OVARIAN CATEGORIES, FOLLICLES AND OOCYTES ANALYSIS OF BUFFALO IN VIEW OF *IN VITRO* PRODUCTION OF EMBRYOS

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CERTIFICATE

This is to certify that the thesis entitled **"OVARIAN CATEGORIES, FOLLICLES AND OOCYTES ANALYSIS OF BUFFALO IN VIEW OF *IN VITRO* PRODUCTION OF EMBRYOS"** submitted to the Department of Animal Nutrition, Genetics and Breeding, Faculty of Animal Science and Veterinary Medicine, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (MS)** in **ANIMAL BREEDING AND GENETICS**, embodies the result of a piece of Bona fide research work carried out by **MUHAMMAD RAKIBUL ISLAM**, Registration No. 13-05311 under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that any help or source of information, received during the course of this investigation has been duly acknowledged.

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Dhaka, Bangladesh

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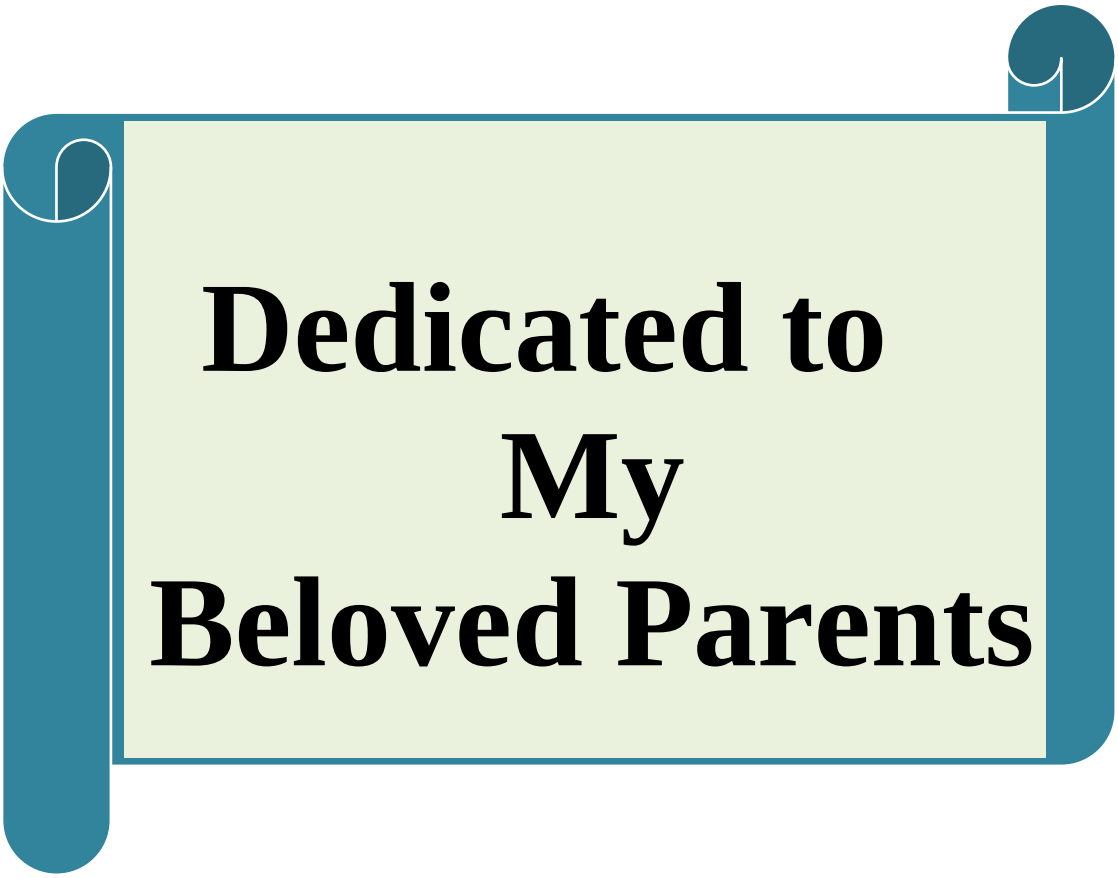
Dedicated to
My
Beloved Parents

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OVARIAN CATEGORIES, FOLLICLES AND OOCYTES ANALYSIS OF BUFFALO IN VIEW OF *IN VITRO* PRODUCTION OF EMBRYOS

ABSTRACT

The present study was carried out at the Department of Animal Nutrition, Genetics and Breeding, Sher-e-Bangla Agricultural University, Dhaka during the period from July 2018 to June 2019. Buffalo ovaries were collected from Slaughter house of Dhaka city. During collection left and right ovaries were identified and recorded and after necessary processing the ovaries were categorized as (i) ovaries without corpus luteum (CL) and (ii) ovaries with corpus luteum (CL). Ovaries were then evaluated on the basis of length (cm), width (cm), weight (g), total number of follicles on the surface of each category ovaries, number of follicles aspirated and total number of COCs. In terms of ovarian categories regarding left and right category, the result obtained from this experiment showed significant ($p<0.01$) difference in the parameters. The highest percentage of CL (27.12±0.11%) was in right ovary compared to left ovaries (3.24±0.11%). The higher length (cm) (2.75±0.06), width (cm) (2.17±0.05), weight (g) (4.74±0.13) was found in right ovaries compared to left ovaries. The number of follicles (aspirated) (5.47±0.16) were also higher in left ovaries compared to right ovaries (4.18±0.16). The normal COCs were higher in case of left ovaries (with a mean of 0.98±0.16 per ovary) compared to right ovaries (with a mean of 0.84±0.16 per ovary). In case of abnormal COCs reverse result was found. In terms of ovarian categories regarding with CL or without CL, 76.47±0.01% ovaries showed without CL where 23.53±0.01% showed ovary with CL. In case of ovarian category regarding with CL and without CL, Significance difference were found in the parameter. The highest length (2.86 ±0.10) and weight (4.73±0.12) were found in with CL ovaries compared to without CL ovaries. In case of width (cm), both in with CL and without CL ovaries were same (2.30±0.01). The number of follicles (aspirated) was distinctly higher in ovaries with CL (5.28 ±0.136) compared to ovary without CL (2.29±0.14). In terms of total COCs, ovaries without CL showed highest number of COCs (1.41± 0.11) compared to the ovaries with CL (1.17±0.11) where the highest numbers of normal COCs (0.55± 0.01) and abnormal COCs (0.86 ±0.03) were found in ovary without CL compared to ovary with CL (normal COCs 0.5 ±0.01 and abnormal 0.54±0.03). Finally, it can be concluded that left ovaries contain more follicles and COCs than right ovaries and also contain more number of normal COCs. Whereas ovaries without CL contain higher number of follicles and normal COCs compared to ovaries with CL. So, higher number of normal COCs found in left and without CL ovaries.

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LIST OF ABBREVIATIONS AND SYMBOLS

AEZ	=	Agro-Ecological Zone
BBS	=	Bangladesh Bureau of Statistics
BCSRI	=	Bangladesh Council of Scientific Research Institute
cm	=	Centimeter
CV %	=	Percent Coefficient of Variation
DAS	=	Days After Sowing
DMRT	=	Duncan's Multiple Range Test
et al.,	=	And others
e.g.	=	exempli gratia (L), for example
etc.	=	Etcetera
FAO	=	Food and Agricultural Organization
g	=	Gram (s)
i.e.	=	id est (L), that is
Kg	=	Kilogram (s)
LSD	=	Least Significant Difference
m^2	=	Meter squares
ml	=	MiliLitre
M.S.	=	Master of Science
No.	=	Number
SAU	=	Sher-e-Bangla Agricultural University
var.	=	Variety
^oC	=	Degree Celceous
%	=	Percentage
NaOH	=	Sodium hydroxide
GM	=	Geometric mean
mg	=	Miligram
Ca	=	Calcium
L	=	Litre
µg	=	Microgram
USA	=	United States of America
WHO	=	World Health Organization
IVP	=	*In Vitro* Production
CL	=	Corpus Luteum
MOET	=	Multiple Ovulation Embryo Transfer
IVM	=	*In vitro* Maturation
IVC	=	*In vitro* Culture

CHAPTER I

INTRODUCTION

Buffalo *(Bubalus bubalis)* is an important part of agricultural economy and it plays a significant role in livestock production. Asian buffalo or Water buffalo is classified under the genus *Bubalus*, species *bubalis*. The *Bubalus bubalis* belongs to the class Mammalia, subclass Ungulata, order Artiodactyla, suborder Ruminantia, family Bovidae, subfamily Bovinae, tribe Bovini, which includes the following three groups: *Bovina* (cattle), *Bubalina* and *Syncerina Syncerina* includes only the species *Syncerus caffer* (the African buffalo). *Bubalina* (the Asian buffalo) includes three species: *Bubalus depressicornis* or Anoa which lives in Indonesia, *Bubalus mindorensis* which lives in the Philippines and *Bubalus bubalis* deriving from the domestication of the *Bubalus arnee*, the Indian wild buffalo (FAO, 2005).

The domestication of this species occurred relatively recently (5000 years ago) compared to the domestication of *Bos taurus* and *Bos indicus* (10000 years ago). Asian buffalo includes two subspecies known as the River and Swamp types, the morphology and purposes of which are different as are the genetics. The River buffalo has 50 chromosomes of which five pairs are submetacentric, while 20 are acrocentric: the Swamp buffalo has 48 chromosomes, of which 19 pairs are metacentric. The difference in the diploid number is only apparent. In fact, the large Swamp buffalo chromosome 1 originated from tandem fusion translocation between the River buffalo chromosome 4 (telomeres of p-arm) and 9 (centromere) (Di Berardino and Iannuzzi, 1981).

The buffalo (*Bubalus bubalis*) population in the world is actually about 168 million head: 161 million can be found in Asia (95.83 percent); 3 717 million are in Africa, almost entirely in Egypt (2.24 percent); 3.3 million (1.96 percent) in

South America, 40 000 in Australia (0.02 percent); 500 000 in Europe (0.30 percent) (FAO, 2005).

In the year 2003, Bangladesh had 772764 buffalo head owned by 270228 holdings representing 1.52 percent of the total holdings in the country. The average buffalo head per holding was 2.67 (Faruque, 2003). Bangladesh now has about 1485000 buffaloes that are being used for draught or dairy purposes (BBS, 2018). These buffalo are found in the Bramhaputra-Jamuna flood plain of central Bangladesh, the Ganges-Meghna flood plain of southern Bangladesh and in institutional herds. Bangladesh has milk/dairy buffaloes of the Swamp crossbred and River types such as the Murrah and Nili-Ravi. The occurrence of crossbred dairy buffaloes indicates that the genetic improvement programme has been operative and is still running (Faruque, 2000).

Reproductive efficiency is the primary factor affecting productivity and is hampered in female buffalo by (1) inherent late maturity, (2) poor estrous expression in summer, (3) distinct seasonal reproductive patterns and (4) prolonged calving intervals. Reproductive efficiency can be improved by introducing embryos produced *in vitro* (Raza *et al*., 2001).

Oocytes are the main raw materials for *in vitro* embryo production (IVP) experiments. So, slaughter house ovaries can be an economic source for the collection of oocytes (Asad *et al*., 2016). Oocyte quality has long been considered as a main limiting factor for IVP. Therefore, the success of any IVP program in buffalo production, either *in vitro* fertilization (IVF) or in vitro culture (IVC) of embryos largely depends on the continuous supply of quality oocytes in optimum quantity.

Ovaries were categorized as right, left, with corpus luteum (CL) and without CL group. Ovaries were then evaluated on the basis of weight (gm), length (cm), width (cm), total number of follicles on the surface of each categorized ovaries,

number of follicles aspirated, total number of COCs, normal COCs and abnormal COCs.The oocytes from ovaries without CL had greater developmental competence than ovaries with CL (Tasripoo *et al*., 2005). Jamil *et al*. (2008) reported that a significantly higher oocyte recovery rate was obtained from the ovaries of buffalo collected during the peak- breeding season and from those without CL. In addition, Warriach and Chouhan (2004) observed that the oocytes of buffalo with >3 layers of cumulus cells showed higher maturation rates than oocytes with partial or no cumulus cells and oocytes co-cultured with cumulus cells but did not differ from oocytes having 1-2 layers of cumulus cells. The degeneration rates were higher for oocytes with partial or no cumulus cells than those surrounded by healthy cumulus cell layers. This result reveals that buffalo oocytes with intact layers of cumulus cells show better IVM rates than oocytes without cumulus cells and the co-culture of poor quality oocytes with cumulus cells improves their meiotic competence (Jamil *et al*., 2008).

Over a decade, there were a lot of researches done towards the implementation of embryo technologies to fasten the genetic manipulation of livestock which involves multiple ovulation and embryo transfer (MOET), *in vitro* embryo production (IVP), cloning, and transgenesis (Asad 2015; Saha *et al.,* 2014; Sreenivas*etal.* 2014; Freitas and Melo, 2010).From those mentioned, IVP has becoming more popular method of producing embryos from slaughter house derived ovaries (Hoque*et al*., 2011). The IVEP system involves at least four steps, namely (i) evaluation of ovaries, efficient collection and grading of oocytes; (ii) *in vitro* maturation (IVM) of these oocytes; (iii) *in vitro* fertilization (IVF) of the matured oocytes; and (iv) *in vitro* culture (IVC) of the resulting embryos (Freitas and Melo, 2010). Nowadays, IVP is becoming a useful tool for maximizing the number of offspring from valuable buffalo, producing calves from infertile and slaughtered buffalo, and producing commercial production (Sianturi, 2001; Hyttel *et al*., 1997; Sirad and Blondin, 1996).

To produce embryos by *in vitro* techniques, it is necessary to recover the oocytes and undergo maturation of oocytes, fertilize, and develop those developing zygotes to blastocyst stage so that they can be transferred to the recipient (Hoque *et al.,* 2012).COCs contains the oocytes and in recent years, the percentage of oocytes reaching the blastocyst stage by *in vitro* techniques still varies. In some cases, the low development of IVM oocytes is related to their quality at the beginning of maturation. Mondal (2008) has reported that higher average number of good quality oocytes was recovered from ovaries without corpus luteum compared to the ovaries with corpus luteum, which thus, can be effectively used for IVM and IVF. In addition, it has been shown that oocytes with at least four layers of cumulus cells have good result for IVM and IVF (Yang *et al.*, 1993).

Buffalo is numerically and economically very important and promising animal genetic resources in the developing countries like Bangladesh. Thus, genetic improvement of buffaloin Bangladesh could be made by planned artificial insemination (AI) with frozen semen, multiple ovulation and embryo transfer (MOET) and *in vitro* production (IVP) of embryos. To produce good embryos, quality oocyte is obligatory. Though lot of ovaries are waste in slaughter house but it may be a good source of quality livestock production which can full fill the existing scarcity of meat, milk and skin. Ovary collection, evaluation and grading technique results in rapid genetic gain of outstanding females. It complements the exploitation of superior males through artificial insemination program. So, *in vitro* production of embryo from slaughterhouse ovaries might be considered as a low cost and sustainable technique in Bangladesh condition (Rahman, 2003). Embryos can be produced from the oocytes collected from the ovaries of the females that are usually being slaughtered in slaughterhouse for meat purpose, and the embryos thus produced can be transferred to the recipient females.

In Bangladesh, *in vitro* techniques in buffalois a recent concept but a great deal of work has been, still going on to standardize IVP techniques followed by IVM and

IVF (Ferdous, 2006; Islam *et al*., 2007; Mondal *et al*., 2008; Hoque, 2009). A critical goal for mass production of buffaloembryo production is the recovery of a large number of oocyte with high developmental competence. With some other factors successful embryo production is also depend on oocyte quality.

So, the present study was undertaken with a view to collection and evaluation of buffaloslaughter house ovaries, follicles and COCs to create vast opportunities to conduct the research work in the area of IVP of buffaloembryos with the following objectives:

1. To evaluate the ovaries based on recovery of follicles and oocytes analysis.
2. To categories the ovaries according to number of follicles, oocytes used for *In Vitro* production of embryos.

CHAPTER II

REVIEW OF LITERATURE

Buffalo is one of the most important livestock species populated largely in tropical and sub-tropical countries (Das and Khan, 2010). A lot of ovaries are waste in slaughter house but it may be a good source of quality livestock production which can full fill the existing scarcity of meat, milk and skin. Ovary collection, evaluation and grading technique results in rapid genetic gain of outstanding females. Regarding the present study, review of literature has been done under the following sub-headings:

2.1 Ovarian categories

Ovaries were categorized as right, left, with corpus luteum (CL) and without CL group. Ovaries were then evaluated on the basis of weight (gm), length (cm), width (cm), total number of follicles on the surface of each categorized ovaries, number of follicles aspirated, total number of COCs, normal COCs and abnormal COCs (Asad *et al*., 2016).

Asad *et al*. (2016) collected goat ovaries and categorized as right, left, with corpus luteum (CL) and without CL group. And also categorized on the basis of weight (gm), length (cm) and width (cm). The length (cm) of right ovaries (1.19±0.09) was found significantly ($p<0.05$) higher than left ones (1.15±0.04). Other parameters, including width, weight and total number of COCs aspirated per ovary did not differ significantly ($P<0.05$) between right and left ovaries.When compared the ovaries in between with-CL and without-CL group, significantly ($p<0.05$) higher number of normal COCs (1.12±0.07) were found in without-CL group with an increase of length (1.17±0.01).

Patra *et al.* (2013) conducted study on 50 native buffaloes of Odisha to evaluate ovarian biometry and found non significantly higher average weight (g) of the right ovary (2.36 ± 0.13) than the left ovary (2.17 ± 0.11).

Kachiwal *et al.* (2012) studied ultrasonographic biometry of the ovaries of pregnant Kundhi buffaloes. The average weight of ovaries with corpus luteum during 1^{st}, 2^{nd}, 3^{rd} and 4^{th} months of pregnancy was 4.6 ± 0.345, 5.90 ± 1.134, 6.10 ± 1.179, and 6.50 ± 1.139 g respectively. While, the average weight of ovaries of non-gravid uterus of same buffaloes was 2.7 ± 0.345, 3.6 ± 1.140, 3.9 ± 1.149 and 4.2 ± 1.093 g during 1^{st}, 2^{nd}, 3^{rd} and 4^{th} month of pregnancy respectively.

Hasanzadeh and Sadeghinejad (2012) collected ovaries of 24 adult (2 to 4 year age) apparently healthy, non pregnant and cyclic buffaloes, *i.e.* 12 specimens during summer (4 pairs in each month of season), and 12 specimens during winter (4 pairs in each month of season). The study revealed that the ovaries were ellipsoid in shape and weighed 3.5 ± 0.2 g. Further, the right ovaries were significantly heavier and larger than the left ovaries.

Khandoker *et al.* (2011) recorded weight of buffalo ovaries. Among 136 ovaries (consisting 68 on each side *i.e.* left and right) a number of 93 belonged without CL and others with CL. Non-significant ($P<0.05$) difference was found in the weight of left (2.87 ± 0.32 g) and right ovaries (3.59 ± 0.31 g). While, the weight was significantly ($P<0.05$) higher in ovaries with CL (3.64 ± 0.18 g) than those of without CL (2.73 ± 0.12 g).

Leal *et al.* (2007) collected buffalo ovaries from a slaughterhouse and transported to the laboratory in saline solution at 36° C. The means of weight, length, width and height of the ovary were 3.83 g (n=84), 2.27 cm (n=84), 1.08 cm (n=84) and 1.56 cm (n=84), respectively.

Neelam and Saigal (2005) studied the gross morphological observations pertaining to weight, volume and size of the paired ovaries in 53 adult buffaloes during luteal

and follicular phases of reproductive cycle. The average weight and size of right and left ovaries did not differ significantly neither in follicular nor in luteal phase. The ovaries during luteal phase had significantly higher values for weight, volume, length and breadth but not for the thickness. The ovaries in buffaloes aging 3.5 to 7 years were found to be heavier and larger in size than that of the older animals; probably due to the higher ovarian activity in younger animals.

Gupta *et al.* (2003) collected ovaries of mature buffaloes (n=121) from local slaughter house. Weight of the ovaries was recorded at the time of oocyte retrieval. Ovaries were classified into three categories *i.e.* ovaries with corpus luteum, ovaries without corpus luteum and pooled ovaries (all ovaries irrespective of the presence of corpus luteum). The mean ovarian weight of pooled ovaries in mature buffaloes observed was 2.69 ± 0.12 g. There was significant ($P<0.005$) difference between the weights of ovaries with CL (3.57 ± 0. 22 g) and without CL (2.17 ± 0.11 g).

2.2 Follicles and oocytes analysis

2.2.1 Follicular count

Patra *et al.* (2013) reported that the mean count of different sized follicles in right ovary and their total count was slightly higher numerically than the left ovary which was statistically non significant in buffaloes. The average number of large, medium and small follicles of right ovary was recorded to be 0.70 ± 0.07, 1.60 ± 0.12 and 6.26 ± 0.37, respectively with a total of 8.54 ± 0.42, similarly the left ovary possessed 0.68 ± 0.07, 1.52 ± 0.13 and 6.10 ± 0.32, large, medium and small follicles with a total of 8.30 ± 0.63.

Makwana *et al.* (2012) conducted the study on abattoir ovaries of Surti buffalo during peak breeding season. In their experiment, the total number of follicles recorded from 408 ovaries was 1369 and the overall mean number of follicles per ovary was found to be 3.36 ± 1.33 ± 0.05 (39.66), 1.32 ± 0.05 (39.44) and 0.70 ±

0.04 (20.90) respectively.

Aziz *et al.* (2012) collected a total of 741 buffalo ovaries and observed 2983 follicles on collected ovaries (average 4.03 follicles per ovary).

Pirestantui *et al.* (2011) collected cow ovaries immediately after slaughter and divided into three categories based on their cyclic status, which included: the presence of a large follicle (LF), the presence of a corpus luteum (CL) and ovaries without LF or CL (WLCF). The highest average oocytes collected per ovary were related to the CL (22), WLCF (21.4) and LF groups (20.8) respectively.

Khandoker *et al.* (2011) recorded a number of 916 follicles (7.25 ± 0.31/left ovary; 6.22 ± 0.32/right ovary) on the surface of the 136 buffalo ovaries and 806 follicles were aspirated from the surface of both the (right and left) ovaries and among them 385 were obtained with a mean of 5.66 ± 0.25 per ovary from right and 421 from left ovaries with a mean of 6.19 ± 0.24 per ovary. In addition, from aspirated 806 follicles, 630 were obtained from 93 ovaries without CL (Follicular phase) and 176 from 43 ovaries with CL (Luteal phase). The significantly higher ($P<0.05$) number of follicles were aspirated per ovary from ovaries without CL (6.78 ± 0.18) than with CL (4.09 ± 0.26).

Mistry and Dhami (2009) selected buffalo ovaries without functional corpus luteum and found an average number of follicles of small, medium and large size per ovary as 0.82, 0.48 and 0.24 respectively, with an overall mean of 1.55. The distribution of small, medium and large follicles was found to be 53.18, 31.12 and 15.70 percent respectively.

Amer *et al.* (2008) reported 6.8 follicles in type III ovaries (without CL), followed by 5.2 in type II ovaries (with regressed CL) and 4.4 in type I ovaries (having functional CL) in buffalo.

Singh *et al.* (2001) studied the effect of presence of corpus luteum on oocyte recovery in buffaloes and reported that the presence of corpus luteum in the ovary at the time of recovery significantly affected availability of total oocytes.

Abdoon and Kandil (2001) reported that the presence of a CL stimulates the development of significantly higher ($P<0.01$) number of ovarian follicles which produced a significantly higher ($P<0.05$) number of good quality oocytes.

2.2.2 Oocytes retrieval rate

2.2.2.1 Effect of corpus luteum

El-Naby *et al.* (2013) collected oocytes by aspiration method and studied the effect of presence or absence of CL on oocyte recovery in Egyptian buffaloes and reported non-significant differences between oocyte yield in ovaries with CL (2.37 ± 0.14 no. of oocytes/ovary; 298 no. of oocytes recovered out of 126 no. of ovaries) and without CL (2.27 ± 0.02 no. of oocytes/ovary; 244 no. of oocytes recovered out of 106 no. of ovaries sliced).

Singh *et al.* (2012) examined 367 buffalo ovaries to know the effect of presence or absence of corpus luteum over the ovary on oocyte recovery rate by slicing method. They recovered significantly ($P<0.05$) greater number of oocytes per ovary (3.31 ± 0.36) when the CL was absent compared with ovaries on which CL was present (1.01 ± 0.05). They concluded that the effect of presence vs absence of CL on the ovaries had significant effect on recovery rate of buffalo oocytes.

Rao and Mahesh (2012) observed that the luteal phase buffalo ovaries (having CL) yielded lower numbers of oocytes (3.00 ± 0.34) compared to non-luteal phase (no CL) ovaries (5.16 ± 0.41).

Makwana *et al.* (2012) observed significantly ($P<0.05$) greater number of oocytes per ovary when the CL was absent (3.77 ± 0.14) compared with ovaries on which CL was present (2.70 ± 0.12) in Surti buffalo.

Boonkong *et al.* (2012) collected bovine oocytes by aspiration method. Oocytes obtained from ovaries with CL and without CL, were recovered and determined as recovery rate prior to *in vitro* culture. The results revealed that the recovery rates of bovine oocytes were not significantly different between ovaries with CL (58.54%; 72 oocytes out of 123 follicles) and without CL (43.54%; 64 oocytes out of 147 follicles).

Khandoker *et al.* (2011) collected follicles from buffalo ovaries and found higher numbers of COCs in ovaries without CL (2.63 ± 0.12 per ovary) than ovaries with CL (1.81 ± 0.17 per ovary). Again, when the COCs were classified in normal and abnormal groups, the significantly higher ($P<0.05$) number of normal COCs was found in ovaries without CL than those ovaries with CL with the mean of 1.71 ± 0.08 and 0.86 ± 0.12 follicles per ovary, respectively, and the reverse trend was found in abnormal group (0.92 ± 0.09 and 0.95 ± 0.13 follicles per ovary respectively).

Mistry and Dhami (2009) collected oocytes from buffalo ovaries without functional corpus luteum by slicing method and a total of 1409 oocytes were recovered from a total of 456 ovaries, with an average recovery rate of 3.09 oocytes per ovary.

Gupta *et al.* (2007) studied the effect of the presence of corpus luteum on the recovery rate of large preantral follicles in buffalo and found significantly ($P<0.05$) higher yield of large preantral follicles from the ovaries with corpus luteum (8.05 ± 0.88 per ovary) than for the ovaries without corpus luteum (4.57 ± 0.43 per ovary).

Rahman *et al.* (2003) collected the follicles by slicing technique from three categories of bovine ovary obtained from local slaughterhouse. Type-I, having functional corpus luteum (CL); type-II, CL in almost regressed condition and type-III without CL. The average number of follicles per ovary was 4.37, 5.28 and

6.48, respectively. Significantly higher (P<0.01) number of follicles was obtained from type-III ovaries.

Gupta *et al.* (2003) studied oocyte recovery rates in relation to morphology and weight of the ovaries (n=121) in buffaloes. The recovery rates of oocytes from ovaries with CL and ovaries without CL were 3.40 ± 0.44 and 3.42 ± 0.52, respectively. On pooled basis, the recovery rate was 3.41 ± 0.36. Statistical analysis showed no significant difference (P>0.05) in the recovery rates among the three different classes of ovaries, indicating the presence of CL does not affect the recovery rate.

Raza *et al.* (2001) reported that the ovaries of Nili-Ravi buffalo with CL yielded significantly lower (P<0.05) good quality and total oocytes (2.63 and 3.76 per ovary) than the ovary without CL (4.48 and 5.88 per ovary).

Samad and Raza (1999) reported that the ovaries with CL yielded significantly lower (P<0.05) total oocytes per ovary (3.94 ± 0.28) than ovaries without CL (5.90 ± 0.39) in buffaloes with scoring method.

Kumar *et al.* (1997) collected 250 ovaries from slaughtered buffaloes and they obtained low average yield of oocytes per ovary (P<0.05) bearing CL (4.08) than those non-bearing CL (6.55).

Das *et al.* (1996) assessed the effect of the presence of a CL on oocyte recovery from buffalo ovaries obtained at an abattoir and reported that buffalo ovaries bearing CL had significantly (P<0.05) lower mean number of oocytes as well as the quality of the oocytes following aspiration.

2.2.2.2 Effect of oocyte collection techniques

El-Naby *et al.* (2013) harvested the oocytes in Egyptian buffaloes from all visible ovarian follicles by aspiration using 18-gauge needle fixed on 10 ml disposable syringe filled with 1 ml of the aspiration medium. They found that the mean

oocyte recovery rate per ovary was nearly similar during spring (2.57 ± 0.23; 306 no. of oocytes recovered out of 123 no. of ovaries) (high ovarian activity) and summer (2.60 ± 0.21; 307 no. of oocytes recovered out of 113 no. of ovaries) (low ovarian activity) seasons.

Singh *et al.* (2012) recovered oocytes from buffalo ovaries by slicing method and obtained 2.28 ± 0.07 overall mean numbers of oocytes per ovary.

Rao and Mahesh (2012) studied the comparative efficacy of three harvesting techniques *viz.*, the aspiration, puncture and slicing methods on oocyte recovery in buffalo ovaries obtained from a local abattoir. Among the three collection methods, the slicing technique yielded the highest number of total oocytes (7.98 ± 0.70) followed by the puncture (3.46 ± 0.31) and the aspiration methods (2.38 ± 0.19). The mean oocyte recovery was 4.60 ± 0.33.

Quintana *et al.* (2012) extracted cumulus-oocyte complexes from buffalo ovaries using two methods, the follicular puncture (FP) with 18G×1½ needle and surgical dissection (SD) of follicles using an ophthalmic micro-scalpel. The results showed significant differences in the average of COCs recovery and their quality when compared to FP and SD methods. By the FP method, the average 2.7 COCs per ovary were recovered. While, the SD method achieved 6.3 COCs per ovary recovery rate.

Makwana *et al.* (2012) collected buffalo follicular oocyte by slicing method and reported 3.01 ± 0.10 overall mean numbers of oocytes per ovary.

Kulasekhar *et al.* (2012) reported that the oocyte recovery rate per ovary was 2.91, 1.53 and 1.89 in slicing, aspiration and post aspiration slicing, respectively, in pluriparous buffaloes. A total of 1092 oocytes were retrieved from 498 ovaries with an average of 2.19 oocytes per ovary.

Chandrahasan *et al.* (2012) used different collection techniques for oocytes

recovery in buffaloes. They obtained 2.71, 3.10 and 5.69 mean number of oocytes from each ovary by aspiration, puncture and dissection methods, respectively. The difference between dissection and other two methods was statistically highly significant.

Aziz *et al.* (2012) retrieved immature buffalo oocytes through follicular aspiration using an 18 gauge needle attached to a 10 ml disposable syringe. Total 2983 follicles were aspirated, of which 1563 immature eggs or oocytes were retrieved giving 52.39 per cent retrieval success.

Aziz *et al.* (2012) recovered immature buffalo oocytes through follicular aspiration and they obtained 2.11 average oocytes per ovary.

Mehmood *et al.* (2011) compared oocyte recovery methods *i.e.* aspiration vs. slicing in buffaloes. They recorded better ($P<0.05$) COCs recovery with the slicing method (2.2 COCs/ovary) than with aspiration (0.9 COCs/ovary).

Masudul Hoque *et al.* (2011) obtained significantly higher ($P<0.01$) number of COCs/ovary in puncture (4.22) and slicing (4.14) followed by aspiration (3.28) technique in goat.

Khandoker *et al.* (2011) obtained buffalo follicular oocytes from left and right ovaries by aspiration method and reported that the collected COCs were higher in left ovaries (2.42 ± 0.14 per ovary) compared to right ovaries (2.32 ± 0.12 per ovary).

Mohammed and Hatif (2010) collected bovine ovaries from abattoir and they obtained 71 oocytes from 80 ovaries (0.89 oocyte/ovary) by aspiration of follicles.

Palanisamy *et al.* (2009) found significantly higher ($P<0.01$) average yield of oocytes per ovary by slicing (4.2 ± 0.33) than by follicle aspiration (1.9 ± 0.25) method in buffalo.

Mistry and Dhami (2009) studied follicular size and oocytes recovery rate from buffalo ovaries by slicing method and concluded that slicing of the ovarian follicle was a convenient and effective method for collecting a high yield of buffalo follicular oocytes for *in vitro* maturation and embryo production.

Kalleshwarappa *et al.* (2009) collected buffalo ovaries from slaughterhouse and oocytes were aspirated with 18-gauge needle from all visible follicles. 6362 ovaries were used for the study and 4999 (78.6%) oocytes were harvested with 0.8 oocytes recovered per ovary.

Nandhi and Kumar (2008) utilized aspiration method for oocyte collection in buffaloes and reported significantly higher oocytes/ovary (3.4 ± 0.8 vs 1.8 ± 0.7) in breeding seasons than in low or non-breeding seasons.

Jamil *et al.* (2008) evaluated the comparative efficacy of oocyte collection methods on the recovery rate of oocytes in buffaloes. They reported significantly ($P<0.05$) higher number of oocytes recovered/ovary in dissection (2.31) than puncture (1.46) and aspiration (1.21) methods.

Das and Santra (2008) recovered 586 oocytes from 417 cattle ovaries (0.71 oocyte/ovary) by three different methods. They found 1.36, 1.04 and 1.75 oocytes/ovary in aspiration, puncturing and slicing methods respectively.

Arangasamy *et al.* (2008) retrieved buffalo oocytes by aspiration of 2-8 mm diameter follicles that appeared on the surface of ovaries and recovered 244 (67.97%) culturable and 129 non-culturable oocytes. The total number of oocytes and the number of culturable oocytes recovered per ovary were 0.74 and 0.50respectively.

Wang *et al.* (2007) studied the influence of the oocyte collection methods (slicing, puncture, aspiration I and II) on oocyte recovery efficiency in Holstein cow. In the slicing method, the whole ovary was chopped into small pieces with a surgical

blade. In the puncture method, the whole ovarian surface was punctured by 18G needle. In other 2 aspiration methods, collected oocytes by aspirating from the visible follicles using an 18-g needle attached to a 5 ml syringe (aspiration I) or using a constant negative pressure (-80 mmHg) with a vacuum pump (aspiration II). They found that slicing (9.6) and puncture (9.7) yielded a larger number of oocytes per ovary than other two aspiration methods (aspiration I and II were 5.8 and 5.6respectively) ($P<0.05$).

Singh and Dhanda (2007) collected buffalo ovaries of unknown reproductive status from abattoir and subjected to mechanical aspiration of oocytes. The average oocyte recovery per ovary was 1.90 ± 0.17.

Leal *et al.* (2007) recovered the Cumulus-Oocyte Complexes (COCs) by aspiration of 2 to 8 mm follicles from buffalo ovaries and found 4.24 average recovery of total oocytes per ovary.

Hussain *et al.* (2005) collected cattle and buffalo oocytes by slicing method to study the effect of season on average number of culturable oocytes recovered from cattle and buffalo ovaries. In their experiment, the average number of good quality cumulus oocyte complexes recovered in cattle and buffalo were 3.9 ± 0.21 (654 no. of oocytes recovered from 172 ovaries sliced) and 1.9 ± 0.14 (272 no. of oocytes recovered from 134 ovaries sliced) per ovaryrespectively. The number of COCs recovered in different seasons were significantly ($P<0.05$) lower in summer in cattle (3.41 ± 0.15 vs 4.4 ± 0.07) and buffalo (1.6 ± 0.16 vs 2.2 ± 0.1).

Sianturi *et al.* (2002) recovered significantly more oocytes per ovary by slicing technique than that of by aspiration (29.38 vs 12.02 oocytes/ovary; $P<0.01$) from cow ovaries.

Raza *et al.* (2001) recovered follicular oocytes by aspiration and scoring methods from Nili-Ravi buffaloes. In aspiration method ovarian follicles were aspirated from ovaries with an 18-guage needle fitted with a 10 mL syringe. While, in

scoring method the surface of ovaries was scored with a sterile surgical blade, with instant rinsing and tapping the ovary to release oocytes in a sterile petri-dish. They found that the scoring method was an appropriate method for high recovery of good quality oocytes per ovary as it yielded 3.85 oocytes per ovary than aspiration method (1.76 oocytes per ovary).

Wani *et al.* (2000) recovered significantly ($P<0.05$) higher average number of sheep oocytes per ovary by puncture (9.5 ± 0.45) and slicing (9.5 ± 0.40) than by the aspiration method (6.8 ± 0.30).

Wani *et al.* (1999) noted that the puncture of whole ovarian surface by an 18-gauge hypodermic needle is more efficient than the slicing method, as the later method produced more debris thus, hindering in the identification of sheep oocytes.

Samad and Raza (1999) obtained 5.02 ± 0.66 total oocytes per ovary in normal breeding season and 4.86 ± 0.88 total oocytes per ovary in low breeding season in buffaloes by scoring method. Statistically the difference was non-significant.

Datta and Goswami (1998) found that the total number of oocytes recovered per ovary was significantly ($P<0.01$) lower using aspiration method than slicing and dissection methods in buffalo, but processing of aspiration required less time than those of slicing and dissection methods.

Cruz (1998) reported that average yield of immature cumulus oocytes complexes was 1.33 per ovary using *in vivo* aspiration with ultrasound guided needle in non super-ovulated buffalo.

Kumar *et al.* (1997) found that the mean number of buffalo oocytes recovered by slicing was significantly higher (6.25/ovary) than that recovered by follicle puncture (3.1/ovary) or aspiration (2.35/ovary).

Das *et al.* (1996) compared efficacy of three methods for oocytes collection from

buffalo ovaries. Oocytes were collected by slicing (n=131), follicle puncture (n=86) and follicle aspiration (n=80). Slicing yielded significantly more oocytes (5.7/ovary) than follicle puncture (2.6/ovary) or aspiration (1.7/ovary).

Totey *et al.* (1992) reported that overall yield of the oocytes recovered by aspiration of buffalo ovaries was quite low compared with that in cattle. The maximum number of usable oocytes recovered per ovary using follicles of 2-6 mm diameter was 0.4. Average number of total oocytes per ovary was 0.7 and 40.8 per cent of the oocytes (0.3 per ovary) were atretic.

Suzuki *et al.* (1992) obtained 536 oocytes from 518 follicles of 2-4 mm diameter, obtaining 1.3 oocytes/ovary by aspiration method in buffalo.

2.3 Grading of oocytes

El-Naby *et al.* (2013) evaluated Egyptian buffalo oocytes morphologically according to the criteria of cumulus investment and classified into four classes (Grade-A; completely invested with cumulus cell layers, Grade-B; partially invested with cumulus cells, Grade-C; denuded oocytes and Grade-D; degenerated oocytes). The ovarian samples collected during summer months were characterized by a significantly ($P<0.05$) higher percent of degenerated oocytes (class D) and lower number of good quality oocytes than those collected during spring months (19.43 ± 3.12 vs. 10.28 ± 1.94 and 72.77 ± 2.28 vs. 81.69 ± 2.95, respectively). In addition, they found 16.02 ± 1.20 per cent degenerated and 73.59 ± 1.41 per cent good quality oocytes when CL was present on ovaries, while the corresponding figures when CL was absent were 21.91 ± 3.84 per cent and 68.29 ± 1.96 per cent respectively.

Singh *et al.* (2012) studied different grades of oocytes recovered by slicing method in buffaloes and found significantly higher ($P<0.05$) mean numbers of oocytes per ovary of grade A (2.92 ± 0.14) and grade B (2.49 ± 0.13) followed by grade C (1.66 ± 0.09) than that of grade D (1.32 ± 0.10) oocytes.

Shanthi *et al.* (2012) collected 708 oocytes from 120 sheep ovaries using slicing technique with a mean recovery rate of 5.9 oocytes per ovary. The mean number of A, B and C grade oocytes retrieved by slicing technique were 47.17 ± 1.64, 54.17 ± 1.85 and 6.67 ± 0.61, respectively. There was significant difference ($P<0.05$) between the groups.

Rao and Mahesh (2012) recorded that out of total buffalo oocytes recovered in three oocyte collection techniques 1.77 ± 0.14 (38.46%), 1.28 ± 0.10 (27.75%), 1.55 ± 0.12 (33.79%) and 3.05 ± 0.23 (66.21%) were of good, fair, poor and culture grade oocytes, respectively. Mean number of good, fair, poor and culture grade oocytes in aspiration technique were 0.84 ± 0.10 (35.08%), 0.77 ± 0.09 (32.46%), 0.77 ± 0.09 (32.46%) and 1.61 ± 0.14 (67.54%), respectively. While, mean number of good, fair, poor and culture grade oocytes in puncture technique were 1.25 ± 0.17 (36.14%), 1.02 ± 0.11 (29.52%), 1.19 ± 0.14 (34.34%) and 2.27 ± 0.22 (65.66%), respectively. Whereas, mean number of good, fair, poor and culture grade oocytes in slicing technique were 3.23 ± 0.30 (40.47%), 2.04 ± 0.23 (25.59%), 2.71 ± 0.27 (33.94%) and 5.27 ± 0.50 (66.06%) respectively.

Makwana *et al.* (2012) reported significantly higher ($P<0.05$) mean number of buffalo oocytes per ovary of grade A (0.82 ± 0.04) and B (0.79 ± 0.04) followed by grade D (0.76 ± 0.05) than grade C (0.64 ± 0.04) oocytes in slicing method.

Kulasekhar *et al.* (2012) graded oocytes collected by different oocyte recovery methods from buffaloes. The yield of grade A oocytes were 53.92, 42.57 and 46.72 in slicing, aspiration and post aspiration slicing, respectively. While, the yield of grade B oocytes were 28.87, 20.92 and 29.10, respectively. The yield of grade C and D oocytes were 15.69 and 9.52; 11.27 and 11.24; and 13.93 and 10.25 in slicing, aspiration and post aspiration slicing methods, respectively. The pooled data revealed that there was significant ($P<0.01$) difference between the grades of oocytes where higher per cent of grade A oocytes were retrieved followed by

grade B, C, and D.

Chandrahasan *et al.* (2012) compared aspiration, puncture and dissection methods for quality of oocytes collected in each method in buffaloes. Mean number of first quality oocyte recovery was 1.42, 1.67 and 3.30 in aspiration, puncture and dissection methods, respectively. The number of oocytes in second and third quality was 0.87, 1.13, 1.02 and 0.42, 0.28 and 1.37 by aspiration, puncture and dissection methods, respectively. Though the percentage of first quality oocyte did not differ in all the three methods, the third quality oocytes were higher in dissection method than in other two methods.

Mohammed Abd-Allah (2011) isolated oocytes by puncturing the antral follicles (2-8 mm) using an 18-gauge needle. The released immature buffalo oocytes were scored for granulose-oocyte cell adhesion as: C+ for granulose-enclosed oocytes, C+/- for partially granulose-enclosed oocytes, (whenever there were granulosa cell-free regions on the oocyte surface), C- for granulose free oocytes. The total numbers of recovered immature oocytes from fresh ovaries were 500. Among them 350 (70%) were scored as C+; 110 (22%) were scored as C+/- and 40 (8%) were scored as C-.

Mehmood *et al.* (2011) studied the conditions for *in vitro* maturation (IVM) of primary oocytes without conventionally used fetal calf serum and hormones in order to reduce the cost of laboratory produced buffalo embryos. Comparisons were made between oocyte recovery methods (aspiration vs. slicing) and IVM in medium 199 (static culture method vs. flux culture method) supplemented with 106 granulosa cells/ml that contained either estrus buffalo serum (EBS) or fetal calf serum (FCS). Recovery methods were compared according to yield, i.e. cumulus oocyte complexes per ovary (COCs/ovary), the expansion rate (% of COCs that expanded), and nuclear maturation rate (% of germinal vesicle breakdown [GVBD]), following IVM for 2224 h. *In vitro* maturation methods

(static culture with EBS or FCS and Flux culture with EBS or FCS) were compared on the basis of the expansion rate and *in vitro* fertilization rate (cleavage rate). COC recovery with the slicing method (2.2 COCs/ovary) was better ($P<0.05$) than with aspiration (0.9 COCs/ovary). However, the IVM rate was better ($P<0.05$) based on expansion (86% vs. 63%) and GVBD (85% vs. 62%) with aspiration than with the slicing method. The cleavage rate (37%) was significantly better with the static culture containing EBS than with the static culture with FCS or the flux culture with either EBS or FCS. It was concluded that aspiration of oocytes and subsequent IVM with static culture containing EBS would be a potential method to reduce the cost of laboratory produced buffalo embryos.

Masudul Hoque *et al.* (2011) obtained significantly higher ($P<0.01$) number of abnormal COCs/ovary in puncture (2.38) and slicing (2.22) followed by aspiration (0.80) technique in goat. In contrast, the number of normal COCs/ovary was significantly higher ($P<0.01$) in aspiration (2.48) followed by slicing (1.91) and puncture (1.85) techniques.

Khandoker *et al.* (2011) collected COCs by aspiration of follicles from buffalo ovaries and classified them as normal and abnormal groups. They found the highest numbers of normal COCs per ovary from left ovary (1.54 ± 0.12) than from right (1.33 ± 0.11) ovary. While, the highest numbers of abnormal COCs per ovary were found in right (0.98 ± 0.11) than that of left (0.88 ± 0.10) ovary.

Francesco *et al.* (2011) stated that at Italian latitudes, buffalo *(Bubalusbubalis)* is a seasonally polyestrous species, showing an improved reproductive efficiency when daylight decreases (autumn). The aim of his study was to evaluate the influence of the season on buffalo oocyte recovery rate, on oocyte quality, assessed on morphological basis, and developmental competence after *in vitro* fertilization. For this purpose, buffalo ovaries were collected from a local abattoir

and the oocytes obtained by aspirating the follicles were evaluated, classified and if considered of good quality, devolved to the different procedures of IVEP. In general, no differences were found in terms of oocyte recovery per ovary among seasons, but interestingly, the percentage of small oocytes was higher ($P<0.05$) during spring and summer (0.9±0.1 and 0.9±0.2) compared to autumn and winter (0.3±0.1 and 0.2±0.1). Both cleavage and embryo rate increased during the period from October to December (71.7±3.1 and 26.5±2.1 respectively) compared to the period from April to June (58.0±2.4 and 18.8±1.6 respectively), thus reflecting the *in vivo* reproductive behavior. Nevertheless, it is worth emphasizing that transferrable embryos were produced *in vitro*, even during the unfavorable season, but with decreased efficiency. They suggested to avoid the oocyte collection during spring when planning OPU trials in order to save resources and improve the benefits/costs ratio.

Sadhan *et al.* (2010) made an attempt to find out the effect of season on oocyte maturation and embryo development in buffalo during low breeding season when environmental temperature was high. Oocytes were isolated from oocytes brought from local slaughter house during different months, viz. March (group 1), April (group 2) and May (group 3), when the environmental temperature was high. Isolated oocytes during different months were matured in TCM-199 with 10% FBS supplemented with follicular fluid and FSH (0.5 μg/ml). Matured oocytes were fertilized by co incubating capacitated frozen thawed buffalo semen. After co incubation the oocytes were cultured and cleavage rate and embryo development was noted. A total number of 1340 buffalo ovaries were collected from local slaughterhouse, from which 1080 culturable cumulus oocytes complexes (COCs) were recovered. The overall recovery rate of buffalo oocytes was 0.85 oocyte/ovary. The maturation rate of oocytes was 68.00 to 87.57%. The cleavage rate as well as morula stage embryo development in March (group 2), April (group 2) and May (group 3) were of 8.27 and 2.5, 5.6 and 3.1, 8.18 and 5.4%,

respectively. The average cleavage rate and morula stage embryo development were 9.60% and 4.60%.

Leal *et al.* (2010) collected ovaries of 86 buffaloes and 95 cows from slaughterhouses and transported to the laboratory in saline solution at 36°C. The cumulus-oocyte complexes (COCs) were recovered by follicular aspiration, and only grades I and II COCs were selected and matured in TCM-199 supplemented with 10% fetal calf serum, sodium pyruvate, LH, FSH, estradiol, gentamicin and cysteamine for 22-24 h. A total of 714 and 1983 COCs were recovered from buffaloes and cows, respectively. In the buffaloes, the recovery rates of each COC categories (grade I: 25.9%, grade II: 30.7%, grade III: 10.2%, denuded: 18.6% and expanded: 14.6%) were lower than in cows (31.8, 30.6, 15.4, 4.5 and 17.7%, respectively) according to Mann-Whitney test ($p<0.05$). The percentage of bubaline oocytes that reached metaphase II (63.4% - 242/396) was lower than that of bovine oocytes (67.8% - 696/1234) under the same laboratory conditions. These differences observed in all the analyses indicated that each species had peculiar physiological characteristics.

Das and Santra (2010) aspirated cattle follicular oocytes from apparently non-atretic surface follicle (3 to 8 mm diameter) with a 19 gauge hypodermic needle attached to a 5 ml disposable plastic syringe containing oocyte collection medium and categorized into A grade (COC with > 5 layers of cumulus cells), B grade (COC with 3-5 layers of cumulus cells), C grade (COC with < 3 layers of cumulus cells) and D grade (COC with partial layer of cumulus cells). Out of 407 total oocytes, 89 oocytes were graded as A (21.86%), 95 as graded B (23.34%), 100 as graded C (24.57) and 123 as graded D (30.22%).

Palanisamy *et al.* (2009) reported higher recovery rate of culturable buffalo oocytes (A and B grade) by slicing method (2.4 ± 0.36) than by aspiration method (0.8 ± 0.16).

Mistry and Dhami (2009) obtained a total of 456 ovaries of Surti buffaloes from the local slaughter house and were transferred within 12 hours to the laboratory in normal saline at 30-35°C. The ovaries selected were without functional corpus luteum. An average number of follicles of small, medium and large size found per ovary were 0.82, 0.48 and 0.24 respectively, with an overall mean of 1.55. The distribution of small, medium and large follicles was found to be 53.18, 31.12 and 15.70% respectively. The slicing method of oocyte recovery employed yielded a total of 1409 oocytes from these ovaries, with an average recovery rate of 3.09 oocytes per ovary. The average recovery of grade A, grade B and grade C oocytes was 1.02, 1.22 and 0.85 respectively. The study demonstrated that slicing of the ovarian surface follicle is a convenient and effective method for collecting a high yield of buffalo follicular oocytes for *in vitro* maturation and embryo production.

Manjunatha *et al.* (2009) carried out a study to examine the effect of season on *in vivo* oocyte recovery and embryo production in non-descriptive, Indian River buffaloes *(Bubalus bubalis).* Ovum pick up (OPU) was conducted twice a week for 8 weeks during peak (October-March) and low (April-September) breeding season in live buffaloes (n=6). The recovered oocytes were graded and only grade A and grade B oocytes were used for *in vitro* production (IVP) of embryos. The mean number of follicles observed per animal per session did not differed ($P<0.05$) between animals or between puncture sessions in both low and peak breeding seasons. Higher ($P<0.05$) number of follicles were observed (4.8+/-0.2 versus 3.1+/-0.3) and punctured (4.0+/-0.2 versus 2.4+/-0.2) during peak breeding season when compared to low breeding season. Oocytes recovered (1.6+/-0.1 versus 1.0+/-0.3) per animal per session were higher ($P<0.05$) in peak breeding season than low breeding season. During the peak breeding season, the blastocyst yield per animal per session (0.3+/-0.4 versus 0.18+/-0.4) was higher ($P<0.05$) than the low breeding season. However, season did not significantly affect the percentage of oocytes suitable for IVP (grade A+B) and blastocyst production rate.

They concluded that the efficiency of OPU combined with IVP was higher during the peak breeding season than the low breeding season in buffaloes.

Kalleshwarappa *et al.* (2009) aspirated buffalo oocytes with 18-gauge needle from all visible follicles. They obtained 2153 (43.0%) morphologically good quality, healthy, evenly granulated cytoplasm, cumulus enclosed oocyte complexes of culturable oocytes (0.4 culturable oocytes per ovary).

Nandhi and Kumar (2008) utilized aspiration method for oocyte collection in buffaloes and reported significantly higher oocyte recovery rate of good (1.5 ± 0.6 vs 0.7 ± 0.6) oocytes/ovary in breeding season than in low or non-breeding season. However, no significant differences in recovery rates of average (1.0 ± 0.3 vs 0.5 ± 0.2) oocytes/ovary and poor (0.9 ± 0.3 vs 0.6 ± 0.2) oocytes/ovary were observed in breeding and low or non-breeding seasons. Further, the recovery rate of good (0.3 ± 0.1 vs 1.3 ± 0.4) oocytes/ovary was significantly lower from ovaries without dominant follicles than that with dominant follicles. Similarly, recovery rate of poor (0.6 ± 0.2 vs 0.2 ± 0.1) oocytes/ovary was significantly higher from ovaries with dominant follicles than that without dominant follicles. However, no significant difference in recovery rates of average (0.5 ± 0.2 vs 0.5 ± 0.1) oocytes/ovary was observed from ovaries with and without dominant follicles.

Jamil *et al.* (2008) conducted studies to evaluate the comparative efficacy of oocyte collection methods, season (low and peak breeding season), and ovarian status (presence or absence of corpus luteum) on the recovery rate and quality of recovered buffalo follicular oocytes. Results revealed that 675, 441 and 363 oocytes were recovered by dissection, puncture and aspiration. A significantly higher ($P<0.05$) oocyte recovery rate was obtained from the ovaries collected during the peak-breeding season and from those without corpus luteum. Dissection method yielded the highest percentage (36.74%) of type 1 oocytes, followed by puncture (32.87%), and aspiration (19.83%) methods. They concluded that the

method of oocyte collection, season and ovarian status at the time of collection significantly affect the recovery of usable buffalo oocytes for *in vitro* fertilization program.

Das and Santra (2008) recovered cattle oocytes by three different methods. The A, B, C and D grades of oocytes recovered in aspiration method were 20.69, 25.00, 32.76 and 21.55%; in puncturing method were 27.93, 25.23, 31.53 and 15.32%; in slicing method were 23.05, 27.16, 30.45 and 42.34%, respectively. In the puncturing method, highest percentage of A grade oocytes were recovered but in slicing method highest percentage of B grade oocytes were recovered. In aspiration method, maximum number of C grade oocytes were recovered, which is not significantly different from the other 2 methods. The recovery rate of denuded oocytes (D grade) was the highest in slicing method. Based on findings, they concluded that the slicing of ovary is comparatively the best method for oocyte recovery from cattle ovaries followed by aspiration and puncturing methods.

Wang *et al.* (2007) reported that the number of the highest quality oocytes (grade A) per ovary was significantly higher in slicing (4.2) and puncture (4.6) methods than in other methods (aspiration I and II were 1.2 and 1.4, respectively) ($P<0.05$) in Holstein cow.

Singh and Dhanda (2007) collected buffalo oocytes by mechanical aspiration. Further shorting and scanning of oocytes yielded 53.47 ± 5.96 per cent grade-I oocytes and 46.52 ± 5.96 per cent grade-II oocytes.

Yadav (2005) reported the mean oocyte recovery from buffalo ovaries by aspiration of surface follicles to be 2.41±0.29 whereas, the culturable grade oocyte recovery in the study was 2.10±0.21.

Das *et al.* (2005a) studied the oocyte recovery rate per ovary to assess the oocyte potential in buffalo ovaries using aspiration techniques. A total of 1137 ovaries were collected during 13 yielded 923 oocytes aspirated from surface follicles,

which were further classified into A and B type. It was shown that the overall oocyte recovery and recovery of type A and type B oocytes were 0.81, 0.43 and 0.37 per ovary respectively. The maximum recovery rate was in the winter months and was lower in the summer period due to the seasonal impact on the reproductive physiology of the animals.

Rahman *et al.* (2003) collected bovine oocytes by slicing techniques from ovaries which were categorized in to Type-I, having functional corpus luteum (CL); type-II, CL in almost regressed condition and type-III without CL. The Cumulus-Oocyte-Complexes (COCs) collected from each follicle further classified into 4 grades. The average number of grade-A COCs was 1.71, 2.85 and 3.57 for type-I, type-II and type-III respectively. The average number of grade-B COCs was 0.71, 1.42 and 1.85 respectively. The average number of grade-C COCs was 0.42, 0.57 and 0.28 respectively. The average number of grade D COCs was 1.28, 0.42 and 1.71 respectively. Significantly higher ($P<0.01$) number of grade-A and B COCs were obtained from type-III ovaries. The number of grade C COCs did not vary significantly ($P>0.01$) among the type. Grade-D COCs was significantly ($P<0.01$) higher in number in type-III ovaries as an exception of the usual expectation.

Gupta *et al.* (2003) classified the oocytes retrieved from abattoir derived buffalo ovaries into three categories i.e. ovaries with corpus luteum (CL), ovaries without CL and pooled ovaries. Correlation coefficient was calculated between the ovarian weights and the oocyte recovery rates for all the three categories of ovaries. Ovarian weight of ovary with CL was significantly more than that of ovary without CL. There was a positive correlation between the ovarian weights and the oocyte recovery rates in all the three categories of ovaries.

Sianturi *et al.* (2002) recorded non significant difference between the percentage of acceptable bovine oocytes (A and B oocytes) of total oocytes, obtained from aspiration (27.71% and 40.66%) and slicing (33.8% and 34.02%) method.

Raghu *et al.* (2002a) collected oocytes from ovaries of buffaloes of different ages (prepubertal, adult and aged), cultured and inseminated *in vitro* with 9-10 million sperm/ml in BO media. The maturation rate,cleavagerate and embryo development were analysed by chi-square test. The recovery and maturation rates of oocytes from ovaries of prepubertal and adult buffaloes were significantly higher than the oocytes from aged buffaloes. The cleavage rate and embryo development were significantly higher in oocytes from ovaries of adult buffaloes than ovaries from prepubertal and aged buffaloes.

Naik *et al.* (2002) co-related the oocyte diameters with the follicular size and the quality of oocytes recovered from buffalo ovaries. The oocyte diameter collected from follicle size 3-5mm and >5 mm were 152.24μm and 159.6μm. The percentage of culturable oocytes obtained from >5mm follicle size were higher as compared to follicle size of 3-5 mm. A positive co-relationship was observed between the oocytes diameter, follicle size and culturable oocytes recovered from buffalo ovarian follicles.

Gupta and Sarma (2002) conducted studies to assess the recovery of oocytes from buffalo ovaries collected from the local abattoir in Bangalore, Karnataka, India during breeding (April to September) and non-breeding (October to March) seasons. 22 buffalo ovaries were collected from April 1997 to March 1998. The ovarian weight, number of follicles, and number of corpora lutea were recorded. Oocytes were retrieved from the ovaries by aspiration and slicing. No significant differences ($P>0.05$) in the ovarian weight, number of follicles, number of corpora lutea, and number of oocytes recovered were observed between the two seasons.

Wani *et al.* (2000) found higher percentage of good quality sheep oocytes in the aspiration method (64.4%) compared to the puncture (54.7%) or slicing (54.3%) methods Abdoon and Kandil (2001) found that slicing of buffalo ovaries produced a higher number of fair, poor and denuded oocytes than aspiration method.

Samad and Raza (1999) reported that the ovaries with CL yielded significantly lower ($P<0.05$) good quality oocytes (A, B and C grade) per ovary (2.85 ± 0.35) than ovaries without CL (4.52 ± 0.52) in buffaloes with scoring method.

Das *et al.* (1996) recovered better quality oocytes (good and fair) by slicing (2.6/ovary) than by puncture (1.3/ovary) or aspiration (0.9/ovary) from buffalo ovaries. However, follicle puncture took the least time (1.5 min) compared with aspiration (1.6 min) and slicing (2.8 min). In their study slicing was found to be the overall best oocyte collection method among the three tested.

CHAPTER III

MATERIALS AND METHODS

The present investigation on "Ovarian categories, follicles and oocytes analysis of buffalo in view of *in vitro* production of embryos" was conducted at the Animal Nutrition, Genetics and Breeding Laboratory at Sher-e-Bangla Agricultural University, Dhaka-1207. In this chapter a short description of the location of the experimental, climatic condition, materials used for experiment, design and methods of the experiment, method of data collection and statistical analysis have been presented.

3.1 Geographical location of research laboratory

Experimental laboratory was located at 90°22/ E longitude and 23°41/ N latitude and altitude of 8.2 m above the sea level.

3.2 Collaboration

The present work was conducted in collaboration with Municipal slaughter house of Dhaka city, Laboratory of Animal Nutrition Genetics and Breeding, Sher-e-Bangla Agricultural University Farm, Dhaka-1207, Bangladesh.

3.3 Chemicals and culture media

All media and chemicals from M/S Sigma Aldrich Chemicals Co. (St. Lois, MO, USA) and Himedia Company were used unless otherwise mentioned. 0.22 μm, 0.45 μm filters, disposable petridishes (large 90 mm, small 35 mm diameter) and 15 ml graduated tubes of Axiva brand were used.

3.4 Design of the study

3.4.1 Working environment

All the procedures were carried out in highly sterile condition under laminar air flow cabinet to avoid any bacterial and fungal contaminations.

3.4.2 Sterilization procedure

All the glassware was sterilized by hot air oven for at least one hour at 160°C. The culture plates, culture bottles, petri dishes, centrifuge tubes, syringes were exposed to ultra violet light for at least 15 minutes before use.

3.4.3 Preparation of different media

The stock solutions used in the study were prepared by using milli Q water (Integral 5, Millipore). All the working solutions/media excluding Oocyte Collection Medium (OCM) were kept for 3-4 hours in CO_2 incubator for quenching before use. The stock of all media were stored at 4°C and used within fifteen days of preparations. All the culture media were sterilized by filtration through 0.22 μm filter and stored at 4°C and used within one week.

3.5 Collection and Processing of ovaries

Buffalo ovaries of unknown reproductive history were collected from local slaughterhouse. The ovaries were kept in collection vial containing 0.9% physiological saline (5 lac IU. of penicillin and 100 mg of streptomycin were added per liter of saline on the day of collection) in a thermo flask at 25-30^{o}C and transported to the laboratory within 4-5 hours. The ovaries were then transferred in the sterilized petridishes containing same saline and rinsed thoroughly by physiological solution at room temperature and marked as right and left ovary (right and left ovary was tagged during slaughter with the help of butcher). The presence or absence of corpus luteum (CL) was also recorded.

3.6 Evaluation of ovary

3.6.1 Measurement of length, width and weight

The length and width of ovaries (right and left ovaries; ovaries with CL and ovaries without CL) were measured with the help of a slide calipers and expressed in cm (Plate 1 and 2). Weight of individual ovary was measured by placing them on a digital balance and was recorded in a tabular form (Plate 3).

Plate 1. Measurement of length of ovary

Plate 2. Measurement of width of ovary

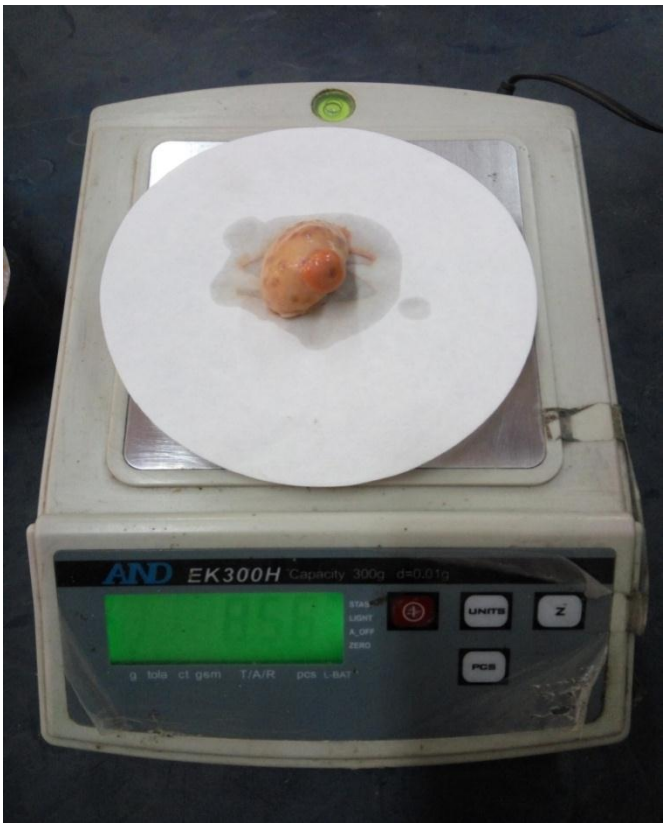


Plate3. Measurement of weight of ovary

3.6.2 Follicles counting on the surface of the ovary

There are many follicles on the surface of both ovaries. The number of visible follicles on the surface of different category of ovaries were counted and recorded accordingly. The highest count of visible follicles was 8 in number where the lowest was 2.

3.6.3 COCs aspiration and grading

The ovaries were washed 2-3 times in saline solution at room temperature. They were then placed in a beaker and kept in a water bath at 30^0C. One ovary was picked up in hand. The 10 ml syringe was loaded with 1.0-1.5ml of PBS (Sigma,

USA) and the needle (18G) was put in the ovary parenchyma near the vesicular follicles of 2-6 mm diameter and all follicles were aspirated near the point. After aspirating the follicles from one ovary, the aspirated follicular materials were transferred slowly into a 90-mm petridishes, avoiding damage to the cumulus cells and the COCs were searched and graded under microscope at low magnification. The COCs were then classified into 4 grades (Plate 4 and 5) according to the slight modification of the method of Khandoker *et al*. (2001), where grade A: oocytes completely surrounded by cumulus cells; grade B: oocytes partially surrounded by cumulus cells, grade C: oocytes not surrounded by cumulus cells and grade D: degeneration observed both in oocytes and cumulus cells. Grade A and B were considered as normal COCs and grade C and D as abnormal. In the mean time another petridishes of D-PBS was prepared for pooling COCs and the COCs were picked up with an appropriate glass micropipette. The tip diameter of the pipette was checked under the microscope to ensure COCs, which could be easily aspirated without damaging the cumulus cells. Then the COCs were washed 2-3 times into D-PBS.

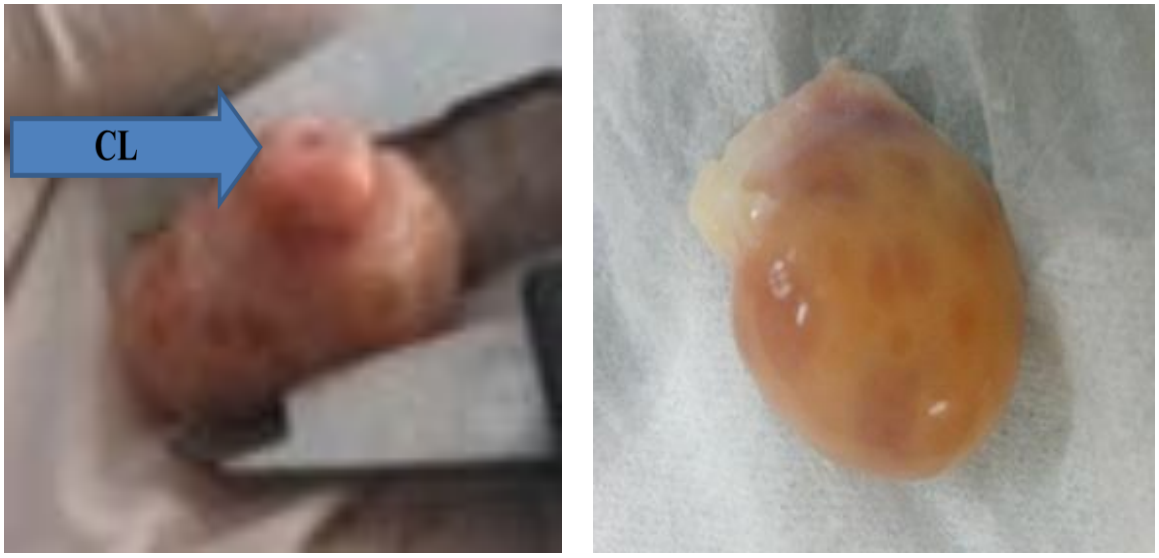
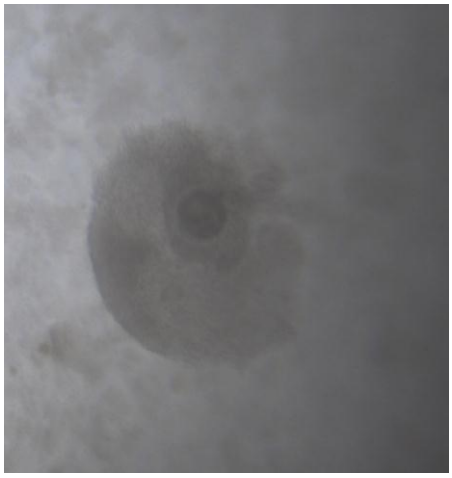
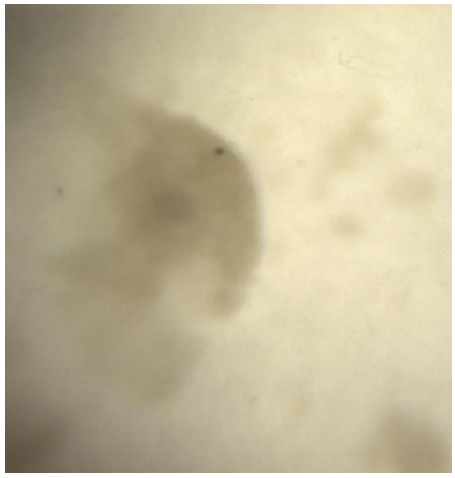


Plate 4: Ovary with CL and without CL

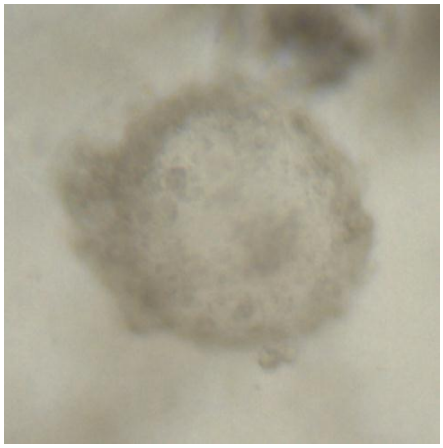


Grade-A



Grade-B

Plate 5. Normal COCs with Grade-A and Grade-B



Grade-C

Grade-D

Plate 6. Abnormal COCs with Grade-C and Grade-D

3.7 Statistical analysis

The data pertaining to various aspects with and without CL *viz*., ovarian weights, follicular counts, oocyte retrieval rate, oocyte recovery rate; with different oocytes collection techniques *viz*., oocyte retrieval rate, oocyte recovery rate; grading of oocytes were suitably tabulated and analyzed using SAS statistics software. The differences among the parameter means were performed using DNMRT. (Duncan's New Multiple Range Test).

3.8 Precautionary measures

Following precautionary measures were adopted during the course of the study:

1. Sterile techniques were used during handling of media.
2. All bench tops and working surfaces were sterilized with ethyl alcohol.
3. All the glass ware, plastic ware and media used were sterile.
4. The glass ware, plastic wares and other instruments employed in this experiment were not used in any other experiment.
5. The petridishes were places in the deeper portion of the incubator to reduce exposure to change in temperature and gas concentration.
6. The pipette tips were always filled and emptied at least once with the medium.
7. When transferring oocytes or embryos from one medium to another, the oocytes/embryos were transferred with the minimum possible medium.

CHAPTER IV

RESULTS AND DISCUSSION

The present work on ovarian categories, follicles and oocytes analysis of buffalo in view of *in vitro* production of embryos was conducted at the Department of Animal Nutrition, Genetics and Breeding, Sher-e-Bangla Agricultural University, Dhaka.The influence of presence or absence of corpus luteum on ovarian size, width, weight, follicular counts, oocyte retrieval rate and oocyte recovery rate was studied.

Buffalo ovaries were collected from different slaughter house of Dhaka city and left and right ovaries were recorded and tagged. The ovaries were then classifiedinto two types, the ovaries without corpus luteum (CL) that is in follicular phase and with CL that is in luteal phase. Among 102 ovaries (51 left and 51 right), CL present 27.12% in right ovaries and 3.24% in left ovaries. The average length, width, weight, number of follicles observed and aspirated and collected COCs from left and right ovaries are summarized in Table 1 to 6.

4.1 Ovarian categories regarding left and right category

4.1.1 With or without corpus luteum (CL)

Significant variation was found on ovary with corpus luteum (CL) or without corpus luteum (CL) in both left and right ovaries (Figure 1). Results showed that the highest percentage of CL (27.12%) was found in right ovary where the lowest percentage of CL (3.24%) was found in left ovary. Similarly, the highest percentage of CL (94.76%) was found in left ovary where the lowest percentage of CL (72.88%) was found in right ovary.

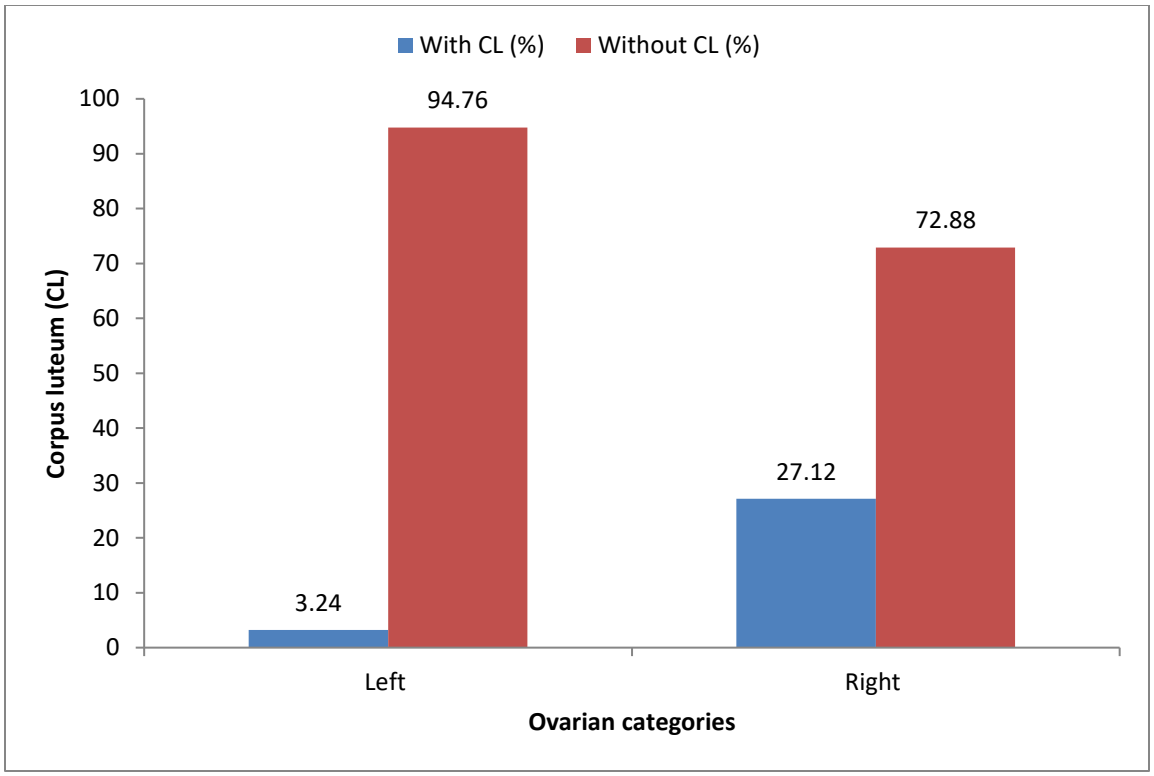


Figure 1. Ovarian categories of buffaloes with or without corpus luteum (CL) on left and right ovaries collected from slaughter house

Follicular growth initiation is one of the most important and least understood aspects of ovarian biology and represents a major challenge for experimental study. Changes in the local microenvironment such as the p^H and hormonal concentration probably occur as the follicles evolve in to the primary stage but these were probably effect rather than causes (Webb *et al.*, 1999). Due to the absence of CL in the without CL group the negative effect of progesterone on anterior pituitary might not be functional in this ovary. So the highest number of COCs in this category other than CL functional group explains the role of hormonal balance on cow folliculogenesis. Within the category, the highest number of normal COCs than that of abnormal COCs further supports the above statement. The CL-absent group ovaries explain the role of progesterone on buffalo follicular degeneration.

4.1.2 Length of ovary (cm)

Among different parameters obtained from different category of ovaries the mean length (cm) was significantly varied between left and right ovaries (Table 1 and Figure 2). The mean length was distinctly higher in case of right ovaries (2.75±0.056) compared to left ovaries (2.27±0.056) which almost similar the previous study Islam *et al.* (2007).

From the collected goat ovaries and categorized as right, left, with corpus luteum (CL) and without CL group. And also categorized on the basis of length (cm), width (cm) and weight (gm). The length (cm) of right ovaries (1.19±0.09) was found significantly ($p<0.05$) higher than left ones (1.15±0.04) which almost similar to the previous study of Asad *et al*. (2016).

4.1.3 Width of ovary (cm)

Width of ovary obtained from different category of ovaries (left and right), the mean width (cm) was significantly varied (Table 1 and figure 2). The highest mean width was obtained in right ovaries (2.17±0.051) where the lowest was found in left ovaries (1.77±0.051).

From collected buffalo ovaries from a slaughter house and transported to the laboratory in saline solution at 36°C. The means of weight, length, width and height of the ovary were 3.83 g (n=84), 2.27 cm (n=84), 1.08 cm (n=84) and 1.56 cm (n=84) respectively which supports the previous study of Leal *et al.* (2007).

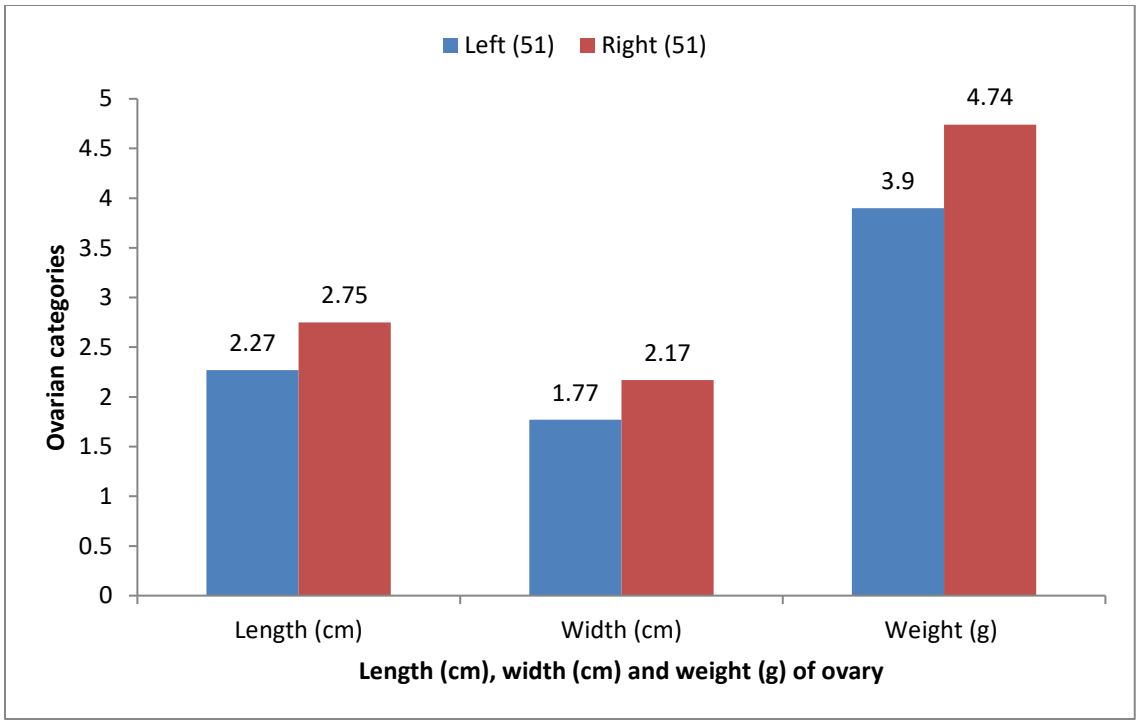


Figure 2. Ovarian categories of buffaloes regarding on length, width and weight of ovary collected from slaughter house

4.1.4 Weight of ovary (g)

Significant variation was found on weight of ovary obtained from different category of ovaries (left and right) (Table 1 and Figure 2). Results indicated that the highest mean weight was observed in right ovaries (4.74±0.13) where the lowest mean weight was found in left ovaries (3.90±0.13).

Study on 50 native buffaloes of Odisha to evaluate ovarian biometry and found non significantly higher average weight (g) of the right ovary (2.36 ± 0.13) than the left ovary (2.17 ± 0.11) was conducted by Patra *et al.* (2013).

Among 136 ovaries (consisting 68 on each side *i.e.* left and right) a number of 93 belonged without CL and others with CL. Non-significant ($P<0.05$) difference was found in the weight of left (2.87 ± 0.32 g) and right ovaries (3.59 ± 0.31 g) was recorded the weight of buffalo ovaries by Khandoker *et al.* (2011).

Table 1. Ovarian categories regarding on length, width and weight of ovary collected from slaughter house

Ovarian categories	Length (cm) (Mean ±SE)	Width (cm) (Mean ±SE)	Weight (g) (Mean ±SE)
Left (51)	$2.27^{b} \pm 0.06$	$1.77^{b} \pm 0.05$	$3.90^{b} \pm 0.13$
Right (51)	$2.75^{a} \pm 0.06$	$2.17^{a} \pm 0.05$	$4.74^{a} \pm 0.13$
CV(%)	16.03	18.33	21.61

Means with different superscripts differ significantly from each other within the same column ($p<0.01$)

4.1.5 Number of follicles

Variation on number of follicles (total and aspirated) was significant in terms of follicles count in left and right ovaries (Table 2 and Figure 3). The highest total follicles in number was observed in left ovary (6.35±0.16) with highest aspirated follicles (5.47±0.16). Again, the lowest total follicles count was found in right ovary (6.16±0.16) with lowest aspirated follicles (4.18±0.16).

The mean count of different sized follicles in right ovary and their total count was slightly higher numerically than the left ovary which was statistically non-significant in buffaloes. The average number of large, medium and small follicles of right ovary was recorded to be 0.70 ± 0.07, 1.60 ± 0.12 and 6.26 ± 0.37 respectively with a total of 8.54 ± 0.42, similarly the left ovary possessed 0.68 ± 0.07, 1.52 ± 0.13 and 6.10 ± 0.32 respectively large, medium and small follicles with a total of 8.30 ± 0.63 was reported from the study of Patra *et al.* (2013).

Table 2. Ovarian categories of buffaloes regarding on number of follicles collected from slaughter house

Ovarian categories	Number of follicles	
	Total (Mean ±SE)	Aspirated (Mean ±SE)
Left	$6.35^{b} \pm 0.16$	$5.47^{b} \pm 0.16$
Right	$6.16^{a} \pm 0.16$	$4.18^{a} \pm 0.16$
CV (%)	26.88	34.61

Means with different superscripts differ significantly from each other within the same column ($p<0.01$)

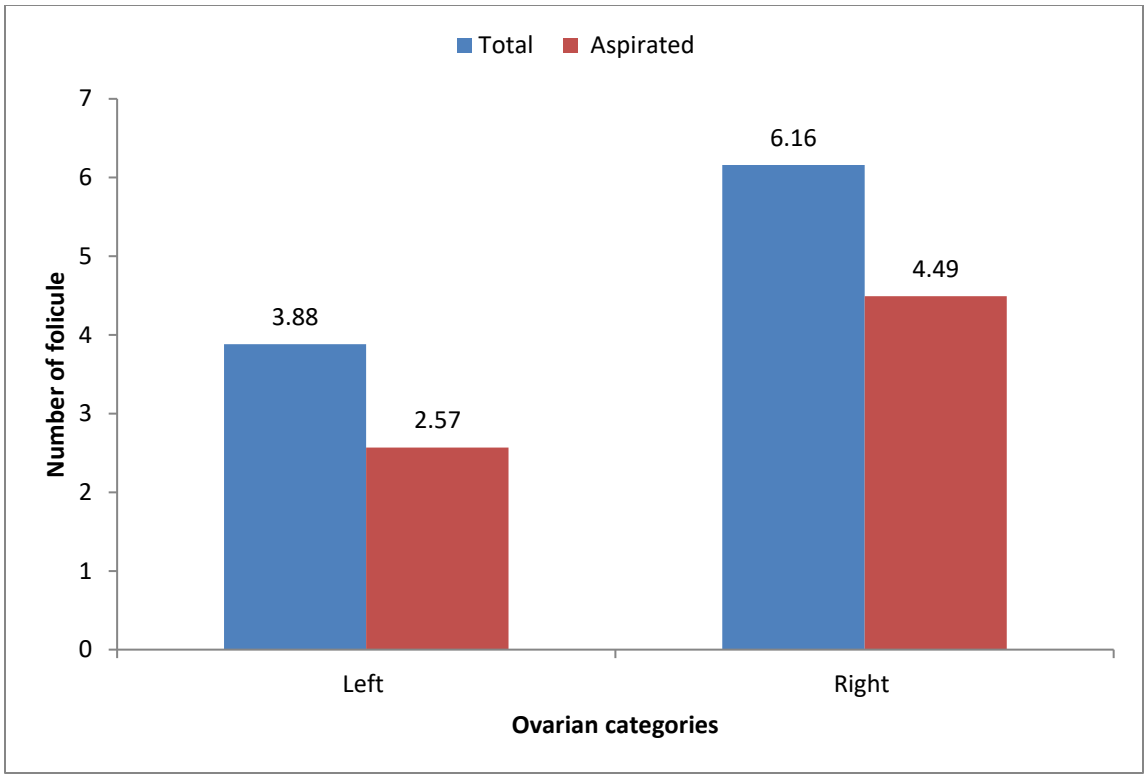


Figure 3. Ovarian categories of buffaloes regarding on number of follicles collected from slaughter house

4.1.6 Grading of COCs

Grading of COC's was done in two types as normal and abnormal. Presence of total COCs with normal and abnormal was significant in left and right ovaries (Table 3 and Figure 4).It was observed that normal COCs was higher than abnormal COCs both in left and right ovaries. Results revealed that the highest normal COCs (0.98±0.13) was found in left ovary where the lowest normal COCs (0.84±0.13) was observed in right ovary. Similarly, the highest abnormal (0.75±0.11) was found in right ovary where the lowest abnormal COCs (0.57±0.11) was observed in left ovary. In terms of total COCs, the highest (1.57±0.17) was found in left ovary where the lowest (1.54±0.17) was observed in right ovary. When the COCs were classified in normal and abnormal groups, the highest numbers of normal COCs were found in left than that of right ovary, which supports the previous result of Islam (2005).

Table 3. Ovarian categories of buffaloes on grading of COCs collected from slaughter house

Ovarian categories	Grading of COCs		
	Normal (Mean ±SE)	**Abnormal (Mean ±SE)**	**Total (Mean ±SE)**
Left	$0.98^{a} \pm 0.134$	$0.57^{b} \pm 0.107$	$1.57^{a} \pm 0.17$
Right	$0.84^{b} \pm 0.134$	$0.75^{a} \pm 0.107$	$1.54^{b} \pm 0.17$
CV (%)	105.04	116.14	77.81

Means with different superscripts differ significantly from each other within the same column ($p<0.01$)

There was significantly higher ($P<0.05$) mean number of buffalo oocytes per ovary of grade A (0.82 ± 0.04) and B (0.79 ± 0.04) followed by grade D (0.76 ± 0.05) than grade C (0.64 ± 0.04) oocytes in slicing method which belongs to the previous study of Makwana *et al.* (2012).

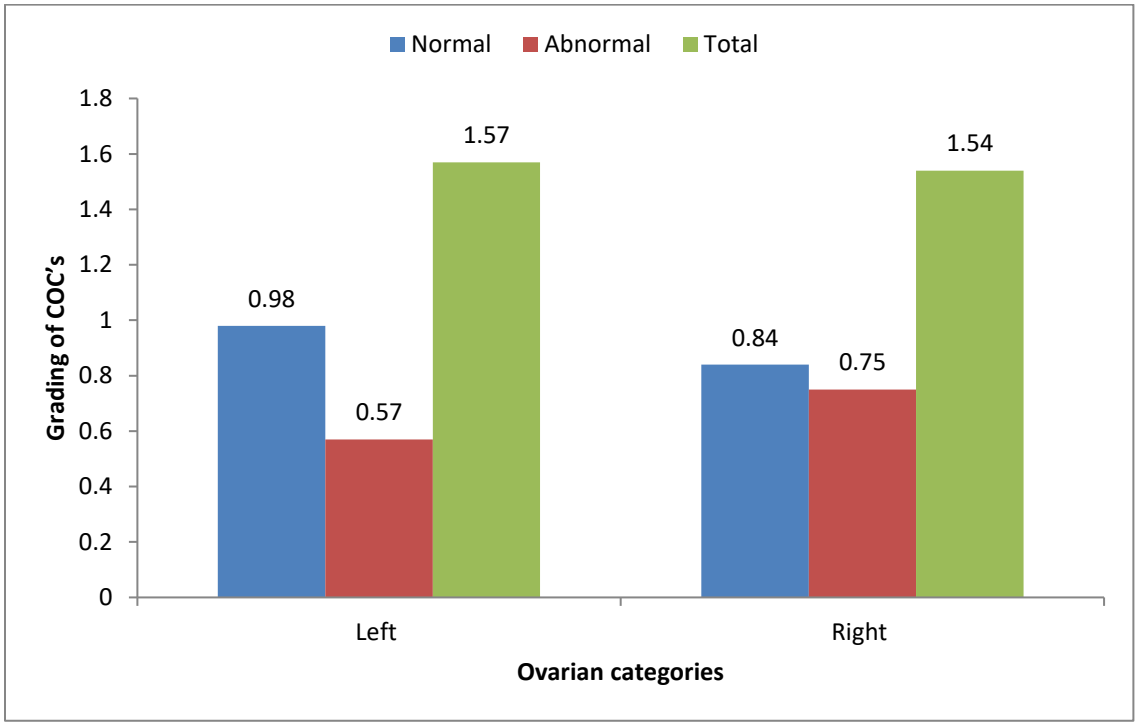


Figure 4. Ovarian categories of buffaloes regarding on grading of COCs collected from slaughter house

4.2 Ovarian categories regarding with CL or without CL

4.2.1 Ovary with CL or without CL

The less number of CL present group ovaries were found in this experiment because less reproductive performer buffaloes are usually slaughtered and most of them might be non-cyclic. So, there had been the possibility to get more non-cyclic ovaries from the slaughterhouse during random sampling. The cause of highest number of follicles found in without CL group ovaries than those of with CL group were not understood well and did not fit the endocrinological explanation.

Significant variation on was found on corpus luteum (CL) in terms of presence or absence (Figure 5). Results showed that the ovary with CL was in 23.5±0.01% where ovary without CL was in 76.47± 0.01%.The causes of higher number of follicles found in ovaries without CL than those of CL containing group were understood well as it fits the endocrinological explanation. Various factors that might influence oocyte recovery revealed that non-luteal phase ovaries yielded significantly higher number of oocytes compared to luteal phase ovaries.

The collected goat ovaries and categorized as right, left, with corpus luteum (CL) and without CL group. When compared the ovaries in between with CL and without CL group, significantly ($p<0.05$) higher number of normal COCs (1.12±0.07) were found in without CL group with an increase of length (1.17±0.01) which was almost similar of the previous study of Asad *et al*. (2016).

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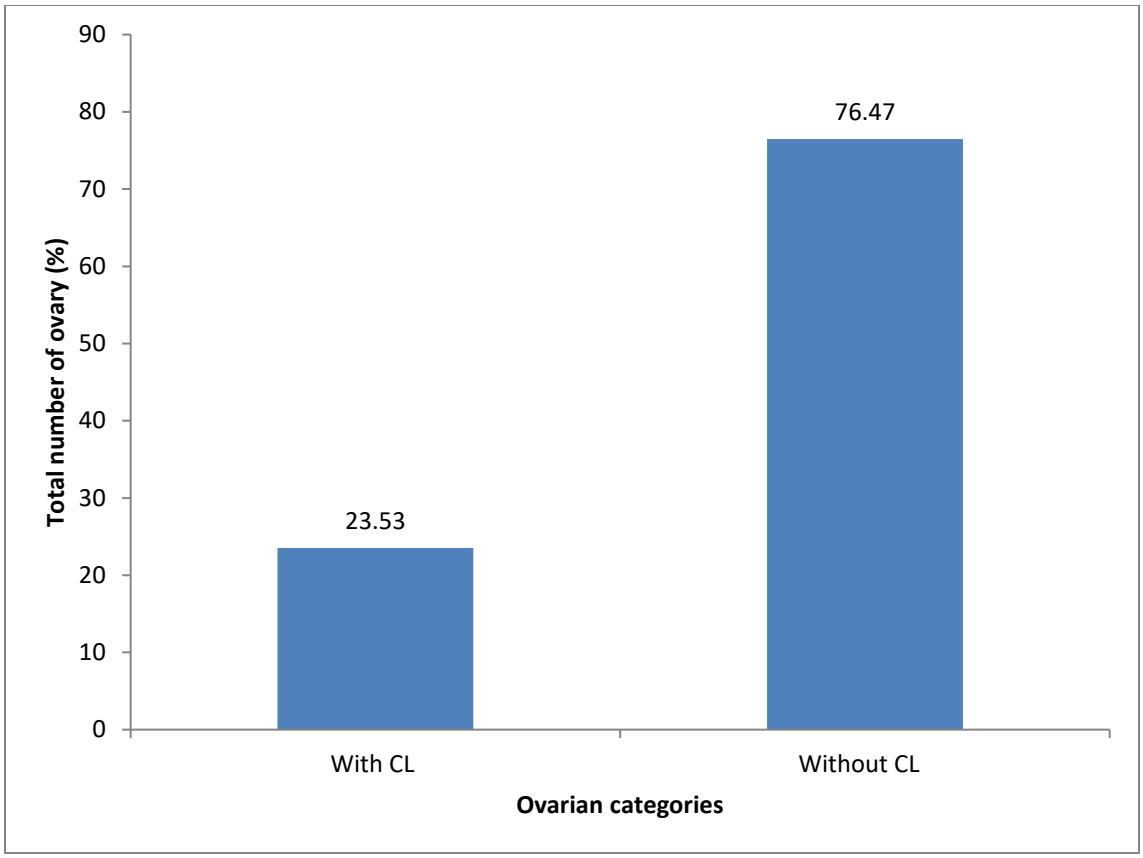

Figure 5. Ovarian categories of buffaloes regarding with CL or without CL collected from slaughter house

4.2.2 Length of ovary (cm)

Among different parameters obtained from different category of ovaries the mean length (cm) was not significantly varied between ovary with CL or ovary without CL (Table 4 and Figure 6). However, the mean length was higher in case of ovary with CL (2.86±0.10) compared to ovary without CL (2.82±0.10).

The ovaries during luteal phase had significantly higher values for weight, volume, length and breadth but not for the thickness. The ovaries in buffaloes aging 3.5 to 7 years were found to be heavier and larger in size than that of the older animals; probably due to the higher ovarian activity in younger animals which found in the previous study of Neelam and Saigal (2005).

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Table 4. Ovarian categories of buffaloes regarding with CL or without CL on length, width and weight of ovary collected from slaughter house

Ovarian categories	Length (cm) (Mean ±SE)	Width (cm) (Mean ±SE)	Weight (g) (Mean ±SE)
With CL	2.86 ±0.10	2.30±0.01	4.73^{a} ± 0.12
Without CL	2.82±0.10	2.30±0.01	4.36^{b} ± 0.12
CV(%)	18.56	8.78	23.54

Means with different superscripts differ significantly from each other within the same column ($p<0.01$)

4.2.3 Width of ovary (cm)

Width of ovary obtained from different category of ovaries (with CL or without CL). The mean width (cm) was not significantly varied (Table 4 and Figure 6). The mean width of both ovary with CL or without CL were same and that was 2.30±0.01 cm.

From a study of collected goat ovaries and categorized as right, left, with corpus luteum (CL) and without CL group. And also categorized on the basis of weight (gm), length (cm) and width (cm). The length (cm) of right ovaries (1.19±0.09) was found significantly ($p<0.05$) higher than left ones (1.15±0.04). Other parameters, including width, weight and total number of COCs aspirated per ovary did not differ significantly ($P<0.05$) between right and left ovaries almost similar to Asad *et al*. (2016).

4.2.4 Weight of ovary (g)

Significant variation was found on weight of ovary obtained from different category of ovaries (with CL or without CL) (Table 4 and Figure 6). Results indicated that the highest mean weight was observed in ovary with CL (4.73±0.12) where the lowest mean weight was found in ovary without CL (4.36±0.12).

Among 136 ovaries (consisting 68 on each side *i.e.* left and right) a number of 93 belonged without CL and others with CL. Non-significant ($P<0.05$) difference was

found in the weight of left (2.87 ± 0.32 g) and right ovaries (3.59 ± 0.31 g). While, the weight was significantly (P<0.05) higher in ovaries with CL (3.64 ± 0.18 g) than those of without CL (2.73 ± 0.12 g) was almost similar to the study of Khandoker *et al.* (2011).

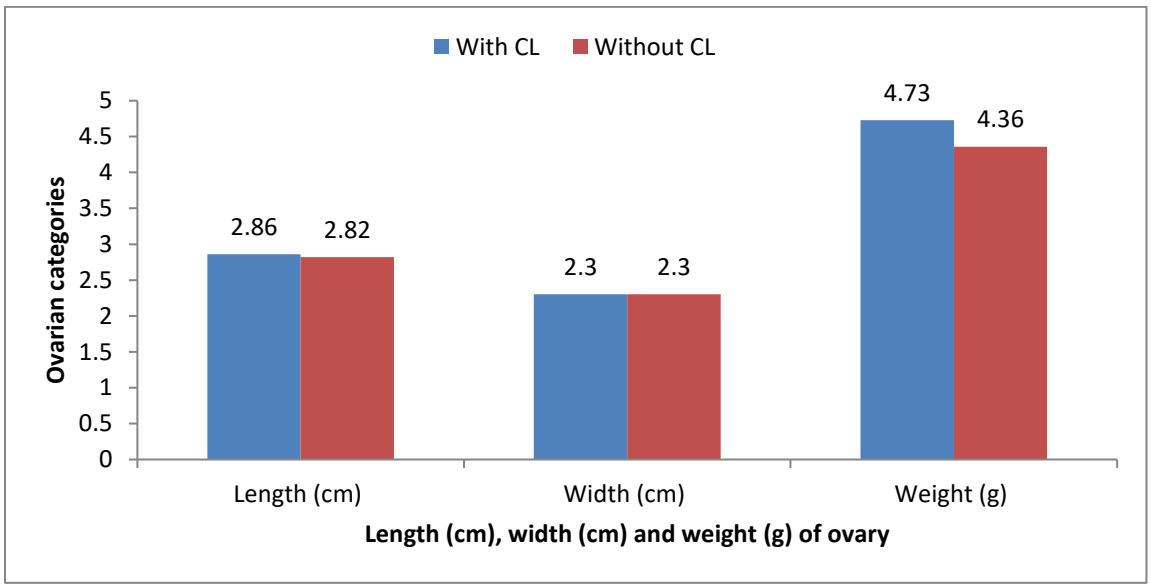

Figure 6. Ovarian categories of buffaloes regarding with CL or without CL on length of ovary collected from slaughter house

4.2.5 Number of follicles

Variation on number of follicles (total and aspirated) was significant in terms of follicles count in with CL or without CL (Table 5 and Figure 7). The highest total follicles in number was observed in without CL ovary (7.60±0.14) with highest aspirated follicles (5.28±0.14). Again, the lowest total follicles count was found in CL present ovary (4.29±0.14) with lowest aspirated follicles (2.29±0.14).

A study was reported that the presence of a CL stimulates the development of significantly higher (P<0.01) number of ovarian follicles which produced a significantly higher (P<0.05) number of good quality oocytes by Abdoon and Kandil (2001).

Table 5. Ovarian categories of different buffaloes regarding with or without CL on number of follicles collected from slaughter house

Ovarian categories	**Number of follicles**	
	Total (Mean ±SE)	**Aspirated (Mean ±SE)**
With CL	$4.29^{b} \pm 0.14$	$2.29^{b} \pm 0.14$
Without CL	$7.60^{a} \pm 0.14$	$5.28^{a} \pm 0.14$
CV(%)	28.27	25.44

Means with different superscripts differ significantly from each other within the same column ($p<0.01$)

Collected cow ovaries immediately after slaughter and divided into three categories based on their cyclic status, which included: the presence of a large follicle (LF), the presence of a corpus luteum (CL) and ovaries without LF or CL (WLCF). The highest average oocytes collected per ovary were related to the CL (22), WLCF (21.4) and LF groups (20.8), respectively found in the previous study of Pirestani *et al.* (2011).

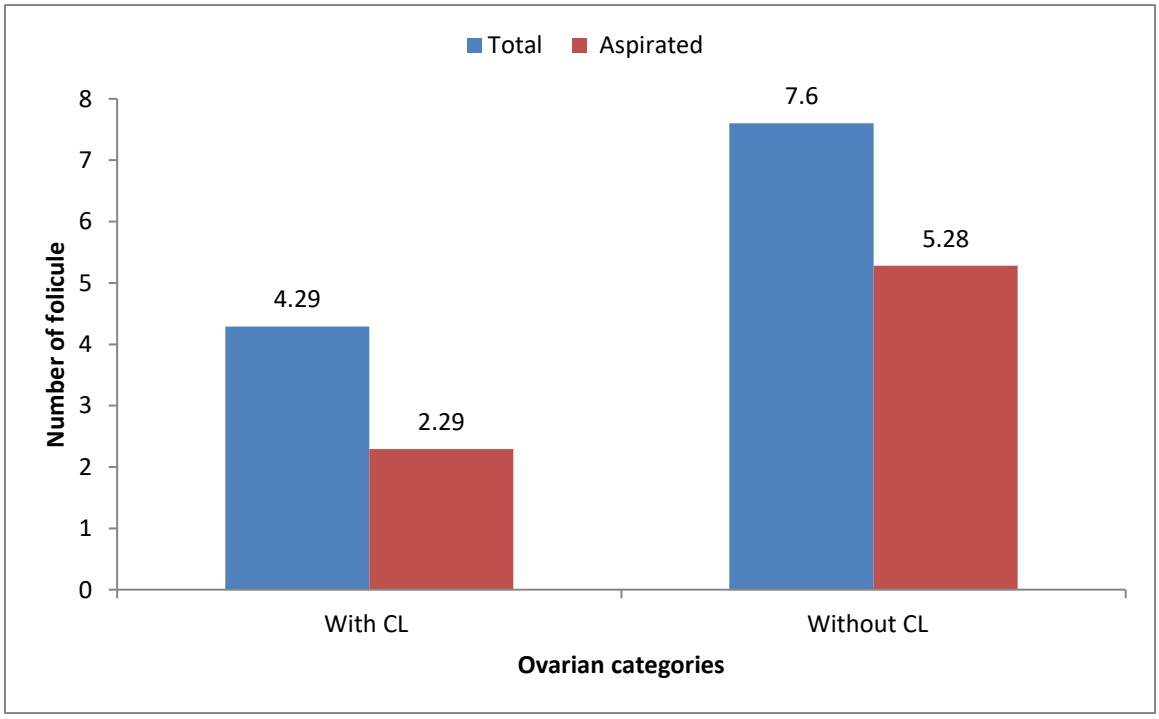

Figure 7. Ovarian categories of buffaloes regarding with CL or without CL on number of follicles collected from slaughter house

It is well established that all female mammals are born with a large store of follicles which rapidly declines as puberty approaches but whether this early losses represent a mechanism of physiological wastage is not definitely known. Follicle growth initiated is one of the most important and least understood aspects of ovarian biology and represents a major challenge for experimental study. Changes in the local micro environment such as the pH and hormonal concentration probably occur as the follicles evolve into the primary stage but these are probably effects rather than causes (Webb *et al*., 1999). Growth initiated of follicles has variously been attributed with hormonal triggers (gonadotropins), chiastic process (fluctuation in the internal signal follicle) and external inhibitory control growing follicles (Webb *et al*., 1999). The balance between the gonadotropins (FSH and LH) and steroid (estrogen and progesterone) might be the important criteria in this process. The highest number of follicles that are found in ovaries without CL in the present study might reflect the optimum level of gonadotropins and steroid. This result was comparable with the observation of Wang *et al*. (2007) who harvested oocytes from ovary by aspiration (2.9) collection techniques. It also supports the findings of Singh *et al*. (2013) in goat.

4.2.6 Grading of COC's

Presence of total COCs with abnormal COCs was significant in with CL or without CL ovaries where normal COCs was not significant (Table 6 and Figure 8). It was observed that normal COCs was lower than abnormal COCs both in CL present or absent ovaries. Results revealed that the highest normal COCs (0.55±0.01) was found in with CL ovary where the lowest normal COCs (0.50±0.01) was observed in with CL ovary. Similarly, the highest total number of COCs (1.41±0.11) was found in without CL ovary where the lowest abnormal COCs (1.17 ±0.11) was observed in with CL ovary.

Table 6. Ovarian categories of buffaloes regarding with CL or without CL on grading of COCs collected from different slaughter house

Ovarian categories	Grading of COCs		
	Normal (Mean ±SE)	Abnormal (Mean ±SE)	Total (Mean ±SE)
With CL	0.50± 0.01	0.54 [b] ± 0.03	1.17 [b] ± 0.11
Without CL	0.55± 0.01	0.86 [a] ± 0.03	1.41[a] ± 0.11
CV (%)	37.55	42.56	52.66

Means with different superscripts differ significantly from each other within the same column ($p<0.01$)

In terms of total COCs, the highest (1.41±0.108) was found in without CL ovary where the lowest (1.17±0.108) was observed in with CL ovary. When the COCs were classified in normal and abnormal groups, the highest numbers of normal COCs were found in without CL ovary than that of with CL ovary. When the COCs were classified in normal and abnormal groups, the highest numbers of normal COCs were found in left than that of right ovary, which almost similar to the previous result of Islam (2005).

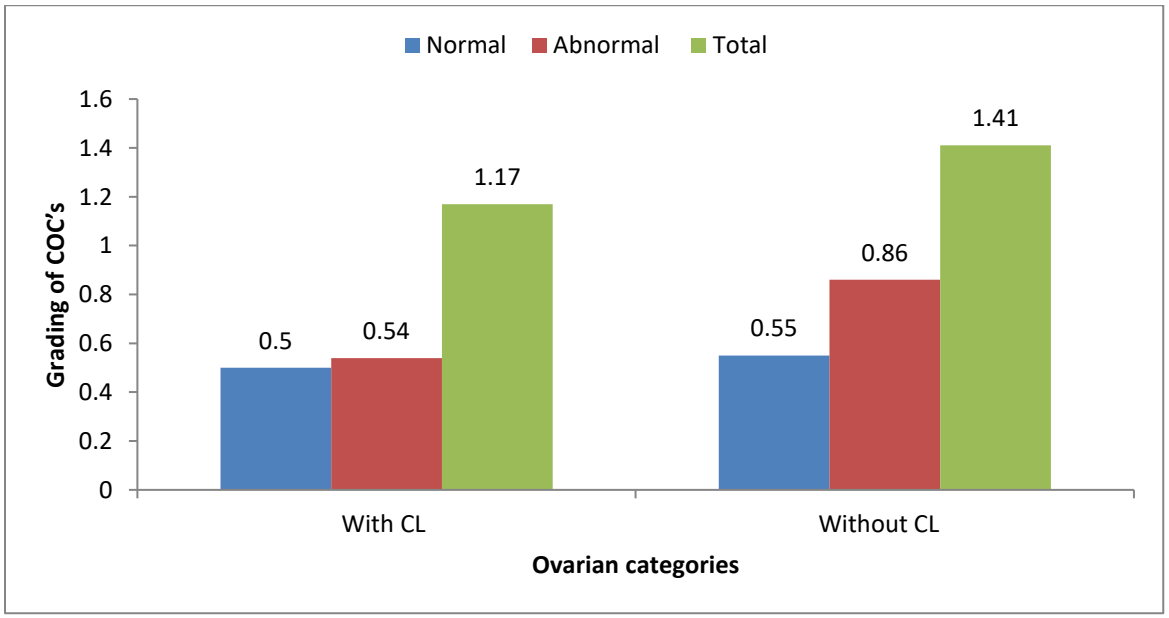

Figure 8. Ovarian categories of buffaloes regarding with CL or without CL on grading of COCs collected from slaughter house

Age, season, nutritional status (body condition) and cyclicity of animals at the time of slaughter, size and functional status of follicles, method of oocyte retrieval has some of the factors that might contribute to be recorded variation in oocyte quality (Nandi *et al*., 2001; Zoheir *et al*., 2007; Amer *et al*., 2008). In terms of quality of oocytes, Ferdous (2006) reported that the numbers of normal COCs were found to be significantly higher ($p < 0.05$) in 2 to 6 mm diameter follicles than others. In the ovaries without CL, the negative effect of progesterone on anterior was not functional in this types of ovaries.

CHAPTER V

SUMMARY AND CONCLUSION

The research was conducted at the Department of Animal Nutrition, Genetics and Breeding, Sher-e-Bangla Agricultural University, Dhaka. It was carried out with a view to ovarian categories, follicles and oocytes analysis of buffalo in view of *in vitro* production of embryos.

Buffalo ovaries were collected from Slaughter house of Dhaka city. During collection left and right ovaries were identified and recorded and after necessary processing the ovaries were categorized as (i) ovaries without corpus luteum (CL) and (ii) ovaries with corpus luteum. Ovaries were then evaluated on the basis of length (cm), width (cm), weight (g), total number of follicles on the surface of each category ovaries, number of follicles aspirated, total number of COCs.

In terms of ovarian categories regarding left and right category, the result obtained from this experiment showed significant ($p<0.01$) difference in the parameters between left and right ovaries. The highest presence of CL (27.12±0.11%) was in right ovary compared to left ovaries (3.24±0.11%). In case of without CL ovary reverse result was found. The length (cm) was distinctly higher in case of right ovaries (2.75±0.05) compared to left ovaries (2.27±0.05).The width (cm) was higher in right ovaries (2.17±0.05) compared to left ovaries (1.77±0.05).The weight (g) was distinctly higher in right ovaries (4.74±0.13) compared to left ovaries (3.90±0.13).The number of follicles (aspirated) was also distinctly higher in case of right ovaries (4.49±0.17) compared to left ovaries (2.57±0.17).

The collected COCs were higher in case of left ovaries (with a mean of 1.57±0.17 per ovary) compared to right ovaries (with a mean of 1.54±0.17 per ovary). When the COCs were classified in normal and abnormal groups, the highest numbers of normal COCs were found in left (0.98±0.13) than that of right (0.84±0.13) ovary.

In case of abnormal COCs reverse result was found.

In terms of ovarian categories regarding with CL or without CL, the result obtained from this experiment showed significant ($p<0.01$) difference in the parameters between with CL and without CL ovaries. Results showed that 76.47±0.01% ovaries showed CL absent where 23.53±0.01% showed CL present.

The length was higher in with CL ovaries (2.86 ±0.103) compared to without CL ovaries (2.82±0.10). The width (cm) was same both in with CL or without CL ovaries (2.30±0.01). In case of mean weight of with CL ovaries showed highest weight (4.73±0.12) compared to without CL ovaries (4.36±0.12). The number of follicles (aspirated) was also distinctly higher in case of without CL ovaries (5.28 ±0.136) compared to with CL ovaries (2.29±0.136).

The collected total COCs were higher in case of without CL ovaries (1.41± 0.11) compared to with CL ovaries (1.17±0.11). When the COCs were classified in normal and abnormal groups, the highest numbers of normal COCs were found in without CL ovary (0.55± 0.01) than that of with CL (0.50± 0.01) ovary. In case of abnormal COCs the highest (0.86 ±0.03) was found in without CL ovaries and the lowest (0.54 ±0.03) was found in with CL ovaries.

Finally, It can be concluded that left ovaries contain more follicles and COCs than right ovaries and also contains more number of normal COCs. Whereas without CL ovaries contain higher number of follicles and normal COCs compared to with CL ovaries. So, higher number of normal COCs found in left and without CL ovaries.

REFERENCES

Abdoon, A.S., Kandil, O.M., Otoi, T. and Suzuki, T. (2001). Influence of oocyte quality, culture media and gonadotropins on cleavage rate and development of *in vitro* fertilized buffalo embryos. *Arch. Tierz. Dummerstorf*.**44**: 157.

Adlak, S.A., Khillare K.P., Pawshe, C.H. and Mude, S.W. (2008b). Influence of commercially available follicle stimulating hormone on *in vitro* maturation of buffalo oocytes. *Buffalo Bull*.**27**:302-304.

Adlak, S.A., Khillare, K.P., Pawshe, C.H. and Mude, S.W. (2008a). Influence of serum and hormones on *in vitro* maturation of buffalo oocytes. *Vet. World*.**1**:243-244.

Adlak, S.A., Khillare, K.P., Pawshe, C.H. and Mude, S.W. (2009). Influence of buffalo oestrus serum (BES) and fetal bovine serum (FBS) in the presence of oestradiol (E2) and FSH on nuclear maturation of buffalo oocytes. *Buffalo Bull*.**28**:25-28.

Amar-Nath, Sharma, V., Dubey, P.K., Pratheesh, M.D., Gade, N.E., Saikumar, G. and Sharma, G.T. (2013). Impact of gonadotropin supplementation on the expression of germ cell marker genes (MATER**,** ZAR1**,** GDF9, and BMP15**)** during *in vitro* maturation of buffalo *(Bubalus bubalis)* oocytes. *In vitro Cell Develop. Biol*.**49**:34-41.

Amer, H.A., Hegab, A.O. and Zaabal, S.M. (2008). Effects of ovarian morphology on oocyte quantity and quality, granulose cells, *in vitro* maturation, and steroid hormone production in buffaloes. *Anim. Reprod*.**5**: 55-56.

Amer, H.A., Hegab, A.O. and Zaabal, S.M. (2008). Effects of ovarian morphology on oocyte quantity and quality, granulosa cells, *in vitro* maturation, and steroid hormone production in buffaloes. *J. Anim. Reprod*.**5**: 55-62.

Arangasamy, A., Singh, L.P. and Chauhan, M.S. (2008). Effect of Heparin and Gelatin binding buffalo seminal plasma proteins on sperm-egg binding assay. *Indian J. Anim.Reprod.* **29**(2): 126-132.

Asad, L., Rahman, A.N.M.I., Hossain, M.M. and Akter, M. (2016). Ovarian category, follicles and oocytes analysis of Goat ovaries in view of *in vitro* production of embryos. *Int. J. Anim. Res*.**1**(1): 27-34.

Asad, L.Y. (2015). Effect of bovine serum albumin and follicular fluid on *in vitro* maturation, fertilization and development of goat embryos. Ph D.

Aziz, F., Mehboob, Z., Jalali, S., Shami, S.A. and Qureshi, I.Z. (2012). Effect of organophosphate pesticide malathion on *in-vitro* oocyte maturation, fertilization and embryo development of Nili Ravi buffalo *(Bubalus bubalis). J. Anim. Plant Sci*.**22(**3 Suppl.): 147-152.

Barakat, I.A.H. and Alhimaidi, A.R. (2012a). Fine structure of Egyptian buffalo oocytes (*Bubalus bubalis*) during different *in vitro* maturation periods using transmission electron microscopy (TEM). *African J. Biotech.***11**:11354-11365.

Barakat, I.A.H., El-Ashmaoui, H.M., Barkawi, A., Kandeal, S.A. and El-Nahass, E. (2012b). Ultrastructural study of Egyptian buffalo oocytes before and after *in vitro* maturation. *African J. Biotech*.**11**:7592-7602.

Barakat, I.A.H., Kandeal, S.A., El-Ashmaoui, H.M., Barkawi, A. and El-Nahass, E. (2012c). Effect of medium type and luteinizing hormone (LH) on *in vitro* maturation of Egyptian buffalo oocytes. *African J. Biotech.***11**:4620-4630.

Barakat, I.A.H., Kandeal, S.A., El-Ashmaoui, H.M., Barkawi, A. and EL-Nahass, E. (2012). Effect of medium type and luteinizing hormone (LH) on *in vitro* maturation of Egyptian buffalo oocytes. *African J. Biotech.***11**(20): 4620-4630.

BBS (Bangladesh Bureau of Statistics). (2018). Statistical Bulletin, Bangladesh. Statistics Division. Ministry of Planning. Government of the Peoples Republic of Bangladesh. Dhaka.

Beheshti, R., Mohammadi-Roshandeh, A., GiasiGhalehkandi, J., Ghaemmaghami, S. and Houshangi, A.F. (2011). Effect of antioxidant supplements on *in vitro* maturation of bovine oocyte. *Adv. Environ. Biol.*,**5**(7): 14731475.

Boonkong, S., Navanukraw, C., Thammasiri, J., Jaikan, W. and Uriyapongson, S. (2012). Effect of culture media on fertilization rate in bovine and caprine embryos production. *Int. J. Environ. Rural Dev*. **3**(2): 56-60.

Cajuday, L.A., Herrera, A.A. and Duran, D.H. (2012). Effect of retinoic acid on the development of water buffalo embryos *in vitro.* Philippine. *J. Vet. Anim. Sci.***38**:107-116.

Chandrahasan, C., Selvaraju, M. and Ravikumar, K. (2012). Different collection techniques for oocytes recovery and their *in vitro* maturation in buffaloes. *Indian Vet. J.***89**(1): 82-84.

Cruz, L.C. (1998). Reproductive biotechniques in water buffaloes. *World Anim. Prod.Conr.*Seol, Korea, Symposium Series No. 1.

Das, B.C., Madan, M.L., Manik, R.S. and Sarkar, M. (2005a). Seasonal impact on per ovarian oocyte retrieval rate in buffalo. *Environ. Ecol.***23**:472-474.

Das, G.K. and Khan, F.A. (2010). Summer anoestrus in buffalo-a review. *Reprod. Domes. Anim*. **45**: 483-494.

Das, G.K., Jain, G.C., Solanki, V.S. and Tripathi, V.N. (1996). Efficacy of various collection methods for oocyte retrieval in buffalo. *Theriogenology,* **46**: 1403-1411.

Das, S.K. and Santra, A. (2008). Comparative efficiency of different oocytes recovery methods from cattle ovaries. *Indian J. Anim. Sci.***78**(3): 277-278.

Das, S.K. and Santra, A. (2010). *In vitro* development of cattle embryos in modular incubator chamber. *Indian Vet. J.***87**(8): 748-750.

Datta, T.K. and Goswami, S.L. (1998). Feasibility of harvesting oocytes from buffalo *(Bubalus Bubalis)* ovaries by different methods. *Buffalo J.***2**: 277-284.

Deneke, Y., Nanda, T. and Yadav, P.S. (2012). Comparative study on the effect of BSA and FCS as a supplement in TCM-199 on the *in vitro* maturation of buffalo oocytes. *Indian J. Anim. Res***.46**:298-301.

Deneke, Y., Nanda, T., Yadav, P.S., Singh, I. and Prasad, G. (2008). Buffalo oocyte vitrification and their post-thaw potential for *in vitro* fertilization. *Indian Vet. J.***85** (8): 816-818.

Devaraj, M., Manjunatha, B.M., Gupta, P.S.P. and Ravindra, J.P. (2007). Effect of cysteamine in the IVM medium on buffalo embryo development. *Proc. XXIII Annual convention of the ISSAR and national symposium*, Veterinary college, Bhubaneswar, Orissa, India, pp. 101 (Abstr.).

Di-Berardino, D. and Iannuzzi, L. (1981). Chromosome banding homologies in Swamp and Murrah buffalo. *J. Hered*.**72**: 183-188.

Di-Francesco, S., Boccia, L., Di Palo, R., Esposito, G., Attanasio, L., De Rosa, A. and Gasparrini, B. (2007). Influence of temperature and time during ovary transportation on *in vitro* embryo production efficiency in the buffalo species *(Bubalus bubalis). Ital. J. Anim. Sci*.**6** (Suppl. 2): 755-758.

El-Naby, Al-shimaa Al-H. H., Mahmoud, Karima G.H.M., Ahmed, Y.F., Abouel-Roos, Mahmoud E.A. and Abdel-Ghaffar, A.E. (2013). Effect of season of

the year and ovarian structures on oocytes recovery rate, quality and meiotic competence in Egyptian buffaloes. *Global Veterinaria,***10** (4): 408-412.

FAO. (2005). Production Year Book. Food and Agricultural Organization of the United Nations Rome, Italy. FAOSTAT statistical database. http://faostat.fao.org.

Faruque, M.O. (2000). Identification of the best genotype of buffalo for dairy purpose in Bangladesh and to improve their productivity. Research report: 1-80.

Faruque, M.O. (2003). Buffalo production system in Bangladesh. Proc. of the Fourth Asian Buffalo Congress, New Delhi, India, 25 to 28 Feb.: 31-35.

Ferdous, J. (2006). Collection, grading and evaluation of goat cumulus oocyte-complexes (COCs) in view of in vitro maturation, fertilization and culture. Master of Science Thesis, Department of Animal Breeding and Genetics. Bangladesh Agricultural University, Mymensingh.

Ferdous, J. (2006): Collection, grading and evaluation of goat cumulus oocyte-complexes (COCs) in view of *in vitro* maturation, fertilization and culture. Master of Science Thesis, Department of Animal Breeding and Genetics. Bangladesh Agricultural University, Mymensingh.

Francesco, S.,Di-Boccia, L., Campanile, G., Di-Palo, R., Vecchio, D., Neglia, G., Zicarelli, L. and Gasparrini, B. (2011). The effect of season on oocyte quality and developmental competence in Italian Mediterranean buffaloes ***(****Bubalus bubalis).Anim. Reprod. Sci*.**123**:48-53.

Freitas, V.J.F. and Melo, L.M. (2010). In vitro embryo production in small ruminants. Revista. Brasileira de Zootecnia 39: 404-413. Genetics, Bangladesh Agricultural University.

Gaspmini, B., Neglia, G., Di Palo, R., Campanile, G. and Zicarelli, L. (2000). Effect of cysteamine during *in vitro* maturation on buffalo embryo development. *Theriogenology*,**54**:1537-l 542.

Gomez, E., Royo, L., Duque, P., Carneiro, G., Hidalgo, C., Goyache, F., Lorenzo, P.L., Alvarez, I., Facal, N. and Diez, C. (2003). 9-cis-Retinoic Acid during *in vitro* maturation improves development of the bovine oocyte and increases midkine but not IGF-I expression in cumulus granulosa cells. *Mol.Reprod. Dev.***66**: 247-255.

Gupta, P.S.P. and Sarma, P.V. (2002). Seasonal variation in the yield of oocytes from buffalo ovaries. *Indian J. Anim. Sci.***72**:1117-1118.

Gupta, P.S.P., Nandi, S., Raghu, H.M., Ravindranatha, B.M. and Sarma, P.V. (2003). Oocyte recovery rates in relation to morphology and weight of the ovaries in buffaloes. *Indian J. Anim. Res.***37** (2): 147 -148.

Gupta, P.S.P., Ramesh, H.S., Nandi, S. and Ravindra, J.P. (2007). Recovery of large preantral follicles from buffalo ovary: Effect of season and corpus luteum. *Anim.Reprod. Sci.***101** (1): 145-152.

Gupta, P.S.P., Ravindra, J.P., Raghu, H.M. and Nandi, S. (2006). Enhancement of *in vitro* maturation rate of oocytes in buffalo with a protein fraction obtained from the ovarian follicular fluid of buffalo. *Indian J. Anim. Sci.***76**:208-213.

Hammam, A.M., Whisnant, C.S., Elias, A., Zaabel, S.M., Hegab, A.O. and Abu-El Naga, E.M. (2010). Effect of media, sera and hormones on *in vitro*

maturation and fertilization of water buffaloes *(Bubalus bubalis). J. Anim. Vet. Adv.***9** (1): 27-31.

Hasanzadeh, S. and Sadeghinejad, J. (2012). Histomorphometry of ovarian follicular growth and atresia in summer and winter seasons in Iranian river buffalo. *Indian J. Anim. Sci.***82** (5): 472-476.

Hoque, S.A.M. (2009). Effect of collection techniques and goat follicular fluid on *in vitro* maturation and fertilization of oocytes. MS Thesis. Dep. Anim. Breed. Genetic. Bangladesh Agricultural Univ. Mymensingh.

Hoque, S.A.M., Khandoker, M.A.M.Y., Kabiraj, S.K., Asad, L.Y., Fakruzzaman, M. and Tareq, K.M.A. (2012). Effect of goat follicular fluid on *in vitro* production of embryos in Black Bengal goats. *J. Appl. Anim. Sci*. **2**: 287-294.

Huque, Q.M.E., Fouzder, S.K. and Islam, R. (2010). Buffalo production system in Bangladesh and its economic return based on input output. Proc. 9thWorl. Buffalo Congr., Argentina. P. 1014-1018.

Hussain, R., Nematullah and Akhter, S. (2005). Effect of season on average number of culturable oocytes recovered from cattle and buffalo ovaries. *Pakistan J. Zool*,**37** (4): 281-283.

Hyttel, P., Fair, T., Callesen, H. and Greve, T. (1997). Oocyte growth, capacitation and final maturation in cattle. *Theriogenogy*. **47**: 23-32.

Islam, M.R., Khandoker, M.A.M.Y., Aforoz, S., Rahman, M.G.M. and Khan, R.I. (2007). Qualitative and quantitative analysis of goat ovaries, follicles and oocytes in view of *in vitro* production of embryos. *J. Zhejiang Univ*. **8**: 465-469.

Jamil H, Samad HA, Qureshi ZI, Rehman N and Lodhi LA (2007). Effect of bull and sperm preparation method on *in vitro* fertilization of buffalo oocytes. *Pak. Vet. J.* ***27***:29-34.

Jamil, H., Samad, H.A., Qureshi, Z.I., Najeeb-ur-Rehman and Lodhi, L.A. (2008). Harvesting and evaluation of riverine buffalo follicular oocytes. *Turkish J. Vet. Anim. Sci.***32**:25-30.

Jamil, H., Samad, H.A., Qureshi, Z.I., Rehman, N. and Lodhi, L.A. (2007a). Effect of bull and sperm preparation method on *in vitro* fertilization of buffalo oocytes. *Pakistan Vet. J.***27** (1): 29-34.

Jamil, H., Samad, H.A., Qureshi, Z.I., Rehman, N.U. and Ladhi, L.A. (2008). Harvesting and evaluation of riverine buffalo follicular oocytes. *Turk. J. Vet. Anim. Sci*. **32**: 25-30.

Jamil, H., Samad, H.A., Rehman, N., Qureshi, Z.I. and Lodhi, L.A. (2011). Effect of somatic cell types and culture medium on *in vitro* maturation, fertilization and early development capability of buffalo oocytes. *Pak. Vet. J.***31** (2): 105-108.

Kachiwal, A.B., Sheikh, B.A., Sheikh, S.A., Qureshi, T.A. and Memon, M.A. (2012). Ultrasonographic biometry of the ovaries of pregnant Kundhi buffaloes. *J. Buffalo Sci.***1** (2): 188-192.

Kalleshwarappa, G.M., Malakar, D., Chauhan, M.S. and Majumdar, A.C. (2009). Isolation, identification and immunogenic characterization of secretory proteins of embryos and in blood circulation during estrus and in early pregnant buffalo. *Indian J. Anim. Sci.***79** (8): 764-772.

Khandoker, M. Reza, M.M.T., Asad, L.Y., Saha, S., Apu, A.S. and Hoque S.A.M. (2011). *In vitro* maturation of buffalo oocytes and fertilization by cattle spermatozoa. *Bang. J. Anim. Sci.***41** (1): 6-12.

Khandoker, M., Jahan, N., Asad, L., Hoque, S., Ahmed, S. and Faruque, M. (2011). Qualitative and quantitative analysis of buffalo ovaries, follicles and oocytes in view of *in vitro* production of embryos. *Bang. J. Anim. Sci*.**409** (1-2): 2327.

Khandoker, M.A.M.Y., Imai, K., Takahashi, T. and Hashizume, K. (2001). The role of gelatinase on follicular atresia in the bovine ovary. *J. Biol Reprod*. **65**: 726-732.

Kulasekhar, T., Solmon Raju, K.G. and Sadasiva Rao, K. (2012). Efficacy of *in-vitro* maturation of bubaline oocytes in TCM 199, TCM 199 + PMSG and follicular fluid. *The Indian J. Anim.Reprod*.**33** (1): 33-36.

Kumar, A., Solanki, V.S., Jindal, S.K., Tripathi, V.N. and Jain, G.C. (1997). Ooocyte retrieval and histological studies of follicular population in buffalo ovaries. *Anim.Reprod. Sci*.**47**: 189-195.

Kumar, D., and Purohit, G.N. (2004). Effect of epidermal and insulin like growth factor-1 on cumulus expansion, nuclear maturation and fertilization of buffalo cumulus oocyte complexes in simple serum free media DMEM and Ham's F-10. *Vet. Arhiv*.**74**:1325.

Kumar, D., Anand, T. and Chauhan, M.S. (2008). *In vitro* development of buffalo embryos using simple media. *The Indian Vet. J*.**85** (8): 819-821.

Kumar, P., Verma, A., Roy, B., Rajput, S., Ojha, S., Anand, S., Yadav, P.,Arora, J., De, S., Goswami, S.L. and Datta, T.K. (2012). Effect of varying glucose concentrations during *in vitro* maturation and embryo culture on efficiency of *in vitro* embryo production in buffalo. *Reprod. Domest.Anim.***47**:269-273.

Leal, L.S., Moya-Araujo, C.F., Fernandes, C.B., Martins, L.R., Landim-Alvarenga, F.C. and Oba, E. (2010). Evaluation of recovery, quality and *in*

vitro nuclear maturation of oocytes obtained from buffalo and bovine ovaries. *Revista Vet.* **21**:892-894.

Leal, L.S., Oba, E., Fernandes, C.B., Moya, C.F., Martins, L.R., Martin, I. and Landim-Alvarenga, F.C. (2007). Ovarian morphometric characterization and *in vitro* maturation of oocytes obtained from buffalo (*Bubalus bubalis*) ovaries - partial results. *Ital. J. Anim. Sci.***6** (Suppl. 2): 804-806.

Lilly-Indira, R., Palanisamy, A., Meenambigai, T.V., Satheshkumar, S., Rangasamy, S. and Kumanan, K. (2010). *In vitro* development of buffalo embryos in different media. *The Indian Vet. J.***87** (11): 1171-1172.

Mahmoud, K.G.M. and Nawito, M.F. (2003). Cytogenetic evaluation of *in vitro* matured buffalo oocytes in different culture conditions. *Egyptian J. Vet. Sci.***37**:105-116.

Makwana, P.M., Shah, R.G., Singh, R.P. and Dhami, A.J. (2012). Influence of different categories of follicles and presence of CL on recovery rate, quality and quantity of buffalo oocytes. *Indian J. Anim.Reprod.***33** (02): 36-40.

Manjunatha, B.M., Devaraj, M., Gupta, P.S.P. and Ravindra, J.P. (2007a). Effect of season on *in vivo* oocyte recovery and *in vitro* embryo production in Indian buffaloes *(Bubalus bubalis). Proc. XXIII Annual convention of the ISSAR and national symposium,* Veterinary college, Bhubaneswar, Orissa, India, pp. 100 (Abstr.).

Manjunatha, B.M., Ravindra, J.P., Gupta, P.S., Devaraj, M. and Nandi, S. (2009). Effect of breeding season on *in vivo* oocyte recovery and embryo production in non-descriptive Indian river buffaloes *(Bubalus bubalis). Anim. Reprod. Sci.***111**:376-383.

Masudul Hoque, S.A., Kabiraj, S.K., Yahia Khandoker, M.A.M., Mondal, A. and Tareq, K.M.A. (2011). Effect of collection techniques on cumulus oocyte

complexes (COCs) recovery, *in vitro* maturation and fertilization of goat oocytes. *African J. Biotech.***10** (45): 9177-9181.

Mehmood, A., Anwar, M., Andrabi, S.M.H., Afzal, M. and Naqvi, S.M.S. (2011). *In vitro* maturation and fertilization of buffalo oocytes: the effect of recovery and maturation methods. *Turk. J. Vet. Anim. Sci.***35** (6): 381-386.

Mishra, A., Chandra, V. and Taru Sharma, G. (2010). Effect of epidermal growth factor on *in vitro* maturation of buffalo oocytes and subsequent development with insulin-like growth factor 1 and B-mercaptoethanol. *Indian J. Anim. Sci*.**80** (8): 721-724

Mistry, C.N. and Dhami, A.J. (2009). Studies on follicular size and oocytes recovery rate from buffalo ovaries by slicing method. *Indian J. Field Vet*.**5** (2): 23-26.

Mistry, C.N., Dhami, A.J., Suthar, V.S., Rank, D.N. and Joshi, C.G. (2010). Effect of different concentrations of bovine serum albumin on *in vitro* maturation of buffalo oocytes. *Indian J. Anim. Reprod*. **31**:75-78.

Mohammed, S.H. and Hatif, S.A. (2010). Bovine oocyte maturation collected from abattoir and *in vitro* fertilization. *Al-Anbar J. Vet. Sci*.**3** (2): 84-88.

Mohammed-Abd-Allah, S. (2009). *In vitro* production of buffalo embryos from stepwise vitrified immature oocytes. *Vet.Italiana*,**45** (3): 425-429.

Mohammed-Abd-Allah, S. (2011). Rescue of oocytes from early antral follicles isolated from cryopreserved buffalo ovaries using an *in situ* oocyte cryopreservation method: competence to undergo maturation, fertilization and development *in vitro. Buffalo Bul*.**30** (1): 45-54.

Mondal, M.A., Khandoker, M.A.M.Y., Mondal, M.A., Rahman, A.H.M.S., Apu, A.S. and Pervage, S. (2008). *In vitro* production of goat embryos in Bangladesh. *Bangladesh. J. Anim. Sci*. **37**: 1-9.

Naik, V.S., Deopurkar, V.L. and Gulavane, S.U. (2002). Micrometry of buffalo oocytes recovered from different size of ovarian follicles. *Indian J. Anim. Reprod*.**23**:63-64.

Nandhi, S. and Kumar, V.G. (2008). Effect of breeding seasons and presence of a dominant follicle on quality of oocytes. *The Indian Vet. J*.**85** (3): 270-271.

Nandi, S., Chauhan, M.S. and Palta, P. (2001). Effect of environmental temperature on quality and developmental competence *in vitro* of buffalo oocytes. *Vet. Rec*.**148**:278-279.

Nandi, S., Chauhan, M.S. and Palta, P. (2001): Effect of environmental temperature on quality and developmental competence *in vitro* of buffalo oocytes. *J. Vet. Res*.**148**: 278-279.

Nandi, S., Gupta, P.S.P., Ravindranatha, B.M. and Sarma, P.V. (2003). Use of cystic follicular fluid for buffalo oocyte maturation *in vitro***.** *Indian J. Anim. Res*.**37**:40-43.

Nasiri, Y. and Beheshti, R. (2012). Effect of p-mercaptoethanol on *in vitro* maturation on oocyte of murrah buffalo. *Res. Opinions Anim. Vet. Sci*.**2**:377-380.

Neelam, B. and Saigal, R.P. (2005). Gross morphological studies on the ovaries of Indian buffalo *(Bubalus bubalis). J. Res*.**42** (2): 224- 227.

Palanisamy, A.,Rangasamy, S.,Satheshkumar, S. and Kumanan, K. (2009). Effect of cysteamine supplementation in semi defined media on *in vitro* production of buffalo embryos. *The Indian J. Anim.Reprod*.**30** (2): 36-38.

Pankaja, H., Kumar, V.G. and Nandi, S (2010). Effect of different media on buffalo oocyte maturation. *Indian Vet.J.***87**:886-889.

Patra, B.K., Mohanty, D.N., Das, S., Das, R.K., Panda, S.K. and Sahoo, L. (2013). Biometrical indices of ovary and surface follicular distribution of native swamp buffaloes of coastal Odisha. *The Indian J. Vet. Res.***22** (1): 67-69.

Pawshe, C.H., Kale, S.R., Ingawale, M.V. and Deshmukh, S.G. (2010). *In vitro* maturation and fertilization of denuded buffalo oocytes with cumulus cell monolayers. *Buffalo Bul.***29** (4): 256-269.

Pirestani, A., Hosseini, S.M., Hajian, M., Forouzanfar, M., Moulavi, F., Abedi, P., Gourabi, H., Shahverdi, A., TaqiDizaj, A.V. and Nasr Esfahani, M.H. (2011). Effect of ovarian cyclic status on *in vitro* embryo production in Cattle. *Int.J. Fert.Steril.***4** (4): 172-175.

Prasad, C.S., Palanisamy, A., Gomathy, V.S., Satheshkumar, S., Thangavel, A. and Raj, G.D. (2013). Effect of TCM-199 and synthetic oviductal fluid (SOF) medium and cysteamine supplementation to *in vitro* maturation media on maturation, cleavage rate and subsequent embryonic development of buffalo oocytes. *Buffalo Bul.***32** (3): 182-188.

Quintana, M.D., Campos, P.E.C., Herrera, P., Gallego, C. and Padron, E. (2012). Comparison of two recollection methods of immature oocytes for *in vitro* fertilization IVF derived from *Bubalus bubalis* females sent to slaughter. *Rev. Salud. Anim.***34** (1): 53-56.

Raghu, H.M., Nandi, S. and Reddy, S.M. (2002b). Effect of insulin, transferrin, selenium and epidermal growth factor on development of buffalo oocytes to the blastocyst stage *in vitro* serum-free semi defined media. *Vet.Rec.***151**:260-265.

Raghu, H.M., Nandi, S., Ravindranatha, B.M. and Reddy, SM. (2002a). Effect of age of donor buffalo on the recovery rate and developmental potential of buffalo oocytes *in vitro***.** *Indian J. Anim. Sci.***72**:586-587.

Rahman, M.G.M., Goswami, P.C., Khandoker, M.A.M.Y., Tareq, K.M.A. and Ali, S.Z. (2003): Collection and grading of bovine cumulus-oocyte-complexes (COCs) from slaughterhouse ovaries in Bangladesh. *Pakistan* J. *Biol. Sci*. **6**: 2054-2057.

Rahman, M.G.M., Goswami, P.C., Yahia Khndoker, M.A.M., Tareq, K.M.A. and Ali, S.Z. (2003). Collection of bovine cumulus-oocyte-complexes (COCs) from slaughter house ovaries in Bangladesh. *Pakistan J. Biol. Sci*.**6**(24): 2054-2057.

Rao, M.M. and Mahesh, Y.U. (2012). Efficacy of different harvesting techniques on oocyte retrieval from buffalo ovaries. *Buffalo Bul*.**31**(4): 209-213.

Ravindranatha, B.M., Nandi, S., Gupta, P.S.P and Sarma, P.V. (2001). Comparison of three different media on maturation of buffalo oocytes *invitro. Indian J. Anim. Sci.***71**:841-843.

Ravindranatha, B.M., Nandi, S., Gupta, P.S.P. and Sarma, P.V. (2002). *In vitro* effects of different levels of commercially available PMSG on buffalo oocyte maturation. *Buffalo J.***18**:101-107.

Raza, A., Samad, H.A., Rehman, N.U. and Zia, E.U.H. (2001). Studies on in vitro maturation and fertilization of Nili-Ravi buffalo follicular oocytes. *Int. J. Agri. Biol*. **3**: 503-506.

Raza, A., Samad, H.A., Rehman, N.U. and Zia, E.U.H. (2001). Studies on *in vitro* maturation and fertilization of Nili-Ravi buffalo follicular oocytes. *Int.J. Agric. Biol*.**3** (4): 503-506.

Reena, D., Balasubramanian, S. and Devanathan, T.G. (2012). *In vitro* production of parthenogenetic embryos by chemical activation in buffaloes (*Bubalus bubalis). The Indian Vet. J.***89*** (3): 12-13.

Reena, D., Devanathan, T.G. and Balasubramanian, S. (2007). Influence of Folligon on *in vitro* maturation of buffalo oocytes. *Indian Vet. J.***84**:264-266.

Sabasthin, A., Nandi, S., Kumar, V.G., Shankare Gowda, P.S.U. and Murthy, V.C. (2013). Effect of sera of normal cycling, pregnant and repeat breeding buffaloes (*Bubalus bubalis*) on *in vitro* maturation of buffalo, sheep and goat oocytes. *Asian Pacific J.Reprod.***2** (2): 110-113.

Sadhan, B., Mehre, P.V. and Majumdar, A.C. (2010). Effect of season on oocyte maturation and embryo development in buffalo. *Indian J. Anim. Sci.***80**:618-620.

Saha, S., Khandoker, M.A.M.Y., Asad, L.Y., Reza, A.M.M.T. and Hoque, A. (2014). Effect of fresh and frozen semen on in vitro fertilization and subsequent development of goat embryos. *Iran. J. Appl. Anim.Sci.* 4: 325-330.

Saini, M.S. and Dhanda, O.P. (2008). Development of *in-vitro* produced Caprine embryos in various culture systems. *Indian J. Anim. Res,***42** (2): 122-124.

Samad, H.A. and Raza, A. (1999). Factors affecting recovery of buffalo follicular oocytes. *Pakistan Vet. J.***19** (2): 56-59.

Sekhar, T.K., Raju, K.G.S. and Rao, K.S. (2012). Efficacy of *in-vitro* maturation of bubaline oocytes in TCM 199, TCM 199+PMSG and follicular fluid. *Indian J.Anim. Reprod.***33**:33-36.

Shang, J.H., Huang, Y.J., Zhang, X.F., Huang, F.X. and Qin, J. (2007). Effect of P- mercaptoethanol and buffalo follicular fluid on fertilization and subsequent embryonic development of water buffalo *(Bubalus bubalis)* oocytes derived from *in vitro* maturation. *Ital. J. Anim. Sci.***6** (Suppl. 2): 751-754.

Shanthi, G., Reena, D., Balasubramanian, S., Rangasamy, S. and Veerapandian, C. (2012). Selection of sheep oocytes using brilliant cresyl blue test. *The Indian Vet. J.***89** (6): 26-27.

Sianturi, R.G. (2001). In vitro production of embryos from abattoir-derived cattle oocytes. Master of Science Thesis. Universiti Putra Malaysia.

Sianturi, R.G., Thein, M., Wahid, H. and Rosnina, Y. (2002). Effect of collection technique on yield of bovine oocytes and the development potential of oocytes from different grades of oocytes. *JITV*.**7**(3): 188-193.

Simon, L., Reena, D., Rangasamy, S., Manokaran, S. and Veerapandian, C. (2003). Meiotic maturation of buffalo oocytes in defined *in vitro* conditions. *Buffalo J*.**21**:259-267.

Singh, L.R., Rao, K.S. and Mohan.K,M, (2013): Oocyte Retrieval Methods, Grade and Percentage of Oocytes in Goats. *Int. J. Mol. Vet. Res*. **3**(2):4- 6.

Singh, R.P., Shah, R.G. and Tank, P.H. (2012). Influence of different quality of buffalo oocytes on *in vitro* maturation and fertilization. *Indian J. Anim. Reprod*.**33**:9-13.

Singh, R.P., Shah, R.G. and Tank, P.H. (2012). Influence of different quality of buffalo oocytes on *in-vitro* maturation and fertilization. *The Indian J. Anim. Reprod*.**33** (1): 09-13.

Singh, S. and Dhanda, O.P. (2007). *In vitro* ovum pick-up from slaughtered buffaloes ovaries using mechanical device. *Indian Buffalo J.*,**5** (1 & 2): 46-48.

Singh, S., Dhanda, O.P. and Malik, R.K. (2001). Effect of the presence of corpus luteum on oocyte recovery and subsequent *in vitro* maturation and fertilization in buffaloes. *Asian-Australian J. Anim. Sci.*,**14**(12): 1675-1677.

Singh, V. and Mishra, A.K. (2007). Effect of growth factors on *in vitro* maturation and *in vitro* culture of buffalo oocytes. *Proc. XXIII Annual convention of the ISSAR and national symposium*, Veterinary college, Bhubaneswar, Orissa, India, pp. 103-104 (Abstr.).

Sirard, M.A. and Blondin, P. (1996). Oocyte maturation and IVF in cattle. *Anim. Reprod. Sci*. **42**: 417-426.

Soren, S., Chakravarty, P., Dutta, A., Sarmah, B.C., Dutta, D.J., Sinha, S. and Bhuyan, S. (2013). Production of embryos following *in vitro* fertilization of oocytes recovered from slaughterhouse ovaries of indigenous cattle. *The Indian Vet. J.*,**90** (3): 108-109.

Sreenivas, D., Kaladhar, D.S.V.G.K., Yarla, N.S., Thomas, V.M., PalniSamy, A. Vadlapudi, V.R. and Preethi, R. (2014).In vitro production of sheep embryos in CR1aa medium supplemented with L-Ascorbic Acid. *J Tissue Sci Eng*. **5**: 131.

Suchitra, B.R., Nandi, S., Gupta, P.S.P., Honnappa, T.G. and Ravindra, J.P. (2012). Influence of cadmium on buffalo oocyte and embryo development *in vitro. Indian J. Anim. Sci*.**82**:292-293.

Sudano, M.J., Mattos, M.C.C., Fernandes, C.B., Mazieiro, R.R. and Landim-Alvarenga, F.C. (2010). *In vitro* production of bovine embryos using Sigma

antioxidant supplement®, a tocopherol and L-ascorbic acid. *Anim. Reprod.***7** (1): 42-48.

Suzuki, T., Singla, S.K., Sujata, J. and Madan, M.L. (1992). *In vitro* fertilization of water buffalo follicular oocytes and their ability to cleave *in vitro*. *Theriogenology*.**38**: 1187 - 1194.

Tasripoo, K., Srisakwattana, K., Suthikrai, W., Chethasing, S. and Kamonpatana, M. (2005). Potential uses of buffalo oocytes from ovaries with CL and without CL for in vitro maturation and fertilization. *Buffalo J*. **21**: 221-228.

Totey, S.M., Singh, G., Taneja, M., Pawshe, C.H., Talwar, G.P. (1992). *In vitro* maturation, fertilization and development of follicular oocytes from buffalo *(Bubalus bubalis). J. Reprod. Fertil.***95**: 597-607.

Wakle, S.S., Bakshi, S.A. and Deopurkar, V.L. (2002). *In vitro* maturation of buffalo oocytes in TCM- 199 and DPBS media supplemented with BSA. *Indian Vet. J.***79**:28-29.

Wang, Z., Yu, S. and Xu, Z. (2007). Effects of collection methods on recovery efficiency, maturation rate and subsequent embryonic developmental competence of oocytes in Holstein cow. *Asian-Aust. J. Anim. Sci.*,**20** (4): 496500.

Wang, Z.G., Yu, S.D. and Xu, S.R. (2007): Effects of collection methods on recovery efficiency, maturation rate and subsequent embryonic developmental competence of oocytes in Holstein cow. Asian. *Aust. J. Anim. Sci*. **20**: 496-500.

Wani, N.A., Wani, G.M., Khan, M.Z. and Salahudin, S. (2000). Effect of oocyte harvesting technique on *in vitro* maturation and *in vitro* fertilization in sheep. *Small Rum. Res.***36**: 63-67.

Wani, N.A., Wani, G.M., Khan, M.Z. and Sidiqui, M.A. (1999). Effect of different factors on the recovery of oocytes for *IVM-IVF* procedures in sheep. *Small Rum. Res.***34**: 71-76.

Warriach, H.M. and Chohan, K.R. (2004). Thickness of cumulus cell layer is a significant factor in meiotic competence of buffalo oocytes. *J. Vet. Sci*. **5**: 247-251.

Webb, R., Campbell, B.K.,Garveric, H.A. and Gong, J.G. (1999): Molecular mechanisms regulating follicular recruitment and selection. *J. Reprod. Fert*. **45**: 123-126.

Yadav, P.S., Khanna, S., Hooda, O. K. and Sethi, R. K. (2000). Effect of oviductal cell co-culture on cleavage and development of buffalo IVF embryos. *Asian- Aus. J. Anim. Sci*.**13** (7): 894-896.

Yadav, P.S., Singh, I. and Sethi, R.K. (2008). *In vitro* cleavage rates of homologous oocytes for fertility evaluation of buffalo bulls. *The Indian Vet. J*. **85** (7): 725-727.

Yadav, R.C.S. (2005). Developmental competence of bubaline cumulus oocyte complexes cryopreserved by vitrification using two cryoprotectants glycerol and ethylene glycol. *Ph.D Thesis* Rajasthan Agricultural University, Bikaner, Rajasthan, India.

Yang X, S Jiang and RH Foote (1993). Bovine oocyte development following different oocyte maturation and sperm capacitation procedures. *Mol. Reprod. Dev*. **34**: 94-100.

Zoheir, K.M.A., Abdoon, A.S.,Mahrous, K.F., Amer, M.A., Zaher, M.M., Li-Guo-Yang and El-Nahass, E.M. (2007): Effect of season on the quality and *in vitro* maturation rate of Egyptian buffalo *(Bubalus bubalis*) oocytes. *J. Cell Anim. Biol*. **1**(2): 29-33.

APPENDIX

Appendix 1.Significant level of ovarian categories of buffaloes with CL or without CL collected from slaughter house

Sources of variation	Degrees of freedom	Mean square of corpus luteum (CL)	
		Present (%)	Absent (%)
Ovary	1	3.52	2.64
Error	100	0.113**	0.104**

* = Significant at 5% level ** = Significant at 1% level

Appendix 2.Significant level of ovarian categories of buffaloes on length of ovary collected from slaughter house

Sources of variation	Degrees of freedom	Mean square of length (cm)
Ovary	1	5.86
Error	100	0.162**

* = Significant at 5% level ** = Significant at 1% level

Appendix 3.Significant level of ovarian categories of buffaloes on width of ovary collected from slaughter house

Sources of variation	Degrees of freedom	Mean square of width (cm)
Ovary	1	4.16
Error	100	0.13**

* = Significant at 5% level ** = Significant at 1% level

Appendix 4.Significant level of ovarian categories of buffaloes on weight of ovary collected from slaughter house

Sources of variation	Degrees of freedom	Mean square of weight (g)
Ovary	1	17.98
Error	100	0.871**

* = Significant at 5% level ** = Significant at 1% level

Appendix 5.Significant level of ovarian categories of buffaloes on number of follicles collected from slaughter house

Sources of variation	Degrees of freedom	Mean square of number of follicles	
		Total	Aspirated
Ovary	1	131.92	94.15
Error	100	1.32**	1.24**

* = Significant at 5% level ** = Significant at 1% level

Appendix 6.Significant level of ovarian categories of buffaloes regarding on grading of COCs collected from slaughter house

Sources of variation	Degrees of freedom	Mean square of grading of COCs		
		Normal	Abnormal	Total
Ovary	1	0.48	0.794	0.0098
Error	100	0.917*	0.582*	1.471*

* = Significant at 5% level ** = Significant at 1% level

Appendix 7. Significant level of ovarian categories of buffaloes regarding with CL or without CL collected from slaughter house

Sources of variation	Degrees of freedom	Mean square of percent corpus luteum (CL)
Ovary	1	4.56
Error	100	0.126**

* = Significant at 5% level ** = Significant at 1% level

Appendix 8. Significant level of ovarian categories of buffaloes regarding with CL or without CL on length of ovary collected from slaughter house

Sources of variation	Degrees of freedom	Mean square of length (cm)
Ovary	1	7.26
Error	100	0.103^{NS}

* = Significant at 5% level ** = Significant at 1% level

Appendix 9. Significant level of ovarian categories of buffaloes regarding with CL or without CLon width of ovary collected from slaughter house

Sources of variation	Degrees of freedom	Mean square of width (cm)
Ovary	1	3.28
Error	100	0.057^{NS}

* = Significant at 5% level ** = Significant at 1% level

Appendix 10. Significant level of ovarian categories of buffaloes regarding with CL or without CL on weight of ovary collected from slaughter house

Sources of variation	Degrees of freedom	Mean square of weight (g)
Ovary	1	21.73
Error	100	1.036**

* = Significant at 5% level ** = Significant at 1% level

Appendix 11. Significant level of ovarian categories of buffaloes regarding with CL or without CL on number of follicles collected from slaughter house

Sources of variation	Degrees of freedom	Mean square of number of follicles	
		Total	Aspirated
Ovary	1	76.84	101.67
Error	100	1.352**	1.507*

* = Significant at 5% level ** = Significant at 1% level

Appendix 12. Significant level of ovarian categories of buffaloes regarding with CL or without CL on grading of COCs collected from slaughter house

Sources of variation	Degrees of freedom	Mean square of grading of COCs		
		Normal	Abnormal	Total
Ovary	1	0.388	1.107	1.376
Error	100	0.344^{NS}	0.362*	1.081*

* = Significant at 5% level ** = Significant at 1% level