IN VITRO MATURATION OF PORCINE OOCYTES

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ABSTRACT

An experiment was conducted in the Plant Physiology Laboratory at the Faculty of Sustainable Agriculture, Universiti Malaysia Sabah throughout June until July 2017. The purpose of the experiment was to examine the effects of bovine serum albumin (BSA) and polyvinyl alcohol (PVA) on the cumulus expansion and nuclear maturation of porcine oocytes. Porcine ovaries were collected from the Sandakan Municipal Council pig slaughterhouse in Mile 8, suitable cumulus-oocyte-complexes (COCs) were aspirated from ovaries with corpus luteum present (CL+) and subsequently matured in tissue culture media 199 (TCM-199) containing either 5% BSA or 4% PVA. The COCs were then incubated at 37.5°C at 20% oxygen (O_2) and 5% carbon dioxide (CO_2) for 48 hours. The cumulus expansion was observed at 24 and 48 hours under an inverted light microscope whilst the nuclear maturation was observed after 48 hours once the oocytes were mechanically denuded, stained with aceto-orcein stain and viewed under an inverted light microscope at 100x magnification. From this experiment, we have found that oocytes matured in BSA (10.8% and 37.8%) had more oocytes expanding compared to PVA (2.2% and 2.2%) for cumulus expansion at 24 hours and 48 hours respectively. The nuclear maturation of oocytes in BSA (43.2%) at 48 hours that had achieved germinal vesicle break down (GVBD) was also significantly higher than PVA (3.7%) however, both mediums did not support nuclear maturation past GVBD. This concludes that BSA has a better potential for *in vitro* maturation usage in porcine oocytes as compared to PVA.

PEMATANGAN OOSIT BABI SECARA *IN VITRO*

ABSTRAK

Satu penyelidikan telah dijalankan di Makmal Fisiologi Tumbuhan di Fakulti Pertanian Lestari, Universiti Malaysia Sabah sepanjang bulan Jun hingga Julai 2017. Tujuan penyelidikan ini adalah untuk mengkaji kesan serum albumin lembu (BSA) dan polivinil alkohol (PVA) terhadap pengembangan sel kumulus dan pematangan nuclear oosit babi. Ovari babi telah dikumpulkan dari rumah penyembelihan babi Majlis Perbandaran Sandakan di Batu 8, kompleks kumulus-oosit (COC) yang sesuai disedut keluar dari ovari yang mempunyai korpus luteum (CL+) dan kemudiannya dimatangkan dalam media pengkulturan tisu 199 (TCM-199) yang mengandungi sama ada 5% BSA atau 4% PVA. COC kemudian diinkubasi pada 37.5°C pada 20% oksigen (O_2) dan 5% karbon dioksida (CO_2) selama 48 jam. Pengembangan kumulus diperhatikan pada 24 dan 48 jam di bawah mikroskop cahaya terbalik manakala pematangan nuclear diperhatikan selepas 48 jam dan oosit akan dibuang sel kumulusnya dan diwarnakan dengan pewarna aceto-orcein dan diperiksa di bawah mikroskop cahaya terbaik pada pembesaran 100x. Dari penyelidikan ini, kami mendapati bahawa oosit dimatangkan di dalam BSA (10.8% dan 37.8%) mempunyai lebih banyak oosit yang sel kumulusnya berkembang berbanding dengan PVA (2.2% dan 2.2%) untuk perkembangan kumulus pada 24 jam dan 48 jam masing-masing. Pematangan nuklear oosit dari BSA (43.2%) pada 48 jam yang mencapai pecahan vesikel germinal (GVBD) juga jauh lebih tinggi berbanding dengan PVA (3.7%) walau bagaimanapun, kedua-dua medium ini tidak dapat menyokong pematangan nuklear selepas GVBD. Ini membawa kepada kesimpulan bahawa BSA mempunyai potensi yang lebih baik untuk kegunaan pematangan *in vitro* bagi oosit babi berbanding dengan PVA.

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LIST OF SYMBOLS, UNITS AND ABBREVIATIONS

°C	Degrees Celcius
ART	Assisted Reproductive Technology
AVA	Agri-Food and Veterinary Authority of Singapore
BSA	Bovine Serum Albumin
CL	Corpus Luteum
CL-	Ovaries without Corpus Luteum
CL+	Ovaries with Corpus Luteum
CO_2	Carbon Dioxide
COC	Cumulus-Oocyte Complex
DVS	Department of Veterinary Services
FAO	Food and Agriculture Organization of United Nations
FCS	Fetal calf serum
FSA	Faculty of Sustainable Agriculture
FSH	Follicle stimulating hormone
GV	Germinal vesicle
GVBD	Germinal vesicle break down
hCG	Human chorionic gonadotropin
IVEC	*In Vitro* Embryo Culture
IVEP	*In Vitro* Embryo Production
IVF	*In Vitro* Fertilization
IVM	*In Vitro* Maturation
IVMFC	*In vitro* maturation, fertilization and culture
JAKIM	Department of Islamic Development Malaysia
LH	Luteinising hormone
LO	Left Ovary
M199	Media 199
MAPK	Mitogen activated protein kinase
MPF	Modern Pig Farming
NaCl	Sodium Chloride
NCSU23	North Carolina State University media 23
O_2	Oxygen
PBS	Phosphate Buffer Saline
PFA	Pig farming area
PGC	Primordial germ cell
PVA	Polyvinyl Alcohol
PVP	Polyvinyl pyrrolidone
RO	Right Ovary
rpm	Revolutions per minute
SOF	Synthetic oviduct fluid
TCM-199	Tissue Culture Media 199
UMS	Universiti Malaysia Sabah

CHAPTER 1

INTRODUCTION

1.1 Introduction

The domesticated pig or hog (*Sus scrofa domesticus*) is an omnivorous mono-gastric mammalian animal from the Suidae family of even-toed ungulates. Pigs were domesticated all over Eurasia and had originated from two separate ancestors, the European hogs (*Sus scrofa*) descended from European wild boars and the Asiatic hogs (*Sus indicus*) descended from unknown origins (Darwin, 1868). In Malaysia, the natural pig population is derived from indigenous wild boar species but these are not used for production of pork though they are occasionally hunted for their meat by wild game hunters. The commercial pig industry in Malaysia mainly relies on imported strains of commercialised pigs such as the Landrace, Large White and Duroc.

The production of pigs in the world is the largest compared to other livestock and the consumption of their meat is the highest according to the FAO (2009). The production of pigs in Malaysia is the second largest compared to other livestock species. Malaysia is a tropical Islamic country in South-East Asia and has a population of 61.3% Islamic population leaving a mere 38.7% of pork consuming population. However, as of 2014, the non-Muslim Malaysians consumption of pork and its products exceeds the consumption of beef or mutton at a per capita consumption rate of 18.8 kilogrammes of pork per year. Despite Malaysian pig farms having a high output, Malaysia only has a self-sufficiency of 95.66% as of 2014 and has shown a decreasing trend since 2011 (DVS, 2015a). Statistics show that Malaysians import more pork meat than they export which shows that the population demand is much greater than reported (FAO, 2013).

Regarding the production of pigs, their reproductive anatomy and physiology is a key aspect which contributes greatly to their prolificacy. The ovaries in a female pig have the site of germ cell (oocyte) formation and sustains its growth until it is released during ovulation. The female germ cells develop in the ovaries prior to birth but most of these germ cells do not mature until they undergo puberty and are lost through follicle atresia (Krakauer and Mira, 1999). As the animal ages, more oocytes succumb to apoptosis with only few maturing into primary follicles and fertilizing. Pincus and Enzmann (1935) concluded from their research on rabbits that these oocytes can be harvested and successfully matured outside of their usual environment within the ovarian follicle by *in vitro* maturation (IVM) and successfully fertilised by *in vitro* fertilization (IVF). Despite that, the ovary is known to produce follicles of differing maturities and after ovulation a corpus luteum (CL) is formed. According to Shabankareh (2015), the CL creates a negative effect on the developmental competence in small and medium sized oocytes. As not all ovaries possess the CL, a simple selection of ovaries must be carried out for best results.

Assisted Reproductive Technology (ART) can be defined as "all treatments or procedures that include the *in* vitro handling of both human oocytes and sperm, or embryo, for the purpose of establishing a pregnancy" according to Zegers-Hochschild *et al.* in 2009. IVM is a form of ART which takes pre-antral follicles from fresh or living ovaries and matures it in a medium, and its use was documented as an alternative that can replace the commonly practiced ovarian stimulation methods (Hashimoto, 2009). Success in the use of IVM oocytes in IVF was documented in cattle (Carolan *et al.*, 1994), goats (Martino *et al.*, 1994), sheep (Wang *et al.*, 1998; Wani *et al.*, 2000), pigs (Tsafiri and Channing, 1974; Hirao *et al.,* 1994) and various other animals. Studies in Malaysia only consisted of research in swamp buffaloes (Jainudeen *et al.*, 1993) goats (Abdullah *et al.*, 2007), and abattoir-derived ovaries from cattle (Sianturi, 2001). Still, research of IVM need to be carried out and documented in Malaysia in the case of pigs or boars.

Through domestication and breeding practices, the pig production industry has become a lucrative industry for producing meat for human and animal consumption, pharmaceuticals, health supplements, quality leather, fibres, glue and even commercial fertilizers. Pigs are also used as models by students the medical field for translational research and play a vital role in the further research and understanding of stem cells and organ transplantation (Kobayashi *et al.*, 2012). As stated in an overview by Aigner *et al.* in 2010, pigs were selected to be genetically modified and used as a model for the study of various human chronic diseases such as diabetes mellitus. Transgenic pigs are also the focus in the production of embryonic stem cells which are immuno-compatible with human recipients as well as for the development and testing of drugs intended for human use (Feng *et al.*, 2015). This makes the transgenic pig industry highly valued as the related research would greatly benefit mankind in the future.

It can be concluded that the pig is an optimal unit for reproductive research purposes as the production of pigs for transgenic and translational purposes is crucial in the development and advancement of science. Despite the many advancements in this field, the cost of using a single IVF cycle in developed countries for the production of a live birth in humans can be expensive (Chambers *et al.*, 2009) implicating that its use in livestock may not be as cost-efficient as presumed. Thus, further research must be carried out in order to discover alternatives or replacements to reduce these costs. Furthermore, the potential of developing IVM technologies in Malaysia is very promising as the country is still developing and any contribution towards academic or scientific advancement, or even in securing the nation's food supply is well received. Coupled with the need to greatly improve the agricultural sector and more specifically livestock, IVM will be a boost towards agricultural sustainability.

1.2 Justification

The production of pigs in Malaysia is almost self-sufficient however, Malaysia also holds the potential to become an international exporter of pork to neighbouring countries, specifically Singapore and thus requires an even greater amount of production. As the limitations of pig production can be overcome with the use of ARTs, more studies must be conducted in this field of research. Despite that, the use of ARTs and production of IVM oocytes is still not popularised in Malaysia except for the use of artificial insemination. This is due to the lack of information and development regarding the purpose of ART and the fact that most Malaysian farmers are not properly educated with the possible prospects of using these technologies.

Furthermore, the availability of pig ovaries is in abundance if compared to other local livestock such as cattle and goats. This can be due to the shortage of breeding stock in cattle and goats whereby most of the female animals are kept for breeding purposes and are rarely slaughtered unless necessary. In comparison, gilts are slaughtered by the hundreds on a daily basis in Malaysia as there is sufficient amount of breeding stock which allows premature gilt ovaries to be readily available.

This study of IVM oocyte production and the comparison between the maturation media can be a foundation for further improvements in IVF and later on the production of *in vitro* embryo production (IVEP). Local researchers will also benefit from the results obtained using locally reared and slaughtered pig ovaries as results may vary depending on rearing methods and climate.

1.3 Objective

The objective of this study is to determine the effects of Polyvinyl Alcohol (PVA) and Bovine Serum Albumin (BSA) on the *in vitro* maturation of porcine oocytes collected from slaughterhouse pig ovaries.

1.4 Hypothesis

H_0 : There is no significant difference in the cumulus expansion and nuclear maturation of the oocytes matured with PVA and BSA.

H_a : There is a significant difference in the cumulus expansion and nuclear maturation of the oocytes matured with PVA and BSA.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

There are a total of three main topics in this literature review. The first topic is a review on the general production of pigs in Malaysia and the needs for reproductive biotechnology. The second topic is a review on the physiology and development of the female reproductive organs and gametes in mammals. The third topic reviews the stages of *in vitro* embryo production from maturation until culture. The final topic reviews the process of collecting and maturing oocytes from slaughterhouse animals.

2.2 Pig Production in Malaysia

The pig production industry has been showing improvements due to the ground-breaking new technologies and management systems over the years. Efforts for the conversion of rural-area or smallholder pig farms into a centralized Modern Pig Farming (MPF) zone exclusively for the production of pork and pork products. This plan will be carried out in hopes that the Malaysian pork industry becomes more self-sustainable and eco-friendly practices of farming (Gardir and Wai, 2014).

2.2.1 Malaysian Pig Population and Distribution

The pig production in Malaysia as shown in table 2.1 (DVS Malaysia, 2015a) shows the standing population of 2014 to be 1.8 million heads of pigs which is the highest among the mammalian livestock species. Despite the stable production of pigs in Malaysia, a slight decreasing trend in pig production can be observed from 2012 to 2015 in table 2.2 (DVS Malaysia, 2015a). It also shows that Peninsular Malaysia is the largest pig producers by region and Perak as the largest producer of pigs by states in 2014 as stated in Table 2.3 (DVS Malaysia, 2015a). As the Chinese population in Malaysia is distributed into states such as Johor, Penang, Selangor and Perak with higher urbanization (Wan-Ibrahim and Zainab, 2014), it is proven that the distribution of pigs in Malaysia is highly influenced by the racial distribution of ethnic groups in Malaysia.

Table 2.1 The livestock population in Malaysia for 2014 by region.

WILAYAH *Region*	KERBAU *Buffalo*	LEMBU *Cattle*	KAMBING *Goat*	BEBIRI *Sheep*	BABI *Swine*
S.Malaysia *P. Malaysia*	61,687	662,818	363,768	138,127	1,425,371
Sabah [P]	52,450	68,105	50,650	2,050	82,552
Sarawak [P]	7,122	15,860	14,980	2,258	336,180
Jumlah *Total*	**121,259**	**746,783**	**429,398**	**142,435**	**1,844,103**

Source: DVS Malaysia, 2015a

Table 2.2 The livestock population in Malaysia by region in 2011-2015

JENIS TERNAKAN *Livestock Type*	2011	2012	2013	2014	2015 [E]
KERBAU *(Buffalo)*					
S.Malaysia *(P. M'sia)*	70 623	65,858	64 218	61,687	61,621
Sabah	50,080	51 850	52 369	52,450	52 975
Sarawak	7 502	7,277	7,059	7,122	6 908
Jumlah *(Total)*	**128,205**	**124,985**	**123,646**	**121,259**	**121,504**
LEMBU *(Cattle)*					
S.Malaysia *(P. M'sia)*	691,049	663,563	667 869	662,818	665,143
Sabah	62,930	65,694	67,997	68,105	70,493
Sarawak	14 731	15,120	15 631	15,860	16 396
Jumlah *(Total)*	**768,710**	**744,377**	**751,497**	**746,783**	**752,032**
KAMBING *(Goat)*					
S.Malaysia *(P. M'sia)*	413 359	394,905	368 774	363,768	372,090
Sabah	51,470	53,010	50 584	50,650	52,342
Sarawak	14 615	14,595	14 844	14,980	15,235
Jumlah *(Total)*	**479,444**	**462,510**	**434,202**	**429,398**	**439,667**
BEBIRI *(Sheep)*					
S.Malaysia *(P. M'sia)*	121,796	127,671	137,718	138,127	135,795
Sabah	2,049	2,070	2,089	2,050	2,069
Sarawak	2 567	2,182	2,111	2,258	2,185
Jumlah *(Total)*	**126,412**	**131,923**	**141,918**	**142,435**	**140,049**
BABI *(Swine)*					
S.Malaysia *(P. M'sia)*	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
Jumlah *(Total)*	**1,816,557**	**1,851,842**	**1,842,953**	**1,844,103**	**1 828,860**

P Sementara *(Provisional)* E : Anggaran *(Estimate)*

Source: DVS Malaysia, 2015a

Table 2.3 Livestock population by states in Malaysia for 2014

NEGERI *State*	Kerbau *Buffalo*	Lembu *Cattle*	Kambing *Goat*	Bebiri *Sheep*	Babi *Swine*	Ayam *Poultry*	Itik *Duck*	Burung Unta *Ostrich*	Puyuh *Quail*	Rusa *Deer*
Perlis	233	8.225	5,713	1,296	30	1.210.282	12.050	n a	3250	265
Kedah	7,757	68.596	49,353	10 764	720	47 664.337	707,200	57	480,070	907
Pulau Pinang	566	16.091	11,005	1,540	311,791	12.452.669	495,200	n a	150,255	145
Perak	11.669	53,007	38,077	4,374	517.163	35.256.700	6,725,600	25	65,750	4.877
Selangor	1,460	22 858	23,536	3.256	273,630	20.540 364	28,118	200	155,710	506
N. Sembilan	3 286	44.574	40.986	20 230	861	19.320 355	25.660	105	286.140	1.555
Melaka	3,740	27.935	39,507	10.710	47,195	19,690.989	124.862	25	76,000	615
Johor	3 458	106.085	49,817	26 942	270 056	63.663.879	890,039	307	1,090,800	1 681
Pahang	14.125	129 255	36.145	17.825	3.400	14.133 621	17.500	n a	399,250	2.900
Terengganu	9.268	86,317	26,519	6,065	n.a	6.400.977	24.349	n.a	93,245	324
Kelantan	6.125	97.425	42.855	35,125	525	1.845.850	58,255	n a	28,100	205
W. Persekutuan	n a	450	255	n a	n a	n a	n a	n a	n a	95
Jumlah S. M'sia *Total For P. M'sia*	**61.687**	**662,818**	**363,768**	**138.127**	**1.425.371**	**242,120,023**	**9,108,833**	**719**	**2.838.570**	**13,995**
Sabah P	52,450	68.105	50,650	2 050	82,552	5,571.223	45,506	n a	n a	n a
Sarawak P	7.122	15.660	14.960	2,258	336.180	40.613 010	347.333	n s	155,630	2 652
JUMLAH BESAR *Grand Total*	**121.259**	**746.783**	**429.398**	**142,435**	**1.844,103**	**288.304.256**	**9.501,672**	**719**	**2,994,200**	**16.647**

P . Sementara (Provisional) n a : Tiada maklumat (*Not available*)

Source: DVS Malaysia, 2015b

2.2.2 Potential and Constraints of Pig Production in Malaysia

Although there lies a potential in the pig production industry in Malaysia, it is hindered by Malaysia's political instability due to heavy Islamic influence and the general dislike of the industry by the country's main ethnic population (Neo, 2009). The main potential of the Malaysian pig industry lies in the export of fresh and frozen pork meat to the neighbouring country of Singapore, with its most recent approval of pork imports from Sarawak in 2014 (AVA, 2015).

As Malaysia is an Islamic country with only 38.7% of non-Muslims which may consume pork produce, all food and medical products face strict regulations involving Halal resources. As stated in the Trade Descriptions (Definition of Halal) Order 2011 and 2012, food or goods described or labelled as halal is used to indicate it is safe for the consumption of Muslims and does not contain anything impure according to the Shariah law and Fatwa, which also includes the use of any pig parts or by-products (JAKIM, 2015). This regulation restricts all products, including by-products such as pig manure as fertilizers, and therefore, greatly limits the marketing of pig products in Malaysia. Similarly, heavy regulation of pig farming practices is found in Malaysia in order to reduce its impact on the Muslim population. Not much emphasis or assistance has been given to the pig industry by the government agricultural departments and its history is full of conflicts between pig farmers its governing states (Xu, 2014).

The pig industry is affected by land zoning whereby farms which are opposed by the local public are generally forced into closure or relocation. As of 1991 due to endorsement by the government, a pig farming area (PFA) has been allocated in order to centralise the farming of pigs and to establish a more systematic management and development (Loh, 2002). Pig farms are also undesirable in areas with a majority Muslim population which limits the land available.

The matter of pig effluent management is a major constraint as most traditional pig farmers conduct their waste into nearby water bodies and rivers. Many farms that do not follow the state guidelines are forcibly closed such as those in Malacca (Utusan Melayu, 1999). The pig industry also faces the problem of its dependency on global feed resources and most of the pig feed are imported in the form of raw materials such as corn and soybean.

2.3 Mammalian Ovaries and Development

The ovaries are the site of germ cell and follicle development, ovulation, and for the production of female sex hormones. The ovary is formed from the coelomic epithelium, found on the centre of the foetal kidney or known as mesonephros, which becomes increasingly thicker over time. Zamboni *et al.* (1979) states that the mesonephros has been shown to contribute cells to the coelomic epithelium in order to form the ovary before any occurrence of sexual differentiation in the foetal gonads. Then, the migration of the primordial germ cells (PGC) begins from the endoderm towards the developing foetal gonads (Mintz, 1957). During the migration phase, the number of PGCs multiply from few hundreds to several thousand depending on the species (Byskov, 1986). After sexual differentiation occurs in the foetal gonads, a group of gonadal ridge epithelial-like cells or GREL proceeds to form the genital ridge and pre-granulosa cells (Hummitzsch *et al.*, 2013) which then combine with the oogonia to form the oogonia-pre-granulosa cell complex which is responsible for steroid production.

As the development of the gonad proceeds further, the pre-granulosa cells begin to isolate the oogonia using cytoplasmic extensions that form on the pre-granulosa cells

(Sawyer *et al.*, 2002). When the ovary is well formed, two-waves of cell recruitment occur, the first wave for the formation of the pre-pubertal follicles and the second wave for the formation of post-pubertal follicles (Sawyer *et al.*, 2002). The follicles formed from the first wave will contribute until the onset of puberty and may even extend further than that. The incidence of germ cell meiosis begins in the foetal stages in cattle (Erickson, 1966), sheep (Mauleon and Mariana, 1976) and mice (Borum, 1961) with the changes beginning from the cortex of the ovary and extending outwards. This coincides with follicle formation within the ovaries and subsequently, the multiplication of PGCs begin to wane and germ cells become apoptotic.

2.3.1 Types of Ovaries

Typical mammalian ovaries undergo changes during the oestrus cycle and during pregnancy until lactation. These changes in ovarian types allows scientists to categorise based on different factors. Ovaries can be categorised based on morphological characteristics of the ovarian follicles which are grape-type, honeycomb-type and intermediate-type (Dufour *et al.*, 1985). As shown in Figure 2.1, the grape-type ovaries (A and C) have large follicles at the ovarian surface with smaller follicles deeper within the ovary compared to honeycomb-type ovaries (B and D) which have small follicles on the surface and inside the ovary. Any other ovary with characteristics that do not conform to the grape-type or honeycomb-type ovaries are categorised as intermediate-type.

In another study, ovaries were categorised into three distinct stages which are: type-I ovaries which have a fully functional CL, type-II ovaries which have a CL that is almost regressed, and type-III ovaries without CL (Rahman *et al.*, 2003). Rahman *et al.* claimed in 2003 that ovaries without a CL was shown to have a significantly higher number of follicles compared to that of ovaries with a functioning CL or with an almost regressed CL. Figure 2.2 shows the ovaries of pre-pubertal gilts which indicate the presence (B) and absence (A) of the CL in a study which was done to evaluate the morphology of ovaries at different cycle stages (Oberlender *et al.*, 2014).

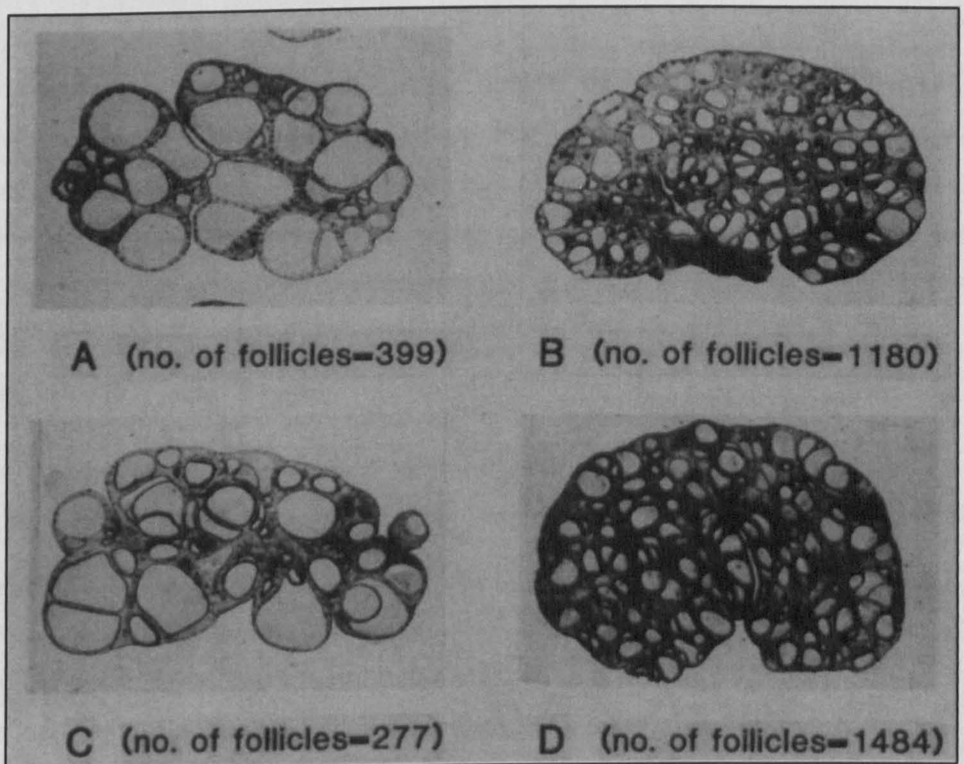

Figure 2.1 Gilt ovaries categorised into types based on morphological characteristics. (A and C) Grape-type ovaries have visibly less follicles; (B and D) Honeycomb-type ovaries have a large amount of follicles.

Source: Dufour *et al.*, 1985

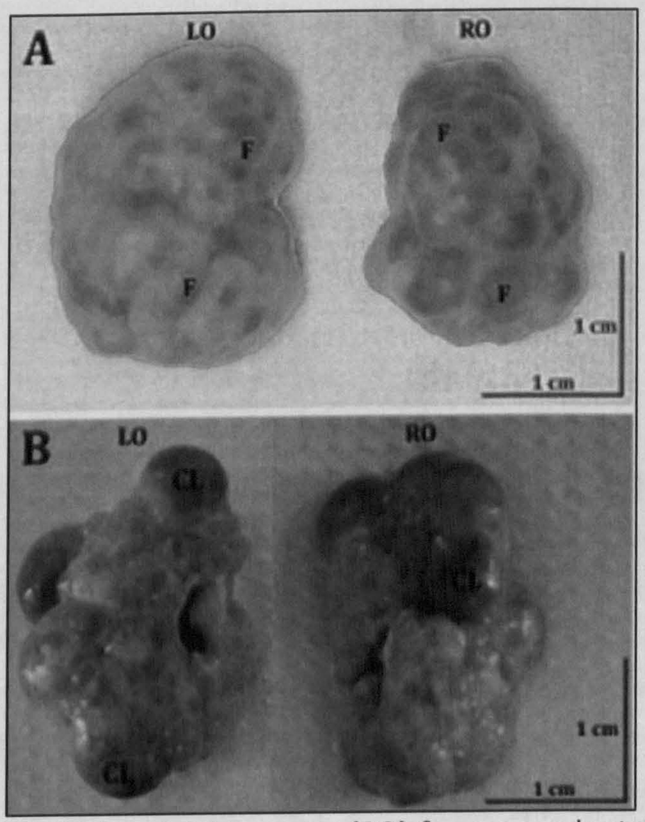

Figure 2.2 The left (LO) and right ovaries (RO) from pre-pubertal gilts. (A) Ovaries without CL; (B) Ovaries with CL.

Source: Oberlender *et al.*, 2014

The type of ovary may also influence the number of follicles and COCs that can be recovered from the ovary. In a study which categorized ovaries based on the presence and absence of CL, ovaries with the absence of CL are more likely to have a higher total COC recovery rate as well as the recovery rate for normal COCs (Khandoker *et al.*, 2016). Therefore, the categorization of ovarian types is crucial in order to obtain a higher number and higher quality of oocytes.

2.3.2 Growth and Development of Oocytes

Pig oocytes, similar to most mammals, are developed through oogenesis during the prenatal phase of the piglet. These immature oocytes form the oocyte reserve which slowly depletes as the pig ages due to ovulation and atresia from failure to ovulate. These oocytes are encased in an ovarian follicle found all over the ovary surface and centre of the ovary. A study by Grant *et al.* (1989) found that the ovarian follicles collected from gilts which are earlier in the oestrus cycle (day 16) are smaller than those collected from gilts later in the cycle (day 20) and also showed that the size increases progressively. However, the same study also showed the number of follicles present on the ovary decreased progressively as the size of follicles increased.

In another study, it was noted that the growth stages of the oocyte is related to the size of the oocyte with larger oocytes producing better developmental potential in bovine ovaries (Arlotto *et al.*, 1996) which implies that oocytes collected later in the cycle were more fertile.

2.3.3 Cumulus-Oocyte-Complexes (COCs)

The COC is a complex formed from an oocyte and its surrounding granulosa cells which form the cumulus. This complex is important for the development of meiotic competence in the oocyte as the cumulus cells play an important role. There exists a cell-to-cell communication between the oocyte and the cumulus cell through which the presence of FSH induced the cumulus cells to begin the production of a diffusible meiosis activating substance which managed to inhibit the meiotic arrest caused by Hypoxanthine and induced the oocyte in contact to undergo meiotic maturation (Byskov *et al.*, 1997).

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