

**STUDIES ON BIOCHEMICAL COMPOSITION OF  
FOLLICULAR FLUID AND OOCYTE  
CHARACTERISTICS IN BUFFALOES**

By

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**2006**

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**Mr. L. PRABHAKAR** has satisfactorily prosecuted the course of research and that the thesis entitled "**STUDIES ON BIOCHEMICAL COMPOSITION OF FOLLICULAR FLUID AND OOCYTE CHARACTERISTICS IN BUFFALOES**" submitted is the result of original research work and is of sufficiently high standard to warrant its presentation to the examination. I also certify that the thesis or part there of has not been previously submitted by him for a degree of any University.

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This is to certify that the thesis entitled "STUDIES ON BIOCHEMICAL COMPOSITION OF FOLLICULAR FLUID AND OOCYTE CHARACTERISTICS IN BUFFALOES" submitted in partial fulfillment of the requirements for the degree of **MASTER OF VETERINARY SCIENCE** for Sri Venkateswara Veterinary University, is a record of the bonafide research work carried out by Mr. L. PRABHAKAR under my guidance and supervision. The student's Advisory committee has approved the subject of the thesis.

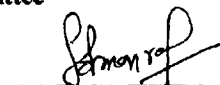
No part of the thesis has been submitted for any other degree or diploma. The author of the thesis has duly acknowledged all the assistance and help received during the course of investigation.




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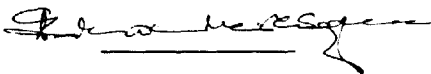
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## DECLARATION

I, Mr. L.PRABHAKAR hereby declare that the thesis entitled **STUDIES ON BIOCHEMICAL COMPOSITION OF FOLLICULAR FLUID AND OOCYTE CHARACTERISTICS IN BUFFALOES** submitted to Sri Venkateswara Veterinary University for the degree of '**MASTER OF VETERINARY SCIENCE**' is the result of the original work done by me. It is further declared that the thesis or any part thereof has not been published earlier in any manner.

Date : 12.02.2007

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### **ABSTRACT**

Studies on biochemical composition of follicular fluid and oocyte characteristics in buffaloes were undertaken at Department of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary Science, Rajendranagar, Hyderabad-30. A total of 890 ovaries were collected from January to August 2006 and used for the present investigation.

The average measurements of length, width, height, weight and size of left ovary were  $2.04 \pm 0.05$ ,  $1.27 \pm 0.04$ ,  $1.41 \pm 0.04$  cm,  $2.72 \pm 0.15$  g and  $2.25 \pm 0.21$  cc, respectively and corresponding values for the right ovary were

2.38±0.07, 1.44±0.07, 1.63±0.06 cm, 3.74±0.28 g and 3.35±0.36 cc, respectively.

The mean number of visible follicles on the surface of left and right ovary was 3.87±0.23 and 3.66±0.28. The mean length, diameter and weight of corpus luteum were 1.10±0.06, 0.86±0.04 cm and 0.88±0.04 g, respectively.

The biochemical indices in follicular fluid of various sized follicles were estimated in the present study as follows. Total protein (10.61±0.46 g per cent), albumin (2.95±0.17 g per cent), globulin (7.69±0.39 g per cent), albumin: globulin ratio (0.51±0.06 g per cent), cholesterol (48.36±2.05 mg per cent) and alkaline phosphatase (13.05±0.59 KAU).

The mineral concentration in follicular fluid of different sized follicles was as follows. Calcium (5.79±0.33 mg/dl), phosphorous (14.26±0.27 mg/dl), sodium (82.25±1.85 ppm), copper (0.57±0.05 ppm), zinc (6.11±0.41 ppm) and iron (1.35±0.05 ppm).

The total number of oocyte yield in aspiration, dissection and slicing techniques were 3.20±0.16, 3.10±0.10 and 5.70±0.13, respectively. The total number of oocyte yield in follicular and luteal phases of estrous cycle was 5.31±0.12 and 3.88±0.13, respectively.

Among the total oocytes recovered 52.94, 25.88 and 21.18 per cent were good, fair and poor quality of oocytes in the present study. The mean diameter of good, fair and poor quality of oocytes were 135.65±2.52, 111.7±2.84 and 94.55±2.22  $\mu$ , respectively.

**It was concluded that the biometrics of ovary and corpus luteum were variable during the stages of estrous cycle and the number of follicles present on the ovary was not significantly differed among the phases of estrous cycle.**

**It was also concluded that the biochemical and mineral content in follicular fluid of buffaloes was playing a major role as the size of the follicle increases and the mean number of oocytes retrieved was higher in slicing technique and from the ovaries under luteal phase of estrous cycle.**

## ABBREVIATIONS

$\mu$	:	Micron (s)
dl	:	Deci litre(s)
GnRH	:	Gonadotrophin Releasing Hormone
cm	:	Centimeter
mm	:	Milli meter
g	:	Gram
Kg	:	Kilogram(s)
LH	:	Leutenizing hormone
FSH	:	Follicle Stimulating Hormone
mg	:	Milligram(s)
Min	:	Minute(s)
U	:	Unit(s)
Fig	:	Figure
I.U	:	International units
ml	:	Milli litre(s)
KAU	:	King Armstrong unit(s)
ppm	:	Parts per million
$^{\circ}$ C	:	Degree centigrade
RPM	:	Rounds per minute
mg%	:	Milli gram per cent
mg/dl	:	Milli gram per deci litre
DPBS	:	Dulbeccos Phosphate Buffer Saline
$\mu$ g	:	Micro gram
NS	:	Normal saline
g/dl	:	grams per deciliter

# ***INTRODUCTION***

## **Chapter I**

### **Introduction**

---

Buffalo has been an integral part of livestock agriculture in Asia for over 5000 years producing draft power, milk, meat and hides. More than half of India's milk is produced from buffaloes. Most Asian countries are agrarian with 60–80% of the population related to farm operations in one way or another. Livestock has been an integral component of traditional agriculture since centuries on record. In Asia, buffalo played a pivotal role in overall social development through contributions to milk, meat, hides and draft power for agricultural operations. Not only that, they are an easily 'convertible currency' and a reliable 'living bank' to serve the immediate needs of the rural masses in several communities.

Of all domestic animals, Asian buffalo holds the greatest promise and potential for production (Cockrill, 1994). Over the centuries, natural and human selection has produced a strong, hardy animal that can thrive on fairly

low-quality feed and forage. The goal of genetic improvement by dairy cattle is to produce the best possible genotype which will operate at top efficiency to which it is subjected, in order to bring the greatest possible profit to the dairy man.

The reproductive performance of buffalo remained much lower than in cattle (Dobson & Kamonpatana, 1986), and with population on decline in certain countries, some of the breeds are already at the verge of extinction. The Food and Agriculture Organization (FAO, 2000) has rightly termed buffalo as important but 'an asset undervalued'.

Of the two generic types, African (*Syncerus caffer*) and Asian (*Bubalus*), the later had three species, of which *Bubalus ami*, the Indian wild buffalo, was domesticated and named as *Bubalus bubalis*. Seals in Indus valley are suggestive of buffalo domestication approximately 5000 years ago. Some ancient writings and sculptures indicated it in 2500–2100 bc. From their home in India, they were carried for meat, milk and draft to Egypt by Arabic invaders in the 9th century and subsequently to Europe by pilgrims and crusaders in the middle ages (Saadulla, 1998). They have now spread to 38 countries in Asia, Europe, Africa, South America and Australia.

Follicular fluid contains numerous biochemical components that are essential for ovarian physiology, including steroidogenesis, follicle growth and ultimate maturation of oocytes, ovulation and oviductal transport of the oocytes.

Follicular fluid functions to provide nourishment to the oocytes and granulosa cells by facilitating transport of specialized nutrients from plasma. The dynamic constituents of this follicular fluid reflect both biochemical and endocrinological activity of the follicle, thereby facilitating its role as a conductor of growth and development through the reproductive cycle (Edwards, 1974). As is the case with any biological fluid, the physical characteristics of follicular fluid are the product of its chemical composition and reflect its isolation from physiological events occurring outside its environment. In a follicle, physiologic factors are influenced principally by the properties of the tissues, which separate follicular fluid from circulation. The permeability of the follicular wall to water and biochemical components originating from blood can markedly alter the composition of follicular fluid.

In addition, the ability of granulosa cells to secrete both stimulating and inhibitory factors as a result of metabolic processes can modify the composition of follicular fluid. These factors influence physical parameters such as osmolarity, colour,  $P^H$ , viscosity and volume of the fluid originating within the developing follicle (Fisch *et al.*, 1990).

It was originally proposed by (Harter, 1948) that follicular fluid was merely a simple transudate of blood that accumulates between the layers of the granulosa cells in growing follicles. It is now known that the follicular fluid contains a large variety of components of serum along with an array of secretions, which are synthesized by the specialized cells of the follicular

microenvironment. As a result, the components of follicular fluid change during growth and expansion of each follicle (Wise, 1987).

Follicular fluid is slightly viscous solution with a P<sup>H</sup> reported to be 7.4, similar to that of plasma. Investigators have reported various elements of follicular fluid to be similar to those found in serum, with only few differences (Edwards 1974 and Luck *et al.*, 2001).

Hence, an attempt is made to study the following objectives:

1. To know the biochemical components of follicular fluid in buffaloes.
2. To record the biometrics of ovaries in buffaloes.
3. To compare the efficacy of various oocyte retrieval methods in buffaloes.
4. To record the micrometry of buffalo oocytes.
5. To grade the oocytes based on morphology.

**REVIEW  
OF  
LITERATURE**

**Chapter – 2**  
**Review of literature**

The available literature on biometrics of ovaries, number of visible follicles, follicular fluid composition, corpus luteum biometrics and oocyte recovery were reviewed along with cows as the literature is meager on buffaloes.

**2.1 Biometrics of ovaries:**

The available literature on biometrics of ovaries of buffaloes were depicted in the table as follows

S.No	Left ovary					Right ovary					Breed	Author and year
	L	W	H	W	S	L	W	H	W	S		
1.	2.38 ±0.06	1.58 ±0.06	1.23 ±0.07	2.73 ±0.25	-	2.28 ±0.10	1.52 ±0.06	1.20 ±0.06	2.56 ±0.23	-	-	Demodaran, (1958)
2.	2.44	1.34	1.58	3.32	-	2.36	1.37	1.64	3.13	-	Buffalo	Bhalla, (1964)
3.	2.97	1.37	1.13	3.66	-	2.91	1.39	1.17	3.81	-	Murrah buffalo	Sane et al. (1964)
4.	3.13	1.44	1.15	3.87	-	3.12	1.49	1.19	4.01	-	Jaffri buffalo	Sane et al. (1965)
5.	3.09	2.29	-	5.37	-	3.31	2.42	-	6.34	-	Heifer	Drennan and Macpherson (1966)
6.	2.68 ±0.38	1.69 ±0.39	1.57 ±0.31	4.2 ±0.14	-	2.58 ±0.44	1.84 ±0.38	1.65 ±0.27	4.53 ±0.35	-	Egyptian buffalo	Ahmed and Abdel hafl (1968)
7.	-	-	-	-	1.623 ±0.47	-	-	-	-	1.568 ±0.375	Pre pubertal buffalo	Gurdial Singh and Singh (1988)
8.	2.48	1.67	1.46	3.71	-	2.44	1.74	1.47	3.77	-	Murrah buffalo	Parkale and Hukeri, (1989)
9.	2.50 ±0.06	1.44 ±0.05	0.84 ±0.15	3.80 ±0.12	-	2.53 ±0.05	1.45 ±0.04	0.85 ±0.4	3.53 ±0.10	-	Heifers African Zebu cattle	Chauhan and Alhaji, (1990)
10.	2.81 ±0.02	1.56 ±1.01	1.04 ±0.01	-	-	2.84 ±0.12	1.63 ±0.02	1.05 ±0.01	-	-	Cows of African Zebu cattle	
11.	1.96	1.07	1.38	2.54	-	2.09	1.17	1.47	2.72	-	Surti Buffalo	Pamar and Mehta (1992)
12.	2.15 ±0.07	0.71 ±0.006	1.21 ±0.04	2.16 ±0.14	-	2.17 ±0.05	1.72 ±0.04	1.23 ±0.04	2.15 ±0.16	-	Buffalo	Ramu, (2000)
13.	2.00 ±0.04	1.30 ±0.03	1.51 ±0.03	2.77 ±0.06	-	2.06 ±0.03	1.35 ±0.03	1.60 ±0.02	2.86 ±0.06	-	Murrah buffalo	Chandrasahsan and Rajasekaran (2004)
14.	-	-	-	2.65 ±0.14	-	-	-	-	2.53 ±0.15	-	Buffalo	Gupta et al. (2004)
15.	2.36 ±0.08	1.90 ±0.05	1.54 ±0.06	-	-	2.13 ±0.08	1.68 ±0.08	1.43 ±0.04	-	-	Murrah	Kumar et al. (2004)
16.	1.8 * ±0.6	2.7 * ±0.7									Zebu cattle	Lamorde and Kumar, (1978)
17.				2.72*							buffalo	Jindal et al. (1997)
18.	2.14* ±0.02	1.41* ±0.02	1.20* ±0.03	2.30* ±0.09							Tarai buffalo	Suresh Kumar et al. (2002)

L: length; W: width; H: height in cm; W: weight in g ; S: size in cc ; \* : Mean values

## CHAPTER-III

### MATERIALS AND METHODS

The present experiment was conducted at the poultry farm, college of veterinary science, Tirupati to study the effect of sheanut extract inclusion on the performance of layers.

#### 3.1 Source Of Sheanut Extract

Sheatnut extract was procured from Food fat and fertilizers factory located at Tadepalligudem in West Godavari District of Andhra Pradesh.

#### 3.2 Chemical Analysis

Samples of sheanut extract and experimental diets were analysed for proximate composition as per A.O.A.C (1990). ME content of sheanut extract was estimated by prediction equation (Janssen, 1989). The crude protein content and ME values of other ingredients were taken from table values of Panda *et al* (1990) except for ME of fishmeal by Narahari (1996) and for ME of Soybean meal by Saxena (1999). The saponin content in sheanut extract was estimated by the method of Yosioka (1974).

**cycle. While, Marian *et al.* (1968) noticed that the follicular growth was rapid up to 1 mm size and development from 1 to 2 mm was continuous in cows.**

**Dufour *et al.* (1971) and Fogwell *et al.* (1977) stated that the ovary with corpus luteum was found to have more large sized follicles than the ovary bearing corpus albicans. Kruij (1982) reported that follicle size of >10 mm in diameter were present in all days of estrous cycle except during the first 3 days and on day 9, 10, 11 and 12 of estrous cycle in cows. Moor *et al.* (1984) recorded the mean number of follicles on the ovarian surface was to be 8-10 in cows.**

**In a study on follicular population in cycling and non-cycling buffalo heifers, Danell (1987) observed the average number of follicles over 2.00 mm in diameter to be 16.8 and 23.6, respectively. El-wishy *et al.* (1988) noticed 10-20 mm sized follicles during the follicular phase of the cycle on the ovaries and the diameter of largest follicle in follicular, metestrous and di estrous phases of estrous cycle were recorded to be  $13\pm 3$ ,  $8\pm 3$  and  $11\pm 3.6$  mm, respectively in cows. Kalmath (2002) observed that more number of medium and large sized follicles during colder months (December to January) than during hot months (March and April) of the year.**

**In Tarai breed of buffaloes, Suresh kumar *et al.* (2002) noticed that the mean number of visible follicles (>2mm) per ovary was to be  $4.12\pm 1.321$  in India. Chandrahasan and Rajasekaran (2003) observed 4.72 follicles on the surface of ovary during the month of November in buffalo cows. While in Murrah breed of buffaloes, the total number of follicles on left and right**

ovaries was 3.45 and 3.83, respectively (Chandrasekaran and Rajasekaran, 2004).

Gupta *et al.* (2004) recorded that the mean total number of all sized follicles on the buffalo ovaries of left and right ovary was  $5.12 \pm 0.31$  and  $4.97 \pm 0.31$ , respectively.

The average number of aspirable follicles per ovary was  $7.33 \pm 3.8$  from ovaries having no dominant structure and  $3.67 \pm 2.2$  from ovaries having dominant follicles (Abdel Razek and Ali, 2005). The total number of follicles per ovary was 2.15, 1.9, 1.8 and 1.5 and the number of follicles sized  $>9\text{mm}$  per ovary was to be 0.27, 0.43, 0.42 and 0.37 during the months of spring, summer, autumn and winter seasons, respectively (Osman and Shehata, 2005).

### **2.2.2 Follicular fluid:**

The follicular fluid was aspirated from the different sized follicles present on the ovary. The biochemical and mineral profiles were estimated.

#### **2.2.2.1 Total protein**

Krishna murthy *et al.* (1986) observed that the protein concentration was 7.50g/dl in follicular fluid of buffalo. Younis *et al.* (1988) recorded that the total protein concentration was decreased with increasing the follicular size. Parmar and Mehta, (1991) reported that the overall concentration of total protein in all seasons was 9.78, 10.64, 9.35 and 10.25 g/ 100 ml in 1-4, 5-8, 9-12 and above 12 mm sized follicles, respectively. Similarly, the albumin

concentration was 3.40, 2.95, 3.33 and 4.10 in 1-4, 5-8, 9-12 and above 12 mm sized follicles, respectively and the overall albumin: globulin ratio was 0.58 in Surti breed of buffaloes.

Eissa, (1995) stated that the total protein levels were recorded to be higher at proestrous than met estrous or diestrous and reached peak values during estrous. Eissa, (1996) recorded that the total protein levels were to be 8.64, 12.44, 6.53 and 6.83 gm/lit in follicular fluid during Proestrous, Estrous, Metestrous and Diestrous, respectively in buffalo cows in Egypt.

Madan Mohan *et al.* ( 1997) recorded that the total protein concentration in small(6mm), medium(6-10mm) and large(11-16mm) sized follicles of left and right ovaries was 6.90 vs 7.2, 6.25 vs 7.0 and 6.60 vs 7.0 g per cent, respectively in electrophoretic analysis . With respect to albumin levels, 4.00 vs 4.40, 3.70 vs 4.20 and 3.95 vs 4.30 g per cent in small, medium and large sized follicles were noticed in follicular fluid of left and right ovaries, respectively. While the albumin: globulin ratio was 1.40 vs1.49, 1.45 vs 1.57 and 1.49 vs 1.60 in small, medium and large sized follicles of left and right ovaries.

Zeitoun, (2002) stated that the total protein and albumin concentrations in follicular fluid of cows were 3.4 and 2.1 g / dl. On the other hand, Mishra *et al.* (2003) stated that the concentration of protein decreased significantly from small to medium and from medium to large sized follicles. Thangavel and Mohammed Nayeem, (2004) recorded that the total protein concentration in

small, medium and large healthy follicles was 6.51, 6.48 and 6.28 g/ dl, respectively in buffalo follicular fluid.

Das *et al.* (2005) recorded that the mean concentration of total protein in pooled follicular fluid samples was  $4.86 \pm 0.41$  g% in buffalo in India. In Japan, Iwata *et al.* (2006) noticed that the mean total protein and albumin concentration in follicular fluid of bovine ovaries was  $7.5 \pm 0.1$  and  $3.4 \pm 0.1$  g/ dl.

#### 2.2.2.2 Cholesterol

Lutwak –Mann, (1954) noticed that the cholesterol concentration was 26.6 mg/ 100 ml in bovine follicular fluid. While in India, Krishnamurthy *et al.* (1986) recorded that the mean cholesterol concentration was 30.00 mg/ dl in buffalo follicular fluid. Younis *et al.* (1988) concluded that the cholesterol concentration was higher in medium than in small follicles.

Parmar and Mehta, (1991) recorded that the overall mean concentration of total cholesterol was 156.56, 153.19, 149.82 and 148.14 mg / 100 ml in 1-4mm, 5-8mm, 9-12mm and >12mm follicles, respectively in follicular fluid of Surti breed of buffalo. Patel *et al.* (1991) observed that the total cholesterol levels were to be 53.45 and 69.86 mg per cent in follicular fluid of pre ovulatory and follicular phases, respectively in the same breed of buffaloes.

Leroy *et al.* (2004) reported that the total cholesterol increased from small to large follicles. Thangavel and Mohammed Nayeem (2004) recorded

the concentration of cholesterol in small, medium and large follicles were 74.93, 80.77 and 74.90 mg per cent, respectively in buffaloes. In Japan, Iwata *et al.* (2006) noticed that the mean total cholesterol concentration in follicular fluid of bovine ovaries was to be  $74.5 \pm 4.5$  mg / dl.

### **2.2.2.3 Alkaline phosphatase**

Krishnamurthy *et al.* (1986) recorded that the mean alkaline phosphatase activity was 30.50 Bodansky units / dl in follicular fluid of buffaloes. Henderson and Cupps, (1990) stated that alkaline phosphatase activity was greater during the pre ovulatory phase of the estrous cycle than during other phases in cattle.

Parmar and Mehta, (1991) observed that the alkaline phosphatase activity was 15.52, 17.72 and 18.93 KA units during winter summer and monsoon seasons, respectively in follicular fluid collected from 1-4 mm sized follicles of Surti breed of buffaloes. But, Eissa, (1995) reported that the alkaline phosphatase activity was highest during diestrous phase of estrous cycle.

Eissa, (1996) recorded alkaline phosphatase activity was to be 135.33, 142.62, 131.54 and 198.44 IU / lit in follicular fluid of proestrous, estrous, met estrous and diestrous, respectively in buffalo cows of Egypt. Madan mohan *et al.* (1998) reported that the alkaline phosphatase activity was 14.00 and 27.00 KAU for small; 2.05 and 11.13 KAU for medium; 1.94 and 7.14 KAU for large follicles of left and right ovaries, respectively.

In Egypt, Zeitoun, (2002) reported that the alkaline phosphatase activity was to be 112.5 IU / lit in cyclic ovaries of buffalo cows. Mishra *et al.* (2003) stated that the alkaline phosphatase decreased progressively as follicle size increased in caprines. While, Iwata *et al.* (2006) noticed that the mean alkaline phosphatase activity in follicular fluid of bovine ovaries was  $230.6 \pm 14.4$  IU / lit.

### **2.2.3 Minerals:**

The mineral profiles in the follicular fluid were found to play a major role in maturation of oocyte in cattle.

#### **2.2.3.1 Calcium**

The Calcium concentration in cow follicular fluid was 8.6 mg / 100 ml (Lutwak –Mann, 1954); and  $7.9 \pm 0.3$  mg / dl (Iwata *et al.*, 2006). Eissa, (1995) stated that the calcium levels were higher at proestrous than metestrous or diestrous and reached peak values during estrous.

In buffaloes, Krishna murthy *et al.* (1986) and Bhardwaj *et al.* (1998) reported that the calcium concentration was to be 16.18 mg / dl and 24.8  $\mu$ g / g in the follicular fluid. Jagjit kaur *et al.* (1997) recorded that the Calcium concentration in follicular fluid of small, medium and large follicles was  $6.9 \pm 1.1$ ,  $5 \pm 1.6$  and  $8 \pm 1.2$   $\mu$ g / ml, respectively in buffaloes in India.

During proestrous, estrous, metestrous and diestrous the Calcium levels were noticed to be  $9.13 \pm 1.31$ ,  $10.5 \pm 1.50$ ,  $6.12 \pm 0.64$  and  $6.52 \pm 0.51$  mg

per cent, respectively in follicular fluid collected from follicles on the ovary (Eissa, 1996). Baszczyk *et al.* (2004) stated that the calcium concentration were similar in all sized follicles.

#### 2.2.3.2 Phosphorus

In cows, the inorganic Phosphorus was recorded to be 5.8 mg / 100ml in follicular fluid of follicles of cows by Lutwak –Mann, (1954). Krishna Murthy *et al.* (1986) reported that the inorganic Phosphorus concentration in Murrah buffalo follicular fluid was 12.60 mg / dl in India.

Eissa, (1995) noticed that the concentrations of phosphorus profile was observed to be increased markedly during the early stage of the cycle and tended to be decreased as the follicle size increased. During proestrous, estrous, diestrous and met estrous the phosphorus levels were recorded to be  $6.20 \pm 0.81$ ,  $9.32 \pm 1.34$ ,  $5.43 \pm 0.41$  and  $5.71 \pm 0.60$  mg%, in follicular fluid of follicles present on the ovary in buffalo cows, respectively (Eissa, 1996).

Jindal *et al.* (1997) reported that the phosphorous concentration was recorded to be 53.5 mg per cent in buffaloes. Iwata *et al.* (2006), recorded the inorganic phosphorous level was to be  $10.5 \pm 0.4$  mg / dl in follicular fluid of cows.

#### 2.2.3.3 Sodium

The Sodium concentration in fresh follicular fluid of cow was to be 252 mg / 100 ml Lutwak –Mann, (1954).

Krishna Murthy *et al.* (1986) reported that the Sodium concentration in Murrah breed of buffalo follicular fluid in estrous cycle was 362.00 mg / dl in India. While Jagjit kaur *et al.* (1997) recorded that the Sodium concentration in follicular fluid of small, medium and large sized follicles was  $25\pm 2.4$ ,  $25\pm 2.9$  and  $75\pm 4.2$   $\mu\text{g} / \text{ml}$ , respectively in buffaloes and in cyclic buffaloes, the sodium concentration of follicular fluid was 66.7  $\mu\text{g} / \text{g}$  (Bhardwaj *et al.*, 1998).

Bordoloi *et al.* (2001) reported that the Sodium concentration in small, medium and large follicles were  $115.67\pm 1.90$ ,  $119.67\pm 3.48$  and  $134.0\pm 1.8$  meq / lit, respectively in goat ovary. In small and large follicles, the Sodium concentration was recorded to be  $84.0\pm 5.87$  and  $97.25\pm 9.69$  meq / lit, respectively in caprines by Thakur *et al.* (2003).

#### 2.2.3.4 Copper

Jagjit kaur *et al.* (1997) stated that the Copper concentration in follicular fluid of small, medium and large sized follicles was 1.25, 1.25 and 0.83  $\mu\text{g} / \text{ml}$ , respectively in buffaloes. While in cyclic buffaloes Bhardwaj *et al.* (1998) recorded that the Copper concentration in follicular fluid as 0.40  $\mu\text{g} / \text{g}$  in India.

In Caprines, Bordoloi *et al.* (2001) recorded that the Copper concentration in small, medium and large sized follicles were to be 10.49, 9.31 and 7.73 mg per cent, respectively in India.

#### 2.2.3.5 Zinc

The Zinc concentration in follicular fluid of small, medium and large follicles was 514.7, 604.1 and 264.1  $\mu\text{g} / \text{ml}$ , respectively in buffaloes as recorded by Jigjit kaur *et al.* (1997). While in cyclic buffaloes Bhardwaj *et al.* (1998) noticed the Zinc concentration in follicular fluid as 6.88  $\mu\text{g} / \text{g}$  in cyclic Indian buffaloes.

In Caprines, Bordoloi *et al.* (2001) recorded the Zinc concentration as 3.49, 4.60 and 2.56 mg % in small, medium and large sized follicles, respectively.

#### 2.2.3.6 Iron

Jigjit kaur *et al.* (1997) reported that the Iron concentration in follicular fluid of small, medium and large follicles was 1.14, 13.5 and 8.3  $\mu\text{g} / \text{ml}$ , respectively in buffaloes. On the other hand, Bhardwaj *et al.* (1998) recorded Iron concentration as 1.72  $\mu\text{g} / \text{g}$  in follicular fluid of cyclic buffaloes (*Bubalus bubalis*).

In Caprines, Bordoloi *et al.* (2001) recorded that the Iron concentration in small, medium and large sized follicles were to be 3.40, 4.70 and 2.29 mg %, respectively.

#### 2.3 Corpus luteum

Mc Nutt, (1924) observed the colour of corpus luteum in cows was yellow or orange yellow and sometimes muddy dirty yellow. On the contrary, Asdell, (1955) observed that the colour was brown first and gradually turned

to less brown. El-sheikh and Abdel hadi, (1970) observed the colour of corpus luteum was brown to pale yellow in buffaloes. Similarly, Ireland *et al.* (1980) reported that the colour of corpus luteum was changed from red to brown, tan to orange and light yellow to white during the estrous cycle. Napolean, (1989) reported the colour of corpus luteum as bright red, tan, grey and reddish brown in pro estrous, estrous, met estrous and di estrous, respectively.

Mc Nutt, (1924) stated that the shape of the fully formed corpus luteum to be either globular or oblong in cow and the diameter of the corpus luteum in cow was 22-30 mm and the young corpus luteum diameter was 6-8 mm. Hafez, (1955) recorded the diameter of buffalo corpus luteum was to be 1.2, 1.5 and 1.0 cm in early diestrous, mid diestrous and late di estrous, respectively.

On the other hand, El-sheikh and Abdel-hadi, (1970) stated that the shape of the buffalo ovary was oval, occasionally spherical and mostly buried in the ovarian stroma. Luktuke and Rao, (1962) noticed the length and breadth of corpus luteum were to be maximum during 10-15 days of estrous cycle and reduced to 1.22 - 1.23 cm during 16-20 days of estrous cycle. The average diameter of the corpus luteum was measured to be 16.85 mm in buffaloes (El-sheikh and Abdel hadi, 1970).

Ireland *et al.* (1979) reported the mean corpus luteum weight was to be  $1.00 \pm 0.26$ ,  $3.35 \pm 0.13$ ,  $3.61 \pm 0.11$  and  $1.34 \pm 0.14$  g, during pro estrous, estrous, met estrous and di estrous, respectively. In another study, the mean weight of the corpus luteum was noticed to be 0.8, 3.4, 3.6 and 3 g and the

size of corpus luteum was recorded to be 0.5-1.5; 1.6-2.0; 1.6-2.0 and 1 cm in pro estrous, di estrous, met estrous and di estrous, respectively by Ireland *et al.* (1980).

Napoleon, (1989) recorded that the length and diameter of the corpus luteum was 0.57, 1.45, 1.25, 1.08 and 0.63, 1.05, 0.86 and 0.85 cm, respectively in four stages of the estrous cycle in buffaloes.

## **2.4 Oocytes:**

On scanning of literature, the oocytes were recovered by employing slicing, aspiration and dissection techniques. The micrometry of oocytes was measured to be variable.

### **2.4.1 Number of oocytes**

In Poland, Lucyna and ZDzislaw, (1984) recorded that the mean number of normal oocytes recovered from 2-6 mm follicles in heifers, younger and older cows were 4.63, 5.24 and 3.89, respectively. while, Bottcher *et al.* (1989) reported that the per cent of first quality oocytes were 60 in rupture of isolated follicles, 14 in aspiration and 0 in dissection methods. The recovery of oocytes by needle puncture and dissection methods was noticed to be  $10.0 \pm 4.4$  and  $16.0 \pm 7.1$ , respectively by Sato *et al.* (1990). Lonergan *et al.* (1991) studies revealed that the recovery of oocyte per ovary was 18.7 by dissection and 14.6 by aspiration method and more number good quality of

**oocytes were recovered by dissection when compared to aspiration technique.**

**In India, Selvaraj *et al.* (1992) recorded that the oocyte recovery per cent was 81.4, 87.0 and 89.9 in <0.5, 0.5 and > 0.8 cm follicles, respectively from the ovaries of buffaloes procured from slaughter house. The per cent of good quality oocytes observed were 44.7, 28.3 and 22.5 in <0.5, 0.5 and > 0.5 cm sized follicles, respectively in buffalo ovaries.**

**Takagi *et al.* (1992) noticed that the number of follicular oocytes with an intact and unexpanded cumulus was averaged as  $17.8 \pm 1.9$  and  $14.3 \pm 1.9$  in aspiration and post aspiration mincing, respectively in bovine ovaries.**

**Das *et al.* (1992) recorded that the good quality oocytes recovered was 2.6, 1.3 and 0.9 in slicing, follicle puncture and follicle aspiration, respectively. In another study by Das *et al.* (1996) recorded the oocyte recovery per ovary was to be 5.7, 2.6 and 1.7 in slicing, follicle puncture and follicle aspiration methods, respectively in buffalo ovaries of India.**

**While, Khan *et al.* (1997) observed that the average number of oocytes recovered was 5.15 and 3.85 in aspiration and slicing, respectively in buffalo ovaries collected from slaughterhouse in Pakistan. Similarly, in India Khan *et al.* (1997) recorded that the morphologically good oocytes were 3.30 and 1.76 per ovary in aspiration and slicing methods in slaughtered buffalo ovaries.**

**Baruha *et al.* (1998) recorded that the mean number of oocytes recovered per ovary was 3.63 and 4.89 in aspiration and dissection methods, respectively in Indian cattle. While, Das and Mohd Amanullah, (1998)**

reported that the oocyte recovery per ovary was 1.08, 1.06 and 0.78 in January, February and March months, respectively in buffaloes in India.

Datta and Goswami, (1998) recorded that the number of oocytes recovered per ovary was 0.59, 1.11 and 1.35 in slicing, aspiration and dissection methods respectively in buffalo ovaries in India. Likewise, Deepak sharma *et al.* (2000) noticed that the average mean number of culturable oocytes identified on the basis of gross morphology in aspiration, individual follicle isolation and whole ovary slicing were 0.26, 0.88 and 0.68 per pair of ovaries, respectively in buffaloes.

Nandi *et al.* (2000) recorded an average of 1.17 oocytes per ovary by aspiration of ovarian follicles from ovaries of buffalo and the number of oocytes recovered from ovaries with corpus luteum and without corpus luteum was 1.06 and 1.25, respectively. While, Yadav *et al.* (2000) reported that the oocyte recovery per ovary was 0.86, 0.56 and 0.57 during cool, hot and moderate hot seasons, respectively in buffalo ovaries.

Suresh Kumar and Maurya, (2000) observed that the oocyte recovery per ovary was 3.09 and 1.89 in slicing and aspiration methods in buffalo ovaries, respectively. Ali and Abdel Razek, (2001) and Hamam *et al.* (2001) reported that the average number of recovered oocytes per ovary was 2.72 and 3.33 per ovary, respectively in Egyptian buffaloes.

Raza *et al.* (2001) reported that the good quality oocytes were 3.85 and 1.76 per ovary by slicing and aspiration methods, respectively. On the other hand, Sajjan singh *et al.* (2001) recorded that the average number of

oocytes recovered per ovary with and without corpus luteum was 0.41 and 0.67, respectively in buffalo ovaries. While Zeron *et al.* (2001) noticed that the oocyte collection per ovary was 7.5 and 5.0 during winter and summer seasons, respectively from bovine ovaries in Israel.

Naik *et al.* (2002) reported that out of 169 oocytes recovered from (3-5 mm follicles) 39.05 per cent were good, 39.05 per cent were fair and 19.52 per cent were of poor quality oocytes. Similarly out of 136 oocytes that were recorded from (> 5 mm follicles) 40.44 per cent - good, 47.05 per cent - fair and 12.5 per cent - of poor quality in buffalo ovaries. Palanisamy *et al.* (2002) recorded the mean number of follicles aspirated in the categories of <3, 3-5 and >5 mm size was 2.75, 1.02 and 0.72, respectively and the mean number of oocytes recovered was 1.22, 0.51 and 0.31, respectively in buffalo ovaries.

Sianturi *et al.* (2002) recorded that the total number of oocytes recovered during aspiration and slicing techniques were 12.02 and 29.38, respectively in bovines of Indonesia. Amongst the yielded oocytes, the good quality oocytes were 8.21 and 19.65 during aspiration and slicing methods, respectively in bovines.

Wakle *et al.* (2002) reported that the overall per cent of good, fair and poor quality oocytes were 25.84, 36.2 and 37.78, respectively in different sized ovarian follicles in ovaries of buffaloes. Wakle *et al.* (2002) reported that the overall oocyte recovery per cent was 73.23 in buffalo ovaries in India and the per cent of good quality oocytes recovered from follicles of larger diameter was higher compared to follicles of smaller diameter.

Gupta *et al.* (2004) observed that the mean number of oocytes recovered were to be 3.14 and 3.23 in right and left buffalo ovaries, respectively. But in Pakistan, Warriach and Chohan, (2004) recorded an average of 0.78 oocytes per ovary.

Abdel Razek and Ali, (2005) recorded that the slicing technique ( $5.00 \pm 2.3$ ) significantly yielded higher number of oocytes per ovary than the aspiration technique ( $3.90 \pm 2.4$ ) and the number of oocytes with more than five cumulus layers was to be 62.1 vs 15.3 per cent in slicing and aspiration methods, respectively. In the same study, the average number of recovered oocytes per ovary was  $5.75 \pm 2.4$  and  $2.79 \pm 2$  from ovaries with no dominant structure and from ovaries with dominant follicle, respectively in buffaloes of Egypt. Likewise, the ovaries with corpus luteum revealed  $4.0 \pm 3.7$  number of aspirable follicles and  $3.22 \pm 3.1$  oocytes was recovered per ovary. Higher number of oocytes with more than 5 cumulus layers was collected from ovaries with or with out dominant follicle was to be 28.9 and 5.1 per cent, respectively.

Das *et al.* (2005) reported that overall oocyte recovery and recovery of type A and type B oocytes were to be 0.81, 0.43 and 0.37 per ovary, respectively. Fassi Fihri *et al.* (2005) recorded that the oocyte yielded in aspiration, puncture and slicing of ovaries were to be 2.59, 3.10 and 6.25, respectively in cows in Morocco.

The number of intact oocytes was 92.3, 88.9 and 71.8 % in medium, large and small follicles (Osman and Shehata, 2005). However in Japan,

Iwata *et al.* (2006) recorded the mean number of oocytes collected was to be  $18.9 \pm 1.7$  from bovine ovaries.

#### **2.4.2 Micrometry of oocytes**

In India, Selvaraj *et al.* (1992) reported that the mean oocyte diameter was 77.9, 90.6 and 95.1  $\mu$  in <0.5, 0.5 and > 0.5 cm sized follicles in buffalo ovaries.

Naik *et al.* (2002) reported that the oocytes retrieved from 3-5 mm and >3mm sized follicles measured to be 152.24  $\mu$  and 159.6  $\mu$  in buffaloes. But In pre pubertal Indian buffaloes (*Bubalus bubalis*), the micrometry of oocytes retrieved from primordial, primary, secondary and graffian follicles were 22.00, 22.73, 38.13 and 79.20  $\mu$ m, respectively (Bhardwaj and Roy, 2004). In another study, Bhardwaj and Roy, (2006) recorded the oocyte diameter in primordial, primary, secondary and small sized graffian follicles were 22.93, 22.94, 33.00 and 91.40  $\mu$ m, respectively in ovaries of Indian buffalo.

***MATERIALS  
AND  
METHODS***

## **Chapter – 3**

### **Materials and methods**

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The present investigation was carried out at the Department of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary Science, Rajendranagar, Hyderabad during the period January 2006 to August 2006.

#### **3.1 Source of material**

The female non gravid genitalia of pluriparous buffaloes irrespective of stage of estrous cycle were collected randomly from Municipal slaughter house, Hyderabad. The ovaries were collected in pairs immediately after slaughter and labeled separately as left and right. For estimation of biochemical indices of follicular fluid, the ovaries were transported to the laboratory in normal saline containing Gentamycin (50 µg / ml of NS) within

90 minutes in thermacol box containing ice cubes. Another group of ovaries were transported to the laboratory at 30-38 °C temperature within 90 minutes.

### 3.2 Stage of Estrous Cycle

The ovaries were examined for presence of corpus luteum and follicles to assess the stage of estrous cycle as per the technique adopted by Ireland *et al.* (1980).

**Table: Showing classification of estrous cycle based on the appearance of corpus luteum in buffaloes.**

Stage of estrous cycle	Appearance of corpus luteum
I. Pro estrous	Corpus luteum was red in colour. No visible vasculature on corpus luteum was noticed.
II. Estrous	Apex of the corpus luteum was red or brown and the internal part was orange in colour.
III Met estrous	On bisection, the entire corpus luteum was orange in colour.
IV. Di estrous	Corpus albican/s and one large follicle were observed.

### 3.3 Ovaries

The ovaries were washed twice with normal saline in the laboratory in order to remove blood clots if any present on the surface of the ovaries and used for the present study.

### 3.3.1 Biometrics

Ovaries were thoroughly examined for the gross anatomy and functional status of reproduction. The diameter between the two extremities of the ovary was considered as the length. The ovarian width was recorded as the greatest distance between lateral and medial surfaces. The thickness of the ovary was recorded from middle portion of the ovary. The Length, Width and Thickness of the ovaries were measured by using Vernier calipers and expressed in cms. The weight of the ovary was recorded by using digital balance after trimming off extraneous tissue and expressed in gms. The size of the ovary was calculated by using the formula given by Gurdial Singh and Singh, (1988) as given below.

$$\text{Size of the ovary} = \frac{\text{Length} \times \text{Width} \times \text{Height}}{6} \text{ (cm)}$$

### 3.3.2 Corpus luteum

The shape, colour and measurements of corpus luteum were recorded. After observing the colour, the external visible portion of corpus luteum was squeezed out from the ovary and weight was recorded. The corpus luteum was divided into two sagittal sections, the length and width was measured with Vernier calipers. The distance between widest portions was represented as the diameter of corpus luteum.

### 3.3.3 Follicles

The number of visible follicles of various sizes existed on the surface of the individual ovary were recorded. The diameters of visible follicles present on individual ovary were recorded with the help of vernier calipers. Based on the external diameter of follicle, the follicles were classified as per the method adopted by Chandrahasan and Rajasekaran, (2004).

**Table: Showing the details of classification of follicles present on ovary in buffaloes.**

S.No	Measurement (mm)	Grade
1.	< 2	Very small
2.	3-5	Small
3.	6-10	Medium
4.	>10	Large

### 3.4 Follicular fluid

The follicular fluid was collected with the help of glass and insulin syringes according to the size of the follicle in the present study. Medium to large sized follicles were aspirated with 18 gauge hypodermic needle attached to 5 ml glass syringe for collection of follicular fluid. The small to very small sized follicles were aspirated by using insulin syringe.

### **3.4.1 Biochemical indices**

The follicular fluid was subjected to centrifugation at 1500 rpm at 5 °C for 5 minutes to remove cells and debris. The supernatant fluid was transferred in to Appendorf tubes. The collected follicular fluid from respective size of follicle was preserved at -20 °C till the estimation of biochemical profiles. The levels of total protein, albumin, cholesterol, alkaline phosphatase activity and minerals in follicular fluid were estimated in the present study.

#### **3.4.1.1 Total Protein**

The total Protein and Albumin levels were estimated in follicular fluid by adopting the procedure of modified Biuret End Point Assay. After estimation of total Protein and Albumin levels, Globulin levels were arrived by subtracting Albumin levels from Total Protein levels. After calculation of Globulin levels, the Albumin and Globulin ratio was obtained by adopting the method of Brar, (2002).

Three test tubes were taken and labeled as blank (B), standard (S) and test (T). Ten  $\mu$ l of follicular fluid and 1 ml of reagent 1 were added to test tube (T). For standard, 10  $\mu$ l of reagent 2 and 1000  $\mu$ l of reagent 1 was added. For blank 1000  $\mu$ l of reagent 1 was added and mixed well and kept at room temperature for 5 min and the absorbance was measured at 578 nm in spectrophotometer.

The total protein and Albumin in follicular fluid was calculated by using the formula

$$\text{Total protein (g/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times 6.5$$

$$\text{Albumin (g/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times 4$$

#### 3.4.1.2 Total cholesterol

The total cholesterol level in follicular fluid was obtained by using standard method of Wybenga and Pileggi's method, (1970).

Three test tubes were taken and labeled as blank (B), standard (S) and test (T). 0.5 ml of cholesterol reagent was added to each test tube and 0.025 ml of distilled water to blank, 0.025 ml of standard to standard and 0.025 ml of follicular fluid was added to test. Test tubes were mixed well and kept the tubes in water bath for exactly 60 sec and they were allowed to run under cool tap water. The absorbance was measured against blank at 560 nm with the help of spectrophotometer.

The total cholesterol concentration was calculated by using the formula

$$\text{Total cholesterol (mg \%)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times 200$$

#### 3.4.1.3 Alkaline phosphatase

The Alkaline phosphatase activity in follicular fluid was estimated by using standard method of Kind and King's method.

Four test tubes were taken and labeled as blank (B), standard (S), control (C) and test (T). Working buffered substrate and deionised water was added to B, S, C and T at the rate of 1 and 3.1 ml each. After 3 minutes of incubation 0.1 ml of follicular fluid was added to T and 0.1 ml of phenol standard to S was added and again incubated for 15 minutes. Colour reagent (2) was added to all the test tubes and finally 0.1 ml of follicular fluid was added to C test tube.

The absorbance was measured in spectrophotometer at 510 nm.

$$\text{ALP activity (KA units)} = \frac{\text{Absorbance of Test} - \text{Absorbance of Control}}{\text{Absorbance of Standard} - \text{Absorbance of Blank}} \times 10$$

#### 3.4.2 Minerals

The macro minerals viz., calcium, phosphorus and trace minerals viz., copper, sodium, zinc and iron in follicular fluid was estimated.

##### 3.4.2.1 Calcium

The calcium level in follicular fluid was estimated by adopting the procedure of O-Cresolphthalein Complexone, End point Assay.

Three test tubes were taken and labeled as blank (B), standard (S) and test (T). 20 µl of follicular fluid and 1000 µl of working calcium reagent were added to test. For standard, 20 µl of reagent 3 and 1000 µl of working calcium reagent were added. For blank 1000 µl of working calcium reagent was added and mixed well and incubated at room temperature for 5 min and the absorbance was measured at 578 nm in spectrophotometer.

$$\text{Calcium concentration (mg/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times 10$$

#### 3.4.2.2 Phosphorus

The phosphorus level in follicular fluid was obtained by using modified metol method by using Qualigens kit.

Three test tubes were taken and labeled as blank (B), standard (S) and test (T). 1 ml of catalyst reagent, 1 ml of Molybdate reagent, 0.1 ml of deionized water and 1 ml of Metol reagent was added to blank. For standard 1 ml of catalyst reagent, 1 ml of Molybdate reagent, 0.1 ml of standard and 1

ml of Metol reagent was added. 1 ml of catalyst reagent, 1 ml of Molybdate reagent, 0.1 ml of follicular fluid and 1 ml of Metol reagent was added. All the tubes were mixed well and kept at room temperature for 5 minutes and the absorbance was measured against 680 nm in Spectrophotometer.

Absorbance of Test

Phosphorus concentration in follicular fluid (mg %) = ----- X 5

Absorbance of Standard

### 3.4.3 Trace minerals

Mineral content in follicular fluid was determined as per the method described by Sandel, (1950) and modified by Arenza *et al.* (1977) utilizing Atomic Absorption Spectrophotometer. Accordingly, one ml of follicular fluid was digested with 20 ml triacid mixture (Concentrated Nitric acid 9 parts, concentrated sulphuric acid 2 parts and concentrated perchloric acid 1 part) on hot plate at 180-200 °C temperature till the dense white colour fumes of triacid mixture appeared. Then it was transferred to a 50 ml volumetric flask by several washings through Whatman filter paper No. 42. Washing of each sample was done by double distilled water and made up to the final volume of 50 ml and then each sample was transferred to a separate sterilized plastic vials. Thereafter, the clear solution was processed in Atomic Absorption Spectrophotometer (Perkin Elmer A Analyst 100) with standard solution of

different concentrations of elements in order to estimate the final concentration of minerals. The concentrations of trace elements were expressed as ppm.

### **3.5 Ovaries**

The ovaries collected for oocyte retrieval were processed separately. The extraneous tissue of the ovaries was removed in the laboratory.

#### **3.5.1 Oocyte collection**

The oocytes were retrieved by three methods as outlined by Suresh kumar and Mourya (2000).

##### **3.5.1.1 Slicing**

The ovaries were held firmly with forceps in a Petridish having DPBS and were sliced with an ordinary blade fixed in the artery forceps and made the ovary in to the possible thin sections. The Pieces of ovarian stroma were transported to fresh washing media in another Petridish, rinsed and discarded.

### **3.5.1.2 Aspiration**

The ovaries were held firmly with forceps and the follicular fluid was aspirated through 0.22 micron syringe filter fitted to 5 ml syringe. The filters were rinsed with phosphate buffer saline into disposable Petridishes.

### **3.5.1.3 Dissection**

The ovaries were dissected bluntly with the help of forceps and isolated the individual follicles. The follicles were kept in Petridish containing DPBS and were ruptured.

The media containing oocytes obtained by 3 different techniques were observed under the stereo zoom microscope (Olympus, Japan). The total number of oocytes of different grades were counted and recorded.

### **3.5.2 Grading**

The oocytes collected were graded according to the classification adopted by Leibfried and First, (1979).

**Table: Showing the grading of oocytes based on cumulus cellular investment in buffaloes.**

S.No	Cumulus	Score
1.	Complete cumulus oophorus was present in more than three layers thickness.	Good
2.	Partial cumulus present either not completely surrounding the oocyte or less than 3 layer thickness.	Fair
3.	Cellular investment absent.	Poor

### **3.5.3 Micrometry**

The oocytes of different grades were measured with the help of micrometer. The micrometry stage was calibrated to eye piece and standardized. One unit of eye piece micrometry is equal to 3.5  $\mu$ . The diameter of an oocyte was calculated by using the formula.

The micrometry was recorded in terms of diameter of intact cumulus cells was recorded.

### **3.6 Statistical analysis**

The data collected were subjected to appropriate methods of statistical analysis as per Snedecor and Cochran (1994).

# ***RESULTS***

## **Chapter - 4**

### **Results**

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The results of the present study were explained with the help of 11 tables and 14 figures.

#### **4.1 Biometrics of ovaries**

The biometry of the ovaries of buffaloes deserves much attention, the reason being the most important germ plasm comes out of slaughtered buffaloes. The biometrics of ovaries is presented in Table 1.

##### **4.1.1 Length**

The mean length of left ovary and right ovary was  $2.04 \pm 0.05$  and  $2.38 \pm 0.07$  cm, respectively. However there was no significant difference between the left and right ovaries as well as between the phases of estrous cycle (Table 1 and Fig.1).

#### **4.1.2 Width**

The mean width of left and right ovary was  $1.27\pm 0.04$  and  $1.44\pm 0.07$  cm, respectively without any significant difference between left and right ovary and among the stages of estrous cycle (Table 1 and Fig.2).

#### **4.1.3 Height**

The mean height was  $1.41\pm 0.04$  and  $1.63\pm 0.06$  cm, for the left and right ovaries, respectively. No significant difference was recorded in the present study (Table 1 and Fig.3).

#### **4.1.4 Weight**

The mean weight of left ovary was  $2.72\pm 0.15$  g and right ovary was  $3.74\pm 0.28$  g. The weight of left ovary was significantly lower during the pro estrous period and significantly higher during estrous, met estrous and di estrous periods (Table 1). Where as the weight of right ovary was not significantly differed among the phases of estrous cycle.

In pro estrous and met estrous the weight of the right ovary was significantly ( $p < 0.05$ ) higher when compared to the left ovary. But there was no significant difference in estrous and diestrous phases of estrous cycle. (Table 1 and Fig.4).

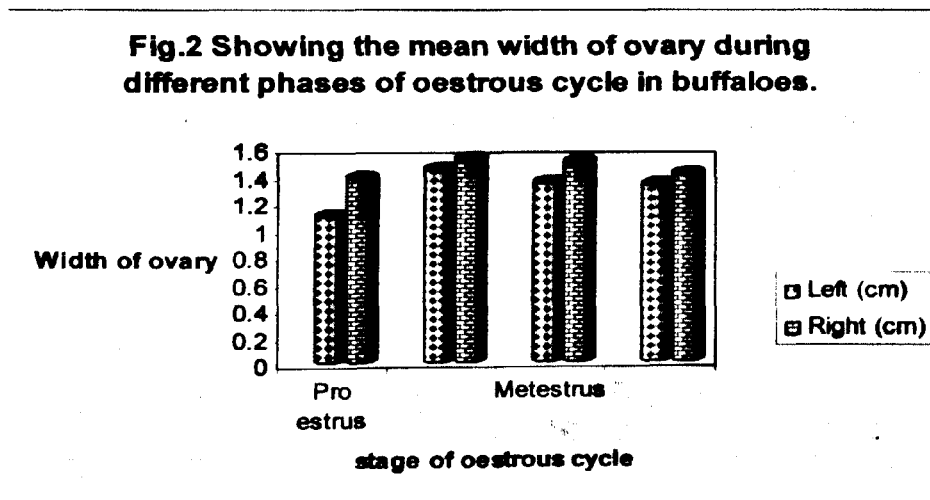
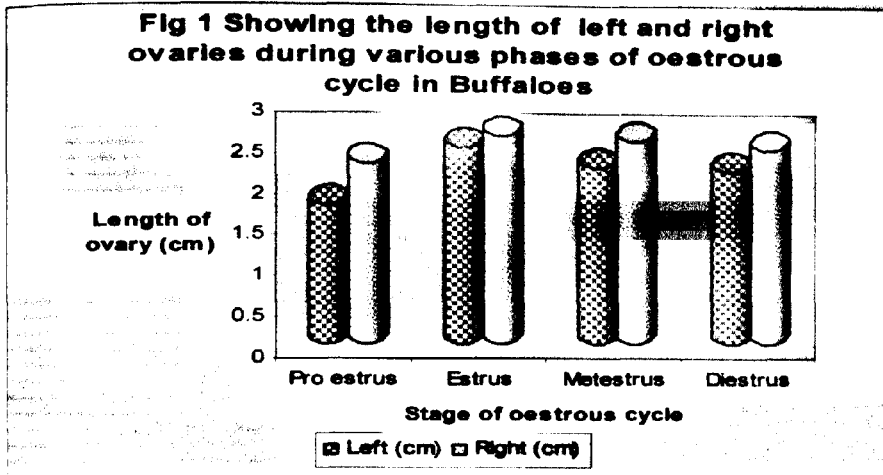
S.No	Stage of E.C	Length (cm)		Width (cm)		Height (cm)		Weight (g)		Size (cc)	
		L	R	L	R	L	R	L	R	L	R
1.	PE	1.68 <sup>a*</sup> ±0.04	2.19 <sup>a*</sup> ±0.14	1.09 <sup>a*</sup> ±0.04	1.37 <sup>a*</sup> ±0.13	1.14 <sup>a*</sup> ±0.04	1.50 <sup>a*</sup> ±0.12	1.93 <sup>a*</sup> ±0.16	3.41 <sup>a*</sup> ±0.57	1.17 <sup>a*</sup> ±0.10	2.71 <sup>a*</sup> ±0.58
2.	E	2.39 <sup>a*</sup> ±0.09	2.52 <sup>a*</sup> ±0.15	1.43 <sup>a*</sup> ±0.10	1.51 <sup>a*</sup> ±0.15	1.69 <sup>a*</sup> ±0.10	1.79 <sup>a*</sup> ±0.14	3.58 <sup>b*</sup> ±0.36	3.86 <sup>a*</sup> ±0.58	3.47 <sup>b*</sup> ±0.53	4.11 <sup>b*</sup> ±0.89
3.	ME	2.14 <sup>a*</sup> ±0.05	2.46 <sup>a*</sup> ±0.15	1.33 <sup>a*</sup> ±0.08	1.48 <sup>a*</sup> ±0.14	1.51 <sup>a*</sup> ±0.08	1.67 <sup>a*</sup> ±0.14	3.01 <sup>b*</sup> ±0.20	4.01 <sup>a*</sup> ±0.58	2.32 <sup>a*</sup> ±0.25	3.68 <sup>a*</sup> ±0.82
4.	DE	2.11 <sup>a*</sup> ±0.10	2.37 <sup>a*</sup> ±0.14	1.32 <sup>a*</sup> ±0.11	1.39 <sup>a*</sup> ±0.12	1.41 <sup>a*</sup> ±0.11	1.57 <sup>a*</sup> ±0.12	2.74 <sup>b*</sup> ±0.32	3.67 <sup>a*</sup> ±0.53	2.39 <sup>a*</sup> ±0.54	2.91 <sup>a*</sup> ±0.59
5.	Avg	2.04 ±0.05	2.38 ±0.07	1.27 ±0.04	1.44 ±0.07	1.41 ±0.04	1.63 ±0.06	2.72 ±0.15	3.74 ±0.28	2.25 ±0.21	3.35 ±0.36

PE: Pro estrous; E: Estrous; ME: Met estrous; DE: Di estrous

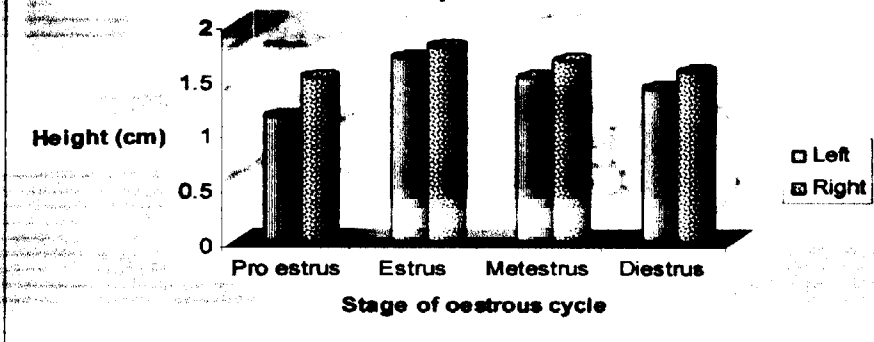
Alphabets for column comparison

Symbols for rows comparison

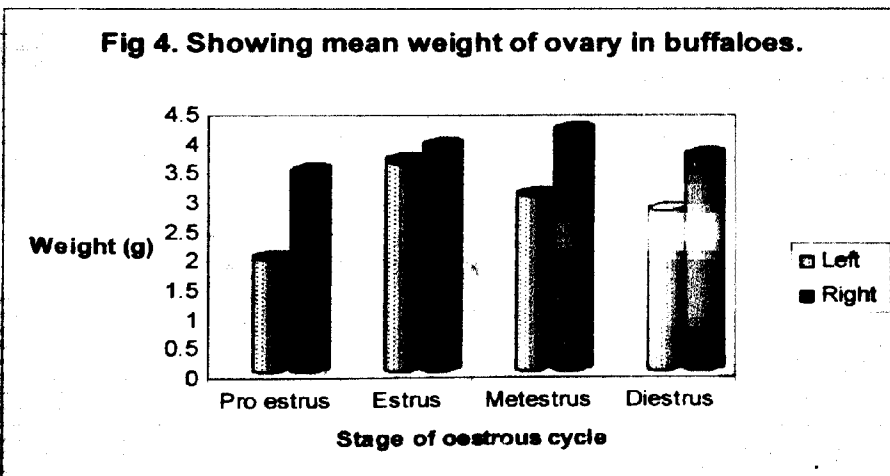
Figures with same alphabet or symbol are not significant (p<0.05)

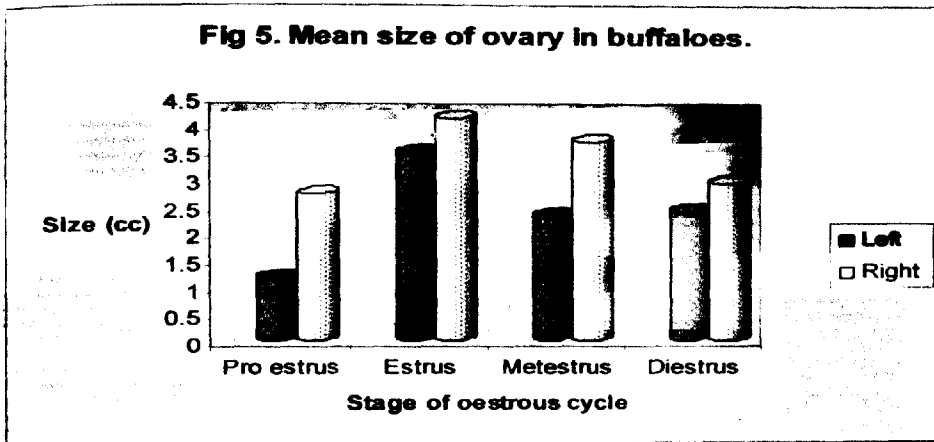


**Fig3. Showing the mean height of ovary in buffaloes.**



**Fig 4. Showing mean weight of ovary in buffaloes.**



**Fig 5. Mean size of ovary in buffaloes.**

#### **4.1.5 Size**

Among the stages of estrous cycle, the size of left and right ovaries was significantly ( $p < 0.05$ ) higher in estrous followed by metestrous, diestrous and proestrous stage of estrous cycle. However, the sizes of left and right ovary were significantly differed during proestrous and metestrous period (Table 1 and Fig.5).

#### **4.2 Number of visible follicles**

The mean number of different sizes of follicles recorded in the present investigation was depicted in Table 2.

The mean number of  $< 2$ mm size visible follicles observed on left and right ovaries was  $2.21 \pm 0.13$  and  $2.57 \pm 0.20$ , respectively and no significant difference was noticed between left and right ovaries (Table 2 and Fig.6).

The mean number of 3-5mm follicles present on left and right ovaries was  $0.96 \pm 0.16$  and  $0.64 \pm 0.11$ , respectively without any significant difference between left and right ovaries in the present investigation (Table 2 and Fig.6).

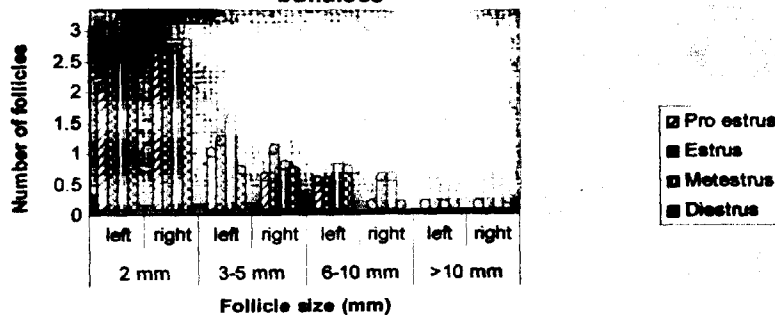
The number of 6-10 mm visible follicles noticed on the left ovary was significantly ( $p < 0.05$ ) higher than the right ovary. The mean number of follicles present on left and right was  $0.49 \pm 0.08$  and  $0.23 \pm 0.08$ , respectively (Table 2 and Fig.6).

The mean number of follicles sized  $> 10$ mm was  $0.21 \pm 0.05$  and  $0.23 \pm 0.06$  on left and right ovaries, respectively without any significant

**Table 2. Number of visible follicles (Mean  $\pm$  S.E) present on left and right ovaries of buffaloes**

No	Stage of Estrous cycle	Size of follicles(mm)									
		<2		3-5		6-10		>10		Total	
		Left	Right	Left	Right	Left	Right	Left	Right	Left	Right
	Pro estrous	1.93 $\pm 0.25$	2.55 $\pm 0.39$	0.86 $\pm 0.25$	0.45 $\pm 0.25$	0.39 $\pm 0.14$	0.00 $\pm 0.00$	0.00 $\pm 0.00$	0.00 $\pm 0.00$	3.18 $\pm 0.36$	3.00 $\pm 0.57$
2.	Estrous	2.38 $\pm 0.26$	2.55 $\pm 0.31$	1.05 $\pm 0.34$	0.91 $\pm 0.21$	0.38 $\pm 0.13$	0.45 $\pm 0.21$	0.48 $\pm 0.11$	0.45 $\pm 0.16$	4.29 $\pm 0.47$	4.36 $\pm 0.34$
3.	Met estrous	2.40 $\pm 0.24$	2.55 $\pm 0.43$	1.40 $\pm 0.50$	0.64 $\pm 0.24$	0.73 $\pm 0.23$	0.45 $\pm 0.21$	0.13 $\pm 0.09$	0.36 $\pm 0.15$	4.67 $\pm 0.53$	4.00 $\pm 0.57$
4.	Di estrous	2.29 $\pm 0.24$	2.64 $\pm 0.51$	0.57 $\pm 0.17$	0.55 $\pm 0.21$	0.57 $\pm 0.20$	0.00 $\pm 0.00$	0.29 $\pm 0.13$	0.09 $\pm 0.09$	3.71 $\pm 0.45$	3.27 $\pm 0.66$
5.	Average	2.21 $\pm 0.13$	2.57 $\pm 0.20$	0.96 $\pm 0.16$	0.64 $\pm 0.11$	0.49 $\pm 0.08$	0.23 $\pm 0.08$	0.21 $\pm 0.05$	0.23 $\pm 0.06$	3.87 $\pm 0.23$	3.66 $\pm 0.28$

**Fig 6. Mean number of follicles present on the ovary in buffaloes**



**difference between left and right ovaries and phases of estrous cycle (Table 2 and Fig.6).**

The overall mean number of follicles present on the left and right ovary was  $3.87 \pm 0.02$  and  $3.66 \pm 0.28$ , respectively.

#### **4.3 Biometrics of corpus luteum**

The mean length of corpus luteum was  $1.10 \pm 0.06$  cm. However the length was significantly ( $p < 0.05$ ) lower ( $0.69 \pm 0.05$ ) and higher ( $1.49 \pm 0.05$ ) in proestrous and estrous phases of cycle, respectively in the present study (Table 3).

The mean diameter of corpus luteum was  $0.86 \pm 0.04$  cm. The diameter was significantly ( $p < 0.05$ ) low in proestrous stage of estrous cycle when compared to other phases of estrous cycle.

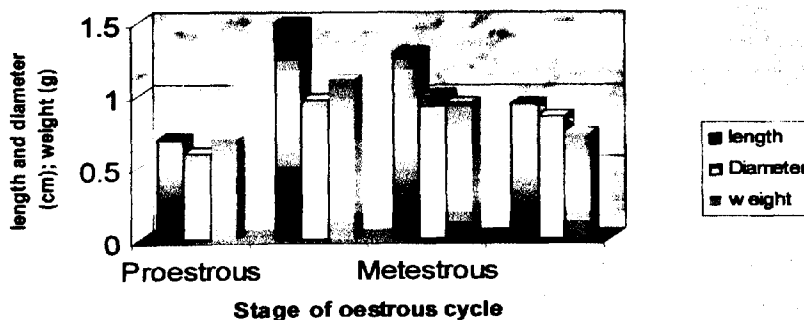
The mean weight of corpus luteum was  $0.88 \pm 0.04$ . The weights were comparable in proestrous and diestrous phases but in estrous and met estrous phases, the weight of corpus luteum was significantly ( $p < 0.05$ ) higher (Table 3 and Fig 7). The weight of corpus luteum was significantly ( $p < 0.05$ ) lower in proestrous and diestrous stage of estrous cycle when compared to estrous and metestrous phases of estrous cycle.

The colour of the corpus luteum was dark red in proestrous, red in estrous, tan in metestrous and tan white in diestrous phases of estrous cycle in buffaloes (Table 3).

**Table 3. Biometrics of corpus luteum (Mean±SE) during various phases of estrous cycle of buffaloes**

S.No	Stage of cycle	Biometrics			
		Length (cm)	Diameter (cm)	Weight (g)	Colour
1.	Pro estrous	0.69 <sup>ab</sup> ±0.05	0.6 <sup>a</sup> ±0.05	0.70 <sup>ab</sup> ±0.05	Dark red
2.	Estrous	1.49 <sup>g</sup> ±0.05	0.97 <sup>de</sup> ±0.07	1.11 <sup>ef</sup> ±0.06	Red
	Met estrous	1.29 <sup>f</sup> ±0.07	0.99 <sup>de</sup> ±0.06	0.95 <sup>de</sup> ±0.09	Tan
4.	Di estrous	0.94 <sup>cde</sup> ±0.06	0.85 <sup>bcd</sup> ±0.08	0.74 <sup>abc</sup> ±0.05	Tan - white
5.	Average	1.10 ±0.06	0.86 ±0.04	0.88 ±0.04	

**Fig.7 Showing mean length, diameter and weight of corpus luteum in buffaloes**



#### **4.4 follicular fluid**

The biochemical composition of follicular fluid was presented from Table 4 and 5 and figures.

##### **4.4.1 Total protein**

The mean protein level in <2 to >10 mm sized follicles  $10.61 \pm 0.46$  was g per cent but there was no significant difference in the protein profiles in relation to size of follicle in buffaloes (Table 4).

In the present study, the mean albumin level in follicular fluid was  $2.95 \pm 0.17$  g per cent. The albumin level was significantly ( $p < 0.05$ ) higher in 2mm and 6-10mm sized follicles when compared to 3-5 and >10 mm sized follicles (Table 4 and Fig.8).

The globulin level in follicular fluid of buffalo ovaries was  $7.69 \pm 0.39$  g per cent. There was no significant difference between the size of follicle and globulin profiles (Table 4).

The albumin and globulin ratio was  $0.51 \pm 0.6$  without any significant difference between the sizes of follicles (Table 4).

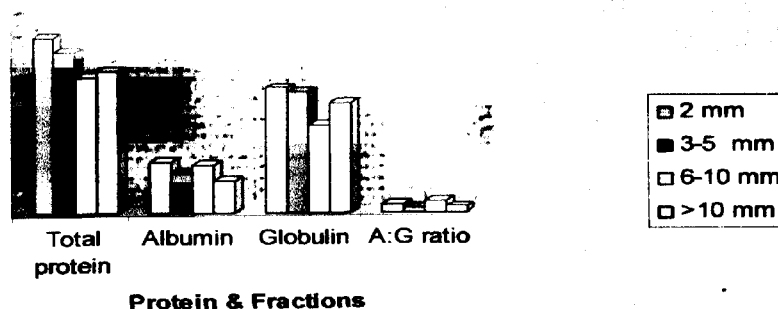
##### **4.4.2 Cholesterol**

The mean cholesterol level in follicular fluid was  $48.36 \pm 3.05$  mg per cent. The follicular fluid levels was significantly ( $p < 0.05$ ) lower in >10 mm

**Table 4. Protein, Albumin, Globulin profiles (Mean  $\pm$ S.E) and Albumin: Globulin ratio in follicular fluid of buffaloes.**

S.No	Size of Follicle(mm)	Total Protein (g %)	Albumin (g %)	Globulin (g %)	A/G ratio
1.	2	12.14 $\pm 1.14$	3.49 <sup>b</sup> $\pm 0.43$	8.65 $\pm 1.01$	0.59 $\pm 0.13$
2.	3-5	11.10 $\pm 0.78$	2.75 <sup>ab</sup> $\pm 0.22$	8.35 $\pm 0.60$	0.34 $\pm 0.02$
3.	6-10	9.36 $\pm 0.81$	3.34 <sup>b</sup> $\pm 0.36$	6.02 $\pm 0.63$	0.73 $\pm 0.17$
4.	>10	9.83 $\pm 0.84$	2.23 <sup>a</sup> $\pm 0.19$	7.60 $\pm 0.77$	0.38 $\pm 0.07$
5.	Total	10.61 $\pm 0.46$	2.95 $\pm 0.17$	7.69 $\pm 0.39$	0.51 $\pm 0.06$

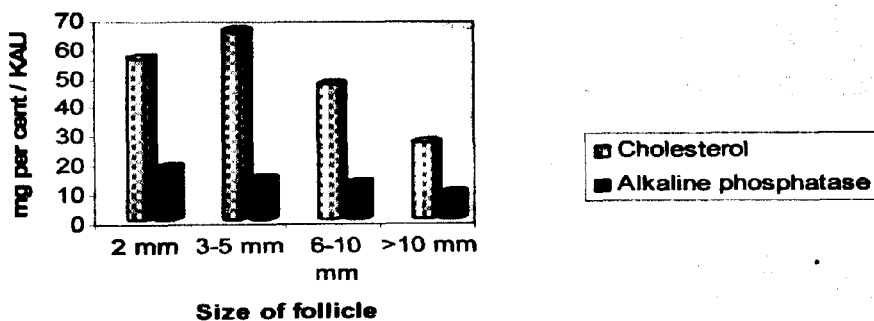
**Fig 8. Showing protein and its fraction profiles in follicular fluid of buffaloes.**



**Table 5. Cholesterol and ALP activity (Mean  $\pm$ S.E) in follicular fluid of follicles of different sizes in buffaloes**

S.No	Follicle size (mm)	Cholesterol (mg %)	Alkaline Phosphatase (KAU)
1.	2	55.67 <sup>c</sup> $\pm 2.91$	17.84 <sup>d</sup> $\pm 0.84$
2.	3-5	65.01 <sup>d</sup> $\pm 3.12$	13.69 <sup>c</sup> $\pm 1.00$
3.	6-10	46.48 <sup>b</sup> $\pm 2.32$	12.22 <sup>b</sup> $\pm 0.95$
4.	>10	26.27 <sup>a</sup> $\pm 1.81$	8.45 <sup>a</sup> $\pm 0.93$
5.	Total	48.36 $\pm 2.05$	13.05 $\pm 0.59$

**Fig 9. Showing mean cholesterol (mg %) and ALP (KAU) activity in follicular fluid of buffaloes.**



sized follicles and significantly higher in 3-5 mm sized follicles. But in 2 mm sized follicles and 6-10mm sized follicles the cholesterol level was significantly higher than >10mm sized follicles (Table 5 and Fig.9).

#### **4.4.3 Alkaline phosphatase**

In the present study, the mean alkaline phosphatase activity was  $13.05 \pm 0.59$  KAU in follicular fluid activity was significantly ( $p < 0.05$ ) followed by 3-5 mm ( $13.69 \pm 1.00$  KAU), 6-10 mm ( $12.22 \pm 0.95$  KAU) and >10 mm ( $8.45 \pm 0.93$  KAU) (Table 5 and Fig.9).

#### **4.5 Mineral profiles**

The profiles of calcium, phosphorous, sodium, copper, zinc and iron in follicular fluid of buffaloes was recorded in the present study.

##### **4.5.1 Calcium**

The average calcium level was  $5.79 \pm 0.33$  mg/dl in follicular fluid of buffaloes. The calcium profile was significantly ( $p < 0.05$ ) higher in >10mm sized follicles and significantly lower in 6-10 mm sized follicles (Table 6 and Fig.10).

#### **4.5.2 Phosphorus**

The mean phosphorus level was  $14.26 \pm 0.27$  mg/dl in follicular fluid of buffaloes. Significantly ( $p < 0.05$ ) higher levels of phosphorus recorded in <2mm follicles followed by 3-5 mm follicles, >10mm sized follicles and 6-10mm sized follicles (Table 6 and Fig.10).

#### **4.5.3 Sodium**

The average sodium level in follicular fluid was  $82.25 \pm 1.85$  ppm. There was no significant difference between the size of follicles and level of sodium in follicular fluid (Table 6 and Fig.10).

#### **4.5.4 Copper**

The mean copper profile in follicular fluid was  $0.57 \pm 0.05$  ppm in the present study. The copper level was lower in 6-10mm sized follicles ( $0.31 \pm 0.04$  ppm) and 2mm ( $0.45 \pm 0.05$  ppm). However significantly ( $p < 0.05$ ) higher levels were recorded in 3-5mm ( $0.74 \pm 0.08$  ppm) and 10 mm ( $0.79 \pm 0.14$  ppm) in follicles of buffaloes (Table 6 and Fig.10).

#### **4.5.5 Zinc**

The mean zinc level was  $6.11 \pm 0.49$  ppm in follicular fluid of buffaloes. Higher levels of zinc was recorded in >10mm size follicles (Table 6 and Fig.10).

#### **4.5.6 Iron**

In follicular fluid the mean iron level was  $1.35 \pm 0.05$ . There was no significant difference between the follicle size and iron levels (Table 6 and Fig.10).

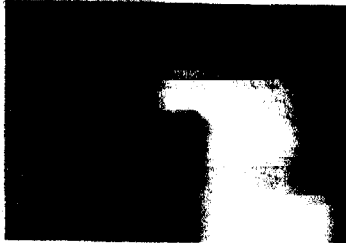
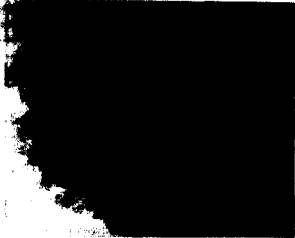
#### **4.6 Oocytes**

The oocytes were recorded from the ovaries procured from slaughtered buffaloes by aspiration, dissection and slicing techniques in the present observation.

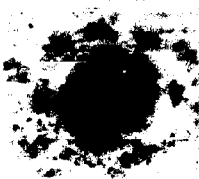
##### **4.6.1 Number of oocytes**

The average mean number of oocytes recorded was significantly ( $p < 0.05$ ) higher in slicing technique ( $5.70 \pm 0.13$ ) where as significantly ( $p < 0.05$ ) lower in aspiration ( $3.20 \pm 0.16$ ) and dissection ( $3.10 \pm 0.10$ ) techniques. Significantly higher number of good quality of oocytes was retrieved by slicing technique rather than aspiration and dissection techniques (Table 7 and Fig.11 and 12).

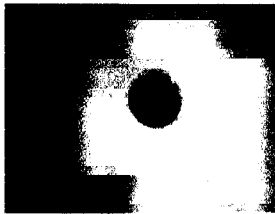
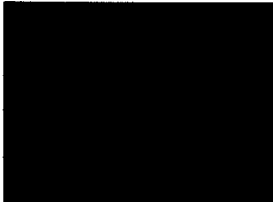
There was no significant difference between the yield of fair quality oocyte and method of retrieval. However, significantly higher number of poor quality oocytes was retrieved in aspiration and slicing techniques (Table 7 and Fig.11 and 12).



Good quality oocytes



Fair quality oocytes

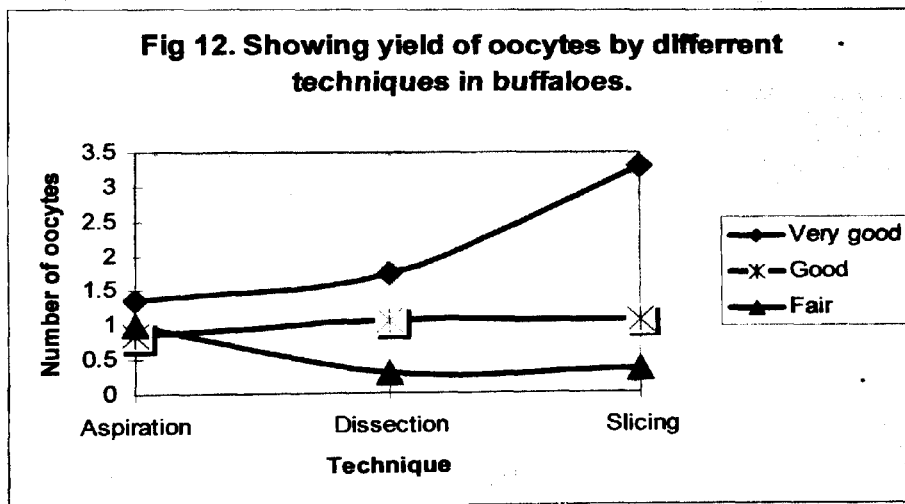


Poor quality oocytes

Fig. 11. Figures of good, fair and poor quality oocytes in buffaloes

**Table 7. Number of oocytes (Mean  $\pm$  S.E) retrieved by different methods from buffalo ovaries**

S.No	Method of retrieval	Grading of oocytes			
		Good	Fair	Poor	Total
1.	Aspiration	1.35 <sup>a</sup> $\pm 0.24$	0.85 $\pm 0.15$	1.00 <sup>b</sup> $\pm 0.21$	3.20 <sup>ab</sup> $\pm 0.16$
2.	Dissection	1.75 <sup>ab</sup> $\pm 0.18$	1.05 $\pm 0.15$	0.30 <sup>a</sup> $\pm 0.13$	3.10 <sup>a</sup> $\pm 0.10$
3.	Slicing	3.30 <sup>c</sup> $\pm 0.15$	1.05 $\pm 0.15$	1.35 <sup>b</sup> $\pm 0.18$	5.70 <sup>c</sup> $\pm 0.13$
4.	Average	2.13 $\pm 0.15$	0.98 $\pm 0.08$	0.88 $\pm 0.11$	4.00 $\pm 0.17$



#### **4.6.2 Phases of estrous cycle and oocyte yield**

The mean number of oocytes retrieved from the ovaries under follicular phase ( $5.31 \pm 0.12$ ) was significantly ( $p < 0.05$ ) higher than the ovaries under luteal phase ( $3.88 \pm 0.13$ ) of buffaloes of the present study (Table 8 and Fig.13).

Higher number of good quality oocytes was retrieved in follicular phase than luteal phase. But there was no significant difference between fair and poor quality of oocytes and the phases of estrous cycle (Table 8 and Fig.13).

#### **4.6.3 Grading of oocytes**

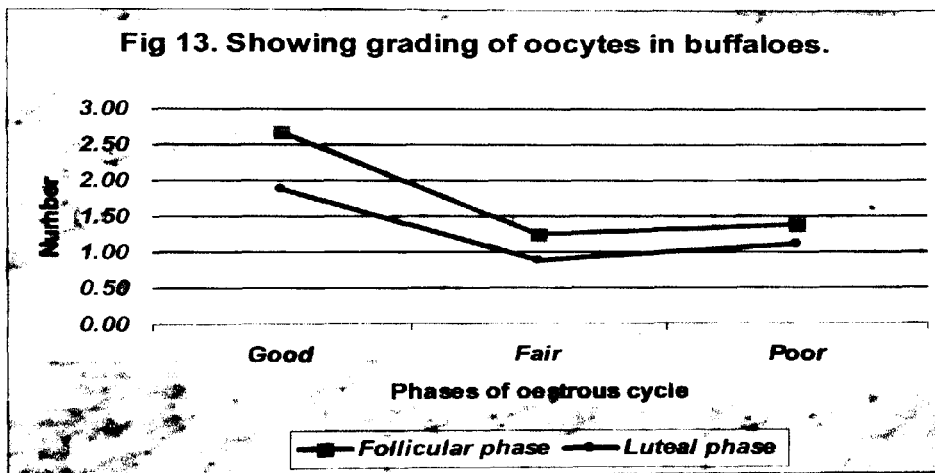
A total number of 255 oocytes were recovered by aspiration, dissection and slicing methods. The per cent of oocytes retrieved was high in slicing technique (44.71) followed by dissection (30.20) and aspiration (25.10) in the present study (Table 9 and Fig.11 and 14).

Among the total oocytes recovered, 52.94, 25.88 and 21.18 per cent were good, fair and poor quality of oocytes, respectively in the present study. Under aspiration technique, higher per cent of (42.19) good quality oocytes followed by poor (31.25) and fair (26.56) quality of oocytes (Table 9 and Fig.11 and 14).

While in slicing technique, 57.89, 23.68, and 18.42 per cent were good, poor and fair quality oocytes retrieved. Where as, in dissection technique 54.55 per cent - good quality, 36.36 per cent - fair and 9.09 per cent - poor

**Table 8. Cumulus layer investment of oocyte (Mean  $\pm$ S.E) during follicular and luteal phases of estrous cycle in buffaloes**

S.No	Stage of Estrous Cycle	Type of investment			
		Good	Fair	Poor	Total
1.	Follicular phase	2.69 <sup>a</sup> $\pm 0.28$	1.25 <sup>a</sup> $\pm 0.25$	1.38 <sup>a</sup> $\pm 0.20$	5.31 <sup>a</sup> $\pm 0.12$
2.	Luteal phase	1.88 <sup>b</sup> $\pm 0.31$	0.88 <sup>a</sup> $\pm 0.15$	1.12 <sup>a</sup> $\pm 0.29$	3.88 <sup>b</sup> $\pm 0.13$



quality of oocytes were retrieved from ovaries of buffaloes (Table 9 and Fig.11 and 14).

#### **4.6.4 Micrometry of oocytes**

Significantly ( $p < 0.05$ ) higher diameter ( $135.65 \pm 2.25$ ) of good quality oocytes was observed in the present study (Table 10). The mean diameter of fair and poor quality of oocytes was  $111.7 \pm 2.84$  and  $94.55 \pm 2.22 \mu$ , respectively. There was significant ( $p < 0.05$ ) difference observed in the oocyte size among good, fair and poor quality of oocytes.

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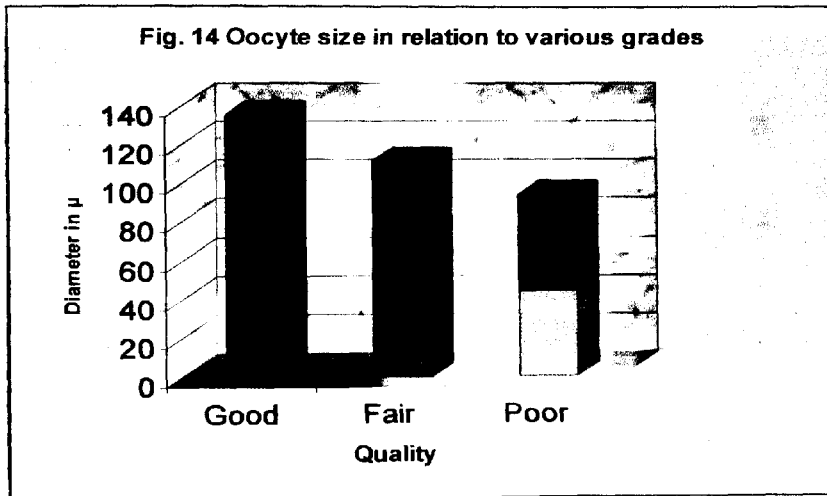
**Table 9. Details of per cent of different grades of oocytes retrieved in buffaloes**

S.No	Method of collection	Good	Fair	Poor	Total number
1.	Aspiration	27 (42.19)	17 (26.56)	20 (31.25)	64 (25.10)
2.	Slicing	66 (57.89)	21 (18.42)	27 (23.68)	114 (44.71)
3.	Dissection	42 (54.55)	28 (36.36)	7 (9.09)	77 (30.20)
4.	Total	135 (52.94)	66 (25.88)	54 (21.18)	255 (100)

Figures in parenthesis are angular values

**Table 10. Oocyte size (Mean  $\pm$ S.E) in relation to various grades**

S.No	Grade	Diameter of oocyte
1.	Good	135.65 <sup>c</sup> $\pm 2.52$
2.	Fair	111.7 <sup>b</sup> $\pm 2.84$
3.	Poor	94.55 <sup>a</sup> $\pm 2.22$



# ***DISCUSSION***

## Chapter V

### Discussion

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It is most essential to acquainted with normal dimensions of genitalia of mature female for the proper gynaeco, clinical assessment of individual buffalo (Parkale and Hukeri, 1989). Moreover, the knowledge of the biometry of genital tract is necessary in artificial breeding operation and in diagnosis of infertility and its treatment (Kumar *et al.* 2004).

The mean length of left ovary ( $2.04\pm 0.05$  cm) and right ovary ( $2.38\pm 0.07$  cm) in buffaloes of present study (Table 1) was inline with studies of Damodaran, (1958), Bhalla, (1964), Kumar *et al.* (2004) and Suresh kumar *et al.* (2001). On contrary, Sane *et al.* (1964), Sane *et al.* (1965), Grennon and Mc pherson, (1966), Ahmad and Abdul, (1968), Parkale and Hukeri,

(1991) and Chauhan and Alhaji, (1990) recorded higher length of ovaries than the present study. But, Lamborde and Kumar, (1978), Chandarahasan and Rajasekaran, (2004), Ramu (2000) and Parmar and Mehta, (1992) noticed shorter lengths than the present study.

The width of the ovary (Table 1) of the present study was in concurrence with the studies of Sane *et al.* (1965) and Suresh Kumar *et al.* (2001) in Jaffri and Tarai breed of buffaloes, respectively. Higher mean width level of ovary than the width recorded in the present study was recorded by Bhalla, (1964), Sane *et al.* (1964), Ahmad and Abdul hadi, (1968), Parkale and Hukeri (1991), Kumar *et al.* (2004) in buffaloes of respective regions.

The mean height of the ovary (Table 1) recorded in the present study was nearer to the studies of Parkale and Hukeri, (1991). However, higher height reported by Kumar *et al.* (2004) in Murrah breed of buffalo and lower in Tarai breed of buffalo by Suresh kumar *et al.* (2004).

The weight of the ovary (Table 1) recorded was ranged from  $2.72 \pm 0.15$  cm (left ovary) to  $3.74 \pm 0.28$  cm (right ovary). Similar studies also conducted by Bhalla (1964), Sane *et al.* (1964), Ramu (2000), Parmar and Mehta (1992), Jindal *et al.* (1997), Chandrasekaran and Rajasekaran, (2004) and Gupta *et al.* (2004) in buffaloes. But the heavier ovaries were recorded in Egyptian buffaloes by Ahmad and Abdul hadi, (1968).

The size of the ovary was  $2.25 \pm 0.21$  cc in left and  $3.35 \pm 0.36$  cc in right ovary (Table 1). On the other side, Gurdial singh and Singh, (1988) recorded lower size than the present study in prepubertal buffaloes.

The variation in the biometrics attributed to season, breed (Parmar and Mehta, 1992), random sampling of ovaries and differences in the health and the reproductive status (Suresh kumar *et al.*, 2002) and nutrition status of animal (Suresh kumar *et al.*, 2002). The variations in biometrics might also be attributed to the difference in breed, age, parity and reproductive status at the time of slaughter (Kumar *et al.*, 2004).

The mean number of follicles present on the left and right ovary (Table 2) was in concurrence with Abdul Razak and Ali (2005), and Chandrahasan and Rajasekaran, (2003) in buffaloes. But more number of follicles was recorded by El-wishy (1988), Chandrahasan and Rajasekaran (2003), Gupta *et al.* (2004).

The appearance of number of visible follicles might be depending on season as reported by Kalmath, (2002) and Osman and Shehata (2005), might be due to their faster turnover between development and atresia (Parmar and Mehta, 1992), environmental temperature (Chandrahasan and Rajasekaran, 2003), Nutrition and Endocrine profiles or presence of corpus luteum.

To understand the interaction of the constituents of follicular fluid with follicular cells and oocytes for their development and maturation, examination of total protein and albumin concentration is essential (Bordoloi *et al.*, 2003).

The total protein level in follicular fluid was  $10.61 \pm 0.46$  g per cent (Table 4) in follicular fluid of buffaloes which was in agreement with the studies of Parmar and Mehta, (1991). The total protein concentration was progressively decreased as the follicle size increases (Fig.8) in the present study was inline with the studies of Younis *et al.*, (1988), Mishra *et al.*, (2003) and; Thangavel and Mohammed Nayeem, (2004) who also stated that total protein concentration was decreased with increase in follicle size.

However, Krishna Murthy *et al.*, ( 1986), Madan mohan *et al.*, (1997), Zeitoun *et al.*, (2002), Das *et al.*, (2005) and Iwata *et al.*, (2006) reported lower levels of total protein than the present study. The source of protein in follicular fluid originates both from plasma and from cells within the follicle (Mc Natty, 1978) but the cellular contribution is unknown. Since preovulatory swelling was dependant on increasing capillary clearance (Bjersing and Cajander, 1974), a relatively higher concentration of total protein in pre ovulatory follicle of buffaloes could be due to the stretched pore phenomenon reported by Parving, (1975).

Higher levels of proteins are required during early development of follicles (Madan mohan *et al.*, 1997). Slightly lower total protein concentration in follicular fluid with increased size of the follicles was probably due to utilization of protein for metabolic activities of the follicular cells during

steroidogenesis or due to change in permeability of the follicular wall for protein (Bordoloi *et al.*, 2003).

The mean albumin level was  $2.95 \pm 0.17$  g per cent (Table 4) which was inconcurrence with Parmar and Mehta, (1991). But higher albumin levels than the present study was reported by Madan Mohan *et al.* (1997). The albumin level was significantly differed among the follicle size. Significantly higher albumin levels were recorded in 2 mm and 6-10 mm sized follicles rather than 3-5 and >10 mm sized follicles in buffaloes (Fig.8).

The Globulin level in follicular fluid was  $7.69 \pm 0.39$  g per cent (Table 4) but there was no significant difference found among different sized follicles. The globulin in follicular fluid play a role in fertilization by acting on spermatozoa and destroy or kill the weak defective spermatozoa and there by enhancing the action of healthy spermatozoa which would otherwise result in fertilization failure (Madan Mohan *et al.*, 1997). Lower level of globulin in follicular fluid of different classes of follicle might be due to non transportation from the blood to the follicular fluid during transudation process (Bordoloi *et al.*, 2003).

The albumin: globulin ratio was higher in 6-10 and 2 mm sized follicles and lower in 3-5 and >10 mm sized follicles (Table 4). However there was no significant difference among the size of follicle. On the contrary, higher albumin:globulin ratio was recorded by Ahmed *et al.* (1998) than the present study. The variation might be due to stage of estrous cycle (Eissa, 1995 and 1996), follicle size (Younis *et al.*, 1998), season (Parmar and Mehta, 1991),

permeability change of capillaries and limiting membrane of ovarian follicles ( Madan Mohan *et al.*, 1997).

The mean cholesterol level in follicular fluid was  $448.36 \pm 2.05$  mg per cent (Table 5). On the other side, lower cholesterol profiles than the present study was recorded by Lutwak-Mann, (1954) and Krishna murthy *et al.* (1986). But higher levels of cholesterol reported than the present observation was by Parmar and Mehta (1991), Patel *et al.* (1991) and Iwata *et al.* (2006).

The cholesterol level was found to be significantly higher in medium sized follicles (Fig.9) viz., 3-5 mm ( $65.01 \pm 3.12$  mg per cent), than the small sized viz., 2 mm ( $55.67 \pm 2.91$  mg per cent) and large sized follicles >10 mm ( $26.27 \pm 1.81$  mg per cent). Similar trend was also reported by Younis *et al.* (1998) and Thangavel and Mohammed Nayeem, (2004). But vice versa was recorded by Patil *et al.* (1991) and Leroy *et al.* (2004).

The cholesterol variation might be correlating to the synthesis of estradiol  $17 \beta$  in the growing follicles (Thangavel and Mohd Nayeem, 2004 and Thakur *et al.*, 2003).

The mean alkaline phosphatase was  $13.05 \pm 0.59$  KAU in follicular fluid of buffaloes (Table 5). But Eissa, (1996) and Parmar and Mehta, (1991) reported higher activity of alkaline phosphatase than the present study.

However, the activity of alkaline phosphatase was found to be fluctuating as per the season as well as follicle size and stage of estrous cycle (Fig.9) as reported by Parmar and Mehta, (1991), Eissa, (1995) and Eissa, (1996). The alkaline phosphatase activity was significantly and progressively

decreased as the follicle size increased. The present observation was in alignment with the studies of Mishra *et al.* (2003) in caprines who stated that the alkaline phosphatase activity was decreased progressively as follicle size increased.

The presence of higher levels of alkaline phosphatase in the follicular fluid of small follicles suggests the growth and functional status of the follicle. Wise, (1987) suggested that higher activity of alkaline phosphatase in follicular fluid was correlated with follicular progesterone, androgens, dehydroepiandrosterone and testosterone level. The alkaline phosphatase activity in follicles is also associated with follicular atrophy (Mc natty, 1978). It indicated that higher activity of alkaline phosphatase might be due to the action of gonadotropins on the follicular cell function. Further, Goode *et al.* (1966) reported a positive correlation between alkaline phosphatase and steroidogenesis in bovine ovaries. The alkaline phosphatase in the ovarian follicles was seemed to be phosphorylate steroid binding receptors and alter the binding property of steroid hormones with those receptors (Wise, 1987).

Calcium is reported to play important role in sensitizing tubular genitalia for the action of hormones (Bhardwaj *et al.*, 1998). Mineral elements influence the physiology of reproduction through their action as metallo proteins. Deficiency of minerals leads to impaired reproductive performance in mammals (Sikka, 1992).

The mean calcium level in follicular fluid was  $5.79 \pm 0.33$  mg/dl (Table 6) which was similar to the studies of Jagjit kaur *et al.* (1997) and Eissa, (1996).

The calcium level in the present study was ranged from  $4.60 \pm 0.59$  to  $7.20 \pm 0.43$  mg/dl as per the size of follicle. However, Lutwak-Mann, (1954), Krishna murthy, (1986) and Bhardwaj *et al.* (1998) reported higher levels than the present study in follicular fluid.

The calcium level was found to be significantly higher levels in 2 mm and large sized > 10 mm size ( $7.20 \pm 0.43$  mg/dl) than the 3-5 or 6-10 mm sized follicles (Fig.10). Similar trend was also reported by Eissa, (1995) who stated that the calcium levels were higher in Pro estrous than met estrous or di estrous and reached peak values during estrous in buffaloes. But Baszczyk *et al.* (2004) contradicting the trend who stated that the calcium concentration was similar in all sized follicles.

Higher concentration of calcium in the follicles might be associated with proliferation of granulosa and theca cells; and thus indirectly reflected the steroidogenic capabilities of the growing follicle. It was also considered as gonadotropic regulation of ovarian steroidogenesis (Veldhuis *et al.*, 1984). The markedly higher calcium concentration in follicular fluid during the early stages of the estrous cycle might be playing a crucial role in steroidogenic capabilities of the growing follicle (Wise, 1987).

The mean phosphorous level was  $14.26 \pm 0.27$  mg/dl (Table 6) and found to progressively decrease from smaller to medium sized follicles and again increased in larger (>10 mm) sized follicles (Fig.10).

But, higher (Jindal *et al.*, 1997) and the lower phosphorous levels than the present study was recorded by Lutwak- Mann (1954), Krishna murthy *et*

*et al.* (1986), Eissa *et al.* (1996) and Iwata *et al.* (2006). The variation in phosphorous level might be due to variation in metabolic activity in follicles of different sizes (Bordoloi, 2001 and Thangavel and Mohd Nayeem, 2004) and sample size (Jagjit kaur *et al.*, 1997).

The mean sodium, copper, zinc and iron in follicular fluid was  $82.25 \pm 1.85$ ,  $0.57 \pm 0.05$ ,  $6.11 \pm 0.41$  and  $1.35 \pm 0.05$  ppm in follicular fluid of present study (Table 6). Higher sodium levels than the present investigation was recorded by Lutwak-Mann, (1954) and Krishna murthy, (1996) and lower levels by Jagjit kaur *et al.* (1997) and Bhardwaj *et al.* (1998). However, there was no significant difference in sodium levels of follicular fluid among the different sized follicles in the present study. On contrary, Jagjit kaur *et al.* (1997) reported lower levels of sodium in small and medium and higher levels in large size follicles in buffaloes.

The sodium concentration might be related to the viability of follicles and most probably linked to active follicular synthesis of estrogen (Wise, 1987). The sodium levels indicate the metabolic activity of granulose cells of osmotic pressure and developing follicle thus the sodium level directly reflects the number of granulose cells present (Jagjit kaur *et al.*, 1997). Changes in sodium concentration reflect the homeostatic dynamism operating in follicles at different stages of development, maturation and maintenance of metabolism.

Higher copper concentration was reported by Jagjit kaur *et al.* (1997). The copper concentration was significantly higher in 3-5 and >10 mm sized

follicles when compared to 2 and 6-10 mm sized follicles (Fig.10).The increased copper concentration could be correlated with the increased estrogen levels which presumably relates to the increases in ceruloplasmins in synthetase (Sharma and Sharma, 1997). The copper levels were noticed to be correlated with the estrogen secretion of follicles (Bordoloi *et al.*, 2001).

The variations observed in different categories of follicles were possible due to the synergistic effect of progesterone and estrogen (Sangha *et al.*, 1993 and Sharma and Sharma, 1997) as well as mobilization of copper in erythrocuprin, hepatocuprin, cytochrome C oxidase, tyrosine monoamine oxidase, ascorbic acid oxidase and a number of co-enzymes in dehydrogenases required in fatty acid catabolism (Sharma and Sharma, 1997).

The zinc deficiency was reported to cause defects in prostaglandin metabolism in ruminants (Ei-Tohmy *et al.*, 1989). Further, it was also observed that the zinc concentration was associated with progesterone and estrogen profiles in normal ovaries and found fall in zinc concentration as the fall in progesterone, estrogen levels in the ovary (Underwood, 1997). The deficiency of zinc, reported to delay in postpartum conception (Saxena and Gupta, 1995).

Iron plays a significant role and its deficiency observed to affect the response of ovarian receptors to estrogen hormone. The iron concentration was found to be increased with serum FSH and decreased with serum LH (Janakiraman and Mehta, 1988). It was also reported that the higher iron

profile was associated with increased output of FSH and decreased output of LH (Desai *et al.*, 1982). The variation in the iron levels might be attributed to the variation in estrogen and progesterone profiles due to increased haemodynamic pulses in the vascular shunt of reproductive tract (Hidiroglow, 1979) and vascular shunt of the developing follicles (Sangha *et al.*, 1993).

The increase in iron concentration was noticed to correspond with the development of smooth musculature around the pre ovulatory follicles (Ishwar *et al.*, 1987) and increased biosynthetic activities of the follicle (Sangha *et al.*, 1993).

In ovulatory follicles, the iron decline might be due to ischemia leading to the rupture of follicle wall at stigma, a vascular bed deficient portion (Sharma and Vats, 1998). These findings further suggest that the variations in iron concentration were in accordance with the haemodynamic changes observed in the follicular wall during follicular development and maturation; and opined that the changes were regulated by intra follicular factors (Guraya *et al.*, 1991). Low concentrations of trace elements may be warning of subsequent infertility in the animal hence, further detailed studies are required to elucidate the precise role of these elements in ovarian physiology.

Several authors ( Bottcher *et al.*, 1989, Sato *et al.*, 1990, Takagi *et al.*, 1992, Das *et al.*, 1992, Das *et al.*, 1996, Khan *et al.*, 1997, Baruha *et al.*, 1998, Datta and Goswamy, 1998, Deepak sharma *et al.*, 2000, Nandi *et al.*, 2000, Suresh kumar and Mourya, 2000, Raza *et al.*, 2001, Abdul razak and Ali, 2005 and Fassi Fihri, 2005) employed techniques like follicle puncture,

dissection, slicing, aspiration and post aspiration mincing to recover the oocytes from the ovaries in cattle.

The mean number of oocytes recovered per pair of ovaries was  $4.00 \pm 0.17$  which was in agreement with the studies of Lusiana and Zdzlaw and Smorag, (1984) in Poland and Khan *et al.* (1997). Significantly higher number of oocytes was recovered by slicing technique ( $5.70 \pm 0.13$ ) than the aspiration ( $3.20 \pm 0.16$ ) and dissection ( $3.10 \pm 0.10$ ) techniques (Table 7). Among the quality of oocytes significantly higher number of good quality oocytes was recovered by slicing technique ( $3.30 \pm 0.15$ ) followed by dissection ( $1.75 \pm 0.18$ ) and aspiration ( $1.35 \pm 0.24$ ) techniques in the present study (Fig.12). While with respect to fair quality of oocytes, there was no significant difference between the techniques employed for the recovery of oocytes. Likewise, significantly higher number of poor quality oocytes was yielded in aspiration and slicing technique rather than dissection technique.

Higher number of oocytes by Bottcher *et al.* (1989), Sato *et al.* (1990), Lonergan *et al.* (1991), Takagi *et al.* (1992), Zeiroun *et al.* (2001) and Iwata *et al.* (2006) and lower number of oocytes was retrieved by Das *et al.* (1992), Mohd Amanullah, (1998), Nandi *et al.* (2000), Yadav *et al.* (2000), Hamam *et al.* (2000), Palanisamy *et al.* (2002), Gupta *et al.* (2004), Warriach and Chouhan, (2004) and Das *et al.* (2005) than the present study.

In slicing method similar number of oocytes was retrieved by Das *et al.* (1996). But, lower number of oocytes recovered by slicing technique than the present study by Khan *et al.* (1996) and Khan *et al.* (1997), Datta and Goswamy (1998), Deepak sharma *et al.* (2000), Suresh kumar and Mourya (2000), Raza *et al.* (2001) and Abdul razak and Ali, (2005). Where as, higher number of oocytes was retrieved by Sianturi *et al.* (2002) and Das *et al.* (2005).

The mean number of oocytes retrieved by aspiration technique was  $3.20 \pm 0.16$  which was nearer to the observations of Baruha *et al.* (1998), Abdul razak and Ali, (2005) and Fassi fihri *et al.* (2005). Among the recovered oocytes  $1.35 \pm 0.24$  (good),  $0.85 \pm 0.15$  (fair) and  $1.00 \pm 0.21$  (poor) oocytes were recovered in the present study (Fig.12).

However, higher number of oocytes recovered by aspiration by Lonergan *et al.* (1991), Takagi *et al.* (1992), Khan *et al.* (1997), Khan *et al.* (1997) and Sianturi *et al.* (2002) than the present study. Where as, lower number of oocytes by aspiration technique was recorded by Das *et al.* (1992), Das *et al.* (1996), Datta and Goswamy (1998), Nandi *et al.* (2000), Suresh kumar and Mourya (2000), Raza *et al.* (2001) and Palanisamy *et al.* (2002).

The mean number of oocytes recovered by dissection method was  $3.10 \pm 0.10$  which is in collaboration with the studies of Fassi and Fihri, (2005). Among the oocytes recovered the good, fair and poor quality oocytes were  $1.75 \pm 0.18$ ,  $1.05 \pm 0.15$  and  $0.30 \pm 0.13$ , respectively (Fig.12). Where as, higher number was recorded by Lonergan *et al.* (1991) and Baruha *et al.* (1998) and

lower number of oocytes by Das *et al.* (1992), Das *et al.* (1996) and Datta and Goswamy, (1998) in dissection technique.

The present study concluded that the higher number of oocytes was recovered by slicing technique followed by aspiration and dissection techniques (Fig.12). But, Sato *et al.* (1990), Lonergan *et al.* (1991), Das *et al.* (1992), Baruha *et al.* (1998), Datta and Goswamy (1998), Fassi and Fihri, (2005) noted that the dissection was yielded higher number of oocytes rather than the aspiration method which is contradicting the present study. Many authors (Das *et al.*, 1992, Das *et al.*, 1996, Suresh kumar and Mourya 2000, Raza *et al.*, 2001, Sianturi *et al.*, 2002, Abdul razak and Ali, 2005 and Fassi and fihri *et al.*, 2005) recovered more number of oocytes by slicing technique than the dissection and aspiration techniques which is supporting the present study where higher number of oocytes were recovered by slicing technique.

On the other side, Khan *et al.* (1997), Khan *et al.* (1997), Datta and Goswamy, (1998) and Deepak Sharma, (2000) reported the yield of oocytes were more by aspiration and dissection techniques rather than the slicing technique. The variation in the yield of oocyte retrieval might be attributed to individual skill as well as the season, the structures present on the ovary and number of observations per day.

Slightly higher number of oocytes (Table 8) was retrieved from the ovaries under follicular phase ( $5.31 \pm 0.12$ ) than the luteal phase ( $3.88 \pm 0.13$ ). Similarly, significantly higher number of good, fair and poor quality oocytes

were recovered from the ovaries under follicular phase rather than the luteal phase (Fig.13) which is inline with the studies of Selvaraj *et al.* (1992), Palanisamy *et al.* (2002), Wakle *et al.* (2002) and Abdul razak and Ali, (2005) who reported higher number of good quality oocytes when the larger sized follicles present on the ovary. In the same manner, Nandi *et al.* (2000) and Sajjan singh *et al.* (2001) reported the recovery of lower number of oocytes collected from the ovaries having corpus luteum. The variation in the yield of oocytes might be due to small number of observations and season of collection (Sajjan singh *et al.*, 2000 ; Yadav *et al.*, 2000 and Amanullah, 1998).

In the present study, 52.94 per cent retrieved were good, 25.88 per cent were fair and 21.18 per cent were poor quality oocytes (Table 9). The retrieval of good quality oocytes were higher in slicing technique (57.89 per cent) followed by dissection technique (54.55 per cent) and aspiration technique (42.19 per cent).

With respect to fair quality of oocytes, higher per cent were retrieved in dissection (36.36) followed by aspiration (26.56) and slicing (18.42). The yield of poor quality oocytes was lower in dissection (9.09 per cent) and higher in aspiration (31.25 per cent) and slicing technique (23.68 per cent).

The variation in the yield of quality of oocytes might be attributed to the technique dependant. As in aspiration, hypodermic needle was used to aspirate the contents of the follicle which might have disturbed the cumulus investment while entering into the bore of the needle. Like wise in slicing

technique, the blade was used which might have cut the cumulus investment resulted in yielding of higher percentage of poor quality in the slicing and aspiration techniques.

The mean diameter of oocytes was higher than the reports of Selvaraj *et al.* (1992). While, the diameter of oocyte in the present study was lower than the reports of Naik *et al.* (2002). The variation in the oocyte size might be due to collection of oocytes from various sized follicles in the buffalo.

# *SUMMARY*

## Chapter VI Summary

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The present study entitled "studies on biochemical composition of follicular fluid and oocyte characteristics in buffaloes" was carried out in the department of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary Science, Rajendranagar, Hyderabad.

The length, width, height and weight of ovaries were recorded in the present study. There was no significant difference in length, width and height of buffalo ovaries between follicular and luteal phases of estrous cycle.

The weight of left ovary was significantly ( $p < 0.05$ ) lower during the proestrous period ( $1.93 \pm 0.16$ ) and significantly higher during estrous ( $3.58 \pm 0.36$ ), metestrous ( $3.01 \pm 0.20$ ) and diestrous ( $2.74 \pm 0.32$ ) phases of estrous cycle. Where as, the right ovary was significantly ( $p < 0.05$ ) heavier

when compared to left ovary in proestrous ( $3.41 \pm 0.57$ ) and met estrous ( $4.01 \pm 0.58$ ) phases of estrous cycle.

The size of left and right ovaries was significantly higher in estrous, diestrous and proestrous phases of estrous cycle. The mean number of different sized follicles present on left and right ovary was  $3.87 \pm 0.02$  and  $3.66 \pm 0.28$ , respectively. The mean length, diameter and weight of corpus luteum were  $1.10 \pm 0.06$ ,  $0.86 \pm 0.04$  and  $0.88 \pm 0.04$ , respectively. There was significant ( $p < 0.05$ ) difference recorded among the phases of estrous cycle in the biometrics and corpus luteum.

The colour of corpus luteum was dark red to tan colour in different phases of estrous cycle.

The mean total protein, albumin, globulin and albumin: globulin ratio was  $10.61 \pm 0.46$ ,  $2.91 \pm 0.17$ ,  $7.69 \pm 0.39$  and  $0.51 \pm 0.06$  g per cent in different sized follicles. The albumin level was significantly ( $p < 0.05$ ) differed between the sizes of the follicle. The mean cholesterol level in the follicular fluid was  $48.36 \pm 2.05$  mg per cent with significant ( $p < 0.05$ ) difference between the sizes of follicle. The mean alkaline phosphatase activity was  $13.05 \pm 0.59$  KAU in follicular fluid with significant difference between sizes of follicle.

The mean calcium, phosphorous, sodium, copper, zinc and iron profiles were  $5.79 \pm 0.33$ ,  $14.26 \pm 0.27$  (mg per cent),  $82.25 \pm 1.85$ ,  $0.57 \pm 0.05$ ,  $6.11 \pm 0.41$  and  $1.35 \pm 0.05$  ppm in follicular fluid. There was significant ( $p < 0.05$ ) difference in calcium, phosphorous and copper profiles in follicular fluid collected from different sized follicles.

The mean number of oocytes retrieved in follicular and luteal phases was  $5.31 \pm 0.12$  and  $3.88 \pm 0.13$ , respectively with significant ( $p < 0.05$ ) difference between the phases of estrous cycle. The per cent of oocytes retrieved in aspiration, slicing and dissection were 25.10, 44.71 and 30.20, respectively.

The mean diameter of good, fair and poor quality of oocytes was  $135.65 \pm 2.52$ ,  $111.7 \pm 2.84$  and  $94.55 \pm 2.22$   $\mu$ , respectively in the present investigation.

The present study concluded that

1. The biometrics of ovary was variable during the stages of estrous cycle.
2. The number of follicles present on the ovarian surface was not significantly differed between the phases of estrous cycle.
3. The biometrics of corpus luteum was variable with the phases of estrous cycle.
4. The biochemical and mineral profiles are playing a major role as the follicle size increases.
5. The mean number of oocytes retrieved was higher in slicing technique and from the ovaries under follicular phases of estrous cycle.

**Future investigations are required**

1. Compare the micrometry of oocytes after vitrification of oocytes.
2. To compare the maturation of good, fair and poor quality oocytes collected from different sizes of follicles.
3. To know the detailed composition of minerals in follicular fluid and their role in maturation of oocyte.
4. To compare the oocyte yield in the ovary stimulated by gonadotropins
5. To study the collection of oocytes by ovum pickup unit.

# ***LITERATURE CITED***

## LITERATURE CITED

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