RELATIVE EFFICIENCY OF OOCYTES COLLECTION TECHNIQUES AND EVALUATION FROM GOAT OVARIES

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JUNE, 2020

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Registration No. 13-05455

A Thesis
Submitted to the Department of Animal Nutrition, Genetics and Breeding
Sher-e-Bangla Agricultural University, Dhaka
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE (MS)
IN
ANIMAL BREEDING AND GENETICS
Semester: January-June, 2020

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CERTIFICATE

This is to certify that the thesis entitled ***"RELATIVE EFFICIENCY OF OOCYTES COLLECTION TECHNIQUES AND EVALUATION FROM GOAT OVARIES"*** *submitted to the Department of Animal Nutrition, Genetics and Breeding, Sher-e-Bangla Agricultural University, Dhaka in partial fulfillment of the requirements for the degree of* ***MASTER OF SCIENCE (MS) in ANIMAL BREEDING AND GENETICS,*** *embodies the results of a piece of bona fide research work carried out by* ***MD. ABDUR RAIHAN RATUL,*** *Registration No.****13-05455*** *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.

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ACKNOWLEDGEMENTS

The author seems it a much privilege to express his enormous sense of gratitude to the almighty Allah for there ever ending blessings for the successful completion of the research work.

The author wishes to express his gratitude and best regards to his respected Supervisor, ***Prof. Dr. Lam Yea Asad****, Department of Animal Nutrition, Genetics and Breeding, Sher-e-Bangla Agricultural University, Dhaka, for her continuous direction, constructive criticism, encouragement and valuable suggestions in carrying out the research work and preparation of this thesis.*

The author wishes to express his earnest respect, sincere appreciation and enormous indebtedness to his reverend Co-supervisor, ***Prof. Dr. Mufazzal Hossain****, Department of Animal Nutrition, Genetics and Breeding, Sher-e-Bangla Agricultural University, Dhaka, for his scholastic supervision, helpful commentary and unvarying inspiration throughout the research work and preparation of the thesis.*

The author feels to express his heartfelt thanks to the honorable Chairman, ***Associate Prof. Dr. Mofassara Akter,*** *Department of Animal Nutrition, Genetics and Breeding along with all other teachers and staff members of the Department of Animal Nutrition, Genetics and Breeding, Sher-e-Bangla Agricultural University, Dhaka, for their co-operation during the period of the study.*

The author feels proud to express his deepest and endless gratitude to all of his course mates and friends to cooperate and help him during taking data from the experiment and preparation of the thesis. The author wishes to extend his special thanks to his lab mates, class mates and friends for their keen help as well as heartiest co-operation and encouragement.

The author expresses his heartfelt thanks to his beloved parents, Elder Sister and Brother and all other family members for their prayers, encouragement, constant inspiration and moral support for his higher study. May Almighty bless and protect them all.

The Author

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ABSTRACT

The present study was carried out at the Department of Animal Nutrition, Genetics and Breeding, Sher-e-Bangla Agricultural University, Dhaka during the period from January 2019 to December 2019. Goat ovaries were collected from Slaughter house of Dhaka city. After necessary processing the ovaries were categorized as right, left, ovaries without corpus luteum (CL), ovaries with corpus luteum (CL). Ovaries were then evaluated on the basis of length (cm), width (cm), weight (g), total number of follicles on the surface of each category ovaries, number of follicles aspirated, total number of cumulus-oocyte-complexes (COCs), normal and abnormal COCs. The length (cm) of right ovaries (1.31±0.04) was found significantly ($p<0.05$) higher than the left ones (1.18±0.04). The number of normal COCs (Grade A and Grade B) were found significantly ($p<0.01$) higher in left ovaries (2.14±0.08 and 1.65±0.08) then right ovaries (0.36±0.08 and 0.23±0.08) respectively. The number of abnormal COCs (Grade C and Grade D) were found significantly ($p<0.01$) higher in right ovaries (1.67±0.09 and 2.20±0.08) than left ovaries (0.33±0.09 and 0.34±0.08) respectively. Other parameters, including width, weight, total number of COCs did not differ significantly ($p<0.05$) between right and left ovaries. When compared between the ovaries with CL and without CL group significantly ($p<0.01$) higher number of normal COCs (Grade A and Grade B) were found without CL group (1.21±0.07 and 0.90±0.07) then with CL group (0.32±0.09 and 0.29±0.09) respectively. The number of abnormal COCs (Grade C and Grade D) were found significantly ($p<0.01$) higher in with CL group (0.82±0.09 and 1.18±0.09) then without CL group (0.31±0.06 and 0.39±0.07) respectively). Total number of follicles were found significantly ($p<0.01$) higher in without CL group (6.79±0.29) then with CL (5.84±0.21) group and number of follicles aspirated were found significantly ($p<0.05$) higher in without CL group (3.79±0.14) then with CL group (3.62±0.20). An increase of length (1.47±0.09) and total number of COCs (2.81±0.11) were found in without CL group, but decrease of length (1.34±0.14) and total number of COCs (2.62±0.16) in with CL group. An increase of width (0.74±0.05) in with CL group then without CL group (0.72±0.04). When COCs per ovary was compared among the collection techniques significantly ($p<0.01$) higher number of total COCs per ovary was yielded by slicing (22.20±2.40), followed by puncture (13.20±1.86) and aspiration (10.37±1.86). The number of normal COCs (Grade A and Grade B)were found significantly ($p<0.05$) higher in aspiration (3.80±0.60 and 3.33±0.42) followed by slicing (3.67±0.78 and 3.20±0.54) and puncture (2.20±0.60 and 1.40±0.42) respectively. The number of abnormal COCs (Grade C and Grade D) were found significantly ($p<0.01$) higher in slicing (7.00±1.02 and 8.33±0.98) followed by puncture (5.00±0.79 and 4.60±0.76) and aspiration (1.40±0.79 and 1.80±0.76).The maximum percent yield of normal COCs were higher in aspiration followed by slicing and puncture techniques. On the other hand, left ovaries contain more normal COCs and higher number of follicles than right ovaries; without CL ovaries contain higher number of follicles and normal COCs than with CL ovaries. Finally, it can be concluded that higher number of normal COCs found in left ovaries, without CL ovaries and aspiration technique is better for quality COCs.

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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
BBG	=	Black Bengal goat
BBS	=	Bangladesh Bureau of Statistics
B.C.	=	Before Christ
BCSRI	=	Bangladesh Council of Scientific Research Institute
BO	=	Brackett and oliphant
BSA	=	Bovine serum albumen
BW	=	Birth weight
CIRG	=	Central institute for Research on Goats
Ca	=	Calcium
CL	=	Corpus Luteum
cm	=	Centimeter
COCs	=	Cumulus-oocyte-complexes
CV %	=	Percent Coefficient of Variation
DAS	=	Days After Sowing
DF	=	Degree of freedom
DLS	=	Department of Livestock Services
DM	=	Dry matter
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
FBS	=	Fetal bovine serum
FCS	=	Fetal calf serum
FF	=	Follicular fluid
FSH	=	Follicle stimulating hormone
g	=	Gram (s)
GDP	=	Gross Domestic Product
GV	=	Germinal vesicle
GVBD	=	Germinal vesicle break down
GLM	=	General linear model
GM	=	Geometric mean

hCG	=	Human chorionic gonadotropin
i.e.	=	id est (L), that is
IVC	=	*In vitro* Culture
IVF	=	*In vitro* Fertilization
IVP	=	*In Vitro* Production
IVM	=	*In vitro* Maturation
Kg	=	Kilogram (s)
L	=	Litre
Lbs	=	Pound
LH	=	Luteinizing hormone
LSD	=	Least Significant Difference
m^2	=	Meter squares
mg	=	Milligram
ml	=	Milliliter
MOET	=	Multiple Ovulation Embryo Transfer
MS	=	Mean Square
NaOH	=	Sodium hydroxide
NBF	=	Nucleus Breeding Flock
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
SS	=	Sum of Squares
TCM	=	Tissue culture medium
var.	=	Variety
µg	=	Microgram
USA	=	United States of America
Viz.	=	Namely
WHO	=	World Health Organization

CHAPTER I

INTRODUCTION

Reproduction is an obligatory part of livestock production. In spite of the fact that livestock plays a major important role (13.46%) in agricultural sector (Livestock Economic Statistics, 2018-19) in the national economy of Bangladesh. But the genetic potentiality of native livestock is inadequate. Due to low genetic potentiality, native livestock cannot fulfill the demand of milk and meat of our country. This problem is the most crucial constraints of livestock population in Bangladesh. 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). Follicular oocytes could be matured *in vitro* and used for *in vitro* fertilization for producing great quantity of embryos (Agarawal and Suzuki, 1992). *In vitro* techniques are powerful tools for studying physiology of maturation, fertilization, development of pre-implantation embryos and increasing production as it gives access to micromanipulation of embryos. For such studies, a large number of *in vitro* produced embryos are needed, which in turn need larger number of oocytes only. To produce good embryos, quality oocyte is obligatory. Oocytes are the raw material for *in vitro* production (IVP) experiments. Ovaries from slaughtered animals are the cheapest and the most abundant source of primary oocytes. Though lot of ovaries are waste in slaughter house but it may be a good source of quality livestock production which can fulfil the existing scarcity of meat, milk and skin. Ovary collection, evaluation and grading technique results in rapid genetic gain of outstanding females. The total number of oocytes obtained per ovary is varied with different techniques. Therefore, for efficient *in vitro* production of embryo from ovaries procured from abattoirs is necessary to develop technique that can maximize the oocyte recovery.

Goat is the first ruminant livestock after dog that domesticated around 8000-11000 B.C. (Luikart *et al*., 2006). Goats are economically very significant and promising animal genetic resources in the developing countries like Bangladesh. Goat play vital role in the economy of Bangladesh (Kosgey, 2004) with the potential to provide high quality meat, milk and skin. Since it provides a good source of milk, fiber and skin, it is popularly known as the "poor man's cow" (MacHugh and Bradley,2001). Goats have become more significant in the rural economy even throughout the country, while dairy cattle and poultry industry are making significant impact as a provider of animal protein in the country (Lebbie, 2004). Government of Bangladesh has given special emphasis on Black Bengal goat for farmers to reduce the poverty with targeting the millennium development goals (MGDs) achievement. Out of world goat population, about 90% goats are found in developing countries. Goat population in Bangladesh is the fourth highest in Asia and constitutes nearly 11.79% of the total population (FAO, 2013). The average number of goats per household is about 2.31 in Bangladesh (Faruque, 2010). Goats are the best convertor of low-quality roughage, green grasses, shrubs and various tree leaves which are provided. Farmers keep cattle, chicken, with goats and also produce agricultural products. They like to rear goat due to it require less feed and capital than others. About 41% of the total income provided by goats in farmer house (Husain, 1993). It also serves as a store of value and instant cash asset (Morand-Fehr *et al*., 2004).

Embryo transfer (ET) technology is well established in cattle but due to poor ovulatory response, the application of this method is not yet familiar in goat. Embryo transfer refers to the technique by which fertilized ova are collected from the reproductive tract of a genetically superior female (donor) and transfer to genetically inferior female (recipient). This technique results in rapid genetic gain of outstanding female, which complement the utilization of superior males through artificial insemination (AI) program. The embryo transfer technology can be successfully done by three ways, i) multiple ovulation and embryo transfer (MOET), ii) *in vivo* ovum pick up (OPU) and iii) oocyte recovery from slaughtered animal. In slaughterhouse, the does which are slaughtered for meat purpose,

oocytes can be collected from the ovaries of those does by different oocyte collection techniques like aspiration, slicing and puncturing. However, the necessity of genetically superior embryos is unavoidable, given the obligation for genetic improvement of livestock. Hence, we need to enhance the system to make genetically superior embryo available for transfer (Danilda, 2000).

A substitute to conventional superovulation procedure is *in vitro* production (IVP) of embryos. This technology allows the anticipated supply of embryos from ovaries of slaughtered females or from selected live animals, via repeated recovery of primary oocytes. This technology does not only offer optimization of high-quality dams, but also allows the preservation and rapid multiplication of genetically superior characters by making embryos available for cloning, sexing and nuclear transfer (Danilda, 2000).

In vitro production (IVP) of embryos indicates the use of laboratory techniques to generate embryos. This process usually refers retrieval of oocytes from the ovaries of a female, *in vitro* maturation (IVM) of oocytes, *in vitro* fertilization (IVF), *in vitro* culture (IVC) of presumptive zygotes to the morula or blastocyst stage of embryo development (Brackett *et al*., 1982). Collection and processing of ovaries, evaluation of ovaries, collection and grading of oocytes, then IVM, IVF and IVC of resulting zygotes are integral part of successful IVP which has created new era for mass research on modern reproduction techniques in farm animals. For IVP, the efficient collection technique and evaluation of cumulus-oocyte-complexes (COCs) are the initial steps to be done. Rahman *et al*. (1997) reported that the right ovary is heavier (0.90g) than the left ovary (0.85g). On the other hand, the length of right ovary reverses to the result of left ovary. Several techniques such as aspiration of total follicular material, puncturing of individual isolated follicles with subsequent isolation of the cumulus-oocyte-complexes (COCs) and slicing of the ovaries have been described to obtain immature oocytes from slaughterhouse ovaries. Techniques such as slicing of the ovaries, flushing the follicles with phosphate buffered saline (PBS) or puncturing the isolated follicles may increase the number of recovered oocytes as compared with that aspiration of follicular materials (Alm *et al*., 1994). The average

number of good quality oocytes recovered from ovaries without corpus lutea, which can be effectively used for IVF (Kumar *et al*., 2004). According to Salim (2004) the average numbers of normal follicles reported significantly higher in normal breeder than acyclic, cyclic but not conceived postpartum anestrous in Black Bengal does.

Although a lot of work has been done regarding evaluation of ovaries, collection of COCs from slaughterhouse ovaries, grading of oocytes, IVM, IVF of the oocytes and IVC of resulting zygotes throughout the world. Several methods like aspiration, slicing, puncture have been used for harvesting oocytes from slaughterhouse ovaries of farm animals. 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., Wang *et al*., 2007) in abroad. But limited work has so far been undertaken about relative efficiency of oocyte collection technique in goat. The slaughterhouse goat ovaries can be an economic source of oocytes for IVM, IVF and IVC. Efficiency of oocyte collection techniques, grading and evaluation of COCs are the basics for IVM, IVF, IVC and MOET.

The best collection technique of COCs and refined evaluation procedure of collected COCs of goat yet to be done. From that stand point this present research work has been undertaken with the following objectives:

1. To compare different parameters between right and left ovary; ovaries with corpus luteum and without corpus luteum.
2. To observe the oocyte recovery rate between the ovaries with corpus luteum and without corpus luteum.
3. To observe the relative efficiency of oocyte collection techniques.

CHAPTER II

REVIEW OF LITERATURE

Generous research works have been performed in different countries of the world related to oocyte recovery in different ruminant species like cattle, goat, sheep, buffalo. But in Bangladesh, this kind of research works have been done to a very little extent. However, some of the related findings of research work carried out in different countries of the world are reviewed in this chapter. The review of literature concerning the studies presented under the following heads:

2.1 Ovarian categories,

2.2 Grading of cumulus-oocyte-complexes (COCs),

2.3 Effect of oocyte collection techniques.

2.1 Ovarian categories

The evaluation of ovaries, the efficient collection and grading of cumulus oocyte complexes (COCs) are most important for IVP of embryos. A number of experiments have been carried out in this regard are summarized as follows:

Gabr *et al.* (2019) studied on the ovarian biometry, oocyte yield and oocyte quality of Baladi goats. Ovaries were collected by slicing from slaughter houses and classified with or without CL during breeding (September-December) and non-breeding (March-July) seasons. Results showed that ovarian weight and biometry (length, width and thickness) were higher in breeding than in non-breeding season, but the differences were significantly only for width. Number of follicles and oocytes/ovary ($P<0.001$) as well as number/ovary and proportion of oocytes at compact ($P<0.0001$) and denuded ($P<0.05$) stage were higher in breeding season than in non-breeding one. Number of degenerated oocytes/ovaries was not affected significantly by season, but its proportion was lower ($P<0.001$) in breeding

than in non-breading season. Number/ovary and proportion of partial denuded oocytes and proportion of denuded oocyte were not affected significantly by breeding season. Weight and biometry of ovaries was higher on ovaries with CL than in non-bearing ones without CL. Only ovarian width was higher ($P<0.001$) by 38% in with CL than in without CL group. Ovaries bearing CL had higher ($P<0.05$) total follicles and oocyte yield/ovary ($P<0.01$) as well as oocyte recovery rate ($P<0.05$) than without CL ovaries. Number of compact, denuded and partial denuded oocytes/ovary was not affected by CL bearing. Number of compact oocytes tended to be greater on ovaries without than with CL. Number of degenerated oocytes/ovaries was higher ($P<0.05$) on with CL ovaries. Proportion of all oocyte categories was not affected by bearing CL. Finally, the effect of interaction between breeding season and bearing CL on all parameters studied was not significant.

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).

Naby *et al.* (2013) collected oocytes by aspiration method and studied the effect of presence or absence of CL on oocyte recovery in Egyptian goats 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).

Kachiwal *et al.* (2012) studied ultrasonographic biometry of the ovaries of pregnant jamunapari goats. The average weight of ovaries with corpus luteum during 1st, 2nd, 3rd and 4th 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 1st, 2nd, 3rd and 4th month of pregnancy, respectively.

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.

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

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

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).

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

g. Further, the right ovaries were significantly heavier and larger than the left ovaries.

Khandoker *et al.* (2011) obtained caprine follicular oocytes from left and right ovaries by aspiration method and 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).

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 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.

Ferdous (2006) 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 as obtained from CL-absent group of ovaries while no significant variation was found in the number of follicles measuring <2mm and >6mmdiameter in CL present and absent group of ovaries. Normal ovaries were found to be significantly higher in number of 2-6 mm diameter.

Lassala *et al.* (2004) conducted study on a group of goats during synchronization of estrus. The study revealed that ovarian follicular dynamics and fertility are unaffected by the presence or absence of a corpus luteum.

Izquierdo *et al*. (2002) experimented on prepubertal goat oocytes and matured in TCM-199 and reported that no significant difference been found in embryo development between oocytes obtained from prepubertal and adult goats.

2.2 Grading of cumulus-oocyte-complexes (COCs)

Das *et al.* (2018) aspirated goat 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%).

Deal *et al.* (2017) collected ovaries of 86 goatss and 95 cows from slaughterhouses and transported to the laboratory in saline solution at 36°C. The cumulus-oocyte complexes (COCs) were recovered by follicular aspiration, and only grades I and II COCs were selected and matured in TCM-199 supplemented with 10% fetal calf serum, sodium pyruvate, LH, FSH, estradiol, gentamicin and cysteamine for 22-24 h. A total of 714 and 1983 COCs were recovered from goats and cows, respectively. In the goats, the recovery rates of each COC categories (grade I: 25.9%, grade II: 30.7%, grade III: 10.2%, denuded: 18.6% and expanded: 14.6%) were lower than in cows (31.8, 30.6, 15.4, 4.5 and 17.7%, respectively) according to Mann-Whitney test ($p<0.05$). The percentage of caprine oocytes that reached metaphase II (63.4% - 242/396) was lower than that of bovine oocytes (67.8% - 696/1234) under the same laboratory conditions. These differences observed in all the analyses indicated that each species had peculiar physiological characteristics.

El-Naby *et al.* (2017) evaluated Egyptian goat 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.

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.

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.

Kharche *et al*. (2009) collected 1313 goat ovaries from the local slaughterhouse and transported within 4 hours to the laboratory in warm saline (37ºC), containing 100 IU penicillin-G and 100µg streptomycin sulphate per ml. Oocytes were retrieved by follicular puncture from the goat ovaries. Recovered oocytes were graded as excellent (A), good (B), fair (C) and poor (D) quality, depending on their cumulus investment and cytoplasmic distribution. They reported that the overall average recovery of goof quality oocytes for IVM was 1.91 per ovary.

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, 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 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 was ($p<0.05$) in CL-absent group (1.30±0.07) of ovaries than 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).

Salim (2004) conducted on reproductive tract of four categories (category 1= acyclic, category 2= cyclic but not conceived; category 3= post-partum anestrus and category 4= normal breeder and kidder. Black Bengal does to monitor the cause 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.

Rahman *et al*. (2003) classified the ovaries in three categories on the basis of the state of CL and reported that the average number of follicles harvested per ovary was 4.37, 5.28 and 6.48 in type I, II and III respectively. Higher number grade A and B COCs was obtained from type III ovaries

Crozet *et al*. (1995) studied on oocytes from follicles of three different sizes (small: 2-3mm; medium: 3.1-5mm; large: >5mm) and reported that oocytes from small and medium follicles yielded a significantly lower proportion of hatched blastocysts (0% and 0.3% respectively) than did those from large follicles and from ovulated oocytes (15% and 34% respectively).

2.3 Effect of oocyte collection techniques

Majeed *et al.* (2019) studied the comparative efficacy of three harvesting techniques *viz.*, the aspiration, puncture and slicing methods on oocyte recovery in goat ovaries obtained from a local abattoir. While the recovery rate of oocyte by using aspiration and puncture methods were significantly ($P<0.05$) higher than the oocyte recovery rate via slicing. 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.

Shaikh *et al.* (2015) conducted an experiment on *in vitro* production of caprine embryos. They harvested oocytes through follicle dissection, slicing and aspiration techniques. In follicle dissection technique they categorized 55.20%, 25.80%, 19.00% oocytes/ovary as good, fair, poor respectively. In slicing technique, they categorized 61.90%, 20.60%, 17.50% oocytes/ovary as good, fair, poor respectively. In aspiration technique, they categorized 58.10%, 22.60%, 19.30% oocytes/ovary as good, fair, poor respectively.

Yelisetti *et al* (2013) conducted an experiment on effect of harvesting technique on recovery rate and in vitro development of caprine follicular oocytes for in vitro procedures. was aimed at assessing efficacy of 3 harvesting methods on the quantity and quality of oocytes recovered for assisted reproduction procedures in goat. The average total number of oocytes recovered per ovary was significantly higher by slicing (6.54±0.39) and puncture (6.59±0.39) than by the aspiration method (4.09±0.19). However, the percentage of good quality oocytes was higher in the puncture method (71.56%), compared to the aspiration (71.27%) or slicing (61.61%) methods. The oocyte recovery was significantly lower in CL containing ovaries than that of ovaries without CL in aspiration (2.92 vs 4.57), puncture (5.89 vs 6.78) and slicing (5.40 vs 7.02) methods. However, the presence of CL did not affect the oocytes ability to reach the MII stage (75.31% vs 76.67%). The side (right or left) of the ovary not showed any significant effect on mean of total oocyte recovery and other grades of oocytes. However, the large sized ovaries were yielded significantly higher number of oocytes than smaller ovaries. The results showed that the rates of COCs that reached the metaphase-II (M-II) stage were 79.88, 78.09 and 72.63% in aspiration, puncture and slicing techniques, respectively. It was concluded that though oocyte recovery, and in vitro developmental rates did not vary significantly between puncture and slicing methods, yet puncture method was found to be superior due to low debris content and recovery of a greater number of culture grade oocytes. Oocyte recovery by aspiration from small sized ovaries was difficult due to a smaller number of visible follicles. Puncture method can be used as an alternative to slicing and aspiration for oocyte recovery in goat.

Mohan *et al.* (2013) undertaken an experiment to assess the relative efficiency of three different collection techniques of percentage and grades of oocytes in goats of Hyderabad. The mean oocyte recovery from aspiration technique, 2.15 ± 0.31 and 2.9 ± 0.46 in dissection technique and 6.55 ± 0.57 and 4.51 ± 0.46 in slicing technique. The mean oocyte recovery rate was 1.5 ± 0.22 and 1.0 ± 0.33 under aspiration technique, 2.58 ± 0.47 and 2.43 ± 0.36 under dissection technique and 5.26 ± 0.46 and 6.3 ± 0.71. The mean numbers of oocytes of different grades recovered were 0.34 ± 0.03, 0.62 ± 0.07 and 1.37 ± 0.09 in

aspiration, dissection and slicing techniques, respectively. Among the different grades of oocytes, significantly higher per cent of A (27.78) and B (38.89) grade oocytes were retrieved by aspiration and dissection technique when compared to slicing technique. With respect to C and D grade oocytes, significantly higher numbers of oocytes were retrieved by slicing technique than the aspiration and dissection techniques. Out of 437 oocytes retrieved in the present study, significantly higher per cent (50.57) yield was by slicing technique, followed by dissection technique (37.07) and aspiration (12.53).

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

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

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

Chandrahasan *et al.* (2012) used different collection techniques for oocytes recovery in goats. They obtained 2.71, 3.10 and 5.69 mean number of oocytes from each ovary by aspiration, puncture and dissection methods, respectively. The difference between dissection and other two methods was statistically highly significant.

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

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

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

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

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

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

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

Wang *et al.* (2007) studied the influence of the oocyte collection methods (slicing, puncture, aspiration I and II) on oocyte recovery efficiency in Boer goat. In the slicing method, the whole ovary was chopped into small pieces with a surgical blade. In the puncture method, the whole ovarian surface was punctured by 18-g needle. In other 2

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

Kumar *et al.* (2004) conducted an experiment on the ovaries from goats (20), 1-4-year-old were collected from local abattoir. The oocytes (558) thus recovered by aspiration of visible follicles followed by slicing of the same ovaries were categorized and subjected to micrometry under binocular compound microscope using ocular micrometer calibrated with stage micrometer. The average diameter of categories 1, 2, 3 and 4 oocytes, with zona pellucida was 172.59± 0.94 μm, 164.00 ± 0.78 μm, 157.08 ± 1.22 μm and 149.66 ± 1.31 μm respectively. The category 4 oocytes recovered from aspiration (157.97 ± 1.35 μm) showed statistically significant increase in diameter compared to oocytes recovered from slicing (147.01 ± 1.58 μm). 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. Based on the micrometric dimensions it was concluded that good quality oocytes (1, 2) were larger in size as compared to poor quality categories (3, 4) and can be effectively used for in vitro fertilization.

Raza *et al.* (2001) recovered follicular oocytes by aspiration and scoring methods from native goats. In aspiration method ovarian follicles were aspirated from ovaries with an 18-guage needle fitted with a 10 mL syringe. While, in scoring method the surface of ovaries was scored with a sterile surgical blade, with instant rinsing and tapping the ovary to release oocytes in a sterile petri-dish. They found that the scoring method was an appropriate method for high recovery of good quality oocytes per ovary as it yielded 3.85 oocytes per ovary than aspiration method (1.76 oocytes per ovary).

Wani *et al.* (2000) harvested oocytes by three different methods like puncture, aspiration, slicing and the oocytes were cultured in TCM-119 supplemented with 10% fetal calf serum,

0.1 IU/ml human menopausal gonadotrophin-5 and 50 IU/ml penicillin at 38.5ºC under 5% CO_2 for 24-26 hour. They reported that overall maturation rate was 62.5%, 61.7% and 66.3% for puncture, aspiration and slicing respectively and no significant ($p>0.05$) difference was found among different harvesting techniques. After fertilization of matured oocytes in medium TCM_199 supplemented with 4 mg/ml BSA, 50 IU/ml penicillin and 50 IU/ml heparin the rates of oocytes penetration were found to be 52.0, 54.4 and 51.3% respectively for those three methods and there was an insignificant ($p>0.05$) difference among the results.

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.

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

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

Das *et al.* (1996) compared efficacy of three methods for oocytes collection from goat ovaries. Oocytes were collected by slicing (n=131), follicle puncture (n=86) and follicle aspiration (n=80). Slicing yielded significantly more oocytes (5.7/ovary) than follicle puncture (2.6/ovary) or aspiration (1.7/ovary).

Pawshe *et al*. (1994) directed a series of experiment on the recovery methods of goat oocytes by using 3 methods: aspiration, puncturing, slicing. They concluded that average number of oocytes recovered per ovary was significantly higher by aspiration (2.7±0.15) than by puncturing (2.2±0.13) or by slicing (2.7±0.12) method. They also reported that significantly more good quality usable oocytes covered with compact cumulus cells were

obtained by slicing (0.9±0.06) than by aspiration (0.5±0.03) and the percentages of oocyte maturing, fertilizing and developing *in vitro* differed significantly among recovered methods.

Mogas *et al*. (1992) conducted the effect of recovery method on the number and type of oocytes obtained for IVM. It was found that aspiration yielded a higher number (4.6) of total oocytes per ovary than slicing (4.0), puncture (3.9). They also observed that higher number of normal oocytes per ovary was obtained by aspiration (3.7) than slicing (2.7) and puncture (2.6) method.

CHAPTER III

MATERIALS AND METHODS

The present experiment on "Relative efficiency of oocytes collection techniques and evaluation from goat ovaries" was operated at Departmental Laboratory of Animal Nutrition, Genetics and Breeding at Sher-e-Bangla Agricultural University, Dhaka-1207 from January 2019 to December 2019. In this chapter, a short description of the arrangement of the laboratory, materials and medium used for COCs collection, design and methods of the experiment, method of data collection and statistical analysis have been presented.

3.1 Arrangement of the laboratory

At first all the essential permanent either electrical power operated or digital instruments were properly installed or examined for good condition. These were rectified, reinstalled and eventually purified and sterilized with 70% alcohol. All the reusable equipment was 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 Permanent equipment

- Phase contrast microscope with USB 2.0 Camera
- Digital slide calipers
- Digital micro pipette
- Weighing balance
- Autoclave machine
- Centrifuge machine
- Dryer
- Water bath
- Laminar air flow cabinet

- Hot air oven

3.1.2 Reclaimable equipment

1. Glassware

- Measuring cylinder
- Beaker
- Petri dishes (90 mm)
- Test tube (10 ml)
- Conical flask
- Pasteur pipette
- Bottles for media
- Glass micropipette

2. Collection vial (for ovary collection)

3. Thermo Flask at 25°C to 30°C

4. Essential Surgical toolkits

3.1.3 Single use equipment

- 10 ml syringes
- 18 G and 19 G needles
- Disinfected rubber gloves
- Culture dishes (35 mm)

3.1.4 Chemicals, Reagents and Media

1. Chemicals and reagents

- Distilled water

[other chemicals and reagents are listed in the composition tables (section 3.2.2)]

2. Media

- 0.9% Physiological saline solution
- Dulbecco's phosphate buffered saline (D-PBS) solution

3.2 Design of the study

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

Table 1. Composition of physiological saline

Material	Amount (g)	Preparation
NaCl	9	Dissolved in 100ml distilled water

[**Note:** The saline was autoclaved before use and on the day of collection, 1000mg of gentamycin were added per liter of saline solution]

Table 2. Composition of Dulbecco's phosphate buffered saline (D-PBS) solution

A-Solution

Material	Amount (g)	Preparation
NaCl	4.00	Dissolved in 400ml distilled water
KCl	0.1	
Na_2HPO_4	0.575	
$KH_2\ PO_4$	0.1	

B-Solution

Material	Amount (g)	Preparation
$CaCl_2$	0.05	Dissolved in 400ml distilled water

C-Solution

Material	Amount (g)	Preparation
$MgCl_2.6H_2O$	0.05	Dissolved in 400ml distilled water

[**Note:** The 3 solutions were autoclaved separately and mixed to prepare the final D-PBS]

3.3 Collection and processing of ovaries

3.3.1 Preparation for ovary collection

0.9% physiological saline of NaCl was ready for washing of ovaries. The saline was sterilized in autoclave and kept in refrigerator for future use. One the day of collection, 5 lac iu of penicillin and 100 mg of streptomycin were mixed with per liter of saline solution. The solution was warmed at 25°C to 30°C and put in a thermos box to maintain this temperature during transporting the ovaries from slaughterhouse to the laboratory. Dulbecco's phosphate buffered saline (D-PBS) solution was also made by adding one pack of PBS salt (Sigma Chemical Co., USA) in one liter of distilled water. Then it was sterilized in autoclave and stored in a refrigerator for further use. The composition of D-PBS is shown in table 2.

3.3.2 Collection of ovaries and trimming

Ovaries from goats of unknown reproductive record were accumulated from local slaughter house. The representative photograph of the ovaries is displayed in (Plate 1). The ovaries were kept in collection vial containing 0.9% physiological saline in a thermo flask at 25°C to 30°C and carried to the laboratory within 4 to 5 hours of slaughter. The ovaries were then transferred to the sterilized petridishes containing same saline. In the laboratory each ovary was trimmed to remove the surrounding tissues and overlying bursa (Plate 2). The ovaries were rinsed minutely thoroughly by physiological saline solution at 25°C and recorded as with or without corpus luteum (Plate 3 & 4). Each ovary was treated to three washings in D-PBS and two washings in oocyte harvesting medium (D-PBS+ 1.50 IU/ml Penicillin) as described by Wani *et al.*, 2000.

Plate 1. Ovaries after collecting from slaughter house.

Plate 2. Trimming of ovary.

Plate 3. Ovaries with CL.

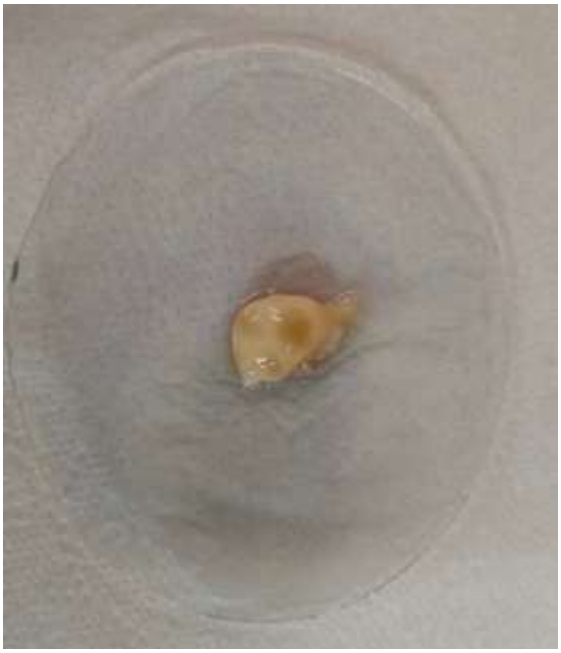

Plate 4. Ovary without CL.

3.4 Evaluation of ovary

3.4.1 Measurement of length, width and weight

The length and width of ovaries (right and left ovaries; ovaries with CL and without CL) were measured with the help of a slide calipers and expressed in cm (Plate 5 and 6). Weight of individual ovary was measures by placing them on a digital balance and recorded in a tabular form (Plate 7).

Plate 5. Measurement of length of ovary.

Plate 6. Measurement of width of ovary.

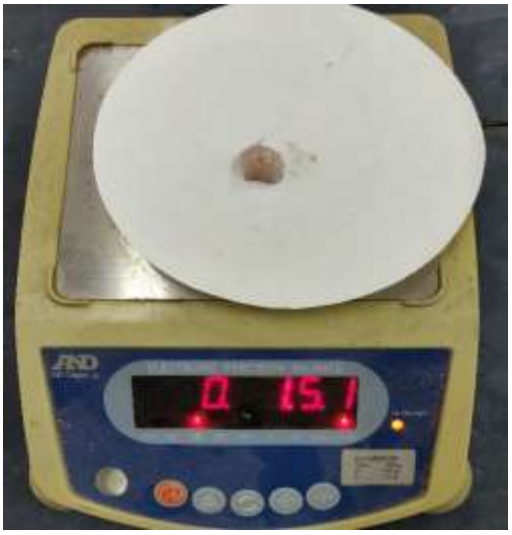

Plate 7. Measurement of weight of ovary.

3.4.2 Oocyte harvesting techniques

After fundamental washing, each ovary was treated individually and the oocytes harvested by the following three techniques as illustrated by Wani *et al*.,2000.

Puncture: Ovaries were kept in a 90-mm petridish containing 5 ml of oocytes harvesting medium, held with the help of forceps and the entire ovarian surface was punctured with an 18-gauge hypodermic needle (Plate 8). in this oocyte collection technique, the ovary was held completely dipped in the medium.

Slicing: Ovaries were put in a 90-mm petridish holding 5 ml of the oocyte harvesting medium, held with the support of forceps. Incisions were done along the entire ovarian surface using a scalpel blade (Plate 9). In this harvesting technique, the ovary was also kept completely immersed in the medium.

Aspiration: The 10-ml syringe was filled with D-PBS (1.0-1.5ml) and the needle (18 G) was kept in the ovarian parenchyma close to the vesicular follicles and all 2-6 mm diameter follicles were aspirated near the point at the same time (Plate 10). After aspiration the follicular materials were transferred gradually into a 90-mm petridish and protecting the cumulus cells from damage.

Plate 8. Puncture technique.

Plate 9. Slicing technique.

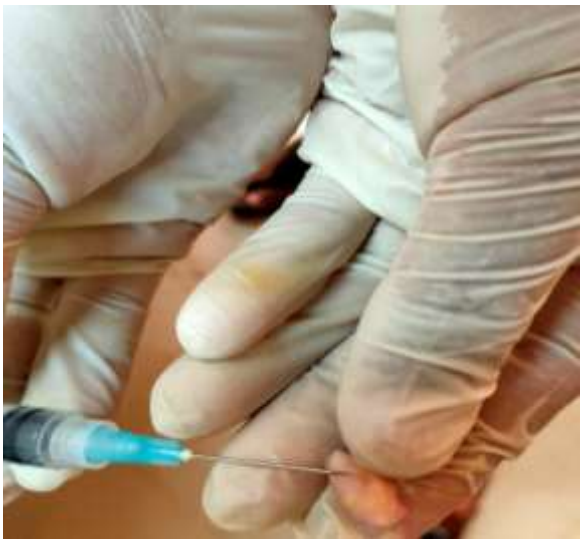

Plate10. Aspiration technique.

3.5 Grading of Cumulus-oocyte-complexes (COCs)

In all the three oocyte harvesting techniques, the petridish were set undisturbed for 5 minutes, allowing the oocyte to settle down. Excess media was removed by using a syringe without hampering the oocytes at bottom of petridish and observed under an inverted digital microscope at 10 x magnification. After that counting the total number of oocytes which were harvested. The COCs were classified into four grades on the basis of cumulus cells and nucleus as described by Khandoker *et al*. (2001), in brief given below;

- **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 (Plate 11). The grade C and D were considered as abnormal COCs (Plate 12). The number of different grades of COCs in each category noted.

Grade-A

Grade-B

Plate 11. Normal COCs with Grade-A and Grade-B

Grade-C

Grade-D

Plate 12. Abnormal COCs with Grade-C and Grade-D

3.6 Statistical analysis

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

3.7 Precautionary measures

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

1. Sterile techniques were used during collection and grading of oocytes.
2. All working surfaces were sterilized with ethyl alcohol.
3. All the glass ware, plastic ware and media used were sterile.
4. The glass ware, plastic wares and other instruments recruited in this experiment were not used in any other experiment.
5. The pipette tips were always filled and emptied at least once with the medium.
6. When transferring oocytes from one medium to another, the oocytes were transferred with the minimum possible medium.

CHAPTER IV

RESULTS AND DISCUSSION

The present work on relative efficiency of oocyte collection techniques and evaluation from goat ovaries was conducted at the Department of Animal Nutrition, Genetics and Breeding, Sher-e-Bangla Agricultural University, Dhaka. The impact of right and left ovary; with or without corpus luteum on ovarian length, width, weight, follicular counts, grading of COCs was studied. The influence of oocyte collection techniques like aspiration, slicing, puncture on COCs recovery rate and grading of COCs was also studied.

Goat ovaries were collected from different slaughter house of Dhaka city. Then ovaries were recorded as right and left; with corpus luteum (CL) and without CL. The ovaries without CL that is in follicular phase and with CL that is in luteal phase**.**68 right ovaries and 68 left ovaries; 69 ovaries without CL and 34 ovaries with CL were used during this experiment. Besides these oocytes from 192 ovaries were harvested through different oocyte collection techniques like aspiration, slicing, puncture. 64 ovaries were harvested through aspiration technique and 64 ovaries were harvested through slicing technique and 64 ovaries were harvested through puncture technique. The average length, width, weight, number of follicles observed and aspirated and collected COCs from left and right ovaries; ovaries with CL and without CL, total collected COCs from different collection technique are summarized in Table 3 to 5.

Table 3. Qualitative and quantitative parameters in right and left ovaries

Ovary (*n*)	Weight (g) (mean±SE)	Length (cm) (mean±SE)	Width (cm) (mean±SE)	Total number of visible follicles (mean±SE)	Number of follicles aspirated (mean±SE)	Collected COCs per Ovary (mean±SE)				
						Normal		Abnormal		Total
						Grade A	Grade B	Grade C	Grade D	
Total (136)	1.14±0.26	1.24±0.04	0.88±0.03	6.68±0.26 (929)	4.75±0.21 (687)	1.25±0.08 (173)	0.89±0.08 (122)	0.99±0.09 (243)	1.27±0.08 (174)	4.46±0.17 (712)
Right (68)	1.14±0.26	1.31^{a}±0.04	0.90±0.03	6.54±0.26 (451)	4.74±0.21 (327)	0.36^{b}±0.08 (25)	0.23^{b}±0.08 (15)	1.67^{a}±0.09 (117)	2.20^{a}±0.08 (151)	4.46±0.19 (308)
Left (68)	1.13±0.26	1.18^{b}±0.04	0.86±0.03	6.83±0.26 (478)	4.77±0.21 (360)	2.14^{a}±0.08 (148)	1.65^{a}±0.08 (107)	0.33^{b}±0.09 (126)	0.34^{b}±0.08 (23)	4.46±0.15 (404)

Mean values in the same column with different superscripts (a, b) differ significantly at $p<0.05$.

SE= Standard error.

Figure in the parenthesis indicates the total number.

4.1 Ovarian categories regarding right and left ovary

4.1.1 Measurement of length (cm), width (cm) and weight (g) of ovary

Among different parameters obtained from different category of ovaries the mean weight (g) and width (cm) were non-significant between right and left ovaries and the mean length (cm) significant between right and left ovaries (Table 3 and Figure 1). The mean weight (g), length (cm) and width (cm) were clearly higher in case of right ovaries (1.14, 1.31 and 0.90 respectively) compared to left ovaries (1.13, 1.18, 0.86 respectively), which supports the previous studies of Asad *et al.* (2016), who reported that 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.

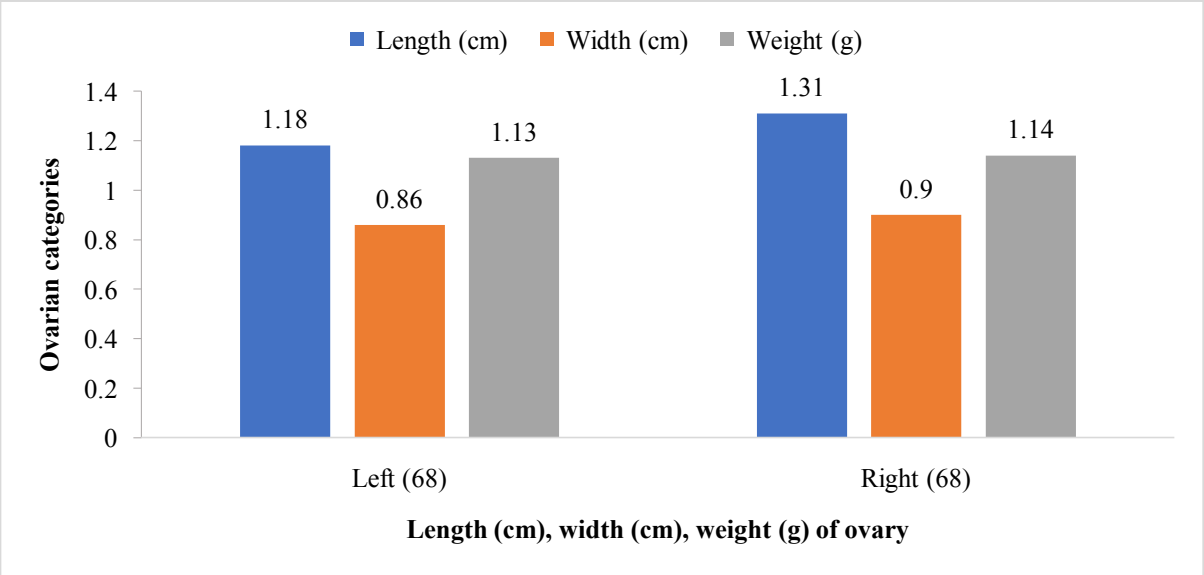

Figure 1. Ovarian categories of goats in respect of length, width and weight on right and left ovaries collected from slaughter house.

4.1.2 Number of follicles in total and aspirated

Variation on number of follicles (total and aspirated) was significant in terms of follicles count in between right and left ovaries (Table 3 and Figure 2). The highest number of follicles in total was observed in left ovary with a mean of 6.83 compared

to right ovary with a mean of 6.54. The highest number of follicles was aspirated in left ovary with a mean of 4.77 compared to right ovary with a mean of 4.74.

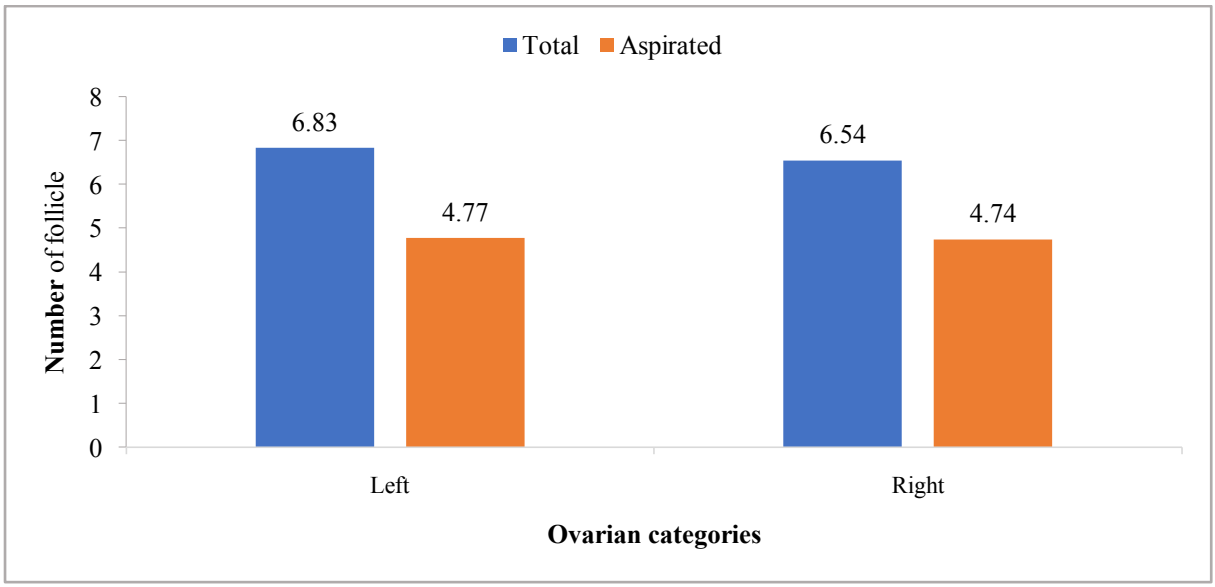

Figure 2. Ovarian categories of goats in respect of number of follicles in total and aspirated on right and left ovaries collected from slaughter house.

4.1.3 Grading of COCs

Presence of total COCs with normal and abnormal was significant in left and right ovaries (Table 3 and Figure 3). The grade A and grade B were considered as normal COCs. The grade C and grade D were considered as abnormal COCs. The number of normal COCs were found significantly higher ($p<0.01$) in left than that of right ovary. Results explained that the highest number of normal COCs (Grade A and Grade B) were found in left ovary with the mean of 2.14 and 1.65 respectively. Distinctly the lowest number of normal COCs (Grade A and Grade B) were found in right ovary with the mean of 0.36 and 0.23 respectively. The number of abnormal COCs were found significantly higher ($p<0.01$) in right than that of left ovary. The number of abnormal COCs (Grade C and Grade D) were found in right ovary with the mean of 1.67 and 2.20 respectively where the lowest abnormal COCs (Grade C and Grade D) was observed in left ovary with the mean of 0.33 and 0.34 respectively. The number of total COCs were found almost same in left and right ovary with the mean of 4.46.

Highest numbers of normal COCs were found in left than that of right ovary, which 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).

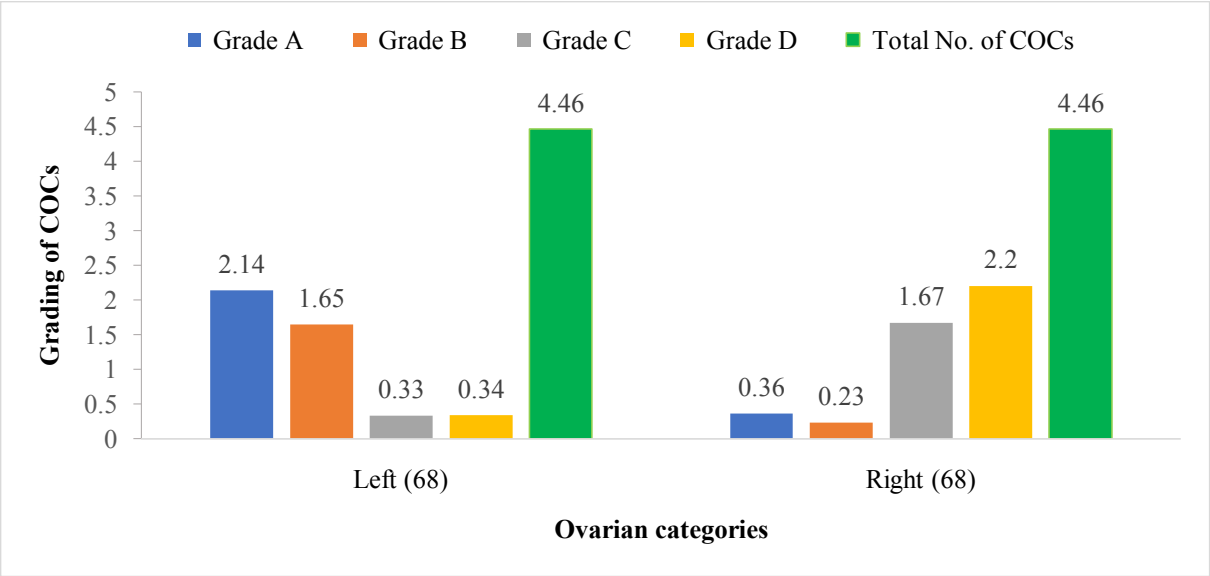

Figure 3. Ovarian categories of goats on grading of COCs on right and left ovaries collected from slaughter house.

4.2 Ovarian categories regarding with CL or without CL

4.2.1 Ovaries with CL or without CL

The less reproductive performer goats 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.

Table 4. Qualitative and quantitative parameters in With CL and without CL groups ovaries

Ovary (*n*)	Weight (g) (mean±SE)	Length (cm) (mean±SE)	Width (cm) (mean±SE)	Total number of follicles (mean±SE)	Number of follicles aspirated (mean±SE)	Collected COCs per Ovary (mean±SE)				
						Normal		Abnormal		Total
						Grade A	Grade B	Grade C	Grade D	
Total (103)	0.73±0.03	1.38±0.08	1.05±0.07	6.15±0.17 (682)	3.73±0.11 (420)	0.92±0.05 (104)	0.70±0.05 (79)	0.48±0.05 (52)	0.64±0.06 (69)	2.75±0.09 (300)
With CL (34)	0.74±0.05	1.34±0.14	1.25^{b}±0.13	5.84^{b}±0.21 (273)	3.62^{b}±0.20 (154)	0.32^{b}±0.09 (19)	0.29^{b}±0.09 (16)	0.82^{a}±0.09 (30)	1.18^{a}±0.09 (43)	2.62±0.16 (109)
Without CL (69)	0.72±0.04	1.47±0.09	0.96^{a}±0.09	6.79^{a}±0.29 (409)	3.79^{a}±0.14 (266)	1.21^{a}±0.07 (85)	0.90^{a}±0.07 (63)	0.31^{b}±0.06 (22)	0.39^{b}±0.07 (26)	2.81±0.11 (191)

Mean values in the same column with different superscripts (a, b) differ significantly at $p<0.05$.

CL- Corpus Luteum

SE= Standard error.

The parenthesis indicates the total number.

4.2.2 Measurement of length (cm), width (cm) and weight (g) of ovary

Significant variation was found on different parameters on ovary with CL and without CL (Table 4 and Figure 4). Results explained that the mean weight (g) and width (cm) were clearly higher in case of ovaries with CL (0.74 and 1.25 respectively) than ovaries without CL (0.72 and 0.96 respectively). On the other hand, the mean length (cm) were found higher in the ovaries without CL (1.47) than the ovaries with CL (1.34).Those results support the previous study of Asad *et al.* (2016), who reported that the mean weight (g) and width (cm) were clearly higher in case of ovaries with CL (0.72 and 0.81respectively) than ovaries without CL (0.66 and 0.76 respectively). They also reported that the mean length (cm) were found higher in the ovaries without CL (1.17) than the ovaries with CL (1.16).

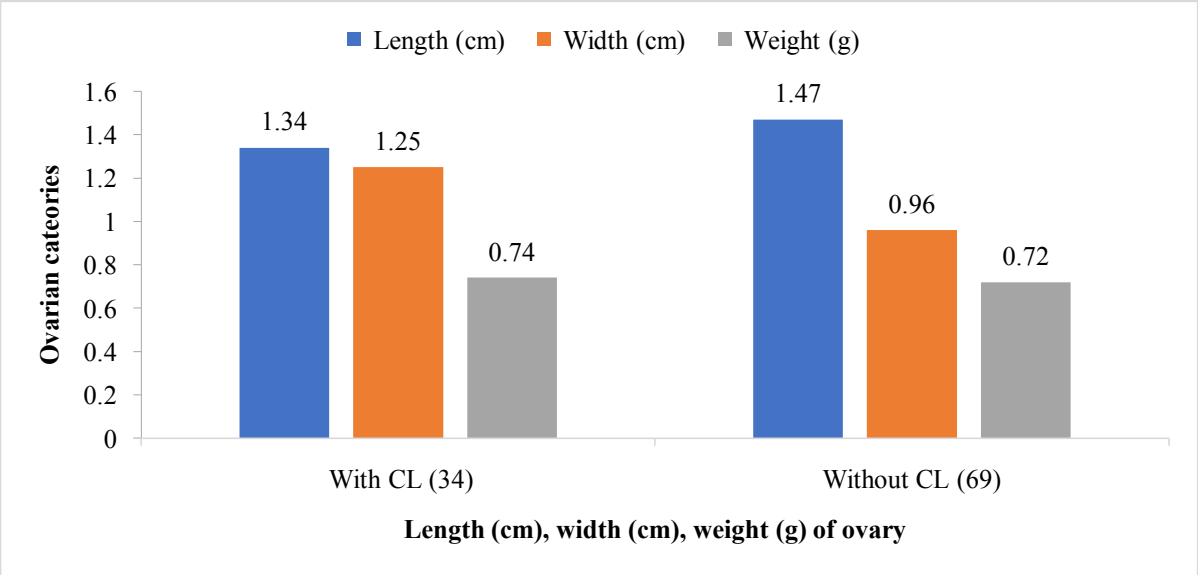

Figure 4. Ovarian categories of goats in respect of length, width and weight on ovaries with CL and without CL collected from slaughter house.

4.2.3 Number of follicles in total and aspirated

Variation on number of follicles (total and aspirated) was significant in terms of follicles count in between ovaries with CL and without CL (Table 4 and Figure 5). The number of follicles in total was significantly higher ($p<0.01$) observed in ovaries without CL with a mean of 6.79 compared to ovaries with CL with a mean of 5.84. The number of follicles was aspirated significantly higher ($p<0.01$) in ovaries without

CL with a mean of 3.79 compared to ovaries with CL with a mean of 3.62. Those results support the previous study of Asad *et al.* (2016) who reported that total number of follicles and total number of follicles aspirated were higher in ovaries without CL with the mean (5.21 and 2.74 respectively) than ovaries with CL with the mean (5.11 and 2.69 respectively).

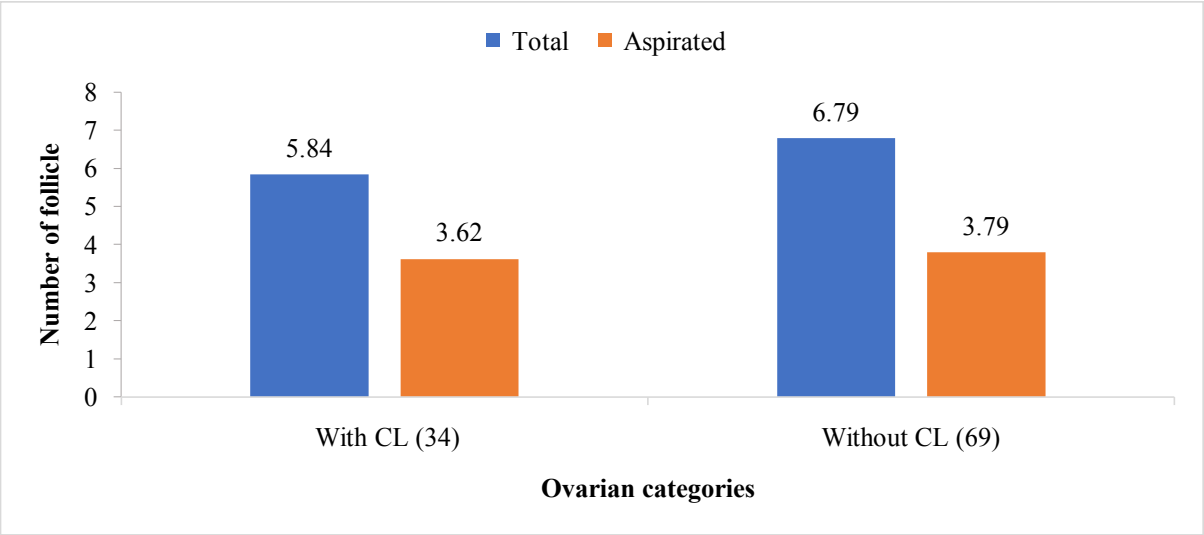

Figure 5. Ovarian categories of goats in respect of number of follicles in total and aspirated on ovaries with CL and without CL collected from slaughter house.

4.2.4 Grading of COCs

Presence of total COCs with normal and abnormal was significant in ovaries with CL and without CL (Table 4 and Figure 6). The grade A and grade B were considered as normal COCs. The grade C and grade D were considered as abnormal COCs. Results explained that the highest number of normal COCs (Grade A and Grade B) were found in ovaries without CL with the mean of 1.21 and 0.90 respectively. Distinctly the lowest number of normal COCs (Grade A and Grade B) were found in ovaries with CL with the mean of 0.32 and 0.29 respectively. Similarly, the highest number of abnormal COCs (Grade C and Grade D) were found in ovaries with CL with the mean of 0.82 and 1.18 respectively where the lowest abnormal COCs (Grade C and Grade D) were observed in ovaries without CL with the mean of 0.31 and 0.39 respectively. The number of total COCs were highest in ovaries without CL with mean of 2.81 than ovaries with CL with a mean of 2.62.Those results support the previous study of Asad

et al. (2016) who reported that the highest number of abnormal COCs were found in ovaries with CL with the mean of 1.02 in comparison with lowest number of abnormal COCs were found in ovaries without CL with the mean of 0.87. They also reported that the highest number of normal COCs were found in ovaries without CL with the mean of 1.12 in comparison with lowest number of normal COCs were found in ovaries with CL with the mean of 0.88.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.

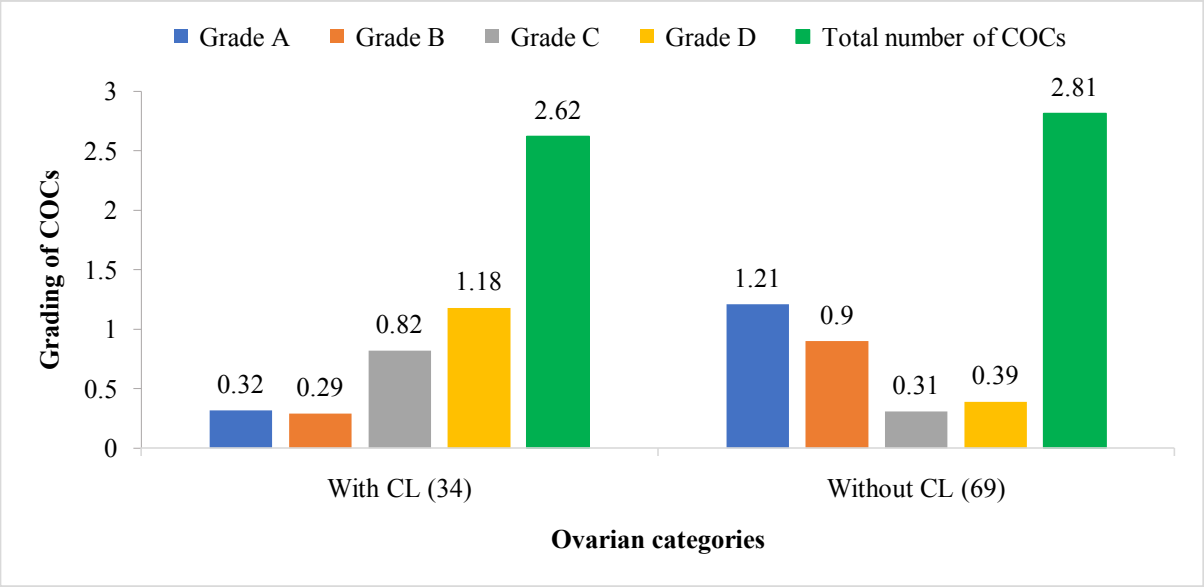

Figure 6. Ovarian categories of goats on grading of COCs on ovaries with CL and without CL collected from slaughter house.

Table 5. Oocyte collection techniques, number of cumulus-oocyte-complexes (COCs) and types of COCs harvested

Oocyte collection techniques	Total number of oocytes	Collected COCs/ovary (mean±SE)				Total number of COCs(mean±SE)
		Grade A	Grade B	Grade C	Grade D	
Slicing	64	3.67^{a} ±0.78 (11)	3.20^{a}±0.54 (10)	7.00^{a}±1.02 (21)	8.33^{a}±0.98 (25)	22.20^{a}±2.40 (67)
Puncture	64	2.20^{b}±0.60 (11)	1.40^{b}±0.42 (7)	5.00^{a}±0.79 (25)	4.60^{b}±0.76 (23)	13.20^{b}±1.86 (66)
Aspiration	64	3.80^{a} ±0.60 (19)	3.33^{a}±0.42 (16)	1.40^{b}±0.79 (7)	1.80^{c}±0.76 (9)	10.37^{b}±1.86 (51)

Mean values in the same column with different superscripts (a, b, c) differ significantly at $p<0.05$

SE= Standard error.

Figure in the parenthesis indicates the total number.

4.3 Effect of collection techniques on COCs recovery

Presence of total COCs with normal and abnormal was significant in different techniques of slicing, puncture and aspiration (Table 5 and Figure 7). The grade A and B were considered as normal COCs. The grade C and D were considered as abnormal COCs. Total number of 67, 66, 51 COCs were collected by slicing, puncture and aspiration techniques respectively from each of 64 ovaries. The results revealed that slicing and puncture yielded significantly higher ($p<0.01$) number of total COCs per ovary (22.2 and 13.20 respectively) than aspiration (10.37). But the highest number of normal COCs (Grade A and B) were found ($p<0.05$) in aspiration (3.80 and 3.33 respectively) than slicing (3.67 and 3.20 respectively) and puncture (2.20 and 1.40 respectively) techniques. The total number of abnormal COCs including Grade C and D were significantly lower ($p<0.01$) in aspiration (1.40 and 1.80 respectively) compared to puncture (5.00 and 4.60 respectively) and slicing (7.00 and 8.33 respectively) techniques.

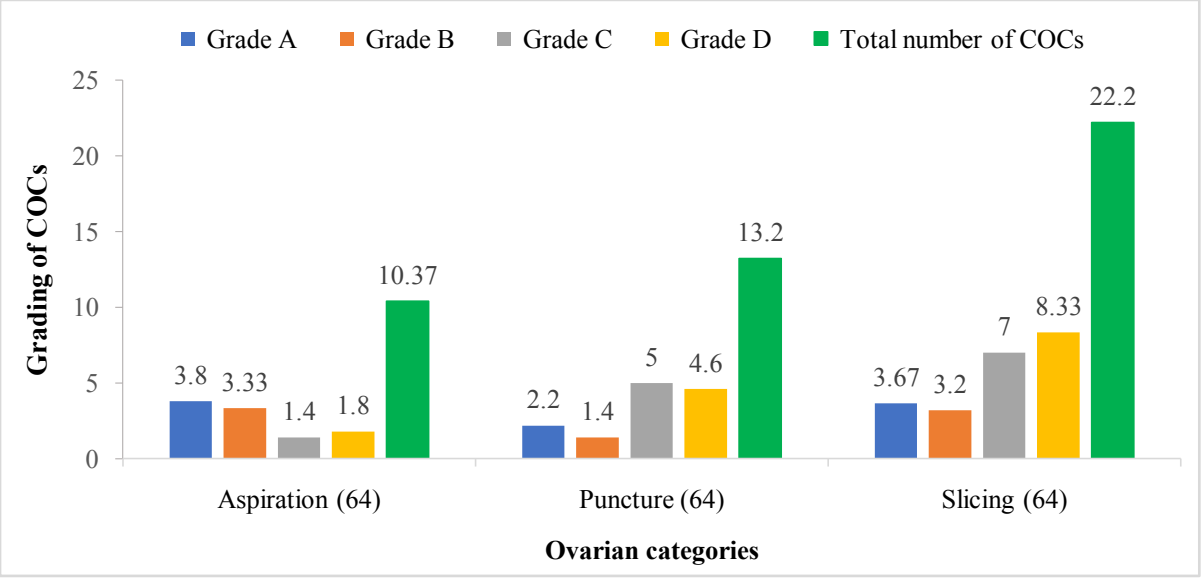

Figure 7. Grading and number of COCs in different oocyte harvesting techniques in goats.

In the aspiration technique, COCs were collected from 2-6 mm diameter of surface follicles using a hypodermic needle with 10 ml syringe. In case of puncture, the whole ovarian surface was punctured by hypodermic needle. In slicing technique, incisions

were given along the whole ovarian surface using a scalpel blade. i.e. all sizes of surface follicles were harvested. Thus, the lowest number of COCs recovered by the aspiration method may be due to the presence of some follicles embedded deeply within the cortex, which are released by puncture or slicing of the ovary. Ferdous (2006) reported that normal COCs were found to be significantly higher ($p<0.05$) in 2-6 mm diameter follicles than others. Moreover, puncture and slicing techniques involve in production of more debris which might intervened on the searching of oocytes under the microscope and also required more washing than aspiration. As a result, a number of COCs were deprived from cumulus cells due to repeated washing and ultimately results a lower number of normal COCs compared to aspiration at the final observation. The lower number of normal COCs with higher number of abnormal COCs in case of puncture and slicing than those of aspiration (Table 5 and Figure 7) might be due to this reason.

Those results support the previous study of Rao and Mahesh (2012). They reported that among the three collection methods, the slicing technique yielded the highest number of total oocytes (7.98 ± 0.70) followed by the puncture (3.46 ± 0.31) and the aspiration methods (2.38 ± 0.19).

Those results also support the previous study of Mehmood *et al.* (2011) who reported that compared oocyte recovery methods *i.e.,* aspiration vs. slicing in goats. They recorded better ($P<0.05$) COCs recovery with the slicing method (2.2 COCs/ovary) than with aspiration (0.9 COCs/ovary).

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

The result of this study was comparable with the observation of Wang *et al.* (2007) who harvested oocytes from ovary of Boer goat by one of the four collection techniques (slicing, puncture, aspiration I and aspiration II) and graded COCs as good,

fair, poor. They reported that slicing and puncture of the ovaries yielded a higher ($p<0.05$) number of oocytes per ovary (6.3 and 5.8 respectively) compared to aspiration I (2.9) and aspiration II (3.1) but the good quality COCs per ovary were significantly higher ($p<0.05$) in aspiration I (3.9) and aspiration II (3.6) than slicing (2.4) and puncture (2.1). Wani *et al.* (2000) reported that slicing (9.5±0.4) and puncture (9.5±0.4) yielded significantly ($p<0.05$) more COCs per ovary than aspiration (6.8±0.3) in sheep but the percentage of good quality oocytes was higher in the aspiration method (64.4%), compared to the puncture (54.7%) or slicing (54.3%) was also in accordance with the results of present study.

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). Different methods have been used for collecting oocytes from the ovaries of cattle, goat and sheep but most usually used method in goat are aspiration, puncture and slicing (Wang *et al*., 2007). From the above result it can be concluded that aspiration is the best oocyte collection technique from 2-6 mm diameter vesicular follicles by an18-gauge hypodermic needle for harvesting normal COCs (Grade A and B) in comparison with slicing, puncture from goat ovaries

CHAPTER V

SUMMARY AND CONCLUSION

The research was carried out by the Department of Animal Nutrition, Genetics and Breeding, Sher-e-Bangla Agricultural University, Dhaka. It was carried out with a view to finding out the best oocyte collection techniques and also to evaluate the slaughter house goat ovaries, COCs depending on some parameters.

Goat ovaries were collected from different slaughter house of Dhaka north city corporation area. Goat ovaries were categorized as right, left, with corpus luteum (CL) and without CL group. Ovaries were then evaluated on the basis of length (cm), width (cm), weight (g), total number of follicles on the surface of each categorized ovaries, number of follicles aspirated,total number of COCs, normal COCs and abnormal COCs.

In terms of ovarian categories regarding left and right category, the result obtained from this experiment showed difference in the parameters between left and right ovaries. The length (cm) was significantly ($p<0.05$) higherin case of right ovaries (1.31±0.04) compared to left ovaries (1.18±0.04). But no significant ($p<0.05$) differences were found in the width (cm) and weight (g) of right (0.90±0.03 and 1.14±0.26)and left ovaries (0.86±0.03 and 1.13±0.26) respectively. In left ovaries with an increase of total number of follicles (6.83±0.26) and number of follicles aspirated (4.77±0.21) but decrease in total number of follicles (6.54±0.26) and number of follicles aspirated (4.74±0.21) were found in right ovaries.The number of normal COCs (grade A and grade B) were found significantly ($p<0.01$) higher in left ovaries than right ovaries. Grade A (2.14±0.08) and grade B (1.55±0.08) were higher in left ovaries in comparison with right ovaries grade A (0.36±0.08) and grade B (0.23±0.08). On the other hand, abnormal COCs (grade C and grade D) the reverse results were found in right ovaries than that of left ovaries. Abnormal COCs were significantly ($p<0.01$) higher in right ovaries than left ovaries.

Grade C (1.67±0.09) and grade D (2.20±0.08) were higher in right ovaries in comparison with left ovaries grade C (0.33±0.09) and grade D (0.34±0.08). Total number of normal COCs were higher in left ovaries with the mean (4.46±0.19) than right ovaries with the mean (4.34±0.19).

In terms of ovarian categories regarding with CL and without CL, the result obtained from this experiment showed difference in the parameters between with CL and without CL ovaries. The length (cm) washigher in ovaries without CL (1.47±0.09) compared to ovaries with CL (1.34±0.14).The width (cm) was significantly ($p<0.05$) higher in ovaries with CL (1.25±0.13) compared to ovaries without CL (0.96±0.09). The weight (g) was higher in ovaries with CL (0.74±0.05) compared to ovaries without CL (0.72±0.04).Total number of follicles were found significantly ($p<0.01$) higher in ovaries without CL (6.79±0.29) in comparison with ovaries with CL (5.84±0.21). The number of follicles aspirated were found significantly ($p<0.05$) higher in ovaries without CL (3.79±0.14) in comparison with ovaries with CL (3.62±0.20). The collected COCs in total were found higher in ovaries without CL (2.81±0.11) in comparison with ovaries with CL (2.62±0.16). When the COCs were classified in normal and abnormal groups, the number of normal COCs (grade A and grade B) were found significantly ($p<0.01$) higher in ovaries without CL than ovaries with CL. Grade A (1.21±0.07) and grade B (0.90±0.07) higher in ovaries without CL in comparison with ovaries with CL grade A (0.32±0.09) and grade B (0.29±0.09). The number of abnormal COCs (grade C and grade D) were found significantly ($p<0.01$) higher in ovaries with CL than ovaries without CL. Grade C (0.82±0.09) and grade D (1.18±0.09) higher in ovaries with CL in comparison with ovaries without CL grade C (0.31±0.06) and grade D (0.39±0.07).

When oocytes recovery per ovary was compared among the collection techniques, significantly ($p<0.01$) higher number of total COCs per ovary was yielded by slicing method (22.20±2.40), followed by puncture (13.20±1.86) and aspiration (10.37±1.86) method. But significantly($p<0.05$) higher number of normal COCs (grade A and grade B) per ovary were observed in aspiration (3.80±0.60 and 3.33±0.42 respectively) than

slicing (3.67±0.78 and 3.20±0.54 respectively) followed by puncture (2.20±0.60 and 1.40±0.42 respectively). On the other hand, the number of abnormal COCs (grade C and grade D) per ovary significantly ($p<0.01$) lower in aspiration (1.40±0.79 and 1.80±0.76 respectively) compared to slicing (7.00±1.02 and 8.33±0.98 respectively) and puncture (5.00±0.79 and 4.60±0.76 respectively).The overall yield of COCs per ovary was highest with slicing than puncture followed by aspiration techniques. The reason for more COCs yield per ovary in slicing could be attributed to the fact that by slicing, oocytes from surface follicles as well as follicles of deeper cortical stroma are released whereas by puncture and aspiration oocytes from surface follicles alone are released. The maximum yield of grade A and grade B oocytes were observed by aspiration than slicing followed by puncture techniques. While the grade C and grade D oocytes were retrieved by slicing than puncture followed by aspiration.

Finally, it can be concluded that yield of normal COCs was highest with aspiration followed by slicing and puncture. Whereas yield of abnormal COCs was highest with slicing followed by puncture and aspiration. Thus, aspiration is the best oocyte collection technique for oocyte recovery from slaughter house goat ovaries.

In Bangladesh, a few works have been done on goat oocyte collection and evaluation techniques. For a complete story, it is essential to do further research.

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APPENDICES

Appendix I: Collected raw data for different parameters acquired from different category ovaries in the experiment

Right Ovary SI. No.	Weight (g)	Length (cm)	Width (cm)	TNFS	NFA	Grade A	Grade B	Grade C	Grade D	Total no. of COCs
1	1.5	1.428	0.91	8	5	0	0	1	3	4
2	0.9	1.3	1.078	5	5	0	0	2	1	3
3	0.43	1.15	0.758	5	4	0	0	1	2	3
4	1.19	1.619	1.06	4	4	0	0	2	2	4
5	0.87	1.333	1.03	5	4	1	0	1	2	4
6	0.25	1.151	0.723	8	6	0	0	2	3	5
7	0.64	1.474	0.861	4	4	1	0	2	1	4
8	0.61	1.447	0.89	8	7	0	1	2	3	6
9	0.89	1.32	0.806	4	3	0	1	0	2	3
10	0.67	1.196	0.87	5	4	0	0	2	2	4
11	0.77	1.396	0.869	7	5	1	0	2	2	5
12	0.83	1.33	0.977	9	7	0	1	3	2	6
13	0.35	1.275	0.923	13	8	1	0	2	4	7
14	1.01	1.62	1.085	8	5	0	0	2	2	4
15	0.52	1.265	0.876	7	6	1	0	2	3	6
16	0.21	0.849	0.763	7	4	0	1	1	2	4
17	0.3	1.301	0.787	5	4	0	0	2	1	3
18	0.27	1.115	0.774	3	3	0	0	1	2	3
19	0.33	1.203	0.804	7	5	1	0	2	3	6
20	0.22	0.846	0.71	4	2	0	0	1	1	2
21	0.88	1.586	1.005	4	4	0	0	2	2	4
22	0.54	1.28	0.893	7	6	1	0	2	3	6
23	0.35	1.243	0.834	8	6	0	0	2	3	5
24	0.88	1.975	0.876	4	3	0	0	1	2	3
25	4.6	0.523	0.18	6	4	1	0	1	2	4
26	3.7	0.479	0.266	5	3	0	0	2	1	3
27	14.4	0.928	0.589	4	4	1	0	1	2	4
28	10.7	0.998	0.487	8	7	0	0	2	4	6
29	0.93	1.385	1.35	9	7	0	1	3	2	6
30	0.51	1.376	0.807	7	5	0	0	2	2	4
31	0.66	1.29	1.033	5	5	1	0	2	3	6
32	0.88	1.581	1.121	11	7	1	0	3	2	6

Right Ovary SI. No.	Weight (g)	Length (cm)	Width (cm)	TNFS	NFA	Grade A	Grade B	Grade C	Grade D	Total no. of COCs
33	0.59	1.315	0.904	8	6	0	0	2	4	6
34	1.61	1.701	1.402	5	3	0	0	2	1	3
35	0.86	1.476	1.048	7	5	0	1	2	2	5
36	0.94	1.371	1.156	5	4	1	0	1	2	4
37	0.27	0.982	0.704	5	3	0	0	2	1	3
38	0.45	1.177	0.82	6	4	0	1	1	2	4
39	0.86	1.653	0.754	5	4	1	0	0	2	3
39	0.61	1.382	1.112	10	7	1	0	2	3	6
40	1.05	1.799	0.971	4	3	0	1	0	2	3
41	0.42	1.272	0.727	3	3	0	0	0	2	2
42	0.31	1.191	0.8	6	4	1	0	1	2	4
43	2.42	2.071	1.488	10	7	0	1	2	4	7
44	0.3	1.032	0.798	5	4	0	0	2	2	4
45	1.21	1.497	1.078	7	5	1	0	0	3	4
46	0.55	1.245	0.819	11	8	1	0	4	2	7
47	1.43	1.764	1.205	9	5	0	0	3	2	5
48	0.57	1.41	0.979	5	3	1	0	0	2	3
49	0.62	1.361	0.891	12	9	0	1	3	5	9
50	0.2	0.888	0.682	7	6	0	1	3	2	6
51	0.82	1.016	0.823	6	4	0	0	2	1	3
52	0.3	1.095	0.762	9	5	1	0	2	2	5
53	1.2	1.688	1.131	7	4	1	0	1	2	4
54	0.23	1.053	0.903	3	2	0	0	1	1	2
55	0.84	1.525	1.068	4	4	0	1	1	2	4
56	0.69	1.416	0.961	9	6	1	0	3	2	6
57	0.93	1.517	1.049	8	4	0	1	0	3	4
58	0.42	1.105	0.782	6	5	0	1	2	2	5
59	0.22	1.11	0.811	9	6	1	0	2	2	5
60	0.34	1.013	0.855	5	4	0	0	2	2	4
61	0.58	1.269	1.006	9	7	0	1	2	4	7
62	0.59	1.215	1.119	6	4	1	0	0	3	4
63	1.4	1.89	1.125	7	5	1	0	2	2	5
64	0.59	1.395	0.961	6	4	0	0	2	2	4
65	1.01	1.581	1.04	11	9	1	1	4	2	8
66	0.66	1.436	0.912	4	2	0	0	0	2	2
67	0.32	1.08	0.718	5	3	0	0	2	1	3
68	0.36	1.03	0.838	3	2	0	0	1	1	2

Left Ovary SI. No.	Weight (g)	Length (cm)	Width (cm)	TNFS	NFA	Grade A	Grade B	Grade C	Grade D	Total no. of COCs
1	0.65	1.14	0.755	6	5	2	2	0	1	5
2	0.78	1.162	0.683	5	4	2	0	1	0	3
3	0.42	1.089	0.661	8	6	2	1	0	1	4
4	0.35	1.019	0.816	3	2	1	1	0	0	2
5	0.64	1.359	0.867	9	7	3	2	0	1	6
6	0.21	0.927	0.671	4	3	2	0	1	0	3
7	0.49	1.074	0.887	7	6	3	2	0	1	6
8	0.74	1.326	0.891	5	4	2	1	1	0	4
9	0.29	1.216	0.827	3	3	2	0	1	0	3
10	0.55	0.95	0.785	8	6	3	2	1	0	6
11	0.46	1.286	0.803	7	6	3	2	0	0	5
12	0.45	1.084	0.816	8	5	2	3	0	1	6
13	0.15	1.476	1.193	13	10	4	3	0	1	8
14	0.2	1.085	0.923	5	4	2	1	1	0	4
15	0.48	1.153	0.991	8	6	3	2	0	1	6
16	0.59	1.334	0.842	7	3	2	1	0	0	3
17	0.21	0.965	0.614	5	4	1	2	1	0	4
18	0.9	1.363	1.082	6	4	3	1	0	0	4
19	0.32	1.004	0.806	8	4	2	1	1	0	4
20	1.09	1.491	1.138	6	4	1	3	0	0	4
21	0.45	1.338	1.154	9	7	4	2	0	1	7
22	2.9	0.205	0.229	6	3	2	0	0	0	2
23	3.7	0.223	0.26	5	3	1	2	0	0	3
24	15.2	0.37	0.189	8	5	2	2	0	1	5
25	2.5	0.334	0.245	6	4	2	1	1	0	4
26	3	0.629	0.24	7	4	2	0	1	0	3
27	3.8	0.563	0.354	5	3	1	2	0	0	3
28	10.7	0.998	0.487	8	5	3	1	0	1	5
29	0.69	1.24	1.009	12	9	4	2	0	1	7
30	0.78	1.329	0.945	7	3	2	1	0	0	3
31	0.65	1.29	1.048	8	6	2	2	1	0	5
32	0.41	0.997	0.924	5	3	2	0	1	0	3
33	0.76	1.278	0.857	7	5	2	2	0	1	5
34	1.09	1.571	1.345	8	5	2	2	0	0	4
35	0.58	1.4	0.859	5	3	1	2	0	0	3
36	0.44	1.243	0.924	6	4	2	1	0	0	3
37	0.34	1.054	0.74	4	3	1	1	0	0	2
38	0.7	1.448	0.941	6	3	2	1	0	0	3

Left Ovary SI. No.	Weight (g)	Length (cm)	Width (cm)	TNFS	NFA	Grade A	Grade B	Grade C	Grade D	Total no. of COCs
39	0.64	1.389	1.006	11	9	4	2	1	0	7
40	1.17	1.557	1.148	9	7	3	2	0	1	6
41	0.55	1.281	0.868	8	6	3	2	0	1	6
42	0.45	1.056	0.752	7	3	2	1	0	0	3
43	0.76	1.335	1.013	7	5	2	2	0	1	5
44	0.41	1.114	0.806	5	4	2	1	1	0	4
45	0.62	1.156	0.827	8	5	2	2	1	0	5
46	0.61	1.234	0.944	5	3	2	0	1	0	3
47	1.16	1.461	1.184	7	5	2	3	0	0	5
48	0.67	1.195	1.093	12	10	4	3	0	1	8
49	0.65	1.358	1.064	8	6	3	2	1	0	6
50	0.94	1.458	0.967	5	3	2	1	0	0	3
51	0.57	1.2	0.989	6	4	2	1	0	1	4
52	0.77	1.465	1.094	7	5	2	2	0	0	4
53	0.69	1.3	0.952	7	6	3	2	0	1	6
54	0.27	0.897	0.715	6	5	2	2	1	0	5
55	0.31	0.957	0.687	7	4	1	2	0	0	3
56	0.38	1.242	0.858	6	4	2	2	0	0	4
57	0.89	1.487	1.207	11	8	4	3	1	0	8
58	0.42	1.302	0.786	5	3	1	1	0	0	2
59	0.71	1.41	1.078	6	5	2	1	0	1	4
60	0.33	1.197	0.768	9	6	3	2	1	0	6
61	0.84	1.429	0.96	7	5	2	2	0	1	5
62	0.23	1.041	0.759	4	3	1	1	0	0	2
63	0.63	1.205	0.997	5	2	1	0	1	0	2
64	0.87	1.589	1.064	6	3	1	2	0	0	3
65	1.23	1.552	1.327	10	6	2	3	0	1	6
66	0.4	1.571	0.81	6	5	2	1	1	0	4
67	0.37	1.159	0.793	5	3	1	1	0	0	2
68	0.47	1.273	0.889	8	6	2	2	0	1	5

Appendix II: Collected raw data for different parameters acquired from different category ovaries in the experiment

Ovary SI. No.	Corpus luteum (present /absent)	Weight (g)	Length (cm)	Width (cm)	TNFS	NFA	Grade A	Grade B	Grade C	Grade D	Total no. of COCs
1	A	0.97	1.464	1.072	5	3	2	0	1	0	3
2	A	0.92	1.605	0.973	4	3	1	1	0	0	2
3	A	0.63	1.476	0.978	6	4	2	1	0	1	4
4	A	0.78	0.986	0.757	4	3	1	1	0	1	3
5	A	0.81	1.015	0.679	7	5	1	2	0	1	4
6	A	0.89	1.631	1.063	5	4	2	1	0	1	4
7	A	1.03	1.762	1.106	3	2	1	1	0	0	2
8	A	0.67	1.059	0.932	7	5	2	1	1	0	4
9	A	1.15	1.428	1.257	6	4	2	0	1	0	3
10	A	0.44	1.281	0.878	5	4	1	2	0	0	3
11	A	0.75	1.365	0.906	4	3	2	0	1	0	3
12	A	0.49	1.225	0.798	5	3	0	2	0	1	3
13	A	0.26	1.02	0.66	4	2	1	0	1	0	2
14	A	0.81	1.34	1.211	7	4	2	2	0	0	4
15	A	0.39	1.258	0.908	6	4	1	2	0	0	3
16	A	1.1	1.516	1.252	5	4	2	1	0	0	3
17	A	0.47	1.375	0.841	3	2	0	1	0	0	1
18	A	0.94	1.42	1.069	8	3	1	1	0	0	2
19	A	1.17	1.691	0.954	7	5	2	1	0	1	4
20	A	0.84	1.601	1.111	6	4	0	2	1	0	3
21	A	1.05	1.654	1.092	7	5	1	2	0	1	4
22	A	0.95	1.529	1.056	8	4	2	0	1	0	3
23	A	0.32	1.162	0.702	7	4	2	1	1	0	4
24	A	1.05	1.392	1.067	4	2	1	1	0	0	2
25	A	0.22	1.111	0.773	3	3	0	1	0	0	1
26	A	0.16	1.088	0.637	5	3	1	1	0	0	2
27	A	0.5	1.322	0.849	6	4	1	1	1	0	3
28	A	0.9	1.299	1.058	3	2	0	1	0	0	1
29	A	0.75	1.385	0.879	5	3	1	1	0	0	
30	A	0.53	1.212	0.939	7	5	2	1	1	0	4
31	A	0.58	1.263	0.869	5	3	1	0	0	1	2
32	A	0.44	1.045	0.792	6	4	2	1	0	1	4
33	A	0.52	1.188	0.981	5	3	1	0	1	0	2
34	A	0.85	1.479	0.986	7	4	2	0	1	1	4

Ovary SI. No.	Corpus luteum (present /absent)	Weight (g)	Length (cm)	Width (cm)	TNFS	NFA	Grade A	Grade B	Grade C	Grade D	Total no. of COCs
35	A	0.41	1.09	0.873	8	5	1	1	0	1	3
36	A	0.35	0.904	0.746	6	3	1	1	0	1	3
37	A	0.98	1.763	1.08	4	3	1	1	0	0	2
38	A	0.51	1.247	0.816	6	4	2	0	0	1	3
39	A	0.5	1.162	0.926	8	5	1	1	0	1	3
39	A	0.51	1.089	0.958	9	6	1	2	1	0	4
40	A	1.47	1.689	1.462	6	5	1	1	0	1	3
41	A	0.77	1.342	0.904	5	4	1	1	0	1	3
42	A	1.18	1.61	1.148	5	3	1	1	0	0	2
43	A	0.65	1.369	0.993	4	3	1	1	0	0	2
44	A	1.76	1.774	1.254	7	4	1	1	0	1	3
45	A	0.83	1.449	1.057	6	4	1	0	1	1	3
46	A	1.62	1.639	1.242	9	6	2	1	1	0	4
47	A	0.96	1.545	1.104	5	4	1	0	1	0	2
48	A	0.53	1.319	0.896	7	5	2	1	0	1	4
49	A	0.63	1.351	0.888	4	2	1	0	0	0	1
50	A	0.98	1.304	1.128	8	4	1	1	0	0	2
51	A	0.77	1.286	1.005	7	4	1	1	0	1	3
52	A	0.38	1.248	0.834	8	5	1	1	1	0	3
53	A	0.57	1.415	0.925	4	3	1	0	0	0	1
54	A	1.37	1.573	1.287	5	3	1	1	0	1	3
55	A	1.21	1.617	1.143	4	2	1	0	0	0	1
56	A	0.95	1.34	1.219	6	4	2	1	0	1	4
57	A	0.76	1.597	1.018	8	5	1	1	1	0	3
58	A	0.48	1.219	0.776	4	2	0	1	0	0	1
59	A	0.56	1.176	0.873	5	4	1	1	0	1	3
60	A	1.08	1.532	1.137	7	5	2	1	1	0	4
61	A	0.33	0.945	0.73	4	3	1	0	0	0	1
62	A	0.55	1.128	0.937	6	4	1	1	1	0	3
63	A	0.53	1.131	0.807	7	5	1	1	0	1	3
64	A	0.61	1.409	0.941	7	4	2	0	0	1	3
65	A	0.5	1.31	0.689	11	7	1	2	1	1	5
66	A	0.38	1.121	0.675	7	5	2	1	1	0	4
67	A	0.48	1.302	0.885	6	3	1	1	0	0	2
68	A	0.73	1.129	0.802	6	4	1	1	0	1	3
69	A	0.56	1.107	0.782	5	3	1	1	0	0	2

Ovary SI. No.	Corpus luteum (present /absent)	Weight (g)	Length (cm)	Width (cm)	TNFS	NFA	Grade A	Grade B	Grade C	Grade D	Total no. of COCs
1	P	0.7	1.284	1.164	12	7	1	0	2	1	4
2	P	0.59	9.05	8.36	6	2	0	0	1	1	2
3	P	0.74	1.25	1.191	9	5	0	1	1	1	3
4	P	0.45	1.039	0.864	8	3	0	0	1	2	3
5	P	0.62	0.906	0.848	4	2	0	0	1	0	1
6	P	0.69	1.001	0.835	7	3	0	0	1	1	2
7	P	0.71	1.208	1.141	9	5	0	1	0	2	3
8	P	0.79	1.402	0.967	7	4	1	0	1	1	3
9	P	0.86	1.501	1.052	4	2	0	0	1	1	2
10	P	0.61	1.08	1.045	5	3	0	1	0	2	3
11	P	0.77	1.145	1.035	5	2	0	0	1	1	2
12	P	0.64	1.207	1.075	7	3	0	0	2	1	3
13	P	0.78	1.254	1.167	9	4	0	0	1	1	2
14	P	0.81	1.297	1.27	7	3	0	0	1	1	2
15	P	0.36	1.059	0.952	6	4	0	1	0	1	2
16	P	0.89	1.478	1.284	5	3	1	0	1	0	2
17	P	0.68	1.352	0.964	8	5	1	0	0	2	3
18	P	0.49	1.222	0.876	4	3	1	0	0	1	2
19	P	0.52	1.235	0.92	5	2	0	0	0	1	1
20	P	0.82	1.228	1.185	7	4	0	1	2	0	3
21	P	0.75	1.095	0.835	5	3	0	0	1	2	3
22	P	0.73	1.091	0.831	6	4	1	0	0	2	3
23	P	1.47	1.689	1.226	5	3	0	1	1	0	2
24	P	1.04	1.431	1.178	4	2	0	0	1	1	2
25	P	0.57	1.228	0.925	7	4	1	0	0	2	3
26	P	0.74	1.248	1.161	8	6	1	0	1	1	3
27	P	0.99	1.388	1.208	8	5	0	1	1	2	4
28	P	0.62	1.125	0.996	7	3	1	0	1	0	2
29	P	0.56	1.295	0.989	9	6	1	0	1	2	4
30	P	0.47	1.218	0.873	8	4	0	1	0	2	3
31	P	0.95	1.336	1.28	5	2	0	0	1	1	2
32	P	0.6	1.091	0.947	6	4	0	1	0	2	3
33	P	0.92	1.297	0.972	9	3	1	0	2	0	3
34	P	0.53	1.233	0.921	10	5	0	1	1	2	4

Oocyte Harvesting Technique	Total No. of Ovaries	Grade A	Grade B	Grade C	Grade D	Total no. of COCs
Slicing	28	4	5	7	12	28
Slicing	24	5	3	10	7	25
Slicing	12	2	2	4	6	14
Puncture	12	2	1	4	6	13
Puncture	10	1	2	5	3	11
Puncture	16	3	1	7	5	16
Puncture	12	2	1	3	5	11
Puncture	14	3	2	6	4	15
Aspiration	16	5	3	2	2	12
Aspiration	10	2	4	1	1	8
Aspiration	18	6	4	2	3	15
Aspiration	8	3	2	0	2	7
Aspiration	12	3	3	2	1	9

Appendix III: Analysis of variance (ANOVA) for weight (gm) in different right and left ovary

Source of Variation	Degrees of freedom	Sum of Squares	Mean Square	F-Value	Level of significance
Between category	1	0.000	0.000	0.00	0.995NS
Within category	136	643.089	4.728		
Total	137	643.089			

NS= Not significant

Appendix IV: Analysis of variance (ANOVA) for length (cm) in different right and left ovary

Source of Variation	Degrees of freedom	Sum of Squares	Mean Square	F-Value	Level of significance
Between category	1	0.597	0.597	6.63	0.011*
Within category	136	12.246	0.090		
Total	137	12.843			

* Significant ($p<0.05$)

Appendix V: Analysis of variance (ANOVA) for width (cm) in different right and left ovary

Source of Variation	Degrees of freedom	Sum of Squares	Mean Square	F-Value	Level of significance
Between category	1	0.067	0.067	1.25	0.265NS
Within category	136	7.341	0.053		
Total	137	7.409			

NS= Not significant

Appendix VI: Analysis of variance (ANOVA) for total no. of follicle in different right and left ovary

Source of Variation	Degrees of freedom	Sum of Squares	Mean Square	F-Value	Level of significance
Between category	1	2.898	2.898	0.60	0.439NS
Within category	136	655.072	4.816		
Total	137	657.971			

NS= Not significant

Appendix VII: Analysis of variance (ANOVA) for no. of follicle aspirated in different right and left ovary

Source of Variation	Degrees of freedom	Sum of Squares	Mean Square	F-Value	Level of significance
Between category	1	0.028	0.028	0.01	0.921NS
Within category	136	399.594	2.938		
Total	137	399.623			

NS= Not significant

Appendix VIII: Analysis of variance (ANOVA) for Grade-A COCs in different right and left ovary

Source of Variation	Degrees of freedom	Sum of Squares	Mean Square	F-Value	Level of significance
Between category	1	109.630	109.630	231.18	0.0001**
Within category	136	64.492	0.474		
Total	137	174.123			

**significant ($p<0.01$)

Appendix IX: Analysis of variance (ANOVA) for Grade-B COCs in different right and left ovary

Source of Variation	Degrees of freedom	Sum of Squares	Mean Square	F-Value	Level of significance
Between category	1	60.007	60.007	137.48	0.0001**
Within category	136	59.362	0.436		
Total	137	119.369			

**significant (p<0.01)

Appendix X: Analysis of variance (ANOVA) for Grade-C COCs in different right and left ovary

Source of Variation	Degrees of freedom	Sum of Squares	Mean Square	F-Value	Level of significance
Between category	1	62.673	62.673	114.69	0.0001**
Within category	136	74.318	0.546		
Total	137	136.992			

**significant (p<0.01)

Appendix XI: Analysis of variance (ANOVA) for Grade-D COCs in different right and left ovary

Source of Variation	Degrees of freedom	Sum of Squares	Mean Square	F-Value	Level of significance
Between category	1	120.586	120.586	254.29	0.0001**
Within category	136	64.492	0.474		
Total	137	185.079			

**significant (p<0.01)

Appendix XII: Analysis of variance (ANOVA) for total no. of COCs in different right and left ovary

Source of Variation	Degrees of freedom	Sum of Squares	Mean Square	F-Value	Level of significance
Between category	1	0.463	0.463	0.19	0.660NS
Within category	136	324.811	2.388		
Total	137	325.275			

NS= Not significant

Appendix XIII: Analysis of variance (ANOVA) for weight (gm) in different with CL and without CL group of ovaries

Source of Variation	Degrees of freedom	Sum of Squares	Mean Square	F-Value	Level of significance
Between category	1	0.009	0.009	0.11	0.744NS
Within category	102	8.871	0.086		
Total	103	8.880			

NS= Not significant

Appendix XIV: Analysis of variance (ANOVA) for length (cm) in different with CL and without CL group of ovaries

Source of Variation	Degrees of freedom	Sum of Squares	Mean Square	F-Value	Level of significance
Between category	1	0.377	0.377	0.61	0.437NS
Within category	102	63.244	0.620		
Total	103	63.621			

NS= Not significant

Appendix XV: Analysis of variance (ANOVA) for width (cm) in different with CL and without CL group of ovaries

Source of Variation	Degrees of freedom	Sum of Squares	Mean Square	F-Value	Level of significance
Between category	1	1.972	1.972	3.67	0.048*
Within category	102	54.851	0.537		
Total	103	56.823			

*significant ($p<0.05$)

Appendix XVI: Analysis of variance (ANOVA) for total no. of follicle in different with CL and without CL group of ovaries

Source of Variation	Degrees of freedom	Sum of Squares	Mean Square	F-Value	Level of significance
Between category	1	20.708	20.708	6.84	0.010**
Within category	102	308.830	3.027		
Total	103	329.538			

**significant ($p<0.01$)

Appendix XVII: Analysis of variance (ANOVA) for no. of follicle aspirated in different with CL and without CL group of ovaries

Source of Variation	Degrees of freedom	Sum of Squares	Mean Square	F-Value	Level of significance
Between category	1	0.646	0.646	0.49	0.048*
Within category	102	135.815	1.331		
Total	103	136.461			

*significant ($p<0.05$)

Appendix XVIII: Analysis of variance (ANOVA) for Grade-A COCs in different with CL and without CL group of ovaries

Source of Variation	Degrees of freedom	Sum of Squares	Mean Square	F-Value	Level of significance
Between category	1	18.157	18.157	59.31	0.0001**
Within category	102	31.226	0.306		
Total	103	49.384			

**significant ($p<0.01$)

Appendix XIX: Analysis of variance (ANOVA) for Grade-B COCs in different with CL and without CL group of ovaries

Source of Variation	Degrees of freedom	Sum of Squares	Mean Square	F-Value	Level of significance
Between category	1	8.400	8.400	27.33	0.0001**
Within category	102	31.358	0.307		
Total	103	39.759			

**significant ($p<0.01$)

Appendix XX: Analysis of variance (ANOVA) for Grade-C COCs in different with CL and without CL group of ovaries

Source of Variation	Degrees of freedom	Sum of Squares	Mean Square	F-Value	Level of significance
Between category	1	5.934	5.934	21.60	0.0001**
Within category	102	28.026	0.274		
Total	103	33.961			

**significant ($p<0.01$)

Appendix XXI: Analysis of variance (ANOVA) for Grade-D COCs in different with CL and without CL group of ovaries

Source of Variation	Degrees of freedom	Sum of Squares	Mean Square	F-Value	Level of significance
Between category	1	14.309	14.309	43.53	0.0001**
Within category	102	33.526	0.328		
Total	103	47.836			

**significant (p<0.01)

Appendix XXII: Analysis of variance (ANOVA) for total no. of COCs in different with CL and without CL group of ovaries

Source of Variation	Degrees of freedom	Sum of Squares	Mean Square	F-Value	Level of significance
Between category	1	0.884	0.884	1.04	0.309
Within category	102	86.615	0.849		
Total	103	87.500			

NS= Not significant

Appendix XXIII: Analysis of variance (ANOVA) for Grade-A COCs in different oocyte harvesting techniques

Source of Variation	Degrees of freedom	Sum of Squares	Mean Square	F-Value	Level of significance
Between category	2	7.425	3.712	2.03	0.018*
Within category	10	18.266	1.826		
Total	12	25.692			

*significant (p<0.05)

Appendix XXIV: Analysis of variance (ANOVA) for Grade-B COCs in different oocyte harvesting techniques

Source of Variation	Degrees of freedom	Sum of Squares	Mean Square	F-Value	Level of significance
Between category	2	10.564	5.282	6.09	0.018*
Within category	10	8.666	0.866		
Total	12	19.230			

*significant (p<0.05)

Appendix XXV: Analysis of variance (ANOVA) for Grade-C COCs in different oocyte harvesting techniques

Source of Variation	Degrees of freedom	Sum of Squares	Mean Square	F-Value	Level of significance
Between category	2	65.723	32.861	10.53	0.003**
Within category	10	31.200	3.120		
Total	12	96.923			

**significant (p<0.01)

Appendix XXVI: Analysis of variance (ANOVA) for Grade-D COCs in different oocyte harvesting techniques

Source of Variation	Degrees of freedom	Sum of Squares	Mean Square	F-Value	Level of significance
Between category	2	80.410	40.205	14.03	0.001**
Within category	10	28.666	2.866		
Total	12	109.076			

**significant (p<0.01)

Appendix XXVII: Analysis of variance (ANOVA) for total no. of COCs in different oocyte harvesting techniques

Source of Variation	Degrees of freedom	Sum of Squares	Mean Square	F-Value	Level of significance
Between category	2	283.425	141.712	8.23	0.007**
Within category	10	172.266	17.226		
Total	12	455.692			

**significant (p<0.01)