EVALUATION OF SLAUGHTERHOUSE PIG OVARIES AND RECOVERY OF CUMULUS-OOCYTE-COMPLEXES (COCs)

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ABSTRACT

Pig production is important in Malaysia as 40 % of Malaysian eat pork. As population increases, the demand of pork also increases. However, the pig production in Malaysia has shown a slightly decreasing trend. To boost up the production, *in vitro* embryo production (IVEP) is used. However, there is lack of information on IVEP for porcine in Malaysia. Therefore, the objectives of this study are to determine the relationship between ovarian types, quality of slaughterhouse pig ovaries and the recovery rate and quality of recovered COCs. This research was conducted at Plant Physiology Laboratory, Faculty of Sustainable Agriculture, Universiti Malaysia Sabah, Sandakan Campus from May 2017 until July 2017 to evaluate the slaughterhouse pig ovaries and recovery of the cumulus-oocyte-complexes (COCs) from the ovaries. The pig ovaries were collected from local pig slaughterhouse and marked left and right. The ovaries were grouped into ovary with corpus luteum (CL+) and without corpus luteum (CL-). Evaluations based on weight of ovaries and number of visible follicles were done in the laboratory. The COCs were aspirated with 25G needle attached to a 10 mL syringe. Aspirated materials were observed under light microscope with magnification of X100 and X400 graded into 4 grades: (a) Grade A: oocyte homogenously surrounded by cumulus cells; (b) Grade B: oocyte partially surrounded by cumulus cells; (c) Grade C: Oocyte not surrounded by cumulus cells; (d) Grade D: degeneration appeared both in oocyte and cumulus cells. Grade A and Grade B were considered as normal COCs, while Grade C and D were graded as abnormal COCs. The number of follicles and COCs for each grade were analysed by using mean ± SE and also inserted into t-test to identify variations between groups. The recorded percentages of ovaries that had been retrieved from slaughterhouse was 3.57% (six) CL+ ovaries and 96.43% (162) CL- ovaries. The number of surface follicles was found comparatively (P>0.05) higher in left ovary (82.75±3.56) than right ovary (79.25±3.15). The number of normal COCs (Grade A and Grade B) found comparatively (P>0.05) higher in left ovary (6.74±0.57 and 5.35±0.42 respectively) than right ovary (6.23±0.52 and 5.20±0.35 respectively). There was no significant different between weight of left ovary and right ovary. When ovaries compared between CL+ and CL-, CL+ ovary (3.42g±0.64g) was significantly (P<0.05) heavier than CL- ovary (2.67g±0.07g). The number of abnormal COCs in CL+ ovary (6.50±1.12) was significantly higher (P<0.05) than CL- ovary (6.40±0.41). Number of surface follicles and number of normal COCs also significant higher (P<0.05) in CL- ovaries than CL+ ovaries. From the experiment, it is found that COCs retrieved from left and right ovaries had no significant differences and CL- ovaries contributed better quality of COCs. Therefore, the finding suggested that the any position of ovary without corpus luteum might be suitable to obtain good quality COCs for *in vitro* embryo production (IVEP).

Penilaian Ovari Babi Dari Rumah Sembilih Dan Pengumpulan Kompleks Cumulus Oosit (COC)

ABSTRAK

Pengeluaran babi adalah penting di Malaysia kerana 40% daripada jumlah penduduk di Malaysia makan daging babi. Peningkatan jumlah penduduk yang makan daging babi menyebabkan permintaan daging babi semakin meningkat. Walau bagaimanapun, pengeluaran babi di Malaysia semakin menurun. Untuk meningkatkan pengeluaran, pengeluaran embrio babi secara *in vitro* (IVEP) mesti digunakan. Walau bagaimanapun, terdapat kekurangan maklumat mengenai IVEP untuk babi di Malaysia untuk dijadikan rujukan. Oleh itu, objektif kajian ini adalah untuk mengetahui hubungan antara jenis ovari dengan kualiti ovari babi dari rumah sembelih, dan kadar pengumpulan kompleks cumulus oosit (COC). Kajian ini telah dijalankan di Makmal Fiosiologi Tumbuhan, Fakulti Pertanian Lestari, Universiti Malaysia Sabah, Kampus Sandakan dari Mei 2017 hingga Julai 2017 untuk menilai ovari babi dari rumah sembelih dan pemulihan COC yang dikumpul dari ovari tersebut. Ovari telah dikumpul dari rumah sembelih babi tempatan dan ditanda sebagai ovari kiri dan ovari kanan. Ovari tersebut juga telah dibahagikan kepada ovari mempunyai corpus luteum (CL+) dan ovari yang tidak mempunyai corpus luteum (CL-). Penilaian berdasarkan berat ovari dan bilangan folikel kelihatan pada pemukaan ovari telah dijalankan di makmal. COC telah diaspirasi dengan menggunakan 25G jarum dan 10 mL jarum suntik. Bahan diaspirasi telah dinilai di bawah mikroskop cahaya dengan pembesaran sudut X100 dan X400 dan telah digredkan ke dalam empat gred: (a) Gred A: oosit dikelilingi oleh sel-sel cumulus; (B) Gred B: sebahagian oosit dikelilingi oleh sel-sel cumulus; (C) Gred C: oosit tidak dikelilingi oleh sel-sel cumulus; (D) Gred D: degenerasi didapati dalam sel-sel oosit dan kumulus. Gred A dan Gred B dianggap sebagai normal COC, manakala Gred C dan Gred D dianggap sebagai COC yang tidak normal. Bilangan folikel dan bilangan COC bagi setiap gred telah dianalisis dengan menggunakan min ± SE dan juga t-test untuk mengkaji variasi antara kumpulan. Peratusan CL+ ovari yang direkodkan ialah 3.57% (enam) dan peratusan CL- ovari ialah 96.43% (162). Bilangan folikel permukaan didapati lebih tinggi ($P<0.05$) di ovari kiri (82.75 ± 3.56) daripada ovari kanan (79.25±3.15). Bilangan COC normal ovari kiri (6.74 ± 0.57 dan 5.35 ± 0.42) lebih tinggi ($P>0.05$) daripada ovari kanan (6.23 ± 0.52 dan 5.20 ± 0.35). Tiada perbezaan yang signifikan antara berat ovari kiri dan ovari kanan. Apabila berat ovari CL + dan CL- dibandingkan, ovari CL + (3.42g ± 0.64g) adalah lebih berat daripada ovari CL- (2.67g ± 0.07g). Bilangan COC yang tidak normal dalam ovari CL+ (6.50 ± 1.12) jauh lebih tinggi daripada ovari CL- (6.40 ± 0.41). Bilangan folikel permukaan dan bilangan COC juga menunjukkan perbezaan yang ketara di antara ovari CL + dan CL-. Berdasarkan kajian ini, tiada perbezaan antara COC yang didapat dari ovari kiri dan ovari kanan, dan ovari CL- menyumbangkan kualiti COC yang lebih banyak. Oleh itu, kajian ini mencadangkan bahawa mana-mana kedudukan ovari tanpa CL mungkin sesuai untuk memiliki COC penemuan yang berkualiti tinggi untuk pengeluaran embrio *in vitro* (IVEP).

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%	Percent
±	Plus or Minus
ART	Assisted Reproductive Technologies
CL	Corpus Luteum
CL-	Corpus Luteum Absent
CL+	Corpus Luteum Present
COCs	Comulus-Oocyte-Complexes
DVS	Department of Veterinary Services Malaysia
FSH	Follicle Stimulating Hormone
G	Gauge
g	Gram
IVC	*In Vitro* Culture
IVEP	*In Vitro* Embryo Production
IVF	*In Vitro* Fertilization
IVM	*In Vitro* Maturation
LH	Luteinizing Hormone
mL	Millilitre
mm	Millimetre
°C	Degree Celsius
rpm	Revolutions per Minute
SE	Standard Error
USDA	United States Department of Agriculture

CHAPTER 1

INTRODUCTION

1.1 Introduction

Pig (*Sus domesticus* or *Sus scrofa domesticus*) is a type of livestock that also known as swine. It is mainly reared for its meat, which is called pork. Forty (40) percent of the Malaysia population, which is 29.6 million, are consuming pork and the self-sufficient of pork in Malaysia is only about 95.66% in 2014 (DVS, 2015). The production of pork was 232,000 metric tons in 2014, and total import of pork was 9,000 metric tons (USDA, 2014). This record is shown in Table 1.1 (USDA, 2014). This indicate that there are demands on pork, as the pork consumers consume 18.8 kilogrammes of pork per capita annually. Therefore, pig production is a very important industry and needed to be developed in Malaysia.

Table 1.1 Pork supply and demand in Malaysia for 2012-2014

	2012	2013	2014
Beginning Stocks (MT)	1,000	1,000	1,000
Production (MT)	232,000	231,000	232,000
Total Imports (MT)	8,450	8,700	9,000
Total Supply (MT)	241,450	240,700	242,000
Total Exports (MT)	0	0	0
Total Domestic Consumption (MT)	240,450	239,7000	241,000
Ending Stocks (MT)	1,000	1,000	1,000
Total Distribution (MT)	241,450	240,700	242,000

Source: USDA, 2014

Another reason for improving pig production is because pig is used worldwide for medical field. Due to the high similarity of pig and human anatomy and physiology, pig is used for medical researches as a translation model (Kobayashi *et al.*, 2012). A translation model is an animal that is used to test out different types of medication before the medication is introduced to human beings. Other than that, pig also plays important role in xenotransplantation. Xenotransplantation is a process of transplanting tissues or organs across species. The transplanted tissues, however, may be rejected by human body. Thus, genes of pig are altered or replace with human genes to allow the growth of human tissues inside the pig bodies.

In order to do researches on altering the genes in pigs, improve production of pigs is required by using the assisted reproduction techniques. These assisted reproduction techniques include in vitro maturation, fertilisation and culture (IVMFC), which is also known as *in vitro* production (IVP). *In vitro* production has been developed well during the past two decades (Katska-Ksiazkiewicz *et al.*, 2007), for the purpose of transgenic animals.

In vitro production consists of four steps. The first steps of IVP is evaluation of ovaries, and collection and grading of oocytes. After the collection and grading of oocyte, the most suitable oocyte will be used for *in vitro* maturation (IVM), then *in vitro* fertilisation (IVF), and lastly *in vitro* culture (IVC) (Freitas and Melo, 2010). These procedures require specific conditions in order to be successful.

In vitro production fully utilize the ovaries of subfertile or dead animals (Deuleuze *et al.*, 2009). Atresia, a process of degeneration of female germ cells (oocytes), often happens before fertilisation can be occurred (Krakauer and Mira, 1999). This causes the potential reproduction of a female to be reduces. Therefore, IVP that uses recovery methods to harvest all the oocytes from the ovary, can help to reduce waste of the female germ cells. The ovaries are usually obtained from abattoir, because it is cheaper and less techniques and procedures are required. Moreover, pig ovaries are inedible and has no other uses. This benefits both the industry of IVP and production of pigs.

Although IVP fully utilize the potential of ovaries, the rate of successful maturation may be as low as 15.6% (Ellenbogen *et al.*, 2011). There are many factors that may be the causes the failure of IVP (Gunasheela *et al.*, 2011). This include the use of different culture media, duration of *in vitro* maturation, different recovery techniques of oocyte, quality of collected oocytes, and the quality of ovaries. In Malaysia, there is no research done on types of pig ovaries that will affect the quality of ovaries and oocytes, and success rate of IVP.

Quality of ovaries can be affected by weight and size of ovaries, number of visible follicles, grades of oocytes, presence of corpus luteum, ovarian location and non-ovarian factors, such as breed of animals, state of pregnancy, and age of the animals (Kouamo *et al.*, 2014). Mondal *et al.* (2008) mentioned that the ovaries that are absence of corpus luteum will have good quality of oocytes. Therefore, types of ovaries can be correlated to the quality of cumulus-oocyte-complexes (COCs) collected.

Grading of COCs can be graded into four grades: Grade-A, Grade-B, Grade-C and Grade-D (Khandoker *et al.*, 2016). Grade-A COCs have the oocyte completely surrounded by cumulus cells, Grade-B COCs have oocyte partially surrounded by cumulus cells, Grade-C COCs have oocytes that are not surrounded by cumulus cells, and Grade-D COCs have degenerated oocytes and cumulus cells. Grade-A and Grade-B COCs are considered normal, while Grade-C and Grade-D are abnormal COCs.

Cumulus-oocyte-complexes (COCs) can be collected through recovery techniques. There are several recovery techniques, which include blunt dissection techniques, slicing, dissecting and aspiration techniques (Hammad *et al.*, 2014). In this study, aspiration method was used. This is because aspiration technique is more suitable for porcine *in vitro* embryo production (Marques *et al.*, 2015). The oocytes were aspirated with sterile syringe with a 25 gauge needle attach to it.

Good quality COCs that are recovered by using aspirated will increase success rate of *in vitro* maturation, *in vitro* fertilization, and *in vitro* culture. Therefore, population of pigs can be boosted by exploiting the potential of female reproduction in pigs.

1.2 Justification

There will always be a demand for pork in Malaysia, as there are non-Muslim residents staying here. Moreover, as the population increases, the demands will also be higher. To fulfil the demands, *in vitro* embryo production (IVEP) is needed, as this is one of the cheapest and fastest way to increase population of the pigs. Unfortunately, there is no specific research that had been done on evaluation of slaughterhouse pig ovaries and recovery of cumulus-oocyte-complexes (COCs) in Malaysia. There is lack of information about which ovary should be chosen for IVEP. This research, which evaluate slaughterhouse pig ovaries and the recovery rate of COCs, may help in the future researches and improvement of pig production. This research can serve as the baseline and reference for obtaining good quality of oocytes, and subsequently improve IVEP for future commercial and research uses.

1.3 Objectives

The objectives of this study are:

1. To determine the relationship of ovarian type and quality of slaughterhouse pig ovaries.
2. To determine the relationship between quality of slaughterhouse pig ovaries and recovery rate and quality of recovered COCs.

1.4 Hypotheses

H_{01} : There is no difference on the position of ovaries, follicular number, and recovery of COCs.

H_{A1} : There is difference on the position of ovaries, follicular number, and recovery of COCs.

H_{02} : There is no difference on the presence of corpus luteum, follicular number, and recovery of COCs.

H_{A2} : There is difference on the presence of corpus luteum, follicular number, and recovery of COCs.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

There were several studies that had been reported on the ovarian type, quality of cumulus-oocyte-complexes (COCs) and recovery or collection techniques of COCs. The related findings are reviewed in this chapter. This chapter consists of reviews of status of pig production in Malaysia, female reproduction of pigs, ovarian types and the effects, short review about *in vitro* embryo production (IVEP), and recovery of COCs. Status of pig production in Malaysia is reviewed to explain the needs and importance of this study. The importance of this study will be revealed through reviewing IVEP in this chapter, as because there are lacking of knowledge about quality of ovaries and recovery of COCs in Malaysia, which will consequently affect the success rate of IVEP. Most of the literature being reviewed is related to other mammals, such as rats, goats and cattle, because most of the researches about IVEP is done on goats and cattle.

2.2 Pig Production

According to Gardir and Wai (2014), Malaysia has 544 pig farms with standing pig population (SPP) of 1.8 million. The Department of Veterinary Services (DVS) of Malaysia had encouraged the farmer to keep their pigs in closed house system through Modern Pig Farming (MPF) system and also implement breeding plans to introduce farmer better management system, environmental protection to sustain the pig industry in Malaysia, and also to improve production (Gardir and Wai, 2014). However, Table 2.1 shows that the population of pigs in 2014 is 1,844,103, and is estimated to be 1,828,860 in 2015, which is a decline in production of pig (DVS, 2016). Table 2.2 shows the number of animals from 2011 to 2015 (DVS, 2016). From Table 2.2, we can clearly see that the population of pig had maintain at 1.8 million for the past 5 years. As the population of residents in Malaysia is increasing, the demand for pork is also increasing. In the year 2013, the self-sufficiency level of pork is only 96.31%, and now, the demand is becoming greater (Gardir and Wai, 2014). Therefore, alternative ways to improve production of pig is through the implement of assisted reproductive technologies (ART) in pig production system. One of the ART is *in vitro* embryo production (IVEP).

Table 2.1 Livestock population in Malaysia in 2014

Region	Buffalo	Cattle	Goat	Sheep	Swine
Peninsular Malaysia	61,687	662,818	363,768	138,127	1,425,371
Sabah	52,450	68,105	50,650	2,050	82,552
Sarawak	7,122	15,860	14,980	2,258	336,180
Total	121,259	746,783	429,398	142,435	1,844,103

Source: Department of Veterinary, 2016

Table 2.2 Pig population in Malaysia from 2011 to 2015

Location	2011	2012	2013	2014	2015^E
Peninsular Malaysia	1,395,815	1,437,354	1,425,310	1,425,371	1,406,426
Sabah	87,625	87,700	82,472	82,552	77,630
Sarawak	333,117	326,788	335,171	336,180	344,804
Total	1,816,557	1,851,842	1,842,953	1,844,103	1,828,860

Source: Department of Veterinary, 2016

2.3 Female Reproduction of Pigs

Reproduction of female pigs is important in producing the female gametes and gestating their offspring. Therefore, it is necessary to acquire knowledge about female reproduction of pigs, in order to improve production of pigs.

Pig is known for its high reproductive performance, as it has high prolificacy with high ovulation rate (Peltoniemi *et al.*, 2007). Therefore, the uterine horns of the pig is long and look like loop of intestines to allow it to carry 8 to 10 piglets in a litter (Miller, 2004). The most important structure of female reproduction system is ovary (Krishna *et al.*, 2015).

2.3.1 Pig Ovaries

Ovaries, the female gonads, are the primary structure of female reproduction. It serves to produce eggs (which is also known as oocytes), hormones, steroids that develop secondary sexual characteristics, and support pregnancy. Basically, ovaries of female pigs are berry-shaped (Dufour *et al.,* 1988; Schwarz *et al.,* 2013). Figure 2.1 shows the grape type ovaries with uterus of pig (Singleton and Diekman, 2017).

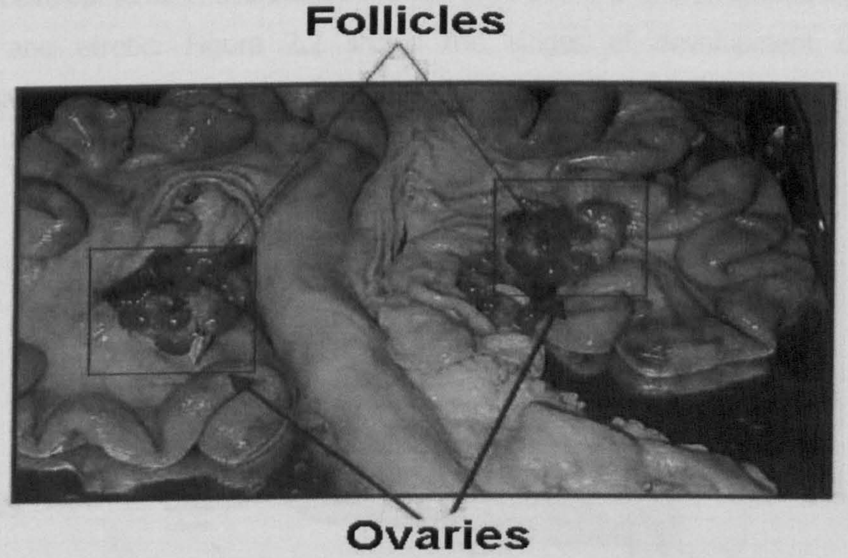

Figure 2.1 Photographic image of pig ovaries with visible follicles
Source: Singleton and Diekman (2017), Purdue University

Ovaries are located at both sides of the uterus (Reece, 2009). Suspensory ligament of ovary is attached to the uterine tube at the end of uterus. (Rahilly *et al.*, 2008). Ovulation occur to the Graafian follicle to rupture, and release ova into a funnel-like structure that collect and direct ova into oviduct (Hollandbeck and Foley, 1964).

According to Krishna *et al.* (2015), the ovaries consists of germ cells and supporting cells, hormone producing cells, blood vessels and nerves.

Two of the main functional structures of ovary play vital roles in reproduction of female. These main functional structures are ovarian follicles and corpus luteum.

2.3.2 Ovarian Follicles

Each germ cell in the ovary is surrounded by the supporting cells, which are also known as thecal cells or granulosa cells (Krishna *et al.,* 2015). This combination form a specific spherical structure, which is known as follicle. Ovarian follicles exist in different sizes. The diameter of follicles of pig ovaries that are weighed around 2.3 g can range from 1.9 mm to 7.8 mm (Chiou *et al.,* 2004), but can go up to 9 mm and above, depending on maturation stage of the animals. Follicles can be categorized into different stages of follicular development. These stages include primordial, primary, secondary, tertiary, Graafian and atretic. Figure 2.2 shows the stages of development of follicles (Encyclopædia Britannica, 2015).

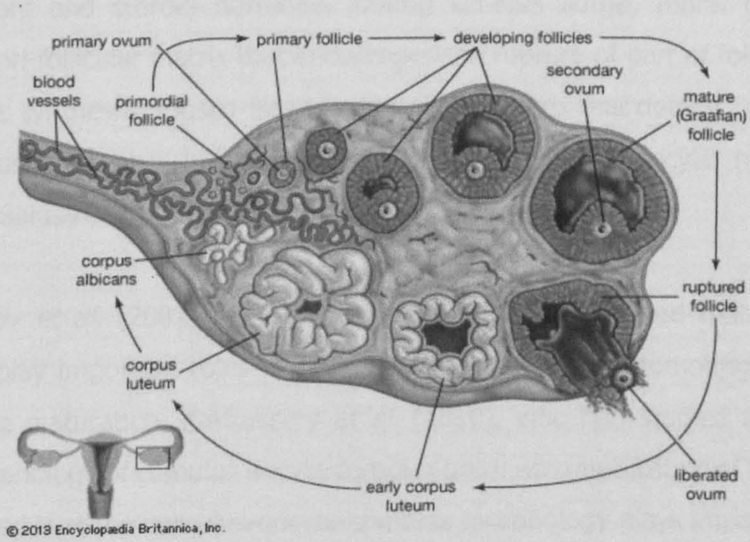

Figure 2.2 Development of follicles and ovulation
Source: Encyclopædia Britannica, Inc., 2015

Different stages of follicles will have different characteristics. Primordial follicle has the oocyte surrounded by single layer of spindle shaped granulosa cells and basement membrane. Primary follicle has the oocyte surrounded by single layer of cuboidal granulosa cells with basement membrane. Secondary follicle has the number of granulosa cells increase to two to eight layers surrounding the oocyte. Theca layers are formed by stroma cells outside the basement membrane. Tertiary follicle contains antra which is surrounded by the granulosa cells, and the theca layers divided into theca interna and theca externa. Finally, Graafian follicle is extend of tertiary follicle. The antra have joined become one large fluid filled cavity (antrum). The granulosa cells inside the antrum surround primary oocyte forming cumulus oophorus. Oocyte is at its maximum size within Graafian follicle (Krishna *et al.*, 2015).

There are a lot of follicles present in pig ovaries. However, most of the oocytes are lost at various stages of growth in ovary, and only small number of oocytes are able to be ovulated (Hirao *et al.,* 1994; Guthrie, 2005). This is why *in vitro* embryo production is important to reduce wastage of potential follicles for reproduction.

2.3.3 Cumulus-Oocyte-Complexes (COCs)

Cumulus cells are granulosa cells that surround the oocyte. Particularly, mural granulosa cells are the granulosa cells that directly in contact with follicle wall. Due to follicle stimulation hormone (FSH), the mural granulosa cells will synthesize luteinizing hormone (LH) receptors and steroid hormone. During LH-FSH surge, mural granulosa cells synthesize peri-follicular matrix that encourages the rupture of part of follicular cell wall. Cumulus cells synthesize musco-elastic extracellular matrix that detach COC from follicle wall. Cumulus cells also provide suitable environment for oocyte maturation and fertilisation (Salustri, 2000).

Byskov *et al.* (2002) and Hurk and Zhao (2005) reported that cumulus cells involve and play important roles in production of maturation promoting factors (MPF) during oocyte maturation. Kakkassery *et al.* (2010), who had studied about effect of different morphology of cumulus oocyte complex on *in vitro* maturation of bovine oocytes, also mentioned that the cumulus-oocyte-complex morphology plays important role in *in vitro* maturation of bovine oocytes. Oocytes with three or more layers of cumulus cells have better maturation than oocytes lesser than two layers of cumulus cells, or denuded oocytes.

In *in vitro* embryo production (IVEP), COCs are used instead of pure oocyte. This is because the cumulus cells play important roles in *in vitro* maturation (IVM). Only with the presence of cumulus cells, the foetus will be able to be produced normally after *in vitro* fertilisation (IVF) (Vanderhyden and Armstrong, 1989).

2.3.4 Corpus Luteum

Corpus Luteum (CL) may or may not appear on surface of ovaries. This is because, it is formed after the rupture of mature follicle (Graafian follicle). It is the remaining in ovary after ova is released into infundibulum. The main function of CL is secreting progesterone. Progesterone is important in preparing the uterus lining for initial stage of pregnancy (Krishna *et al.,* 2015). Lutz (2009) also stated that the remaining of ruptured Graafian follicle that form CL is actually granulosa cells and thecal cells.

2.4 *In Vitro* Embryo Production

In vitro Embryo Production (IVEP) is one of the assisted reproductive technologies (ART), which maximise exploitation of the potential of female reproduction. There are four steps involve in *in vitro* embryo production (IVEP). The first step is recovery of oocytes from follicles. Then follow by *in vitro* maturation (IVM), *in vitro* fertilisation and lastly *in vitro* culture (IVC) (Freitas and Melo, 2010).

All female mammals are born with large amount of follicles, and are lost while approaching to puberty (Islam *et al.,* 2007). Oocytes that are lost during growth or maturation process may have potential to reproduce offspring. *In vitro* production of embryo is useful to reduce the waste of potentially usable oocyte (Hirao *et al.,* 1994). However, Ellenbogen *et al.* (2011) reported that among the 1224 oocytes that they had undergo IVM, only 15.6% were able to mature up. Gunasheela *et al.* (2011) later did some modifications on IVM and IVF, and had successfully found out that by using large follicles, the success rate for IVM and IVF increased to 72.22% and 75.44% respectively. This means that there are still room for improvement in this field.

Gunasheela *et al.* (2011) also mentioned in their published article that there are many factors, such as different culture media, duration of *in vitro* maturation, different recovery techniques of oocyte, quality of collected oocytes, and the quality of ovaries may affect the success rate of maturation. Grabowska *et al.* (2016) stated that the quality of oocytes also maybe depends on the sexual maturity of the animals. They said that the effectiveness of *in vitro* embryos obtained from gilts are lower compared to embryo obtained from sow. This is because of the greater sensitivity to environmental factors and lower cytoplasmic content in oocyte. They also mentioned that the oocyte obtained from gilts are smaller in size, indicating to have smaller volume of cytoplasm and lower amount of cellular components that is required for embryonic development. This is supported by Kouamo *et al.* (2014), who stated that quality of ovaries can be affected by ovarian and non-ovarian factor, which include presence of corpus luteum, state of pregnancy of the animal, and age of the animal.

Islam *et al.* (2007) stated there is variation among species. They did not obtain high significance result for variation in left and right ovaries, which differed from the previous few reported journal articles. Additionally, only 2.55 COCs were collected from one goat ovaries in their study. They mentioned that this result was lower than previous result reported in ovine and bovine. Therefore, more modifications, improvement and knowledge about ovaries and COCs are needed to increase success rate of IVEP.

Since there are variations among species and lack of study of pig ovaries in Malaysia, this study is very important, so that the IVEP specifically for pig production can be improved. Moreover, Shabankareh *et al.* (2015) stated that oocyte quality determines the rate of blastocyst production, and also related to ovaries quality which oocytes will be recovered from.

2.5 Slaughterhouse Pig Ovaries

For successful *in vitro* embryo production, good quality of oocyte is necessary (Shabankareh *et al.,* 2015). And since a lot of ovaries are wasted in slaughterhouse, because ovaries are inedible, slaughterhouse ovaries can be good source for oocytes (Asad *et al.,* 2016). Thus, studies must be done on slaughterhouse pig ovaries to obtain information and knowledge about good ovaries, which will affect quality of oocyte and subsequently improve the fundamental of *in vitro* embryo production.

Some studies had been carried out to determine the factors of slaughterhouse pig ovaries that will affect results of IVEP. Islam *et al.* (2007) emphasized that ovaries without presence of CL has high number of superior quality of COCs and can be used for IVP of goat embryos. However, he also stated that there may be variation among species.

Grabowska *et al.* (2016) stated that ovaries from gilt is low in number of corpus luteum and the size of oocytes, which correlated with the age and sexual maturity of

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