COLLECTION AND EVALUATION OF CUMULUS-OOCYTE-COMPLEXES (COCs) FROM GOAT SLAUGHTERHOUSE OVARY IN VIEW OF IN VITRO PRODUCTION OF EMBRYO

NUR ARIANI BINTI DZULKARNAIN

DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE DEGREE OF BACHELOR OF AGRICULTURAL SCIENCE WITH HONOURS

LIVESTOCK PRODUCTION PROGRAMME (HG36)
FACULTY OF SUSTAINABLE AGRICULTURE
UNIVERSITI MALAYSIA SABAH
2016

UNIVERSITI MALAYSIA SABAH

BORANG PENGESAHAN TESIS

JUDUL: Collection and Evaluation of Cumulus-Oocyte-Complexes (COCs) from Goat Slaughterhouse Ovary In vion of In Vitro Production of Embryo

IJAZAH: Sarjana Muda Sains Pertanian dengan Kepujian

SAYA: NUR ARIANI BINTI DZULKARNAIN SESI PENGAJIAN: 2012 - 2016
(HURUF BESAR)

Mengaku membenarkan tesis *(LPSM/~~Sarjana/Doktor Falsafah~~) ini disimpan di Perpustakaan Universiti Malaysia Sabah dengan syarat-syarat kegunaan seperti berikut:-

1. Tesis adalah hak milik Universiti Malaysia Sabah.
2. Perpustakaan Universiti Malaysia Sabah dibenarkan membuat salinan untuk tujuan pengajian sahaja.
3. Perpustakaan dibenarkan membuat salinan tesis ini sebagai bahan pertukaran antara institusi pengajian tinggi.
4. Sila tandakan (/)

☐	SULIT	(Mengandungi maklumat yang berdarjah keselamatan atau kepentingan Malaysia seperti yang termaktub di AKTA RAHSIA RASMI 1972)
☐	TERHAD	(Mengandungi maklumat TERHAD yang telah ditentukan oleh organisasi/badan di mana penyelidikan dijalankan)
☑	TIDAK TERHAD	

PERPUSTAKAAN
UNIVERSITI MALAYSIA SABAH

Disahkan oleh:
NURULAIN BINTI ISMAIL
*LIBRARIAN
UNIVERSITI MALAYSIA SABAH

(TANDATANGAN PENULIS)
Alamat Tetap: 44/162 Lorong 8, Gedong Lalang Ampangan, 70400 Seremban, Negeri Sembilan Darul Khusus

(TANDATANGAN PUSTAKAWAN)

(NAMA PENYELIA)
PROF. DR. M.A.M. YAHIA KHANDOKER
PENSYARAH
FAKULTI PERTANIAN LESTARI
UMS KAMPUS SANDAKAN

TARIKH: 8/1/2016

TARIKH: 8/1/2016

Catatan:
*Potong yang tidak berkenaan.
*Jika tesis ini SULIT dan TERHAD, sila lampirkan surat daripada pihak berkuasa/organisasi berkenaan dengan menyatakan sekali sebab dan tempoh tesis ini perlu dikelaskan sebagai SULIT dan TERHAD.
*Tesis dimaksudkan sebagai tesis bagi Ijazah Doktor Falsafah dan Sarjana Secara Penyelidikan atau disertai bagi pengajian secara kerja kursus dan Laporan Projek Sarjana Muda (LPSM).

DECLARATION

I hereby declare that this dissertation is based on my original work except for citations and quotations which have been truly acknowledged. I also declare that no part of this dissertation has been previously or concurrently submitted for a degree of this or any university.

NUR ARIANI BINTI DZULKARNAIN

BR12110082

11 JANUARY 2016

VERIFIED BY

1. PROF. DR. M. A. M. YAHIA KHANDOKER
   SUPERVISOR

PROF. DR. M.A.M. YAHIA KHANDOKER
PENSYARAH
FAKULTI PERTANIAN LESTARI
UMS KAMPUS SANDAKAN

2. PROF. DR. ABDUL RASHID BABA
   CO-SUPERVISOR

PROF. DR. ABDUL RASHID BABA
KETUA PROGRAM HG36
FAKULTI PERTANIAN LESTARI
UMS KAMPUS SANDAKAN

ACKNOWLEDGEMENT

First of all, all praises is to Allah and to Him alone which He guided me, showed me the way to overcome all the obstacles appeared throughout the process of writing this dissertation. He also lighted my path, inspired me and gave me surplus energy to eventually finish the writing of this dissertation.

I would like to extend my sincere gratitude to my supervisor, Prof. Dr. M. A. M. Yahia Khandoker and my co-supervisor, Prof. Dr. Abdul Rashid Baba for their continuous constructive guidance, correction, comments and invaluable knowledge regarding this dissertation. Without their help and dedicated involvement throughout the process, this paper would have never been accomplished.

A great thanks goes to Prof. Dr. Wan Mohamad Wan Othman, dean of Faculty of Sustainable Agriculture, UMS for his invaluable support and encouragement until this dissertation able to finish.

I am also very grateful for the continuous and dedicated involvement from Dr. Nurul Aini Fitria Kaliwon, veterinary officer of Faculty of Sustainable Agriculture. Without her help, this thesis might experience difficulty in getting the sample.

I would also like to thank Tuan Hj. Yussuf Nannong, owner of the Hafiz Farm Sdn. Bhd, Lahad Datu, Sabah and his staff especially Mr. Mahadir Sultan for giving me the permission to take the goat ovaries sample from his slaughterhouse. Without his permission, this thesis can never been done successfully.

My deep appreciation goes to my beloved parents, Mr. Dzulkarnain b. Shamsuddin and Mdm. Nor Fazilla bt. Mohd. Zain for their continuous support, help and encouragements from the beginning until the end. They are my inspiration to keep me strong and never give up to face any obstacles appeared before success.

My special gratitude also goes to my friends especially Nur Farah Atiqah bt Yahya, Tatiana Imrah bt Roslan, Maryam bt Abdullah for their great help during the collection and processing of the goat ovaries taken from the slaughterhouse.

I would also like to express my deepest gratitude to those who are directly and indirectly involved throughout the finishing this dissertation. I realized that in the process of gaining knowledge, I cannot stand on my own without these people mentioned above because in every success person, there are always those people who stand behind to assist. Thank You!

ABSTRACT

The evaluation of ovaries, efficient collection and grading the cumulus oocyte complexes (COCs) is the initial step involve in the production of embryo *in vitro*. The objectives of this study were to evaluate the ovaries and recovery rate of COCs from goat slaughterhouse ovary and to find out the relationship between the number of follicles dissected and cumulus oocyte complexes (COCS) recovery. From the observation of the ovarian types, it was found that 100% of ovaries were to ovary without CL group. In each and every ovarian surface, the number of visible follicles was counted and the follicles were collected by blunt dissection technique. Via blunt dissection, scissors and forceps were used to dissect individual follicles from the ovaries, and then the follicular materials were harvested individually in the culture dish. The follicular materials collected was observed under microscope to categorize the COCs as grade A (oocyte homogenously surrounded with cumulus cells), grade B (oocyte partially surrounded with cumulus cells), grade C (oocyte not surrounded at all with cumulus cells) and grade D (degeneration observed both in oocyte and cumulus cells). Grade A and grade B were considered as normal COCs while grade C and grade D were considered as abnormal COCs. The results were analysed using mean ± SE with the help of Microsoft Office Excel 2013. From the total of 159 visible follicles, 117 follicles were able to be dissected. Among the follicles dissected, the total 66 COCs collected and classified into two groups; normal (Grade A and B) group and abnormal (Grade C and D) group. It shows that all the visible follicles not able to be dissected which is normal. However, the number of normal COCs per ovary (4.9 ± 0.6) observed is higher than the number of abnormal COCs per ovary (1.7 ± 0.6). The average recovery rate of COCs per ovary was 56.7 %. From the total, the recovery rate for the normal COCs (42.4 %) was higher than the recovery rate for the abnormal COCs (14.3 %). The COCs recovery increased as the number of follicle dissected increased which reflected that higher number of follicles positively effected on the increasing of COCs recovery. From this study, it can be concluded that only the non-cyclic female goat were slaughtered in the slaughterhouse. Mostly, the non-reproductive performing animals were slaughtered which increase the possibility to get the non-cyclic ovaries from the slaughterhouse during the sample collection and remarkable number of normal COCs is possible to collect by blunt dissection technique to initiate the in vitro production of embryo experiment.

Pengumpulan dan Penilaian kompleks kumulus oosit (COCs) daripada ovari kambing dalam pandangan pengeluaran embrio secara *in vitro*

ABSTRAK

Penghasilan embrio melalui kaedah in vitro melibatkan beberapa langkah permulaan dan penting seperti penilaian ovari, pengumpulan oosit secara berkesan dan pengklasifikasian kompleks kumulus oosit (COCs). Objektif kajian ini adalah untuk menilai keadaan ovari dan kadar penemuan COCs dari ovari kambing yang disembelih dan juga untuk mengetahui hubungan di antara bilangan folikel yang dibedah dan penemuan kompleks kumulus oosit (COCs). Melalui pemerhatian, didapati jenis ovari yang diperolehi dari rumah sembelih adalah 100% ovari yang tidak mempunyai korpus luteum. Setiap bilangan folikel di permukaan ovari dikira dan folikel tersebut dibedah menggunakan kaedah pembedahan. Gunting dan forsep telah digunakan untuk membedah setiap dan kesemua folikel dibedah secara individu melalui kaedah pembedahan dan diletakkan ke dalam gelas Petri. Kemudian, folikel yang dikumpul diteliti di bawah pandangan mikroskop untuk mengklasifikasikan COCs sebagai Gred A (oosit dikelilingi sel-sel kumulus dengan seragam), Gred B (sebahagian oosit dikelilingi sel-sel kumulus), Gred C (oosit tidak dikelilingi sel-sel kumulus), dan Gred D (degenerasi dilihat pada oosit dan sel-sel kumulus). Gred A dan B diklasifikasikan sebagai COCs normal manakala Gred C dan D diklasifikasikan sebagai COCs tidak normal. Keputusan kajian dianalisis mennggunakan min ± SE dengan pengggunaan system Microsoft Excel 2013. Daripada 159 jumlah keseluruhan folikel yang dapat dilihat, hanya 117 folikel yang dapat dibedah. Di antara folikel yang dibedah, 66 jumlah COCs berjaya dijumpai dan diklasifikasikan kepada dua kumpulan; COCs normal (Gred A dan B) kumpulan dan COCs tidak normal (Gred C dan D). Ini menunjukkan jumlah folikel yang dilihat tidak dapat keseluruhannya dibedah adalah satu situasi yang normal dalam kajian ini. Walaubagaimanapun, di dalam kajian ini jumlah COCs normal pada setiap ovari adalah dilihat lebih tinggi (4.9 ± 0.6) berbanding jumlah COCs tidak normal pada setiap ovari (1.7 ± 0.6). Purata kadar penemuan COCs pada setiap ovari adalah 56.7 %. Daripada jumlah tersebut, purata kadar penemuan COCs yang bersifat normal adalah lebih tinggi (42.4 %) berbanding dengan COCs yang bersifat tidak normal (14.3 %). Penemuan COCs meningkat selari dengan peningkatan bilangan folikel yang dibedah, ia menunjukkan bahawa jumlah folikel yang tinggi memberi kesan yang positif kepada penemuan COCs. Melalui kajian ini, dapat disimpulkan bahawa hanya kambing betina yang tidak produktif akan disembelih di rumah penyembelihan. Kebanyakannya, binatang yang kurang berprestasi dalam pembiakan akan disembelih meningkatkan kemungkinan untuk mendapatkan ovari yang tidak mempunyai korpus luteum sewaktu pengumpulan sampel dari rumah penyembelihan dan jumlah normal COCs yang luar biasa adalah mampu dikumpul melalui teknik pembedahan dengan menggalakkan kematangan dan persenyawaan kajian melalui kaedah in vitro.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS, UNITS AND ABBREVIATIONS

%	Percent
<	Less than
>	More than
°C	Degree Celsius
µg	Microgram
µm	Micrometre
BSA	Bovine serum albumin
CC	Cumulus cell
CL+	Corpus luteum present
CL-	Corpus luteum absent
COCs	Cumulus-oocyte-complexes
FSH	*Follicle-stimulating hormone*
G	Gauge
GnRH	Gonadotropin-releasing hormone
IVC	*In vitro* culture
IVEP	*In vitro* embryo production
IVF	*In vitro* fertilization
IVM	*In vitro* maturation
LH	Luteinising hormone
ml	Milliliter
mm	Millimetre
NaCl	Sodium chloride
rRNA	Ribosomal ribonucleic acid
SE	Standard error
UMS	Universiti Malaysia Sabah
vs	Versus
ZP	Zona pellucida

LIST OF FORMULAE

CHAPTER 1

INTRODUCTION

1.1 Introduction

In vitro production (IVP) of goat embryos is a rapidly advancing field and it has been improved greatly during the past two decades (Kątska-Książkiewicz *et al.*, 2007). The production of valuable transgenic goats, capable of producing substances of pharmaceutical and technological value in their milk and meat, has encouraged the development of *in vitro* techniques which able to support the propagation of this animal. Before this latest development in gametes and embryo cellular biology, the field of molecular embryology of farm animals has been poorly explored due to limited availability of suitable experimental material at an acceptable cost. For this reason, the *in vitro* techniques for the production of mammalian embryos have received great attention and support in recent years (Holtz, 2005) by using the oocytes collected from the slaughterhouse derived ovaries with low cost.

Basically, there are four basic steps of *in vitro* production of embryos; (a) the evaluation of ovaries, efficient collection and grading the oocytes; (b) the *in vitro* maturation (IVM) of these oocytes; (c) the *in vitro* fertilization (IVF) of the matured oocytes; and (d) the *in vitro* culture (IVC) of the resulting embryos (Freitas and Melo, 2010). It is necessary to recover the oocytes and undergo maturation of oocytes, fertilize, and develop those developing zygotes to blastocyst stage in order to produce embryos by *in vitro* techniques before they can be transferred to the recipient. There is still varies in the percentage of oocytes reaching the blastocyst stage by *in vitro* techniques done in the recent years. In some cases, the quality of the oocytes at the beginning of maturation is related to the lower development of IVM oocytes.

Following the first successful fertilization *in vitro* of bovine oocytes (Brackett *et al.*, 1982) in which *in vivo* matured bovine oocytes have been used, researchers have focused on IVF of *in vitro* matured mammalian oocytes (Sirard and Bilodeau, 1990). Efficiency of IVP depends on several factors, such as transport time and temperature from the abattoir to the laboratory (Yang *et al.*, 1990), follicle size (Blondin and Sirard, 1995), developmental stage of oocyte (Hagemann *et al.*, 1999), oocyte diameter (Hyttel *et al.*, 1997), composition of media (Lonergan *et al.*, 1997), hormones (Zuelke and Brackett, 1990), serum and protein supplementation to the basic culture medium (Avery *et al.*, 1998). Mondal (2008) has concluded that the higher average number of good quality oocytes was recovered from ovaries without corpus luteum compared to the ovaries with corpus luteum, which thus, can be effectively used for IVM and IVF. In addition, it has been shown that oocytes with at least four layers of cumulus cells have good result for IVM and IVF (Yang *et al.*, 1993).

Therefore, we should undertake the program on this issue for developing the modern and sustainable techniques in Sabah region of Malaysia. IVM, IVF of the goat oocytes and IVF of the resulting embryos also performed in developing country (Asad, 2015). Moreover, recent advances in biotechnology have enabled in University of Malaya to produce cloned caprine blastocysts (Abdullah and Wan Khadijah, 2010). However, the technology allows the predictable supply of embryos from ovaries of slaughtered females or from lives selected animals, via repeated recovery of primary oocytes. To date, this technique has given considerable success in West Malaysia (Kwong *et al.*, 2012). 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. But till now, IVP program in goat is obscure in Sabah region of Malaysia. So, there exists vast opportunity to conduct the research in the area of IVP of goat embryos in Sabah region.

There are two suitable techniques of oocytes collection that are commonly and can be used; (a) aspiration and (b) blunt dissection. However, as one of my colleague (Nur Farah Atiqah, 2016) had performed research on both types of cumulus oocytes complexes (COCs) collection techniques from cattle slaughterhouse ovaries and reported that blunt dissection technique is more effective compared to aspiration.

1.2 Justification

Nowadays, human depends on meat and milk of livestock as their part of food source. Even though the demand for the goat is not very high compared to the cattle demand, but the supply is still below the expectations. In Sabah, there is still a limitation of information so far been found on the evaluation of goat ovaries from slaughterhouse and follicle size to get quality cumulus oocyte complexes (COCs) in view of *in vitro* production of embryo. Therefore, this experiment needs to be conducted to determine the quality of ovary (corpus luteum present or absent) and number of follicles on the surfaces of those which are most suitable to produce higher number of good quality COCs. Furthermore, by successful start with this work at FPL, UMS Sandakan campus would create a new avenue of modern Animal Biotechnological research like *in vitro* embryo production, cloning, sexing and nuclear transfer.

1.3 Objective

1. To evaluate the recovery rate of cumulus oocyte complexes (COCs) from goat slaughterhouse ovary.

2. To find out the relationship between the number of follicles dissected and COCS recovery.

1.4 Hypothesis

H_0: There is no relationship between the number of follicles dissected and COCS recovery.

H_a: There is the relationship between the number of follicles dissected and COCS recovery.

CHAPTER 2
LITERATURE REVIEW

Previous researches done in this scope of study especially in the qualitative and quantitative of ovary, follicles and COCs which had been the basic step to proceed to the successful IVP in goat embryo. There were several research done on the collection techniques of COCs but in a place like Sabah where getting slaughterhouse goat ovaries is really difficult. No research work so far been performed in this topic. The related findings of research work carried out in different countries of the world are reviewed in this chapter.

2.1 Goat Ovaries

The ovaries are the primary sex organ in female goat which produce the ova (eggs) and secrete the female reproductive hormones such as progesterone and estrogen. Follicles in the ovaries, containing primary oocytes, develop in successive waves and until some will rupture and release a secondary oocyte during ovulation. The released egg passes through the oviduct to join with spermatozoa, whereas the ruptured follicle transforms into a corpus luteum (CL). The development of the follicle is under the control of gonadotropins (follicle stimulating hormone - FSH, and luteinizing hormone - LH) released by the pituitary gland (Wildeus, 2000). Following ovulation, the luteinized follicle (CL) secrets progesterone, which prepare the uterus for a possible pregnancy, and suppresses the secretion of gonadotropins to halt further follicular development. Failure to establish pregnancy will result in the release of prostaglandin from the non-pregnant uterus, which will regressed the corpus luteum and allow a new cycle to proceed (Wildeus, 2000). Fertilization requires the proper timing of insemination and ovulation, as spermatozoa remain viable for only 12 hours in the female reproductive tract, and the life span of the ovulated egg is limited to 12-24 hours.

2.2 Goat Oocyte

Like other mammals, the primary oocytes of goat become arrested at the diplotene stage of meiosis at birth *in vivo*. However, they are capable of resuming meiosis spontaneously when removed from their follicles and cultured *in vitro* (Gilchrist and Thompson, 2007). Before any oocyte can be expected to be able to mature *in vitro*, it must be visualized as being normal. Normal oocytes should have cumulus cell (CC) investment surrounding the zona pellucida (ZP), absence of cracked ZP and absence of vesicles in the ooplasm. The presence of more and compact layers of CCs is considered better. A good goat oocyte will appear golden, golden-yellow or brownish in color and has granulated appearance in the ooplasm (Rajikin *et al.,* 1994). The size of an oocyte is also important for the attainment of maturation. De Smedt *et al.* (1992) showed that 86% of goat oocytes from follicles 2 to 6 mm in diameter progressed to MII, whereas only 24% of oocytes from follicles 1-1.8 mm attained that stage. A good oocyte also has ooplasm which fills the entire part of vitelline space (Rajikin *et al.*, 1994).Significantly ($p<0.001$) higher percentages of goat oocytes were matured when they were surrounded by more than five layers of CCs than those with less than five CC layers and denuded oocytes (Rahman *et al.*, 2006).

2.3 Ovarian type

There are two types of ovaries that can be found such as ovary with corpus luteum (CL) and ovary without CL. CL can be found on the ovarian surface which is the structure formed on the ovary when an egg is shed. The CL is a reproductive gland that produces progesterone, needed for the establishment and maintenance of pregnancy. In goats, the CL is the sole source of progesterone throughout the pregnancy (Charles Esson, n.d.).The CL has a much thicker wall than a follicle with much denser texture. There is dark red in appearance of the outside of a CL and the cross section reveals a bright yellow to yellow-orange interior (Anonymous, n.d.).

If the female goats which mate in the first two to three days during the mating season do not fall pregnant, they will return for service five days later. These short oestrous cycles are very common in goats. Therefore, most does will have been mated, and be pregnant, by the tenth day (Charles Esson, n.d.). However, if the goats is not

pregnant or in the other word not productive anymore, hormone prostaglandin is released and cause regression of CL, resulting in declining of progesterone production. That is why, when ovaries are collected from slaughterhouse, there are low possibilities of finding CL present than CL absent in the ovaries. This is supported by Asad (2015), found 200 out of 275 ovaries collected from slaughterhouse were without CL and only 75 with CL. Saha *et al.* (2014) also observed that higher number of ovaries belonged without CL which is 150 from 195 ovaries collected.

Figure 2.1 Representative photographs showing (A) ovaries without corpus luteum and (B) ovaries with corpus luteum
Source Nur Farah Atiqah, 2016

2.3.1 Effect of Type of Ovary on the Number of Follicles

Burns (2002) stated the decrease in progesterone cause for an increase in GnRH and GnRH stimulates the release of follicle stimulating hormone (FSH) in order to stimulate the release of LH. FSH has the function of stimulating rapid growth of follicles on the ovary. That is why, ovaries without CL in which having low progesterone, have high number of follicles.

The previous finding of Asad (2015) supports the statement strongly in which higher number of visible follicles (5.17±0.13 vs 3.98±0.19 per ovary) were found in without CL group than those of with CL group of goat ovaries. In case of buffalo ovaries, Khandoker *et al.* (2011) mentioned that from a total of 806 aspirated follicles, 630 were obtained from ovaries without CL and 176 from ovaries with CL, showing higher number of follicles observed and obtained from ovaries without CL. There is also previous experiment on the collection and evaluation of cumulus-oocyte-complexes (COCs) from slaughterhouse goat ovaries and reported that the ovaries without corpus luteum has the higher number of follicles (Islam *et al.*, 2007).

2.3.2 Effect of Type of Ovary on Quantitative and Qualitative of COCs

According to Nandi *et al.* (2000) the oocyte recovery rate decreased when ovaries had a corpus luteum. This was due to restriction of follicular development as lutein cells occupy most of the ovary (Kumar *et al.*, 1997). The dominant follicle was usually observed in the corpus luteum-bearing ovary, and the other follicles were very small and remained mostly inaccessible. Agrawal (1992) reported goat ovaries containing a corpus luteum yielded a lower number of oocytes and also a lower proportion of usable oocytes than ovaries without a corpus luteum.

There is a research done in evaluation of buffalo ovaries, follicles and COCs with the view of IVP. The length, width and weight of ovaries with CL were higher ($p<0.05$) whereas, number of observed follicles, aspirated follicles, number of COCs and number of normal COCs were significantly ($p<0.05$) higher in ovaries without CL than ovaries with CL (Khandoker *et al.*, 2011).

There is a study done by using the goat ovaries from the local abattoir by Kharche *et al.* (2009) which 1313 goat ovaries were obtained and transported to the laboratory within 4 hours in warm saline (37 °C), containing 100 IU penicillin-G and 100 µg streptomycin sulfate per mL. As studied, oocytes were graded as excellent (A), good (B), fair (C) and poor (D) quality, depending on their cumulus investment and cytoplasmic distribution. They found that the overall average recovery of good quality oocytes for IVM was 1.91 per ovary.

Other research also done by Mondal *et al.* (2008) via evaluation of the goat ovaries and categorized them on the basis of corpus luteum (CL) present and absent. The COCs were harvested by aspiration method and graded as A, B, C and D where grade A and B was considered as normal while C and D as abnormal. They reported that significantly higher ($p<0.05$) number of follicles of 2-6 mm diameter (5.25) and COCs (1.96) was obtained from CL-absent group of ovaries compared to CL present group (3.94 and 1.54 respectively). There is no significant variation was found in the number of follicles measuring <2mm and >6mm diameter in CL present and absent group of ovaries. However, the average number of normal COCs per ovary was significantly higher

($p<0.05$) in CL-absent group (1.30±0.07) than CL-present group (0.68) but the average number of abnormal COCs was higher in CL-present group (0.66) than absent group.

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

Previous study conducted by Islam *et al.* (2007) used goat ovaries from the slaughterhouse and categorized them as right, left, corpus luteum (CL)-present and absent group and evaluated on the basis of weight (g), length (cm), width (cm), number of follicles. Aspiration methods were applied to harvest the oocytes. In the study stated that the left ovaries contained comparatively higher number of normal COCs [(1.06) per ovary] than right ovaries [(1.03) per ovary]. The similar trend was found in total number of follicles [(4.51) vs (4.30) per ovary] and follicles aspirated [(2.55) vs (2.52) per ovary]. However, the total COCs per ovary was almost similar in both ovaries [right and left: (1.85) and (1.85) per ovary, respectively]. Islam *et al.* (2007) also concluded that higher number of total COCs [(1.87) vs (1.76) per ovary], total number of follicles [(4.45) vs (4.16) per ovary], and follicles aspirated [(2.55) vs (2.48) per ovary] and normal COCs [(1.12) vs (0.76) per ovary] were found in CL-absent group than those of CL-present group ovaries.

2.3.3 Effect of Collection Techniques on COCs

Commonly, there are three methods that usually used in collecting the cumulus oocyte complexes (COCs) such as puncture, slicing and aspiration. The research had been conducted shown that puncture and slicing techniques yielded significantly higher number of COCs collected from the ovary compared to aspiration technique (Hoque *et. al*, 2012). However, by aspiration technique the COCs recovery is higher followed by slicing and then punctures. The research concluded that the number of COCs were denuded from cumulus cells (CC) in punctures and slicing might due to repeated washing

and ultimately resulted in a lower number of normal COCs when compared to aspiration at the final observation.

The result of the previous research (Hoque *et. al.*, 2012) was comparable with the previous 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). Wang *et al.* (2007) 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) when compared to aspiration I (2.9) and aspiration II (3.1). Furthermore, 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%), when compared with the puncture (54.7%) or slicing (54.3%) in ewe.

The researcher, Pawshe *et al.* (1994) concluded a series of experiment on the recovery method of goat oocytes by using three methods: aspiration, puncturing and slicing and they concluded that average number of oocytes recovered per ovary was significantly higher by aspiration (2.7) than by puncturing (2.2) or by slicing (2.4). As reported, there were significantly more good-quality usable oocytes covered with compact cumulus cells were obtained by slicing (0.9) than by aspiration (0.5) and the percentages of oocytes maturing, fertilizing and developing *in vitro* differed significantly among recovery methods.

From this chapter, it is possible to conclude that there are a remarkable number of researches so far been conducted on mammals all over the world in terms of ovaries type which give effects on COCs quality for *in vitro* embryo production (IVEP) purposes. To perform research on farm animal *in vitro* production of embryos, source of ovary to collect the COCs and efficient method of collection is important. In Sabah, very few slaughterhouse is available and getting ovary is really difficult. So, if the researcher want to conduct research on embryo development, it is obviously necessary to establish the efficient collection technique of quality COCs.

CHAPTER 3
METHODOLOGY

3.1 Site of Experimentation and Duration of Study

The study was conducted at the Anatomy and Physiology Laboratory, Faculty of Sustainable Agriculture, Universiti Malaysia Sabah (UMS) Sandakan Campus from September 2015 until October 2015.

3.2 Materials

3.2.1 Chemicals and Reagent

In this study, the 0.9% physiological saline (or 0.9% sodium chloride) and the 5% bovine serum albumin (BSA) (Sigma, USA) were used. The microscope also was used during the study. Disposable materials used in this study are Petri dishes, slide, scissors, forceps and filter paper.

3.2.2 Animal

The animals that were used in this study are female goats sacrificed at slaughterhouse. The reason for the animals to be culled at the slaughterhouse may vary according to the policy of the farm. Usually, those culled animals are those older in age and/or finisher animal.

3.3 Methods

3.3.1 Collection and Processing of Ovaries

Goat ovaries were collected from a local slaughterhouse Hafiz Farm Slaughter House at Lahad Datu, Sabah and put in collection vial containing 0.9% physiological saline that was warmed at 25-30°C and kept in a polystyrene box to maintain this temperature during transporting the ovaries, according to Mondal *et al.* (2008). The ovaries were transported to the laboratory within 4 to 5 hours of slaughter. The ovaries were then transferred to sterilized Petri dish and rinsed thoroughly by physiological saline at 25°C. Each ovary was trimmed to remove the surrounding tissues and overlying bursa.

3.3.2 Evaluation of Ovaries

The ovaries were then observed and categorized as corpus luteum present (CL+) and corpus luteum absent (CL-) groups (Figure 2.1) and the number of both types of ovaries were recorded (Mondal *et al.*, 2008). Number of follicles were observed and counted from the surface of the ovaries. The number of follicles from the surface of the ovary were recorded.

3.3.3 Collection and Grading of Cumulus-Oocyte-Complexes (COCs)

For this study blunt dissection technique was used which individual follicles were dissected from the ovaries by using scissors and forceps. Then, the follicular materials were harvested individually in the Petri dish. A small amount of physiological saline solution (0.9% NaCl) with 5% bovine serum albumin (BSA) was added on the slide after the follicular materials is collected followed by observing under microscope to grade the COCs.

The follicular materials collected were observed under microscope at low magnification. The COCs were graded according to the method of Khandoker *et al.* (2001) into 4 grades, grade A: oocytes homogenously surrounded by cumulus cells; grade B: oocytes partially surrounded by cumulus cells; grade C: oocytes not surrounded at all with cumulus cells and grade D: degeneration occurs both in oocytes and cumulus

REFERENCES

Anonymous. n.d. Reproduction Management. *Anatomy and Physiology.* Plain City

Asad, L.Y. 2015. *Effect of Bovine Serum Albumin and Follicular Fluid on In Vitro Maturation, Fertilization and Development of Goat Embryos.* Doctoral Thesis. Bangladesh Agricultural University

Abdullah, R.B. and Wan Khadijah, W.E. 2010. Caprine Reproductive Technologies in Malaysia, *Reproductive Biotechnology* **7(1)**: 21

Agrawal, K. P. 1992. Factors Affecting Oocyte Recovery from Caprine Ovaries of Abattoir Origin. In: Recent Advances in Goat Production. R. R. Lokeshwar, ed., Proc. 5th Int. Conf. Goats. 1207-1210.

Avery, B., Bavister, B.D. and Greve, T. 1998. Development of bovine oocytes, *in vitro* matured in a chemically defined protein-free medium, supplemented with different amino acid formulations. *Theriogenology.* **49**: 306-310.

Blondin, P. and Sirard, M. A. 1995. Oocyte and Follicular Morphology as Determining Characteristics for Developmental Competence in Bovine Oocytes. *Molecular Reproduction Development* **41**: 54- 62.

Bracket, B. G, Bousquet, D., Boice, M. L, Donawick, W. J., Evans, J. F., and Dressel, M. A. 1982. Normal Development Following *In Vitro* Fertilization in the Cow. *Biology Reproduction* **27**: 147-158.

Burns, P. D. 2002. *The Dairy Cow Heat Cycle.* Doctoral Thesis. Colorado State University

Callesen H, Greve T, Christensen F. 1987. Ultrasonically guided aspiration of bovine follicular oocytes. *Theriogenology* **27**: 217.

Charles Esson, n.d., Reproductive Cycle of Goats, Sheep and Wool Branch, South Perth.

De Smedt, V., Crozet, N., Ahmed-Ali, M., Martino, A., and Cognie, Y. 1992. *In Vitro* Maturation and Fertilization of Goat Oocytes. *Theriogenology* **37**: 1049-1060.

Ferdous, J. 2006. Collection, Grading and Evaluation of Goat Cumulus-Oocyte-Complexes (COCs) in View of *In Vitro* Maturation, Fertilization and Culture. M.S. Thesis, Department of Animal Breeding and Genetics. Bangladesh Agricultural University, Mymensingh.

Freitas, V. J. F. and Melo, L. M. 2010. *In Vitro* Embryo Production in Small Ruminants. *Revista Brasileira de Zootecnia* **39**: 404-413

Gilchrist, R.B. and Thompson, J.G. 2007. Oocyte Maturation: Emerging Concepts and Technologies to Improve Developmental Potential *in Vitro*. *Theriogenology*, **67**: 6-15.

Hagemenn, L.J., Beaumont, S.E., Berg, M., Donnison, M.J., Ledgard, A., and Peterson, A.J. 1999. Development during Single IVP of Bovine Oocytes from Dissected Follicles: Interactive Effects of Estrous Cycle Stage, Follicle Size and Atresia. *Molecular Reproduction Development* **53**: 451.

Hafez, E. S. E. 1993. *Reproduction in Farm Animals.* 5th Edition. Lea and Febriger: Philadelphia.

Holtz, W. 2005. Recent Developments in Assisted Reproduction in Goats.*Small Ruminant Research* **60**: 95-110.

Hoque, S.A.M., Kabiraj, S.K., Khandoker, M.A.M.Y., Mondal, M., Tareq, K.M.A. 2012. Effect of Collection on Cumulus Oocytes Complexes (COCs) Recovery, *In Vitro* Maturation and Fertilization of Goat Oocytes, *African Journal of Biotechnology* **10**: 9177-9181.

Hyttel, P., Fair, T., Callesen, H., and Greve, T. 1997. Oocyte Growth, Capacitation and Final Maturation in Cattle. *Theriogenology.* **47**: 23-32.

Islam, M. R., Khandoker, M. A. M. Y., Afroz, S., Rahman, M. G. M. and Khan, R. I. 2007. Qualitative and Quantitative Analysis of Goat Ovaries, Follicles and Oocytes in View of *In Vitro* Production of Embryos. *J. Zhejiang University.* **8**: 465-469.

Kątska-Książkiewicz, L., Opiela, J. and Ryńska, B. 2007. Effects of Oocyte Quality, Semen Donor and Embryo Co-Culture System on the Efficiency of Blastocyst Production in Goats. *Theriogenology.* **68**: 736-744.

Khandoker, M.A.M.Y., Imai, K., Takahashi, T., Hashizume, K. 2001. Role of Gelatinase on Follicular Atresia in the Bovine Ovary. *Biology Reproduction* **65(3)**: 726-732.

Khandoker, M.A.M.Y., Jahan, N., Asad, L., Hoque, S.A.M., Ahmed, S. 2011. Qualitative and Quantitative Analysis of Buffalo Ovaries, Follicles and Oocytes in View of *In Vitro* Production of Embryos, *Bangladesh Journal of Animal Science* **409**: 23-27.

Kharche, S.D., Goel, A.K., Jindal, S.K., Yadav, E.N., Yadav, P., Sinha, R., Sinha, N.K. 2009. Effect of Serum Albumin Supplementation on *In Vitro* Capacitation and Fertilization of Caprine Oocytes. *Small Ruminant Research* **81**: 85-89.

Kwong, P.J., Abdullah, R.B. and Wan Khadijah, W.E. 2012. Increasing Glucose in KSOMaa Basal Medium on Culture Day 2 Improves *In Vitro* Development of Cloned Caprine Blastocysts Produced via Intraspecies and Interspesies Somatic Cell Nuclear Transfer. *Theriogenology* **78**: 921-929.

Kumar, A., Solanki, V. S., Jindal, S. K., Tripathi, V. N., and Jain, G. C. 1997. Oocyte Retrieval and Histological Studies of Follicular Population in Buffalo Ovaries. *Animal Reproduction Science* **47**: 189-195.

Lonergan, P., Khatir, H., Carolan, C. and Mermillod, P. 1997. Bovine Blastocyst Production *In Vitro* after Inhibition of Oocyte Meiotic Resumption for 24h. *Jurnal Reproduction Fertilization* **109**: 355-65.

Mahadir Sultan. Executive Manager of Hafiz Farm Slaughterhouse, Lahad Datu, Sabah. 13 September 2015. Personal Communication.

Mondal, M. A., Khandoker, M. A. M. Y., Mondal M. A., Rahman, A. H. M. S., Apu, A. S., and Pervage, S. 2008. *In Vitro* Production of Goat Embryos in Bangladesh. *Bangladesh Jurnal Animal Science* **37(1)**: 1-9

Mossa F, Leoni GG, Berlinguer F, Succu S, Madeddu M, Bebbere D, Naitana S. 2007. Recovery of COCs from Ovaries with High Follicle Numbers Enhances *In Vitro* Embryo Yield in Sheep. *Animal Reproduction Science* **109(1-4)**: 134-145.

Nandi, S., Chauhan, M. S., Palta, P. 2000. Effect of a Corpus Luteum on the Recovery and Developmental Potential of Buffalo Oocytes. *Veterinary Record* **147**: 580-581.

Nur Farah Atiqah Yahya. 2016. *Evaluation of Bovine Ovaries, Follicles and Cumulus Oocyte Complexes (COCs) in view of In Vitro Production of Embryo.* Bachelor of Science Dissertation. Universiti Malaysia Sabah.

Pawshe, C.H., Totey, S.M., and Jain, S.K. 1994. A Comparison of Three Methods of Recovery of Goat Oocytes for *In Vitro* Maturation and Fertilization. *Theriogenology*, **42**: 117-125.

Rahman, A.N.M.A., Abdullah, R.B. and Wan Khadijah, W.E. 2006. Goat Embryo Development Following *In Vitro* Maturation and Intracytoplasmic Sperm Injection According To Oocyte Grading. *Proceedings of the 11th Biological Sciences Graduate Conference*, December 15-17, 2006, Bangkok, Thailand. 143.

Rajikin, M.H., Yusoff, M., and Abdullah, R.B. 1994. Ultrastructural Studies of Developing Goat Oocytes *In Vitro*. *Theriogenology*, **42**: 1003-1016

Saha, S., Khandoker, M. A. M. Y., Asad, L. Y., Reza, A. M. M. T. and Hoque, A. 2014. Effect of Fresh and Frozen Semen on *In Vitro* Fertilization and Subsequent Development of Goat Embryos. *Iranian Journal of Applied Animal Science* **4(2)**: 325-330

Shirazi, A., Shams-Esfandabadi, N. and Hosseini, S. M. 2005. A Comparison of Two Recovery Methods of Ovine Oocytes for *In Vitro* Maturation. *Small Ruminant Research* **58**: 283-286.

Singh, L. R., Rao, K. S. and Mohan, K. M. 2013. Oocyte Retrieval Methods, Grade and Percentage of Oocytes in Goats. *International Journal of Molecular Veterinary Research* **3(2)**: 4-6

Sirard, M.A. and Bilodeau, S. 1990. Effects of Granulosa Cell Co-Culture on *In Vitro* Meiotic Resumption of Bovine Oocytes. *Jurnal Reproduction Fertilization* **89**: 459-465.

Wani, N.A., Wani, G.M., Khan, M.Z., and Salahudin, S. 2000. Effect of Oocyte Harvesting Techniques on *In Vitro* Maturation and *In Vitro* Fertilization in Sheep. *Small Ruminant Research* **36**: 63-67.

Wang, Z.G., Xu, Z.R., and Yu, S.D. 2007. Effects of Oocyte Collection Techniques and Maturation Media on *In Vitro* Maturation and Subsequent Embryo Development in Boer Goat. *Czech Jurnal Animal Science* **52**: 21-25.

Wildeus, S., 2000, Goat Reproduction, http://www2.luresext.edu/goats/training/reproduction.html. Access on 28 March 2015. Verified on 2 April 2015.

Yang, N.S., Lu, K.H. and Gordon, I. 1990. *In Vitro* Fertilization (IVF) and Culture (IVC) of Bovine Oocytes from Stored Ovaries. *Theriogenology*. **33**: 352.

Yang, X., Jiang, S. and Foote, R. H. 1993. Bovine Oocyte Development Following Different Oocyte Maturation and Sperm Capacitation Procedures. *Molecular Reproduction and Development* **34**: 94-100

Zuelke, K.A. and Brackett, B.G. 1990. Luteinizing Hormone Enhanced *In Vitro* Maturation of Bovine Oocytes With and Without Protein Supplementation. *Biology Reproduction* **43**: 784-787.