools like ultrasonography and laparoscopy.

Jainudeen *et al.* (1983) studied some Swamp buffaloes which calved between August, 1981 and January, 1982. They were maintained in paddocks of established pastures with facilities for wallowing and were fed concentrate ration during the last month of gestation and lactation. Forty five ovarian examinations were conducted on 30 suckled buffaloes between days 40 and 150 post-partum by rectal palpation followed immediately by laparoscopy to ascertain the presence of follicles and/or a corpus luteum in their ovaries. According to them, ovaries of the Swamp buffalo were ovoid and firm. They measured 2 to 3 cm in length, 1 to 1.5 cm from surface to surface and 1 to 2 cm from attachment to the free border and were located within the pelvic cavity, caudal and lateral to the uterine horns. In anestrous buffaloes, the ovaries were less than 10 mm in length, smooth, devoid of structures and with no evidence of progesterone secretion.

2.2 BIOMETRY OF CORPUS LUTEUM

2.2.1 *IN VITRO* STUDY

Ovaries with CL were collected from slaughter house and the CL was manually excised from the ovary. Then the biometry of

that CL was measured. On the basis of the morphological characteristics of the corpus luteum, many workers have classified it into different categories. The important characteristics and different classification of different stages of corpora lutea as given in the reports available in literature are reviewed here.

El-Sheikh *et al.* (1967) studied the average weights of corpora lutea on different stages of estrous cycle in Egyptian buffaloes and reported that it's weight 0.465 gms on day 1 to 2 of the estrous cycle, 1.134 gms on day 3 to 5, 1.798 gms on day 6 to 15, 0.587 gms on day 16 to 20 and 0.473 gms on day 21 to 24 of the estrous cycle.

Luktuke and Rao (1961) studied the genital organs of 50 adult buffalo-cows. They reported that length, width and weight of corpus luteum were 1.19 to 1.52 cm, 1.08 to 1.34 cm and 0.72 gms to 1.54 gms, respectively. While Polding and Lall (1945) reported that in buffalo cows, width of the corpus luteum varies from 1.3 to 2.0 cm.

Shalash and Salama (1960) studied the mean diameter and weight of corpora lutea of diestrous stage and reported that the diameter and weight of corpora lutea in diestrous was 16 $\pm$ 4.2 mm and 1.9 $\pm$ 0.5 gms, respectively. Foley *et al.* (1984) also reported that weight of diestrous corpora lutea to be 5.1 $\pm$ 1.6 gms in cattle.

Luktuke and Roy (1967) reported that corpus luteum in buffaloes on days 6, 12 and 18 of estrous cycle weighed 0.986 $\pm$ 0.09 gms, 2.28 $\pm$ 0.17 gms and 1.176 $\pm$ 0.07 gms, respectively and they observed a positive correlation between CL weight and

endocrine activity. However, the weight of corpora lutea on day 3, 5, 7, 9, 11, 13, 15, 17 and 19 of estrous cycle in virgin heifers was 0.54, 1.88, 4.06, 3.70, 5.54, 4.20, 5.27, 3.53 and 3.05 gms as reported by Mares *et al.* (1962). So from the comparison of these two studies, it is evident that there is great difference in maximum weight of corpus luteum between cattle and buffalo, when maximum weight was 5.54 gms in cattle and 2.285 gms in buffaloes.

Edwards (1962) studied the length and weight of corpus luteum in 14 cycling cattle. The respective values of length and weight were 2.30 cm and 5.71 gms. The weight of corpus luteum removed after first ovulation post-partum was 3.28 $\pm$ 1.40 gms, after second ovulation 4.56 $\pm$ 1.40 gms and after third ovulation, 6.10 $\pm$ 2.20 gms in cattle (Duby *et al.*, 1985).

Corpora lutea has been classified by Ireland *et al.* (1980) into stages I, II, III, IV corresponding to days from 1 to 4, 5 to 10, 11 to 17 and 18 to 20, respectively. The mean weights of stages I, II, III and IV corpora lutea in cattle heifers were 0.8, 8.4, 3.6 and 1.3 gms, respectively, while Donaldson and Hansel (1965) during the histological study of different stage corpora lutea reported the weight of CL increased from 1.3 to 4.6 gms on days 4 to 7 in cattle.

2.2.2 *IN VIVO* STUDY

Biometry of the corpus luteum when measured inside the body of the animal (*in situ*) has direct relevance to know the secretory activity of the animal.

Singh *et al.* (1984) studied the palpable changes of corpora lutea during different stages of estrous cycle in 31 buffalo heifers over a period of 9 month in which 83 estrous cycles of the animals were included and rectal palpation was carried out from 5 days before expected oestrous to 5 days after and on days 7, 9, 10, 11, 12 and 14 of the oestrous cycle. For measuring the size of corpus luteum, the required ovary was secured per rectum and the corpus luteum was palpated between thumbs and fingures. A Vernier Calliper was taken in the other hand. The sliding jaw of the Vernier Calliper was moved backward and forward till the distance between the two jaws coincided with the maximum size (length) of corpus luteum as felt by the inserted hand. The reading of the calliper was noted and the above procedure was repeated three times to obtain the size of CL with accuracy. The average length of the corpus luteum reported by them was 1.11 cm on day of estrus, 1.40 cm on 2 to 5 days, 1.71 cm on 6 to 8 days and 1.74 cm on 9 to 18 days of the cycle.

Different categories and characteristics of different stage corpora lutea in Swamp buffalo from a study by Jainudeen *et al.* (1983) using rectal palpation and laparoscopy indicated that CL-1 (earlier CL) on days 2 to 4 of the cycle were not palpable, CL-II (developing CL) on day 5 to 8 was soft protrusion, CL-III (mature CL) on day 9 to 16 was firm, well defined and CL-IV (regressing CL) on day 17 to 21 was hard.

2.3 CORPUS LUTEUM CONTENT AND PROGESTERONE PRODUCTION

Progesterone concentration in bovine corpus luteum was estimated (Donaldson and Hansel, 1968) by column and adsorption chromatography and spectro- photometry and they reported that progesterone content of corpus luteum increased from 38.0 to

145.0 µg from day 4 to 7 with increase in luteal weight from 1.3 to 4.6 gms but the concentration varies from 30.0 to 31.7 µg/gm tissue.

The concentration of progesterone measured by El-Sheikh *et al.* (1967) in corpus luteum of Egyptian buffaloes with spectrophotometer was 30.45, 32.22, 49.91, 20.39 and 0.00 µg/gm tissue on 1-2, 3-5, 6-15, 16-20 and 21-24 days after ovulation while progesterone concentration in CL of cattle was 24.7, 24.1, 25.8, 35.0, 33.8, 44.7, 7.0 and 14.2 µg/gm on days 3, 5, 7, 9, 11, 13. 15, 17 and 19 after ovulation (Mares *et al.*, 1962). Erb *et al.* (1960) reported that total progesterone concentration in CL of estrous cycle was highest (mean 251 µg) at 14 to 16 days following estrus in bred but non-pregnant cows. They also reported a mean concentration of 69 µg/gm of total progestins at day 2 and 3 following estrous in unbred cows on which approximately 1/3 was 20-B-ol but these mean concentrations obtained were only from three corpora lutea which had a range of 7 to 197 µg of progestin per gland. Similar pattern was reported in corpora lutea in cattle by Gomes *et al.* (1963). The hormone was quantitated by spectrophotometric method and concentration of progesterone estimated was 6.5, 34..7, 22.5, 73.2, 54.9, 32.2, 10.0 and 0.0 µg/gm on days 1, 4, 8, 13, 15, 16, 20 and 21 after ovulation.

2.4 ULTRASOUND STUDY OF OVARY AND CORPUS LUTEUM

Today, it is impossible to conceive of diagnostic unit without ultrasonography in medical science. The application of diagnostic ultrasonography to the veterinary science has not been explored extensively. Very recently, it has been proved to be an effective tool to study the ovarian status both in cattle

(Pierson and Ginther, 1987; Quirk *et al.*, 1986; Reeves *et al.*, 1984; Kastelic *et al.*, 1988) and buffaloes (Manik *et al.*, 1992, 1992, 1992).

Pierson and Ginther (1987) reported the accuracy of diagnostic ultrasonography for assessment of ovarian structures by comparing results of *in vivo* ultrasonography and slices of excised ovary. According to them, there was 100 per cent agreement between ultrasound and slicing results for identification of the corpus luteum bearing ovary and presence of cavities within the luteal glands in Holstein heifers. So diagnostic ultrasonography was determined to be a reliable method of detecting mature corpus luteum and luteal cavities in heifers.

In order to augment rectal palpation, real time B mode ultrasonography, using a 5 MHz transducer was used by Fissore *et al.* (1986) as a diagnostic aid in routine dairy herd fertility work (n = 360) and in approximately 90 other cows immediately prior to slaughter. Cows and heifers were examined during various stages of their estrous cycle over a four month period. The ultrasound images were consistent with rectal palpation and visual examination of dissected specimens.

Kito *et al.* (1986) reported the use of a transrectal real time B mode linear scanner to learn the appearance of a cavity in the corpus luteum in cows. They measured the diameter of the cavity of the corpus luteum which were not spherical by the formula

$$\sqrt{\text{Length} \times \text{Width}}$$

In nulliparous Holstein heifers, ultrasonography was used by Kastelic *et al.* (1990) to measure cross sectional areas of

corpora lutea, central luteal cavities and luteal tissue on days 2, 5, 8 and 11 and daily on days 14 to 21 (pregnant heifers, n = 7) or day 14 to the day of the subsequent ovulation (non-bred and bred pregnant heifers, n = 7 and n = 8). A blood sample for progesterone assay was collected prior to each ultrasound examination. Combined for the three reproductive statuses, luteal tissue area and plasma progesterone concentration increased over days 2, 5, 8 and 11. Luteal tissue area and plasma progesterone concentration over days 19 and 17 were significantly lowered respectively, in non-bred and non-pregnant heifers than in pregnant heifers. According to them, utrasonic assessment of the corpus luteum is a viable alternative to the determination of peripheral progesterone levels for assessment of luteal function in nulliparous Holstein heifers.

Quirk *et al.* (1986) studied the size of corpora lutea using a B mode real time linear scanner array with 5 MHz transducer designed transrectally in heifers undergoing spontaneous and PGF_2-alpha induced luteolysis. According to them, heifers treated with PGF_2 alpha, the size of CL (width x length; mean $\pm$ SD) decreased from 16.5 $\pm$ 1.9 x 26.0 $\pm$ 1.8 mm before PGF_2 alpha to 13.7 $\pm$ 2.9 x 22.3 mm and 12.2 $\pm$ 3.1 x 20.0 $\pm$ 2.8 mm at -2 and -1 days from estrous respectively. In heifers undergoing spontaneous luteolysis, the size of CL decreased from 16.8 $\pm$ 2.8 x 25.8 $\pm$ 2.6 mm on day 15 of the cycle to 12.4 $\pm$ 3.2 x 20.4 $\pm$ 3.9 mm at -2 days from estrous. Decrease in the size of CL in each heifer was coincident with decrease in plasma progesterone concentration.

Using a linear array ultrasound scanner with a 5 MHz transducer, Pierson and Ginther (1984) studied the follicular and luteal status in heifers. The ovaries of five heifers were

monitored by inserting 5 MHz transducer into rectum, removing faecal material, moved along the dorsal surface of the uterine horns, them moved laterally to examine the ovaries. The reproductive tract was not manipulated directly before or during the ultrasound examination. All heifers were examined for a period from three days before an ovulation to three days after the next ovulation. There was a significant difference among days for diameter of the largest follicle and second largest follicle and for the number of follicles 4-6 mm and > 10 mm. The CL became visible approximately three days after ovulation and was identifiable throughout the rest of inter- ovulatory interval. In two of the five heifers, the corresponding corpus albicans was identified for three days after the second ovulation. Two heifers were induced to superovulate and follicular growth was monitored. The ultrasound instrument was judged effective for monitoring and evaluating ovarian follicles and CL in normal and superovulated heifers.

Using ultrasonography during the estrous cycle, no intra-ovarian relationships were found between the ovary containing the corpus luteum and the ovary containing the dominant follicle (n= 165) or between the location of CL and the characters of the dominant follicles (Pierson and Ginther, 1987).

Perry *et al.* (1991) used transrectal ultrasonics to monitor follicle size, ovulation and formation and regression of the CL in 13 Hereford x American Angus sucking cows during resumption of estrous cycles. They reported that concentration of progesterone in serum and diameter of the corpus luteum were similar in first 4 days after 1st and 2nd ovulations. In contrast, from 5 to 8 days after ovulation, serum progesterone was higher and corpus luteum was larger after 2nd than after 1st ovulation.

Adams *et al.* (1991) using transrectal ultrasonography monitored daily the presence and diameter of the corpus luteum for a minimum of 30 days in 59 Llamas. According to them, corpus luteum diameter was significantly correlated (0.83) with plasma progesterone concentration. The corpus luteum reached the maximum diameter on day 5.9 $\pm$ 0.3 and 21.4 $\pm$ 1.2 respectively and the maximum diameter was 12.8 $\pm$ 0.3 and 16.3 $\pm$ 0.3 mm.

Pieterse *et al.* (1990) compared the accuracy of rectal palpation and transvaginal ultrasonography with a 5 MHz linear array for the detection of corpora lutea and follicles in 59 pluriparous cows of unknown reproductive history. The rectal palpation was first carried out to judge the presence of follicles of more than 5 mm diameter and corpora lutea which were classified as young (days 1 to 4), mid cycle (days 5 to 16) or old (days 17 to 21) according to morphological criteria. The cows were then examined for follicles and corpora lutea by ultrasonography and the corpora lutea were classified again directly as young, mid cycle or old according to their appearance. For the detection of mid cycle corpora lutea, the sensitivity and predictive value of rectal palpation were 83.3 per cent and 73.2 per cent and for ultrasonography the sensitivity and predictive value were 80.6 per cent and 85.3 per cent, respectively.

CHAPTER 3

MATERIALS AND METHODS

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3. MATERIALS AND METHODS

3.1 BASIC PRINCIPLES OF ULTRASONOGROPHY

WHAT IS ULTRASOUND

The normal range of sound that human being can perceive is 20 to 20,000 Hz. A sound wave with a frequency higher than 20,000 Hz is called ultrasound.

3.2 FREQUENCY OF ULTRASONIC WAVES

Frequency is understood to mean the number of vibrations (cycles) per second and it's unit is Hertz.

1 MHz = 1 million Hz

3.3 ULTRASONIC PRINCIPLE

Similar to the way Sonar is used to map the ocean floor (Fig. 1), ultrasonography use high frequency sound waves to produce images of soft tissues and internal organs.

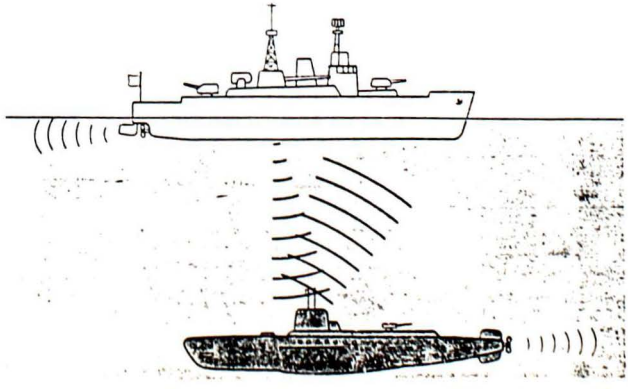


Fig. 1 Sonar, an application of ultrasound.

Fig. 2 shows scheme of the principle. When electric current is applied to each side of a piece of quartz coated with silver, the quartz expands or contracts from its original thickness depending upon polarity of current applied. This phenomenon is called the "Piezoelectric effect" and substance with this property is called Piezoelectric element. When alternating current is applied to each surface of the Piezoelectric element, ultrasound is produced. When the Piezoelectric element is physically compressed by externally applied ultrasound, it produces current. Hence, the Piezoelectric element serves as dual function as both transducer and receiver. Tissues have ability to either propagate or reflect back sound waves to varying degrees. The proportion of sound beams that is reflected or echoed is received by the same Piezoelectric crystals in the transducer, converted to electric impulses and displayed on ultrasound screen as series of grey dots.

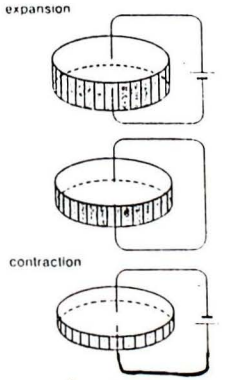


Fig. 2 Piezoelectric effect. A piezoelectric element changes its thickness when an electric current is applied.

3.4 TRANSDUCER

The part of the ultrasonographic equipment which transmits and receives ultrasound on the tissue surface is called as transducer head. Transducer of various frequencies are available

and are selected on the basis of size and location of structure of interest. The most commonly used frequencies in large animals reproduction are 3.5, 5.0 and 7.5 MHz. High frequency provides greater detail, whereas low frequency provides greater tissue penetration.

3.5 ULTRASONOGRAPHIC EQUIPMENT

Most ultrasound scanners used today in reproductive research are B-mode (brightness modality), real time scanner. Here, the image is two-dimensionsal display of dots.

3.6 WHAT IS REAL TIME

If the information from the examination is displayed on the monitor without significant delay, it is called real time. In other words, it refers to the live or moving display in which echoes are recorded and refreshed continuously. Two types of real time, B-mode scanner in veterinary markets are available:

1. Linear array scanner
2. Sector scanner

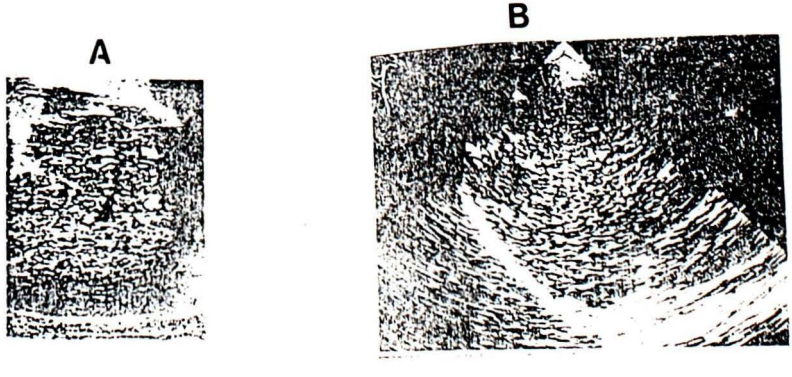


Fig. 3 Ultrasound beam pattern. A. Beam pattern of linear scan. B. Beam pattern of sector scan.

In linear array scanner, the sound waves are emitted perpendicular to the transducer along the row of crystals. The waves produced by each crystal travel parallel to those produced by neighbouring crystals in a linear array. Therefore, resulting image is rectangular.

Sector scan also applies the same principle as linear but the transducer surface is smaller. Beam pattern in such scan is shown in Fig. 3.

3.7 EXPERIMENTAL PROCEDURES IN PRESENT INVESTIGATION

3.7.1 COLLECTION AND TRANSPORT OF OVARIES

The ovaries were collected from the slaughter house which is 130 km from the laboratory. The samples were brought to the laboratory in sterile saline (32^{o} to 37^{o}C) in thermos flask. Ovaries were collected irrespective of age, body condition, season and reproductive status. The ovaries were removed within 2 h of slaughter of animal and transported to lab within 5 to 6 h of slaughter.

3.7.2 SAMPLE PREPARATION

The ovaries were washed in sterile saline and were trimmed clean of any extrenous tissue to have the accurate sonographic image and dimensions.

3.7.3 *IN VITRO* OBSERVATIONS ON OVARIAN BIOMETRY

A total number of 254 ovaries without CL and 107 with CL were taken for the study.

3.7.3.1 Physical methods

The three measures, viz., length, width, height were recorded using dial calliper in millimetre. The length was measured from the anterior to the posterior extremity. The width was measured as the greatest diameter between the lateral and medial surfaces. The height was measured from the attachment to the free border.

The volume was measured according to Archimedes principle. Clean ovaries containing no water drops on its surface were immersed in a measuring cylinder containing fixed volume of water and then the water dispersed by the ovary was taken out by an automatic pipetter and that volume of water gave the volume of ovary. Weight was measured by electrical balance (OHAUS, USA) which weighs upto four digit after decimal.

3.7.3.2 Ultrasound method

The biometry of the same ovaries were measured using ultrasound machine.

3.7.3.2.1 Equipment

Linear array "Tokyo Keiki, LS-1000".

3.7.3.2.2 Transducer frequency

5 MHz

3.7.3.2.3 Water bath method

Ultrasonic sound waves of high frequency can not transmit through air as it is completely reflected by air. So, in water bath technique ovaries were kept immersed in a water bath and transducer is held over the ovary in angle as desired to get clear image. To measure the length and height, the transuducer was held over the ovary in the water bath along the length of the ovary. The transducer was rotated at an angle of 90^{o} to obtain height and width.

3.7.3.2.4 Measurements

The freeze key of the ultrasound instrument was used to freeze the current real time image. In freeze mode the indicator lamp of the key is illuminated. Freezing of image is very important for measurements, detailed study of the ultrasound information and for photographic documentation.

The LS-1000 is equipped with a dual calliper system. Two pairs of cursors are available, the first one consists of a large and a small '+' sign, the second one of a large and a small 'x' sign. They can be moved freely within the ultrasound display by means of the joy stick. The distance between the two sets of callipers is read out at the left side of the screen as follows:

Distance: + ... + = mm

x ..... x = mm

3.7.3.2.5 Volume

The volume of the ovary was calculated from the length (D_1),

width (D_2) and height (D_3) measured through ultrasound technique by the following formula:

$$\frac{4}{3} \pi \times \frac{D_1}{2} \times \frac{D_2}{2} \times \frac{D_3}{2} \times \frac{1}{1000} \text{ (c.c.)}$$

3.7.3.2.6 Image documentation

Representative samples of ovaries were photographed as such keeping a scale and ultrasound images of the same ovaries were taken with the help of camera attached with the machine.

3.7.4 *IN VITRO* OBSERVATION ON CORPUS LUTEUM

The biometry of 87 corpora lutea were measured both by physical and ultrasound method.

3.7.4.1 Ultrasound method

3.7.4.1.1 Equipment

Tokyo Kekei LS-1000.

3.7.4.1.2 Transducer frequency

5 MHz

3.7.4.1.3 Measurements

The length, width and height were measured using water bath technique as detailed earlier in section 3.7.3.2.4. Volume was calculated using the same formula (section 3.7.3.2.5).

3.7.4.2 Physical method

The corpus luteum was manually removed carefully from the ovary with the help of blade and forceps. Using dial calliper, the length of the corpus luteum was measured from free surface to embedded end along the long axis. Width was the diameter of the

corpus luteum. Height was the 3rd dimension of the corpus luteum. Volume was measured by Archimedes principle discussed earlier in section 3.7.3.1. Weight was measured by electrical balance as discussed under section 3.7.3.1.

3.7.4.3 Image documentation

Some representative samples were photographed as such keeping a scale and ultrasound images of the corpus luteum were also taken by the method discussed earlier (3.7.3.2.6).

3.7.5 STATISTICAL ANALYSIS

The mean, standard error were calculated for various dimensions of the ovary and corpus luteum.

3.7.5.1 Correlation coefficient

The correlation coefficient between dimensions of ovaries (with and without corpus luteum), corpus luteum measured by dial calliper and ultrasonography were calculated by the method suggested by Snedecor and Cochran (1967).

$$r = \sqrt{\frac{\left(\Sigma X.Y - \frac{\Sigma X \cdot \Sigma Y}{n}\right)^2}{\left(\Sigma X^2 - \frac{(\Sigma X)^2}{n}\right)\left(\Sigma Y^2 - \frac{(\Sigma Y)^2}{n}\right)}}$$

$$S.E.\ (r) = \sqrt{\frac{1 - r^2}{n - 2}}$$

Significance of correlation coefficient was tested by 't' test:

$$t = \frac{|r_{yx} - 0|}{\sqrt{\frac{1 - r^2_{yx}}{n - 2}}}$$

3.7.5.2 Regression method

Various prediction equations to calculate weight of the ovaries and CL from various dimensions, viz., length, width, height and volume were formulated by regression method.

The following equation was used to predict the value of dependent parameter (Y) on the value of independent variable (X) (Amble, 1975).

$$\hat{Y} = a + b X$$

where,

$\hat{Y}$ = predicted value of dependent trait

a = a constant = $\bar{Y} - b\bar{X}$

$\bar{Y}$ = average value of observations on Y

$\bar{X}$ = average value of observations on X

b = regression coefficient of Y on X

$$b = \frac{\Sigma XY - \frac{\Sigma X \cdot \Sigma Y}{n}}{\Sigma X^2 - \frac{(\Sigma X)^2}{n}}$$

$$S.E.(b) = \sqrt{\frac{(\Sigma Y^2 - \frac{(\Sigma Y)^2}{n} - b\,(\Sigma XY - \frac{\Sigma X . \Sigma Y}{n})}{(n-2)\,(\Sigma X^2 - \frac{(\Sigma X)^2}{n})}}$$

Significance of regression coefficient was tested by 't' test:

$$t = \frac{b}{S.E.\ of\ b}$$

The reliability of the prediction equations was tested using 'F' test.

3.7.6 *IN VIVO* ULTRASONOGRAPHY

To study the various measurements of the ovaries (with and without corpus luteum) and corpus luteum, viz., length, width, volume and weight during different phases of estrous cycle, *in vivo* ultrasonography was performed.

3.7.6.1 Animals

Twenty Murrah buffaloes of 5 to 6 years, apparently free from all clinical abnormalities were selected for present investigation. The body weight of experimental animals ranged between 400 to 500 kg. The animals were maintained in a good managemental condition in the Embryo Biotechnology Centre, National Dairy Research Institute, Karnal.

3.7.6.2 Synchronization of oestrous

Rectal palpation was carried out to detect the presence of corpus luteum in the ovaries of the animals. The animals which contained mature corpus luteum were selected and 25 mg of PGF_2 alpha (Lutalyse, Upjohn, Belgium) was administered intramuscularly and the animals examined for oestrous after 72 hrs. The estrus day was taken as day 0 of the cycle.

3.7.6.3 Equipment

Tokyo Keiki-LS 1000.

3.7.6.4 Transducer frequency

5 MHz

3.7.6.5 Transrectal technique

3.7.6.5.1 Preparation of Animal

Preparation of animal for ultrasound examination was very important. Excessive movement of the animal interferes with the interpretation of ultrasound image. So proper facilities to restrain the animal were adopted. Some animals by its nature were very restless and might prove to be dangerous to both the operator and the machine. Prior to the ultrasound examination, proper evacuation of the rectum was performed as faeces would prevent the good contact between probe and rectal mucosa. There is every chance of entry of air to the rectum during back-racking and if it was allowed, presence of air between the acoustic face and the rectal mucosa would greatly reduce the image quality. So proper care during evacuation of rectum was taken. The back of the animal was washed with water and tail was tied properly to facilitate ultrasound examination.

3.7.6.5.2 Installation of machine

The machine was brought near the animal. The LS-1000 has been designed and constructed to ensure maximum safety for both animal and operator but as with any electrical device, appropriate care like extra earthing was taken in handling the unit. There are three gain controls in the machine which were properly adjusted to compensate for tissue attenuation so that similar echoes from similar interfaces are all displayed with the

same intensity regardless of the depth of a reflecting structure. The machine was placed at the right side of the animal for easy handling. Then the equipment was connected to uninterrupted power supply unit to provide uniform electric current.

3.7.6.5.3 Technique

In buffalo, the uterus is coiled and tortuous and the shape varies with the stage of the estrous cycle. To scan the entire ovary, ultrasonic anatomy was fully conceived. After the proper evacuation and restraining of the animal, rectal palpation was done to locate the position of both the ovaries. The acoustic face of the transducer was covered with coupling gel and was inserted into the rectum. After locating the cervix, the transducer was moved over the dorsal surface of the uterine body and moved slightly from side to side. When the corpus cornual junction reached, the transducer was moved over the dorsal surface of one uterine horn so that the horn is examined in oblique planes. Then the transducer was rotated laterally to examine the ovary. The other ovary was then similarly examined.

3.7.6.5.4 Image quality and examining condition

The production of the ultrasound image and the interpretation of the image by the operator was found to be an interaction of four factors: (1) operator, (2) scanner, (3) environment, and (4) animal. Considerable operator experience is essential for the detection of subtle structures and difference between tissues. The transducer must be manipulated so that the entire ovary is examined. A high quality probe, preferably with at least a 5 MHz frequency, is essential. The scanner should be positioned close to the operator and at eye level to facilitate

same intensity regardless of the depth of a reflecting structure. The machine was placed at the right side of the animal for easy handling. Then the equipment was connected to uninterrupted power supply unit to provide uniform electric current.

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viewing and adjustments, but protected from potential damage. An electric outlet free from electronic interference must be available. The intensity of ambient light should be dim to reduce reflections and to avoid excessive brightness or contrast settings on the viewing screen. The animal must be well restrained as excessive movement will interfere with interpretation of the ultrasound image.

3.7.6.6 Measurements

Measurement mode and freeze mode has been discussed previously (3.7.3.2.4). With the help of these two modes, length and width of the ovaries with and without corpus luteum and of corpus luteum was taken on day 0, day 5, day 12 and day 18 of the estrous cycle. The measurements were recorded in millimeters.

3.7.6.7 Volume and weight

Volume and weight of the ovaries and corpus luteum were calculated with the help of prediction formula.

3.7.6.8 Image documentation

Some representative samples of ovaries and corpus luteum were photographed to record ultrasound images to show the size difference during various phases of estrous cycle.

3.7.7 MAINTENANCE AND CLEANLINESS OF EQUIPMENT

For removing stubborn dirt spots, first the unit was disconnected from the main power source. Loose dust accumulated on the outside of the unit was removed with a soft cloth. For

cleaning the monitor screen, soft non-linting cloth dampened in pure alcohol was used. The scan heads contain delicate ceramic crystals which may be damaged by mechanical shock or fall. Therefore, scan head was always handled with appropriate care. For cleaning of the scan head, ethanol was used.

3.7.8 STATISTICAL ANALYSIS

Mean and standard errors of the various measurements of ovaries and corpus luteum were calculated during different phase of estrous cycle. To see whether there is any significant difference or not between the biometry of left and right ovary, paired 't' test was performed using the following formula.

$$t = \left| \frac{\bar{d} - 0}{\sqrt{\frac{S^2 d}{n}}} \right|$$

Frequency distribution of dimensions of ovaries and corpus luteum was also done.

CHAPTER 4

RESULTS AND DISCUSSION

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4. RESULTS AND DISCUSSION

4.1 *IN VITRO* STUDY

4.1.1 VALIDATION OF ULTRASONIC MEASUREMENT OF OVARY AND CORPUS LUTEUM

The dimensions of ovaries and corpus luteum, viz., length, width and height were measured using physical (dial calliper) and ultrasound method (water bath). Results of the two methods were compared.

There was a linear relationship between length, width and height measured using ultrasound machine and dial calliper for ovaries with and without corpus luteum (Fig. 4). The equation for length was $Y_1 = -0.13 + 1.002\ X_1$ and $Y_2 = -0.144 + 0.99\ X_2$ ($r_1 = 0.99$ and $r_2 = 0.97$; $P < 0.01$). The corresponding values for width were $Y_1 = 3.92 + 0.811\ X_1$ and $Y_2 = -0.18 + 0.99\ X_2$ ($r_1 = 0.86$ and $r_2 = 0.98$; $P < 0.01$). The respective equations for height were $Y_1 = -0.48 + 1.02\ X_1$ and $Y_2 = 0.105 + 0.97\ X_2$ ($r_1 = 0.97$ and $r_2 = 0.97$; $P < 0.01$). Here, Y_1 and Y_2 = length, width, height of the ovary with and without corpus luteum measured by physical method; X_1 and X_2 = length, width and height of ovary with and without corpus luteum measured by ultrasound method.

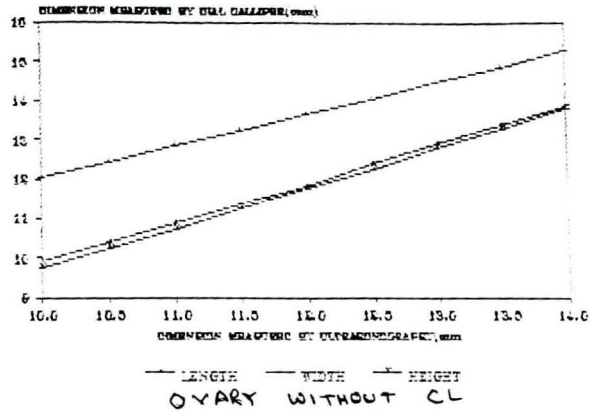
Similarly, a linear relationship between biometry of corpus luteum (length, width and height) measured by two methods (physical vs ultrasound) was observed (Fig. 4). The respective equations are given for length ($Y_1 = 0.66 + 0.97\ X_1$; $r_1 = 0.98$;

$P < 0.01$), width ($Y_2 = 0.44 + 0.94\ X_2$; $r_2 = 0.96$; $P < 0.01$) and height ($Y_3 = -0.45 + 1.006\ X_3$; $r_3 = 0.96$; $P < 0.01$), where Y_1, Y_2 and Y_3 = length, width and height of corpus luteum measured by physical method and X_1, X_2 and X_3 = length, width and height of corpus luteum measured by ultrasound method.

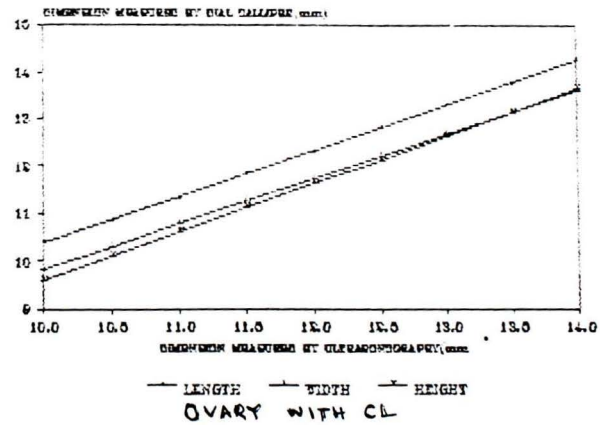
No report is available to compare these results in buffaloes. However, present study confirms that ultrasonic measurements are very close to the actual measurements of ovary and corpus luteum. The little difference may be attributed to the experience of operator, sample preparation (trimming of tissues, etc.) and technique in itself. In agreement, Quirk *et al.* (1986) and Guilbault *et al.* (1991) studied not with biometry of ovary and corpus luteum but with the follicular diameter and confirmed the ultrasonic validation when diameter of the antral cavity measured by ultrasonography and the diameter of the same follicles measured after dissection.

Pierson and Ginther (1988) similarly confirmed the accuracy of diagnostic ultrasonography for assessment of ovarian structures by comparing results of *in vitro* ultrasonography and slicing the excised ovaries. They used a 5 MHz transducer and a high quality scanner. Heifers were slaughtered on day 12 or 14 within 4 hours of ultrasound examination. Then follicular quantitations were made during slicing without prior knowledge of the ultrasound results. High correlation coefficient (0.80 to 0.97) between these two methods and 95 per cent confidence intervals were obtained which indicated that the ultrasound measurements overestimated the number of follicles 2 to 3 mm and $>$ 2 mm by approximately one follicle and this overestimation was slight. Zero was centered within the confidence intervals for number of follicles $\geq$ 4 mm, $\geq$ 7 mm and $\geq$ 11 mm. These results

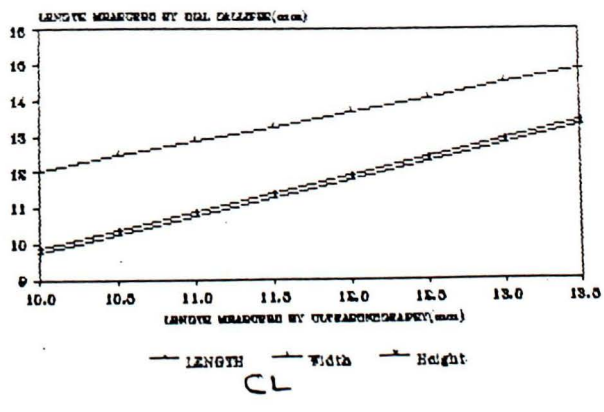
Fig.4 Relationship between dimensions of ovary and CL measured by ultrasonography and dial calliper.



OVARY WITHOUT CL



OVARY WITH CL



CL

demonstrated that diagnostic ultrasonography was a reliable method of identifying and measuring follicles in heifers.

4.1.2 ULTRASONIC IMAGING OF OVARIES

The technically correct, useful ultrasound image production and its interpretation involves the great role of operator and soundness of the system. The characteristic of a tissue determine what proportion of the sound beam are reflected. The reflected portion is represented on the ultrasound image by shades of grey dots, extending from black to white. With this in view, representative samples of ovaries were photographed (coloured) and ultrasound image of same ovaries were taken to have comparative evaluation (Fig. 5). Ovarian border could be distinguished from other ecogenic area which were of different acoustic impedence, thereby, revealing the difference in grey colour and making the image identification possible. The sonographic images were taken by placing the ovaries in water bath. The water should be perfectly clean, stationary, transparent and the bottom of the water bath should be non reflecting to minimise side lobe artifacts, to avoid confusing image. The placement of the transducer, i.e., angle and distance of it from the ovary in the water bath was an art of the operator which determines quality of image, accuracy of proximation of the size of image with that of actual size.

4.1.3 BIOMETRY OF OVARIES

Biometry of the ovaries (with and without corpus luteum) was studied to assess and compare the changes in ovarian dimensions by physical and ultrasound measurements and to develop the formulae by which one can predict the weight of ovary using these

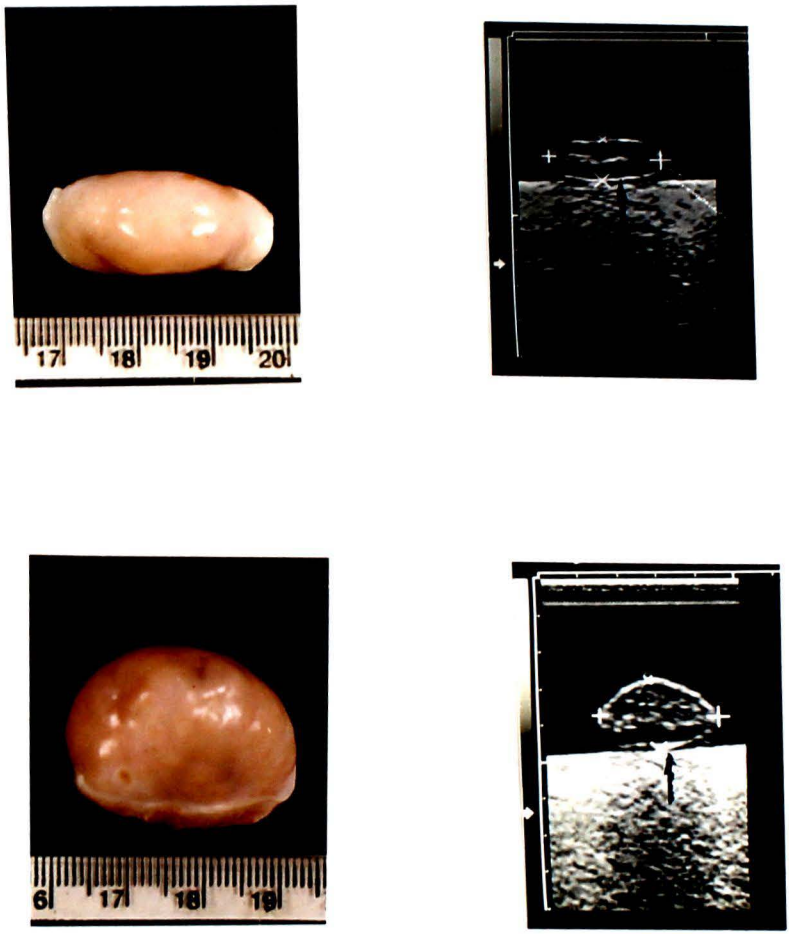


Fig. 5 Photograph showing ovary as such (with scale) and their respective ultrasound image taken using water bath. The ovarian border/stroma has been indicated by arrows.

dimensions. This may facilitate in diagnosing the ovarian status for various reproductive purposes including *in vitro* fertilization.

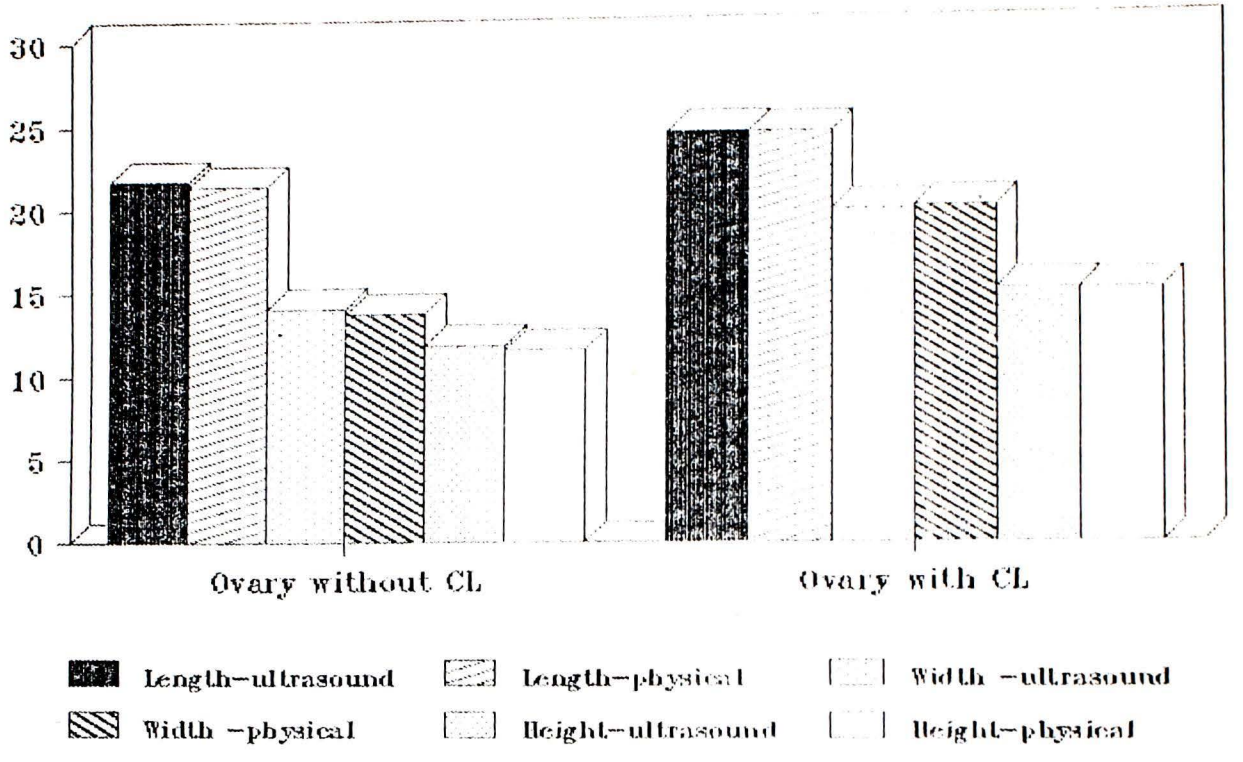
Biometry of the ovaries without CL was done on 254 samples and for ovaries with CL, 107 samples were taken. The average values for length, width, height and weight obtained by physical and ultrasound methods for ovaries with and without CL have been presented in Table 1.

Perusal of data showed very little difference between ultrasound measurements and dial calliper values for both type of ovaries, i.e. with or without CL (Fig. 6). The average values for length, width and height were 21.43 $\pm$ 0.23, 13.67 $\pm$ 0.18 and 11.47 $\pm$ 0.12 mm using dial calliper, respectively, for ovaries without CL. The corresponding values for ultrasonic measurements were 21.72 $\pm$ 0.23, 13.99 $\pm$ 0.18 and 11.73 $\pm$ 0.12 mm. Ovaries with CL had increased dimensions which were quite obvious due to the size of luteal tissue alongwith the ovary and these differences were statistically significant ($p < 0.01$). The mean values using dial calliper were 24.38 $\pm$ 0.42, 19.95 $\pm$ 0.28 and 14.90 $\pm$ 0.23 mm and ultrasound method were 24.45 $\pm$ 0.41, 19.75 $\pm$ 0.29 and 14.94 $\pm$ 0.22 mm. The weight was also more for ovaries with corpus luteum (4.07 $\pm$ 0.12 g) in comparison to ovary without corpus luteum (2.14 $\pm$ 0.06 g). The data when subjected to statistical analysis showed highly significant difference ($P < 0.01$). These results are in agreement with the findings of Luktuke and Bhattacharya (1973) who reported the average weight of functional ovaries with a corpus luteum (4.1 g) and ovaries with developing follicle but without luteal activity (2.4 g). Whereas, Mittal (1991) observed mean weight of ovaries with CL (4.32 g) and without CL (3.60 g). The dimensions and weight of

Table 1. Mean biometry of ovary by physical and ultrasound method *in vitro*

Particular	Sample size	Technique	Length (mm)	Width (mm)	Height (mm)	Weight (by electrical balance) (g)
Ovary without corpus luteum	254	Ultrasound method	21.72 ± 0.23	13.99 ± 0.18	11.73 ± 0.12	2.14 ± 0.06
		Physical method	21.43 ± 0.23	13.67 ± 0.18	11.47 ± 0.12	
Ovary with corpus luteum	107	Ultrasound method	24.45 ± 0.41	19.75 ± 0.29	14.94 ± 0.22	4.07 ± 0.12
		Physical method	24.38 ± 0.42	19.95 ± 0.28	14.90 ± 0.23	

Fig.6.Biometry(mm.) of ovaries compared using ultrasound and Physical method.
In Vitro.



buffalo ovaries are less when compared with cattle. El-Wishy and El-Sayed (1988) made examination of 248 native cows and 658 buffaloes with unknown history. The average minimum and maximum weights were 2.9 g and 6.1 g in buffaloes compared to 3.9 g and 6.9 g in cattle.

Mittal (1991) reported the length and width of ovaries without CL (2.25 ± 0.03 cm and 2.14 ± 0.03 cm) and for ovaries with CL (2.48 ± 0.06 cm and 2.35 ± 0.03 cm) measured by Vernier Calliper which were quite comparable to present findings. Other workers also reported the length, width and thickness with the range of 2.3 to 3.1 cm, 1 to 2 cm and 1 to 1.59 cm in buffaloes (Jainudeen *et al.*, 1983; Sane *et al.*, 1970; Bhalla *et al.*, 1964; Damodaran, 1958; Luktuke and Bhattacharya, 1973; Parkale and Hukeri, 1989).

The average volume of ovaries with and without CL measured by Archimedes principle and ultrasound method has been presented in Table 2. The values were same (3.86 ± 0.11 vs 3.86 ± 0.11) in case of ovaries with CL. However, little difference (0.04) was observed for ovaries without CL (Fig. 7). There was highly significant ($P < 0.01$) relationship for the estimates by these two methods as depicted by correlation coefficient value (Table 2). This further emphasizes the accuracy of ultrasound measurement in relation to actual.

4.1.4 FREQUENCY DISTRIBUTION OF OVARIAN DIMENSIONS

There was a great variation between each dimensions of the ovaries with and without CL. The length, width and height of the ovaries without CL ranged from 14.8 to 34.9 mm, 6.8 to 23.8 mm and 7.5 to 19.1 mm, respectively (Table 3) when measure by dial

Table 2. Mean measurement of volume (c.c.) by physical and ultrasound methods *in vitro*

Particular	Sample size	Technique		Correlation coefficient
		Ultrasound method	Physical method	
Ovary without corpus luteum	254	1.96 ± 0.06	2.00 ± 0.06	0.99**
Ovary with corpus luteum	107	3.86 ± 0.11	3.86 ± 0.11	0.98**

** Significant ($P < 0.01$)

Fig.7 Volume(cc) of ovaries compared using ultrasound & physical method.
In Vitro.

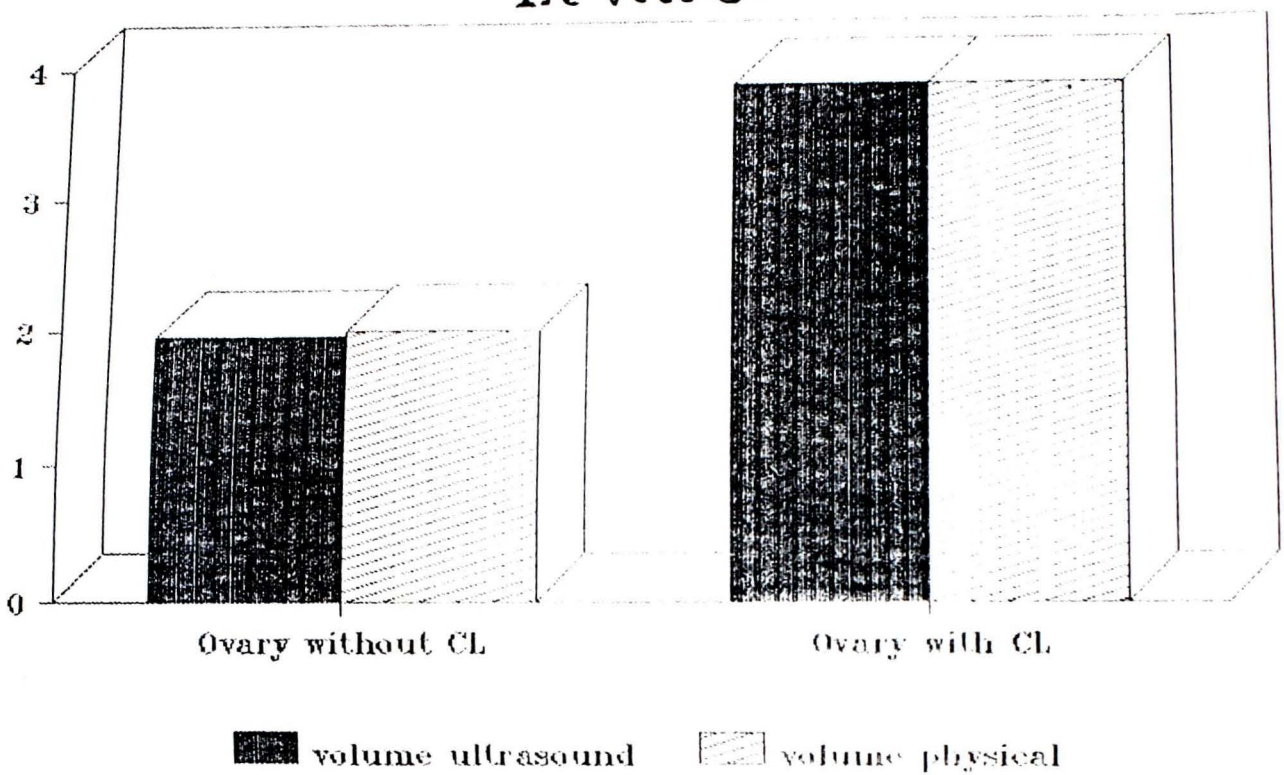


Table 3. Frequency distribution of ovarian (without corpus luteum) dimensions

Class interval	Based on physical method			Based on ultrasound method		
	Length	Width	Height	Length	Width	Height
	(%)					
4 - 7	-	0.29	-	-	-	-
7 - 10	-	4.90	18.10	-	2.35	14.20
10 - 13	-	35.43	57.66	-	28.74	57.08
13 - 16	4.07	40.94	20.32	2.91	40.94	24.40
16 - 19	19.53	11.80	3.14	16.35	14.98	3.93
19 - 22	39.25	3.14	0.78	35.09	7.48	0.39
22 - 25	21.79	3.50	-	25.59	5.51	-
25 - 28	10.80	-	-	14.17	-	-
28 - 31	2.21	-	-	2.75	-	-
31 - 34	1.96	-	-	1.96	-	-
34 - 38	0.39	-	-	1.18	-	-

Table 3a. Frequency distribution summary

Dimensions	Range	Percentage of ovaries in physical method	Percentage of ovaries in ultrasound method
Length	19 - 31	74.05	77.60
Width	10 - 19	88.17	87.00
Height	10 - 22	81.90	85.80

calliper. The corresponding values for ultrasound measure were 15 to 35 mm, 8 to 24 mm and 7 to 19 mm. However, length in 74.05 (dial calliper) and 77.60 (ultrasound) per cent of ovaries were within the range of 19 to 31 mm. The width in 88.17 (dial calliper) and 87.00 (ultrasound) per cent were in the range of 10 to 19 mm. The height in 81.90 (dial calliper) and 85.80 (ultrasound) per cent were in the range of 10 to 22 mm (Table 3a).

The length, width and height of ovaries with CL ranged from 16.5 to 34.5 mm, 13.5 to 26.5 mm and 7.6 to 22.3 mm (Table 4), respectively, measured by dial calliper. The corresponding values for ultrasound method were 17 to 36 mm, 14 to 26 mm and 8 to 23 mm. However, length in 81.26 (dial calliper) and 80.36 (ultrasound) per cent of ovaries were within the range of 19 to 31 mm. The width in 40.31 (dial calliper) and 41.31 (ultrasound) per cent of ovaries were within the range of 10 to 19 mm. The height in 97.21 (dial calliper) and 98.12 (ultrasound) per cent of the ovary were within the range of 10 to 22 mm (Table 4a). The present findings are in line with the results of Bhalla *et al.* (1964) who reported length, width and height of ovaries within the range of 1.27 to 3.95, 0.70 to 3.10 and 0.70 to 2.70 cm, measured by physical method. They further reported that length in 79.50 per cent of the ovaries, width in 89.00 per cent of the ovaries and height in 91 per cent of the ovaries were within the range specified in present investigation. The little differences could be due to the fact that they have not classified the ovaries into two categories, i.e. with and without CL. Goswami *et al.* (1992) also studied the distribution of ovarian dimensions of buffalo cows for length and width and reported that length in 95.90 per cent of the ovaries were within

Table 4. Frequency distribution of ovarian (with corpus luteum) dimensions

Class interval	Based on physical method			Based on ultrasound method		
	Length	Width	Height	Length	Width	Height
	(%)					
4 - 7	-	-	-	-	-	-
7 - 10	-	-	1.86	-	-	0.95
10 - 13	-	-	14.01	-	-	13.08
13 - 16	-	7.47	57.94	-	7.67	49.53
16 - 19	10.28	32.84	22.46	10.54	33.64	32.71
19 - 22	20.42	30.84	2.80	20.56	31.77	2.80
22 - 25	26.14	25.23	0.93	25.93	23.19	0.93
25 - 28	23.49	3.62	-	23.36	3.73	-
28 - 31	11.21	-	-	11.21	-	-
31 - 34	6.60	-	-	6.54	-	-
34 - 38	1.86	-	-	1.86	-	-

Table 4a. Frequency distribution summary

Dimensions	Range	Percentage of ovaries in physical method	Percentage of ovaries in ultrasound method
Length	19 - 31	81.26	80.36
Width	10 - 19	40.31	41.31
Height	10 - 22	97.21	98.12

the range of 10 to 30 mm and width in 96.70 per cent of the ovaries were within the range of 10 to 25 mm.

4.1.5 INTERRELATIONSHIP AMONG OVARIAN DIMENSIONS

Correlation coefficients among dimensions of ovary measured ultrasonographically and between physical and ultrasound method are presented in Tables 6 and 5. Correlation between measures of physical and ultrasound methods were highly significant ($P < 0.01$), thereby, confirming the association of these methods in terms of their accuracy and reliability for both ovaries with and without CL. In agreement to our findings, Pierson and Ginther (1988) reported similar kind of relationship and stressed the reliability of ultrasonography for follicular study. The correlations between all possible combinations of ovarian dimensions measured by ultrasound method were highly significant ($P < 0.01$) for ovary without corpus luteum. However, similar trend was not observed for the ovary with corpus luteum. The difference being that width and length were not correlated and the association of height and width were only significant at 5 per cent level. This can be attributed to the growth of luteal tissue on the ovary on one hand and technique of measuring the width from attached to free border especially in ovaries with protruded CL.

4.1.6 WEIGHT PREDICTION USING ULTRASOUND MEASUREMENTS

There was close association between weight and ultrasonic measurements, thereby emphasizing the need for regression analysis. Therefore, regression analysis was carried out and several prediction equations were formulated to estimate the weight of ovary from all possible combinations of ovarian

Table 5. Correlation coefficients of the dimensions of the ovary between physical and ultrasound methods

Character	Ovary without corpus luteum	Ovary with corpus luteum	Level of significance
Length 1 - Length 2	0.977	0.994	**
Width 1 - Width 2	0.984	0.859	**
Height 1 - Height 2	0.970	0.970	**
Volume 1 - Volume 2	0.990	0.979	**

** Significant ($P < 0.01$)
Suffix 1 denotes measured by physical method
Suffix 2 denotes measured by ultrasound method

Table 6. Interrelationship of the ovarian dimensions measured by ultrasonography

Character	Length	Width	Height	Volume	Weight #
			Without corpus luteum		
Length		0.458*	0.479**	0.744**	0.744**
Width	0.034		0.517**	0.844**	0.833**
Height	0.290**	0.191*		0.779**	0.755**
Volume	0.698**	0.500**	0.691**		0.985**
Weight#	0.686**	0.460**	0.687**	0.976**	
	With corpus luteum				

Measured by electrical balance
** Significant ($P < 0.01$)
* Significant ($P < 0.05$)

dimensions. These equations have been derived separately for both type of ovaries, i.e., with and without CL to have more precise estimation. The weight was predicted in the ovary as a careful observation of the changes in ovarian size in situ, concomitant with the developing CL may facilitate in diagnosing the stage of estrous cycle on one hand and, on the other hand, will generate the data on ovarian weight throughout the estrous cycle for evaluation of the reproductive index of animal. The technique earlier not available in the literature.

The prediction equations estimated for the ovary without corpus luteum were as follows:

1. $Y = -2.289 + 0.204$ (Length) ($R^2 = 55.40\%$)
2. $Y = -1.76 + 0.279$ (Width) ($R^2 = 69.50\%$)
3. $Y = -2.53 + 0.399$ (Height) ($R^2 = 57.14\%$)
4. $Y = -3.51 + 0.125$ (Length) $+ 0.209$ (Width) ($R^2 = 86.12\%$)
5. $Y = -4.014 + 0.136$ (Length) $+ 0.273$ (Height) ($R^2 = 76.09\%$)
6. $Y = -3.429 + 0.202$ (Width) $+ 0.233$ (Height) ($R^2 = 84.00\%$)
7. $Y = -4.35 + 0.099$ (Length) $+ 0.169$ (Width) $+ 0.171$ (Height) ($R^2 = 93.03\%$)
8. $Y = 0.1206 + 1.033$ (Volume)* ($R^2 = 97.06\%$)

* volume calculated by formula =

$$\frac{4}{3}\pi \times \frac{\text{Length}}{2} \times \frac{\text{Width}}{2} \times \frac{\text{Height}}{2} \times \frac{1}{1000} \text{ c.c.}$$

In formulae 1 to 3, weight was predicted considering single dimension. Maximum reliability ($R^2 = 69.57\%$) of the estimate as per statistical analysis was obtained using width. Equations 4 to 6 uses multiple regression analysis and two dimensions with all possible combinations were used to derive weight. Length and

width showed highest (R^2 = 86.12%) reliability with little difference with width and height (R^2 = 84.00%). Formula 7 considers all the three dimensions (length, width, height) and could predict weight with 93.03 per cent reliability. Considering spherical shape of ovary, it was thought to calculate the volume using these dimensions by the formula and then weight was calculated with 97.06 per cent accuracy.

The prediction equations derived for ovary with CL were as follows:

1. Y = -0.5621 + 0.1895 (Length) (R^2 = 47.00%)
2. Y = 0.5805 + 0.1769 (Width) (R^2 = 21.00%)
3. Y = -1.368 + 0.364 (Height) (R^2 = 47.26%)
4. Y = -3.78 + 0.1854 (Length) + 0.1681 (Width) (R^2 = 78.00%)
5. Y = -3.74 + 0.1468 (Length) + 0.2824 (Height) (R^2 = 73.00%)
6. Y = -3.4428 + 0.1312 (Width) + 0.3295 (Height) (R^2 = 59.00%)
7. Y = -5.92 + 1.491 (Length) + 0.1358 (Width) + 0.2453 (Height) (R^2 = 85.00%)
8. Y = 0.23 + 0.995 (Volume)* (R^2 = 95.80%)

* volume calculated using formula discussed earlier

In formula 7, weight was predicted considering all the three dimensions (length, width and height). The reliability of the estimate was 85 per cent. However, when any two measurements were used to derive weight, length and width gave the maximum (78%) reliability. While considering single dimension in formulae 1 to 3, none of the equation could predict weight satisfactorily. Like in the ovaries without CL, the weight in this case could be predicted with reliability of 95.80 per cent by calculation of volume and then weight from it. Perusal of these results reveals that volume is the most reliable dimensions to predict the weight for both types of ovaries, i.e., with or

without CL. The ovaries with CL gave less reliable estimate due to the intereference of the luteal tissue in measuring dimensions of ovary especially with protruded corpus luteum.

To test the efficacy of the formulae derived for estimating ovarian weight, a random sample of 15 ovaries with actual weight was selected for both ovaries with CL and without CL (Table 7). A perusal of results revealed that the formulae were readily adaptable for estimating ovarian weight *in situ* where no method is available except excision of ovary. Therefore, findings are novel contribution to literature and will revolutionize the augmentation of reproduction in buffaloes.

4.1.7 PREDICTION OF VOLUME

Volume can be calculated only using three dimensions. As the volume gave the more reliable estimate, measuring all the three dimensions using ultrasround machine may not be possible for some of the animal as it requires either rotation of transducer at 90^{O} or rotation of ovary *in situ*. It was deemed fit to estimate the volume by using two ultrasonic dimensions which gave the maximum reliability and then to predict the weight using predicted volume. The prediction equation of volume for both types of ovaries were

Ovary without CL

$$Y = -3.498 + 0.122\ (\text{Length}) + 0.203\ (\text{width}) \quad (R^2 = 85.70\%)$$

Ovary with CL

$$Y = -3.7619 + 0.1811\ (\text{Length}) + 0.1617\ (\text{width}) \quad (R^2 = 74.00\%)$$

where, Y = volume

Table 7. Comparative efficacy of various formulae for the prediction of ovarian weight

Ovary without corpus luteum				Ovary with corpus luteum			
Formulae number	Actual weight (g)	Derived weight (g)	Difference (g)	Formulae number	Actual weight (g)	Derived weight (g)	Difference (g)
1	2.27	2.56	0.294	1	5.034	4.756	0.278
2	2.27	2.41	0.140	2	5.034	4.361	0.673
3	2.27	2.55	0.280	3	5.034	4.783	0.251
4	2.27	2.35	0.080	4	5.034	5.182	0.148
5	2.27	2.11	0.160	5	5.034	5.196	0.162
6	2.27	2.45	0.180	6	5.034	5.231	0.197
7	2.27	2.31	0.040	7	5.034	5.093	0.059
8	2.27	2.29	0.020	8	5.034	5.061	0.027

Using the two formulae, the volume of 15 ovaries were calculated using length and width measured with ultrasound method. Weight of the same ovaries (actual weight was known) were calculated from length and width, predicted volume from length and width and volume from three dimensions (length, width and height) (Table 8). It was found that prediction of weight from volume (predicted from length and width) gave more accuracy than from simply length and width.

4.1.8 ULTRASONIC IMAGING OF CORPUS LUTEUM

A representative samples of ovaries with CL, i.e., protruded CL and embedded CL were photographed (coloured) and ultrasound image of the same samples were taken to have comparative evaluation (Fig. 8). The acoustic impedence of the luteal and ovarian tissue were different and so there was a beautiful contrast between these two tissues, thereby facilitating to identify the luteal tissue which appears as grey colour in the middle of the ovary in ultrasound image.

4.1.9 BIOMETRY OF CORPUS LUTEUM

Biometry of the corpus luteum was studied to assess and compare the changes in corpora luteal dimensions by physical and ultrasound methods to estimate the reliability of ultrasonography and to develop the formulae by which weight of corpora lutea could be predicted *in vivo* to judge the status of reproductive cyclicity of the animals.

Biometry of CL was done on 87 samples. The length, width, height of each CL was measured by first ultrasonography by

Table 8. Efficacy of formula to predict volume from two dimensions (length and width) of ovary

Particular	Volume from two dimensions (c.c.)	Volume from three dimensions (c.c.)	Actual volume (c.c)	Weighted from length and width (g)	Weight from volme predicted from two dimensions (g)	Weight from volume predicted from three dimensions (g)	Actual weight (g)
Ovary without corpus luteum	1.93	1.95	1.97	2.25	2.11	2.13	2.15
Ovary with corpus luteum	3.85	3.87	3.89	4.16	4.12	4.08	4.01

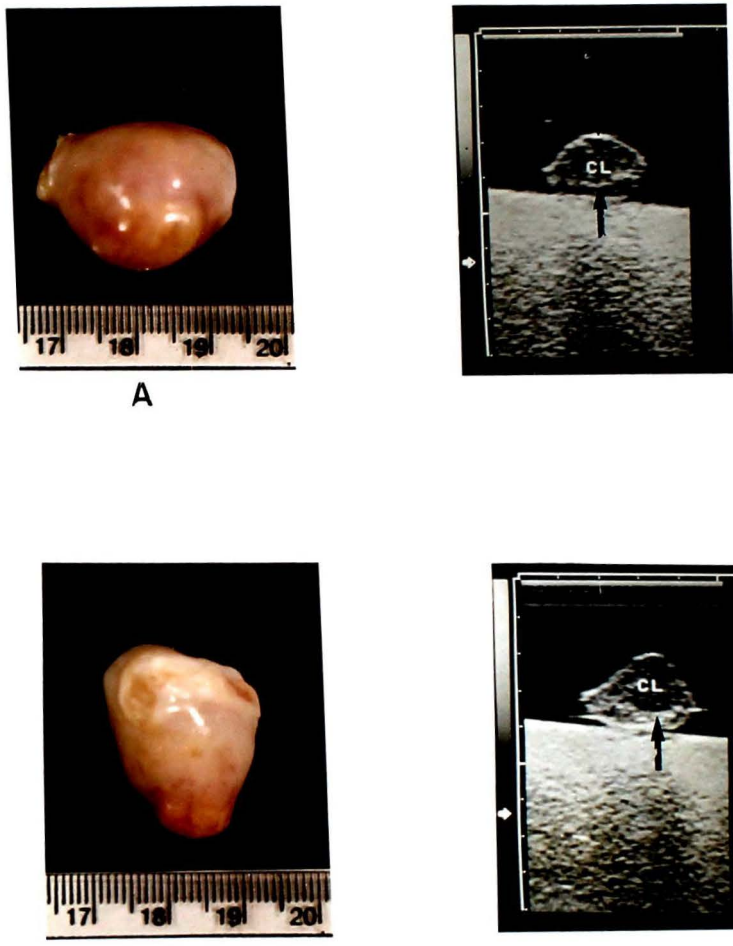


Fig. 8 Photograph showing corpus luteum and their respective ultrasound image taken using water bath. The luteal border has been indicated by arrows. A. Embedded corpus luteum. B. Protruded corpus luteum.

placing the ovary with CL in the water bath and then the CL was manually excised and it's dimensions were measured by dial calliper. Weight was recorded by electrical balance. The length, width, height and weight of CL obtained both in dial calliper and ultrasound methods have been presented in Table 9. The data obtained in this study shows that there was little difference between ultrasound measurement and dial calliper values (Fig. 9). The average length, width, height of CL in dial calliper method was 15.53 ± 0.29 mm, 12.72 ± 0.25 mm, 10.38 ± 0.22 mm, respectively. These parameters in the ultrasound method were 15.81 ± 0.29 mm, 13.00 ± 0.25 mm, 10.77 ± 0.22 mm. The average weight (1.36 ± 0.05 g) of corpora lutea obtained in this study correlate with the corresponding values (0.465 to 1.79 g) reported by El-Sheikh *et al.* (1967) in Egyptian buffaloes.

Luktuke and Rao (1961) reported that length, width, weight of corpus luteum in buffalo-cows as 1.19 cm to 1.52 cm, 1.08 cm to 1.34 cm and 0.72 g to 1.54 g. However, Polding and Lall (1945) reported that in buffalo-cows, width of the corpus luteum varies from 1.3 to 2 cm. In the present study, the length, width and weight were in accordance with Luktuke and Rao (1961) and width was little less than that observed by Polding and Lall (1945). The maximum and minimum weights of corpus luteum in cattle has been reported to be 5.54 g and 0.54 g (Mares *et al.*, 1962). Small size and weight of CL in buffaloes as observed in present findings may be one of the cause of low plasma progesterone values and also it's small size might be possible for creating difficulty in diagnosis of it's correct stage through rectal palpation leading to wrong diagnosis of stage of estrous cycle.

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Table 9. Mean biometry of corpus luteum by physical and ultrasound method *in vitro*

Sample size	Technique	Length (mm)	Width (mm)	Height (mm)	Weight (by electrical balance) (g)
87	Ultrasound method	15.81 ± 0.29	13.00 ± 0.25	10.77 ± 0.22	1.36 ± 0.05
	Physical method	15.53 ± 0.29	12.72 ± 0.25	10.38 ± 0.22	

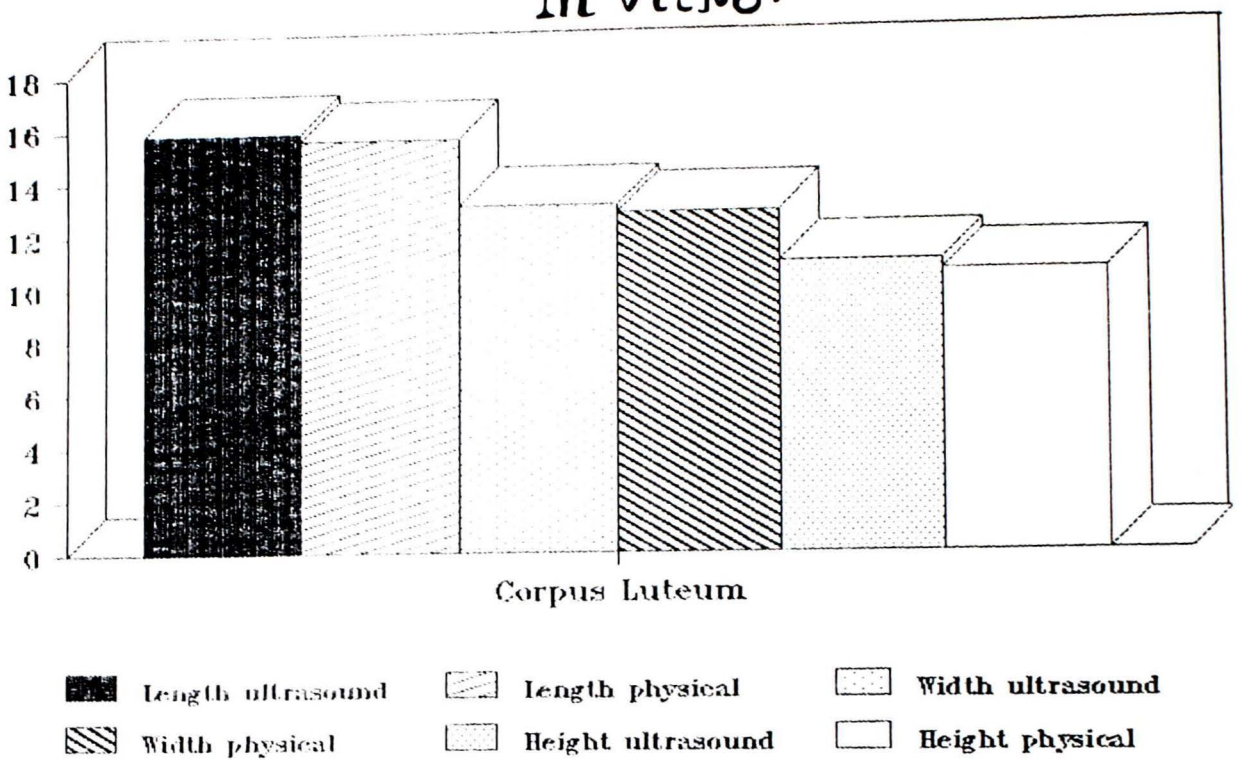
Table 10. Mean measurement of volume of corpus luteum by physical and ultrasound method *in vitro*

Sample size	Technique		Correlation coefficient
	Ultrasound method (c.c.)	Physical method (c.c)	
87	1.20 ± 0.05	1.21 ± 0.05	0.94**

** Significant ($P < 0.01$)

Fig.9.Biometry(mm.) of Corpus luteum compared using ultrasound & phy.method

In Vitro.



The average volume of corpora lutea measured by Archimedes principle and ultrasound method has been presented in Table 10. The values were very close in these two methods (Fig. 10). The average volume of CL in ultrasound method was 1.20 $\pm$ 0.05 c.c. and in the physical method 1.21 $\pm$ 0.05 c.c. There was highly significant ($P < 0.01$) relationship for the estimates by these two methods as depicted by correlation coefficient value (Table 10). In agreement to present findings, El-Sheikh *et al.* (1967) measured the mean volume of CL from 0.04 to 1.50 c.c.

4.2 FREQUENCY DISTRIBUTION OF DIMENSIONS OF CORPORA LUTEA

There was no literature regarding the frequency distribution of dimensions of corpora lutea. However, a great variation was obtained between each dimension of the corpus luteum measured (Table 11). The length of the corpus luteum varied from 8.9 mm to 21.1 mm in dial calliper method and 9 mm to 22 mm in ultrasound method. However, 86.12 per cent and 82.60 per cent of the CL were within the range of 10 to 19 mm in dial calliper and ultrasound method, respectively (Table 11a). The width of CL ranged from 8.9 to 16.5 mm in dial calliper method and 9 to 17 mm in ultrasound method. Whereas, 88.60 and 90.80 per cent of the CL measured lie within the range of 10 to 19 mm. The height of the CL ranged from 4.8 mm to 15 mm in dial calliper method and 5 to 15 mm in ultrasound method. However, 94.26 per cent and 93.74 per cent of the CL were in the range of 7 to 16 mm.

4.2.1 INTERRELATIONSHIP AMONG CORPORA LUTEAL DIMENSIONS

Various correlation coefficients among dimensions of CL measured ultrasonographically and between physical and ultrasound

Fig.10.Volume(cc.) of corpus luteum compared using ultrasound & phy. method.

In Vitro.

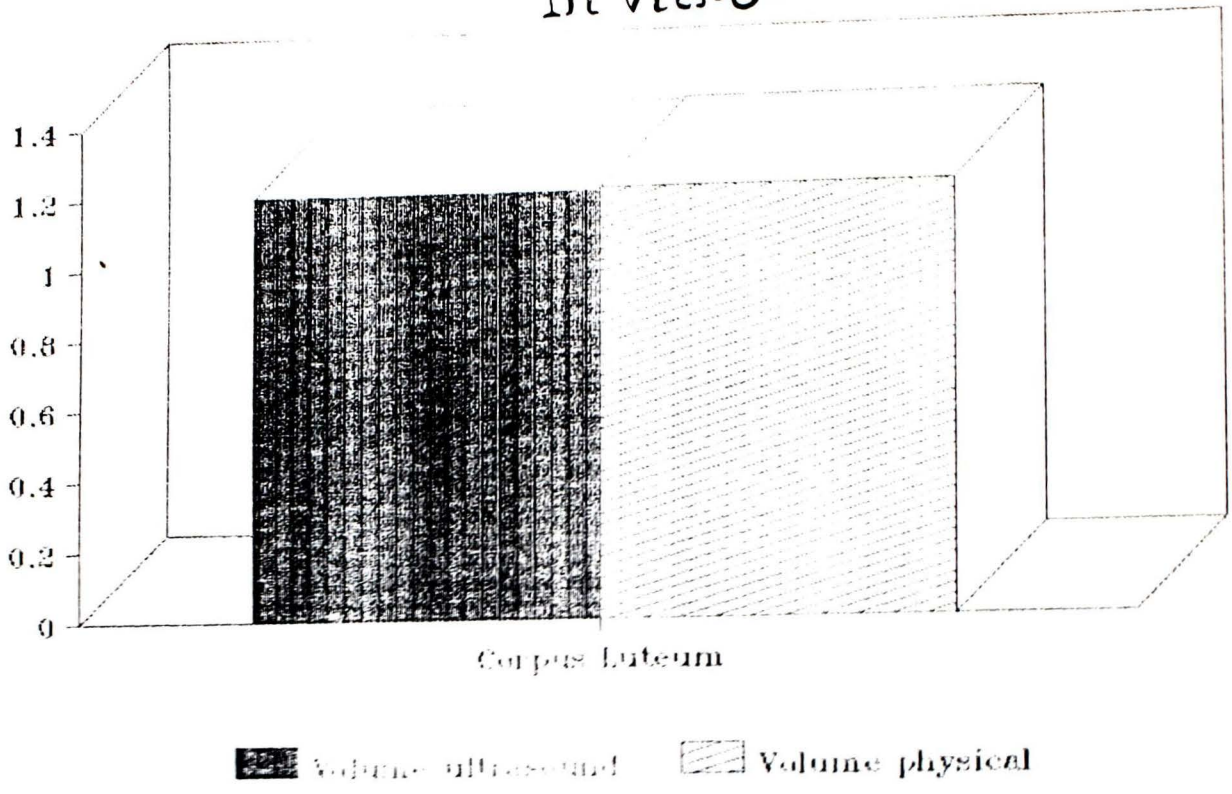


Table 11. Frequency distribution of corpora luteal dimensions

Class interval	Based on physical method			Based on ultrasound method		
	Length	Width	Height	Length	Width	Height
	(%)					
4 - 7	-	-	5.74	-	-	5.12
7 - 10	1.14	11.40	32.18	1.32	8.06	12.14
10 - 13	14.94	48.27	50.68	11.34	36.78	21.83
13 - 16	35.63	32.29	11.40	33.33	39.08	59.77
16 - 19	35.65	8.04	-	37.93	14.94	1.14
19 - 22	12.64	-	-	13.79	1.14	-
22 - 25	-	-	-	2.29	-	-

Table 11a. Frequency distribution summary

Dimensions	Range	Percentage of ovaries in physical method	Percentage of ovaries in ultrasound method
Length	10 - 19	86.22	82.60
Width	10 - 19	88.60	90.80
Height	7 - 16	94.26	93.74

methods have been presented in Tables 12 and 13. Statistical analysis of the correlation coefficients reveals that they were highly significant ($P < 0.01$), thereby, indicating ultrasound technique to be reliable and accurate. In agreement to our findings, Pierson and Ginther (1988) reported that diagnostic ultrasonography was determined to be a reliable method of identifying and measuring follicles and corpora lutea and luteal cavities in heifers as there was 100 per cent agreement between ultrasound and slicing results. The correlations between all possible combinations of corpora luteal dimensions measured by ultrasound method were highly significant ($P < 0.01$). The correlation between volume and weight was highest (0.932).

4.2.2 WEIGHT PREDICTION USING ULTRASOUND MEASUREMENTS

As there was close association between weight and ultrasonic measurements of CL, regression analysis was carried out to develop various prediction equations to estimate the weight of CL from all possible combinations. Weight of the CL is correlated to it's secretory activity due to the fact that profiles of mean weight of the corpus luteum are similar to the profiles of peripheral blood progesterone concentration during the oestrous cycle (Kastelic *et al.*, 1990). Therefore, prediction of weight of CL in live animal is very important tool to diagnose the stages of estrous cycle and it will be a valuable index to know the reproductive functioning of the animal.

Prediction equations estimated for CL were as follows:

1. $Y = -0.94 + 0.141$ (Length) $(R^2 = 60.00\%)$
2. $Y = -0.90 + 0.17$ (Width) $(R^2 = 63.50\%)$
3. $Y = -0.56 + 0.173$ (Height) $(R^2 = 47.80\%)$

Table 12. Interrelationship of the corpora luteal dimensions measured by ultrasonography

Character	Length	Width	Height	Volume	Weight #
Length	1.000	0.481**	0.465**	0.762**	0.779**
Width		1.000	0.571**	0.814**	0.797**
Height			1.000	0.734**	0.691**
Volume				1.000	0.932**
Weight#					1.000

\# Measured by electrical balance
** Significant (P < 0.01)
* Significant (P < 0.05)

Table 13. Correlation coefficients of the dimensions of the corpus luteum between physical and ultrasound methods

Character	Correlation coefficient	Level of significance
Length 1 - Length 2	0.983	**
Width 1 - Width 2	0.967	**
Height 1 - Height 2	0.956	**
Volume 1 - Volume 2	0.942	**

** Significant (P < 0.01)
Suffix 1 denotes measured by physical method
Suffix 2 denotes measured by ultrasound method

4. $Y = -1.70 + 0.098\ (\text{Length}) + 0.117\ (\text{Width})$ $(R^2 = 83.90\%)$

5. $Y = -1.514 + 0.106\ (\text{Length}) + 0.105\ (\text{Height})$ $(R^2 = 74.50\%)$

6. $Y = -1.30 + 0.127\ (\text{Width}) + 0.087\ (\text{Height})$ $(R^2 = 71.80\%)$

7. $Y = -1.86 + 0.008\ (\text{Length}) + 0.01\ (\text{Width}) + 0.012\ (\text{Height})$ $(R^2 = 87.02\%)$

8. $Y = 0.184 + 0.95\ (\text{Volume})^*$ $(R^2 = 88.20\%)$

* volume calculated by formula

$$\frac{4}{3}\pi \times \frac{\text{Length}}{2} \times \frac{\text{Width}}{2} \times \frac{\text{Height}}{2} \times \frac{1}{1000}\ \text{c.c.}$$

Single dimension was considered from equations 1 to 3 and out of these, maximum reliability (63.50%) was achieved using width. Equation 4 to 6 uses two dimensions and covers multiple regression analysis in which weight can be predicted with maximum reliability (83.90%) using length and width. If three dimensions were available, weight could be predicted with a reliability of 87.02 per cent by equation 7. Taking CL as sphere, volume was calculated by the formula stated above and weight could be predicted with maximum accuracy ($R^2 = 88.20\%$) by the equation 8. A go through of these equations reveals that volume was the most reliable dimensions from which weight could be calculated successfully with maximum reliability.

To test the efficacy of the prediction equations for estimation of weight, at random, 15 samples with actual weight were selected and weight predicted from the equations were compared with that of actual (Table 14). The perusal of these results indicate that the equations were highly fitted, unique and could predict weight very close to the actual weight, thus may be used to improve the reproductive efficiency of buffaloes.

Table 14. Comparative efficacy of various formulae to predict the weight of corpora lutea

Formulae number	Actual weight (g)	Derived weight (g)	Difference (g)
1	1.29	1.55	0.26
2	1.29	1.51	0.22
3	1.29	1.56	0.27
4	1.29	1.39	0.10
5	1.29	1.41	0.12
6	1.29	1.45	0.16
7	1.29	1.35	0.06
8	1.29	1.34	0.05

4.2.3 PREDICTION OF VOLUME

Volume is calculated from three dimensions (length, width and height). But under *in vivo* conditions, it is difficult to get all the three dimensions in some cases as it needs rotation of the transducer at 90^{o}C angle or of the ovary *in situ*. So it was thought appropriate to estimate the volume by using two ultrasonic dimensions which gives the maximum reliability and then to predict the weight with more accuracy as in the case of ovary. The prediction equation for volume was:

$$Y = -1.58 + 0.08\ (\text{Length}) + 0.114\ (\text{Width}) \qquad (R^2 = 84.51\%)$$

where, Y = volume

Using this formula, the volume of 15 CLs were calculated using length and width measured ultrasonographically. Weight of the same samples (actual weight was known) were calculated from length and width, predicted volume from length and width and volume from three dimensions (length, width and height) (Table 15). It was found that prediction of weight from volume (predicted from length and width) gave more accuracy than from simply length and width.

4.3 *IN VIVO* STUDY

4.3.1 BIOMETRY OF OVARIES

An application to the *in vitro* results, a study was undertaken to compare it with the *in vivo* ultrasonography. Biometry of ovaries (*in vivo*) was studied during the various

Table 15. Efficacy of formula to predict volume from two dimensions (length and width) of corpora lutea

Volume from two dimensions (c.c.)	Volume from three dimensions (c.c.)	Actual volume (c.c)	Weighted from length and width (g)	Weight from volme predicted from two dimensions (g)	Weight from volume predicted from three dimensions (g)	Actual weight (g)
1.21	1.27	1.25	1.31	1.33	1.35	1.37

phases of estrous cycle. This study is different than earlier report where biometry has been studied after excision of the ovaries. The results so obtained may be very helpful in comparing the biometry of ovaries *in vivo* vs *in vitro* so as to have the exact idea about status of buffalo ovary from slaughter house in reference to IVF results. Moreover, prediction of weight of ovary may facilitate to diagnose the reproductive functioning of the animal.

A cycle of 20 animals were synchronized to record these measurements on 0 day, 5 day, 12 day and 18 day of the cycle by ultrasound machine. Volume was calculated using the prediction equation Y = -3.498 + 0.122 (Length) + 0.203 (Width) for ovary without CL and for ovary with CL Y = -3.7619 + 0.1811 (Length) - 0.1617 (Width). Weight was then predicted by using the formula Y = 0.1206 + 1.033 (volume) for ovary without CL and Y = 0.23 - 0.995 (Volume) in case of ovary with CL. These formulae were derived under *in vitro* study.

The mean values for the biometry of ovaries with or without CL during different periods of cycle have been presented in Table 16 and Fig. 11. The average values, irrespective of the day of cycle, were 23.71 mm, 18.06 mm, 3.44 c.c. and 3.61 g for length, width, volume and weight, respectively, for the ovaries with CL. The corresponding values for ovaries without CL were 22.63 mm, 17.29 mm, 2.69 c.c. and 3.29 g. These results when compared with the biometry of ovaries *in vitro* (Table 1) revealed the fact that ovarian status under *in vivo* conditions was comparatively better than *in vitro* for ovaries without CL (Fig. 12). However, reverse trend was noticed for ovaries with CL. These variations may be attributed to the large difference in sample size *in vitro* vs *in vivo*. However, these results

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Table 16. Mean biometry of ovary *in vivo*

Particular	Day 5	Day 12	Day 18	Mean
Ovary with corpus luteum				
Length (mm)	23.05 ± 0.21	24.13 ± 0.18	23.95 ± 0.17	23.71 ± 0.15
Width (mm)	17.95 ± 0.13	18.20 ± 0.12	18.04 ± 0.14	18.06 ± 0.13
Volume (c.c.)	3.30 ± 0.08	3.61 ± 0.06	3.43 ± 0.07	3.44 ± 0.07
Weight (g)	3.51 ± 0.13	3.77 ± 0.15	3.57 ± 0.18	3.61 ± 0.12
Ovary without corpus luteum				
Length (mm)	21.80 ± 0.22	23.14 ± 0.21	22.97 ± 0.23	22.63 ± 0.21
Width (mm)	17.61 ± 0.13	17.27 ± 0.15	17.01 ± 0.14	17.29 ± 0.12
Volume (c.c.)	2.66 ± 0.09	2.75 ± 0.09	2.68 ± 0.08	2.69 ± 0.07
Weight (g)	3.27 ± 0.14	3.35 ± 0.14	3.25 ± 0.13	3.29 ± 0.14

Fig.11. Biometry(mm)of ovaries measured using invivo ultrasonography.

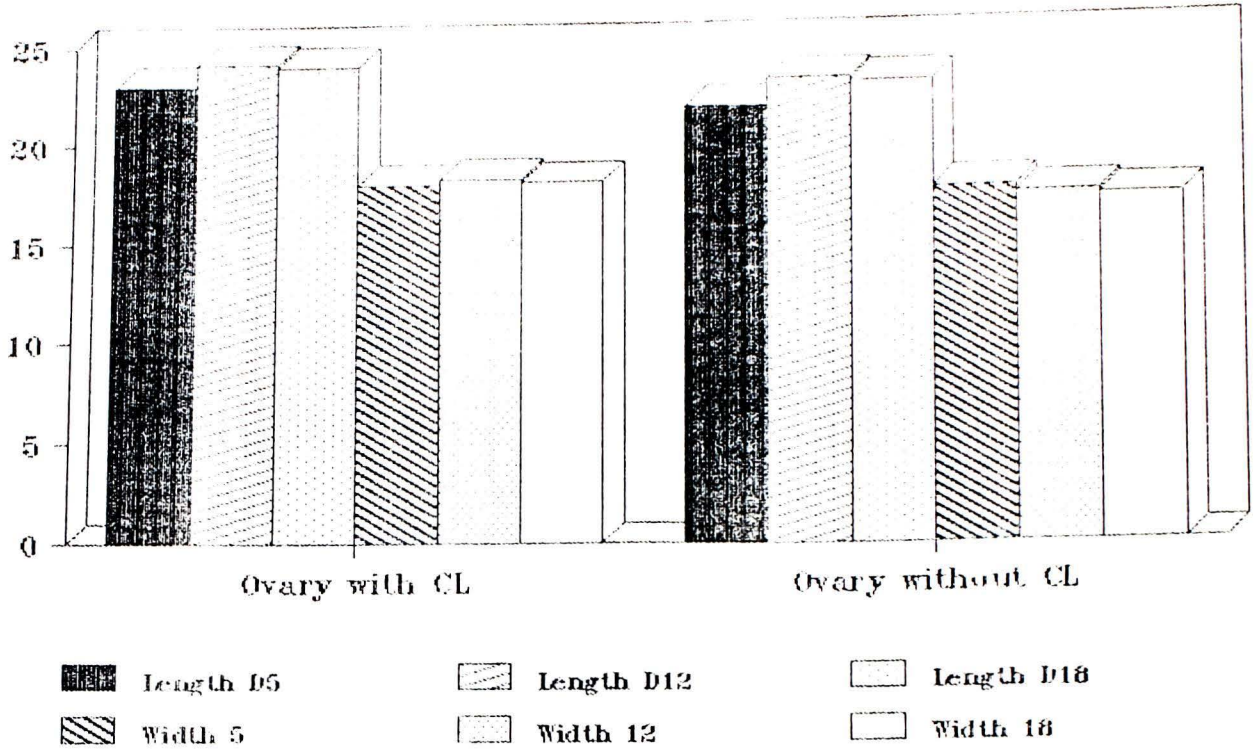
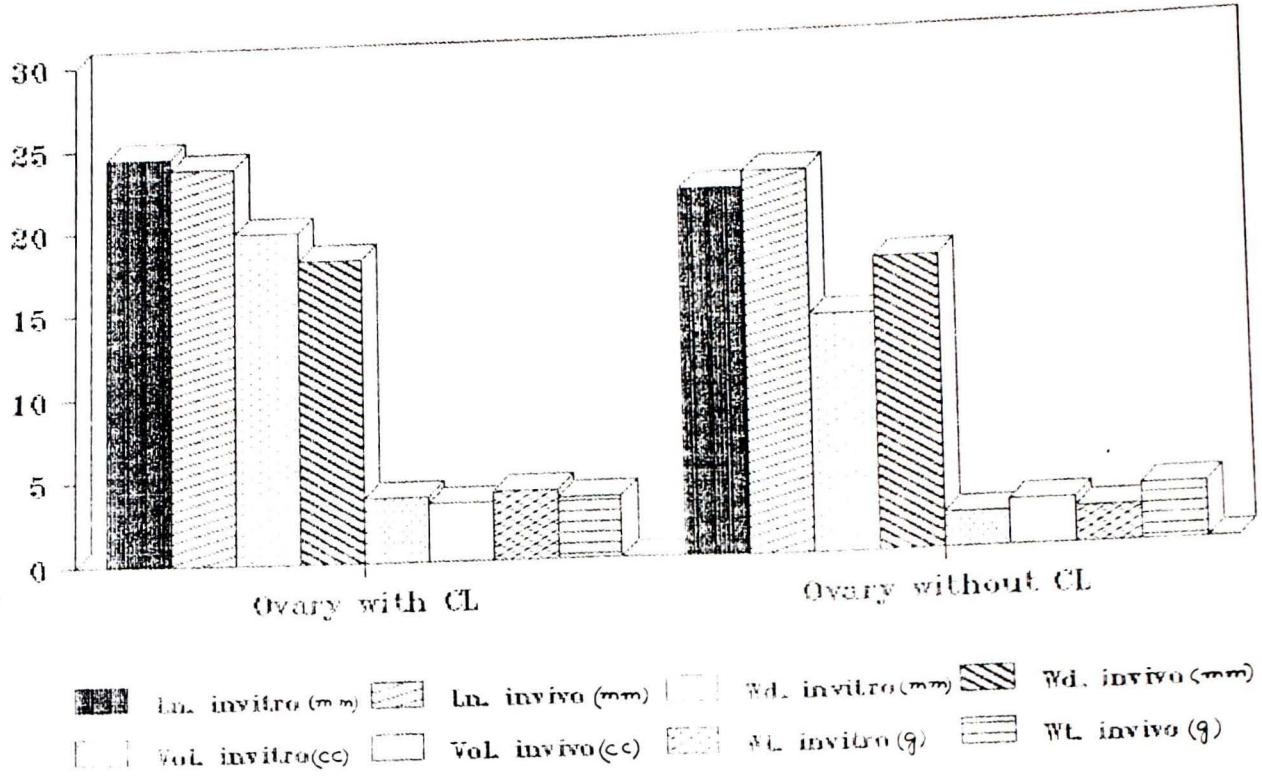


Fig.12.Ultrasonic biometry of ovaries invitro vs. invivo.



definitely conclude that the buffaloes going for slaughter may have poor health with respect to their body confirmation, etc. but their ovarian status in terms of biometry was quite comparable to *in vivo* results, which in turn is very crucial for IVF studies.

The data was further classified with respect to left and right ovary and mean biometry during different days of estrous cycle has been presented in Table 17. The results indicate that there was change in ovarian dimension during different phases of the sexual cycle; the ovaries reaching its maximum size and attaining maximum weight on day 12 of the estrous cycle. These alterations in the size and weight were in accordance with the development and regression of Graafian follicle and the formation, development and involution of the corpora lutea. In accordance with the present findings, Bhattacharya *et al.*(1970) also reported that the size and weight of ovaries reaches maximum during 10 to 15 days after the estrous. The mean weight of the ovaries with CL reported by the same author was 3.63 g on day 5, 3.85 g on day 12, 3.21 g on day 18 and for ovaries without CL, the corresponding values were 2.11 g, 2.27 g and 2.19 g.

The data when subjected to statistical analysis to find degree of variation for ovarian dimension and weight between left and right ovary revealed non-significant difference. In agreement to our findings, various workers (Parkale and Hukeri, 1989; Damodaran, 1958; Luktuke and Rao, 1961) also reported non-significant difference for ovarian dimension in Murrah buffalo between left and right ovary. On the contrary, significant differences were observed at 1 per cent level in the paired observations on thickness and weight of Murrah buffalo ovaries as reported by Sane *et al.* (1970).

Table 17. Mean measurements of ovaries during various phases of estrous cycle

Left ovary	Day estrous	Day 5	Day 12	Day 18	Overall mean
Length (mm)	23.04 ± 0.21	23.55 ± 0.22	23.82 ± 0.22	23.40 ± 0.23	23.45 ± 0.12
Width (mm)	18.23 ± 0.14	17.80 ± 0.13	18.00 ± 0.12	17.17 ± 0.14	17.52 ± 0.13
Volume (c.c.)	2.96 ± 0.09	3.05 ± 0.08	3.15 ± 0.08	2.99 ± 0.07	3.03 ± 0.05
Weight (g)	3.17 ± 0.08	3.28 ± 0.08	3.37 ± 0.07	3.20 ± 0.06	3.25 ± 0.15

Right ovary	Day estrous	Day 5	Day 12	Day 18	Overall mean
Length (mm)	23.08 ± 0.20	23.45 ± 0.21	23.82 ± 0.22	23.58 ± 0.21	23.50 ± 0.17
Width (mm)	16.47 ± 0.16	17.70 ± 0.15	17.44 ± 0.17	17.14 ± 0.16	17.39 ± 0.14
Volume (c.c.)	2.45 ± 0.08	2.89 ± 0.06	3.03 ± 0.08	2.94 ± 0.08	2.86 ± 0.03
Weight (g)	2.67 ± 0.03	3.34 ± 0.05	3.56 ± 0.04	3.38 ± 0.04	3.22 ± 0.13

The length, width and weight reported by Parkale and Hukeri (1989) were 24.8 vs 24.4 mm, 16.7 vs 17.4 mm and 3.71 vs 3.77 g for left and right ovaries, respectively. The corresponding values reported by Bhalla *et al.* (1964) were 24.4 vs 23.6 mm, 13.4 vs 13.7 mm and 3.32 vs 3.18 g. The similar dimensions measured using ultrasound machine in the present investigation were 23.45 vs 23.50 mm, 17.5 vs 17.4 mm and 3.25 vs 3.22 g. These results were quite comparable irrespective of the different techniques used for studying biometry. Because we used ultrasound machine to take the measurements and from these measurements, weight was calculated using formulae derived on *in vitro* observations using ultrasonography. However, results of various authors reported earlier (Parkale and Hukeri, 1989; Bhalla *et al.*, 1964) used traditional methods of studying biometry from slaughter house specimens and the weight by balance. The close association of weight further confirms the accuracy of our formulae when applied to know the weight *in situ*.

4.3.2 SONOGRAPHIC IMAGING

The same ovary of one animal was photographed using ultrasound machine on day 0, day 5, day 12 and day 18. Ovary was echogenic and appeared as grey but follicle, like other fluid filled structures, was nonechogenic and appeared black especially on day 0. When CL was present, the border of the ovarian stroma was not difficult to appreciate because corpus luteum had an echotexture different from that of ovarian stroma. Ultrasound images of CL on various days of the cycle are shown (Fig. 13). A well defined border was visible. A visible characteristic of the CL did not change through the luteal phase of the cycle as it could be seen on day 5, day 12 and even day 18 of the cycle.

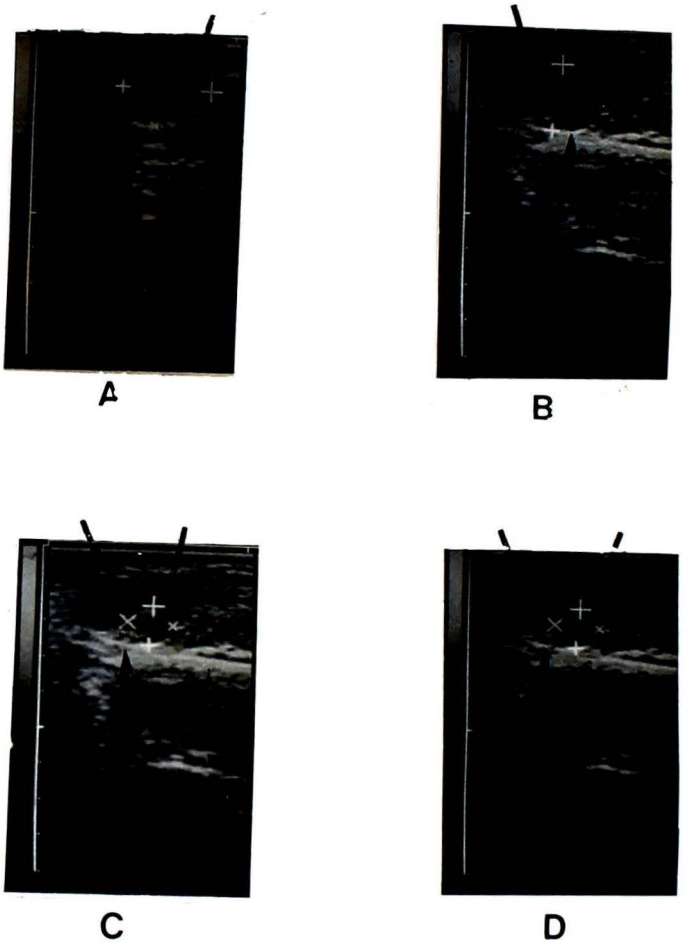


Fig.13 Ultrasound images of ovaries and corpus luteum during different days of oestrous cycle. A. Day of estrous (day 0), B. Day 5, C. Day 12 and D. Day 18. Ovarian/luteal border has been indicated by arrows.

4.3.3 BIOMETRY OF CORPUS LUTEUM

Biometry of the corpus luteum was studied during different phases of estrous cycle. The results of this study may help in comparing the biometry of CL *in vivo* and *in vitro*. Prediction of weight of CL might prove to be an alternate to progesterone estimation during various phases of estrous cycle.

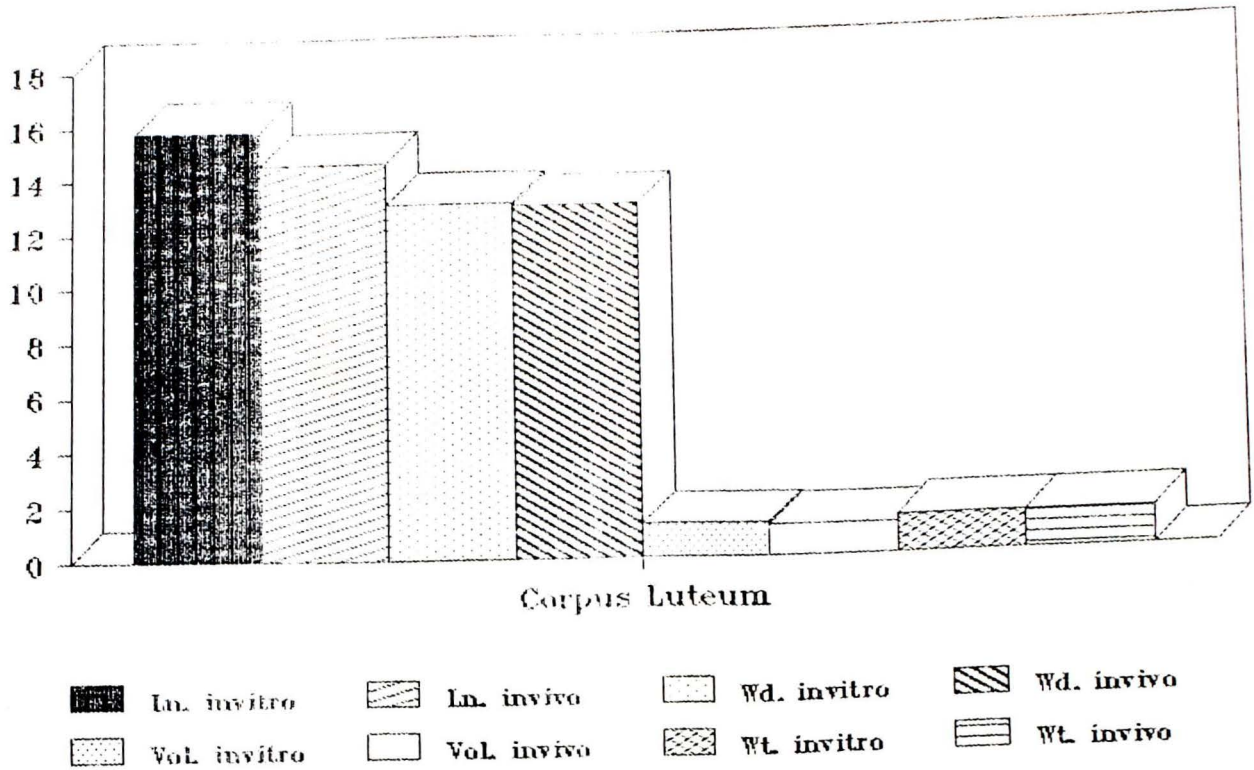
Length and width of the corpus luteum was measured of 20 cyclic animals on day 5, day 12 and day 18 of the estrous cycle. Volume and weight was predicted with the help of prediction equations derived under *in vitro* study, viz., Y = -1.58 + 0.08 (Length) + 0.114 (Width) and Y= 0.184 + 0.95 (Volume), respectively. The average length, width, volume and weight of the CL has been presented in Table 18. The measures were 14.56 $\pm$ 0.16 mm, 12.89 $\pm$ 0.14 mm, 1.11 $\pm$ 0.02 cc and 1.37 $\pm$ 0.08 g. These dimensions were quite comparable to *in vitro* results (Fig. 14), thereby, reconfirming our earlier statement regarding status of buffalo ovaries from slaughter house.

Edwards (1962) reported that the CL in cycling cattle were 2.30 cm and 5.71 g in length and weight, respectively and according to Foley *et al.* (1984) diestrous CL weighted 5.1 $\pm$ 1.6 g in cattle. Luktuke and Rao (1961) reported that in adult buffalo cows the average length, width and weight of CL was 1.19 to 1.52 cm, 1.08 to 1.34 cm and 0.72 to 1.54 g, while Polding and Lall (1945) reported that in buffalo cows, width of the CL varies from 1.3 to 2.0 cm. So the findings of the present investigation were in accordance with Luktuke and Rao (1961) and very close to Polding and Lal (1945).

Table 18. Mean biometry of corpus luteum *in vivo*

Particular	Day 5	Day 12	Day 18	Overall mean
Length (mm)	14.35 ± 0.15	15.56 ± 0.13	12.35 ± 0.14	14.56 ± 0.16
Width (mm)	13.30 ± 0.06	13.34 ± 0.08	11.21 ± 0.09	12.89 ± 0.14
Volume (c.c.)	0.75 ± 0.01	1.67 ± 0.02	0.68 ± 0.02	1.11 ± 0.02
Weight (g)	1.25 ± 0.10	1.78 ± 0.10	1.18 ± 0.10	1.37 ± 0.08

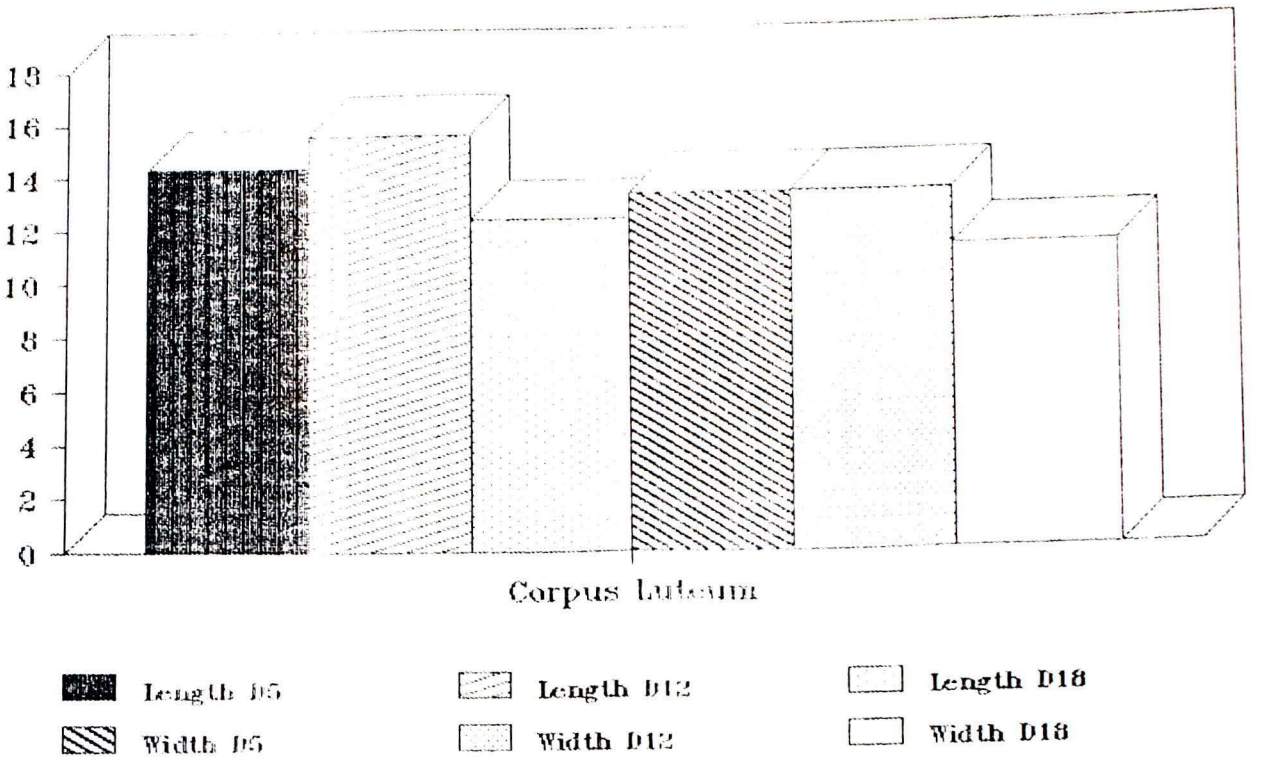
Fig.14 Ultrasonic biometry of corpus luteum invitro vs. in vivo.



The various measurements of corpus luteum during day 5, day 12 and day 18 of the estrous cycle has been also presented in Table 18 and Fig. 15. The average length, width, volume and weight on day 5 was 14.35 ± 0.15 mm, 13.30 ± 0.06 mm, 0.75 ± 0.01 cc and 1.25 ± 0.10 g, on day 12; 15.56 ± 0.13 mm, 13.34 ± 0.08 mm, 1.67 ± 0.02 c.c. and 1.78 ± 0.10 g and on day 18 12.35 ± 0.14 mm, 11.21 ± 0.09 mm, 0.68 ± 0.02 c.c. and 1.18 ± 0.10 g, respectively. The results indicate that the length, width and weight were maximum on day 12 of the cycle and then decreases significantly which was in line with the results of Bhattacharya *et al.* (1970). Singh *et al.* (1984) studied the palpable changes of corpora lutea during different stages of estrous cycle in buffalo heifers and reported that average length of the CL was 1.11 cm on day 0, 1.40 cm on 2 to 5 days, 1.71 cm on 6 to 8 days and 1.74 cm on 9 to 18 days of the cycle.

El-Sheikh *et al.* (1967) studied the average volume and weights of corpora lutea on different stages of estrous cycle in Egyptian buffaloes and reported it's volume and weight on day 3 to 5 of the estrous cycle, 0.75 c.c. and 1.13 ± 0.13 g, on day 6 to 15, 1.597 c.c. and 1.798 ± 0.11 g and on day 16 to 20, 0.308 c.c. and 0.587 ± 0.015 g, respectively. The results of the present study on day 5 and day 12 were quite comparable, however, the volume and weight on day 18 was much higher. The mean diameter and weight of corpora lutea of diestrous stage as reported by Shalash and Salama (1960) and in present investigation were 16.0 ± 4.2 mm vs 1.9 ± 0.5 g and 15.56 ± 0.13 mm vs 1.78 ± 0.10 g, respectively. Luktuke and Roy (1967) reported that corpus luteum in buffaloes on day 6, 12 and 18 of estrous cycle weighted 0.986 ± 0.09 g, 2.28 ± 0.17 g and 1.176 ± 0.07 g, respectively.

Fig.15 Biometry(mm) of corpus luteum using invivo ultrasonography.



CHAPTER 5

SUMMARY

5. SUMMARY

The investigation was carried out to have ultrasonic assessment of biometry of ovary and corpus luteum *in vitro* and *in vivo*. The ovaries obtained from slaughter house were placed in water bath to record ultrasonic measurements. The dimensions of same ovaries were measured using dial calliper. Validation of ultrasonic measurements, correlation coefficients among dimensions of ovary and corpus luteum measured ultrasonographically and between physical (dial calliper) and ultrasound (water bath) methods were studied. This study also involved the biometry of ovaries with and without corpus luteum to assess the changes in dimensions and weight of ovaries due to luteal tissue. The important aspect of this study was to derive the formulae for prediction of weight of ovary and corpus luteum using ultrasound measurements. These formulae were then applied to calculate the weight of ovary and CL *in situ* during different phases of estrous cycle. A total number of 254 ovaries without CL and 107 with CL were taken for study on ovarian dimensions and 87 samples were taken for study of CL dimensions. A cycle of 20 animals was synchronised for *in vivo* ultrasonography.

1. There was a linear relationship between length, width and height measured using ultrasound machine and dial calliper for ovaries with and without corpus luteum. Similarly, a linear relationship between biometry of corpus luteum (length, width and height) measured by two methods (physical vs ultrasound) was observed.

2. The length, width, height and volume measured using ultrasound method were 21.72 ± 0.23 mm, 13.99 ± 0.18 mm, 11.73 ± 0.12 mm and 1.96 ± 0.06 c.c., respectively, for ovaries without CL. The ovaries with CL had increased dimensions ($P < 0.01$) and corresponding values were 24.45 ± 0.41 mm, 19.75 ± 0.29 mm, 14.94 ± 0.22 mm and 3.86 ± 0.11 c.c. The weight of the ovaries without corpus luteum and with corpus luteum was 2.14 ± 0.06 and 4.07 ± 0.12 g, respectively. The ultrasonic length in 77.60 per cent, width in 87.00 per cent and height in 85.80 per cent of the ovaries (without CL) were within the range of 19 to 31 mm, 10 to 19 mm and 10 to 22mm, respectively. The corresponding values for ovaries with CL were 80.36, 41.31 and 98.12 per cent.

3. Correlation between measures of dial calliper and ultrasound method were highly significant ($P < 0.01$) ($r = 0.859$ to 0.994 for various measurements) which confirms the association of these methods in terms of their accuracy and reliability for both types of ovaries with and without CL. This emphasizes that the diagnostic ultrasound was a reliable method. The correlation between all possible combinations of ovarian dimensions (with CL) measured using ultrasound machine were also highly significant ($P < 0.01$; $r = 0.458$ to 0.985). Similar relationship was obtained for ovaries with CL ($r = 0.034$ to .0976) except width and length (no association) and height and width were correlated at 5 per cent level.

4. The weight of the ovaries (without CL) using ultrasound dimensions could be predicted with reliability (R^2) of 55.40 to 69.50 per cent using single dimension, 76.09 to 86.12 per cent using two dimensions and 93.03 to 97.06 per cent using three dimensions at a time. Similarly, weight of ovaries with CL could be predicted with reliablity, R^2 = 21.00 to 47.26 per cent (using

single dimension), R^2 = 59.00 to 78.00 per cent (using two dimensions) and R^2 = 85.00 to 95.80 per cent (using three dimensions). The efficacy of the formulae derived were tested to estimate the ovarian weight on 15 ovaries each for CL and without CL, selected randomly. The actual weight was recorded on electrical balance. The difference in weight by both the methods was 0.020 to 0.673 g depending on the number of variable used to estimate the weight. These formulae were readily adeptable to estimate ovarian weight *in situ* and findings are novel contribution to literature as no method exist to record weight in living animal.

Sonographic recording of all the three dimensions (length, width, and height) inside animal (*in situ*) may not be possible in some animals. Therefore, formulae were derived to predict volume from two dimensions (length and width) for both types of ovaries, i.e. with CL and without CL. It was found that prediction of weight from volume (predicted from length and width) gave more accuracy than from simply length and width.

5. Biometry of CL was done on 87 samples. The ovaries were placed in water bath to record the ultrasonic measurements. The average length, width, height and volume was 15.81 $\pm$ 0.29 mm, 13.00 $\pm$ 0.25 mm, 10.77 $\pm$ 0.22 mm and 1.20 $\pm$ 0.05 c.c., respectively. The average weight as recorded by electrical balance was 1.36 $\pm$ 0.05 g. The corpus lutuem length in 82.60 per cent, width in 90.80 per cent and height in 93.74 per cent were within the range of 10 to 19 mm, 10 to 19 mm and 7 to 16 mm, respectively. The ultrasonic measurements were reliable and accurate because relationship between dimensions of CL measured ultrasonographically and by dial calliper were highly significant ($P < 0.01$; r = 0.942 to 0.983). The correlation coefficient

between all possible combinations of ultrasonic measurement were also highly significant ($P < 0.01$).

6. The weight of corpus luteum could be predicted with maximum reliability (R^2 = 87.02 to 88.20%) using three dimensions. However, with the use of two dimensions and single dimension, the weight was predicted with reliability (R^2) of 71.80 to 83.90 per cent and 47.80 to 63.50 per cent, respectively. The efficiency of these formulae were tested on 15 samples when actual weight was recorded on electrical balance and weight was also predicted using various formulae derived. It was found that equations were highly fitted, unique and could predict weight very close to actual with a difference of 0.05 to 0.27 g depending upon number of variables/formulae used. Like for ovaries, prediction equation was also made to predict volume from two dimensions (length and width), and the results revealed that predicted weight of CL from volume predicted from length and width gave more accuracy than from length and width.

7. A cycle of 20 animals was synchronised to study biometry on day 0, day 5, day 12 and day 18 of the estrous cycle using ultrasound.machine. Weights of the ovaries were predicted using the formulae derived under *in vitro* study, i.e. Y = 0.1206 + 1.033 (volume) for ovary without CL and Y = 0.23 + 0.995 (volume) for ovary with CL. Volume was predicted using equation Y = -3.498 + 0.122 (length) + 0.203 (width) for ovary without CL and Y = -3.7619 + 0.1811 (length) + 0.1617 (width) for ovaries with CL. The average length, width, volume and weight were 23.71 $\pm$ 0.15 mm, 18.06 $\pm$ 0.13 mm, 3.44 $\pm$ 0.07 c.c. and 3.61 $\pm$ 0.12 g, respectively, irrespective of the day of the cycle for ovaries with CL. The corresponding values for ovaries without CL were 22.63 $\pm$ 0.21 mm, 17.29 $\pm$ 0.12 mm, 2.69 $\pm$ 0.07 c.c. and 3.29 $\pm$

0.17 g. These results when compared with *in vitro* biometry concludes that buffalo going for slaughter had ovarian status in terms of biometry quite comparable to *in vivo* results.

8. The ovarian dimension and weight changed during different phases of the sexual cycle and reached its maximum size and weight on day 12 of the estrous cycle. The data when classified with respect to left and right ovary did not reveal significant difference in biometry.

9. The average length, width, volume and weight of CL were 14.56 $\pm$ 0.16 mm, 12.89 $\pm$ 0.14 mm, 1.11 $\pm$ 0.02 c.c. and 1.37 $\pm$ 0.08 g, respectively. Volume and weight were predicted using the equation derived earlier, Y = -1.58 $\pm$ 0.08 (length) + 0.114 (width) and Y = 0.184 + 0.95 (volume) respectively. These dimensions were quite comparable to *in vitro* results that indicate the good status of buffalo ovaries from slaughter house. Like in the ovary, CL biometry also increased with advanced stage of cycle and was maximum during luteal phase (12 day) and then decreased.

This study make use of modern tool of ultrasonography to study ovarian and corpus luteum dimensions and contribute to literature the unique formulae, using them weight of ovary and corpus luteum can now be calculated *in situ*.

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