QUALITATIVE AND QUANTITATIVE ASSESSMENT OF BOVINE OOCYTES

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CERTIFICATE

This is to certify that the thesis entitled **QUALITATIVE AND QUANTITATIVE ASSESSMENT OF BOVINE OOCYTES** submitted to the Department of Animal Production & Management, Sher-e-Bangla Agricultural University, Dhaka in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (MS) in ANIMAL SCIENCE,** embodies the results of a piece of bona fide research work carried out by **MD. ABDULLAH AL ZABER,** bearing Registration No. **12-04918** under my supervision and guidance. No part of this thesis has been submitted for any other degree or diploma in any other institution.

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

Dated:

Dhaka, Bangladesh

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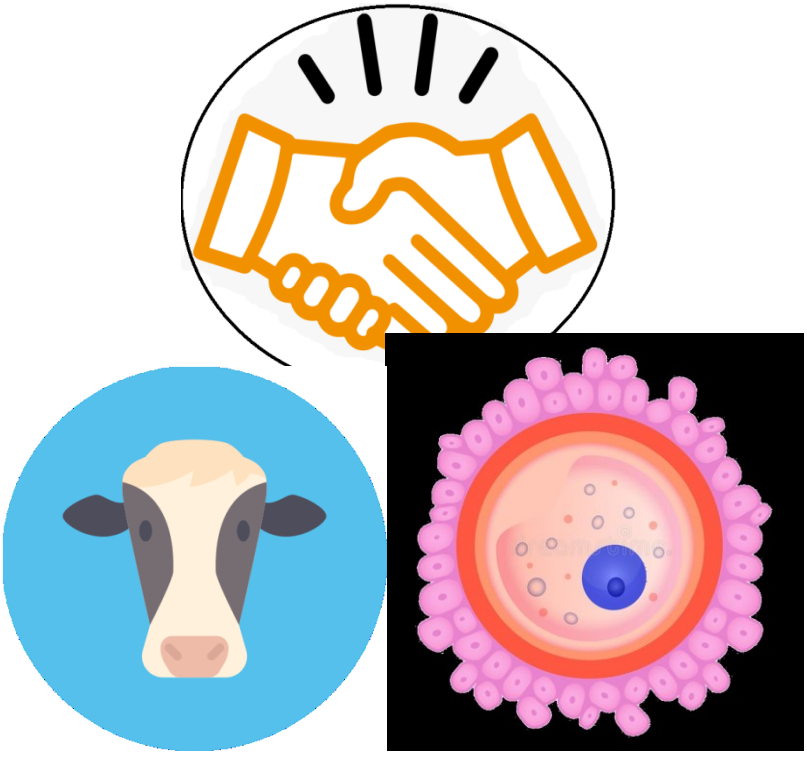
DEDICATED TO
My Beloved Parents



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ABSTRACT

The reproductive development of cattle is must to hasten the production of milk and meat. It relies mostly on the quality of oocytes. Thus the present study was carried out to sort out the superior ovary to be used in further research. Cow ovaries were collected from slaughter house, processed and categorized as right or left and ovaries with or without corpus luteum (CL). Morphology of ovaries were studied 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 cumulus-oocyte-complex (COCs) and then graded as normal and abnormal. Significantly (p<0.05) higher mean weight (3.47±0.34 vs 2.44±0.32), length (1.94±0.06 vs 1.71±0.07) and width (1.36±0.06 vs 1.09±0.05) were found in right ovaries than left. Forty seven per cent of right and 20% of left ovaries were found with CL. Numerically higher number of normal (0.97±0.16 vs 0.67±0.15) and total (1.60±0.18 vs 1.40±0.21) COCs was found in left ovaries than right ovaries whereas abnormal COCs were higher in right ovaries (0.73±0.14 vs 0.63±0.13). On the other hand, in comparison between with and without CL ovaries, significantly (p<0.05) higher mean weight (3.81±0.38 vs 2.53±0.29), length (2.04±0.07 vs 1.72±0.06) and width (1.45±0.06 vs 1.11±0.05) were found in with CL ovaries than without CL. The average number of normal COCs of with CL ovary (0.75±0.19) was lower than that of without CL (0.85±0.13) but they are not significant (p<0.05). Finally, it can be concluded that, oocytes from left ovaries in comparison to right and without CL in comparison to with CL are superior in the context of selection of oocytes for embryo development.

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LIST OFABBREVIATIONS AND SYMBOLS

>	=	Greater than
<	=	Less than
±	=	Plus minus
^{o}C	=	Degree Celsius
%	=	Percentage
AI	=	Artificial insemination
ANOVA	=	Analysis of Variance
AV	=	Artificial vagina
BAU	=	Bangladesh Agricultural University
BBS	=	Bangladesh Bureau of Statistics
B.C.	=	Before Christ
BSA	=	Bovine serum albumen
Ca	=	Calcium
CL	=	Corpus Luteum
cm	=	Centimeter
COCs	=	Cumulus-oocyte-complexes
CV %	=	Percent Coefficient of Variation
DF	=	Degree of freedom
DLS	=	Department of Livestock Services
D-PBS	=	Dulbecco's phosphate buffered saline
DMRT	=	Duncan's Multiple Range Test
DW	=	Distilled water
ET	=	Embryo transfer
et al.,	=	And others
e.g.	=	exempli gratia (L), for example
etc.	=	Etcetera
FAO	=	Food and Agricultural Organization
FF	=	Follicular fluid
FSH	=	Follicle stimulating hormone
g	=	Gram (s)
GDP	=	Gross Domestic Product
GM	=	Geometric mean
i.e.	=	id est (L), that is
IVC	=	*In vitro* Culture
IVF	=	*In vitro* Fertilization

LIST OFABBREVIATIONS AND SYMBOLS (cont'd)

IVP	=	*In vitro* Production
IVM	=	*In vitro* Maturation
Kg	=	Kilogram (s)
L	=	Liter
LH	=	Luteinizing hormone
ml	=	Milliliter
MOET	=	Multiple Ovulation Embryo Transfer
NaOH	=	Sodium hydroxide
No.	=	Number
NS	=	Non-significant
OPU	=	Ovum pick up
PBS	=	Phosphate buffered saline
SAU	=	Sher-e-Bangla Agricultural University
SAS	=	Statistical Analysis
SE	=	Standard Error
µg	=	Microgram
WHO	=	World Health Organization

CHAPTER 1
INTRODUCTION

Bangladesh is an agrarian country. Livestock is an important sub-sector of Bangladesh agriculture. Its contribution to country's Gross Domestic Product (GDP) is about 1.43 percent which is 13.44 percent of agricultural GDP with a growth rate of 3.04 percent (DLS, 2020). This sub-sector is also important from the perspectives of food-security, crop cultivation, poverty reduction, nutrition, and employment generation in the country. Poultry and dairy farming has also certain specific advantages over crops, fisheries and forestry, as they require less land and are least influenced by seasonality (Ali & Hossain, 2014). Half of the national employment indirectly related to this sub-sector whereas twenty percent people are involved in livestock sector as permanent occupation (Ahmed *et al.,* 2008).

Cattle are reared as a household livestock to meet the demand of milk as well as to make money using kitchen waste and grazing mainly. Some commercial farms are also developed in recent couple of decades. The key objective of cattle rearing is to get meat and milk. However, manure, hides, bones and some other byproducts are also found from cattle. According to DLS Bangladesh is sufficient in meat having availability of 126 g meat per day per head instead of 120g of demand and deficit in milk having availability of 175ml milk per day per head instead of 250ml of demand with 412.2 million of livestock population including 24.3 million of cattle (DLS, 2020).

Cattle are the major member of Farm Animal Genetic Resources (FAnGR) of Bangladesh. The main problem of cattle production lies in its very low productivity due to low genetic potentiality. The diseases and disorders of animals also hinder livestock development in our country (Islam *et al*., 2001). Breeding is the major technological improvement process in the dairy industry. A cross-breeding program for cattle was introduced in the country in 1976 with

a view to improving the milk production efficiency of indigenous cows (Shamsuddoha and Edwards, 2000). The genetic improvement of livestock can be achieved by proper utilization of proven sires and dams by following the artificial insemination (AI) with frozen semen and Embryo Transfer Technology (ETT).

Ovaries are primary reproductive organ of female. It supplies germ cells and oocytes as well as maintains reproductive health by producing hormones. Remarkably lower numbers of ovarian follicles is considered as one of the major causes of infertility (Amin *et al*., 2005). The degree of reproductive performance relies upon the interaction of genetic and environmental factors however this performance is particularly susceptible to latter, for example, the seasonal availability of nutrients can affect reproduction considerably (Forcada and Abecia, 2006). Lof *et al*., (2012) stated that ability to accommodate to fluctuation in environment often involves some degree of reproductive failure. Several molecular factors are known to be responsible for the follicular development in mammals (Skinner, 2005). The effect of environmental factors on the development of follicles is not understood well. Animals are exposed to adverse climatic conditions, e.g., acute sun shine, high temperature, humidity, rainfall and cool weather in tropical countries like Bangladesh. The effect of such environmental factors on morphology of follicles has not been understood.

The quality of the immature oocyte is determined by the quality of cumulus oocyte complexes (COCs) and oocyte diameter (Arlotto *et al*., 1996), but the result of aspiration of ovarian follicles are known have varying quality of cells (Lucas *et al*., 2002). The specific cause of the variation of oocyte quality is unknown, but may be caused by a variety of follicle size and oocyte diameter was used, which relates to the process of folliculogenesis.

The conservation of indigenous and endangered species and their faster genetic improvement has been achieved by adopting modern biotechnological tools eg.

Multiple ovulation and embryo transfer (MOET), *in vitro* fertilization (FVF), micromanipulation of gametes, cryopreservation of embryos and gene transfer etc. The refractoriness of the embryos of most domestic species by MOET to culture techniques, high costs and inconsistent outcome have impeded the progress in domestic species of economic interest.

The latest developments in gametes and embryo cellular biology, the field of molecular embryology of farm animals has been poorly explored and genetic improvement of farm animals could be made by planned AI with frozen semen and Estrus Synchronization (ES) (Hoque *et al.,* 2011). After a dramatic development of cellular biology, many research efforts have been moved towards the implementation of embryo technologies involving Multiple Ovulation and Embryo Transfer (MOET), *in vitro* Production (IVP) of embryos, Cloning and Transgenesis to transfer a targeted number of embryos having desired genetic make-up (Hoque *et al*., 2012).

In abroad, quantitative and histological aspects of ovary and ovarian follicles have been studied in sheep (Draincourt *et al*., 1993), bovine (Singh and Adams, 2000), mouse (Satosi and Motoalci, 2004), wapiti (McCorkel *et al*., 2004) and Iranian Lori-Bakhtian Sheep and native goat (Mohammadpour, 2007). However, until today, there is limited study on qualitative, quantitative and histological analysis on the ovary, ovarian follicle and oocytes in cattle.

The method of in vivo collection of oocytes is expensive and the number of oocytes recovered per ovary is very small (Pawshe *et al.,* 1994); whereas ovaries of valuable slaughtered animals "a waste product" are the cheapest and the most abundant source of oocytes for large scale embryo production through *in vitro* maturation and *in vitro* fertilization (Agrawal *et al.,* 1995). Oocytes are the main raw materials for *in vitro* embryo production (IVP) experiments. Therefore, the success of any IVP program, either *in vitro* fertilization (IVF) or *in vitro* culture (IVC) of embryos largely depends on the continuous supply of quality oocytes in optimum quantity.

A number of research works have been performed to compare the efficiency of the oocyte collection techniques in cattle (Katska, 1984, Lonergan *et al*., 1991), sheep (Wahid *et al*., 1992, Wani *et al*., 2000) and goat (Mogas et al.,1997; Wang *et al*., 2007) in abroad.

From that stand point the present research work has been undertaken with the following objectives:

- To assess the quality and quantity of oocytes collected from bovine ovaries
- To find out the superior quality of ovary and oocytes to use in further study
- To help in the making genetic improvement of cattle through *in vitro* embryo production

CHAPTER 2
REVIEW OF LITERATURE

A lot of research works related to collection, recovery and evaluation of oocyte were done in different species of animals like cattle, buffalo, goat and sheep in different countries of the world. But in Bangladesh, reports with this kind of works have been done to a very limited extent. In fact, most of the studies in this field were on goat in our country. However, some of the related findings of research work carried out in different countries of the world are reviewed in this chapter. In view to focus an easy overview the bulk of literature is presented into several sections as follows.

2.1 Overview on Ovary and Oocyte

Ovary is the primary female reproductive organ found in the left and right side of the body. It releases mature oocytes to be fertilized by sperm and secretes various hormones i.e. estrogen, testosterone, inhibin, and progesterone that play a vital role in the menstrual cycle and fertility to regulate successful breeding of species (Colvin *et al*., 2013).

The ovary progresses through many stages beginning in the prenatal period through menopause. Morphological differentiation in ovary starts when gonads are crowed with primordial germ cells (PGCs). These PGCs produce oogonia that gradually becomes primary oocytes and then female gamets in the ovary which is called oogenesis. The process of oogenesis happens in the ovaries and starts three weeks after fertilization in the early fetal development with formation of PGCs (Edson *et al*., 2009), stops at birth and continues during puberty in the course of the reproductive life of the female (Rahman *et al*., 2008).

Oocyte in cattle is a female gametocyte or germ cell involved in reproduction that formed during embryogenesis and develop within individual follicles in the

cortex of the ovary. Dormant primordial follicles become active and undergo progressive development at regular intervals commencing during the late fetal stage and continuing throughout adulthood. Once activated, follicles and oocytes in a cohort either grow to maturation and ovulation or undergo atresia, ultimately depleting the ovaries of germ cells. It takes an estimated 100 days for a follicle and its oocyte to reach the mature ovulatory stage (Britt, 2008).

The oocyte in the Graafian follicle surrounded by many layers of tightly packed cumulus cells is called the cumulus-oocyte complex (COCs). The oocyte is arrested in Meiosis II at the stage of metaphase II and is considered a secondary oocyte. A structural change of COCs before ovulation is known as cumulus expansion. As a result, the tightly compacted granulosa cells transform to an expanded mucoid matrix. According to various studies, cumulus expansion is critical for the maturation of the oocyte because the cumulus complex is the oocyte's direct communication with the developing follicle environment. It also plays a significant role in fertilization, though the mechanisms are not entirely known and are species specific (Yokoo *et al*., 2004; Zhongwei *et al*., 2010).

2.2 Harvesting of Oocytes

In case of *in vitro* production of embryos (IVEP) including *in vitro* maturation and fertilization, the number of good quality oocytes that are harvested from the ovary is essential. Oocytes for IVF are collected from either of three sources i.e. the oviducts soon after ovulation, mature follicles shortly before ovulation or immature and antral follicles usually from abattoir material (Wani, 2002).

2.2.1 Collection of ovaries

Ovaries of abattoir origin are generally used for the production of embryos in domestic animals. The ovaries of slaughtered animals are the cheapest and most abundant source of oocytes for large scale production of embryos through

IVM-IVF (Agrawal *et al.,* 1995). The sheep ovaries obtained from slaughter house were brought to the laboratory in a normal saline solution or Dulbeccos phosphate buffered saline solution (Pugh *et al.,* 1991) at 20°C (Slavik *et al.,* 1992), or at room temperature (Watson *et al.,* 1994; Wani *et al.,* 2000) without any detrimental effect on oocyte. The time interval between collection of ovine ovaries and harvesting of oocytes also varied from 1-2 (Pugh *et al.,* 1991) to 3-4 h (Wani *et al.,* 1999) without any deleterious effect on oocyte maturation. Similarly, Snyder (1977) collected ovine ovaries after slaughter and transported them for about 3h at 30°C, 37°C and 21°C. The proportions of ova maturing were 16.7, 50 and 37.8 per cent respectively, indicating 37°C to be the best temperature for transportation of ovine ovaries. In pigs, storage of ovaries for 2-3 h at 33-35°C was considered to be best (Sato *et al.,* 1977). Likewise, bovine ovaries can be stored for 11 h at 24-25°C (Yang *et al.,* 1990), 8 h at 20°C (Gordon and Lu, 1990) and 8.5 h at 20-35°C (Sato *et al.,* 1977) without any significant effect on the maturation and fertilization of recovered oocyte.

Kharche *et al.,* (2009), Kumar *et al.,* (2004) and Gupta *et al.,* (2012) collected goat ovary from abattoir. Deal *et al.,* (2017) collected ovaries of 86 buffaloes and 95 cows from slaughterhouses and transported to the laboratory in saline solution at 36°C.

2.2.2 Isolation of oocytes

The collection of sufficient number of good quality oocytes from ovaries is a major part of the study. Baldassarre *et al.,* (1996) collected *in vivo* matured oocytes either by surgical or laparoscopic techniques. Both of them were expensive and the number of oocytes recovered per ovary was small as described by Pawshe *et al.,* (1994). Lambert *et al.,* (1986) tried by laparoscopy and Pieterse *et al.,* (1992) tried by transvaginal ultrasound guided techniques to aspirate follicular oocytes in bovines.

Several techniques had been used for the collection of oocytes from ovaries of abattoir origin in cattle (Katska, 1984; Iwasaki *et al.,* 1987; Deal *et al.,* 2017),

goats (Mogas *et al.,* 1992; Pawshe *et al.,* 1994) and sheep (Wahid *et al.,* 1992a b; Wani *et al.,* 1999). Commonly employed methods to recover oocytes from abattoir ovaries were dissection of follicles, aspiration of visible antral follicles, puncturing of follicles, slicing and mincing of ovaries. Follicular dissection was first used to recover ovine follicular oocytes (Crosby *et al.,* 1981; Fukui *et al.,* 1980). Currently, slicing (Wahid *et al.,* 1992a b) and aspiration (Slavik *et al.,* 1992; Watson *et al.,* 1994; Wani *et al.,* 2000) are being employed routinely for oocyte recovery- in sheep.

Deal *et al.* (2017), Mondal *et al*. (2008), Das *et al.,* (2011), Khandoker *et al.* (2011), Boonkong *et al.* (2012), El-Naby*et al.* (2013), Islam *et al.* (2007) and Ferdous (2006) collected COCs by aspiration method for their study on different species whereas Sani *et al.* (2013), Singh *et al.* (2012) and Gabr *et al.* (2019) used slicing method.

After conducting a series of experiment on the recovery methods of goat oocytes Pawshe *et al*. (1994) concluded that average oocytes recovery rate per ovary was significantly higher by aspiration (2.7±0.15) than by puncturing (2.2±0.13) or by slicing (2.4±0.12) method.

Kumar *et al.* (2004) showed after studied on goat that, oocytes recovered by aspiration (157.97±1.35µm) showed statistically significant increase diameter compared to oocytes recovered from slicing (147.01±1.58 µm).

The proportions of goat oocytes that were fertilized and developed to morulae were 82 of 102 (80.4%) and 50 of 102 (49%) respectively for those obtained by aspiration, and 77 of 126 (61.1%) and 27 of 126 (21.4%) for those obtained by mincing according to Keskintepe *et al.* (1994).

Kharche *et al.* (2009) retrieved oocyte by follicular puncture from the goat ovaries. Wang *et al.* (2007) harvested oocytes from ovary of Boer goat and graded COCs as good, fair and poor. According to their study, slicing and puncture of the ovaries yielded (6.3 and 5.8 respectively) significantly higher

(p<0.05) number of oocyte per ovary compared to aspiration I and aspiration II (2.9 and 3.1 respectively). The good quality COCs was significantly higher (p<0.05) in aspiration I and aspiration II (3.9 and 3.6) than slicing and puncture (2.4 and 2.11 respectively).

Aspiration yielded a high number (4.6) of total oocytes per ovary than slicing (3.9) and puncture (4.0) in the study of Mogas *et al.* (1992). They also reported that significantly higher number of normal oocytes per ovary was obtained by aspiration (3.7) than slicing (2.7) and puncture (2.6) method.

Wani *et al.* (2000) subjected 47, 61 and 51 ovaries to puncturing, slicing and aspiration technique, respectively, for recovery of ovine oocytes. He harvested ovine oocytes from and showed that, the average total number of oocytes recovered per ovary was significantly higher by puncture (9.5±0.45) and slicing (9.5±0.40) than by the aspiration method (6.8±0.30) (p<0.05). However, the percentage of good quality oocytes was higher in the aspiration method (64.4%), compared to the puncture (54.7%) or slicing (54.3%) methods. On the contrary, the oocyte recovery rates for puncturing and aspiration of ovine ovaries were 84.9 and 57.6 per cent, respectively, from sheep ovaries (Lorenzo *et al.,* 1999), however, both the methods yielded similar quality oocytes. In goat, slicing yielded more oocytes per ovary (6.05) than dissection (1.71) or aspiration (1.25), however, *in vitro* fertilization capacity of oocyte obtained by slicing method was lower (18.2 vs 29.1%) than those obtained by dissection (Martino *et al.,* 1994). The lower number of oocytes recovered by aspiration may be attributed to the presence of some follicles embedded deep within the cortex, which were released by puncture or slicing of the ovary. Some of the oocytes might even be lost during aspiration of follicles, whereas, slicing or puncturing reduces these chances (Wani *et al.,* 2000).

2.2.3 Grading and selection of oocytes

The grading and selection of oocytes is an important step which determines the success of *in vitro* development of immature oocytes. The presence of a

compact and healthy population of cumulus cells surrounding the oocyte has been universally used to characterize the cultivable quality oocytes and to assess their development potential *in vitro* (Lonergan *et al.,* 1994). Higher *in vitro* maturation, fertilization and cleavage rates had been achieved in compact cumulus enclosed bovine oocytes (Leibfried and First, 1979; Yang and Lu, 1990; Cox *et al.,* 1993). Similarly, selection of immature sheep oocytes had been based on compactness and number of enclosing cumulus layers (Pawshe *et al,* 1994) as well as cytoplasmic characteristics (Wani *et al.,* 1999, 2000). The denuded bovine oocytes had lower frequencies of maturation and fertilization *in vitro* (Kim and Park, 1990; Lorenzo *et al.,* 1995) and none of them progressed to the blastocyst stage (Yang *et al.,* 1990).

Cetica and associates (1999) emphasized that class A oocytes in bovine -*were* most likely to mature *in vitro* as they had a close association with their surrounding cumulus cells. Good and poor graded bovine oocytes showed maturation rate of 75 and 58.8 percent, respectively, indicating better maturation rates of good quality oocytes (Im *et al,* 1995).

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 goats 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).

Size of the follicles/oocytes also influences the quality and developmental competence of recovered oocytes. The oocytes with a larger diameter produce morula and blastocyst at higher rates (Arlotto *et al.,* 1992). Ledda and associates (1999) reported that the meiotic competence of the *in vitro* matured prepubertal and adult sheep oocytes were affected by the follicular size. Non-atretic follicles were dissected from ovaries of prepubertal and adult sheep and depending on follicle diameter were divided in three groups (<1, 1-2 and >2mm). On maturation, a lower percentage of adult and prepubertal oocytes of <lmm reached metaphase-II than those obtained from l-2mm and >2mm groups (70.4 vs 89.5 and 95.5% for adult ovine oocytes and 27.2 vs 79.8 and 81.8% for prepubertal ovine oocvies, respectively). The results indicated that oocytes with the same diameter derived from different follicles showed similar meiotic progression rates.

Echert and Niemann (1995) divided the collected COCs into two morphological categories: COCs with a homogenous evenly granulated cytoplasm possessing at least three layers of compact cumulus cells designated as category I and COCs with less than three layers of cumulus cells and were partially denuded possessing a homogenous evenly granulated cytoplasm as category II and better results were found with the former category of COCs.

Islam *et al.* (2007) collected goat ovaries from the slaughterhouse and categorized as right, left, corpus luteum (CL)-present and absent group and evaluated on the basis of weight (g), length (cm), width (cm), number of follicles. The oocyte was harvested by aspiration method. The left ovaries contained comparatively higher number of normal COCs [(1.06±0.09) per ovary] than right ovaries [(1.03±0.10) per ovary]. The similar trend was found in total number of follicles [(4.51±0.25) vs (4.30±0.23) per ovary] and follicles aspirated [(2.55±0.14) vs (2.52+0.12) per ovary]. But the total COCs per ovary was almost similar in both ovaries [right and left: (1.85±0.12) and (1.85±0.11) per ovary, respectively). Higher number of total COCs [(1.87±0.09) vs (1.76±0.16) per ovary], total number of follicles [(4.45±0.19) vs (4.16±0.37)

per ovary], follicles aspirated [(2.55±0.10) vs (2.48±0.21) per ovary] and normal COCs [(1.12±0.07) vs (0.76±0.14) per ovary] were found in CL-absent group than those of CL-present group ovaries.

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%).

El-Naby *et al.* (2013) evaluated Egyptian buffalo oocytes morphologically according to the criteria of cumulus investment and classified into four classes (A; completely invested with cumulus cell layers, B; partially invested with cumulus cells, C; denuded oocytes and 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.

Sani *et al.* (2013) collected goat 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.

According to Khandoker *et al*. (2011), the COCs were classified into four grades on the basis of cumulus cells and nucleus. In brief, he denoted oocytes completely surrounded by cumulus cells as Grade A, oocytes partially surrounded by cumulus cells as Grade B, oocytes not surrounded by cumulus cells as Grade C and degenerated oocytes and cumulus cells as Grade D. The grade A and B were considered as normal COCs where oocyte was surrounded by cumulus cell. On the other hand, the grade C and D were considered as abnormal COCs where oocyte was not surrounded by cumulus cell.

2.3 Effects on Oocyte

There are several factors that affect the quality and quantity of oocyte in different animal like left or right ovary, size and shape of follicle, oocyte collection technique, physiological condition and age of the donor and the seasonal variation.

2.3.1 Left and right ovary

Khandoker *et al.* (2011) obtained caprine 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).

2.3.2 Size of ovary, follicle and oocyte

The size of the ovary also affects number and quality of oocytes obtained by aspiration of visible follicles. The large ovaries (>5x7x9mm) yielded 8.4±0.4 oocytes/ovary compared to small ovaries (<5x7x9mm) which yielded 6.1±0.5 oocytes/ovary following aspiration (Wani *et* al., 1999). It might be attributed to the low number of visible follicles present on the small ovaries, whereas the large sized ovaries had a good number of visible follicles for aspiration.

The size of the follicle seems to be an important factor in the selection of potential oocytes (reviewed by Sirard *et al*., 2006), involving RNA or protein stores as factors involved in oocyte competence. Increased developmental competence of oocytes has been associated with increased follicular diameter as reported in several studies in various species. In cattle, Lonergan *et al*., (1994) reported a higher proportion of blastocysts obtained from follicles >6 mm compared to 2–6 mm follicles. Similarly, in calves, Kauffold *et al*., (2005) showed an increase in blastocyst production in oocytes coming from follicles with diameter >8 mm than from follicles of <8 mm. It has been suggested that the reason for the differences between the follicular diameters on oocyte quality is due to their content. The follicular fluid (FF) constitutes the microenvironment of the oocyte during follicular maturation and contains molecules involved in nuclear and cytoplasmic maturation, ovulation and fertilization (Yoshida *et al*., 1992). Thereby, Ali *et al*., (2004) illustrated the effect of follicle diameter by the use of bovine follicular fluid obtained from large (>8 mm) and small follicles (2–5 mm) as a supplement of *in vitro* maturation media of bovine oocytes. Results of this study showed that following fertilization and embryo culture, more oocytes reached the blastocyst stage when oocytes were cultured with FF from large follicles compared with FF derived from small follicles. Similarly, in buffalo (Raghu *et al*., 2002), sheep (adult: Cognié *et al*., 1998; prepubertal: Ledda *et al*., 1999b), goats (adult: De Smedt *et al*., 1994; Crozet *et al*., 1995; prepubertal: Romaguera *et al*., 2010) and pigs (Marchal *et al*., 2002) has been reported that

the acquisition of meiotic competence as well as the ability to develop up to the blastocyst stage is acquired sequentially as the follicle enlarges. In prepubertal goats, Romaguera *et al,*. (2010) demonstrated that oocytes from follicles of ≥3 mm showed greater mean oocyte diameter, higher percentages of fragmented DNA cells, higher cleavage rates and greater developmental competence to the blastocyst stage than oocytes from follicles of < 3mm. Palma *et al.* (1993) observed in cattle that the recovery rate of follicles was 25, 27 and 62% for small, middle and large ovaries respectively.

In bovine, Otoi *et al*., (1997) classified oocytes in six categories according to oocyte diameter (<110 μm, 110 to <115 μm, 115 to <120 μm, 120 to <125 μm, 125 to <130 μm and ≥130 μm), and concluded that bovine oocytes have acquired full meiotic competence at a diameter of 115 μm but not yet attained full developmental competence to blastocysts, and that oocytes have acquired full developmental competence at a diameter of 120 μm. In ovine, Shirazi and Sadeghi (2007) reported no significant differences in the percentage of oocytes that reached the MII stage (81, 82, and 84%) with diameters of <110 μm, 110–150 μm, >150 μm, respectively. In buffalo, the rate of *in vitro* blastocyst production was significantly higher in oocytes with diameters greater than 145 μm (Raghu *et al*., 2002a). In prepubertal goats, oocytes were classified by Anguita *et al*., (2007) and Jiménez- Macedo *et al*., (2006), into 4 categories of diameter: <110 μm; 110 to 125 μm; 125 to 135 μm and >135 μm, results of those studies showed that oocytes smaller than 125 μm fertilized by IVF (Anguita *et al*., 2007) and ICSI (Jiménez-Macedo *et al*., 2006), were unable to develop up to blastocyst stage. Anguita *et al*., (2007) observed that the oocyte diameter was positively related to the percentage of oocytes at MII after IVM (0, 21, 58 and 78%, respectively) and the percentage of blastocysts obtained at 8 days post insemination (0, 0, 1.95 and 12.5%, respectively). However, using ICSI to fertilize these oocytes categories, the percentage of ICSI derived blastocysts (blastocysts/injected oocytes) obtained from oocytes of 125–135 μm diameter had similar blastocyst development to oocytes larger than 135

μm (15.9 and 11.1%, respectively) (Jiménez-Macedo *et al*., 2006). This difference between oocyte categories after IVF and ICSI protocols could be explained by the fact that oocytes selected to perform ICSI have completed their nuclear maturation. Also, the lack of polyspermic zygotes by the microinjection could be one other explanation.

2.3.3 Oocyte collection techniques

Majeed *et al.* (2019) reported that, among the three collection methods, aspiration (0.966±0.139) and puncture (0.966±0664) methods recorded a high recovery rate due to the aspiration and puncture considered as the applicable technique for obtaining perfect oocytes production (quality and quantity), while the presence of the ovarian tissue debris in the slicing (0.571±0.320) due to destruction the ova during the examination.

In an experiment on *in vitro* production of caprine embryos, Shaikh *et al.* (2015) showed that, good quality oocytes was found higher in slicing (61.90%) than aspiration (58.10%) and follicle dissection (55.20%). Similar result was found by Datta and Goswami (1998) and Mohan *et al.* (2013). Datta and Goswami (1998) also said that processing of aspiration required less time than those of slicing and dissection methods. Kumar *et al.* (1997), Das *et al.* (1996), Sianturi *et al.* (2002), Das and Santra (2008), Palanisamy *et al.* (2009), Mehmood *et al.* (2011), Kulasekhar *et al.* (2012) and Rao and Mahesh (2012) also recorded slicing as the collection technique with the comparative efficient three harvesting techniques.

On the other hand, Chandrahasan *et al.* (2012) found statistically highly significant difference between dissection (5.69) and other two methods i.e. aspiration and puncture. Jamil *et al.* (2008) also found the similar result. Whereas Wang *et al.* (2007) found that slicing (9.6) and puncture (9.7) yielded a larger number of oocytes per ovary than two aspiration methods (aspiration I and II were 5.8 and 5.6, respectively) ($P<0.05$).

In aspiration method recovery of good quality oocytes per ovary was lower (1.76 oocytes per ovary) than that of scoring method (3.85 oocytes per ovary) according to the study of Raza *et al.* (2001) on goat.

2.3.4 Physiological condition

Many investigators have reported that the stage of estrous cycle (Leibfried and First, 1979; Fukui and Sakuma, 1980; Leibfried-Rutledge *et al,* 1985; Tan, 1990) and pregnancy (Vazta *et al.,* 1992) of the donors did not influence the developmental potential of the isolated oocytes; the selection of ovaries was based on reproduction status, therefore, appears non-significant.

Kachiwal *et al.* (2012) studied ultrasonographic biometry of the ovaries of pregnant Kundi 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 goats 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.

Salim *et al.* (2004) studied on reproductive tract of four-category (category I = acyclic; category 2= cyclic but not conceived; category 3=post partum anestrus and category 4 normal breeder and kidder) Black Bengal does to investigate the causes of infertility and reported that average number of normal follicles were significantly higher in category 4 compared to other category and degenerated follicles were reverse to that of the result of the normal follicles.

Lassala *et al.* (2004) conducted study on a group of goats during synchronization of estrus. The study revealed tha ovarian follicular dynamics and fertility are unaffected by the presence or absence of a corpus luteum. However, presence/absence of corpus luteum (C.L.) on ovaries had marked effect on total number of oocytes recovered. It was reported higher oocyte recovery rates in ovaries without C.L. compared to ovaries with C.L. (10.5±0.2

vs 4.7±0.4 oocytes/ovary respectively). The cause of a lower number of oocytes with a C.L. might be attributed to the fact that C.L. inhibits the growth of follicles and increases their atresia (Hafez, 1993). The functional activity of ovary also had an effect on IVM-IVF and development of follicular oocytes. The maturation rates of oocytes collected from functionally active and inactive ovaries were 76.9 and 7.7 per cent respectively (Im *et al.,* 1995).

Singh *et al.* (2012) examined 367 goat 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 goat oocytes. Study of Mahesh (2012) and Makwana *et al.* (2012) on goat revealed the similar result.

Boonkong *et al.* (2012) collected goat 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 caprine 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). Naby *et al.* (2013) studied 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).

Mondal *et al.* (2008) collected goat ovaries and categorized as corpus luteum (CL) present and absent group. The COCs were harvested by aspiration method and graded as A, B, C and D where grade A and B was considered as normal and C and D as abnormal. They reported that significantly higher ($p<0.05$) number of follicles of 2-6 mm diameter (5.25±0.20) and COCs (1.96±0.09)

was obtained from CL absent group of ovaries than present group (3.94±0.34 and 1.54±0.15 respectively) while no significant variation was found in the number of follicles measuring <2mm and >6mm diameter in CL present and absent group of ovaries. The average number of normal COCs per ovary was significantly higher (p<0.05) in CL-absent group (1.30±0.07) than CL- present group (0.68±0.12) but the average number of abnormal COCs was higher in CL-present group (0.66±0.06) than absent group (0.86±0.11).

Gupta *et al.* (2012) classified the oocytes retrieved from abattoir derived goat 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.

Kumar *et al.* (2004) studied on the ovaries from goats and found that the average diameter of good quality oocytes (categories 1,2) recovered from ovaries without corpora lutea was more as compared to the ovaries with corpora lutea. Kumar *et al.* (2004) concluded that good quality oocytes (category 1, 2) were larger in size as compared to poor qualities (category 3, 4) and can be effectively used for IVF.

Ferdous (2006) was collected COCs by aspiration method and reported that the average number of normal COCs was 1.77 and 2.04 for CL- present and CL-absent group ovaries respectively. Significantly higher number of COCs and follicles of 2-6 mm diameter as well was obtained from CL- absent group of ovaries while no significant variation was found in the number of follicles measuring <2mm and >6mm diameter in CL present and absent group of ovaries. Normal COCs were found to be significantly higher in number of 2-6 mm diameter.

2.3.5 Age of the donor

Izquierdo *et al*. (2002) studied on prepubertal goat oocytes and matured in TCM-199 and reported that no significant differences been found in embryo development between oocytes obtained from prepubertal and adult goats. Thus we can say that there is no effect found of the age of animal on the number and quality of ovine oocytes harvested and maturation, fertilization or cleavage rates (O'Brien *et al.,* 1997). However, a greater number of IVM oocytes developed into blastocysts from adult sheep ovaries, compared to those from lambs. There was, however, no difference in pregnancy rates obtained (O'Brien *et al,* 1997). Oocytes had also been harvested from the ovaries of prepubertal lambs for successful IVM-IVF (Salykbaev *et al.,* 1986; Armstrong *et al.,* 1994).

Oocytes derived from juvenile females show a reduced developmental competence as reported in numerous studies on farm species including bovine (Revel *et al*., 1995; Damiani *et al*., 1996; Khatir *et al*., 1998), ovine (Ledda *et al*., 1997; O'Brien *et al*., 1997b), and porcine (Marchal *et al*., 2002). In caprine, using *in vitro* produced zygotes from laparoscopic ovum pick-up (LOPU), both cleavage and blastocyst development rates of embryos from adult donors have been higher than those from prepubertal donors (90 and 16% vs. 82 and 6%, respectively) (Wang *et al*., 2002a).

Many factors may reduce the development competence of oocytes collected from prepubertal animals. In bovine, the lower developmental ability of oocytes from 3- month-old calves compared with that of cyclic cow oocytes may depend on some defective endocrine environment encountered *in vivo* before the onset of puberty (Revel *et al*., 1995). Gandolfi *et al*., (1998) observed differences in size between calf and cow oocytes (118 μm and 123 μm respectively) and showed that the difference in the developmental competence may be induced because of difference in gene expression abundance between adult and the prepubertal oocytes, showing a reduced

protein synthesis in oocytes and cumulus cells from calves. It has been also reported that in oocytes from prepubertal donors, structural changes are delayed and incomplete and may contribute to failures of appropriate zona pellucida (ZP) changes (reviewed by Slavik *et al*., 2005).

However, other reports suggest that donor age may not be the only important criterion, since oocytes coming from follicles (>3mm diameter) of 45 days old goats have the capacity to *in vitro* develop to blastocyst stage as well as oocytes derived from adult goats (Romaguera *et al*., 2011). Thus, the low embryo development of prepubertal female oocytes could be related to the small number of large follicules in their ovaries, as reported by Martino *et al*., (1994) since only 1.1% of follicles were larger than 3 mm.

For all above mentioned and in order to make use of these prepubertal oocytes more efficiently, it is important to develop culture systems that permit oocytes to acquire the competence for undergoing maturation, fertilization, and development up to blastocyst stage *in vitro* in a similar way than their adult counterparts and those coming from *in vivo*.

2.3.6 Seasonal variation

In ovine, only a limited number of mature oocytes can be collected via superovulation and subsequent ovum pick up (OPU) procedure (Rosa *et al*., 2003; Wani *et al*., 2000; Wang *et al*., 2007). Reproductive seasonality in the ewe was described by Rosa *et al*., (2003) and Harper, (1993).

Khaza (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 goats by scoring method. Statistically the difference was non-significant.

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.

Das *et al.* (2011) studied the oocyte recovery rate per ovary to assess the oocyte potential in goat 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.

The results of a study conducted by Bartlewski *et al*. (2000) have previously showed that the *in vitro* developmental competence of ovine oocytes was influenced by hormonal treatment and the endocrine status of the donor.

On the other hand, Ramsingh *et al.* (2013) reported that ovarian biometrics have a great influence on oocyte grading and recovery rate, and have great fluctuations in goat. The ovarian biometric variations are associated to the breeding, seasonal and nutritional status.

2.4 Conclusion

Ovary is the key organ for reproduction in female. Oocyte plays the vital role in the process. Thus the oocytes along with cumulus cells surrounding it, cumulus-oocyte-complex (COCs), were collected from slaughterhouse, evaluated and graded in laboratory in different study in different species. Different researchers studied on the effects of right and left ovaries on ovary morphology and activity. Presence and absence of corpus luteum found responsible on activity of oocyte. Collection technique has important effects on ovary and oocyte. Effects of hormonal, seasonal and physiological factors as well as age of the animal were recorded on the quality and quantity of oocyte.

CHAPTER III
MATERIALS AND METHODS

The experiment on "Qualitative and Quantitative Assessment on Bovine Oocyte" was conducted at Departmental Laboratory of the Department of Animal Production & Management as well as the laboratory of the Department of Medicine & Public Health and the Department of Animal Nutrition, Genetics & Breeding at Sher-e-Bangla Agricultural University, Dhaka-1207 from January 2019 to December 2019.

3.1 Laboratory Preparation

All the permanent equipments required for the experiment were checked for its viability and accuracy. These were properly installed and/or examined for good condition before use every time. All the reusable equipments were properly washed, sterilized, dried, wrapped with aluminum foil and lastly kept in a cleaned and disinfected chamber until application. All the necessary instruments as well as media, chemicals, reagents were made readily available before beginning the experiments. The lists of above prerequisites are indicated beneath:

3.1.1 Materials

- Phase contrast microscope with USB 2.0 Camera
- Desktop computer
- Slide calipers
- Micropipette
- Digital weighing balance
- Autoclave machine
- Water bath
- Laminar air flow cabinet
- Hot air oven
- Measuring cylinder

- Beaker (250 ml)
- Petri dishes (90 & 60mm)
- Test tube (10 ml)
- Test tube rack
- Conical flask
- Collection vial (for ovary collection)
- Thermo Flask at 25°C to 30°C
- Scissors
- Scalpel
- 10 ml syringes
- 18 G and 19 G needles
- Disinfected rubber gloves
- Face mask
- Glass slide and cover slip

3.2 Preparation before Work

3.2.1 Sterilization procedure in working environment

All procedures were performed in proper sterile condition under laminar air flow cabinet to avoid contaminations. All the glassware was sterilized by hot air oven for at least one hour at 160^0C.

3.2.2 Preparation of COCs collection medium

Dulbecco's phosphate buffered saline (D-PBS) solution was used as COCs collection medium. To prepare the medium, 4gm of NaCl, 0.1gm of KCl, 0.575gm of Na_2HPO4 and 0.1 gm of KH_2PO4 were weighed in the digital balance and taken in a beaker. Then 400ml of distilled water was measured in a measuring cylinder and poured in the beaker. All were mixed thoroughly by a stirrer and finally D-PBS solution was made.

3.2.3 Preparation for ovary collection

0.9% physiological saline of NaCl was prepared to wash the ovary. Prepared saline was sterilized in autoclave and for future use it was kept in refrigerator. 5 lac iu of penicillin and 100mg of streptomycin were mixed with per liter of saline solution on the collection day. During transporting the ovaries from slaughter house to laboratory, the solution was warmed at 25ºC to 30ºC temperature and put in a thermos box to maintain the temperature. Dulbecco's phosphate buffered saline (D-PBS) solution was also made and sterilized in autoclave. Then it was stored in a refrigerator for further use (Plate 3.1).

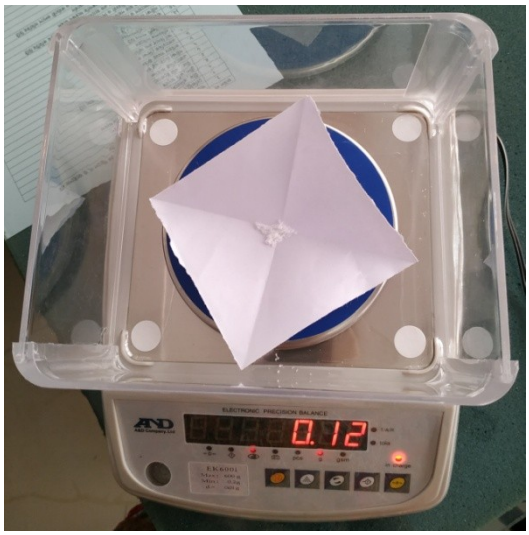


Plate 3.1: Measurement of chemicals to prepare collection medium

3.3 Collection and Processing of Ovaries

At the early morning, ovaries along with female genital tract from sexually matured cows were collected within 30 min of slaughter from the slaughter house at Geneva Camp, Mohammadpur, Dhaka (Plate 3.2 A). They were then transported within 2 hours of slaughter to the laboratory in a vacuum flask containing sterilized phosphate buffered saline (PBS) (pH 7.35) supplemented with 100 IU penicillin G and 100 mg/ml streptomycin at 25-30°C.

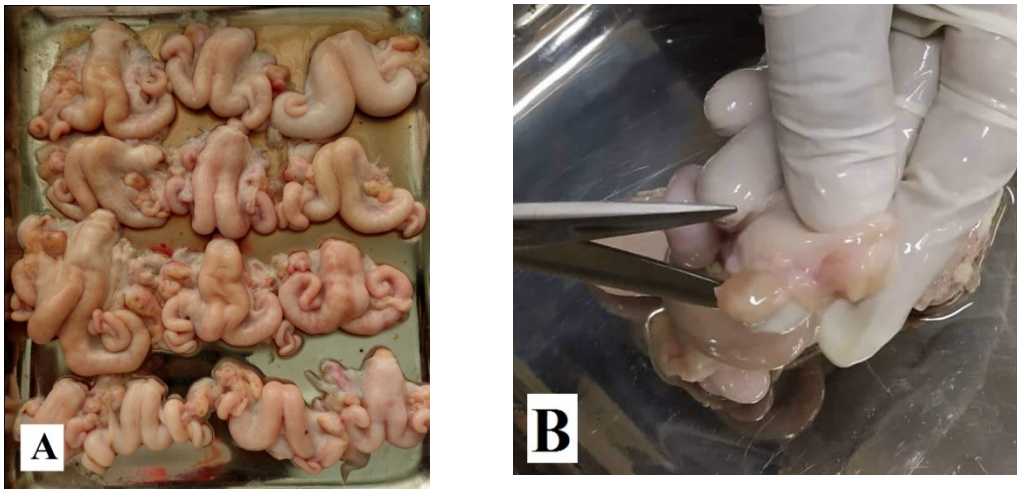


Plate 3.2: A. Collected reproductive tracts, B. Trimming of ovary

At the laboratory, ovaries were separated from other parts by cutting. All the excess materials like adipose tissue, surrounding bursa etc were removed from ovarian surface (Plate 3.2 B). According to Wani *et al.,* (2000) each ovary was washed five times i.e. three washings in D-PBS and two washings in oocytes harvesting medium (DPBS+4mg/ml BSA+1.50 IU/ml Penicillin). After all five washings, ovaries were transferred to sterilized petri dishes and rinsed thoroughly by physiological saline at 25°C before further processing as described by Haque *et al*., 2016.

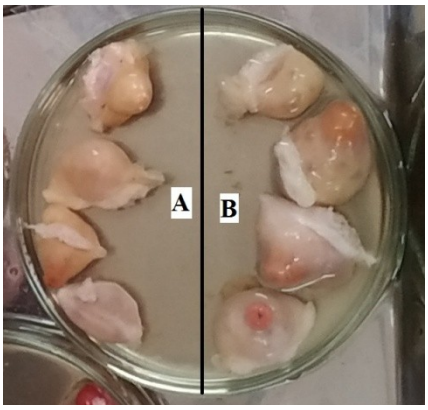


Plate 3.3: Right (A) and left (B) ovaries

3.4 Categories of ovary

Right and left ovaries were kept in different petri dishes (Plate 3.3). Then the ovaries were divided into two groups, i.e. the ovaries with and without a corpus luteum (Plate 3.4) to investigate the influence of the orientation as well as the corpus luteum on the quantity and the quality of COCs recovered per ovary. COCs recovered from each ovary of the two ovary groups were recorded. Some of them were usable and some of them were not.

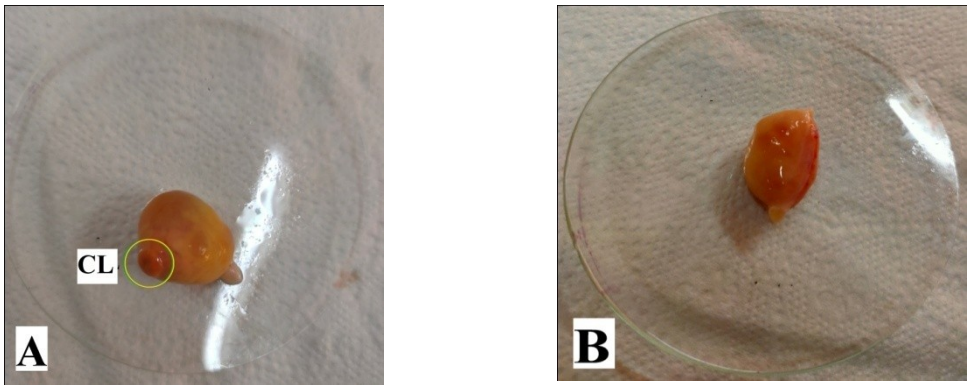


Plate 3.4: Ovaries with (A) and without (B) CL

3.5 Measurement of weight, length and width of ovaries

All the ovaries were weighed individually irrespective of grouping in g in a digital balance (Plate 3.5).

Plate 3.5: Measurement of weight of ovary

Then the length (Plate 3.6 A) and width (Plate 3.6 B) of each ovary were measured in cm with the help of a slide calipers. All data were recorded in tabular form individually and separately.

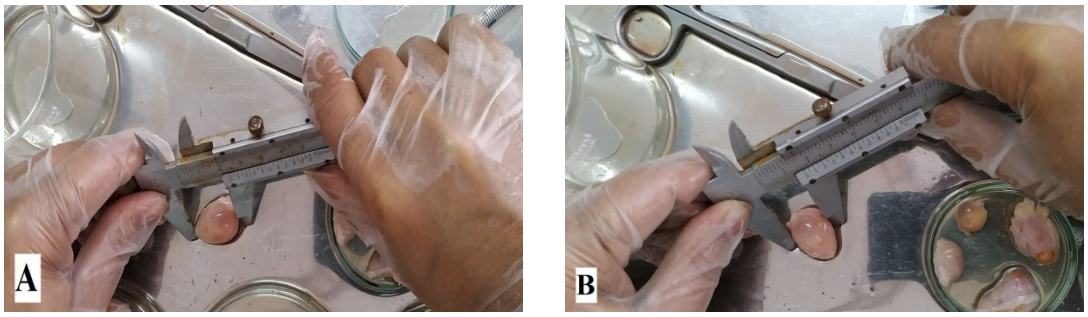


Plate 3.6: Measurement of length (A) and width (B) of ovary

3.6 Counting of follicle on the surface of the ovary

Ovarian surface contains many follicles of different sizes. All the visible follicles were counted manually irrespective of grouping i.e. with or CL, left or right ovary (Plate 3.7). Number of follicles on each ovarian surface was recorded individually in tabular form.

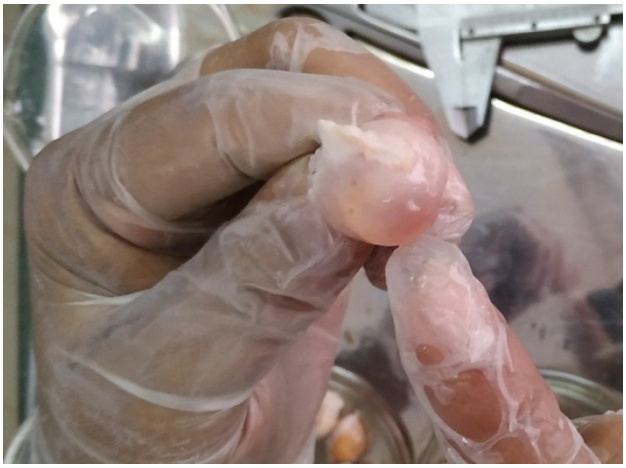


Plate 3.7: Counting of follicle from ovary surface

3.7 Oocytes collection and COCs evaluation

The ovaries were washed 2-3 times in saline solution at 30°C. They were then placed in a beaker and kept in a water bath at 30°C. After fundamental washing, each ovary was treated individually and the oocytes harvested by aspiration techniques (Plate 3.8) as illustrated by Wani *et al*., 2000. Each ovary was individually handled, and oocytes were recovered by following method.

Plate 3.8: Collection of oocyte by aspiration method

The ovary parenchyma near the vesicular follicles (2 to 6 mm diameter) and all 2 to 6 mm diameter follicles were aspirated near the point at the same time with 22G hypodermic needle fixed to 5 ml disposable syringe containing 1-2 ml of D-PBS. The cattle oocyte was aspirated from individual ovary and placed in petri dish containing 1 ml of D-PBS. Number of collected oocytes were recorded after grading.

3.8 Microscopic study and grading of COCs

The petri dish was kept undisturbed for 5 minutes after collection to settle down the oocytes. Unexpected media was discarded by using a syringe without hampering the oocytes at the bottom of the petri dish. The 10 ml syringe was loaded with D-PBS (1.0 -1.5 ml), and the needle (18G) was used to transfer oocytes from petri dish to glass slide. Then it was observed under an inverted

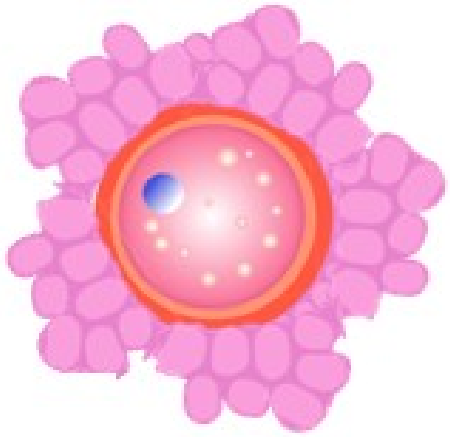
digital microscope at 10x magnification and the total number of harvested oocytes were counted (Plate 3.9).

Plate 3.9: Microscopic examination of oocyte

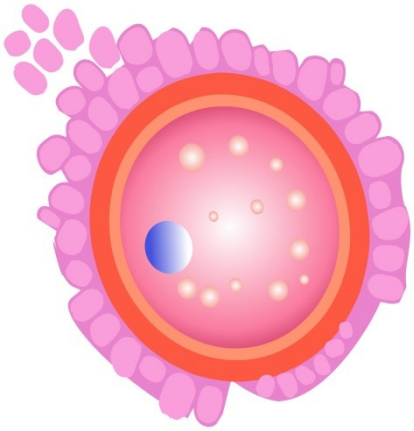
According to Khandoker *et al*. (2001), the COCs were classified into four grades on the basis of cumulus cells and nucleus (Plate 2.1).

- **Grade A:** Oocytes completely surrounded by cumulus cells
- **Grade B:** Oocytes partially surrounded by cumulus cells
- **Grade C:** Oocytes not surrounded by cumulus cells
- **Grade D:** Degeneration observed both in oocytes and cumulus cells

The grade A and B were considered as normal COCs where oocyte was surrounded by cumulus cell. On the other hand, the grade C and D were considered as abnormal COCs where oocytes was not surrounded by cumulus cell. The number of different grades of COCs in each category noted.



Grade A



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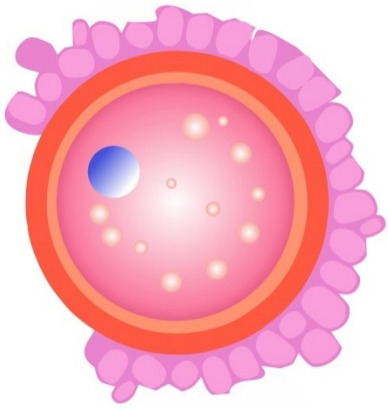
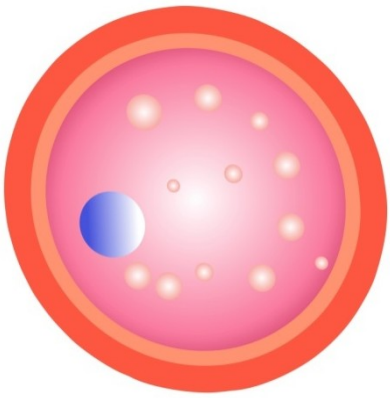
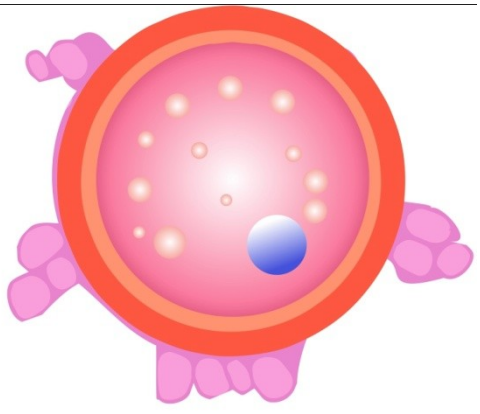


Grade C



Grade D



Grade E

Grade F

Plate 3.10: Graphical representation of oocytes grading adopted from Khandoker *et al*. (2001)

3.9 Statistical analysis

All values were expressed as (Mean±SE). Statistical significance of differences between different parameters was evaluated by using student's t-test. The statistical analysis was done by SPSS program (Version 16.0; SPSS Inc., Chicago, IL, USA).

CHAPTER IV

RESULTS AND DISCUSSION

The present work on assessment of bovine oocyte was conducted at the Department of Animal Production & Management, Sher-e-Bangla Agricultural University, Dhaka. The result of the different parameters is summarized in this chapter.

4.1 Gross Study of the Ovary

Almond-shaped, pale colored ovaries were found at the edge of the mesovarium near the lateral margin of the pelvic inlet (Plate 4.1). May (1970) was reported like this. His report also supported this study in the statement that, follicles of different sizes projected from the surface of each ovary to make the surface irregular. The length of right ovary was numerically higher than that of left ovary (Table 4.1).

The uterine extremity of the ovaries was connected with the extremity of the horn of uterus by a proper ligament of the ovary. There was no demarcation between the horn of the uterus and the flexuous uterine tubes.

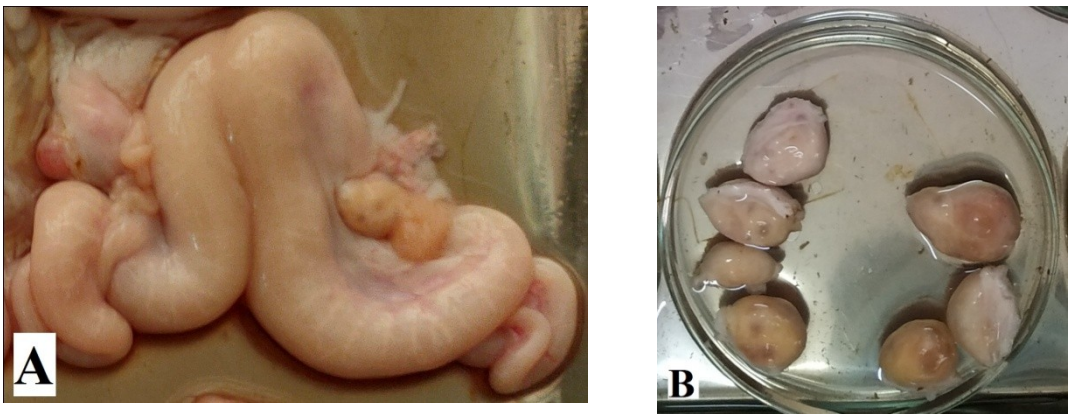


Plate 4.1: Reproductive tract of cow (A) and collected ovaries (B)

4.2 Ovarian Categories Regarding Right and Left Ovary

4.2.1 Ovarian categories in respect to morphology of ovary

Different parameters of the morphology of the ovaries under the current study were found significantly different between right and left ovaries ($p<0.05$). Higher mean weight (g) (3.47±0.34 vs 2.44±0.32), length (cm) (1.94±0.06 vs 1.71±0.07) and width (cm) (1.36±0.06 vs 1.09±0.05) were found in right ovaries than that of left ovaries (Table 4.1 and Figure 4.1).

Right ovaries are more active than left ovaries. Thus they are much bigger than left part in terms of length and width as well as weight (Dangudubiyyam and Ginther, 2019; Rahman *et al*., 1977 and Sarkar, 1993. Similar results were found in goat (Islam *et al*., 2007).

Table 4.1: Ovarian categories in respect to morphology of right and left ovaries

Ovarian categories (*n*)	Weight (g) (mean±SE)	Length (cm) (mean±SE)	Width (cm) (mean±SE)
Right (30)	3.47^{a}±0.34	1.94^{a}±0.06	1.36^{a}±0.06
Left (30)	2.44^{b}±0.32	1.71^{b}±0.07	1.09^{b}±0.05
	*	*	*

Mean values in the same column with different superscripts (a, b) differ significantly at $p<0.05$.
Figure in the parenthesis indicates the total number of ovaries.
n= number of ovary
*= significant, NS= Non significant

But the results of some previous study, Singh *et al.* (1974) did not support this result. They claimed that, there was no significant difference ($p<0.05$) in the parameters of left and right ovaries of goat. Asad *et al.* (2016) found significantly ($p<0.05$) larger right ovaries in terms of length in goat whereas weight and width were found insignificant. Schneebeli and Döbeli, 1991 and Pierson and Ginter, 1987 stated that, ovaries on the right side are bigger and more active in cows.

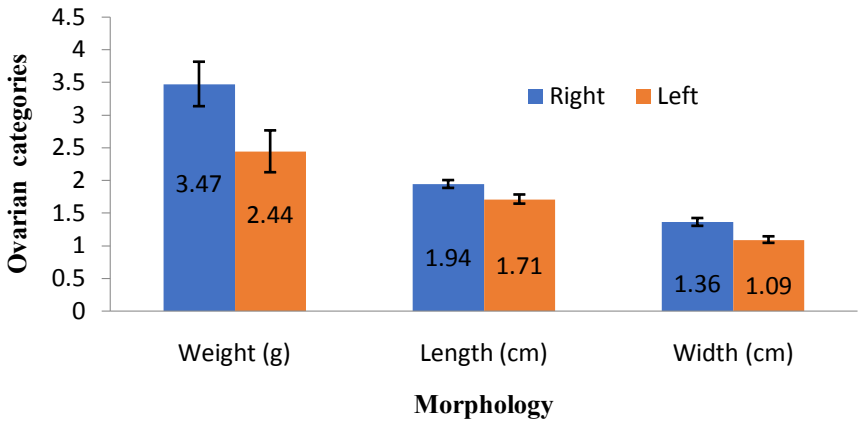


Figure 4.1: Ovarian categories of cow in respect of morphology of right and left ovaries

4.2.2 Ovarian categories in respect to presence and absence of CL

Significant variation was found on corpus luteum (CL) in terms of presence or absence in left and right ovaries (Figure 4.2). A total of 20 ovaries were found with presence of corpus luteum among the total of 60 ovaries. In which, higher portion (24, 70%) was from right ovaries in comparison to left (6, 30%) ovaries. On the other hand, higher without CL ovaries were found in left (16, 60%) than right (14, 40%) among total 40 without CL ovaries.

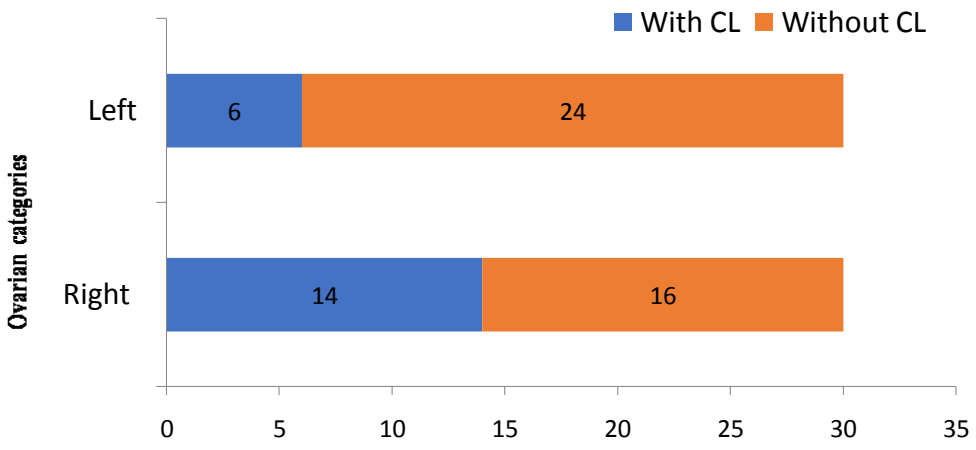


Figure 4.2: Ovarian categories of cow on with or without CL in left and right ovaries

As the right ovaries are more active than the left, right ovaries produce more ova than left. So, more ovulation as well as more CL is a normal phenomenon.

The result was similar to Reece and Turner, 1954; Spriggs, 1945; Erdheim, 1942; Schram, 1937; Clark, 1936 and Casida *et al.,* 1935.

4.2.3 Ovarian categories in respect to number of follicles

Variation on number of follicles (total and aspirated) was not significant in terms of follicles count in left and right ovaries (Table 4.2 and Figure 4.3). The highest number of total follicles was observed in right ovary (6.60±0.63) with highest aspirated follicles (5.00±0.48). Again, the lowest total follicles count was found in left ovary (6.13±0.68) with lowest aspirated follicles (4.33±0.47). Number of follicles as well as aspired follicle count was higher in right ovary than left numerically but they was no statistically significant difference ($p<0.05$).

Table 4.2: Ovarian categories in respect to follicle and COCs in right and left ovaries

Ovarian categories (*n*)	Total number of follicles (mean±SE)	Number of follicles aspirated (mean±SE)	Collected COCs per Ovary (mean±SE)		
			Normal	Abnormal	Total
Right (30)	6.60^{a}±0.63 (198)	5.00^{a}±0.48 (150)	0.67^{a}±0.15 (20)	0.73^{a}±0.14 (22)	1.40^{a}±0.21 (42)
Left (30)	6.13^{a}±0.68 (184)	4.33^{a}±0.47 (130)	0.97^{a}±0.16 (29)	0.63^{a}±0.13 (19)	1.60^{a}±0.18 (48)
	NS	NS	NS	NS	NS

Mean values in the same column with different superscripts (a, b) differ significantly at $p<0.05$.
Figure in the parenthesis indicates the total number of ovaries.
n= number of ovary
*= significant, NS= Non significant

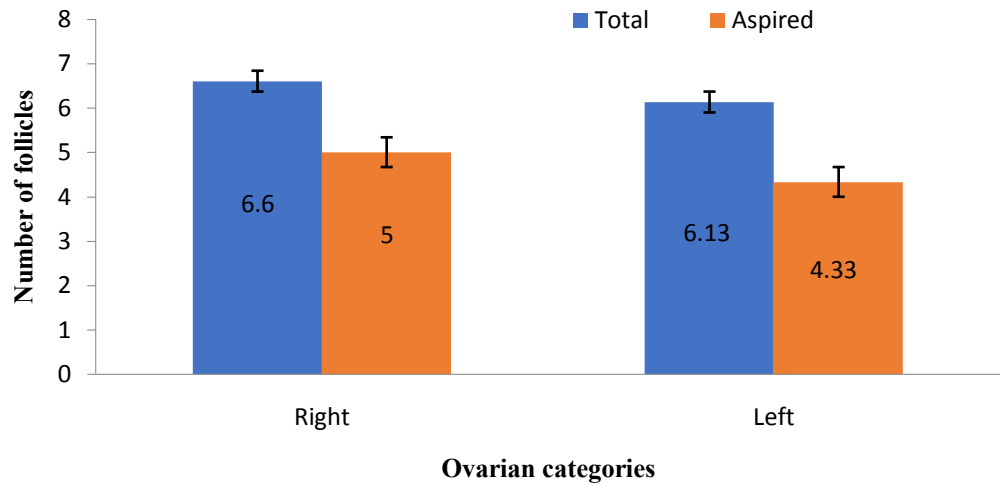


Figure 4.3: Ovarian categories of cow in respect of number of follicles in total and aspirated in right and left ovaries

Right ovaries are more active than left according to Stalfors (1916) who found the right ovary to be more active than the left one. So finding of higher number of follicles in right ovary is a normal phenomenon.

The result of the study supported by the findings of Casida *et al.,* 1948; Nielsen, 1949; Rajakoski, 1960; Kidder *et al.,* 1952; Lagerlof and Boyd, 1953; Perkins *et al.,* 1954 and Morrow *et al.,* 1968.

4.2.4 Grading of COCs

Grading of COCs was done on the basis of cumulus cell diameter. Khandoker *et al*. (2001) classified the COCs into four grades on the basis of cumulus cells and nucleus. Oocytes completely and partially surrounded by cumulus cells were graded as Grade A and Grade B respectively and commonly as normal oocytes. Whereas, oocytes not surrounded by cumulus cells were classified as Grade C and degenerated oocytes and cumulus cells were classified into Grade D. Last two are commonly named as abnormal oocyte (Plate 4.2).

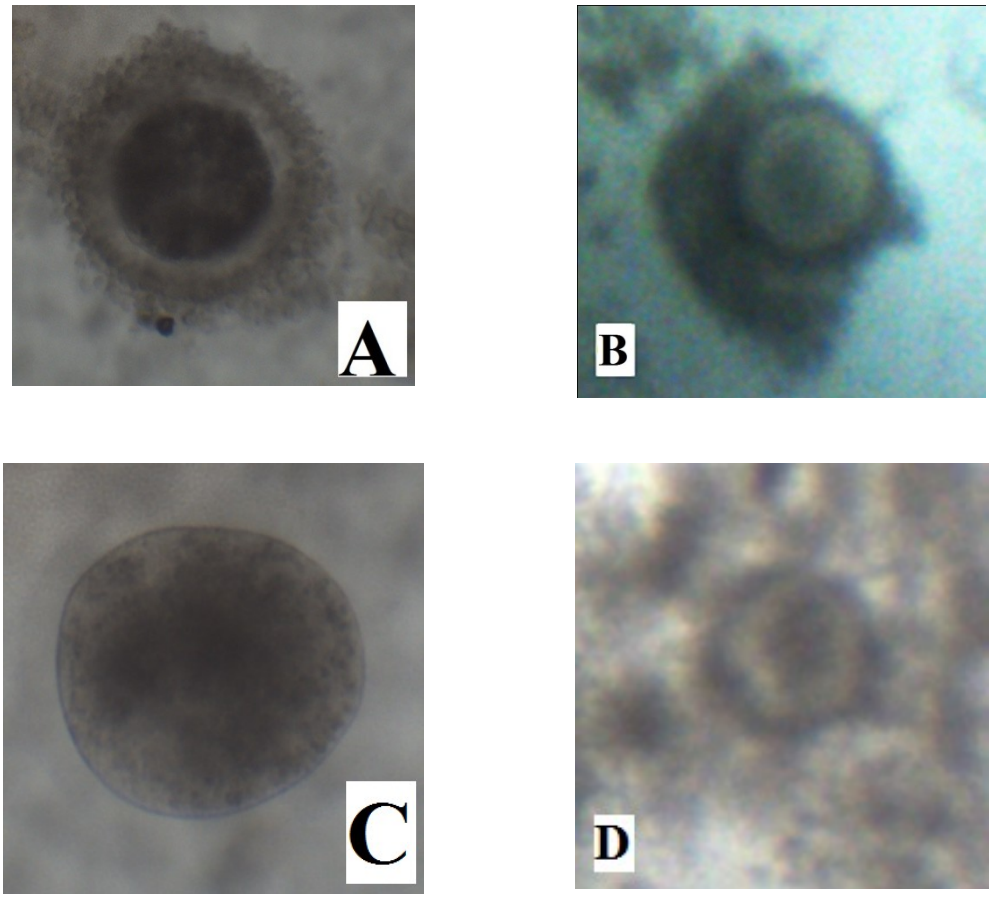


Plate 4.2: Normal oocyte [Grade-A (A) and Grade B (B)] and abnormal oocyte [Grade C (C) and Grade D (D)]

Presence of total COCs with normal and abnormal was not significant in left and right ovaries (Table 4.2 and Figure 4.4). The mean count of normal and total COCs was higher in left ovaries than that of right ovaries numerically. On the other hand, abnormal COCs were higher in right ovaries. But the differences were not statistically significant in cattle ($p<0.05$). The average number of normal and abnormal COCs of right ovary was recorded to be 0.67±0.15 and 0.73±0.14 respectively with a total of 1.40±0.21, similarly the left ovary possessed 0.97±0.16 and 0.63±0.13 normal and abnormal COCs with a total of 1.60±0.18.

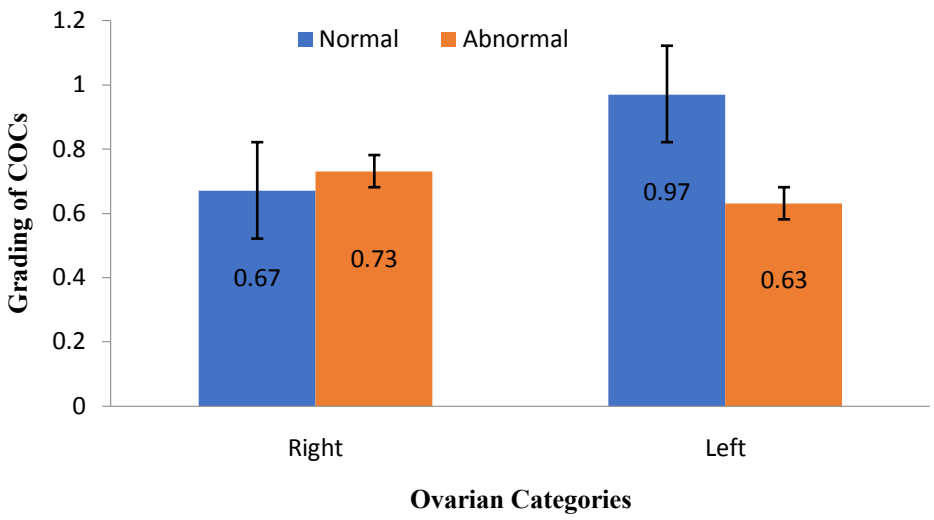


Figure 4.4: Ovarian categories of cows on grading of COCs in right and left ovaries

The cumulus cells (CCs) surrounding the oocytes plays a key role in oocyte maturation and they are known to supply nutrients and energy substrates (Sutton *et al*., 2003).

This result supports the previous result of Khandoker *et al.* (2011) who reported that the collected normal COCs were higher in left ovaries (2.42 ± 0.14 per ovary) compared to right ovaries (2.32 ± 0.12 per ovary).

The result of the study also supports the report of the study of Patra *et al.* (2013) and Islam (2005).

4.3 Ovarian Categories Regarding with or without CL

4.3.1 Ovarian categories in respect to morphology of ovary

Different parameters of the morphology of the ovaries under the current study were found significantly different between presence and absence group of ovaries ($p<0.05$). Higher mean weight (g) (3.81±0.38 vs 2.53±0.29), length (cm) (2.04±0.07 vs 1.72±0.06) and width (cm) (1.45±0.06 vs 1.11±0.06) were found in with-CL ovaries than that of without-CL ovaries (Table 4.3 and Figure 4.5).

Table 4.3: Ovarian categories in respect to morphology of with and without CL ovaries

Ovarian categories (*n*)	Weight (g) (mean±SE)	Length (cm) (mean±SE)	Width (cm) (mean±SE)
With CL (20)	3.81^{a}±0.38	2.04^{a}±0.07	1.45^{a}±0.06
Without CL (40)	2.53^{b}±0.29	1.72^{b}±0.06	1.11^{b}±0.05
	*	*	*

Mean values in the same column with different superscripts (a, b) differ significantly at $p<0.05$.
Figure in the parenthesis indicates the total number of ovaries.
n= number of ovary
*= significant, NS= Non significant

After ovulation, if the ovum fails to contact with sperm a fleshy structure is formed known as corpus luteum, which might give rise to the weight, length and width of ovaries. This result supports the previous result of Sarkar *et al.* (1993), Rahman *et al.* (1977) and Singh *et al.* (1974). Asad *et al* (2016) found significantly ($p<0.05$) higher width and weight in with CL group whereas length was found insignificant.

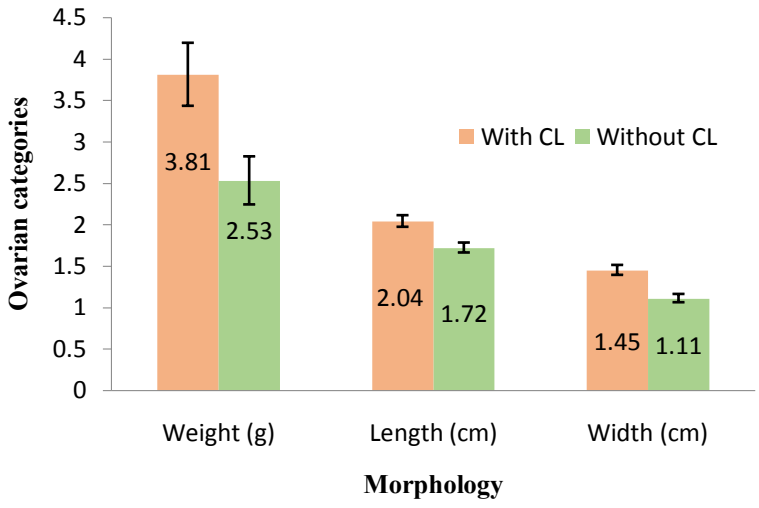


Figure 4.5: Ovarian categories of cow in respect of morphology in with and without CL ovaries

The result is very usual as the hypertrophy of luteinized granulosa cells, hyperplasty of fibroblasts of the connective tissues and vascularity contribute to an increase in size of the CL (Jablonka-Shariff *et al*., 1993). The maximum diameter of CL is reached 6~9 days after ovulation and then regression starts between days 13 and 16 if maternal recognition does not occur (Jablonka-Shariff *et al*., 1993).

4.3.2 Ovarian categories in respect to presence and absence of CL

A total of 20 ovaries were found with presence of corpus luteum among 60 ovaries. Significant variation was found on corpus luteum (CL) in terms of presence or absence (Figure 4.6). Results showed that the CL was present in 33% ovaries where CL was absent in 67% ovaries. 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.

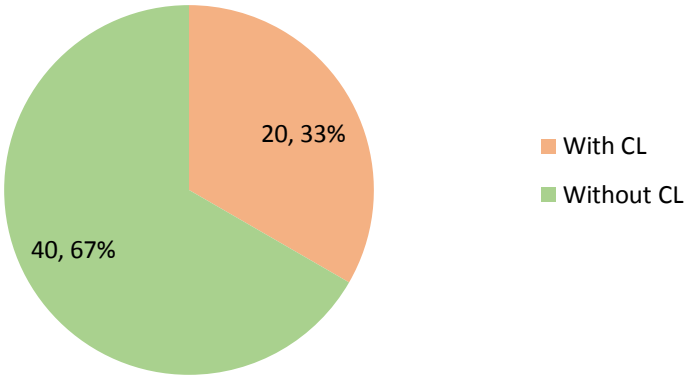


Figure 4.6: Ovarian categories of cow in respect of with and without CL ovaries

It is caused due to the less reproductive performer cows 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 due to absence of hormonal influence during estrus cycle.

Islam (2019) found the similar result in buffalo whereas Hoque (2009); Ferdous (2006) and Islam (2005) found in goat. All of them studied on slaughterhouse derived ovaries of respective species.

4.3.3 Ovarian categories in respect to number of follicles

Variation on number of total follicles was significant in terms of follicle count in with and without CL ovaries (Table 4.4 and Figure 4.7). The highest number of total follicle was observed in without CL ovary (7.03±0.58) with highest aspirated follicles (5.05±0.43). The difference was statistically significant ($p<0.05$). Again, the lowest total follicles count was found in with CL ovary (5.05±0.66) with lowest aspirated follicles (3.90±0.50). Aspired follicle count was higher in without CL ovary than with CL numerically but they were not statistically significant ($p<0.05$).

Table 4.4. Ovarian categories in respect to follicle and COCs in with and without CL ovaries

Ovarian categories (*n*)	Total number of follicles (mean±SE)	Number of follicles aspirated (mean±SE)	Collected COCs per Ovary (mean±SE)		
			Normal	Abnormal	Total
With CL (20)	5.05^{a}±0.66 (101)	3.90^{a}±0.50 (78)	0.75^{a}±0.19 (15)	0.70^{a}±0.18 (14)	1.45^{a}±0.27 (29)
Without CL (40)	7.03^{b}±0.58 (281)	5.05^{a}±0.43 (202)	0.85^{a}±0.13 (34)	0.68^{a}±0.12 (27)	1.53^{a}±0.16 (61)
	*	NS	NS	NS	NS

Mean values in the same column with different superscripts (a, b) differ significantly at $p<0.05$.
Figure in the parenthesis indicates the total number of ovaries.
n= number of ovary
*= significant, NS= Non significant

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).

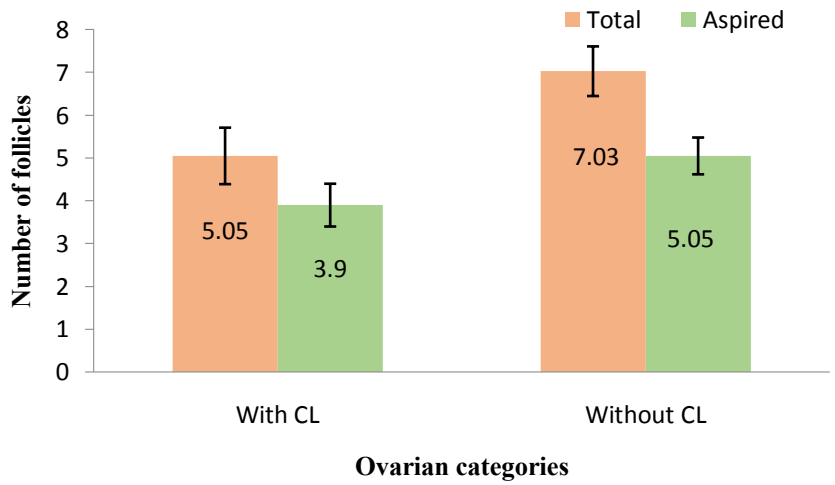


Figure 4.7: Ovarian categories of cow in respect of number of follicles in total and aspirated in with and without CL ovaries

As follicle bears oocyte, so we can say that more follicle contains more oocyte and less follicle contains less oocyte. According to Nandi *et al*. (2000) the oocyte recovery rate decreased when ovaries had a corpus luteum. This is because follicular development is restricted, as lutein cells occupy most of the ovary (Kumar *et al*., 2004). The dominant follicle is usually observed in the corpus luteum-bearing ovary, and the other follicles are very small and remain mostly inaccessible (Gasparrini *et al.,* 2000). Cow (Moreno *et al.,* 1993) and goat (Agrawal *et al.,* 1995) ovaries containing a corpus luteum yielded a lower number of oocytes than ovaries without a corpus luteum. Several researchers have reported that the presence of a corpus luteum yields a lower number of oocytes per ovary and a lower proportion of usable oocytes (Moreno *al.,* 1993). In contrast, Boediono *et al*., (1995) and Das *et al.,* (2010) found no difference in the mean number of oocytes per ovary between corpus luteum-bearing and non-bearing ovaries.

4.3.4 Grading of COCs

Grading of COCs was done in two types as normal and abnormal. Presence of total COCs with normal and abnormal was not significant in with and without CL ovaries (Table 4.4 and Figure 4.8). The mean count of normal and total COCs was higher in without CL ovaries than that of with CL ovaries numerically. On the other hand, abnormal COCs were higher in with CL ovaries. But the differences were not statistically significant in cattle ($p<0.05$). The average number of normal and abnormal COCs of with CL ovary was recorded to be 0.75±0.19 and 0.70±0.18 respectively with a total of 1.45±0.27, similarly the without CL ovary possessed 0.85±0.13 and 0.68±0.12 normal and abnormal COCs with a total of 1.53±0.16. Those results support the previous study of Mondal *et al.,* (2008).

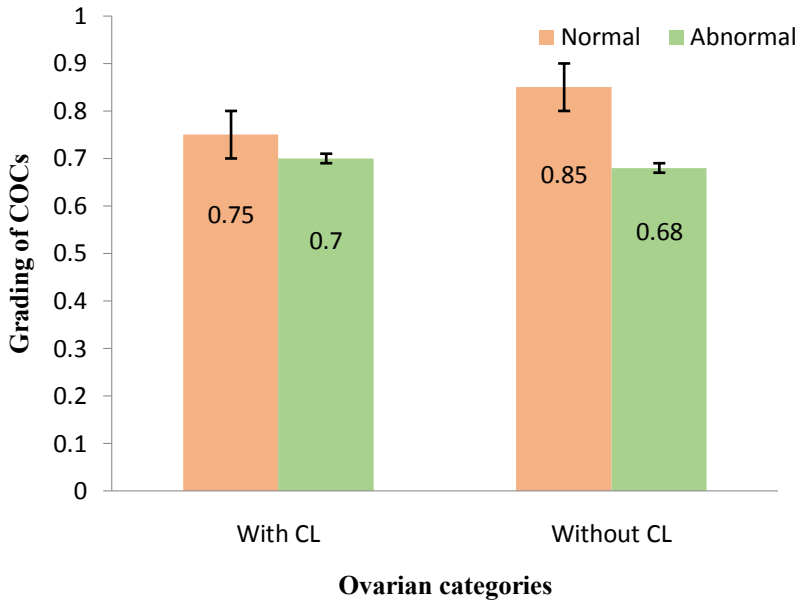


Figure 4.8: Ovarian categories of cows on grading of COC's in with and without CL ovaries

The presence of CL in cyclic female's ovary produces a higher level of progesterone hormone that signals negative response to anterior pituitary gland for the restriction of gonadotrophin secretion and ultimately follicular degeneration occurs by Webb *et al*. (1999). But due to the absence of CL in non-cyclic female, the negative effect of progesterone might not be functional and estrogen-progesterone remains in balanced level which allows follicular growth and oocyte maturation. The higher number of COCs in ovaries without CL than that of ovaries with CL as found in this study explains the role of hormonal balance on goat folliculogenesis.

The findings of with CL group of ovaries explain the role of progesterone on cattle follicular degeneration and further strengthening the previous statement. The results of the number of total and normal COCs per ovary strongly supports the result of the study of Mondal *et al.* (2008), who reported that significantly higher (p<0.01) number of total COCs (3.94 vs 1.30 per ovary) and normal COCs (1.54 vs 0.68 per ovary) were found in without CL group

than those of with CL group of ovaries. In addition, significantly higher (p<0.05) number of abnormal COCs per ovary in with CL group (0.86) than absent group (0.66) as reported by Mondal *et al.,* (2008) is also similar to the present results. Ferdous (2006) reported that normal COCs were found to be significantly higher (p<0.05) in 2-6 mm diameter. Since Mondal *et al.,* (2008) collected COCs by aspiration of 2-6 mm diameter follicles, they obtained lower number of abnormal COCs.

According to Webb *et al.* (1999), growth initiation of follicles has variously been attributed to i) hormonal triggers (gonadotropins). ii) stochastic processes (fluctuation in internal signal molecule) and iii) external inhibitory control from growing follicles. Changes in the local microenvironment such as the pH and hormonal concentration probably occur as the follicles evolve into the primary stage but these are probably effects in the process rather than the causes. The higher number of follicles that were found in without CL group of ovaries in the present study, might reflect the optimum level of gonadotropins and steroids.

Above all, the number and quality of COCs recovered per ovary is a significant consideration for *in vitro* maturation (IVM) and *in vitro* fertilization (IVF) of COCs, *in vitro* production (IVP) of embryos, multiple ovulation and embryo transfer (MOET).

CHAPTER V
SUMMARY AND CONCLUSION

Oocyte is one of the key parts of the reproduction. Female animal provides ovum in fertilization that is the developed and modified state of oocyte. Selection of perfect oocyte is of higher importance. Oocytes are produced and developed in ovary. As oocytes are microscopic structure, we can judge and select ovary as material for reproduction. So, the current research was carried out by the Department of Animal Production and Management, Sher-e-Bangla Agricultural University, Dhaka with a view to finding out the best oocyte in terms of quality and quantity and also to evaluate the slaughter house cattle ovaries and COCs depending on some parameters.

Cattle ovaries were collected from the slaughter house of Geneva Camp, Mohammadpur, Dhaka. After trimming and necessary processing, ovaries were categorized as right, left, with corpus luteum (CL) and without CL group. Ovaries were then evaluated morphologically on the basis of length (cm), width (cm) and weight (g). Quality of the ovary was evaluated in terms of total number of follicles on the surface of each categorized ovaries, number of follicles aspirated, total number of COCs collected, normal COCs and abnormal COCs. Then ovaries were graded regarding the size of cumulus cell diameter and expressed as normal and abnormal.

The study revealed that, right ovary of cattle is more active and morphologically bigger than that of left ovary. Significantly ($p<0.05$) higher mean weight (g) (3.47±0.34 vs 2.44±0.32), length (cm) (1.94±0.06 vs 1.71±0.07) and width (cm) (1.36±0.06 vs 1.09±0.05) were found in right ovaries than left.

One third of the ovaries were containing CL. Among them, 47% (14 out of 30) of right and 20% (6 out of 30) of left ovaries were found with CL. Rest of the ovaries were having no CL. Thus we can say that, more ovulation was occurred from right ovary.

Right ovary (6.60±0.63) contained higher number of follicles on its surface than left (6.13±0.68). Number of aspired follicle was also higher in right ovary (5.00±0.48) than left (4.33±0.47).

Numerically higher number of normal COCs was found in left ovaries (0.97±0.16) than right ovaries (0.67±0.15). Similarly, total number of COCs was found in left ovaries (1.60±0.18) than right (1.40±0.21). On the contrary, abnormal COCs were higher in right ovaries (0.73±0.14) than left ovaries (0.63±0.13). But it was not statistically significant.

On the other hand, in comparison between the ovaries with and without CL group, the result obtained from this experiment showed significantly ($p<0.05$) higher mean weight (g) (3.81±0.38 vs 2.53±0.29), length (cm) (2.04±0.07 vs 1.72±0.06) and width (cm) (1.45±0.06 vs 1.11±0.05) of with CL ovaries than that of without CL group. Addition of an extra fleshy part i.e CL causes difference in morphology.

Observed follicle on the surface of the ovary was significantly higher in without CL group (7.03±0.58) compared to with CL group (5.05±0.68) due absence of hormonal influence. The average number of normal as well as total COCs of with CL ovary (0.75±0.19 and 1.45±0.27 respectively) was lower than that of without CL (0.85±0.13 and 1.53±0.16 respectively) but they are not statistically significant.

Finally, according to above discussion, it can be concluded that, left ovaries contain more normal COCs and lower number of follicles than right ovaries and without CL ovaries contain higher number of follicles and normal COCs than with CL ovaries. Higher number of normal COCs recovered in left ovaries without CL of slaughter house cattle. So, left ovaries having no corpus luteum are suitable for obtaining good quality cumulus-oocyte-complexes (COCs) in experiment for IVM and might for IVF and subsequent IVC.

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.

Agarwal, K. P., Sharma, A., Sexana, C. and Sharma, N. (1995). Chronology of first meiotic events of caprine oocytes matured *in-vitro. Indian J. of Anim. Sci.* **65**:285- 88.

Ahmed, N., Demaine, H. and Muir, J. F. (2008). Freshwater prawn farming in Bangladesh: history, present status and future prospects. *Aquacult. Res.* **39(8)**:806-819.

Ali, A., Coenen, K., Bousquet, D. and Sirard, M. A. (2004). Origin of bovine follicular fluid and its effect during in vitro maturation on the developmental competence of bovine oocytes. *Theriogenology.* **62(9)**:1596- 606.

Ali, Z., and Hossain, I. (2014). Barriers to the Development of Livestock Sub-sector in Bangladesh. **In:** BIDS Policy Brief. BIDS, Dhaka, Bangladesh. Number **1408**:1-8.

Amin, M. R., Salim, M.S., Khandoker, M. A. M. Y. and Hossain, M. M. (2005). Causes of infertility in Black Bengal does investigated by anatomical and histological tools. *Progress. Agric.***16(2)**: 117-124.

Anguita, B., Jiménez-Macedo, A., Izquierdo, D., Mogas, T. and Paramio, M. (2007). Effect of oocyte diameter on meiotic competence, embryo development, p34 (cdc2) expression and MPF activity in prepubertal goat oocytes. *Theriogenology*. **67(3)**:526-536.

Arlotto, T., Leibfried-Rutledge, M. L., & First, N. L. (1992). Correlation of oocyte diameter with time of maturation in bovine oocytes. *Biol. Reprod*., **46(Suppl. 1)**:63.

Arlotto, T., Schwarctz, J. and First, N. (1996). Aspect of follicle and oocyte stage that affect *in vitro* maturation and development of bovine oocytes. *Theriogenology*. **45**: 943-956.

Armstrongs D. T., Irvine, B. and Earl, C. R. (1994). *In vitro* fertilization of follicular oocytes from juvenile lambs and their developmental competence *in vitro* and *in vivo*. **In**: *Biology of Reproduction.* 1603 Monroe ST, Madison, WI 53711-2021: SOC Study Reproduction. Vol.**50**: 189.

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.

Baldassarre, H., Fumus, C. C, Matos, D. G., Pessi, H. and De-Matos, D. G. (1996). *In vitro* production of sheep embryos using laparoscopic folhculocentesis: alternative gonadotrophin treatment for stimulation of oocyte donors. *Theriogenology.* **45**:707-717.

Bartlewski, P. M., Vanderpol, J., Beard, A. P., Cook, S. J. and Rawlings, N. C. (2000). Ovarian antral follicular dynamics and their associations with peripheral concentrations of gonadotropins and ovarian steroids in anoestrous Finnish Landrace ewes. *Anim. Reprod. Sci.* **58(3-4)**:273–91.

Boediono, A., Rajamahendran, R., Saha, S., Sumantri, C. and Suzuki, T. (1995). Effect of the presence of a CL in the ovary on oocyte number, cleavage rate and blastocyst production in vitro in cattle. *Theriogenology*, **43**:169.

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.

Britt, J. H. (2008). Oocyte development in cattle: physiological and genetic aspects. *Revista Brasileira de Zootecnia*, **37(spe):**110-115.

Casida, L. E., Chapman, A. B. and Rupel, I. W. (1935). Ovarian development in calves. *J. Agricult. Res.* **50:**953-960.

Casida, L. E., Heizer, E. E. and Barrett, G. R. (1948). Most calves develop in right horn. Annual report of the director, Wisconsin *Agr. Exp. Sta. Ann., Rpt., Bul.* **480**:59.

Cetica, P. D., Dalit, G. C. and Beconi, M. T. (1999). Study of evaluation criteria used for *in vitro* bovine oocyte selection and maturation. *Biocell: official journal of the Sociedades Latinoamericanas de Microscopia Electronica* **23(2)**:125-133.

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.

Clark, C. F. (1936). Does the right ovary of the bovine function more frequently than the left. *J. Am. Vet. Med. Assoc.* **88:**62-65.

Cognié, Y., Benoit, F., Poulin, N., Khatir, H. and Driancourt, M.A., (1998). Effect of follicle size and of the Fec (B) Booroola gene on oocyte function in sheep. *J Reprod Fertil.***112(2)**:379-386.

Colvin, C. W. and Abdullatif, H. (2013). Anatomy of female puberty: The clinical relevance of developmental changes in the reproductive system. *Clin Anat.* **26(1):** 115–129.

Cox, J. F., Hormazabal, J. and Maria, S. A. (1993). Effect of the cumulus on *in vitro* fertilization of bovine matured oocytes. *Theriogenology* **40(6)**:1257-1267.

Crosby, I. M., Osbom, J. C. and Moor, R. M. (1981). Follicle cell regulation of protein synthesis and developmental competence in sheep oocytes. *J. of Rep. and Fer*. **62:**575-582.

Crozet, N., Ahmad-Ali, M. and Dubos, M. P. (1995). Developmental competence of goat oocytes from follicles of different size categories following maturation, fertilization and culture *in vitro*. *Reproduction*, **103(2)**:293- 298.

Damiani, P., Fissore, R., Cibelli, J., Long, C., Balise, J., Robl, J. and Duby, R. (1996). Evaluation of developmental competence, nuclear and ooplasmic maturation of calf oocytes. *Mol. Rep. and Dev.* **45(4):**521-534.

Dangudubiyyam, S. V., and Ginther, O. J. (2019). Relationship between more follicles in right than left ovary in recently born calves and right ovary propensity for ovulation in cattle. *Reproductive Biology*, **19(4):**363-367.

Das, B. C., Madan, M. L., Manik, R. S. and Sarkar, M. (2011). Seasonal impact on per ovarian oocyte retrieval rate in buffalo. *Environ. Ecol.* **23**:472-474.

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.

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.

De Smedt, V., Crozet, N. and Gall, L. (1994). Morphological and functional-changes accompanying the acquisition of meiotic competence in ovarian goat oocyte. *J Exp Zool.* **269(2):**128-139.

Deal, L. S., Moya-Araujo, C. F., Fernandes, C. B., Martins, L. R., Landim-Alvarenga, F. C. and Oba, E. (2017). Evaluation of recovery, quality and *in vitro* nuclear maturation of oocytes obtained from goat and bovine ovaries. *Revista Vet.* **21**:892-894.

DLS (2020). Livestock economy at a glance 2019-2020 by Department of livestock Services (DLS) Dhaka, Bangladesh. Available at http://www.dls.gov.bd/site/page/22b1143b-9323-44f8-bfd8-647087828c9b/Livestock-Economy.

Draincourt, M. A., Gougeon, D. R. and Thibault, C. (1993). Ovarian Function Reproduction in Mammals and Man. Thibault, MC. Levaseur and R.H.F. Hunter Ellipses (lids.), Paris: 281-305.

Eckert, J., and Niemann, H. (1995). *In vitro* maturation, fertilization and culture to blastocysts of bovine oocytes in protein-free media. *Theriogenology,* **43(7):** 1211-1225.

Edson, M. A., Nagaraja, A. K. and Matzuk, M. M. (2009). The mammalian ovary from genesis to revelation. *Endocr Rev.* **30(6):** 624–712.

El-Naby, A. H. H., Mahmoud, K. G. M., Ahmed, Y. F., Abouel-Roos, M. 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.

Erdheim, M. (1942). The incidence of right and left horn pregnanies in dairy and beef cattle. *J. Am. Vet. Med. Assoc.* **100:**343-344.

Ferdous, J. (2006). Collection, grading and evaluation of goat cumulusoocyte-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.

Forcada, F., & Abecia, J. A. (2006). The effect of nutrition on the seasonality of reproduction in ewes. *Reproduction Nutrition Development*, **46(4):**355-365.

Fukui, Y., & Sakuma, Y. (1980). Maturation of bovine oocytes cultured in vitro: relation to ovarian activity, follicular size and the presence or absence of cumulus cells. *Biology of Reproduction*, **22(3):**669-673.

Gabr, S. A., Nagy, W. M. and Ali, M. A. (2019). Ovarian biometry, oocyte yield and oocyte quality of baladi goats as affected by ovarian status in breeding and non-breeding season. *J. of Anim. And Poul. Prod.* **10(3)**:41-47.

Gandolfi, F., Milanesi, E., Pocar, P., Luciano, A., Brevini, T., Acocella, F., Lauria, A. and Armstrong, D. (1998). Comparative analysis of calf and cow oocytes during *in vitro* maturation. *Mol. Reprod. Dev.* **49(2):**168-175.

Gasparrini, B., Neglia, G., Di Palo, R., Campanile, G., Zicarelli, L. (2000). Effect of cysteamine during in vitro maturation on buffalo embryo development. *Theriogenology.* **54**:1537-1542.

Gordon, I. and Lu, K. H. (1990). Production of embryos *in vitro* and its impact on livestock production. *Theriogenology*. **33**:77-87.

Gupta, P. S. P., Nandi, S., Raghu, H. M., Ravindranatha, B. M. and Sarma, P. V. (2012). Oocyte recovery rates in relation to morphology and weight of the ovaries in buffaloes. *Indian J. Anim. Res.* **37(2)**:147-148.

Hafez, E. S. E. (1993). Folliculogenesis, egg maturation and ovulation. **In**: Reproduction in Farm Animals, Volume 1. Lea and Febriger, Philadelphia. Pp:114-143

Haque, Z., Haque, A., and Quasem, M. A. (2016). morphologic and morphometric analysis of the ovary of black bengal goat (*Capra hircus*). *Int. J. Morphol.* **34(1)**:13-16.

Harper, K. M. (1993). Bovine blastocyst development after *in vitro* maturation in a defined medium with epidermal growth factor and low concentrations of gonadotropins. *Biol Rep. J.* **48(2):**409–16.

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., Kabiraj, S. K., Khandoker, M. A. M. Y., 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. *Afr. J. Biotec.* **10(45)**:9177-9181.

Hoque, S. A. M. (2009). Effect of collection techniques and follicular fluid on *in vitro* maturation and fertilization of oocytes in goat. Master of Science thesis submitted to the Dept. of Animal Breeding and Genetics, Bangladesh Agricultural University.

Im, K. S., Kim, H. J., Chung, K. M, Kim, H. S. and Park, K. W. (1995). Effects of ovary type, oocyte grade, hormone, sperm concentration and fertilization medium on *in vitro* maturation, fertilization and

development of bovine follicular oocytes. *Asian Aus. J. of Anim. Sci*. **8**:123-127.

Islam, M. R. (2005). Studies on collection, *in vitro* maturation and fertilization of goat oocytes. Master of Science thesis submitted to the Dept. of Animal Breeding and Genetics, Bangladesh Agricultural University.

Islam, M. R. (2019). Ovarian categories, follicles and oocytes analysis of buffalo in view of *in vitro* production of embryos. Master of Science thesis submitted to the Dept. of Animal Nutrition, Genetics and Breeding, Sher-e-Bangla Agricultural University.

Islam, M. R., Khandoker, M. A. M. Y., Afroz, 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 University Sci. B*. **8(7)**:465-469.

Islam, M., Shamsuddin, M., Das, P. and Dewan, M. (2001). An outbreak of peste des petits ruminants in Black Bengal goats in Mymensingh, Bangladesh. *Bangl. Vet.* **18**:14-19.

Iwasaki, S., Kono, T., Nakahai, T., Shioyo, Y., Fukushima, M. and Hanada, K. (1987). New methods for the recovery of oocytes from bovine ovarian tissue in relation to *in vitro* maturation and fertilization. *Jap. J. of Anim. Reprod*. **33**:188-191.

Izquierdo, D., Villamediana, P., Lopez-Bejar, M. and Paramio, M. (2002). Effect of *in vitro* and *in vivo* culture on embryo development from prepubertal goat IVM-IVF oocytes. *Theriogenology*. **57(5)**:1431-1441.

Jablonka, S. A., Grazul, A., Redmer, D. and Reynold, L. (1993). Growth and cellular 432 proliferation of ovine corpora lutea throughout the estrous cycle. *Endocrinology,* **133(4):**1871–1879.

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.

Jiménez-Macedo, A., Anguita, B., Izquierdo, D., Mogas, T. and Paramio, M. (2006). Embryo development of prepubertal goat oocytes fertilised by intracytoplasmic sperm injection (ICSI) according to oocyte diameter. *Theriogenology.* **66(5)**:1065-1072.

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.

Katska. K. (1984). Comparison of two methods for recovery of ovarian oocytes from slaughter cattle. *Anim. Rep. Sci.* 7:461-463.

Kauffold, J., Amer, H. A. H., Bergfeld, U., Weber, W. and Sobiraj, A. (2005). The *in vitro* developmental competence of oocytes from juvenile calves is related to follicular diameter. *J. Reprod. Dev.* **51(3):**325-332.

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.

Kharche, S. D., Goel, A. K. Jindal, S. K., Yadav, E. N. Yadav, P., Singh, R. and Sinha, N. K. (2009). Effect of serum albumin supplementation on in vitro capacitation and fertilization of caprine oocytes. *Small Rumin. Res. J.* **81**: 85-89.

Khatir, H., Lonergan, P. and Mermillod, P., (1998). Kinetics of nuclear maturation and protein profiles of oocytes from prepubertal and adult cattle during *in vitro* maturation. *Theriogenology.* **50(6):** 917-929.

Khaza, A., Rehman, N. U. and Zia, E. U. H. (1999). Studies on *in vitro* maturation and fertilization of goat follicular oocytes. *Int. J. Agric. Biol.,* **3(4):**503-506.

Kidder, H. E., Barrett, G. R. and Casida, L. E. (1952). A study of ovulations in six families of Holstein-Friesians. *J. Dairy Sci.* **35:**436-444.

Kim. S. K. and Park, H. K. (1990). Studies on the *in vitro* maturation and fertilization rate of bovine follicular oocytes. *Theriogenology*. **33**:267.

Kulasekhar, T., Solmon Raju, K. G. and Sadasiva Rao, K. (2012). Efficacy of *in-vitro* maturation of goat 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 goat ovaries. *Anim. Reprod. Sci. J.* **47**:189-195.

Kumar, N., Paramasivan, S., Sood, P. and Singh, M. (2004). Micrometry of different category oocytes recovered from goat ovaries. *Ind. J. Anim. Sci*. **74(3):**259-260.

L. Keskintepe, Darwish, G.M., Kenimer, A.T. and Brackett, B.G. (1994). Term development of caprine embryos derived from immature oocytes *in vitro*, *Theriogenology*, **42(3):**527-535.

Lagerlof, N. and Boyd, H. (1953). Ovarian hyperplasia and other abnormal conditions in the sexual organs of cattle of the Swedsh Highland breed: results of postmortem examinations of over 6000 cows. *Cornell Vet.* **43:**52-64.

Lambert, R. D., Sirard, M. A., Bernard, C., Beland, R., Rioux, J. E., Leclerc, P., Menard, D. P. and Bedoya, M. (1986). *In vitro* fertilization of bovine oocytes matured *in vivo* and collected at laparoscopy. *Theriogenology.* **25**:117-133.

Lassala, A., Hernandej, J., Rodriguez, R. and Gutierrez, C. G. (2004). The influence of the corpus luteum on ovarian follicular dynamics during estrous synchronization in goats. *Anim. Reprod. Sci. J.* **84**:3-4.

Ledda, S., Bogliolo, L., Calvia, P., Leoni, G. and Naitana, S. (1997). Meiotic progression and developmental competence of oocytes collected from juvenile and adult ewes. *J. of Reprod. and Fert.* **109(1)**:73- 78.

Ledda, S., Bogliolo, L., Leoni, G. and Naitana, S. (1999). Follicular size affects the meiotic competence of *in vitro* matured prepubertal and adult oocytes in sheep. *Reprod. Nutr. Dev.* **39**:503-508.

Ledda, S., Bogliolo, L., Leoni, G. and Naitana, S. (1999). Production and lambing rate of blastocysts derived from *in vitro* matured oocytes after gonadotropin treatment of prepubertal ewes. *J. Anim. Sci*. **77(8)**:2234-2239.

Leibfried, L. and First, N. L. (1979). Characterization of bovine follicular oocytes and their ability to mature *in vitro*. *J. of Anim. Sci.* **48**:76-86.

Leibfried-Rutledge, M. L., Critser, E. S. and First, N. L. (1985). Fertilization potential of follicular oocytes classified by stage of cycle and size of follicle. *Theriogenology*, **23(5):**753-759.

Lof Marjolein, E., Reed Thomas, E., McNamara John, M. and Visser Marcel, E. (2012). Timing in a fluctuating environment: environmental variability and asymmetric fitness curves can lead to adaptively mismatched avian reproduction. *Proc. R. Soc. B.* **27(9):**3161–3169

Lonergan, P., Monaghan, P., Rizos, D., Boland, M. P. and Gordon, I. (1994). Effect of follicle size on bovine oocyte quality and developmental competence following maturation, fertilization, and culture in vitro. *Molecular reprod. and dev.*, **37(1):**48-53.

Lonergan, P., Vergos, E., Kinis, A., Gallagher, M. and Gordon, I. (1991). The effect of recover method on type of bovine oocytes for in vitro maturation. *Theriogenology*. **35**:231.

Lorenzo, P. C, Niera, M. J., Illera, J. C. and Dlera, M. (1995). Role of EGF, IGF-I sera and cumulus cells on maturation *in vitro* of bovine oocyte. *Theriogenology*. **44**:109-118.

Lorenzo, P. L., Calduch, T., Illera, M. J., Illera, J. C., Picazo, R. A. and Illera, M. (1999). Study of the ultra-structural morphology of sheep oocytes collected for in vitro maturation and fertilization. *Medicina veterinaria*, **16(4):**196-201.

Lucas, X., Martinez, E. A., Roca, J., Vazquez, J., Pastor, L. and Alabart, J. (2002). Relationship between antral follicle size, oocyte diameters and nuclear maturation of immature oocytes in pigs. *Theriogenology*. **58(5):**871-885.

Mahesh, Y. U. (2012). Efficacy of different harvesting techniques on oocyte retrieval from goat ovaries. *Indian J. Anim. Reprod.* **31(4):**209-213.

Majeed A. F., Omar A. A., Saad A., Jassim T., Maadhidy H. and Ahmed K. D. (2019). Effect of collection methods on oocyte recovery rate in goat. *Research J. of Biotechnology*. **14 (1)**:262-264.

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 goat oocytes. *Indian J. Anim. Reprod.,* **33(02):**36-40.

Marchal, R., Vigneron, C., Perreau, C., Bali-Papp, A. and Mermillod, P. (2002). Effect of follicular size on meiotic and developmental competence of porcine oocytes. *Theriogenology*, **57(5):**1523–1532.

Martino, A., Mogas, T., Palomo, M. J. and Paramio, M. T. (1994). Meiotic competence of prepubertal goat oocytes. *Theriogenology*, **41(4):**969-980.

May, N. D. S. (1970). The Anatomy of the sheep: a dissection manual (3rd Edition). University of Queensland Press, St. Lucia, Queensland.

McCorkel, R. B., MacDougall, L. and Adams, G. P. (2004). Ovarian follicle development in wapiti (*Cervus elaphus*) during the anovulatory season. *Theriogenology*. **61(2)**:473-483.

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

Mogas, T., Martino, A., Palomo, M. J. and Paramio, M. T. (1992). Effect of recovery method on the number and type of oocytes obtained for IVM. *J. Reprod. Fertile.* **9**:53.

Mohammadpour, A. A. (2007). Comparative histomorphological study of ovary and ovarian follicles in Iranian Lori-Bakhtiari sheep and indigenous goat. *Pakistan J. Bio. Sci.* **10(4)**:673-675.

Mohan, K., Singh, L. R. and Rao, K. S. (2013). Oocyte retrieval methods, grade and percentage of oocytes in goats. *Int. J. Mol. Vet. Res.* **3(2)**:4-6.

Mondal, 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. *Bang. J. Animal Sci*. **37**:1-9.

Moreno, J. F., Flores-Foxworth, G., Westhusin, M. and Kraemer, D. C. (1993). Influence of pregnancy and presence of a CL on quantity and quality of

bovine oocytes obtained from ovarian follicles aspirated post-mortem. *Theriogenology,* **39**:271.

Morrow, D. A., Roberts, S. J. and Mcentee, K. (1968). Latent effects of pregnancy on postpartum ovarian activity in dairy cattle. *J. Anim. Sci*. **27:**1408-1411.

Nandi, S., Chauhan, M. S. and Palta, P. (2000). Effect of a corpus luteum on the recovery and developmental potential of buffalo oocytes. *Vet. Rec.* **147**:580-581.

Nielsen, F. (1949). Sterility in cattle, especially as a result of uterine infection. Proc. 14th International Veterinary Congress, London, 8-13 August 1949, p105.

O'Brien, J., Beck, N., Maxwell, W. and Evans, G. (1997). Effect of hormone pre- treatment of prepubertal sheep on the production and developmental capacity of oocytes *in vitro* and *in vivo*. *Reprod Fertil Dev*. **9(6):** 625-631.

O'Brien, J., Catt, S., Ireland, K., Maxwell, W. and Evans, G. (1997). *In vitro* and *in vivo* developmental capacity of oocytes from prepubertal and adult sheep. *Theriogenology*. **47(7):**1433-1443.

Otoi, T., Yamamoto, K., Koyama, N., Tachikawa, S. and Suzuki, T. (1997). Bovine oocyte diameter in relation to developmental competence. *Theriogenology*. **48(5):**769-774.

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.

Palma, G. A. (1993). *In vitro* production of cattle embryos from calf oocytes. *Theriogenology*, **39:**278.

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 goats of coastal Odisha. *The Indian J. Vet. Res.* **22(1):**67-69.

Pawshe, C. H., Totey, S. M. and Jain, S. K. (1994). A comparison of three methods of recovery of goat oocytes for *in vitro* maturation and fertilization. *Theriogenology*. **42(1)**:117-125.

Perkins, J. R., Olds, D. and Seath, D. M. (1954). A study of 1,000 bovine genitalia. *Journal of Dairy Science*, **37(10):**1158-1163.

Pierson, R. A. and Ginther, O. J. (1987). Follicular populations during the estrous cycle in heifers. II. Influence of right and left sides and intraovarian effect of the corpus luteum. *Anim. Reprod. Sci.*, **14(3):**177-186.

Pieterse, M. C., Vos, P. L. A. M., Kruip, Th. A. M., Warth, Y. A., Van Beneden, Th. H., Willemse, A. H. and Taveme, M. A. M. (1992). Repeated transvaginal ultrasound guided ovum pick up in ECG treated cows. *Theriogenology*. **37**:273.

Pugh, P. A., Fukui, Y., Tervit, H. R. and Thompson, J. G. (1991). Developmental ability *of in vitro* matured sheep oocytes collected during the non breeding season and fertilized *in vitro* with frozen ram semen. *Theriogenology*. **36:**771-778.

Raghu, H. M., Nandi, S., Ravindranatha, B. M. and Reddy, S. M. (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.

Raghu, H., Nandi, S. and Reddy, S. (2002). Follicle size and oocyte diameter in relation to developmental competence of buffalo oocytes *in vitro*. *Reprod Fertil. Dev.* **14(1-2):**55-61.

Rahman, A. N. M. A., Abdullah, R. B. and Wan-Khadijah, W. E. (2008). Gametogenesis, fertilization and early embryogenesis in mammals with special reference to goat: a review. *J. Bioll. Sci*. **8(7)**:1115-1128.

Rahman, A., Hossain, A., Ahmed, M. V. and Sen, M. M. (1977). Studies on some reproductive performances and biometry of the female genital tract of Black Bengal goat. *J. Anim. Sci*. **47(11):**714-725.

Rajakoski, E. (1960). The ovarian follicular system in sexually mature heifers with special reference to seasonal, cyclical, and left-right variations. *European Journal of Endocrinology*, **34(3_Suppl):**S7-S68.

Ramsingh, L., Sadasivarao, K., Muralimohan, K. (2013). Ovarian biometrics and oocyte grading percentage of yield in local goats of Andhra Pradesh. *Iosr. J. Pharma.* **3(1):**47-49.

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.

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

Reece, R. P., & Turner, C. W. (1938). The functional activity of the right and left bovine ovary. *J. of Dairy Sci.*, **21(1)**:37-39.

Revel, F., Mermillod, P., Peynot, N., Renard, J. and Heyman, Y. (1995). Low developmental capacity of *in-vitro* matured and fertilized oocytes from calves compared with that of cows. *J. Reprod. Fertil.* **103(1):**115-120.

Romaguera, R., Casanovas, A., Morató, R., Izquierdo, D., Catalá, M., Jiménez- Macedo, A., Mogas, T. and Paramio, M. (2010). Effect of follicle diameter on oocyte apoptosis, embryo development and

chromosomal ploidy in prepubertal goats. *Theriogenology*. **74(3):**364-373.

Romaguera, R., Moll, X., Morató, R., Roura, M., Palomo, M. J., Catalá, M. G., Jiménez-Macedo, A., Hammami, S., Izquierdo, D., Mogas, T. and Paramio, M. (2011). Prepubertal goat oocytes from large follicles result in similar blastocyst production and embryo ploidy than those from adult goats. *Theriogenology*. **76(1):**1-11.

Rosa, H. J. D., Bryant, M. J. (2003). Seasonality of reproduction in sheep. *Small Rumin. Res.* **48(3):**155–71

Salim, M. S. (2004). Causes of infertility in Black Bengal does investigated by anatomical and histological tools. Master of Science thesis submitted to the Dept. of Animal Breeding and Genetics. Bangladesh Agril. Univ.

Salykbaev, T., Dombrovskii, N. N., Dzhienbaeva, R. S. and Murzamadiev, A. M. (1986). Ability of oocytes from sheep of different ages to mature when incubated within follicles. *Eksperimential Noi Biologii Akademiya Nauk, Kazakhskoi SSR*, **19**:167-169.

Sani, B., Mehre, P. V. and Majumdar, A. C. (2013). Effect of season on oocyte maturation and embryo development in goat. *Indian J. Anim. Sci.* **80**:618-620.

Sarkar, M. K. (1993). Studies on the incidence of reproductive abnormalities in female goat (*Capra hircus*). Master of Science thesis submitted to the Dept. of Anatomy, Bang. Agril Univ.

Sato, E., Iritani, A. and Nishikawa, Y. (1977). Factors involved in the maturation of pig and cattle follicular oocytes cultured *in vitro*. *Jap. J. of Anim. Reprod.* **23**:12-18.

Satosi, K. and Motoalci, U. (2004). Histological analysis of the critical point in follicular development in mice. *Reprod. Med. Biol*.**3**:141-145.

Schneebeli, J. and M. Döbeli (1991). Untersuchungen über die asymmetrische Verteilung ovarieller Funktionsgebilde beim Rind. *Schweiz Arch. Tierheilk.* **133:**375-381.

Schram, W. (1937). Untersuchungen über links und Rechtsträchtigkeit beim Rinde. *Dent. Tierarzte,* **45**:387-389.

Shaikh, K., Nakhashi, H. and Sharma, V. K. (2015). Advances on *in-vitro* production of caprine embryos. *J. of Inter. Aca. Res.* **3(7)**:2320-5083.

Shamsuddoha, A. K. and Edwards, G. (2000). Dairy Industry in Bangladesh: Problems and Prospects. Proc. AARES 2000 Conference. Pp:1-26.

Shirazi, A. and Sadeghi, N., (2007). The effect of ovine oocyte diameter on nuclear maturation. *Small Rumin Res.* **69(1):**103-107.

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

Singh, J. and Adams, G. P. (2000). Histomorphometry of dominant and subordinate bovine ovarian follicles. *Anat. Rec.* **258(1):**58-70.

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

Singh, S., Bhattacharay, A. and Luktura, S. (1974). Studies on biometry of genital organs of female goat. *Indian Vet J*., **51(1):**81–85.

Sirard, M. A., Richard, F., Blondin, P. and Robert C. (2006). Contribution of the oocyte to embryo quality. *Theriogenology*. **65(1):**126–136.

Skinner, J. (2005) Regulation of primordial follicle assembly and development. *Hum Reprod Update.* **11(5):**461-471.

Slavik, T., Fulka, J. and Goll, I. (1992). Pregnancy rate after the transfer of sheep embryos originated from randomly chosen oocytes matured and fertilized *in vitro. Theriogenology*. **38(4):**749-756.

Slavik, T., Libik, M., Wierzchos, E. and Fulka, J. (2005). An attempt to reduce polyspermic penetration in lamb oocytes. *Folia Biol.* **51(2):**34-39.

Snyder, D. A. (1977). *In vitro* ovum maturation and anestrus ovulation induction in sheep. Ph.D Thesis submitted to Michigan State University.

Spriggs, D. C. (1945). Some experiences of a sterility officer. *Vet. Rec.* **57**:469.

Stalfors, H. (1916). Gestation and sterility in cows. *Monatshefte für praktische Tierheilkunde* **27:**338.

Sutton, M. L., Gilchrist, R. B. and Thompson, J. G. (2003). Effects of *in-vivo* and *in-vitro* environments on the metabolism of the cumulus–oocyte complex and its influence on oocyte developmental capacity. *Human reproduction update*, **9(1):**35-48.

Tan, S. J. (1990). Effect of different estrus stages of ovaries and sizes of follicles on generation of bovine embryos *in vitro. Theriogenology.* **33**:335.

Vazta, G., Machaty, Z., Barandi, Z. S. and Varga, Z. S. (1992). Embryos derived from the *in vitro* fertilization of oocytes of pregnant cows. *Theriogenology*. **37**: 811-815.

Wahid, H., Gordon, I., Sharif, H., Lonergan, P., Monaghan, P. and Gallagher, M. (1992). Effect and efficiency of recovery method for obtaining ovine follicular oocytes for *in vitro* procedures. *Theriogenology.* **37**:318.

Wang, B., Baldassarre, H., Tao, T., Gauthier, M., Neveu, N., Zhou, J., Leduc, M., Duguay, F., Bilodeau, A., Lazaris, A., Keefer, C. and Karatzas, C.

(2002). Transgenic goats produced by DNA pronuclear microinjection of *in vitro* derived zygotes. *Mol. Reprod. Deve.* **63**:437-443.

Wang, Z. G., Xu, Z. R. and Yu, S. D. (2007). Effects of oocyte collection techniques and maturation media on *in vitro* maturation and subsequent embryo development in Boer goat. *Czech J. Anim. Sci.* **52(1):**21-25.

Wang, Z. G., Yu, S. D. and Xu, Z. R. (2007). Effects of collection methods on recovery efficiency, maturation rate and subsequent embryonic developmental competence of oocytes in Holstein cow. *Asian-Australian J. Anim. Sci.* **20(4):**496–500.

Wani, N. A. (2002). *In vitro* maturadcm and *in vitro* fertilization of sheep oocytes. *Small Rumin. Res*. **44:**89-95.

Wani, N. A., Wani, G. M., Khan, M. Z. and Salahudin, S. (2000). Effect of oocyte harvesting techniques on *in vitro* maturation and *in vitro* fertilization in sheep. *Small Rumin. 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 Rumin. Res.* **34**:71-76.

Watson, A. J., Watson, P. H., Wames, D., Walker, S. K., Armstrong, D. T. and Seamark, R. F. (1994). Pre-implantation development *of in vitro* matured and *in vivo* fertilized ovine zygotes: comparison between co-cultured on oviductal epithelial cell monolayers and culture under low oxygen atmosphere. *Biol. of Reprod.* **50**:715-724.

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.

Yang, N. S., Lu, K. H. and Gordon, I. (1990). *In vitro* fertilization (IVF) and culture (IVC) of bovine oocytes from stored ovaries. *Theriogenology*. **33**:352.

Yokoo, M. and Sato, E. (2004). Cumulus–oocyte complex interactions during oocyte maturation. *Int. Rev. Cytol*. **235**:251-91.

Yoshida, M., Ishizaki, Y., Kawagishi, H., Bamba, K. and Kojima, Y. (1992). Effects of pig follicular fluid on maturation of pig oocytes *in vitro* and on their subsequent fertilizing and developmental capacity *in vitro*. *J. Reprod. Fertil.* **95(2):**481-488.

Zhongwei, H. and Dagan, W. (2010). The human oocyte and cumulus cells relationship: new insights from the cumulus cell transcriptome. *Mol. Hum. Reprod.***16(10)**:715–725.