MORPHOMETRIC ASSESSMENT OF OOCYTES RECOVERED FROM OVARIES OF BLACK BENGAL GOAT

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To

My Beloved Parents

DEPARTMENT OF ANIMAL PRODUCTION AND MANAGEMENT

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CERTIFICATE

This is to certify that the thesis entitled **"MORPHOMETRIC ASSESSMENT OF OOCYTES RECOVERED FROM OVARIES OF BLACK BENGAL GOAT"** submitted to the Faculty of Animal Science and Veterinary Medicine**,** 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 bonafide research work carried out by **Falguni Dadok,** Registration no. **12-04745** under my supervision and guidance. No part of this thesis has been submitted for any other degree or diploma.

I further certify that such help or source of information as has been availed of during the course of this investigation has duly been acknowledged.

Dated:
Dhaka, Bangladesh

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LIST OF ACRONYMS

Abbreviation	Full Word
AI	Artificial Insemination
AMP	Adenosine Monophosphate
ANOVA	Analysis of Variance
ATP	Adenosine triphosphate
cAMP	Cyclic Adenosine Monophosphate
cAMP-PDE	Cyclic Adenosine Monophosphate-Phosphodiesterase
CENP-E	Centromere-associated protein E
CL	Corpus Luteum
cm	Centimeter
COCs	Cumulus Oocyte Complexes
DLS	Department of Livestock Services
DNA	Deoxyribonucleic Acid
DPBS	Dulbecco's Phosphate Buffer Solution
d.p.c.	days post-coitum
ES	Estrus Synchronization
et al.	And others
FAO	Food and Agriculture Organization
FF	Follicular Fluid
FSH	Follicle Stimulating Hormone
GDP	Gross Domestic Product
gm	Gram
GV	Germinal Vesicle
GVBD	Germinal Vesicle Breakdown
h	Hour

IAEA	International Atomic Energy Agency
ICSI	Intracytoplasmic sperm injection
IGF-I	Insulin-like Growth Factor I
IVEP	*In Vitro* Embryo Production
IVF	*In Vitro* Fertilization
IVM	*In Vitro* Maturation
IVP	*In Vitro* Production
KCl	Potassium Chloride
KH_2PO_4	Potassium Di Hydrogen Phosphate
LH	Luteinizing Hormone
LOPU	Laparoscopic Ovum Pick-Up
MAP-K	Mitogen-activated Protein Kinase
ml	Milliliter
mm	Millimeter
MOET	Multiple Ovulation and Embryo Transfer
MPF	Maturation Promoting Factor
mRNA	Mitochondrial RNA
MI	Metaphase I
MII	Metaphase II
No.	Number
NaCl	Sodium Chloride
Na_2HPO_4	Di Sodium Hydrogen Phosphate
OD	Ooplasm diameter

OPU	Ovum Pick-Up
PB 1	First polar body
PGCs	Primordial Germ Cells
PK-C	Protein Kinase C
Plk 1	Polo-like kinase 1
PN	Pronuclei
PVS	Perivitellinespace
RNA	Ribonucleic Acid
SAS	Statistical Analysis System
SD	Standard Deviation
ZPO	Zona pellucida
ZPT	Zona pellucida thickness
µm	Micrometer

MORPHOMETRIC ASSESSMENT OF OOCYTES RECOVERED FROM OVARIES OF BLACK BENGAL GOAT

ABSTRACT

Ovary plays an important role in the reproductive biology and biotechnology of female animals. With the aim to study the oocyte morphometry of Black Bengal goat, both right and left ovaries were collected from the slaughter houses of Dhaka city and the research was conducted at Animal Production and Management lab of Sher-e-Bangla Agricultural University. Ovaries were separated from the surrounding tissues, washed thoroughly with distilled water repeatedly and after that washed with normal saline. For each of the samples, gross parameters such as weight, length and width were recorded. The study revealed that the weights of right and left ovary were 0.79±0.17 g and 0.77±0.10 g, respectively. The length of right ovary was 1.36±0.21 cm and 1.39±0.32 cm of left ovary. The widths of right and left ovary were 1.00±0.13 cm and 0.98±0.15 cm, respectively. Oocytes were collected by three methods like dissection, slicing and aspiration. The result showed that slicing methods yield more oocytes with a moderate quality while aspiration methods yield a moderate oocytes count with good quality. The oocyte diameter was 100.21±4.84 µm and cumulus cells (CCs) diameter was 49.77±12.62 µm. The research revealed that the oocytes with larger diameter represent larger CCs diameter as well as good quality for *in vitro* production. These results will be helpful to manipulate ovarian functions, reproductive biology and biotechnology such as *in vitro* maturation (IVM), *in vitro* fertilization (IVF) and *in vitro* embryo production (IVP) in small ruminants.

CHAPTER I

INTRODUCTION

Bangladesh is an agricultural country and livestock plays a vital role in national economy. Goat is a multi-functional animal and contributes greatly to the agrarian economy, especially in areas where crop and dairy farming are not economic. Thus, goat plays an important role in the livelihood of a large proportion of small farmers particularly women, landless and marginal farmers inhabiting geographically isolated areas, who seldom have other means of survival (Choudhury *et al*., 2012). The total livestock population composed of 25.93 million goats, 23.94 million cattle, 1.48 million buffaloes and 3.4 million sheep (DLS, 2017). Among the livestock sector goats are a very important species of livestock in Bangladesh, mainly on account of their short generation intervals, higher rates of prolificacy and the ease with which the goats and their products can be marketed. Goats play an important role in livelihoods of smallholder farmers in Bangladesh as they serve as assets that can be easily liquidated to provide cash in times of need (Akhter *et al*., 2006). Goats are one of the earliest domesticated farm animals (Naderi *et al*., 2008). As indicated by the archaeological proof, they have been associated with man in a symbiotic correlation for up to 10,000 years (Ensminger and Parker, 1986). They proved valuable to man throughout the eternities due to their productivity, small size, and non-competiveness with him for food (Devendra, 1999). About 80% of our population is directly or indirectly involved with agriculture and livestock farming. Twenty percent people are involved in livestock sector as permanent occupation (Ahmed *et al.,* 2008). The goat is called the “Poor man’s cow” in our country and it’s the second important livestock in Bangladesh which plays an important role in the rural economy and earn substantial amount of foreign currency by exporting skin and others byproducts (MacHugh and Bradley, 2001). Each year goat production provides

20,400 metric tons meat, which accounts for 25% of total red meat in Bangladesh (Ferdous *et al*., 2012). The contribution of Livestock in the magnitude of Gross Domestic Product (GDP) is about 16.23 % in Bangladesh. But the diseases and disorders of animals are the most important hindrance towards livestock development in our country (Islam *et al*., 2001). The total population of goat in the world is 861.9 million. Among that in Asia the goat population is 514.4 million, Africa 291.1 million, Northern America 3 million, Central America 9 million, Caribbean 3.9 million, South America 21.4 million, Europe 18 million, Oceania 0.9 million (Aziz, 2010). In Asia most of the goats are found in sub-continent area like India, Bangladesh, Pakistan, Nepal and Myanmar (Shah *et al*., 2004). There are lots of breeds of goat found throughout the world but in Bangladesh the most common goat breeds are Jamunapari and Black Bengal goat (Gall, 1996). Considering the possibilities, a participatory approach in rearing poultry and later goat as a small scale subsistent family enterprise has been promoted by the government and various non-government organizations with the credit and input support since 1980's in this country (Hassan *et al*., 2007). Farmers are now trying to adapt and rear goats under intensive management systems. The husbandry and genotypes of goats, however, need to be developed and adapted to this new system. At the same time, appropriate technologies should also be developed. This success of this work will dictate the prospects of goat production in Bangladesh in the coming years.

Ovary is the key female reproductive organ of all the vertebrates. Goat play a major role in modern agriculture and their size and physiology provide an appropriate model to study a variety of mammalian biological functions, including reproduction, embryology and fetal development. 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 Artificial Insemination (AI) with frozen semen and Estrus Synchronization (ES) (Hoque *et al.*, 2011). After a dramatic development of

cellular biology over the last ten-fifteen years, 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). Considering the poor ovulatory response in small animal the application of MOET is difficult to adopt (Danilda, 2000).

The reproductive physiology of goat is least understood compared to cattle, sheep and pig. Description of goat is usually made as if it is identical with sheep (Smith, 1986). Some work on the morphology, physiology and pathology of reproductive organs of the goat (Epelu-Opio *et al*., 1988; Moreira, *et al*., 1991; Sattar & Khan, 1988; Torres & Badiongan, 1989) has been reported in many countries. But no comprehensive study has yet been undertaken on the ovary of Black Bengal goat in Bangladesh. Therefore, the study was designed to clarify the morphology and morphometry of the ovary of Black Bengal goat. Ovaries are vital organ that supplies the germ cells, oocytes and produce hormones for maintaining reproductive health. Several molecular factors are known to be responsible for the follicle population in fetal or neonatal ovaries (Skinner, 2005). Recently, it is found that remarkably lower numbers of ovarian follicles is one of the major causes of infertility in Black Bengal goats (Amin *et al*., 2005).

Black Bengal goat is the national pride of Bangladesh. The most promising prospect of Black Bengal goat in Bangladesh is that this dwarf breed is a prolific breed, required small area to raise and with the advantage of their selective feeding habit with a broader feed range. It is very popular to consumers for its delicious and tender meat. Its skin is also highly valued in the world market due to some unique features of yielding finest leather that is light in weight and fine in texture. Considering the paramount importance and bright prospects of Black Bengal goat in Bangladesh, goat production level should be maintained properly by increasing fertility and conception rate. To increase the population of goat, the reproduction rate improvement is essential. In mammalian species primordial follicles are the

least developed and most numerous follicles of the ovary. Later on it developed to primary, secondary and graffian follicles, respectively (Banks, 1993). 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 no complete study on qualitative, quantitative and histological analysis on the ovary, ovarian follicle and oocytes in black Bengal goat or indigenous Bengal sheep. Keeping the aforesaid reality in mind the present research work has been undertaken with the following objectives-

-To assess the oocytes morphologically and morphometrically collected from Black Bengal goat ovaries.

-To evaluate the potential quality of oocytes of Black Bengal goat in view of *in vitro* maturation and *in vitro* embryo production.

-To correlate the quality of oocytes and CCs of Black Bengal goat with various aspects and effects.

CHAPTER II

REVIEW OF LITERATURE

2.1 Oogenesis

Ovary is the female reproductive organ which is responsible for differentiation and release of a mature oocyte for fertilization and successful breeding of species. Morphological differentiation of the ovary starts when gonads are populated with primordial germ cells. Oogenesis represents formation of female gametes, when oogonia formed from primordial germ cells (PGCs) reach the stage of primary oocytes. 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). In mammals during the process of oogenesis, oocytes grow and produce great amount of macromolecules and undergo a complex morphological and developmental alteration (Wassarman and Albertini, 1994). These developmental processes need high energy support. Oogenesis begins with a germ cell –oogonia and this cell undergoes series of mitosis to increase in number and ultimately results in up to one to two million cells in the embryo localized in the utmost layers of the fetal ovaries. PGCs appear during the embryo development and originate extra gonadally from yolk sac endoderm and part of the allantois which arises from the posterior part of the primitive sex cords. The PGCs migrate from the epithelial yolk sac through the connective tissue to the genital ridges where they rapidly proliferate. After colonization of the gonad, PGCs undergo a phase of mitotic proliferation with an unfinished cytokinesis and this leads to the creation of germ cell. Sanchez and Smitz (2012) reported that migration, proliferation and colonization of PGCs to the developing gonads are regulated by many factors and depend as well on the

interaction of PGCs and their surrounding somatic cells. Primordial germ cells can be clearly identified at 7.5 days post-coitum (d.p.c.) during gastrulation by their high expression of alkaline phosphatase. Until 12.5 dpc, PGCs stay sexually undifferentiated, when they combine with the mesodermally derived somatic components of the gonads to form ovaries or testes. In pigs, these cells were observed on the surface of the emerging gonads between 24 – 26 days of embryonic 13 development (Bielanska - Osuchowska, 2006). PGCs increase in number through mitotic cell division, later these cells divide meiotically to produce mature oocytes or sperms. The process of oogenesis consists of the proliferation of PGCs, growth and maturation phase.

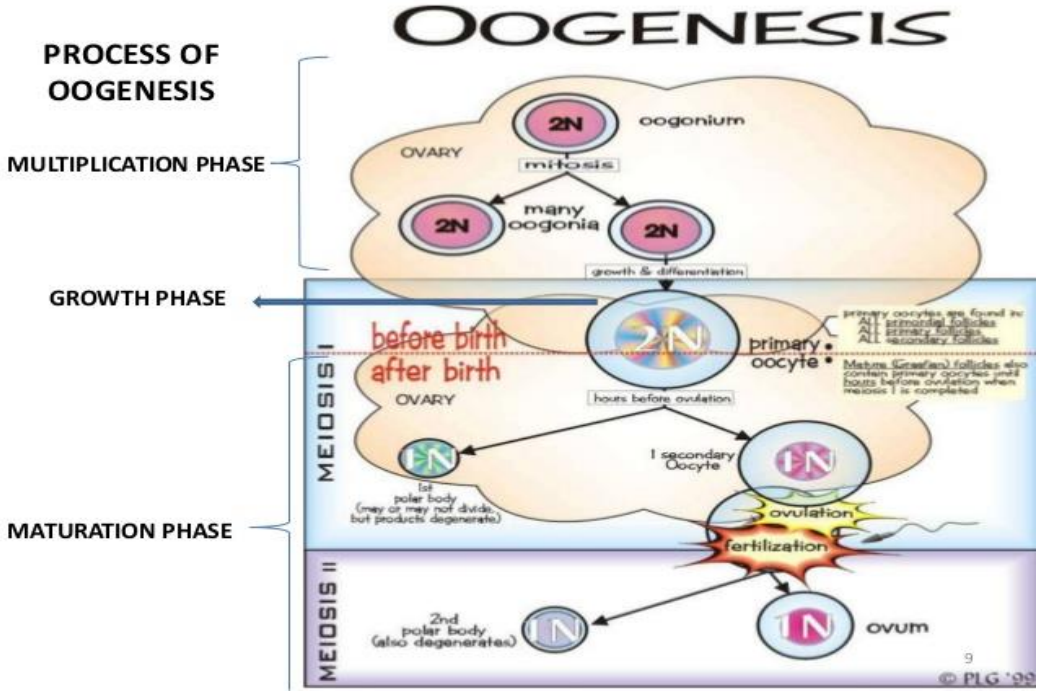

Plate 1: Process of oogenesis

Source: https://image.slidesharecdn.com/biochemicalchangesduringoogenesis131030212110-phpapp01/95/biochemical-changes-during-oogenesis-9638.jpg?cb=1383168143

2.1.1 Proliferation phase

In the first phase of oogenesis, primitive sex cords (medullary cords) were colonized by PGCs which undergo repeated mitotic divisions to form the primordial oocytes known as oogonia-stem cells. Oogonia, surrounded by somatic epithelial cells originating from genital ridge of mesenchymal cells,condense to form individual primordial follicles, which first develop in the inner areas of the ovarian cortex (Guraya, 2008). Then oogonia interrupt mitotic acitivity and enter meiosis (Goto *et al*., 1999). After meiosis has been started, the oogonial germ cell is called a primary oocyte. The primary oocyte is surrounded by a layer of follicular cells forming the primary follicle. Guraya (2008) reported that differentiation of oogonia into oocytes is closely followed up by their congregation with pregranulosa or follicular cells which get segregated from the somatic cells of the ovarian blastema.

In fetal ovary, the primary oocyte progress gradually, it enters meiosis I, over the different stages of prophase I and arrests at the diplotene stage of the first prophase with homologous chromosomes (Mandelbaum, 2000). At birth, all oocytes from growing follicles are arrested at the diplotene stage of prophase I. The prophase of the first meiotic division as long inactive phase lasts from birth to reproductive age. The oocytes persist in the arrested stage until a few hours before ovulation. As generally known, oocytes of vertebrates are arrested in prophase of the first meiotic division for several weeks, months or years. The duration of this period depends on the species. The oocytes arrested in the diplotene stage of the first meiotic prophase are managed by a few determinants: follicle size, cumulus cell communication, gonadotropins, steroids, cAMP, cAMP-PDE, adenylate cyclase, PK-C, MAP-K, MPF and gene expression (Chaube, 2001). Cyclic AMP is the intracellular signaling molecule produced by granulosa cells and transported via gap junctions to the oocytes, which maintains the oocyte in the meiotic arrest (Desai *et al*., 2013).

Marteil *et al*. (2009) reported that the arrest in prophase of meiosis I correlates with the sensitivity to hormone activity which disenables early nuclear maturation of small oocytes. In the process of oogenesis, oocytes go thorugh different cytoplasmic changes. The first cytoplasmic alterations begins at the late diplotene stage of prophase I (Blerkom and Runner, 1984). In this protracted period, proportionally with increasing of their size, oocytes concentrate molecules of mRNA, increase a store of cytoplasmic enzymes, metabolic substrates,lipids and sugars necessary for their maturation and the starting of embryonic development (Marteil *et al*., 2009). In mammalian species, the developmental procedure commences when maternal RNA and protein are concentrated during growth and maturation of oocytes (Telford *et al*., 1990).

2.1.2 Oocyte growth

Oocytes have to grow before they achieve their competence to resume nuclear maturation for subsequent fertilization and cleavage (Kanitz *et al*., 2001). The growth of oocytes represents interactions among the somatic and germ cells on the molecular and cellular level. During growing phase of oocytes, fewest cytological changes take place. Oocytes undergo a complex organization of new genes and products, as well as alteration and distribution of the existing ones. During the follicular development, the growth and differentiation of oocytes are controlled by the activity of granulose cells whose functions are regulated by multiform hormones and factors. For the oocyte growth, the intracellular communication among the oocyte and granulosa cells ensures the nutritional and metabolic support (Guraya, 2008). In the growth phase by means of biochemical reactions and cytoplasmic changes, oocytes gradually acquire competence for nuclear and cytoplasmic maturation (Blerkom and Runner, 1984). Chromosomes of the primary oocyte are composed of two chromatids, and each chromatid contains identical DNA molecules. In this period of growth, the overall level of DNA methylation increases, with the development of follicles from preantral stage to

full maturity that results in inhibition of gene activity. In the growth phase of oogenesis, major changes of cytoplasmic organelles take place. In the cytoplasm of the oocyte, the second rearrangement occurrs. In the growth phase the number and size of the lipid droplets also enlarge. In this phase, mitochondria circulate from perinuclear area and become distributed over the whole cytoplasm (Van Blerkom and Runner, 1984). In the cortical stroma, after exit from the resting stage, the oocyte starts to grow rapidly and its amount is enchanced by accumulating organelles, lipids, proteins and RNAs (Fauser *et al.*, 1999). The content of proteins necessary for this rapid growth is produced by the oocyte and the granulosa cells.

2.1.3 Oocyte maturation

In the process of oogenesis, the competence of mammalian oocytes is influenced by many factors, but one of the most important is oocyte maturation. For adequate fertilization and embryo development and also for implementation of reproductive biotechnologies methods, the procedure of oocyte maturation is greatly important. Development in the embryonic period depends on events occurring during oocyte maturation. In the course of the complex process of maturation, mammalian oocytes achieve gradually the capability for maintenance of following developmental period. The process of maturation and the timing of oocyte meiotic arrest must be firmly managed, because the mature oocytes have a relatively short life time in the female reproductive tract (Mehlmann, 2005). The period of oocyte maturation varies between species. The processes which occur during oocyte maturation are associated with the energy status of oocytes.

Oocyte maturation is a dynamic and highly coordinated process, in which the oocytes finish the first meiotic division, cytoplasmic alteration and prosper to metaphase II (Mehlmann, 2005). During the maturation of oocytes, meiosis I occurs at the time of ovulation and meiosis II at the time of fertilization (Chappel,

2013). When the mature oocyte is exposed to luteinizing hormone (LH) surge, meiosis I is terminated.

Maturation of mammalian oocytes involves nuclear and cytoplasmic maturation and the final result of these processes is a mature oocyte competent for successful fertilization. A few hours before ovulation oocyte develops the ability to be fertilized. The mammalian capacity of oocytes to resume meiosis and undergo nuclear and cytoplasmic changes has a large influence on subsequent process of fertilization and early mammalian development.

2.1.4 Nuclear maturation

The oocyte contains a large germinal vesicle (GV) with a large nucleolus, before maturation starts. Nuclear maturation means the meiotic process of chromosomal reduction to a haploid content, so as to produce a diploid organism upon fusion with sperm (Voronina and Wessel, 2003). Throughout nuclear maturation, redistribution of the cytoskeleton eventuate. This process includes actions correlated with the germinal vesicle breakdown (GVBD), condensation of chromosomes, metaphase I spindle formation, separation of the homologous chromosomes with extrusion of the first polar body and arrest at metaphase II (Josefsberg *et al*., 2000). With the second meiotic division, chromatids separate and the second polar body is formed. Finally, the chromatids remaining in the oocyte decondense and a pronucleus forms. Niimura *et al*. (2002) observed lipid droplets in porcine oocytes and reported that alteration in the size of lipid droplets is associated with nuclear maturation. For completion of nuclear maturation, bovine oocyte need 24 h (Sirard *et al*., 1989), and porcine 44 h (Kim *et al*. 2011). After successful nuclear maturation, an oocyte arrested in metaphase II is formed. In this phase the oocyte is physiologically relevant to finish the second meiotic division in the process of fertilization.

2.1.5 Cytoplasmic maturation

For cellular functions during maturation, fertilization and early embryo development, the cytoplasm of oocytes ensures useful metabolic conditions for the production of energy indispensable in these processes (Cetica *et al*., 2002). Cytoplasmic maturation means both the ultrastructural changes occurring in the oocyte from the GV to the MII stage and the acquisition of developmental competence of the oocyte (Duranthon and Renard, 2001). Because of this characteristic, there is considerable interest in the cytoplasmic maturation of mammalian oocytes. Cytoplasmic maturation is regulated and greatly affected by hormone fluctuations. During maturation, the alteration of hormonal concentration probably has a substantial pertinence for the secretory activity of cumulus cells, a subset of granulosa cells. Accordingly, meiotic or cytoplasmic maturation of oocytes may be affected indirectly by the grade and continuance of hormonal exposure of cumulus cells, surrounding maturing oocytes as cumulus oophorus inside follicle.

Cytoplasmic maturation includes organelle rearrangement, storage of mRNAs, proteins and transcription factors which participate in the process of maturation, fertilization and early embryogenesis (Ferreira *et al*., 2009). The same author classified cytoplasmic maturation into three phases: organelle distribution, cytoskeleton dynamics and molecular maturation.

2.1.6 Maturation *in vivo* and *in vitro*

The liberation of a mature oocyte from the follicle with the competence to support regular ermbryonic development is the final stage of maturation in vivo (Fulka *et al*., 1998). Oocyte maturation in vivo is triggered by the preovulatory surge of gonadotropins via granulose cells (Eppig, 1991) and also cumulus expansion can be induced with FSH or LH hormones (Hillensjo and Channing, 1980). It is probable that spontaneous maturation in vivo lasts longer than in vitro maturation

induced by hormones. Pincus and Enzmann (1935) first described meiotic maturation of mammalian oocytes in vitro in rabbits. A number of studies suggest that oocytes isolated from large antral follicles of many species promptly advance to metaphase II in culture and undergo fertilization in a high percentage of instances. However, their developmental potential after fertilization is low. From these facts, it can be concluded that in vivo conditions are greater in comparison with in vitro. For the provision of energy during maturation, cumulus cells have an effect on the oocyte, because they manage the nutritive repository, across the control of fatty acid lipolysis and synthesis. During in vitro maturation, absence of cumulus cells influence metabolism of lipids and causes substandard maturation (Auclair *et al.*, 2013). Oocytes of different species required different intervals for in vitro maturation. The porcine oocytes achieved metaphase II after 44 h of maturation culture (Abeydeera *et al*., 1998; Gonzales-Figueroa and Gonzales Molfino, 2005), bovine after 24 h in maturation medium (Luna *et al*., 2001; Smiljakovic and Tomek, 2006), human from 24 h to 48 h (Roberts *et al.*, 2002) and equine between 24 and 32 h (Hinrichs *et al*., 1993).

2.1.7 Regulation of oocyte maturation

Mammalian oocytes are arrested at prophase of the first meiotic division and maturation of oocytes starts with the resumption of meiosis. Shortly before ovulation, meiosis resumes as reaction to a surge of luteinizing hormone from the pituitary gland (Mehlmann, 2005). The wave of LH hormone activates ovulation of the oocyte and induces oocyte maturation. Subsequent activation of M-phase promoting factor causes meiosis and process of germinale vesicle breakdown (Desai *et al.*, 2013). The first meiotic division ends with the emergence of two haploid cells, oocyte II and the first polar body and this represents process of ovulation. Consequently, the oocytes undergo the first meiotic division and then

become arrested atmetaphase of the second meiotic division after ovulation and wait for the process of fertilization (Chaube, 2001).

The regulation of meiosis resumption in porcine oocytes is controlled by a few protein kinases such as MPF, MEK/MAP kinase/p90rsk, PKC, cAMP, Connexin-43 and PI 3-kinase. Assembly of meiotic apparatus of porcine oocytes includes MAP kinase/p90rsk, NuMA and γ-tubulin, polo-like kinase 1(Plk1) and CENP-E (Sun and Nagai, 2003). For meiotic resumption, protein synthesis is substantial in mammalian oocytes.

After ovulation, each oocyte continues to metaphase of meiosis II. The second meiotic division begins with the penetration of a viable sperm. Meiosis II is completed only if fertilization occurs, resulting in a fertilized mature ovum and the second polar body. Meiosis is the process whereby diploid oogonia and spermatogonia divide their number of chromosomes, in purpose of subsequent combining with a haploid cell of opposite gender to procreate new diploid individual (Mehlmann, 2005). Then the oocyte extrudes 23 sister chromatids, forming second polar body and a fertilized zygote that has a normal diploid complement of 46 chromosomes. The proces of pulling chromosomes outside of the oocyte, to create the first and second polar bodies, demands important content of energy, which is ensured by ATP from oxidative phosphorylation in the mitochondria.

However, finalization of oogenesis occurs only after a spermatozoon penetrates to a mature, ovulated oocyte (Van Blerkom and Runner, 1984). After fertilization when sperm penetrated into the oocyte, the development that was started during oogenesis is activated and the process of embryogenesis begins. The initial periods of embryogenesis are directed by maternally inherited constituents positioned inside the oocyte. The process of early embryogenesis becomes dependent on the expression of genetic information derived from the embryonic genome when development proceeds and maternally inherited molecules decay (Telford *et al.*, 1990).

2.2 Meiotic competence

The meiotic competence represents the ability to resume and complete the first meiotic division and to arrest at the second meiotic metaphase. The ability to resume and complete the first meiotic division is connected to the process of oocyte growth, this is the reason for the complicated process of meiosis. After isolation and oocyte culture, the oocytes do not sustain spontaneous maturation until the final stage of growth, when oocyte acquire competence for spontaneous maturation *in vivo*. Evaluation of energy cytoplasmic markers during process of *in vitro* maturation and investigation of changes in this section during achievement of meiotic competence for development of oocytes is very important. Beyond meiotic competence, the oocyte has to complete its differentiation by acquiring the ability to support the cytoplasmic maturation and finally the ability to be successfully fertilized and develop into a viable embryo. The final differentiation of meiotically competent oocytes occurs at the end of folliculogenesis. Oocyte capacitation comes in the late period of oocyte differentiation.

Meiotic competence of porcine oocytes has been associated with oocyte size, nucleolar structure and function (Motlik *et al.*, 1984). As generally known, the cells derived from large follicles are more competent compared to those obtained from smaller follicles. In early antral follicles, oocytes become able to resume meiosis but only in growing antral follicles they become able to complete meiosis up to metaphase I and progress to metaphase II (Marchal *et al*., 2002). Porcine oocytes acquire meiotic competence gradually during growth of follicles (Motlik and Fulka, 1986). Marchal *et al.* (2002) reported that porcine oocytes acquired meiotic competence in ovarian follicles with a diameter of 3 mm and more. In growing porcine oocytes, acquisition of meiotic competence depends on their capacity to activate MPF and MAP kinase (Kanayama *et al.*, 2002). The acquisition of meiotic competence during follicular growth was also described in mouse (Eppig and Schroeder, 1989) and ruminant oocytes (Mermillod *et al*., 1999).

2.3 Developmental competence

Developmental competence means the ability of matured oocytes to undergo fertilization, subsequent embryo cleavage, and embryonic development. Internal ability of development is reflected in the quality of oocytes. This is associated to biochemical and molecular processes which support maturation and fertilization of oocytes and enable subsequent embryo development (Gilchrist *et al.,* 2008). The developmental potential of an embryo is reliant on developmental potential of oocyte from which it derives. Mattioli *et al*. (1989) for the first time described *in vitro* developmental competence of porcine oocytes. Many immature oocytes are able to terminate meiosis *in vitro*, but only a small proportion of them is competent to continue development to the blastocyst stage (Krisher and Bavister, 1998). According to Sirard *et al.* (2006), developmental competence of oocytes consists of a few important factors: meiosis resumption, cleavage following fertilization, capability to develop to the blastocyst stage and ability to induce pregnancy and bring it to term in good health. The cumulus cells surrounding the oocyte have an impact on acquisition of oocyte developmental competence.

The quality and developmental competence of mammalian oocytes are characterized by specific cytoplasmic factors: lipids, microtubules, relocation of mitochondria and ATP production. The mature oocytes with high developmental competence have a different lipid structure in comparison with immature oocytes (Ami *et al*., 2011). The study of Kim *et al.* (2001) indicates that the fatty acids located in lipid droplets of bovine oocytes are important for oocyte competence. A low developmental competence is related to deficiency of microtubule network which prevents appropriate relocation of mitochondria (Brevini *et al.,* 2005). Several authors already confirmed differences among morphologically good and poor oocytes indicating that activity and relocation of mitochondria is in correlation with their different deveopmental ability after *in vitro* fertilization. Failure of mitochondrial function and insufficiency of ATP production has been associated to reduced developmental competence (Dalton *et al.,* 2014). The

nuclear transfer experiments indicated that decline of developmental competence was linked with cytoplasmic defects (Mermillod *et al.,* 1998).

The follicle ambience and maternal signals, transmitted via granulosa and cumulus cells, are important for the progressive gain of developmental competence and as a support for oocyte growth (Gilchrist *et al.,* 2008). Khatir *et al.* (1997) reported important information that calf follicular fluid, irrespective of the size and quality of the follicle from which it originates, stimulates the acquisition of developmental competence by adult oocytes during maturation, as does adult follicular fluid, but is inactive on prepubertal oocytes. However they concluded that the follicular environment is not responsible for the low developmental competence of prepubertal oocytes, as these oocytes are unable to respond to the stimulatory components of follicular fluid. Koenig and Stormshak (1993) described differences between pubertal and third-estrous gilts, when embryos delivered during the first-estrus cycle are not fully competent for *in vitro* development. The results of Menino *et al.* (1989) show that the reduced *in vitro* development of embryos collected from gilts mated at the first estrus may be due to an aberration in blastocoel formation and expansion.

The developmental competence of oocytes matured *in vitro* is lower in comparison with oocytes matured *in vivo*, reducing the general effectiveness of *in vitro* maturation process.

According to Rodriguez and Farin (2004), oocytes matured in the presence of gonadotropic hormones have a better developmental competence. The acquisition of developmental competence of bovine oocytes increases with the age of the calves, and full developmental competence of oocytes is accomplished before puberty (Presicce *et al.,* 1997). Volarcik *et al.* (1998) reported that the developmental competence of human oocyte decreases with age.

2.4 Folliculogenesis

The ovarian follicles are basic structural and functional unit of the ovary and they ensure micro-conditions essential for oocyte development and maturation (Abd-Allah, 2010). The process of ovarian follicle development is known as folliculogenesis. The process of folliculogenesis starts during foetal development in many mammalian species, involving porcine (Bielanska-Osuchowska, 2006). The same author reported that the creation of ovarian follicles starts on day 56 p.c., while follicles encircled by a single layer of somatic cells are present on day 106 p.c. During oestrus cycle, porcine females express only one wave of follicular activity (Ratky and Brussow, 1998). During the early phases of gestation, follicles are already present in the bovine fetus (Erickson *et al.,* 1966).

Process of folliculogenesis in different animals has been investigated *in vivo* and *in vitro* in many laboratories. Through this process, an ovarian follicle passes through several stages: primordial-resting, primary, secondary-preantral, tertiary-antral, and the preovulatory-Graafian follicle stage. Rüsse (1983) reported first appearance of primordial, primary, secondary and early antral follicles in bovine fetus. Several million primordial follicles are present in the ovaries during the fetal period containing an oocyte surrounded by layers of somatic granulosa and theca cells (Knight and Glister, 2006; Krisher, 2013). The primordial follicles go through consequential growth and development forming preovulatory follicle containing a mature oocyte (Volarcik *et al*., 1998). The preantral phase is characterized by zona pellucida formation, granulosa cell proliferation, the recruitment of thecal cells to the follicular basal lamina and a dramatic increase in oocyte volume (Pedersen, 1969). Antral follicle is characterized by the existence of antrum in the granulosa and theca externa and a fibrous layer around theca interna. In the preovulatory follicle, the fully grown oocyte has collected nutrient stores, mRNA, proteins and organelles, also great number of mitochondria (Dunning *et al.,* 2014). Immature mammalian oocytes liberated from ovarian follicles proceed in meiosis and complete *in vitro* maturation.

The beginning phase of folliculogenesis starts independently of gonadotrophic hormones (Roche, 1996). However, the oocytes resume meiosis throughout progress from preantral to antral stage and after stimulation by pituitary gonadotropins FSH and LH, the mature oocyte is removed from the follicle and transfer into the oviduct (Uhlenhaut and Treier, 2011). Findings of Tanaka *et al.* (2001) demonstrated that in bovine fetal ovary, the serum concentration of FSH may play an important role as an initiator of early follicular development. After formation of follicular antrum, which roughly corresponds to the final stage of oocyte growth, granulosa cells differentiate into the mural granulosa cells which have a steroidogenic role and cumulus cells which create a close relatedness with the oocyte (Gilchrist *et al.*, 2008).

In mammals, folliculogenesis is a greatly selective process that includes steps of differentiation and proliferation of somatic and germ cells (Hernandez-Medrano *et al.,* 2012). During prospering from the preantral to the antral stage, the follicle is structurally changed to three separate populations of somatic cells: theca cells, granulosa cells and cumulus oophorus (Piotrowska *et al.,* 2013). After activation of growth, the granulosa cells start to proliferate, at the same time the oocyte initiates the growth stage. After this processes of initiation, development of follicle consists of proliferation and differentiation of the granulosa layers and the oocyte development. Roche (1996) reported that inhibins, activin, insulin-like growth factor I (IGF-I) and their binding proteins have direct and indirect effects on granulosa and theca cells that can modulate follicular development and steroidogenesis. During oocyte growth and maturation, the cumulus cells are metabolically linked with the oocyte and these cells are involved in the process of ovulation and fertilization (Tanghe *et al*., 2002).

The quality achievement of oocytes, their maturation, fertilization and development are complex processes and the size of follicles from which oocytes are obtained have a significant influence on their maintenance. Size of the follicle from which the oocyte is derived affects the oocytes ability to resume meiosis and

reach optimum maturation during IVM (Hyttel *et al*., 1997). The meiotic and developmental capability of oocytes is achieved gradually, during development of follicles (Eppig *et al.*, 1994; Schramm and Bavister, 1995). According to Marchal *et al*. (2002), developmental competence rises simultaneously with the size of follicles. The porcine oocytes acquire capability to complete meiotic maturation when they accomplish their full size in antral follicles of approximately 2 mm of diameter and at the same time, the transcriptional activity of oocytes decreases (Motlik *et al.*, 1984).

In vitro embryo production (IVP) represents a method for enhancing the population of genetically valuable animals (Pfeifer *et al.,* 2008). The examination of folliculogenesis is significant for improving of IVP techniques. Machatkova *et al.* (2004) reported that interaction among follicle size and the phase of follicular wave has an impact on embryo production. The production of embryos was primarily affected by the number of oocytes collected from medium follicles and the lower developmental competence of oocytes from small follicles. The production of embryos *in vitro* is usually diminished, which implies that all oocytes are not capable of successful *in vitro* maturation and fertilization. The quality of oocytes and *in vitro* conditions are the primary elements which characterize development of embryo and production of normal offspring.

2.5 *In vitro* maturation (IVM)

Full maturation involves both nuclear and cytoplasmic events that confer on the oocyte the capacity for supporting normal fertilization and early embryonic development (Thibault and Gerard, 1973; Moor and Trounson, 1977). Mammalian oocytes arrested at the germinal vesicle (GV) stage acquire the capacity to resume meiosis during growth of the ovarian follicle from the primary to the preovulatory stage. As removed from antral follicles of females ovaries, cumulus–oocyte complexes (COCs) can be induced to undergo *in vitro* the sequence of events

found during *in vivo* maturation. Matured oocytes reach metaphase II stage (nuclear maturation), accompanying by the molecular and structural changes that allow them to support normal fertilization and embryonic development (cytoplasmic maturation) (Bevers *et al*., 1997; Watson,2006).

2.5.1 Nuclear maturation

Oocytes that are collected from growing follicles for IVEP are blocked at the prophase of the first meiotic division. As soon as they are removed from follicles and transferred to a culture medium, the oocytes can spontaneously resume the meiotic division (Edwards, 1965). The resumption of meiosis is characterized by dissolution of the nuclear membrane and a condensation of the chromatin, a nuclear stage referred as germinal vesicle breakdown (GVBD). Chromosomes are then arranged in metaphase I (MI) stage which is followed by anaphase I to telophase I (AI-TI) transition and oocytes progress to metaphase II (MII) where they remain until their fertilization. Nuclear maturation can be visualized by the extrusion of the first polar body. Studies *in vitro* (Samake *et al*., 2000; Bormann *et al*., 2003), showed that under specific conditions, pre- selected oocytes can resume the nuclear maturation reaching 70–90% of the oocytes the MII stage. Prepubertal goat oocytes recovered from follicles larger than 3 mm (Martino *et al*., 1994), were able to reach the MII stage at a rate of 72 % after IVM.

2.5.2 Cytoplasmic maturation

Embryo development is strongly influenced by events occurring during oocyte maturation. IVM seems to be the limiting factor, because even after selection of a homogenous population of cumulus-oocyte complexes, only 35% will attain full cytoplasmic maturation and possess the competence to produce a viable, transferable blastocyst (Blondin and Sirard, 1995). Cytoplasmic maturation covers all morphologic and molecular events accompanying nuclear maturation after LH

surge in preovulatory follicles and preparing oocyte cytoplasm to successful fertilization and embryo development. As reviewed by Mermillod *et al.,* (2006), cytoplasmic maturation includes well known morphological modifications, such as the migration of cortical granules in the cortical region of the ooplasm. These granules are stored during oocyte growth and release their enzymatic content in the perivitellin space after fertilization. Cytoplasmic maturation also includes the accumulation of mRNA, proteins, alterations to Golgi complexes, mitochondrial accumulation in the ooplasm, and cumulus expansion (Sirard, 2001). Cumulus expansion can importantly be used to microscopically assess the *in vitro* maturation rate of oocytes (Gupta *et al.,* 2005). This store of maternal RNA instill upon the oocyte a capacity to decondense and remodel the sperm head, to form the pronuclei (PN), zygote formation, early embryogenesis and normal fetal development (Mermillod *et al*., 1999; Watson,2006).

2.6 Ovary

The ovary is an organ found in the female reproductive system that produces an ovum. When released, this travels down the fallopian tube into the uterus, where it may become fertilised by a sperm. There is an ovary (from Latin *ovarium*, meaning 'egg, nut') found on the left and right sides of the body. The ovaries also secrete hormones that play a role in the menstrual cycle and fertility. The ovary progresses through many stages beginning in the prenatal period through menopause. It is also an endocrine gland because of the various hormones that it secretes (Colvin *et al*., 2013). The ovaries are considered the female gonads. Each ovary is whitish in color and located alongside the lateral wall of the uterus in a region called the ovarian fossa. The ovarian fossa is the region that is bounded by the external iliac artery and in front of the ureter and the internal iliac artery. The

ovaries are surrounded by a capsule, and have an outer cortex and an inner medulla.

The side of the ovary closest to the fallopian tube is connected to it by infundibulopelvic ligament and the other side points downwards attached to the uterus via the ovarian ligament. Other structures and tissues of the ovaries include the hilum.

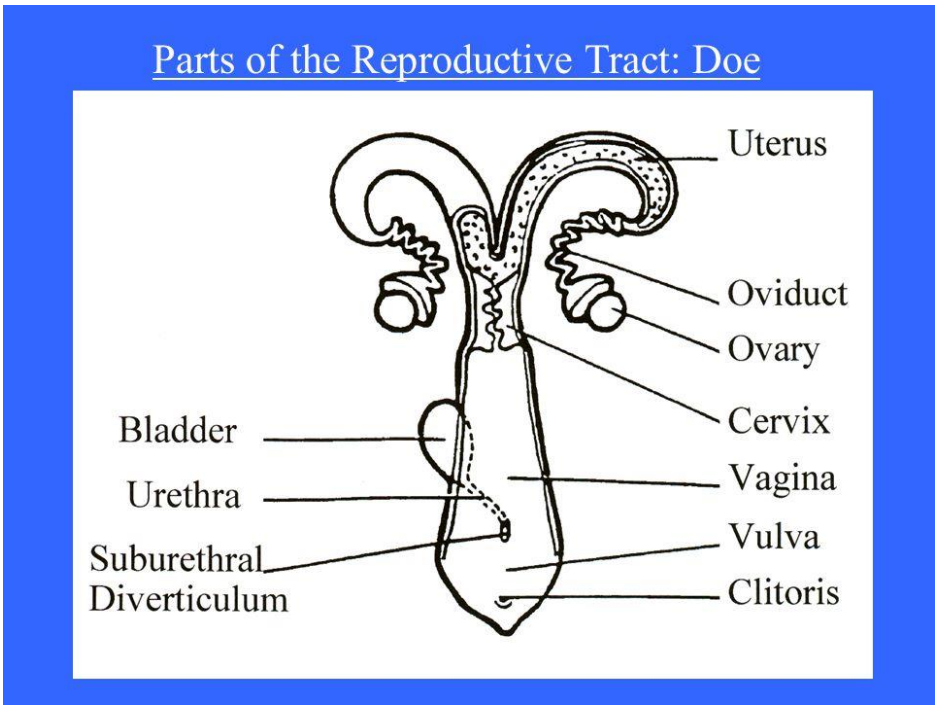

Plate 2: Female reproductive tract of goat

Source:https://www.google.com/search?q=goat+reproductive+system&tbm=isch&source=univ&sa=X&ved=2ahUKEwj9idbBnN7jAhXNfSsKHffEAbYQ7Al6BAgGECQ&biw=1366&bih=657#imgrc=_FtKrS3hO0qhXM:

2.6.1 Function

At puberty, the ovary begins to secrete increasing levels of hormones. Secondary sex characteristics begin to develop in response to the hormones. The ability to produce eggs and reproduce develops. The ovary changes structure and function beginning at puberty (Colvin *et al*., 2013).

2.6.1.1 Gamete production

The ovaries are the site of production and periodical release of egg cells, the female gametes. In the ovaries, the developing egg cells (or oocytes) mature in the fluid-filled follicles. Typically, only one oocyte develops at a time, but others can also mature simultaneously. Follicles are composed of different types and number of cells according to the stage of their maturation, and their size is indicative of the stage of oocyte development.

When the oocyte finishes its maturation in the ovary, a surge of luteinizing hormone secreted by the pituitary gland stimulates the release of the oocyte through the rupture of the follicle, a process called ovulation. The follicle remains functional and reorganizes into a corpus luteum, which secretes progesterone in order to prepare the uterus for an eventual implantation of the embryo.

2.6.1.2 Hormone secretion

At maturity, ovaries secrete estrogen, testosterone, inhibin, and progesterone (Colvin *et al*., 2013). In women, fifty percent of testosterone is produced by the ovaries and adrenal glands and released directly into the blood stream. Estrogen is responsible for the appearance of secondary sex characteristics for females at puberty and for the maturation and maintenance of the reproductive organs in their mature functional state. Progesterone prepares the uterus for pregnancy, and the

mammary glands for lactation. Progesterone functions with estrogen by promoting menstrual cycle changes in the endometrium.

2.7 Oocyte

An oocyte is a female gametocyte or germ cell involved in reproduction. In other words, it is an immature ovum, or egg cell. An oocyte is produced in the ovary during female gametogenesis. The female germ cells produce a primordial germ cell (PGC), which then undergoes mitosis, forming oogonia. During oogenesis, the oogonia become primary oocytes. An oocyte is a form of genetic material that can be collected for cryoconservation. Cryoconservation of animal genetic resources have been put into action as a means of Cytoplasm. Oocytes are rich in cytoplasm, which contains yolk granules to nourish the cell early in development.

2.7.1 Nucleus

During the primary oocyte stage of oogenesis, the nucleus is called a germinal vesicle. The only normal human type of secondary oocyte has the 23rd (sex) chromosome as 23, X (female-determining), whereas sperm can have 23,X (female-determining) or 23,Y (male-determining).

2.7.2 Nest

The space within an ovum or immature ovum is located is the cell-nest.

2.7.3 Cumulus-Oocyte Complex

The cumulus-oocyte complex contains layers of tightly packed cumulus cells surrounding the oocyte in the Graafian follicle. The oocyte is arrested in Meiosis II at the stage of metaphase II and is considered a secondary oocyte. Before ovulation, the cumulus complex goes through a structural change known as cumulus expansion. The granulosa cells transform from tightly compacted to an

expanded mucoid matrix. Many studies show that 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).

2.8 Factors affecting oocyte quality

Good quality oocyte maturation is an essential prerequisite for embryo development. Naturally the oocyte quality is determined by the oocyte's ability to mature, be fertilized and give rise to normal offspring after gestation (Sirard *et al*., 2006). This capacity is acquired during folliculogenesis, as the oocyte grows and during the period of oocyte maturation (Krisher, 2004). Proper oocyte selection in the laboratory is crucial for successful embryo production. A study performed in bovine (Blondin and Sirard,1995) showed that the best indicators of an immature oocyte ability to undergo maturation and embryonic development were the presence of an intact complement of cumulus cell layers surrounding the oocyte and a homogenous appearing cytoplasm. Also, the follicular and oocyte sizes, as well as several factors, such as age of the donor animal, and the media used for maturing the oocytes are some factors that have been linked to the maturational competence of oocytes and have therefore been proposed as selection criteria for oocyte quality, as a key factor in determining the development of embryos to the blastocyst stage (Lonergan *et al*., 2003).

2.8.1 Effect of follicle and oocyte size

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*., 2002a), 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*., 2010a) 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 thefollicle enlarges. In prepubertal goats, Romaguera *et al*,. (2010a) 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.

The comparison of the oocyte diameter is often used as a marker for oocyte maturity or meiotic competence, able to attain their full developmental competence to blastocysts *in vitro*, as there is an intensive synthesis of RNA

during this phase that causes an increase in size. According to Brevini *et al*., (2007), during the oocytes growth, messenger RNAs and proteins of maternal origin are accumulated into the oocyte throughout its growth in the ovary, upon fertilization, several mechanisms are activated that control the appropriate use of such material and prepare for the synthesis of new products supporting fertilization and initiating embryo development. The association between the oocyte diameter and its ability to resume and complete meiotic maturation *in vitro* has been described in several farm animals. 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 > de 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.

2.8.2 Age of the donor

There is a general agreement upon the fact that the use of prepubertal females as oocytes donors plays an important role on *in vitro* embryo production programs, by the reduction of the generation interval and consequently increasing the intensity of breeding. However, 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*etal*.,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). In prepubertal goats, it has been revealed functional deficiencies in cytoplasmic maturation of oocytes, such as altered distribution of cortical granules (Velilla *et al*., 2004) and mitochondria (Velilla *et al*., 2006), disorganization of microtubule and microfilament (Velilla *et al*., 2005) and alteration in total RNA content, p34 (cdc2) and cyclin B1 expression as well as maturation promoting factor (MPF) activity (Anguita *et al*., 2007; 2008). After *in vitro* fertilization, a low incidence of sperm head decondensation at fertilization (Martino *et al*., 1995; Mogas *et al*., 1997b), a high percentage of haploid embryos (Villamediana *et al*., 2001) and, consequently, a low rate of blastocyst production (Izquierdo *et al*., 2002) has been also observed.

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.

Studies in ovine (Earl *et al*., 1994; O'Brien *et al*., 1997a; Ledda *et al*., 1999a) prove a better effectiveness of using this source of oocytes applying a previous stimulation of prepubertal females with exogeneous gonadotropins. A positive effect of gonadotropins on follicular and oocyte size could be the cause of the lack of differences in embryo development between prepubertal and adult oocytes, which allow the oocytes to develop up to blastocyst stage similarly to that obtained when oocytes from adult sheep. Besides, these oocytes showed higher diameter than oocytes from unstimulated lambs. Also in caprine, following ovarian stimulation and laparoscopic recovery, prepubertal and adult goat oocytes

cultured in semi-defined media presented a similar developmental competence to blastocyst stage (Koeman *et al*., 2003).

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*.

2.8.3 Seasonal effect

In ovine, only a limited number of mature oocytes can be collected via superovulation and subsequent ovum pick up (OPU) procedure. Ovaries obtained from slaughtered animals are the most abundant and inexpensive sources of oocytes for in vitro embryo production (IVEP) (Rosa *et al*., 2003; Wani *et al*., 2000; Wang *et al*., 2007). "Reproductive seasonality in the ewe is characterized by changes at behavioral, endocrine and ovulatory levels, in an absolute fashion, giving rise to an annual alternation between two distinct periods; a breeding season, characterized by the succession at regular intervals (mean of 17 days) of estrous behavior and ovulation, if a pregnancy does not develop, and an anestrous season characterized by the cessation of sexual activity" (Rosa *et al*., 2003). Considering that ovine is a seasonal breeder species and a great influence of different seasons on their hormonal and systemic changes, it would be reasonable to consider a combination of seasonal effects with other primary steps as the most important elements may affect the success rate of IVEP (Harper, 1993).

Several factors influence the IVEP and the optimization of in vitro techniques could improve the IVEP efficiency (Harper, 1993). Higher percentages of blastocysts are obtained via in vivo maturation of oocytes compare to in vitro

maturation (IVM). This kind of evidence suggests that in vitro milieu is not sufficient for supporting development and early embryo competence (Belaisch-Allart *et al*., 1985). Due to the significant impact of the seasons on ovine reproductive cycle, it is not uncommon for IVM-IVF systems to endure some deficiencies in non-breeding seasons (Carolan *et al*., 1994). Thus the IVEP yield may increase by optimizing the IVM system (Carolan *et al*., 1994). The ovarian status and different seasons have a great influence on ovine embryo production. 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.

CHAPTER III

MATERIALS AND METHODS

The experiment was conducted at the Laboratory of Animal Production and Management Department, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh.

3.1. Collection of ovaries

Ovaries were collected from local slaughterhouse with their reproductive history being unknown. The ovaries were then recorded as right, left and the presence or absence of corpus luteum (CL) was also recorded. They were then kept in collection vial containing 0.9% physiological saline in a thermo flask and transported to the laboratory. The ovaries were then transferred to sterilized petridishes and rinsed thoroughly by physiological saline before further processing.

Plate 3: Collection of ovaries, A. Reproductive tract collection at slaughter house, B. Female reproductive tract at flask

3.2. Processing of ovaries

The ovaries were then transferred to the Petri-dishes which is sterilized and containing the same saline. The ovaries were rinsed for two times thoroughly by physiological saline solution. Each ovary was trimmed and the surrounding tissues, fat & overlying bursa were removed by dissection.

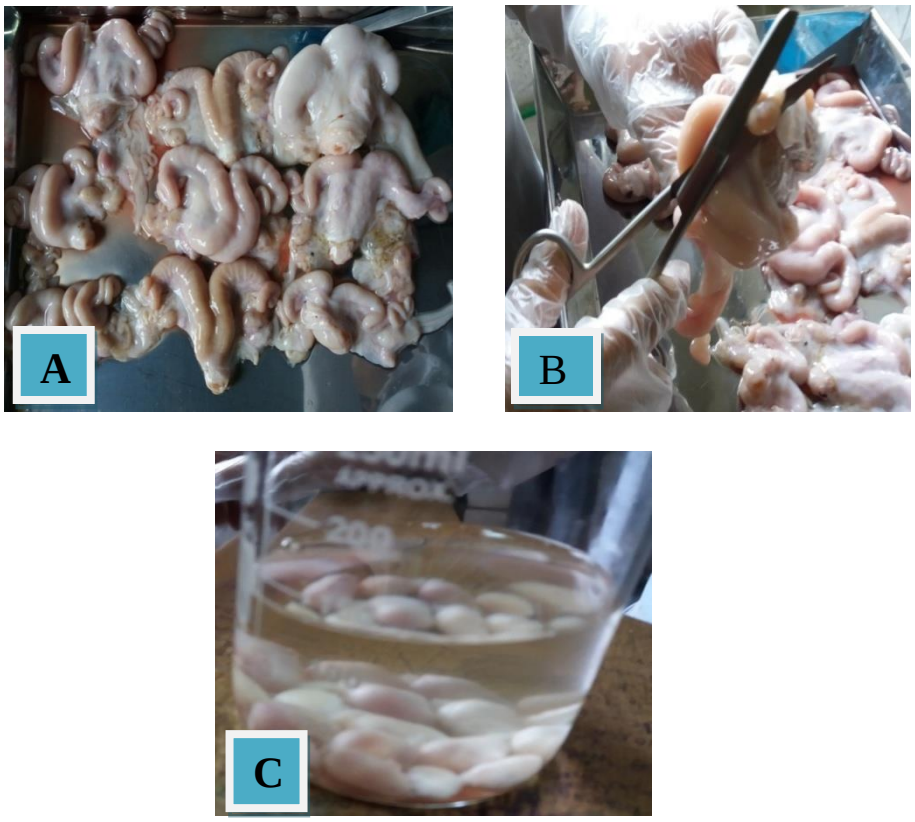

Plate 4: Processing of ovaries, A. Female reproductive tract at steel tray, B. Trimming of ovaries, C. Collected ovaries at PBS containing beaker

3.3. Morphological study of ovaries

After trimming individually, right, left, CL-present and -absent ovaries were weighed in gram by digital balance and was recorded in tabular form. The length and width of the ovaries were measured in cm with the help of a slide caliperse.

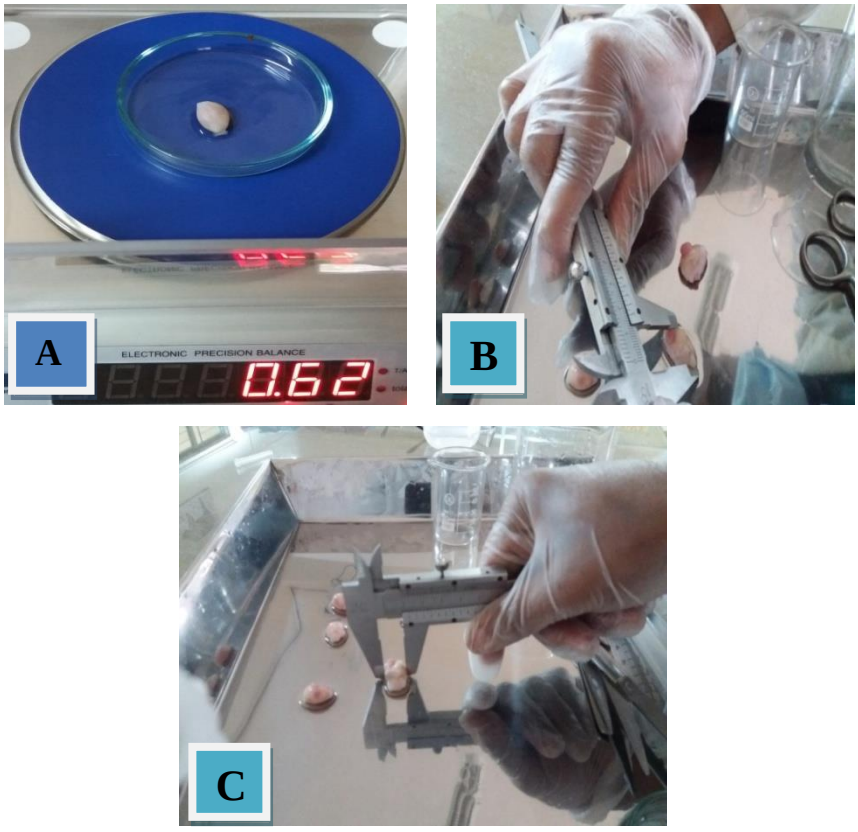

Plate 5: Morphological study of ovaries, A. Measuring weight of ovaries, B. Measuring length of ovaries, C. Measuring width of ovaries

3.4 Oocyte collection

The numbers of visible follicles on the surface of different category of ovaries were counted and recorded. The ovaries were washed 2 to 3 times in saline solution. Each ovary was processed individually and the COCs were harvested by the following three techniques:

3.4.1. Dissection Technique

The ovaries were placed in a sterile glass petridish containing 2 ml of DPBS (NaCl, KCl, Na_2HPO_4, KH_2PO_4). All the visible follicles were carefully subjected to blunt dissection with the help of forceps and the remaining ovarian tissues were removed after a brief rinsing. The follicles were ruptured and the follicular fluid was allowed to flow into the DPBS.

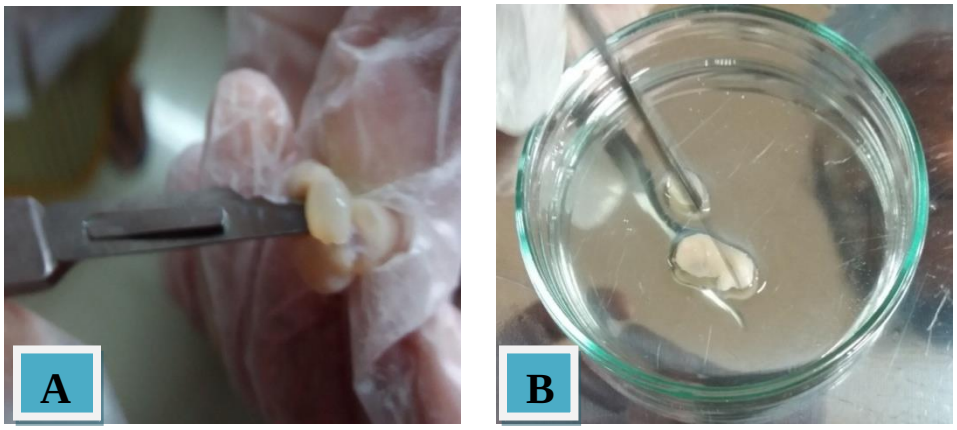

Plate 6: Dissection technique, A. Dissection of follicle by scalpel blade, B. Rupture of follicle by scalpel blade

3.4.2. Slicing Technique

The ovaries were held firmly with the help of forceps in a sterile glass petridish containing 2 ml of DPBS. The ovaries were sliced into possible thin sections with a blade and fixed to the artery forceps. Incisions were given along the whole ovarian surface by using a scalpel blade. The oocytes containing DPBS media were transferred to the petridish and observed under microscope to grade the oocytes.

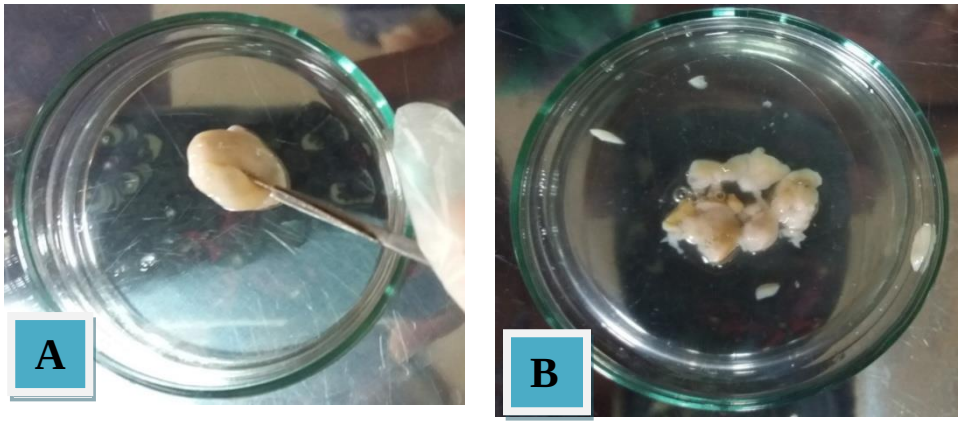

Plate 7: Ovary slicing Technique, A. Incision of ovary, B. Slicing of ovary

3.4.3. Aspiration Technique

Visible follicles were aspirated using an 18 gauge hypodermic needle attached with a sterile 5 ml disposable syringe containing 2ml DPBS. The media along with the collected COCs was then transferred to a 35 mm Petri dish. In all the three techniques, the Petri dishes were kept undisturbed for 5 min, allowing the COCs to settle down.

Plate 8: Aspiration of follicles by needle and syringe

3.5. Oocyte grading

The Petridishes were then examined under an inverted microscope, and the total number of COCs harvested was counted. The oocytes were graded into 4 grades on the basis of cumulus cells and nucleus as described by Khandoker *et al.* (2001): Grade A: oocytes completely surrounded by cumulus cells; Grade B: oocytes partially surrounded by cumulus cells; Grade C: oocytes not surrounded by cumulus cells and Grade D: degeneration observed both in oocytes and cumulus cells. The grade A and B were considered as normal and grade C and D as abnormal oocytes.

3.6. Morphometry of oocytes

The morphometric criteria that comprised the dimensions of each oocyte were as follows: diameter of the whole oocyte, including the zona pellucida (ZPO), zona pellucida thickness (ZPT), ooplasm diameter (OD), the perivitelline space (PVS) area, and PVS diameter. Among the oocytes with different nuclear status, there were no differences in ZPO and ZPT (Islam *et al.*, 2017). The collected oocytes were grouped based on diameter: Group 1 (<95 µm), Group 2 (95-100 µm) and Group 3 (>100 µm). The thicknesses of the cumulus cell layer (C) in COCs were classified into three categories: Category 1 (<30 µm), Category 2 (30-50 µm) and Category 3 (>50 µm).

3.7. Data analysis

Data were presented as mean±standard deviation (SD). The P value less than 0.05 were considered as significant. Data were analyzed with the SAS (Statistical Analysis System) software using one-way Analysis of Variance (ANOVA).

CHAPTER IV

RESULTS AND DISCUSSION

4.1. Gross study of the ovary

The ovaries were found almond-shaped, pale colored structures situated in the edge of the mesovarium near the lateral margin of the pelvic inlet. This report corresponds to the report of Getty (1975); May (1970). Each ovary had an irregular surface by follicles of various sizes projecting from the surface. It also supports their observations. The length of right ovary was numerically higher than that of left ovary (Table 1). Comparatively a lower length for both right and left ovaries of goats was reported by Islam *et al*. (2007). However, a higher length was reported in goats (Mohammadpur, 2007; Adigwe and Fayemi, 2005; Sharma and Sharma, 2004; Smith, 1986). Non significant difference was found between the mean width of right and left ovaries (Table 1, Figure 1). The mean weight of the ovary recorded in the present study was higher than that of other observation (Islam *et al*., 2007). The weight, length and width of the right ovary were 0.79±0.17 g, 1.36±0.21 cm and 1.00±0.13 cm and of the left were 0.77±0.10 g, 1.39±0.32 cm and 0.98±0.15 cm respectively. This observation showed the slight difference with other reports (Sisson and Grossman, 1975; Haque *et al*., 2016). The length of the left and right ovaries was 1.71 ± 0.27 cm and 1.73 ± 0.27 cm respectively in Nigerian goats (Adigwe and Fayemi, 2005). 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 9 A).

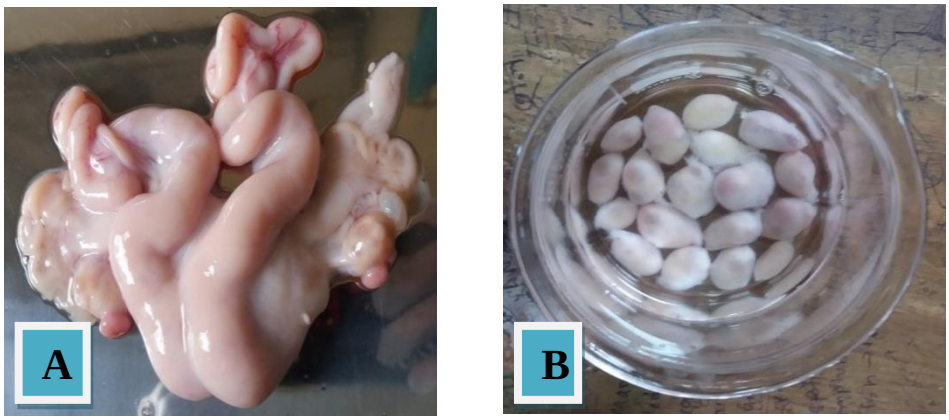

Plate 9: Gross study of ovary, A. Ovary and associated structures, B. Collected ovaries

Table 1: Morphology of ovary:

		Right mean±SD	Left mean±SD	Level of Significance
Morphology of Ovary	Weight(g)	0.79±0.17	0.77±0.10	NS
	Length(cm)	1.36±0.21	1.39±0.32	NS
	Width(cm)	1.00±0.13	0.98±0.15	NS

NS= Non significant

The observation expressed that both left and right ovaries were equally active to normal physiological and/or ovarian activity. Islam *et al.* (2007) worked on goat ovaries and expressed that the mean weight, length and width were found to be higher in right ovaries than those of left ovaries. They concluded that right ovaries were more active than left ones to show normal physiological and/or ovarian activity. These observations differ to that of the present study. Talukder *et al.* (2011) observed that there was no significant (P>0.05) difference in length, width and weight between the left and right ovaries of sheep. The results of some previous study, Singh *et al.* (1974), Mohanunadpour (2007), Asad *et al.* (2016)

supports the findings of present study that there was no significant difference (P>0.05) in the parameters of left and right ovaries of goat.

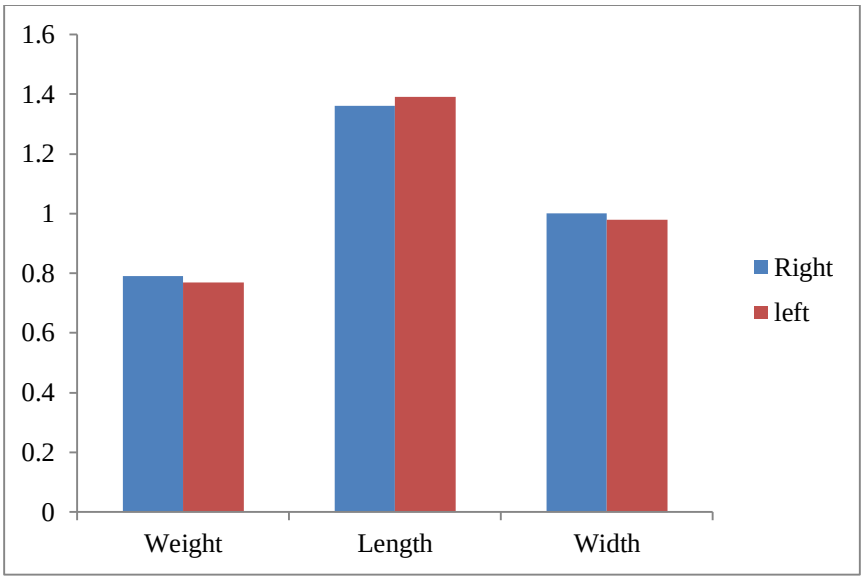

Figure 1: Measurements of right and left ovaries

4.2. Effect of time elapsed from slaughtering to sample processing on oocyte quality

It is better to transport quickly the specimens from abattoir to the place of processing after slaughter. If the samples stored well by cool box under 4-8°C, samples yield a remarkably decreased oocyte numbers upon all three collection methods (Dissection, Slicing, Aspiration) (Table 2). The degree of oocyte damaging, ageing and deterioration was elevated if the time elapsed from the period of slaughtering to processing at the laboratory, as shown in table 2 and figure 2.

Table 2: The effect of time elapsed from slaughtering to specimens processing on oocyte quality

Time after slaughter (Hour)	Oocyte collection rate (%)	Oocyte quality
2	75%	Good
6	69%	Fair
12	62%	Poor
24	55%	Bad

It is noted in the present study that there was a direct effect on elapsed time from the period of slaughtering the donor animals toward time of specimens processing inside the laboratory. As this time prolonged it affects the oocytes quality which interferes with final result. This result agreed with other observations (Lonergan *et al*., 2016). This might be due to many factors affect directly the oocytes quality. Time of slaughter is the more dominant factor that influence the quality. Lihua *et al*. (2010); Saleh (2017) also approved the effect of elapsed time on the oocytes quality that might interfere (impaired) with *in vitro* oocytes maturation (IVM) that yield low quality embryos.

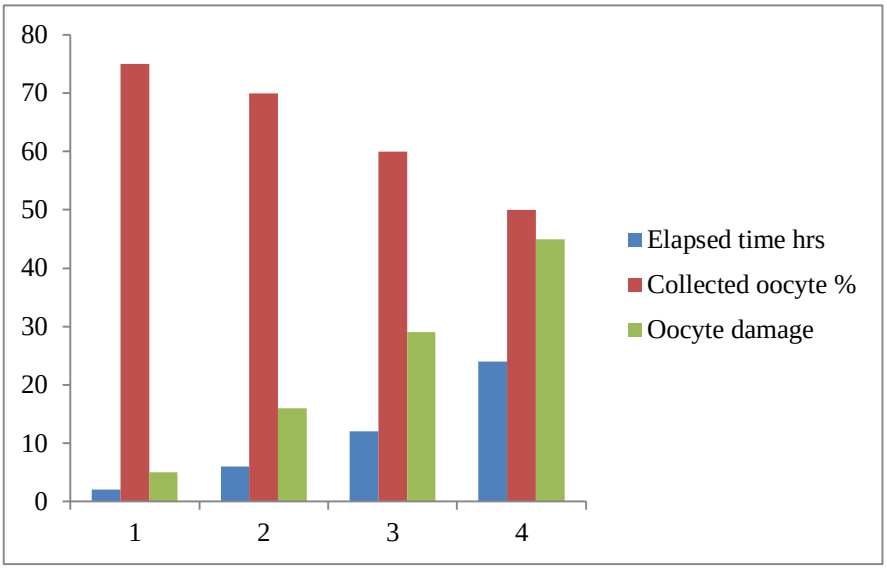

Figure 2: The effect of time elapsed from slaughtering to samples processing on oocyte quality

4.3. Effect of ovary storage temperature on oocyte quality

Abattoir samples must be transported inside container of 4-8°C temperature that protects the samples from damage or spoiling. This might lead to deterioration of samples and then bad or aged quality oocytes which interfere with the following step of IVM and IVF. When ovaries are exposed to temperatures from 37 to 39 °C for 5-6 h, both the maturation rate of oocytes and their potential to develop into blastocysts decrease. The results showed the effect of ovary storage in different temperature from time of collection to the laboratory (Table 3, Figure 3).

Table 3: Effect of storage temperature on oocyte quality:

Storage temperature (ºC)	Oocyte collection rate (%)	Oocyte quality
4-8	75	Good
15-20	69	Fair
25-30	62	Poor
35-40	55	Bad

Blad-Stahl *et al.* (2016) mentioned that and agreed in which any elevation over 4-8°C might affect the process of *in vitro* fertilization or even embryo cleavage later on. The fact that the *in vitro* development potential of oocytes decreases with high temperatures is a problem for experimental studies in laboratories with transport difficulties (Gardner *et al*., 2004; Abe and Shioya, 1996; Yang *et al*., 1990). However, storage of cattle ovaries at 4 °C for 24 h has no effect on the *in vitro* maturation and development of ovaries (Solano *et al*., 1994).

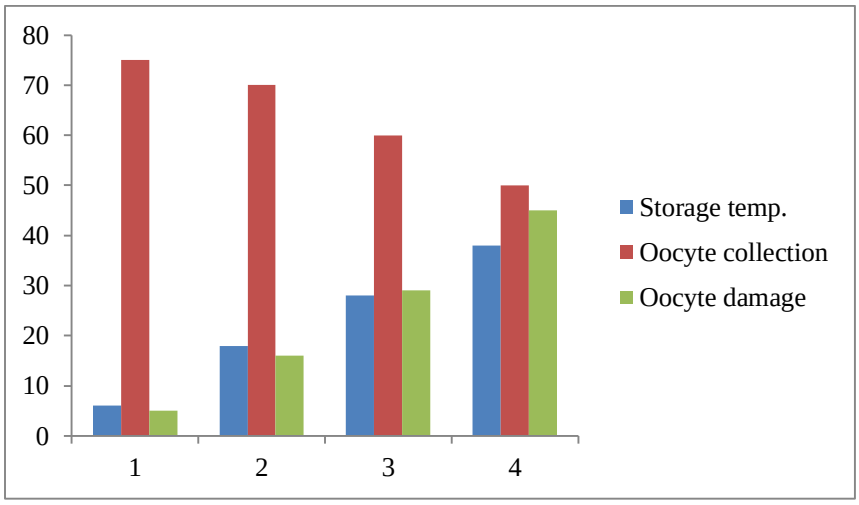

Figure 3: The effect of ovary storage temperature on oocyte quality

4.4: Effect of collection techniques on oocytes recovery

Several methods have been used for harvesting oocytes from slaughterhouse ovaries of farm animals. In the present research, the oocytes were collected by three different methods (Dissection, Slicing and Aspiration). The result of oocytes recovery per ovary by three different techniques of dissection, slicing and aspiration is summarized in Table 4. Total 33, 53 and 48 oocytes were collected by dissection, slicing and aspiration techniques; respectively, from each of 30 ovaries. The results indicate that slicing and aspiration yielded a significantly higher ($p < 0.01$) number of total oocytes than that of dissection method. However, a significantly higher ($p < 0.01$) number of normal graded (A & B) oocytes was observed in aspiration method (68.75%)) than those of dissection (33.33%) and slicing (45.28%) techniques. The most commonly practiced methods of oocytes recovery in goat are puncture and aspiration of visible follicles and follicular dissection (Wang *et al*., 2007). In the aspiration technique, oocytes were collected from 2 to 6 mm diameter of surface follicles using a hypodermic needle with 10 ml syringe. However, in the case of dissection, the surface follices were ruptured by scalpel blade and in case of slicing method, incisions made along the whole ovarian surface using a scalpel blade that is, all sizes of surface follicles that were harvested. Thus, the lower number of oocytes recovered by the dissection method in this experiment may be attributed to the presence of some follicles embedded deeply within the cortex, which can be released by aspiration or slicing techniques used in the ovary.

Table 4: Effect of collection techniques on oocyte quality

Collection Techniques	Total no. of ovaries	Total no. of Oocytes	Oocyte Quality (%)					
			A	B	Total	C	D	Total
Dissection	30	33	4 (12.12%)	7 (21.21%)	11 (33.33%)	13 (39.39%)	9 (27.27%)	22 (66.66%)
Slicing	30	53	15 (28.30%)	9 (16.98%)	24 (45.28%)	10 (18.87%)	19 (35.85%)	29 (54.72%)
Aspiration	30	48	26 (54.17%)	7 (14.58%)	33 (68.75%)	11 (22.92%)	4 (8.33%)	15 (31.25%)

Ferdous (2006) reported that the numbers of normal oocytes were found to be significantly higher in 2 to 6 mm diameter follicles than others ($p < 0.05$). Moreover, dissection and slicing techniques produce more debris which makes problem in searching of oocytes under the microscope and also required more washing as compared to aspiration technique. As a result, a number of oocytes were denuded from cumulus cells due to repeated washing and ultimately resulted in a lower number of normal oocytes when compared to aspiration at the final observation. In the present experiment comparatively more oocytes per ovary was obtained by slicing method, when compared to aspiration or dissection. This is in agreement with the observations of Martino *et al*. (1994), Vijayakumaran (1995) and Wang *et al*. (2007) in goats. The reason could be attributed to the fact that oocytes from surface follicles as well as follicles of deeper cortical stroma are released by slicing method, whereas by puncture and aspiration oocytes from surface follicles alone are released (Das *et al*., 1996 and Pawshe *et al*., 1994). So this experiment points to the fact that as far as the oocyte recovery rate is concerned maximum efficiency is for slicing method than aspiration or dissection. Yield of culturable quality oocyte was highest with aspiration followed by slicing and dissection. This finding is in agreement with the results of Vijayakumaran (1995), Kharche *et al*. (2006) and Wang *et al*. (2007). Pawshe *et al*. (1994) obtained a comparatively lower percentage of culturable quality oocytes by

puncture than the present study. Lower yield of good quality oocytes by slicing method may be due to the damage caused by the blade used for the technique.

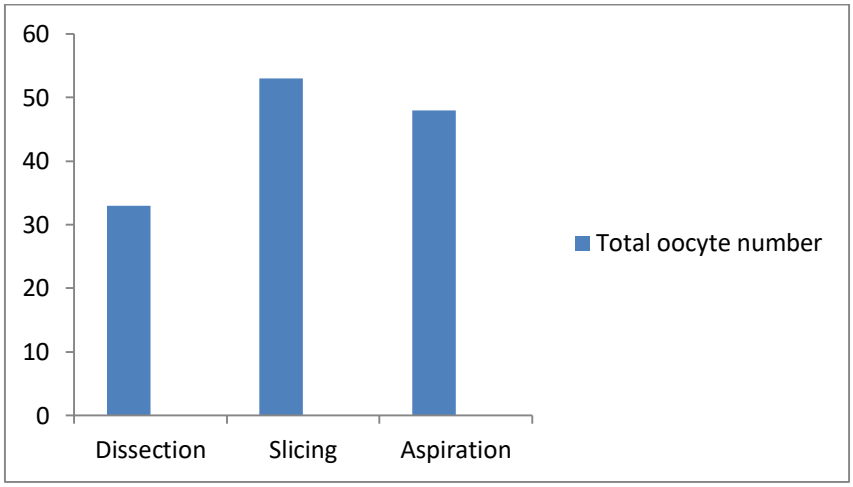

Figure 4: Total oocytes no. in different collection technique

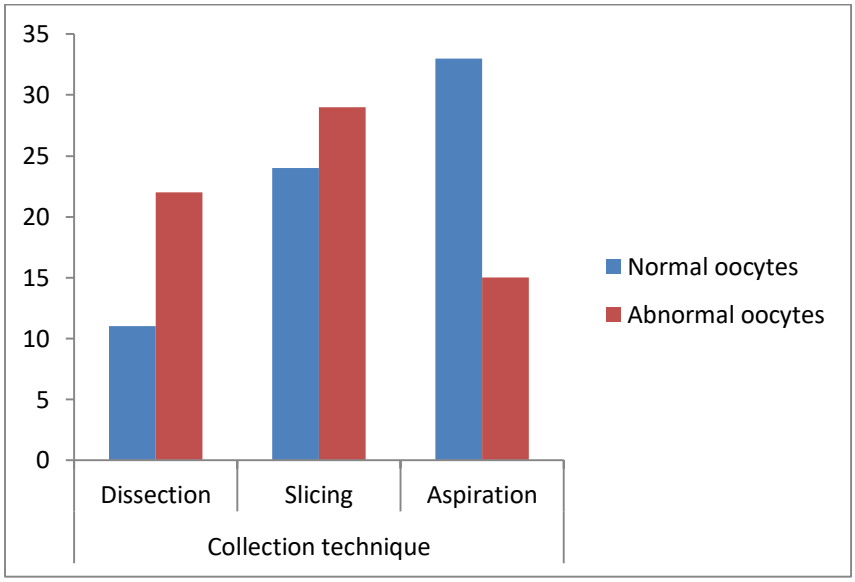

Figure 5: Oocyte quality in different collection technique

In contrast, Shirazi *et al.* (2005) reported that the number of oocytes per ovary for slicing (4.0) and aspiration (3.7) did not differ significantly. Many factors have been found to affect the yield and quality of oocytes viz. breed, season, time interval from collection of ovaries to oocyte harvest, temperature of media for

transport of ovaries, retrieval technique and the criteria used for classification of culture grade oocytes. Nutritional status, stage of estrous cycle and agro-climatic conditions in which goats are reared also influence oocyte yield and quality. This study revealed that among collection techniques, slicing yielded maximum number of oocytes per ovary, while maximum percent yield of culturable quality oocytes obtained by aspiration method.

4.5. Interrelationship between diameter of oocytes and oocytes quality

These results showed an association between oocyte diameter and oocytes quality. During the process of folliculogenesis, the oocyte diameter and layers number of granulose cells increase. As a result, follicle size and follicular fluid accumulation will increase. In the process of folliculogenesis, oocyte diameter growth and will continue to grow after antrum formation until to a certain diameter. The present study showed that there was a remarkable interrelationship between the oocyte diameter and quality (Table 5, Figure 6 and Plate 10).

Table 5: Interrelationship between oocyte diameter and oocytes quality

Group	Oocyte Diameter	No.of Oocyte Total=75	Oocyte Quality (%)					
			A	B	Total	C	D	Total
1	<95 µm	14	2 (14.29%)	4 (28.57%)	6 (42.86%)	6 (42.85%)	2 (14.29%)	8 (57.14%)
2	95-100 µm	34	14 (41.17%)	10 (29.41%)	24 (70.58%)	5 (14.71%)	5 (14.71%)	10 (29.42%)
3	>100 µm	27	13 (48.15%)	9 (33.33%)	22 (81.48%)	3 (11.11%)	2 (7.41%)	5 (18.52%)

The results showed a noticeable correlation between oocytes diameter with quality of oocyte. In Table 5, it is seen that oocytes group 3 (>100 µm) possess good quality oocytes when compared with group 2 (95-100 µm) and group 1 (<95 µm). On the other hand, the abnormal oocytes observed in highest number (57.14%) in the group 1 (<95 µm). These results revealed an association between oocyte diameter and oocyte quality. Research conducted in pigs (Lucas *et al.*, 2002) and in cattle (Otoi *et al.*, 1997) showed the oocytes with a large diameter resulting in a higher maturation rate when compared to oocytes with a small diameter.

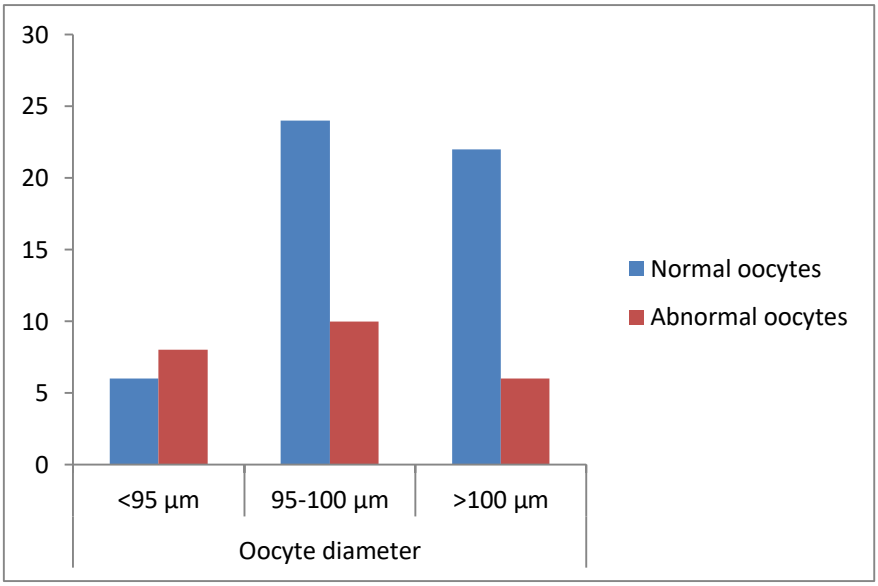

Figure 6: Oocytes quality in different oocyte diameter

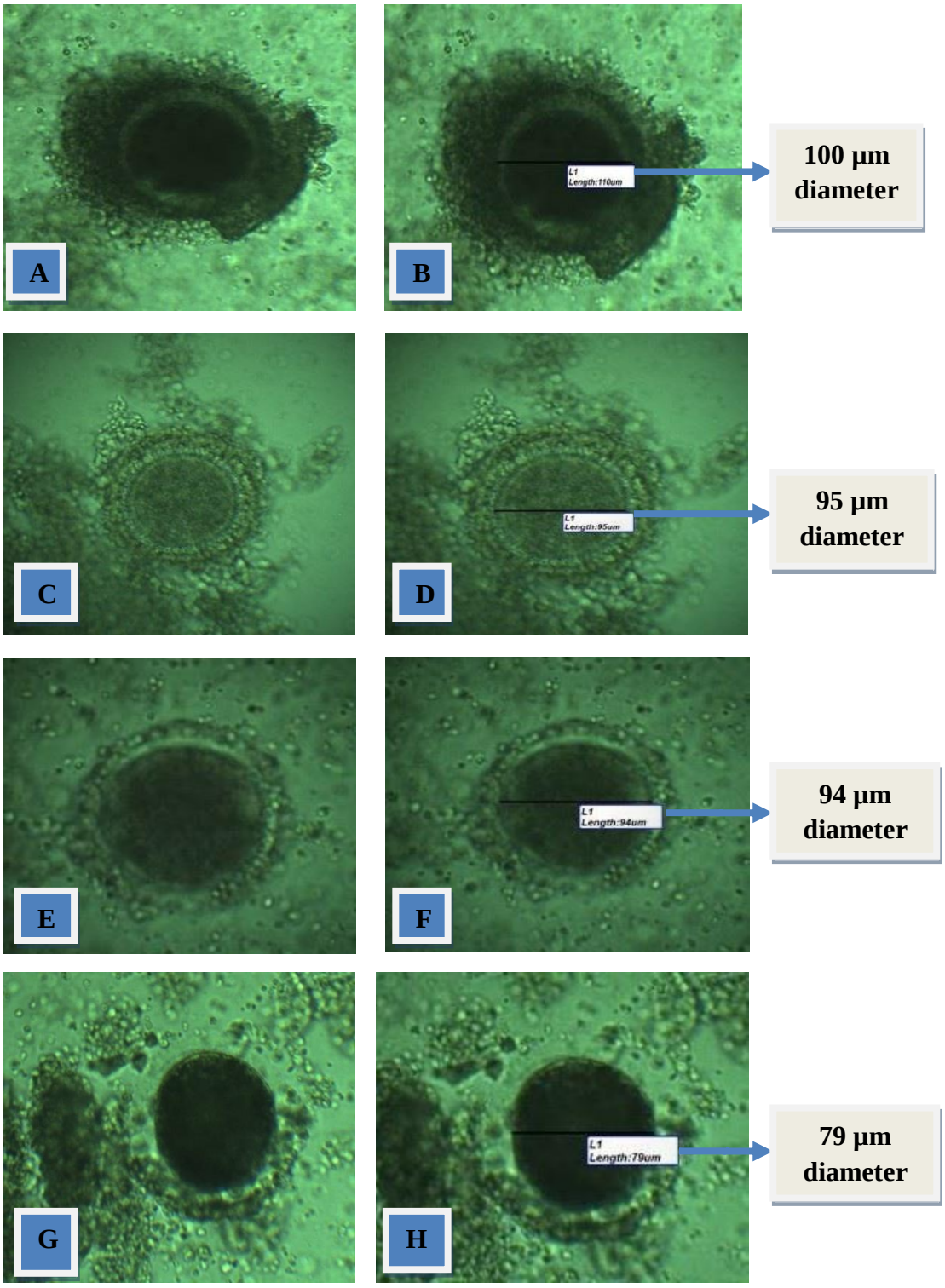

Plate 10: Interrelationship between diameter and quality of oocytes (under 10X objective), A & B. Grade A oocyte, C & D. Grade B oocyte, E &F. Grade C oocyte, G & H. Grade D oocyte

In other studies Haque *et al* (2012) and Da costa *et al* (2013) showed that oocytes with good quality COCs have a higher success rate and embryo development after fertilization. Results of the research showed a close relationship between oocyte diameter with oocyte quality for the ability of oocytes development.

4.6. Relationship between cumulus cells diameter and oocyte quality

The cumulus cells (CCs) surrounding the oocyte plays a key role in oocyte maturation, and they are known to supply nutrients, energy substrates (Sutton *et al*., 2003). To evaluate the effect of cumulus layer thickness on oocyte developmental potential, we first divided the CCs diameter into three categories according to the thicknesses of their surrounding cumulus layer (C). The CCs that were in the category of C>50 µm, had more than three layers of cumulus cells, whereas the CCs that had two or three cumulus cell layers fell into the category of 30-50 µm, and the CCs with less than two layers of cumulus cells fell into the category of C<30 µm. The table 6 showed the relationship of CCs diameter with oocyte diameter and oocyte quality. It was seen that 48.15% of the total (27) oocyte number contain >50 µm diameter oocytes. From the table 6, it was observed that, most of the oocytes having >100 µm diameter represent >50 µm CCs diameter. On the other hand, 57.14% of <95 µm oocytes diameter represent <30 µm. Meanwhile, less diameter CCs (<30 and 30-50 µm) are found more in oocyte diameter group (<95 µm). It was 57.14% and 28.57% than thicker Ccs diameter (>50 µm) which was only 14.29%.

Table 6: Relationship between cumulus cells diameter and oocyte quality:

Group	Oocyte diameter	No. of Oocyte	Cumulus cells diameter		
			<30 µm	30-50 µm	>50 µm
1	<95 µm	14	7.14% (Normal) 50% (Abnormal)	21.43% (Normal) 7.14% (Abnormal)	14.29% (Normal) 0.0% (Abnormal)
2	95-100 µm	34	14.71% (Normal) 14.71% (Abnormal)	23.53% (Normal) 5.88% (Abnormal)	32.35% (Normal) 8.82% (Abnormal)
3	>100 µm	27	11.11% (Normal) 7.41% (Abnormal)	25.93% (Normal) 7.40% (Abnormal)	44.44% (Normal) 3.71% (Abnormal)

Zhou *et al*. (2014) found that all of the CCs with C>50 µm were able to progress to the metaphase II (MII) stage (100%, 111/111), thus showing the highest rate of oocyte nuclear maturation. The CCs with 30-50 µm showed a slightly decreased rate in comparison, with 94.8% (255/269) showing PB1 extrusion ($P<0.05$). The lowest oocyte nuclear maturation rate was observed in the CCs with C<30 µm, with only 85.3% (116/136) displaying PB1 extrusion ($P<0.01$). These data suggest that a thicker cumulus cell layer promotes *in vitro* oocyte maturation. The result showed that, the more diameter containing oocytes bear thicker CCs which suitable for better quality oocytes and reproduction. The function of cumulus cells

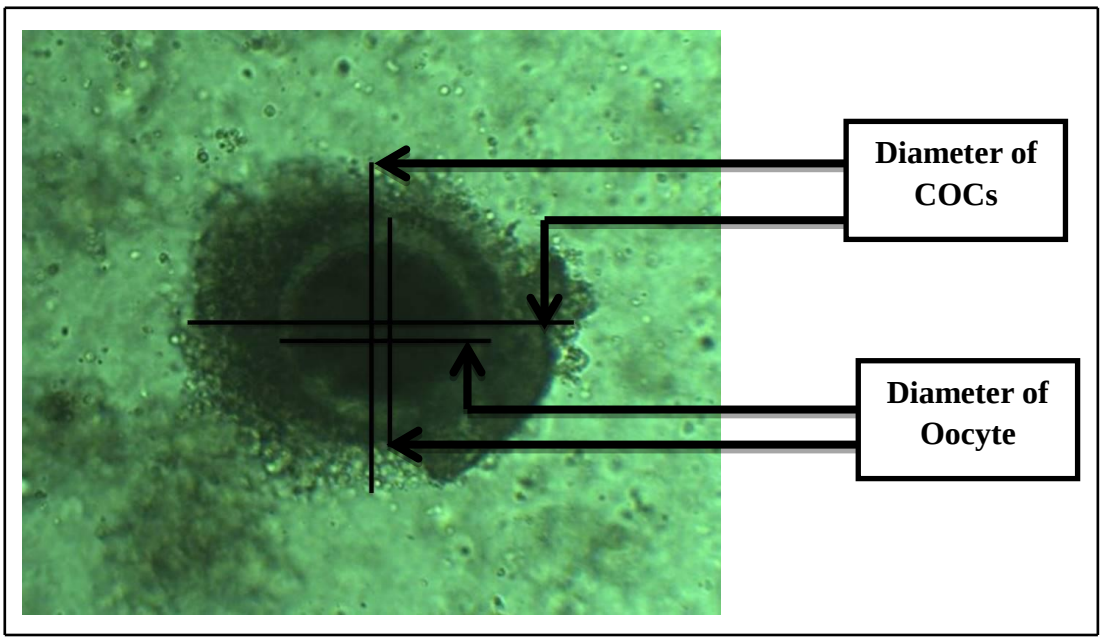

Plate 11: Determination of cumulus cell diameter (Average diameter of COCs-Average diameter of Oocyte)

surrounding the oocyte is exhibited in three stages: follicle growth, oocyte maturation, and fertilization. As one of several important components in the growing follicle, cumulus cells affect the acquisition of oocyte developmental competency (Sutton *et al*., 2003). During follicle development, communication between the cumulus cells and the oocyte provides a mechanism for regulating oocyte growth (Gilula *et al*., 1978). This function of cumulus cells may influence not only the nuclear maturation of oocytes, but also the cytoplasmic maturation (Demant *et al*., 2012). The oocytes that contain fewer cumulus cell layers have a lower potential to undergo maturation, fertilization, and development to an early embryo stage.

CHAPTER V

SUMMARY AND CONCLUSION

Quality of oocytes is a factor, which limits fertility. Of course, sperm plays an important role in the formation of the embryo (number of chromosomes and some factors, which are necessary for fertilization and further epigenetic changes) but the embryo's destiny depends mainly on oocytes. The research was carried out from April, 2018 to December 2018 at the Animal Production and Mnagement laboratory of Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka, Bangladesh to assess the quality of ovary and oocytes for reproduction.

From the present study, it was observed that the dimensions of ovary like length, width and weight of right and left ovaries of Black Bengal goats were not significantly different. The left and right ovaries are equally active for ovarian activity. The result creates a great influence for conducting Black Bengal goat embryo production research in Bangladesh. The present research revealed that the oocyte quality is highly influenced by timing and preservation temperature of sample processing. Time consuming for samples transport as well as the temperature of transporting vessel affect the samples quality. The factors might interfere with good quality embryo which associated with *in vitro* oocytes maturation (IVM), *in vitro* fertilization (IVF) and *in vitro* embryo production (IVP).

It was concluded from the present study that more oocytes with moderate quality were obtained by slicing method while good quality oocytes were collected by aspiration collection method. The overall oocyte yield was highest in slicing technique. But the maximum per cent yield of grade A and B oocytes was observed by aspiration technique. It could be concluded from the present study that aspiration of follicles is the effective technique for oocyte recovery from

slaughterhouse goat ovaries. It is the wise decision that not to discard the ovarian samples after aspiration in which they still gain more oocytes that can obtained by slicing those ovaries.

From the research it was seen that there was a remarkable relationship between the oocyte diameter and quality. The oocytes having larger diameter showed the better quality than the less diameter oocytes. The oocytes of larger diameter represent better quality oocytes which are essential for higher success rate and better embryo development. The research also showed that the larger diameter oocytes present larger diameter cumulus cells with higher percentage of good quality oocytes and these oocytes have greater contribution for *in vitro* production (IVP). It may be concluded that the ovaries of Black Bengal goat should be collected, transported, processed and evaluated with proper temperature and duration. The collected oocytes with good diameter and good cumulus cells possess the better maturation, fertilization and embryo production *in vitro*.

The present study revealed that morphometric assessment of left and right ovaries have a great potentiality to identify the good number of oocytes for *in vitro* studies. This finding creates a great opportunity for conducting further research on Black Bengal goat embryo production that may help to enhance their productivity in Bangladesh.

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