EVALUATION OF BOVINE OVARIES, FOLLICLES AND CUMULUS-OOCYTE-COMPLEXES (COCs) IN VIEW OF *IN VITRO* PRODUCTION OF EMBRYO

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

LIVESTOCK PRODUCTION PROGRAMME
FACULTY OF SUSTAINABLE AGRICULTURE
UNIVERSITI MALAYSIA SABAH
2016

UNIVERSITI MALAYSIA SABAH

BORANG PENGESAHAN TESIS

JUDUL: EVALUATION OF BOVINE OVARIES, FOLLICLES AND CUMULUS-OOCYTE-COMPLEXES IN VIEW OF *IN VITRO* PRODUCTION OF EMBRYO

IJAZAH: IJAZAH SARJANA MUDA SAINS PERTANIAN DENGAN KEPUJIAN (PENGELUARAN TERNAKAN)

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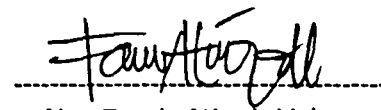

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ACKNOWLEDGMENT

First and foremost, I would like to thank Allah the Almighty for His continuous blessings, loves, and strengths. Immeasurable appreciation and deepest gratitude for the supervision, inspiration, guidance, suggestions and persuasion extended to my supervisor, Prof. M.A.M. Yahia Khandoker, and my co-supervisor, Prof. Dr. Abdul Rashid Baba. Without their assistance and persistent help, this paper would have never been accomplished.

Thanks to Universiti Malaysia Sabah (UMS) for supporting the project and providing some fund for the research entitle Quantitative and Qualitative Evaluation of Bovine Ovaries, Follicles and Cumulus-Oocyte-Complexes (COCs) and *In Vitro* Production of Embryos. Valuable thanks also dedicated to Prof. Dr. Wan Mohamad Wan Othman, Dean of Faculty of Sustainable Agriculture, UMS for his keen encouragement and constructive support. Special appreciation dedicated to Dr. Nurul Aini Fitria Kaliwon, Veterinary Officer of Faculty of Sustainable Agriculture, UMS, and also the staffs involved for their kind help and support throughout the research work.

I would also like to thank Tuan Hj. Yussuf Nannong, owner of Hafiz Farm Sdn. Bhd., Mr. Mahadir Sultan, the Executive Manager and the staffs of Hafiz Farm Slaughterhouse for their kindness in supplying me bovine ovaries that was intended to be used in my study.

I am further grateful and thank all my family members especially my mother, Mdm. Siti Hajar Hashim and my father, Mr. Yahya Ibrahim for their undivided attention, dedication, encouragements, patience, love, and always there besides me through thick and thin. Without my family, none of this could have happened.

My special gratitude also goes to all my friends particularly Miss Nur Ariani Dzulkarnain for her great and warm encouragement and help during ovaries collection as well as during handling the research work. Not to forget Miss Tatiana Imrah Roslan who has been supporting from the beginning until completion of the Final Year Project and Mdm. Yushiana Mansor who have been helping me throughout finishing this dissertation. Special thanks also goes to Mdm. Nurul Syakina Marli and Mdm. Amira, the lab assistants, who were always helping me especially regarding to required lab equipments preparation used during the work.

Finally, I take this opportunity to acknowledge my examiners, lecturers, FYP coordinators and those who are directly or indirectly involved throughout completing this Final Year Project. Thank You.

ABSTRACT

The present study was conducted at the Anatomy and Physiology Laboratory, Faculty of Sustainable Agriculture, Universiti Malaysia Sabah Sandakan Campus from July 2015 until September 2015 to undergo the evaluation of bovine ovaries, follicles and cumulus-oocyte-complexes (COCs) in view of *in vitro* production of embryo. The objectives of this study were to perform the evaluation of bovine slaughterhouse ovary, follicles, and cumulus-oocyte-complexes (COCs) and to compare the effect of collection techniques on the recovery rate of COCs. The collected slaughterhouse ovaries was classified as corpus luteum present (CL+) and corpus luteum absent (CL-) groups. It was found that 62.5% of the ovaries collected were CL- type and only 37.5% were CL+. Observation on follicular number on ovarian surface was done in both types of ovaries. The higher number of visible follicles was found in ovaries without corpus luteum (15.5±2.7 and 45.0±14.3) compared to ovaries with corpus luteum (11.0±2.0 and 35.8±14.5). Two COCs collection techniques were applied which were blunt dissection and aspiration techniques. For the blunt dissection, individual follicles were dissected from the ovaries by using scissors and forceps and the follicular materials were harvested individually in the Petri dish. For the aspiration, the follicles were aspirated by 10 ml syringe attached with 18 gauze needle. The aspirated follicular materials were transferred slowly into a falcon tube, precipitated for 10 minutes, then the upper part was discarded and in the lower part a small amount of 0.9% physiological saline solution with 5% BSA was added and entered into a Petri dish. The follicular materials collected from both techniques were 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 and grade C and grade D were considered as abnormal COCs. The results were analysed using mean ± SE with the help of Microsoft Office Excel 2007. Comparatively higher number of follicles aspirated and dissected from ovaries without CL (11.2±1.8 and 37.8±14.9) than from ovaries with CL (10.5±1.5 and 28.3±15.6). The result further indicated that ovaries without CL contributing more number of total COCs collected per ovary (6.8±1.0) and more normal (A and B grades) COCs (5.7±0.9) than that of ovaries with CL (6.0±2.0 and 4.5±1.5, respectively) in blunt dissection technique and reverse trend was found in aspiration technique. Ovaries without CL were suggested to be suitable for collecting COCs for *in vitro* production of bovine embryos although the reverse trend was found in aspiration technique. Blunt dissection is found more efficient than that of aspiration technique on the harvesting of high number of COCs with recovery rate of 61.6% and 16.5%, respectively. There was also more efficient in the recovery rate of normal (A and B grades) COCs by blunt dissection technique (48.6%) than by aspiration technique (11.7%). The result of this experiment is a preliminary work for planning and execution of future pragmatic research on *in vitro* production of bovine embryos.

ABSTRACT

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Penilaian terhadap Ovari Lembu, Folikel dan Kompleks-Kumulus-Oosit (COCs) dalam Pandangan Pengeluaran Embrio secara In Vitro

ABSTRAK

Kajian ini telah dijalankan di Makmal Anatomi dan Fisiologi, Fakulti Pertanian Lestari, Universiti Malaysia Sabah Kampus Sandakan dari Julai 2015 hingga September 2015 untuk menjalani penilaian terhadap ovari lembu, folikel dan kompleks-kumulus-oosit (COCs) dalam pandangan pengeluaran embrio secara in vitro. *Objektif kajian ini adalah untuk melaksanakan penilaian terhadap ovari lembu sembelihan, folikel dan kompleks-kumulus-oosit (COCs) dan untuk membandingkan kesan teknik pengumpulan terhadap kadar penemuan COCs. Ovari dikumpul dan dikelaskan sebagai kumpulan yang mempunyai korpus luteum (CL+) dan kumpulan ttidak mempunyai korpus luteum (CL-). Didapati bahawa 62.5% daripada ovari tersebut adalah jenis CL- dan hanya 37.5% ovari jenis CL+. Pemerhatian terhadap bilangan folikel pada permukaan ovari dilakukan terhadap kedua-dua jenis ovari. Jumlah folikel lebih banyak ditemui dalam ovari tanpa korpus luteum (15.5±2.7 dan 45.0±14.3) berbanding dengan ovari yang mempunyai korpus luteum (11.0±2.0 dan 35.8±14.5). Dua teknik pengumpulan COCs telah digunakan di dalam kajian ini iaitu teknik pembedahan dan teknik aspirasi. Untuk teknik pembedahan, folikel telah dibedah daripada ovari dengan menggunakan gunting dan forsep dan bahan-bahan folikel diambil secara individu dalam piring Petri. Bagi teknik aspirasi, folikel telah diaspirasi dengan menggunakan 10 ml picagari bersama 18G jarum. Bahan-bahan folikel yang diaspirasi dipindahkan secara perlahan-lahan ke dalam tabung uji dan dibiarkan selama 10 minit. Setelah itu, bahagian atas dibuang dan 0.9% normal saline dan 5% BSA telah ditambah sedikit di bahagian bawah dan dimasukkan ke dalam piring Petri. Bahan-bahan folikel yang dikumpul daripada kedua-dua teknik kemudiannya diperhatikan di bawah mikroskop untuk proses pengklasifikasian COCs mengikut gred Gred A (oosit dikelilingi sel-sel kumulus dengan sepenuhnya), gred B (hanya sebahagian oosit dikelilingi dengan sel-sel kumulus), gred C (oosit langsung tidak dikelilingi dengan sel-sel kumulus) dan gred D (degenerasi diperhatikan pada oosit dan kumulus sel). Gred A dan gred B dianggap sebagai COCs normal dan gred C dan gred D dianggap sebagai COCs tidak normal. Keputusan kajian telah dianalisis menggunakan min ± SE dengan menggunakan sistem perisian Microsoft Office Excel 2007. Secara perbandingan, didapati jumlah folikel yang lebih tinggi diaspirasi dan dibedah dari ovari tanpa CL (11.2±1.8 dan 37.8±14.9) berbanding dari ovari dengan CL (10.5±1.5 dan 28.3±15.6). Melalui hasil kajian, didapati ovari tanpa CL menyumbangkan lebih banyak jumlah bilangan COCs (6.8±1.0) dan lebih banyak gred A dan B COCs (5.7±0.9) berbanding ovari dengan CL (masing-masing 6.0±2.0 dan 4.5±1.5) melalui teknik pembedahan dan trend sebaliknya ditemui melalui teknik aspirasi. Melalui kajian ini, ovari tanpa CL adalah didapati lebih sesuai digunakan untuk mengumpulkan COCs untuk penghasilan embrio lembu secara* in vitro *walaupun trend sebaliknya ditemui dalam teknik aspirasi. Teknik pembedahan didapati lebih cekap berbanding dengan teknik aspirasi dalam penemuan jumlah COCs, dengan kadar penemuan masing-masing 61.6% dan 16.5%.Teknik pembedahan juga lebih cekap dalam kadar penemuan COCs kelas normal (gred A dan B) iaitu sebanyak 48.6% berbanding dengan teknik aspirasi (11.7%). Hasil kajian ini merupakan langkah asas untuk perancangan dan pelaksanaan bagi penyelidikan pragmatik di masa hadapan dalam pengeluaran embrio lembu secara* in vitro.

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LIST OF SYMBOLS, UNITS AND ABBREVIATIONS

%	Percent
°C	Degree Celsius
BSA	Bovine Serum Albumin
CL	Corpus luteum
CL-	Corpus luteum absent
CL+	Corpus luteum present
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
MOET	Multiple ovulation and embryo transfer
NaCl	Sodium chloride
RR	Recovery rate
rRNA	Ribosomal ribonucleic acid
SE	Standard error
UMS	Universiti Malaysia Sabah
vs	Versus
µm	Micrometre

LIST OF FORMULAE

CHAPTER 1

INTRODUCTION

1.1 Introduction

A cow usually produces only single ovum during the period of ovulation. If the ovum is fertilised *in vivo*, a cow only delivers a calf after an average of nine months gestation period. This situation causes slow in bovine's genetic improvement. Hence, over a decade, there is a lot of research done towards the implementation of embryo technologies to fasten the genetic improvement of livestock which involves multiple ovulation and embryo transfer (MOET), *in vitro* embryo production (IVEP), cloning, and transgenesis. From those mentioned, IVEP has becoming popular method of producing embryos from slaughter house-derived ovaries with low cost (Hoque *et al.*, 2011).

The IVEP system involves at least four steps, namely (a) the evaluation of ovaries, efficient collection and grading of 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). According to Sreenivas *et al.* (2014), the first lamb produced by IVM, IVF and IVC of ova was done in 1991. Nowadays, IVEP is becoming a useful tool for maximizing the number of offspring from valuable cows, producing calves from infertile, dead or slaughtered cows, and producing commercial beef cattle in programme for beef production without brood cows. In Malaysia, one of the research studies conducted was by Sianturi (2001) on *in vitro* production of embryos from abattoir-derived cattle oocytes.

To produce embryos by *in vitro* techniques, it is necessary to recover the oocytes and undergo maturation of oocytes, fertilise, and develop those developing zygotes to blastocyst stage so that they can be transferred to the recipient. In recent

years, the percentage of oocytes reaching the blastocyst stage by *in vitro* techniques still varies. In some cases, the low developmental of IVM oocytes is related to their quality at the beginning of maturation. Mondal (2008) has reported that higher average number of good quality oocytes was recovered from ovaries without corpus luteum compared to the ovaries with corpus luteum, which thus, can be effectively used for IVM and IVF. In addition, it has been shown that oocytes with at least four layers of cumulus cells have good result for IVM and IVF (Yang *et al.*, 1993).

There is a huge gap between the demand and supply of milk and meat in Malaysia including in Sabah due to low cattle population and low productivity. In Malaysia, there is still no information so far has been found on the evaluation of bovine slaughterhouse ovaries, follicles, and cumulus-oocyte-complexes (COCs) for IVEP. For successful IVEP of bovine embryos, the evaluation of ovaries, the efficient collection and grading of oocytes is essential. Therefore, this experiment needs to be conducted as an initial work for planning and execution of future pragmatic research on *in vitro* production of bovine embryos to increase cattle productivity. The main purpose of this research is to determine the optimum type of ovary (corpus luteum present or absent) and COCs recovery technique that is most suitable to produce higher number of good quality cumulus-oocyte-complexes (COCs) for IVEP.

1.2 Justification

Human depends on meat and milk of livestock as their part of food source. Nowadays, human population keeps increasing, but the livestock population is not congruent to it. In Malaysia including Sabah, meat and milk supply cannot fulfill the market demand due to low cattle population and productivity. Thus, more research is needed for better livestock productions, particularly on *in vitro* embryo production as it is now widely being used to fasten genetic improvement throughout the world. This research might help in improving animals' production specifically in bovine in terms of *in vitro* embryo production of animals for commercial and future research purpose. This research will open the window for future research on embryogenesis and the results obtained may serve as baseline information in order to produce high quality cumulus-oocyte-complexes (COCs) for *in vitro* embryo production of bovine to improve their population. The analysis of ovarian type (corpus luteum present or absent), follicles and COCs may provide useful information on the mechanisms underlying the oocyte maturation and

fertilisation, and suggest new possibilities to improve the low efficiency of *in vitro* embryo production.

1.3 Objectives

The objectives of this study were:

1. To perform the evaluation of bovine slaughterhouse ovaries, follicles and cumulus-oocyte-complexes (COCs).
2. To compare the effect of collection techniques on the recovery rate of COCs.

1.4 Hypothesis

$H_{o\ (i)}$: There is no difference on the ovarian types, follicular number, and quantity and quality of COCs.

$H_{a\ (i)}$: There is difference on the ovarian types, follicular number, and quantity and quality of COCs.

$H_{o\ (ii)}$: There is no difference on the recovery rate of COCs between the collection techniques.

$H_{a\ (ii)}$: There is difference on the recovery rate of COCs between the collection techniques.

CHAPTER 2

LITERATURE REVIEW

Several research studies have been conducted and reported in the literature on quantitative and qualitative evaluation of ovaries, follicles and cumulus-oocyte-complexes (COCs). Few researches so far had also been reported on the collection techniques of COCs. The related findings of research work carried out in different countries of the world are reviewed in this chapter.

2.1 Bovine Ovaries

The ovaries are the primary organs in a bovine's reproductive tract which produce eggs. Ovaries also produce estrogen and progesterone hormones throughout the different stages of the estrus cycle. Follicles and corpus luteum can be found on the surface of the ovary. Follicles are fluid filled, blister like structures which contain developing oocytes. Normally, there are follicles with variation in size from barely visible to 18 to 20 mm in diameter found on each ovary. Figure 2.1 shows the photograph of bovine ovaries with visible follicles. The largest follicle present is the growing follicle, and is used for ovulation when the bovine comes into heat. Over time, more than 95% of the other follicles on the ovary regress and die without ovulating and are replaced by new growing follicles (DeJarnette and Nebel, n.d.).

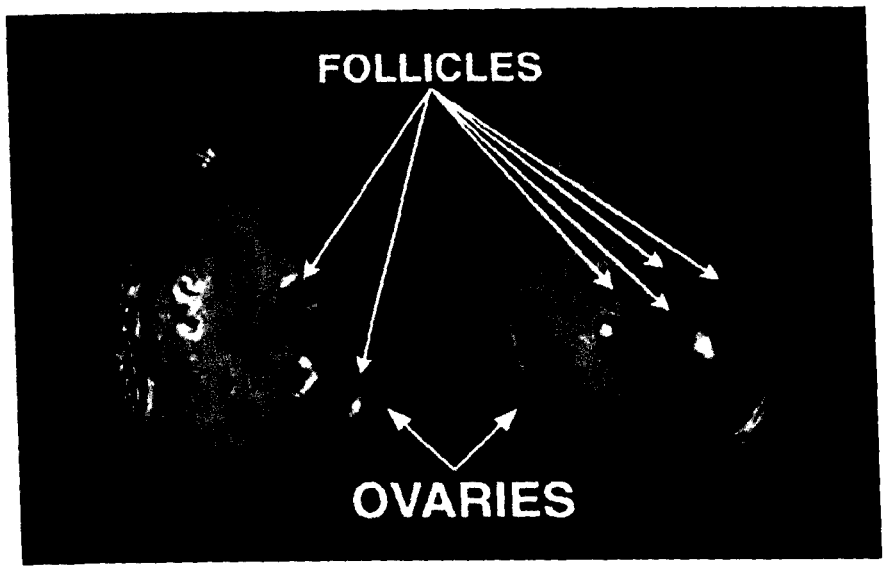

Figure 2.1 Photograph of bovine ovaries with visible follicles
Source: DeJarnette and Nebel, n.d.

2.2 Bovine Oocytes

Oocyte is an immature female sex unit which starts as an oogonium by the process of oocytogenesis, and matures to become fully mature egg cell or ovum. Bovine oocyte is the oocyte produced by bovine female sex organ. Oocytes in cattle are formed during embryogenesis and develop within individual follicles in the cortex of the ovary (Britt, 2008).

There are five levels of oocyte competence as described by Sirard *et al.* (2006). The levels are the ability of the oocyte to: (1) resume meiosis; (2) cleave following fertilisation; (3) develop to the blastocyst stage; (4) induce a pregnancy and bring it to term; and (5) develop to term in good health.

In bovine, the oocyte growth phase includes modulations of organelles and inclusions, and a period of oocyte transcription, which are necessary for the oocyte to achieve meiotic and developmental competence. Nucleolar function (rRNA-synthesis) is activated in the secondary follicle during oocyte transcription and is maintained up to an oocyte diameter of about 110 µm in the 3 mm tertiary follicle. The oocytes undergo meiotic maturation and sustain embryonic development at a diameter of 100 to 110 µm. Capacitation occurs when the oocyte in the dominant follicle undergoes further ultrastructural modifications and attains full developmental competence. The oocyte undergo final maturation up to metaphase II after LH stimulation of the follicle which

undergo final maturation up to metaphase II after LH stimulation of the follicle which is when the culmination of the previous processes and oocyte is equipped with a haploid chromosomal compartment and the cell biological apparatus specialised for fertilisation and initial embryonic development (Hyttel *et al.,* 1997).

According to Sirard and Blondin (1996), the immature oocytes possess differing degrees of competence can be due to factors affecting the oocyte during late folliculogenesis. Different follicular conditions can cause different level of oocyte competence. The oocytes origin are according to five different aspects which are cumulus morphology, follicular size, follicular health, ovarian stimulation and the oocyte handling procedure before the beginning of incubation. Based on the results, it was suggested that the oocyte enters a permissive state when obtained from large and differentiated follicles as in dominance, early atresia or near ovulation.

2.3 Ovarian Type

Corpus luteum (CL) can be found on the ovarian surface. Just after ovulation, remaining cells of the follicle initially formed into corpus haemorrhagicum and then fills the cavity of the ruptured follicle. After that, under the influence of luteinising hormone (LH), the granulosa cells lining the empty follicular cavity begin to multiply and form a corpus luteum (Husvéth, 2011). The CL is a reproductive gland that produces progesterone, needed for the establishment and maintenance of pregnancy. 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 (DeJarnette and Nebel, n.d.). The cross section of bovine ovary with corpus luteum is shown in Figure 2.2.

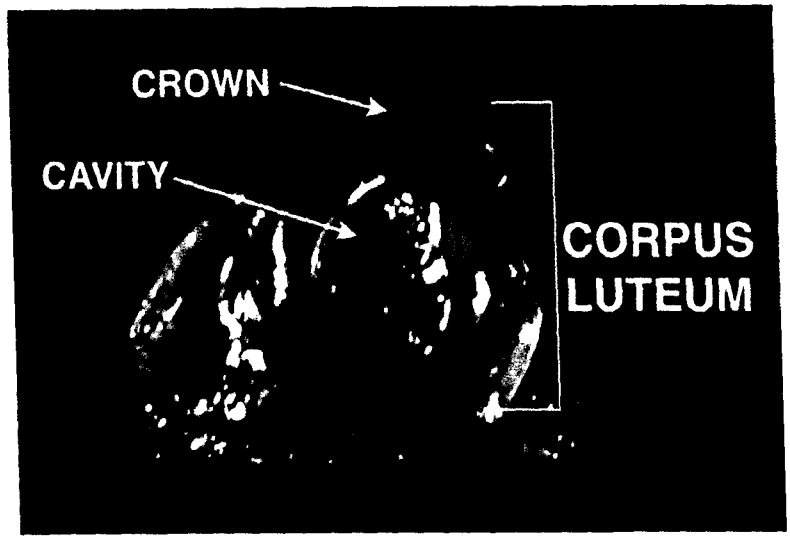

Figure 2.2 Cross section of bovine ovary with a corpus luteum
Source: DeJarnette and Nebel, n.d.

If the cow is not pregnant, hormone prostaglandin is released on day 16 to 18 of the heat cycle, and cause regression of CL, resulting in declining of progesterone production. However, if there is pregnancy, the release of progesterone is blocked by the embryo, thus CL continues secreting progesterone (Burns, n.d.). That is why, when ovaries are collected from slaughterhouse, there are possibilities of CL present or CL absent in the ovaries. Thus, there are two types of ovaries that can be classified which are one with CL and other, without CL.

Usually, in the slaughterhouse, higher number of ovaries obtained having no CL compared to ovaries with CL as higher less reproductively performing cows were slaughtered due to economic reason. Asad (2015) had 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.

2.3.1 Effect of Type of Ovary on the Number of Follicles

According to Burns (n.d.), 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 statement strongly supports the previous finding of Asad (2015) 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. Khandoker *et al.* (2011) mentioned that, in case of buffalo ovaries, 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 with no 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 the Quantity and Quality of Cumulus-Oocyte-Complexes (COCs)

In some cases, the low developmental of IVM oocytes is related to their quality at the beginning of maturation. Ovaries with CL and ovaries without CL give different effects on the quality of cumulus-oocyte-complexes (COCs). According to Asad (2015), it was found that the number of total, normal and abnormal COCs per goat ovary was significantly higher ($p<0.01$) in ovaries without corpus luteum (2.12, 1.42 and 0.69) compared to ovaries with corpus luteum (1.46, 0.81 and 0.73).

An experiment done by Khandoker *et al.* (2011) on evaluation of buffalo ovaries, follicles and COCs with the view of IVP found that the number of observed follicles, aspirated follicles, number of COCs as well as number of normal COCs were significantly ($p<0.05$) higher in ovaries without CL than ovaries with CL.

Mondal *et al.* (2008) had done research on goat ovaries and classified them as corpus luteum absent (type I) and present (type II). 516 follicles were aspirated and among them, 432 were obtained from type I and 84 from type II ovaries. The research found that significantly ($p<0.05$) higher number of follicles were aspirated per ovary in type I than in type II. The average number of normal COCs per ovary was higher in ovaries without CL than ovaries with CL and the average number of abnormal COCs was higher in ovaries with CL than ovaries without CL.

There is also another research found that the CL-absent group ovaries have higher number and superior quality of COCs than CL-present group ovaries. That research also concluded that the CL-absent group ovaries can be used to collect the quality COCs for IVP of goat embryos (Islam *et al.*, 2007).

Those results mentioned before strongly support the result of Kumar *et al.* (2004), who reported that the average number of good quality oocytes recovered from ovaries without corpus luteum was more than the ovaries with corpus luteum, which can be effectively used for IVF. Nandi *et al.* (2000) suggested that the oocyte recovery rate decreased when ovaries had a corpus luteum. Thus, types of ovary at the time of oocyte collection have affected the quality of COCs in animals for use in IVEP program.

2.4 Cumulus-Oocyte-Complexes (COCs)

Cumulus cells are specialized granulosa cells surrounding and nourishing the oocyte. Cumulus cells directly surround oocyte to form cumulus oocyte complex (COC). During the preovulatory period, cumulus cells change from a compact cell mass into a dispersed structure of cells for the synthesis and deposition of a mucoid intercellular matrix, called cumulus expansion. Cumulus expansion influences a variety of fundamental developmental changes during oocyte maturation.

Cumulus cell is important and the absence of cumulus cells or insufficient numbers of cumulus cells impairs embryo production. Cumulus cells are required for the successful maturation of oocytes and needed for fertilization. These cells synthesise an abundant muco-elastic extracellular matrix that helps oocyte extrusion from the follicle, a 20-40 fold increase in the volume of the cumulus mass, and as a selective barrier for sperm (Salustri, 2000).

That is why, only grades A and B COCs are considered as good quality COCs and can be used for further processes of *in vitro* embryo production as grade A COCs is where oocytes homogenously surrounded by cumulus cells and grade B is oocytes partially surrounded by cumulus cells (Khandoker *et al.*, 2001). If there is no cumulus cells surround the oocyte or degeneration occurs, that COCs are not suitable for having successful maturation, fertilisation and embryo production.

2.4.1 Collection Techniques of COCs

Various techniques have been used to collect oocytes from ovaries of bovine. These techniques would improve recovery rates and oocyte quality. Commonly, there are several techniques used which are puncture, slicing, blunt dissection, and aspiration.

Anna (2013) discovered that oocytes obtained from slaughterhouse ovaries using the slicing technique showed a significantly higher ($p<0.05$) number of oocytes per goat compared to the LOPU method from live donors. However, LOPU method shows higher percentage of grade A oocytes compared to slicing technique which had a higher percentage of grade D oocytes.

For the results of study by Singh *et al.* (2013), the overall yield of oocyte was higher in slicing (50.57) followed by dissection (37.52) and the least is by aspiration (20.70) technique. However, the maximum yield of grade A and B oocytes was observed by aspiration technique and dissection technique, while maximum grade C and D oocytes collected from slicing technique.

An experiment was done by Hoque *et al.* (2012) and 1205 COCs were collected by three techniques which are by puncture, slicing, and aspiration from slaughterhouse goat ovaries. Hoque *et al.* (2012) found that there was no significant effect ($p>0.05$) of the collection techniques on *in vitro* maturation and *in vitro* fertilization of oocytes.

According to Hoque *et al.* (2011), puncture and slicing techniques yielded significantly higher number of COCs per ovary as well as number of abnormal COCs per ovary compared to aspiration technique. However, by aspiration technique, the normal COCs recovery is higher followed by slicing and then punctures.

The result of the research of Hoque *et al.* (2011) was compared 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) recovered that slicing and puncture of the ovaries yielded a higher number of oocytes per ovary which are 6.3 and 5.8, respectively when compared to aspiration I (2.9) and aspiration II (3.1).

REFERENCES

Abdel-Khalek, E. A., El-Harairy, M. A., Shamiah, S. M. and Khalil, W. A. 2010. Effect of Ovary Preservation Period on Recovery Rate and Categories of Dromedary Camel Oocytes. *Saudi Journal of Biological Sciences* **17**:231-235

Anna, A. A., Hisham, M. N., Wan Khadijah, W. E. and Abdullah, R. B. 2013. Comparative Study on Goat Oocyte Recovery Methods and Factors Affecting the Quantity and Quality of Oocytes. *Asian Journal of Animal and Veterinary Advances* **8(3)**: 437-448

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

Britt, J. H. 2008. Oocyte Development in Cattle: Physiological and Genetic Aspects. *Revista Brasileira de Zootecnia* **37**: 110-115

Burns, P. D. n.d. The Dairy Cow Heat Cycle. Colorado State University. https://www.cvmbs.colostate.edu/ilm/proinfo/cdn/2002/Heat%20CycleSep02.df. Access on 1 April 2015

DeJarnette, M. and Nebel, R. n.d. Reproductive Anatomy and Physiology of Cattle. Select Reproductive Solutions. http://www.selectsires.com/resources/fertilitydocs/reproductive_anatomy.pdf. Access on 1 April 2015

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

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

Hoque, S. A. M., Kabiraj, S. K., Khandoker, M. A. M. Y., Mondal, A. and Tareq, K. M. A. 2011. Effect of Collection Techniques on Cumulus Oocyte Complexes (COCs) Recovery, In Vitro Maturation and Fertilization of Goat Oocytes. *African Journal of Biotechnology* **10(45)**: 9177-9181

Hoque, S. A. M., Khandoker, M. A. M. Y., Kabiraj, S. K., Asad, L.Y., Fakruzzaman, M. and Tareq, K. M. A. 2012. Effect of Goat Follicular Fluid on In Vitro Production of Embryos in Black Bengal Goats. *Journal of Applied Animal Science* **2**: 287-294

Husvéth, F. 2011. Physiological and Reproductional Aspects of Animal Production: Corpus Luteum. Digitális Tankönyvtár. http://www.tankonyvtar.hu/en/tartalom/tamop425/0010_1A_Book_angol_05_termeleselettan/ch12s05.html. Access on 24 November 2015

Hyttel, P., Fair, T., Callesen, H. and Greve, T. 1997. Oocyte Growth, Capacitation and Final Maturation in Cattle. *Theriogenology* **47(1)**: 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

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

Khandoker, M. A. M. Y., Jahan, N., Asad, L., Hoque, S. A. M. and 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

Kumar, N., Paramasivan, S., Sood, P. and Singh, M. 2004. Micrometry of Different Category Oocytes Recovered from Goat Ovaries. *Indian Journal of Animal Science* **74**: 259-260

Microsoft. 2007. Microsoft Excel [computer software]. Redmond, Washington: Microsoft.

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. *Bang. J. Anim. Sci.* **37(1)**: 1-9

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

Nowshari, M. A. 2004. The Effect of Harvesting Technique on Efficiency of Oocyte Collection and Different Maturation Media on the Nuclear Maturation of Oocytes in Camels (*Camelus dromedaries*). *Theriogenology* **63**: 2471-2481

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

Salustri, A. 2000. Paracrine Actions of Oocytes in the Mouse Pre-Ovulatory Follicle. *International Journal of Developmental Biology* **44(6)**: 591-597

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

Sianturi, R. G. 2001. *In Vitro Production of Embryos from Abattoir-derived Cattle Oocytes.* Degree of Master of Science Thesis. Universiti Putra Malaysia

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 Blondin, P. 1996. Oocyte Maturation and IVF in Cattle. *Animal Reproduction Science* **42(1-4)**: 417-426

Sirard, M. A., Richard, F., Blondin, P. and Robert, C. 2006. Contribution of the Oocyte to Embryo Quality. *Theriogenology* **65**: 126-136

Sreenivas, D., Kaladhar D. S. V. G. K., Yarla, N. S., Thomas, V. M., PalniSamy, A., Vadlapudi, V. R. and Preethi, R. 2014. *In Vitro* Production of Sheep Embryos in CR1aa Medium Supplemented with *L*-Ascorbic Acid. *J Tissue Sci Eng* **5(1)**: 131

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

Webb, R., Campbell, B. K., Garveric, H. A. and Gong, J. G. 1999. Molecular Mechanisms Regulating Follicular Recruitment and Selection. *J. Reprod. Fert.* **45**: 123-126

Yang, 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