QUALITATIVE EVALUATION OF SLAUGHTERHOUSE GOAT OVARIES BY HISTOCHEMICAL STUDY

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ABSTRACT

A histological study of mature goat ovaries was carried out at the Animal Anatomy and Physiology Laboratory, Faculty of Sustainable Agriculture, Universiti Malaysia Sabah (UMS) Sandakan Campus from May 2016 until October 2016. The objectives of this study are to identify the quality of follicles in goat ovaries and to find out the relationship between the quality of follicles and ovarian state. The animals used in this study were 2 adult female goats (doe) with unknown reproductive background that have been slaughtered at Hafiz Farm slaughterhouse in Lahad Datu. The ovaries were categorized as right, left, corpus luteum (CL) present (CL+) and absent (CL-). The categorized ovaries were evaluated physically and histologically by using light microscope. The mean weight (g), length (cm), and width (cm) of right ovary was distinctly higher [(0.67±0.02)g, (1.16±0.03)cm, (0.75±0.15)cm respectively] than the left ovary. On the other hand, the ovary with CL have higher weight and width [(0.69)g, (0.9)cm respectively], while the mean length of ovary without CL were found higher [(1.15±0.02)cm]. The follicles were categorized into few stages which are the primordial, primary, secondary, early antral, antral and preovulatory follicles by histological means. From the classification, higher number of primordial, primary and secondary follicles [(675.5±619.50), (145.0±81.0) and (53.5±38.50) respectively] was found in right ovaries. However, the number of preovulatory follicles counted was found higher in left ovary (7.5±5.5) compared to right. From other point of view, total number of primordial, primary and secondary follicles observed higher in ovary with CL (1295, 226 and 92 respectively) compared to ovary without CL except the preovulatory follicles which is higher (5.33±3.84) than with CL (3). From qualitative evaluation, a high number of abnormal follicles were found in the right ovaries (58.5±52.5) while higher number of normal follicles was found in left ovaries with (60.0±4.0). In respect of CL present or absent, higher number of normal and abnormal follicles were found in ovary with CL [(51 and 111) respectively] compared to ovaries without CL [(43.0±17.16) and (28.67±11.35) respectively]. The present findings revealed that right and left ovaries both have a great potentiality to provide good number of oocytes for in vitro studies. Considering with the effects of CL on ovaries and low number of sample, highest number and normal grade follicle would be collected from ovaries without CL. Moreover, this result can be used to collect quality follicles for oocyte aspiration for IVEP thus creates a great opportunity for conducting further research on goat embryo production especially here in Sabah, Malaysia.

PENILAIAN KUALITATIF OVARI KAMBING DARIPADA RUMAH SEMBELIH SECARA HISTOLOGI

ABSTRAK

Medan kajian histologi ovari kambing matang telah dijalankan di Makmal Anatomi dan Fisiologi Haiwan, Fakulti Pertanian Lestari, Universiti Malaysia Sabah (UMS) Kampus Sandakan dari Mei 2016 sehingga Oktober 2016. Objektif kajian ini adalah untuk mengenal pasti kualiti folikel dalam ovari kambing dan untuk mengetahui hubungan antara kualiti folikel dan keadaan ovari. Haiwan yang digunakan dalam kajian ini ialah 2 kambing betina dewasa (doe) dengan latar belakang pembiakan yang tidak diketahui yang telah disembelih di rumah sembelih Hafiz Farm di Lahad Datu. Ovari dikategorikan sebagai kanan, kiri, corpus luteum (CL) hadir (CL +) dan tidak hadir (Cl-). Ovari dikategorikan telah dinilai dari segi fizikal dan histologi dengan menggunakan mikroskop cahaya. Min berat badan (g), panjang (cm) dan lebar (cm) ovari kanan adalah jelas lebih tinggi [(0.67 ± 0.02) g, (1.16 ± 0.03) cm, (0.75 ± 0.15) cm masing-masing] daripada ovari sebelah kiri. Sebaliknya, ovari dengan CL mempunyai berat dan lebar yang lebih tinggi [(0.69) g, (0.9) cm masing-masing], manakala panjang min didapati lebih tinggi di ovari tanpa CL [(1.15 ± 0.02) cm]. Folikel dikategorikan kepada beberapa peringkat iaitu awalan, pertama, kedua, awal antral, antral dan folikel praovulasi melalui histologi. Dari klasifikasi, nombor yang lebih tinggi kiraan awalan, pertama dan kedua folikel [(675,5 ± 619,50), (145.0 ± 81.0) dan (53.5 ± 38.50) masing-masing] telah dijumpai di dalam ovari kanan. Walau bagaimanapun, bilangan folikel praovulasi didapati lebih tinggi dalam ovari kiri (7.5 ± 5.5) berbanding dengan kanan. Dari sudut pandang lain, jumlah folikel awalan, pertama dan kedua diperhatikan lebih tinggi dalam ovari dengan (masing-masing 1295, 226 dan 92) CL berbanding ovari tanpa CL kecuali folikel praovulasi yang lebih tinggi (5.33 ± 3.84) berbanding dengan CL (3). Daripada penilaian kualitatif, bilangan yang tinggi folikel yang tidak normal ditemui dalam ovari kanan (58.5 ± 52.5) manakala jumlah yang lebih tinggi daripada folikel tidak normal ditemui dalam ovari kiri (60.0 ± 4.0). Berkenaan dengan CL hadir atau tidak hadir, jumlah yang lebih tinggi daripada folikel normal dan tidak normal ditemui dalam ovari dengan CL [(51 dan 111) masing-masing] berbanding dengan ovari tanpa CL [(43.0 ± 17.16) dan (28.67 ± 11.35) masing-masing]. Penemuan ini mendedahkan bahawa ovari kanan dan kiri kedua-duanya mempunyai potensi yang besar untuk memberikan jumlah telur yang baik untuk kajian luar tabie. Mengambilkira kehadiran CL pada ovari dan nombor sampel yang rendah, bilangan tertinggi dan folikel gred biasa boleh diambil dari ovari tanpa CL. Selain itu, keputusan ini boleh digunakan untuk mengumpul folikel berkualiti untuk pengambilan oosit untuk IVEP dan mewujudkan peluang yang besar untuk menjalankan penyelidikan secara lanjut mengenai pengeluaran embrio kambing terutamanya di sini di Sabah, Malaysia.

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±SE	Standard Error
AI	Artificial Insemination
CL	Corpus Luteum
CL-	Corpus Luteum Absent
CL+	Corpus Luteum Present
COCs	Cumulus-Oocyte-Complexes
DVS	Department of Veterinary Services
EGS	Estrous Goat Serum
FF	Follicular Fluid
FSH	Follicular Stimulating Hormone
GEOC	Goat Epithelial Oviduct Cells
IVC	In-Vitro Culture
IVEP	In-Vitro Embryo Production
IVF	In-Vitro Fertilization
IVM	In-Vitro Marturation
IVP	In-Vitro Production
LH	Luteinizing Hormone
MOET	Multiple Ovulation and Embryo Transfer
NAP	National Agriculture Policy
UMS	Universiti Malaysia Sabah
ZP	Zona Pellucida
ZPRs	Zona Pellucida Remnants

CHAPTER 1

INTRODUCTION

1.1 Introduction

As a developing country, Malaysia is facing with growing population that give challenge in increasing agricultural productivity to meet the demand. The Malaysian livestock industry is an important and integral component of the agricultural sector, providing employment and producing useful animal protein food for the population. It contributes about 18 percent to the total food sector agriculture value added and export earnings (NAP, 1998). The industry can be classified into the non-ruminant and the ruminant sub-sectors. It has shown a steady growth over the years mainly because of the active participation of the private sector, particularly in the sub-sectors of poultry, eggs and pork. The ruminant sub-sector, however, is not well developed in spite of the emphasis and priority it has received from the government in its development plans. Cattle, buffalo, goat and sheep constitute the ruminant sub-sector and smallholders are the principal producers within this sub-sector. Malaysia is able to fulfil its own requirements for pork, poultry meat and eggs but has to import milk, beef and mutton. Self-sufficiency for milk, mutton and beef are below 20 percent (DVS, 2015). As a result, the country has seen an increase in its food import bill from RM4.6 billion in 1990 to RM10.0 billion in 1997. Thus, the Third National Agricultural Policy (NAP3, 1998-2010) emphasizes that the further growth of the agricultural sector requires that the nation address the challenge of efficient and optimal utilization of existing resources in order to further improve competitiveness.

Other than strengthening the policy, Malaysia also started to adopt and apply animal biotechnology in livestock production sector with specific research priority on genetic engineering of animals for improved production and quality, improvement of reproductive biotechnologies, development of cheap feedstuff from local resources, novel vaccines and drug delivery system and development of rapid diagnostic kits (Abdullah *et al.*, 2011). Biotechnology can be defined as any technique that uses living organism or substances from such organism to make or modify a product, to improve or develop micro-organism for specific purposes which have been used for thousand years in agriculture (Lebbie and Kagwini, 1996). Moreover, the advance in new biotechnology such as embryo manipulation technology has enhanced the possibilities of manipulating biological systems for the benefit of mankind.

Goat is a multipurpose animal that has been used since long ago for the production of milk, meat, skin, fur, and even for transport in remote area (Sawhney, 1992). Here in Malaysia, demand for goat meat was increasing years by years and this can be proved by the total mutton/chevon consumption every year since 2005 until 2014 (DVS, 2015). They have a rugged physical strength that is able to survive under unfavourable weather conditions and climate. The goat is a prolific breeder with litter size varying from 1 to 4. During the last decade, a number of research papers have appeared on the morphology, histochemistry, biochemistry and physiology of various aspects of folliculogenesis and ovum maturation both in vivo and in vitro (Chauhan and Anand, 1991; Pawshe *et al.*, 1994 a,b; Chauhan *et al.*, 1997). Various reports on the improvement of genetic potential of goats through artificial insemination or through embryo transfer technology had been used (Chauhan and Anand, 1991; Pawshe *et al.*, 1994a, b; Chauhan *et al.*, 1997). In vitro fertilization has recently been involved to the successful development of blastocysts for studying their genetic superiority, polyembryony, production of transgenic embryos and subsequent embryo transfer (Madan *et al.*, 1994; Chauhan *et al.*, 1999).

All this reproductive biotechnology work already been discovered and applied all over the world but still limited here in Malaysia. In fact, there are a lot of major constraints that have been specifically listed out by Madan (2003) such as biodiversity within species and breeds, biotechnologies development in developed countries not suitable for developing countries, lack of trained scientists, technicians and field-workers, absence of mechanism between industry, universities and institutions for technology transfer. Therefore, a lot of studies need to be done for us to implement this technique successfully in our agriculture sector for animal improvement and production with economic returns. One of the preliminary works on IVEP has already been done at undergraduate research level by (Nur Ariani, 2016) and this present study as supportive findings on the quality of follicles by histological evaluation to enhance the productivity of this small ruminant.

In-vitro Embryo Production (IVEP) is also one of the reproductive biotechnology that has started been used in Malaysia. In producing embryo by IVEP, there are two procedures available which is one consists of superovulation, followed by Artificial Insemination (AI) and then flushing of uterus to gather the embryo and the other is In-Vitro Fertilization (IVF) that consists of recovery of eggs from ovaries of the female (Nur Ariani, 2016) then maturing and fertilizing them outside the body until they are ready to be implant into foster females. The benefit of this IVEP is its possibility to produce several progeny from a female in which the average lifetime production of an animal can be increased (Lebbie and Kagwini, 1996). This shows that reproductive biotechnology have an important role in contributing on animal production. For initiating the IVEP experiment, remarkable number of quality oocytes is the prerequisite and a preliminary work on the recovery of quality oocytes towards IVEP had so far been done previously (Nur Ariani, 2016) and stated that corpus luteum absent ovaries contributes higher number of quality oocytes. The present study was conducted for further explanation of egg quality recovered from corpus luteum present or absent of ovaries by histological study.

1.2 Justification

As the demand for animal protein increasing year by year in Malaysia, there is urgently need of reproductive biotechnology implementation such as IVEP to improve the quality and population of livestock. In addition, to overcome the constraints of this technology we firstly need to have clear and concrete knowledge and experiences in animal reproductive biotechnology for a successful animal production. This present study might provide the scientific explanation for obtaining good oocytes from the ovarian follicles by histological evaluation and create the successful clue on IVEP which is able to improve the production of animals thus resolve the issues arising here in Sabah, Malaysia.

1.3 Objective

Considering the above facts and circumstances, the present study was undertaken with the following objectives :

1. To identify the quality of follicles in goat ovaries

2. To find out the relationship between the quality of follicle and ovarian state

1.4 Hypothesis

H_o: There is no difference on the quality of follicles and it relationship with ovarian state of goat ovaries

H_a: There is difference on the quality of follicles and it relationship with ovarian state of goat ovaries

CHAPTER 2

LITERATURE REVIEW

There are several research have been carried out throughout the world related to histochemical study of ovary as well as type of ovary, number of follicle and follicular size which affect cumulus-oocyte-complex (COCs) quality. The related findings of research work carried out in different countries of the world are reviewed in this chapter.

2.1 Ovary

Ovary is the vital reproductive organ in female animals that play a major role in producing the progenies. Ovary is a pair of tiny glands in female pelvic cavity that are located on opposite sides of the uterus and attached to the uterus by ovarian ligament. Ovaries also function in producing estrogen and progesterone hormones during different stages of estrus cycle. In mature ovary, corpus luteum and different size of follicles can be found. This has prompted many investigations on the growth, morphology and morphometry of ovarian follicles (Rajakoski, 1960; Settergren, 1964; Brand and DeJong, 1973; Cahill *et al.*, 1979; Ireland, 1987; Ginther *et al.*, 1989), and changes during the stages of the oestrous cycle (Ireland *et al.*, 1979; Driancourt *et al.*, 1985; Ravindra *et al.*, 1994).

However, most of the studies stated above deals with sheep and cattle, and relatively few information is available on ovarian follicles in goats. Studies on the goat concentrated mainly on ovarian activity around the time of ovulation (Rao and Bhattacharyya, 1980; Akusu *et al.*, 1986) or only on the microscopic follicles visible on the surface of the ovary (Camp *et al.*, 1983). In addidtion, there are lacking in the existing knowledge on follicular systems in goat particularly on the qualitative value of follicles and it relationship with the ovarian state. Thus this study may provide some histological observation on the follicular system of goat.

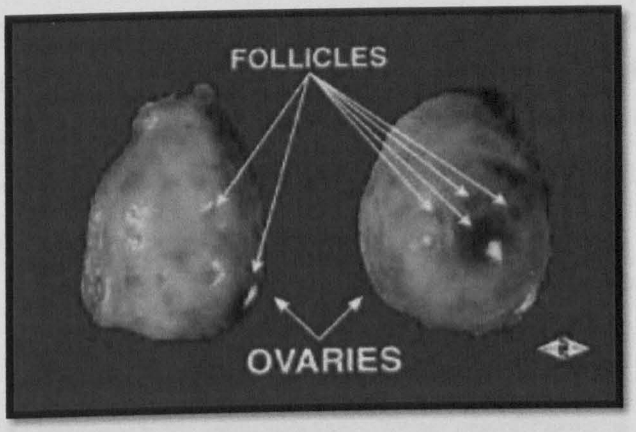

Figure 2.1 : Goat ovaries with visible follicles

Source : Anonymous, n.d.

2.1.1 Ovarian Type

There are two type of ovary which is ovary with corpus luteum (CL+) and without corpus luteum (CL-). Corpus luteum (CL) is a reproductive gland that produces progesterone needed for establishment and maintenance of pregnancy. The CL can be easily found on the ovarian surface and have thicker wall and denser texture than follicles. When the follicle ruptured, bleeding occurs and blood clot forms in its cavity and is called corpus hemorrhagium. The corpus hemorrhagicum is replaced by the CL, which forms rapidly by proliferation of a mixture of theca externa, theca interna and granulosa cells. There is dark red in appearance of the outside of a CL and the cross section reveals a bright yellow to yellow-orange interior (Vernunft *et al.*, 2013).

The presence of CL in does ovary produces a higher level of progesterone hormone that signals a negative response to anterior pituitary gland for the restriction of gonadotropin secretion and ultimately degeneration occurs (Webb *et al.*, 1999). While, the absence of CL in the ovary cause the estrogen-progesterone remains in balance level that allows follicular growth and oocyte maturation. Progesterone secreted by the luteal cells of the CL inhibits estrus and gives the negative feedback on the anterior pituitary to secrete FSH (Hafez, 1993). As a result, the growing follicles regressed and become antretic.

If embryo are present, the release of progesterone is blocked by the embryo, thus CL continues secreting progesterone (Burns, 2002). However, if the doe is not pregnant, direct action of prostaglandin from uterine wall on day 16 to 18 of the heat cycle cause the CL to regress and result in declining of progesterone production. When CL regresses, it will lost its yellowish colour and eventually appearing as a small white scar on the surface of the ovary which is called as corpus albican.

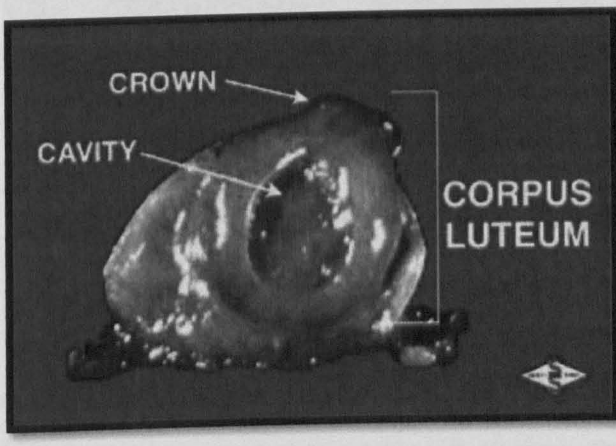

Figure 2.2 : Bisection of goat ovary with corpus luteum

Source : Anonymous, n.d.

2.2 Ovarian Structure

The goat ovaries are oval in shape 0.7 to 1.9 cm long, 0.5 to 0.9 cm in width and 0.2 to 0.4 cm in thickness and weigh 0.8 to 1.2 g (Roberts, 1971; Gretty, 1975; Singh and Prakash, 1988). The bisection of the ovary has revealed 2 demarcated zones, the peripheral zone or cortex or zona parenchymatosa (375 to 490 mm in thickness) and central zone or medulla or zona vasulosa (215 to 305 mm in thickness) (Singh and Prakash, 1988). The cortex contained those tissue layers associated with ovum and hormone production while medulla is composed primarily of blood vessels, nerves and connective tissue. The broad cortex of the goat ovary contains largely the primordial follicles and follicles in various stages of growth and atresia, interstitial gland tubules and some epithelial cords (Sharma *et al.*, 1992). Beneath this lies a thick layer of tunica albuginea composed of coarse aveolar connective tissue with fibroblast cells and collagen fibres oriented parallel to the ovarian surface (Singh and Prakash, 1988). The term albuginea was assigned to it because of its white colour (Ham and Cormack, 1979).

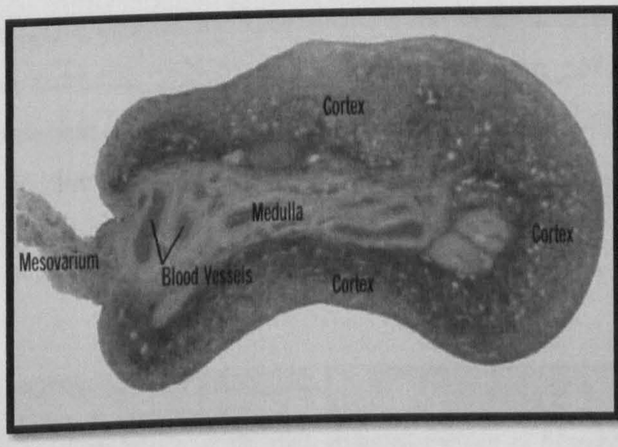

Figure 2.3 : Bisection of goat ovary

Source : Anonymous, n.d.

2.2.1 Follicular Growth

The overall process of follicular growth appears to be similar across mammals where during oestrus, increased secretion of follicular stimulating hormone (FSH) in the pituitary promotes the assemblage of growing follicles in the ovaries. FSH and luteinizing hormone (LH) are both transported to the theca via blood circulation (Richards and Pangas, 2010) and stimulate the production of androgens which are then converted to oestrogen in the granulosa cell layer of the follicle (Bao and Garverick, 1998; Erickson *et al.*, 1979; Silva and Price, 2002). The oestrogens, in turn, enter the blood and down-regulate the production and secretion of FSH in the pituitary (Kumar *et al.*, 1997). As the FSH concentration in the blood circulation subsequently falls, an increasing number of follicles undergoes atresia, and only the one follicle that can best compensate the lower FSH signalling with LH signalling survives and becomes the dominant, ovulating follicle (Xu *et al.*, 1995).

Follicles in an ovary can be found in different size depends on the stage of the follicles. Pedersen and Peters (1968) described that the follicles can be classified as (a) Primordial follicles when an oocyte (o) surrounded by a layer of squamous (flattened) granulosa cells, (b) Primary follicles when an oocyte surrounded by a single layer of predominantly cuboidal granulosa cells, (c) Secondary follicles were surrounded by more than one layer of cuboidal granulosa cells, with no visible antrum, (d) Early antral follicles when they have emerging antral spaces, (e) Antral follicles that possessed a clearly defined antral space, (f) Preovulatory follicles were the largest of the follicular types and possessed a defined cumulus granulosa cell layer, (g) zona pellucida (ZP) of healthy follicles and zona pellucida remnants (ZPRs) representing end-stage atretic follicles, (h) High magnification of ZPR (arrowhead), which is easily distinguished from neighbouring blood vessels.

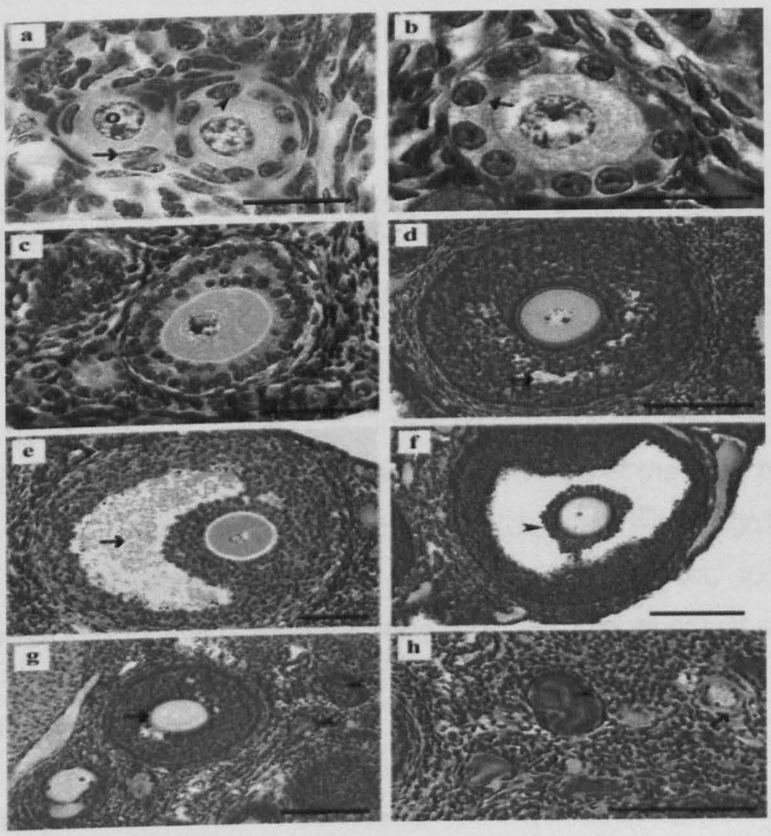

Figure 2.4 : Follicular growth

Source : Pedersen and Peters (1968)

2.2.2 Follicular size

The follicles are present in a wide range of sizes representing various stages of folliculogenesis. Folliculogenesis is the process where ovulatory follicles are developed and the release of one or more mature oocytes at a fixed interval throughout the reproductive life of a female human or animal. Folliculogenesis are governed by the activity of granulosa cells whose activity is regulated by various extra ovarian and intra ovarian hormones and growth factors (Guraya, 1998). The oocyte and follicle structure, composition and organelle distribution show considerable variability during the course of follicular growth and maturation (Sharma and Chowdhury, 1998). Different sizes of follicles can be found in an ovary representing different stages of follicular growth. The diameter of follicles at different stages of growth can be seen in Table 2.1 as discovered by Ariyaratna and Gunawardan (1997).

Table 2.1 : Means (±SD) in µm for growth in relation to follicular growth in the ovary

Stage of follicle	Diameter of oocyte	Thickness of zona pellucida
Primordial follicles (160)	29.6 ± 2.1[a]	
Follicles at antrum formation (42)	80.1 ± 9.6[b]	<1
Vesicular follicles <1 mm (40)	109.6 ± 8.9[c]	2.2 ± 0.3[e]
Vesicular follicles >2 mm (34)	119.0 ± 9.5[d]	3.2 ± 0.3[f]

Source : Ariyaratna and Gunawardan, 1997

The average diameter of the oocyte and the thickness of the zona pellucida in the various stages of follicle formation are given in Table 2.1. There was a significant increase in the oocyte diameter during all stages of follicular development, together with a significant increase in thickness of the zona pellucida at each development stage.

2.2.3 Follicular Fluid

Follicular fluid (FF) can be found between the spaces in membrane granulosa of preantral follicles. It is more prominent and consistent in early phases. As the size of follicles increasing, these small fluid filled spaces forming an antrum. Merker (1961) and Hadek (1963) have envisioned the emptying of large vesicle into the intercellular space seem to be derived from ergastoplasmic vesicle thus suggested that the granulosa cells contribute some substances to the follicular fluid. However, there are some study shows that protein of follicular fluid are directly derived from the blood vascularity of theca interna (Mancini *et al.*, 1963). Following are types of FF that were described in mammals (Guraya, 1985; 1997): (i) primary follicular fluid of membrane granulosa origin, (ii) secondary follicular fluid of blood plasma origin and (iii) follicular fluid having leucocytes, cell debris that plugs ruptured follicle.

Follicular fluid consists mainly of mucopolysaccharides (Zachariae, 1959; Jacoby, 1962) and autoradiographic studies have shown active secretion of these mucopolysaccharides by granulosa cells and high protein turn over (Bjorkman, 1962). Steroid hormones derived from follicles wall can also be found in the FF (Younglai and Short, 1970). The straw coloured follicular fluid viscosity varies with the follicular growth and there appears a complex network of fibrous mucopolysaccharides. The pH of follicular fluid is normally 7.0, whereas pCO2, varies with the stage of follicular growth and species (Guraya, 1985). Physical and chemical characteristic of mucopolysaccharides are impressively changed during preovulatory swelling of follicles thus increase an intrafollicular pressure which leads to ovulation in an explosive manner (Zachariae, 1959).

2.2.4 Theca layer

Theca cells can be seen once a follicle has two or more layer of granulosa cells which is around the time when thecal cells become LH responsive and steroidogenic enzymes are activated (Magoffin and Weitsman, 1994). Thecal cells produce androgens in response to LH, which are then converted into estrogen by (FSH)-induced aromatase in the neighboring granulosa cells of selected growing follicles. The outer and most fibrous layer in Graafian follicle is the theca externa while the inside layer is the theca interna. Theca externa acts largely as cellular barrier, while theca interna as steroidogenic entity (Sharma *et al.*, 1996). The electron and light microscopic analysis of normal theca externa reveals that it comprises fibrous cells, collagenous filaments and a number of blood vessels, and nerve transverse this structure (Guraya *et al.*, 1991; Sharma *et al.*, 1996).

Theca interna consists of elongated to polyhedral cells and a thick bed of capillaries and lipoidal tissue lines this structure (Sharma *et al.*, 1996). Ultrastructurally, the theca interna cells of the goat shows features of steroid-secreting cells (Christensen and Gilim, 1969; Guraya, 1971; 1972 a,b). These features are (a) abundant diffuse lipids (lipoproteins) in the cytoplasm,(b) well-developed cell organelles, especially mitochondria

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