The Journal of Animal and Plant Sciences, 22(3 Suppl.): 2012, Page: 181-188
ISSN: 1018-7081

HISTOLOGICAL STUDIES OF THE BUFFALO ADENOHYPOPHYSIS WITH REFERENCE TO PHASES OF ESTRUS CYCLE

N. Akhtar, G. Nabi, L.A. Lodhi, S. Ali, N. Ahmad, S. U. Rahman and M. Akhtar

Department of Theriogenology, Department of Microbiology and Department of Parasitology, University of Agriculture, Faisalabad, Directorate of Breed Improvement, Government of Punjab, Pakistan
Corresponding Author e-mail: nafees439@yahoo.com

ABSTRACT

To study the histological details of the buffalo adenohypophysis responsible for the production of gonadotropin hormones, 60 healthy and non pregnant buffaloes brought to Faisalabad abattoir were selected. These buffaloes were divided into three groups according to their age, viz. group A= buffalo heifers, group B= buffaloes in lactation 1-3 and group C = buffaloes in lactation 4th or above. These buffaloes were in their estrus, anestrus or diestrus phase. The reproductive tracts of these buffaloes were examined to confirm their stage of estrus cycle. Pituitary glands of selected buffaloes were collected immediately after slaughter. These pituitary glands were round in shape and light pink in colour. Mean length, width and thickness of the pituitaries collected from heifers during anestrus, estrus and diestrus phases showed non-significant differences among them in respect of size and colour. Mean weight of the pituitaries (305±7.5 and 300±7.5 mg) collected from buffaloes of group A and B which were in estrus phase was significantly ($P<0.05$) higher than 270±7.5mg found for buffaloes in group C, and was also significantly ($P<0.05$) higher than for buffaloes of all groups which were in their estrus and diestrus phases. Histological staining procedure i.e. Azan Mallory Heidenhain or Hematoxylin and Eosin stains were used to stain the cut sections of buffalo adenohypophysis. Gonadotrophs population (19.66%) recorded for buffaloes in lactation 1-3 was significantly ($P<0.05$) higher than 14.75 and 13.75% found in adenohypophysis of heifers and buffaloes in lactation 4th or above. Number of maturing (6-10 mm) GF (104) found on the ovaries of heifers was significantly ($P<0.05$) higher than 40 and 15 found for buffaloes in lactation 1-3 and buffaloes in lactation 4th or above. It was concluded that heifers and buffaloes in lactation 1-3 during estrus showed significantly higher gonadotrophs population than buffaloes in lactation 4th or above, indicating higher ovarian activity in young animals.

Key words: Buffalo, Adenohypophysis, Estrus phases, Gonadotrophs.

INTRODUCTION

Buffaloes contribute more than 61% milk and over 50% beef to the total demand of milk and beef in Pakistan (Anonymous, 2011). In spite of the significant contribution of the buffalo to agriculture economy, its lower reproductive efficiency is accepted as a serious constraint (Cockrill, 1980). With more than 14 million heads, the larger commercial dairies milking around 30 or more buffaloes decreased to 10 in number during 1982-1993. This decrease was mainly due to trading losses associated with inadequate management, late maturity, longer calving interval and seasonal anestrus during hot summer (Anonymous, 1993).

The anterior pituitary gland has receptors for GnRH in response to which it secretes follicular stimulating hormone (FSH) and leutinizing hormone (LH). Both of these gonadotropins control and regulate the development of follicles on the ovaries (Roch *et al*., 1992). The pituitary FSH is required for normal reproductive functions in all animals. In the female, FSH is essential for follicular growth. The use of the gonadotropins in cases of true anestrus or early maturity in young females is an important tool to reduce the long calving interval and enhance calf and milk production (Morrow, 1986). Rebeeca and Tortonese (2000) reported higher production of gonadotropins (22.5±3.6%) in the pituitary of sexually active than 9.7±1.2% gonadotropins in the pituitary of sexually inactive animals. They further reported specific FSH or LH cells that dominate during active sexual phase.

The cytological changes which affect the increased or decreased production of such hormones and their effects upon the dependent endocrine glands are still to be explored in buffaloes. The present study was, therefore, planned to investigate the histological and cytological picture of adenohypophysis and to correlate the ovarian status during different phases of estrus cycle in buffaloes.

MATERIALS AND METHODS

Selection of experimental animals: A total of 60 non pregnant buffaloes were selected from those brought to the Faisalabad abattoir. These buffaloes were divided into three groups according to their ages, i.e. group A=buffalo

heifers (n=33), group B=buffaloes in lactation 1-3 (n=15) and group C= buffaloes in lactation 4th or above (n=12). Each selected buffalo was further assigned into three subgroups according to the phase of the estrus cycle, i.e. anestrus, estrus and diestrus (Table 1).

Collection of sample: All the selected buffaloes were rectally palpated before slaughter to confirm their stage of estrus cycle viz. anestrus, estrus and diestrus. The reproductive tracts were immediately collected after slaughtering and were transferred to the laboratory for the confirmation of the rectal findings. Number of Graafian's follicles (GF) present on each ovary (left and right) were counted separately, and each follicle was measured in size by using Vernier Caliper and categorized according to its diameter as 3-5, 6-10 and >10mm (Khan, 1985).

Biometry of the pituitary gland: The pituitary glands were also collected from experimental buffaloes immediately after slaughter. The biometrical observations of each pituitary gland were determined for its size and weight (Muhammad, 1984).

Histological studies: For histological studies the glands were transferred to freshly prepared Bouin's Fixative and were kept overnight in this solution. The glands were then washed and transferred to 70% alcohol. The pituitary tissues were then dehydrated into ascending grades of ethyl alcohol, cleared in Xyline and embedded in paraffin before making blocks. The prepared tissues were sectioned at 5µ, stained with Azan Mallory Heidenhain's or Hematoxylin and Eosin stain and examined under microscope (Humason, 1972).

Statistical analysis: The data for mean weights (±SEM) of pituitary gland were analyzed using general linear model procedure (Steel and Torrie, 1997) in Minitab Statistical software Computer Package (Anonymous, 1991). Duncan's Multiple Range test was applied for multiple means comparisons (Duncan, 1955).

RESULTS

Number of Graafian follicles: The Number of GF on the left ovary of buffaloes in group A, B and C falling in anestrus, estrus and diestrus stage of their estrus cycle are presented in the Table 2. For the left ovary, the total number of the GF (46) were highest on the ovary of anestrus heifers (group-A) with 12, 33 and 1 follicles of 3-5, 6-10 and >10mm diameter, respectively. Whereas anestrus buffaloes, in group C (Lactation 4th or above) showed only 9 GF, with 4, 3 and 2 follicles of 3-5, 6-10 and >10mm diameter, respectively. Anestrus buffaloes in group-B (Lactation 1-3) showed 3, 8 and 0 GF of 3-5, 6-10 and >10mm diameter, respectively.

For the left ovary, the total number (21) of GF were highest in buffalo heifers (Group-A) which were in estrus phase with 6, 11 and 4 follicles of 3-5, 6-10 and >10mm size, respectively. Whereas the lowest number (4) of GF was shown by estrus buffaloes in group C (Lactation 4th or above) with 2, 2 and 0 follicles of 3-5, 6-10 and >10mm diameter, respectively. The buffaloes in estrus in group B (Lactation 1-3) showed 9 GF with 3, 6 and 0 of 3-5, 6-10 and >10mm diameter, respectively.

For the left ovary, the highest number of GF (14) were found in group B (Lactation 1-3) with 4, 10 and 0 follicle of 3-5, 6-10 and >10mm diameter, respectively, and the lowest number (4) of GF was found in buffaloes of group C (Lactation 4th or above) with 1, 2 and 1 follicles of 3-5, 6-10 and >10mm diameter, respectively. Whereas buffaloes heifers (group A) showed 4, 9 and 0 GF of 3-5, 6-10 and >10mm diameter, respectively.

The number of GF on the right ovary of buffaloes in groups A, B and C falling in anestrus, estrus and diestrus stage of their estrus cycle is presented in Table 3. For the right ovary, the total number (47) of GF were highest in anestrus heifers (Group A) with 18, 29 and 0 follicles of 3-5, 6-10 and >10mm diameter, respectively. Whereas anestrus buffaloes in group B (Lactation 1-3) showed lowest number (4) of GF with 1, 3 and 0 of 3-5, 6-10 and >10mm diameter, respectively. Buffaloes in group C (Lactation 4th or above) showed only 5 GF with 2, 1 and 2 of 3-5, 6-10 and >10mm diameter, respectively.

For the right ovary the total number (22) of the GF with 6, 14 and 2 of 3-5, 6-10 and >10mm diameter, respectively, were highest in the estrus heifers (Group A). Whereas the lowest number (6) of GF was shown by estrus buffaloes in group C (Lactation 4th or above) with 1, 3 and 2 follicles of 3-5, 6-10 and >10mm diameter, respectively. The buffaloes in estrus in group B (Lactation 1-3) showed 10 GF with 2, 7 and 1 of 3-5, 6-10 and >10mm diameter, respectively.

For the right ovary, the highest (14) number of GF with 4, 8 and 2 of 3-5, 6-10 and >10mm diameter, respectively, were found in the group A buffaloes (heifers). Whereas the lowest number (6) of GF with 1, 4 and 1 of 3-5, 6-10 and >10mm diameter, respectively, were found in buffaloes in group C (Lactation 4th or above) in diestrus phase of the estrus cycle. Buffaloes in group B (Lactation 1-3) showed 9 GF with 3, 6 and 0 of 3-5, 6-10 and >10mm diameter, respectively.

Gross anatomical observation of the buffalo pituitary: The pituitary gland of the buffaloes was found to be located at the base of the brain and lodged in a bony cavity of sphenoid bone known as sella turcica. The gland was enclosed in a thick capsule having one extension towards the floor of the brain. The diaphragm had an aperture through which infundibulam passed for its attachment with the hypothalamus. The infundibulam was a short cylindrical structure having no visible lumen. In general, the pituitary gland was rounded in shape and light pink in colour.

Weight of the Pituitary gland in buffaloes during different phases of estrus cycle: The mean weight (305±7.5, 300±7.5 and 270±7.5mg) of the pituitary glands (Table 4) collected from buffalo heifers, buffaloes in lactation 1-3 and buffaloes in lactation 4th slaughtered during estrus was significantly (P<0.05) higher than for those collected from buffaloes slaughtered during anestrus and diestrus. Buffalo heifers slaughtered during estrus showed the highest weight of their pituitary glands, whereas the (P<0.05) lower weight (210±6.1mg) was observed for buffalo heifers slaughtered during anestrus. Gross mean weight of pituitary gland was 258±30.82mg. Mean length, width and thickness of pituitary glands were 0.79±0.01, 0.69±0.01 and 0.56±0.01cm, respectively. Non-significant difference was found in size of pituitaries collected from buffaloes slaughtered during anestrus, estrus and diestrus.

Table 1. Group wise distribution of experimental buffaloes

Group	Anestrus	Estrus	Diestrus	Total
Heifer	20	8	5	33
Buffaloes from lactation 1-3	4	6	5	15
Buffaloes in lactation 4th or above	4	3	5	12
Total	28	17	15	60

Table 2: Number of Graafian's follicles of 3-5, 6-10 and >10mm diameter present on the left ovary of buffaloes in groups A, B and C during anestrus, estrus and diestrus phases

Group	No. of buffaloes	No. of GF of 3 sizes			
A - Heifers		3-5 mm	6-10 mm	>10mm	Total
Anestrus	20	12	33	1	46
Estrus	8	6	11	4	21
Diestrus	5	4	9	0	13
B - Lactation 1-3					
Anestrus	4	3	8	0	11
Estrus	6	3	6	0	9
Diestrus	5	4	10	0	14
C - Lactation 4th or above					
Anestrus	4	4	3	2	9
Estrus	3	2	2	0	4
Diestrus	5	1	2	1	4

Table 3. Number of Graafian's follicles of 3-5, 6-10 and >10mm diameter present on the right ovary of buffaloes in groups A, B and C during estrus, anestrus and diestrus phases

Group	No. of buffaloes	No. of GF of 3 sizes			
A - Heifers		3-5 mm	6-10 mm	>10mm	Total
Anestrus	20	18	29	0	47
Estrus	8	6	14	2	22
Diestrus	5	4	8	2	14
B - Lactation 1-3					
Anestrus	4	1	3	0	4
Estrus	6	2	7	1	10
Diestrus	5	3	6	0	9
C - Lactation 4th or above					
Anestrus	4	2	1	2	5
Estrus	3	1	3	2	6
Diestrus	5	1	4	1	6

Table 4. Mean (±SE) weight of pituitary glands collected from buffaloes of groups A, B and C during anestrus, estrus and diestrus phases

Groups	Anestrus	Estrus	Diestrus
A - Buffalo heifers	210 ± 6.1 ^{c}	305 ± 7.5 ^{a}	240 ± 7.5 ^{b}
B - Buffaloes in lactation 1-3	230 ± 7.0 ^{c}	300 ± 7.5 ^{a}	260 ± 7.0 ^{b}
B - Buffaloes in lactation 4th or above	250 ± 7.5 ^{b}	270 ± 7.5 ^{a}	260 ± 7.0 ^{ab}

Functional cytology of adult buffalo adenohypophysis: Photomicrograph in Plate 1 shows a pituitary collected from an adult estrus buffalo. The adenohypophysis was shown on right, neurohypophysis on left and pars intermedia in the centre of section. The adenohypophysis was predominantly populated with acidophils and basophils of intermediate degree of degranulation. Cells containing granules were evident in the acinar arrangements. At higher magnification (Plate 2) delta types of cells were distinguishable, acidophils cells were also seen as large, polygranular, ill defined cytoplasmic outline and were scattered in the cortex. Delta cells were smaller, rounded and sharply outlined; the granules were coarse and irregular in size and shape and were binucleated.

Cytology of adenohypophysis of buffalo in heifers in anestrus, estrus and diestrus: Photomicrograph (plate 3) of adenohypophysis of buffalo heifer slaughtered during anestrus showed degranulated gonadotrophs in distinct acinar arrangement. In another section of adenohypophysis (Plate 4) from estrus buffalo heifer having GF of >10mm diameter on her ovaries showed dark granulated gonadotrophs, cell nuclei were not distinguishable. Photomicrograph (Plate 5) of adenohypophysis of a buffalo heifer slaughtered during diestrus having multiple GF (6-10mm) on her ovaries showed small number of heavily granulating gonadotrophs in non distinct acinar arrangement.

Cytology of adenohypophysis of buffaloes in lactations 1-3 in anestrus, estrus and diestrus: Photomicrograph (Plate 6) of adenohypophysis from a buffalo in lactation 1-3 slaughtered during anestrus showed a few scattered delta basophils. Animals showed very low ovarian activity, GF were < 8mm in diameter. Whereas another section of adenohypophysis (Plate 7) of a buffalo in lactation 1-3 slaughtered during estrus stage at peak ovarian activity with large GF >10mm diameter showed granulated gonadotrophs apposing against the reticular areas in the acini. Another section of adenohypophysis from a buffalo during diestrus stage having multiple GF <10mm in diameter reflecting intermediate ovarian activity showed incomplete degranulated delta cells (Plate 8).

Cytology of adenohypophysis of buffaloes in lactations 4th or above in anestrus, estrus and diestrus: Photomicrograph (Plate 9) of adenohypophysis from a buffalo in lactation 4th or above slaughtered during anestrus, showed granulated and degranulating gonadotrophs whereas in a photomicrograph (Plate 10) of adenohypophysis from estrus buffalo having GF >10mm diameter on her ovaries showed distinctly large granulated gonadotrophs. Clumps of various degranulated cells with prominent nuclei were distinct in acinar arrangement. Photomicrograph (Plate 11) of adenohypophysis of a buffalo in diestrus with multiple GF >10mm in diameter representing intermediate ovarian activity showed well stained scattered and pycnotic binucleated degranulated cells with distinct cytoplasmic outline.

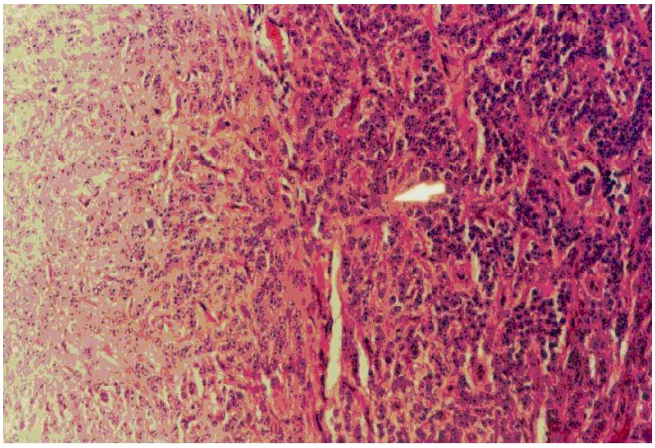

Plate 1. Photomicrograph of a pituitary gland collected from an adult buffalo in estrus. Pars intermedia is marked in the centre of section. Adenohypophysis on the right is populated with acidophils and basophils (H & E Stain X 200).

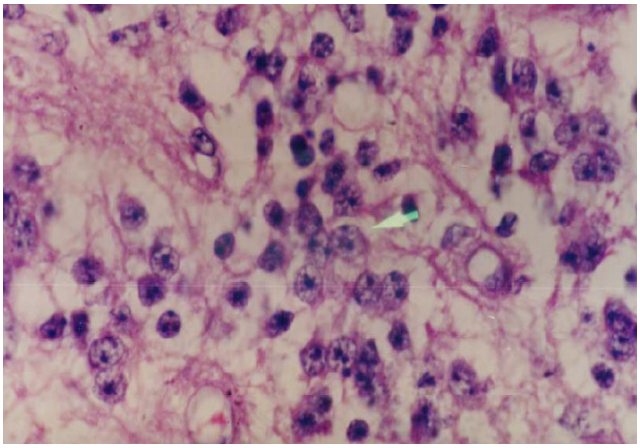

Plate 2. Photomicrograph of an adenohypophysis, gonadotrophs (Delta cells) are seen poly-angular, sharply outlined and bi-nucleated (H & E stain X 1000).

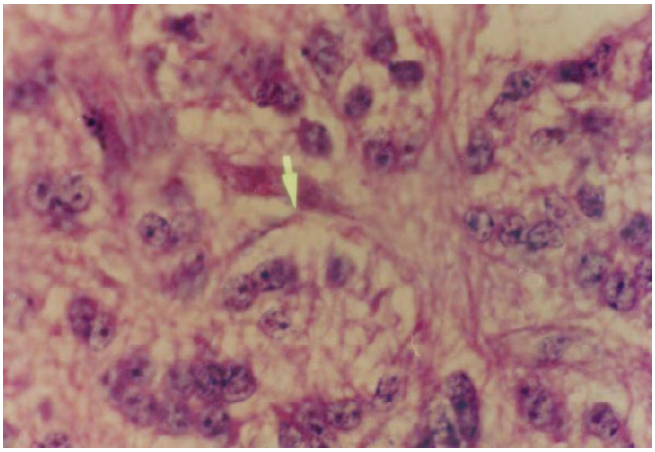

Plate 3. Photomicrograph of adenohypophysis of a buffalo heifer (an-estrus), degranulated gonadotrophs are in acinar arrangement (H & E stain X 1000).

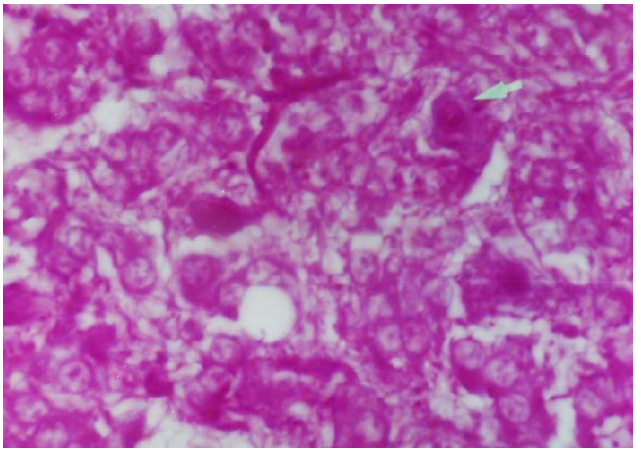

Plate 4: Photomicrograph of adenohypophysis of a buffalo heifer (estrus), dark cells are typical granulated gonadotrophs (Azan Mallory stain X 1000)

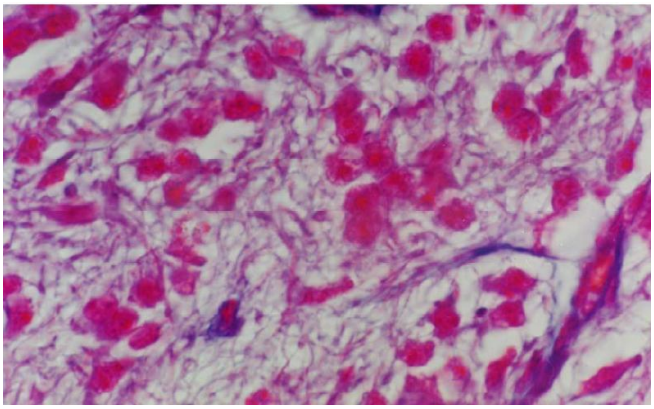

Plate 5: Photomicrograph of adenohypophysis of a buffalo heifer (di-estrus), heavily granulated gonadotrophs are in non-distinct acinar arrangements (Azan Mallory stain X 1000).

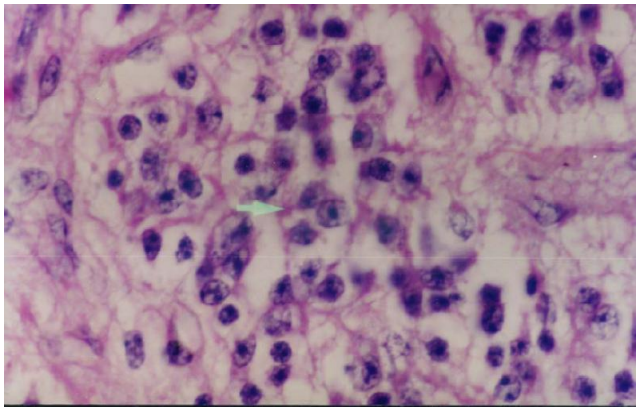

Plate 6: Photomicrograph of adenohypophysis of a buffalo in lactation 1-3 (an-estrus), showing a few scattered delta basophils (H & E stain X 1000).

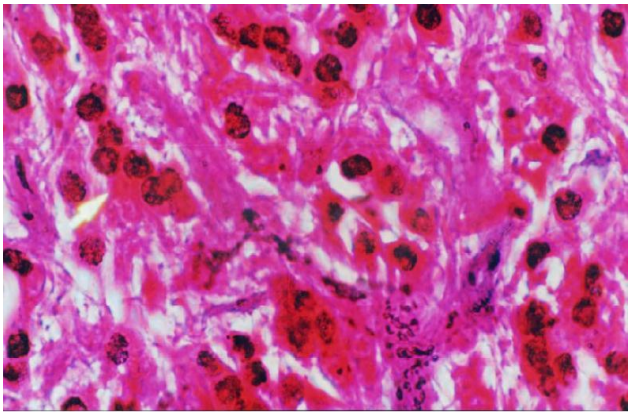

Plate 7: Photomicrograph of adenohypophysis of a buffalo in lactation 1-3 (estrus), granulated gonadotrophs are apposing against the reticular areas in acini (Azan Mallory stain X 1000).

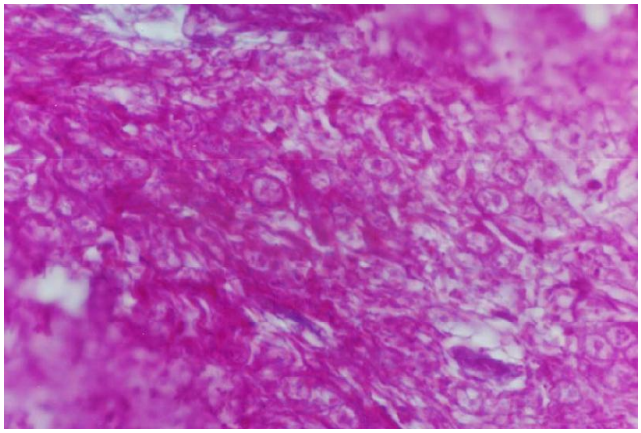

Plate 8: Photomicrograph of adenohypophysis of a buffalo (di-estrus), poorly showing granulated delta cells (Azan Mallory stain X 1000).

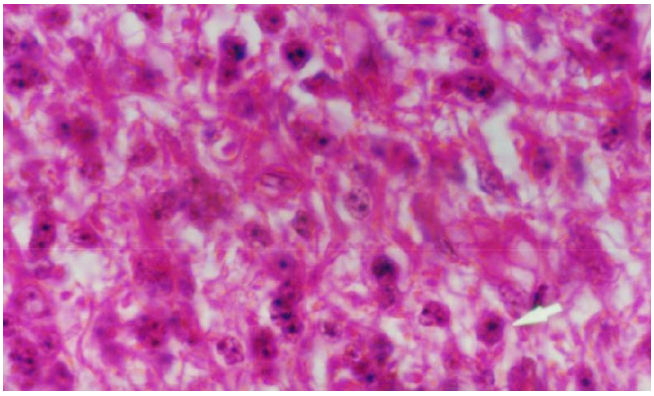

Plate 9: Photomicrograph of adenohypophysis of a buffalo in lactation 4th or above (anestrus), granulated and degranulating gonadotrophs are distinct, cytoplasmic membrane is not prominent, nuclei are stained blue (Azan Mallory stain X 1000).

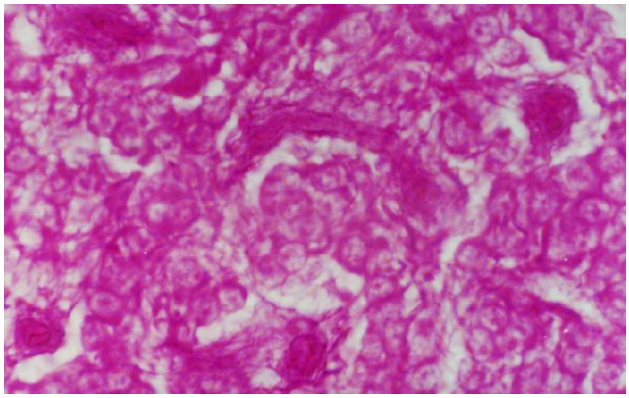

Plate 10: Photomicrograph of adenohypophysis of a buffalo in lactation 4th or above (estrus), clumps of degranulated cells with prominent nuclei are distinct in acinar arrangement (Azan Mallory stain X 1000).

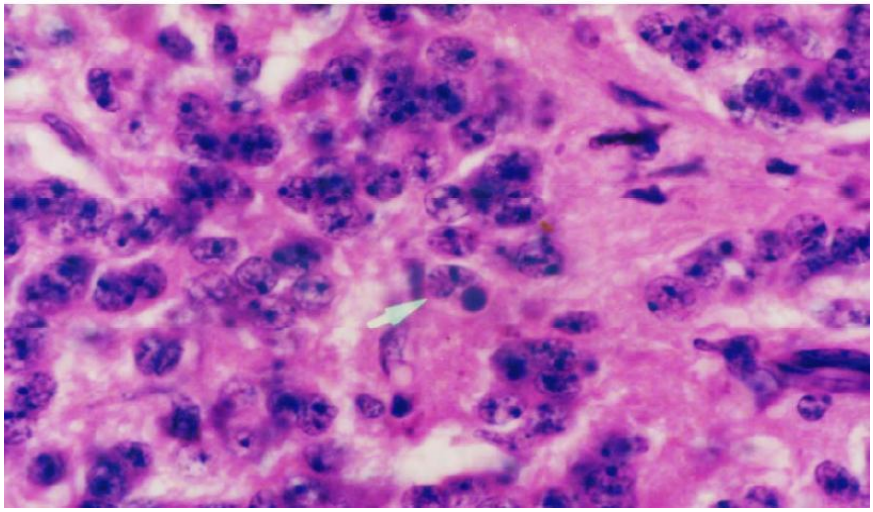

Plate 11: Photomicrograph of adenohypophysis of a buffalo (di-estrus), showing scattered degranulated cells with distinct outline (H & E stain, X 1000).

DISCUSSION

Biometry of the Graafian follicles: The present study revealed non-significant differences in number of GF between right and left ovaries among all three groups i.e. heifer, lactation 1-3 and lactation 4th or above during anestrus, estrus and diestrus. It shows that both of the ovaries would remain equally functional in cyclic buffaloes. However, with the increasing age, number of follicles reduced. Follicular growth and maturation of the ovaries represent a series of sequential sub-cellular and molecular transformation of various components of the follicle such as oocyte, granulosa and theca cells (Hafez, 1987). These are governed by intra ovarian factors, intra follicular factors and hormonal signal that lead to the secretion of the endogenous estrogens, mainly estradiol (Rao *et al.,* 1978; Sato *et al.,* 1982). Follicular growth involves hormonally induced proliferation and differentiation of theca and granulosa cells, leading to increased ability of follicles to produce estradiol and respond to pituitary gonadotropins. Production of estradiol determines which follicle would gain LH response necessary for ovulation and luteinization. Disturbances in responsiveness of the granulosa and theca cells to the gonadotropin signals stimulate and maintain maximum rate of estrogen and androgen biosynthesis, lead to cessation of the follicular growth and initiate the follicular atresia.

Gross anatomical observation of the buffalo pituitary: The pituitary gland of the buffalo was found to be located at the base of the brain, lodged in the body cavity of the sphenoid bone and attached with the hypothalamus by a stalk, as has been reported earlier in bovines (Jubb and McEntee, 1955; Leason and Leason, 1976; Copenhaver *et*

al., 1978; Chakravarthy and Mariappa, 1978). No pathological abnormalities were grossly observed in the pituitary glands of buffaloes. Muhammad (1984) reported 52.63 percent pathological conditions in the pituitaries collected from the adult buffaloes and 11.26 percent abnormal pituitaries in heifers. He further reported that 42.11 percent pituitaries from adult buffaloes had cysts. However, no cyst was seen in the pituitaries of the buffalo heifers during the study. Bassett *et al.* (1951) also reported colloid cyst in 30% glands from dairy cows. They further reported that treatment with stilbestrol also increased the weight of the pituitary gland. Jubb and Kenedy (1970) reported that microscopic and even multiple cysts were more common in the anterior lobe of the pituitary gland of the domestic animals, but sometimes their volume increased from pituitary gland and they might rupture through the capsule. No such finding was observed during the present study. The variation of the results found during different studies might be due to the difference of the species or it might be possible that the previous workers collected the pituitaries mostly from the problem/condemned animals.

Biometry of the buffalo pituitary: Significantly ($P<0.05$) higher (305±7.5mg) mean weight of the pituitary gland was recorded during estrus compared with 210±6.1 and 240±7.5mg found during anestrus and diestrus, respectively in buffalo heifers. Similar results were observed for buffaloes in lactation 1-3 and 4th or above. Namboothripad and Luktuke (1978) reported higher mean weight of the pituitary gland collected from the adult animals than glands from heifers. They also reported that mean weight of the pituitary gland of animals in different phases of the ovarian activity remained the same. However, Ganguli and Yadava (1975) reported lower weight of the pituitary gland during estrus than diestrus.

Functional cytology of the buffalo adenohypophysis: Photomicrograph of pituitary tissue from adult buffaloes showed distinct pars nervosa, pars intermedia and pars distalis (adenohypophysis). Harris and Donovan (1960), Leason and Leason (1976) and Banks (1992) also described similar histological description for the anterior pituitary gland. One subset of these cells produce follicle stimulating hormone and other produce luteinizing hormone, these cells are called gonadotrophs. Basophils stained as Hematoxylin positive granules, whereas acidophils showed eosinophilic granules, and chromophobes were seen as agranular or unstained. The proportion of cell type varies with age, species, sex and physiology. Generally, 50% cells are chromophobes, 40% are acidophils and 10% are basophils (Banks, 1992). Morphologically and functionally these cells were gonadotrophs. The bovine degranulated gonadotrophs were small, round or nearly so, with a sharp cytoplasmic out line and became less distinctive with the advancing degranulation.

During period of secretory inactivity, the granular contents increase and fully replete resting cells, characterized by a small crenated and pycnotic nucleus acentrically situated in a strongly megnata cytoplasm giving the impression of granularity rather than of distinguishable granule or granule fuse into homogeneity. This process may not involve whole cytoplasm uniformly (Jubb and McEntee, 1955). Pearse (1953) studied the cytology of human hypophysis in various physiological and pathological conditions and demonstrated that even in the fetal gland, muco protein containing cells, which are presumed to belong to the basophils series, are visible at the 8th week, considerably antedating the appearance of the earliest acidophils. Basophils containing true beta granules were seen first at the age of 14th week.

Number of gonadotrophs: Sections of adenohypophysis from each group of buffaloes at anestrus, estrus and diestrus were selected to study the gonadotrophs population. Gonadotrophic cell population was compared with the ovarian follicular activity. The results revealed that the gonadotrophs population was higher in the adenohypophysis of the buffaloes showing the maximum size of the GF in heifers in respect of their number. The ovarian activity and number of gonadotrophs were lower in buffaloes in lactation 4th or above. Namboothripad and Luktuke (1978) reported lower number of gonadotrophs in the pituitary of anestrus buffaloes compared with cyclic ones. Total gonadotrophic potency and FSH potency of pituitary of the anestrus buffaloes was significantly lower than that of cyclic animals. This probably was attributed towards failure of maturation and rupture of follicles in the ovary and resultant failure of manifestational estrum.

The results of the present study revealed that number of growing follicles gradually decreased with the age of the animal. Cupps (1991) also demonstrated that follicles were higher in number at the age of 12 months and reduced to only 72 at the age of 15-20 years. Roberts (1971) stated that the number of primordial follicles in cattle remained fairly stable around 140,000 until about 4-6 years of age and then declined rapidly thereafter to 25,000 at 10-14 years and to near zero at 20 years of age. Salisbury *et al*. (1978) cited that the number of primordial follicles in the three months old calf was 75,000; in heifers at 15 to 30 months of age the number was reduced to 21,000 and in old cows it was as low as 2500.

It is concluded that significantly ($P<0.05$) higher weight of the pituitaries and higher gonadotrophs cell population in the adenohypophysis of the estrus buffaloes appears to be indicating the greater activity of the gonadotrophs producing more FSH as evidenced by higher number of mature GF found on the ovaries of slaughtered buffaloes during this phase.

Acknowledgements: We greatly acknowledge Pakistan

Science Foundation, Islamabad, for funding the project titled "Technologies development for the production of Gonadotropins from animal sources"

REFERENCES

Anonymous, (1991). Minitab statistical software, Minitab Inc. 3081 Enterprise Drive, State College, PA 1681-3008.

Anonymous, (1993). Agricultural Research, Livestock Report (IDA CR 2154-Pak) Water and Agriculture Division, Govt. Pakistan, Islamabad.

Anonymous, (2011). Pakistan Economic Survey, Ministry of Finance, Govt. Pakistan, Islamabad.

Banks, W. J., (1992). Applied Veterinary Histology. 3rd Ed. Mosby Year Book Toronto, Canada. 408-413.

Bassett, E. G., C. P. Mcmeekan and K. Wodzicki. (1951). The anterior lobe of the cattle pituitary. I. Quantitative cell type variation in various normal and abnormal sexual conditions. II. Distribution of Colloid. J. Endocrinol. 7:203-214.

Chakravarthy, Y. S. and D. Mariappa, (1975). Histological observations on the pituitary of the Indian buffalo (*Bos bubalis*). Ind. Vet. J. 52:121-124.

Cockrill, W. R., (1980). The ascendant water buffalo, Key domestic animal. World Anim. Rev. 33: 2-13.

Copenhaver, W. M., E. K. Doughlas and L. W. Richard. (1978). Bailey's Text Book of Histology. The Williams and Wilkins Company Baltimore, USA. Pp:694-707.

Cupps, P. T. (1991). Reproduction in Domestic Animals. 4thEd. Academic Press Inc. New York, USA. Pp:119-163.

Duncan, D. B., (1955). Multiple Range and Multiple F-test Biometrics, 11: 1-42.

Ganguli, A. and C. P. Yadava, (1975). Macroscopic observation on pituitary gland of Indian buffalo (*Bos bubalis*). Ind. Vet. J. 52:933-935.

Hafez, E. S. E. (1987). Reproduction in Farm Animals. Lea and Febiger, Philadelphia, USA. Pp:130-167.

Harris, G. W. and B. T. Donovan, (1966). The Pituitary Gland. I: Univ. of California Press, USA.

Humason, G. L. (1972). Animal Tissue Techniques. 3rdEd. Freeman and Company San Francisco, USA.

Jubb, K. V. F and K. McEntee, (1955). Observation on the bovine pituitary gland. II. Architecture and cytology with special reference to basophil cell function. Cornell Vet. 45:574-641.

Jubb, K. V. F. and P. C. Kenedy, (1970). Pathology of Domistic Animals. I: Academic Press, New York, USA. Pp:428-442.

Khan M. Z. (1985). Studies on the biometrical values of normal reproductive organs of female buffaloes along with the incidence and pathology of diseases of the reproductive tract. Ph.D. Thesis Univ. Agri. Faisalabad, Pakistan.

Leason, R. and T. S. Leason, (1976). Histology. 4th Ed. W.B. Saunders Company, Philadelphia, USA. Pp:450-461.

Morrow, D. A., (1986). Current Therapy in Theriogenology**.** 2nd Ed. W.B. Saunders Company, Philadelphia, USA. Pp: 54-87.

Muhammad, G. (1984). Pathological studies on pituitary gland (Adenohypophysis) of the buffalo. MSc Thesis, Univ. Agri. Faisalabad, Pakistan.

Namboothripad, T. R. B. and S. N. Luktuke (1978). Studies on the gonads and hypophysis cerebri of anestrus buffaloes. Kerala J. Vet. Sci. 9:293-310.

Pearse, A. G. E. (1953). Cytological and cytochemical investigation on the fetal and adult's hypophysis in various physiological and pathological stages. J. Pathol. Bactriol. 65 Pp:355-370.

Rao, M. C., J. S. Richards and A. R. Midgley Jr. (1978). Hormonal regulation of ovarian cellular proliferation. Cell, 14 Pp:71-78.

Rebeeca, C.E. and D.J. Tortonese 2000. Characterization and distribution of gonadotrophs in the pars distalis and pars tuberalis of the equine pituitary gland during the estrus cycle and seasonal anestrus. Biol. Reprod., 63 Pp:826–832.

Roberts, S. J., (1971). Veterinary Obstetrics and Genital Diseases. Edwards Brothers Inc., Ann Arbor, U.S.A. 359-361.

Roch, P. C., R. J. Ryan and D. J. Mccornich (1992). Identification of the hormone binding regimes of the luteinization hormone/Human chorionic gonadotrophin receptor using synthetic peptides. Endocrinology, 131 Pp:268-274.

Salisbury, G. W., N. L. Vandemark and J. R. Lodge, (1978). Physiology of Reproduction and Artificial Insemination in Cattle. Freeman and Co, San Francisco, USA, Pp:94-129.

Sato, E., T. Eishibashi and A. Iratani. (1982). Purification and action site of a follicle stimulating hormone inhibator from bovine follicular fluid. J. Anim. Sci., 55:873-877.

Steel, R. G., J. H. Torrie and D. A. Dieky, (1997). Principles and Procedures of Statistics. 3rd Ed. McGraw Hill Book Co. Inc, New York, USA.