Histomorphometry of Golden Hamster Ovaries

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ABSTRACT

Introduction: Genital system is important herd in animal's creation. It is necessary for animal reproduction. Mammal has two sex in genital system such as male and female in separated animals. Animal reproduction destroyed by disorder of genital system. In this case, animals cannot produce an animal similar to them. Finally it cause overthrow a species of animals.

Methods: Histomorphologic characteristic of hamster ovary studied in this research for a human model. 5 right and 5 left ovary of puberty hamster is studied. Studied biometric parameters were length, wildness and thickness.

Results: This research results showed which right hamster ovary had 5/08±1/6mm in length, 3/05 ± 0/56 mm in wide ,and 2/88 ± 0/327 mm in diameter and left ovary had 4/68 ± 1/253 mm in length, 2/2 ± 0/703 mm in wide and 2/4 ± 0/609 mm in diameter. Countering of different type of hamster ovarian follicles were showed which number of primordial follicles were 1628/33± 305/6049, number of primary follicles were 307/33 ± 56/76, number of secondary follicles were 140 ± 60/63, number of tertiary follicles were 9± 0 and number of graafian follicles were 8 ± 3/6. Total count of hamster ovarian follicles was 2192/66± 178/46. Histological studies showed which covering epithelium, parenchyma of hamster ovary is similar to another mammal, and zuna plucida is forming in primary follicles when two cuboidal cell layers are observed around oocytes.

Conclusion: Finally, because hamsters has short living period in comparing to human, therefore they have low ovarian follicles and oocytes but hamster ovarian follicles and oocyte were similar to human follicles and oocyte in histologic characteristics.

Key Words:
Embryology,
Female Genital System,
Hamster,
Histology.

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1. Introduction

Reproduction is a biological occurrence which exhibits a regular recurrence of pattern of activities in a cyclic manner [7]. Rodents comprise the largest and diverse group of mammals, with 1700 different species [2]. Hamster is one of the best established experimental animals and probably among the best popular pets in the western world. The entire laboratory and pet population of hamster originated from a single brother-sister pairing back in 1930. With the acceptation of a single outbreed stock which derived from 12 wild animals brought to the USA in 1971 [1, 9].

Folliculogenesis begins with the recruitment of a primordial follicle into the pool of growing follicles and ends with either ovulation or death by atresia. Folliculogenesis can be divided into two phases. The first phase, known as the prenatal or gonadotropin-independent phase, is characterized by the growth and differentiation of the oocyte. The second, known as the natal or gonadotropin-dependent phase, is characterized by the tremendous increase of the size of the follicle itself. Structure and population of ovarian follicles and oocytes are very important for evaluation of fertility ability in mammals [3, 6, 11, 12].

Number of total count of ovarian follicles depends on the length of female reproductive life approximately. Females have long sexual life; therefore, they have about 300,000 oocytes in each ovary [7]. Number of primordial follicles was reported 2,500 in the mouse, 10,500 in the cattle, and 21,000 in the sow, respectively [12]. The entire oocytes can not be ovulated in reproductive life of a female; therefore, most of the oocytes are degenerating through atresia process [7]. In each sexual cycle, one or more follicles are beginning to growing but one oocyte is ovulated in monopoly birth animals and tow or more oocytes are ovulated in multiple birth animals [4, 7]. Although numerous papers have been published on the structure of rodent ovaries and other mammals [2, 6, 11, 12, 14, 15], but we studied histomorphometry of golden hamster in Iran. The results of this study can be used for future studies on hamster as laboratory animals or production hamster as pet animals.

2. Materials and Methods

The appropriate ethics committee approval was obtained for using animals' ovarian tissues in this study. Five non-parturient female mature hamsters (about 100 g weight) were selected and euthanized by ether under animal right condition. Then, abdominal cavity was opened and ovaries were removed. First, length, width, and thickness of ovaries were measured by Vernier. Then, ovaries were fixed by 10% formalin. Samples were processed by routine histological methods by autotechnicon (JUNG HISTOKINET 2000 Leica). Paraffin ovarian blocks were sectioned as serial section with 7 µm thickness. The sections were stained by Hematoxylin & Eosin and PAS staining method. The obtained ovaries tissue slides were studied by light fluorescent

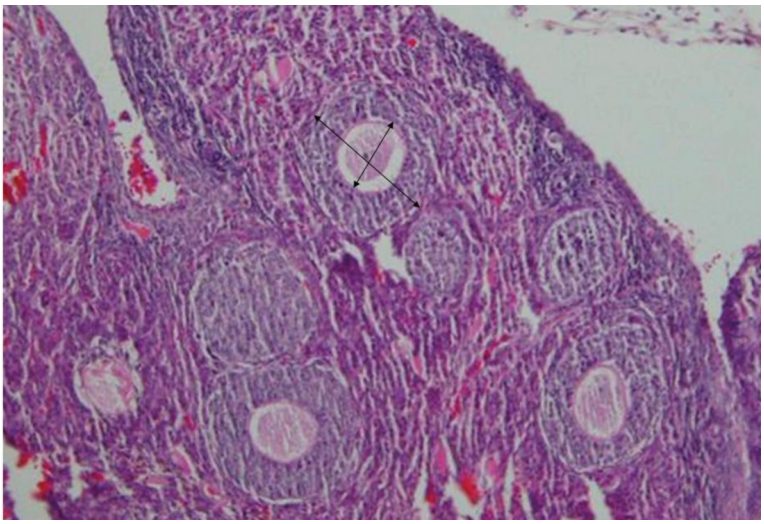

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Figure 1. Ovary of hamster. Larger arrow is showing size of late secondary follicle and smaller arrow is showing size of oocyte.

microscope (Olympus BX 60). Different types of follicles and oocytes were countered in serial section slides, and then histometries of ovaries, follicles and oocytes were captured with the Digital Olympus Camera (DP12 model).

Method of Williams and Erickson (2012) was used for ovarian folliclesnomenclature as following list:

- Primordial follicle (one squamous layer cell around oocyte)
- Primary follicle (one cuboidal layer cell around oocyte)
- Early secondary follicle (two layer cuboidal cell around oocyte)
- Late secondary follicle (more two cuboidal layer cells around oocyte)
- Tertiary follicle (present first hollow space between follicular cells)
- Graafian follicle (a follicle with a complete antrum around oocyte)
- Obtained data were analyzed by Microsoft office excel 2007 16.

3. Results

There was a thick adipose tissue around hamster ovary which adipose tissue removed during tissue processing procedure. Hamster ovaries were observed as grape-shaped in histological sections. Outer tissue layer was a simple cuboidal epithelium (covering epithelium). There was an irregular dens connective tissue under covering epithelium (tunica alboginea). Tunica alboginea was vascularized poorly.

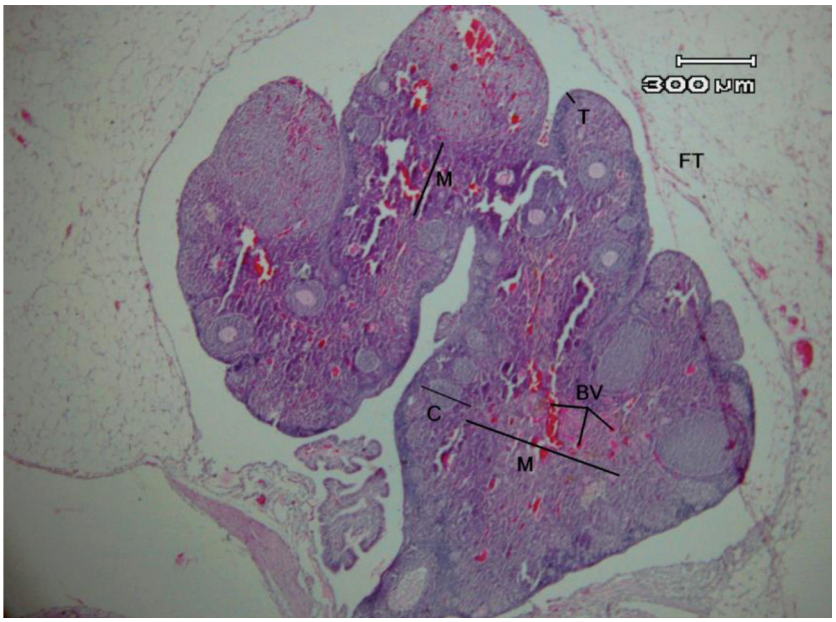

Figure 2. Grap shape hamster ovary. FT, Surrounded fat tissue, T, Tunica albogina, M, Medulla, C, Cortex, BV, Blood vessels.

Two regions were recognized in hamster's ovary parenchyma, cortex and medulla. Cortex was a loose connective tissue with type I collagen fibers and blood vessels. Different types of ovarian follicles were observed in cortex which numbers of follicles are shown in Tables 2 and 3. Medulla was occupied by a loose vascular connective tissue. There were very low connective tissue fibers in medulla.

Table 1 shows biometric parameters of left and right hamster's ovary. Right hamster ovary is bigger than left hamster ovary in all of the biometric factors but only thickness of hamster's ovary (between right and left) has significant difference ($P<0.05$).

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Figure 3. Cortex of Hamster's ovary. C, Covering epithelium that it is simple cuboidal ept., TA, tunica albuginea. BV, Blood vessels. (PAS staining, ×1000)

Table 1. Biometrical results of right and left hamster ovaries by vernier (macroscopic).

Ovary	Length mm	Thickness mm	Width mm
Right	5.08 ±1.1326	3.05 ± 0.5	1.70 ± 2.68
Left	4.68 ±1.572	2.214 ± 0.481	0.398 ± 2.44

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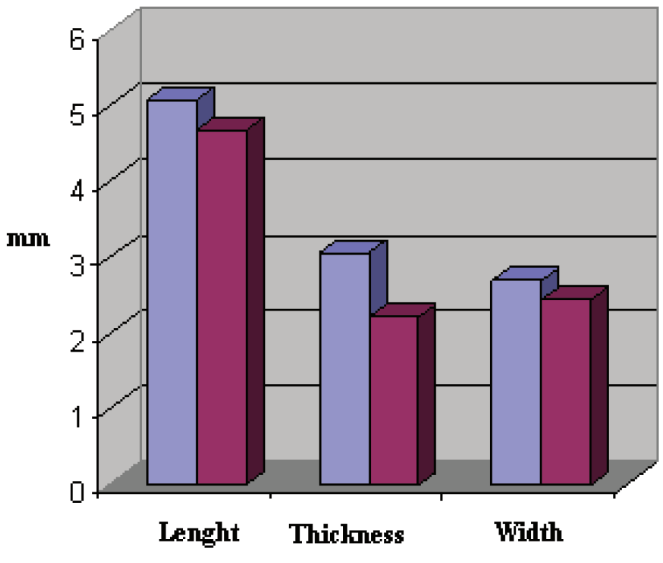

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Figure 4. Comparative diagram of biometric hamster's ovaries parameters. Blue column is right ovary and red column is left ovary.

Table 2. Number of different types follicles in right ovary of hamster.

Type of fullicle	Number	Percent
Primordial follicle	1627.33 ± 305.6049	77.78
Primary	307.33 ± 56.7 3	14.69
Secondary	140 ± 60.63	6.69
Tertiary	9 ± 0	0.43
Graafian	8.33 ± 0.57	0.38
Total	2091.99	100

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Table 3. Number of different types follicles in left ovary of hamster.

Type of fullicle	Number	Percent
Primordial follicle	1707.67± 62.67	78.03
Primary	300 ± 87.246	13.71
Secondary	161.67 ± 58.045	7.36
Tertiary	9.66 ± 2.33	0.44
Graafian	9.33 ± 0.57	0.43
Total	2188.33	100

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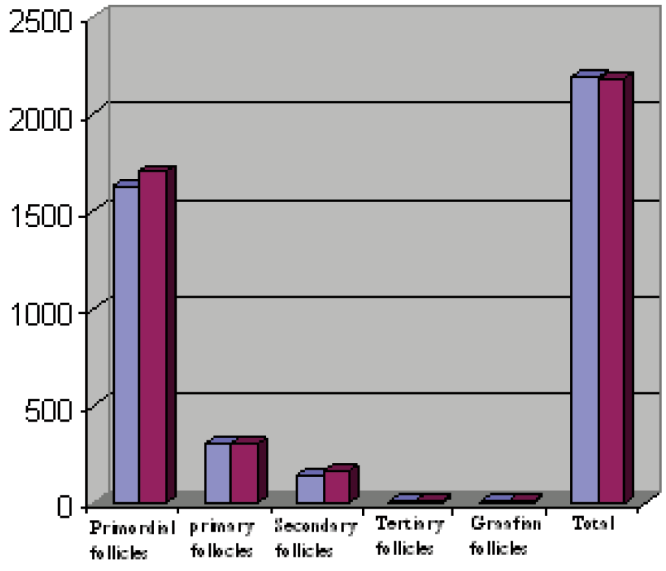

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Figure 5. Coparative diagram of number of different types follicles of left and right hamster's ovaries. Blue column is follicles of right ovary and red column is left ovary's follicles.

Table 4. Size of different types of follicles in ovary of hamster. (If word of a cell to be in another cell, cells don't have any significant different.)

Type of Fullicle	Size (µm)
Primordial	18.375 ± 1.063 a
Primary	32.531 ± 1.540 b
Early Secondary (two layer cell)	42.656 ± 0.749 c
Late Secondary	58.968 ± 1.361 def
Tertiary	62.343 ±1.900 edf
Graafian	66.093 ± 1.346 fde

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Table 5. Size of oocytes in different types of follicles in ovary of hamster . (If word of a cell to be in another cell, cells don't have any significant different.)

Type of Fullicle	Size (µm)
Primordial	5.62 ± 0.668 a
Primary	11.98 ±1.480 b
Early Secondary (two layer cell)	22.26 ± 0.870 c
Late Secondary	39.98 ± 4.34 d
Tertiary	42.38 ± 2.604 ef
Graafian	43.678 ± 0.366 fe

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Primordial follicles had the maximum number (74.23%) and graafian follicles had the minimum number (0.38%), in each two hamster ovaries, left and right. Numbers of graafian follicles of left ovary were more than graafian follicles of right ovary but there were no significant difference ($P<0.05$) between different type of follicles of right and left ovary. Therefore, it shows that hamster's left and right ovaries have equals activities (Table 2 and 3).

Table 4 shows size of different types of follicles in ovary of hamster. Observation of this table showed which primary follicles are smaller than other ovarian follicles and graafian follicles are larger than other follicles. Significant difference ($P<0.05$) was observed between size of primordial follicles, small secondary follicles, large secondary follicles, tertiary follicles, and graafian follicles.

Also, there is significant difference between size of small secondary follicles and large secondary follicles. The information of this table and figure 4 showed that largest size follicles are tertiary and graafian follicles. Significant difference ($P<0.05$) is observed between these follicles and other hamster ovarian follicles but there is no significant difference between size of tertiary and graafian follicles.

Table 5 shows size of oocytes in different types of follicles in hamster ovary. Results of this table showed that primordial follicles oocytes are smaller than other ovarian follicles oocytes. There is significant difference ($P<0.05$) between size of primordial follicle oocytes and small secondary follicles oocytes.

Also, a significant difference is observed between secondary follicles oocytes and large secondary follicles oocytes. There is no significant difference

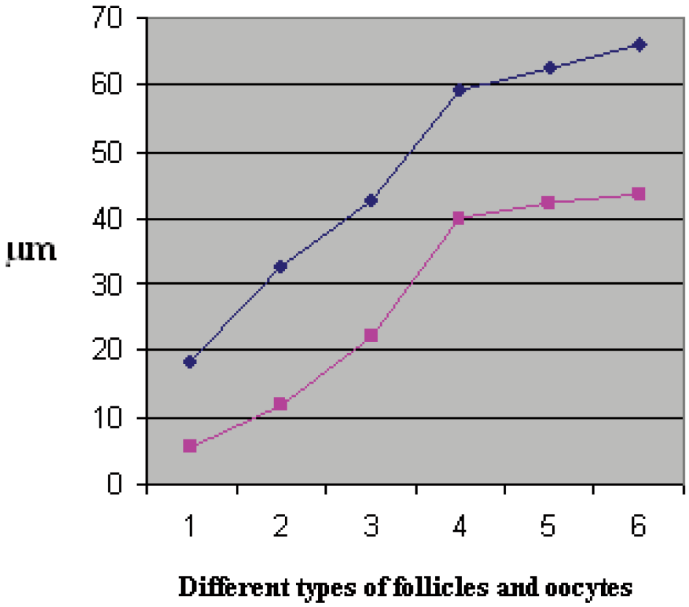

Figure 6. Comparative diagram of size of different types follicles with size of oocytes in different types follicles in hamster's ovaries (Blue curve is size of follicles and red curve is size of oocytes).

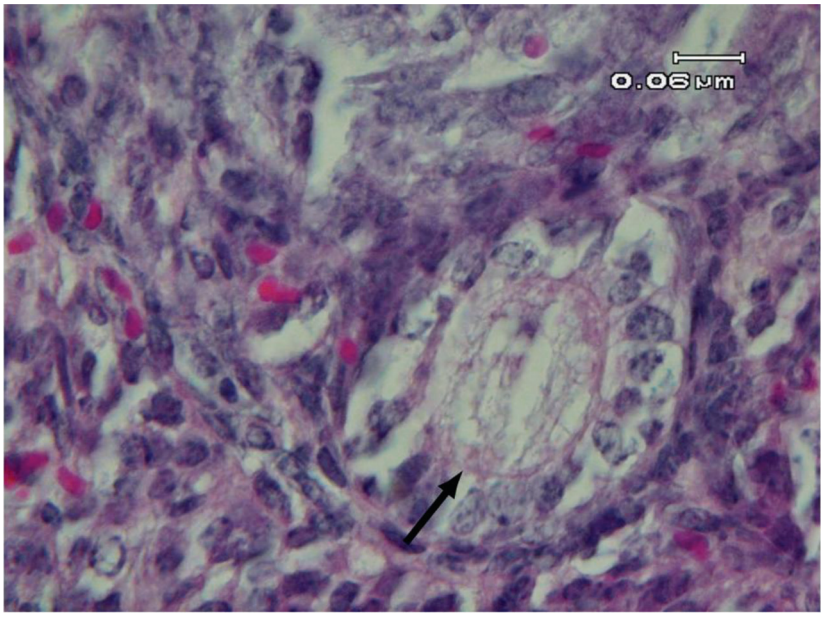

Figure 7. An early secondary follicle with a layer cuboidal cell. Tip of arrow is showing early zona pellucida.(PAS staining, ×1000).

(P<0.05) between large secondary follicles oocytes, tertiary follicles oocytes, and graafian follicles oocytes. Therefore, the results of this study show that hamster's ovary get maximum size in large secondary follicles (Figure 5). Also, it was appeared that increase of size of follicles similarly shows the increase in the size of its oocytes.

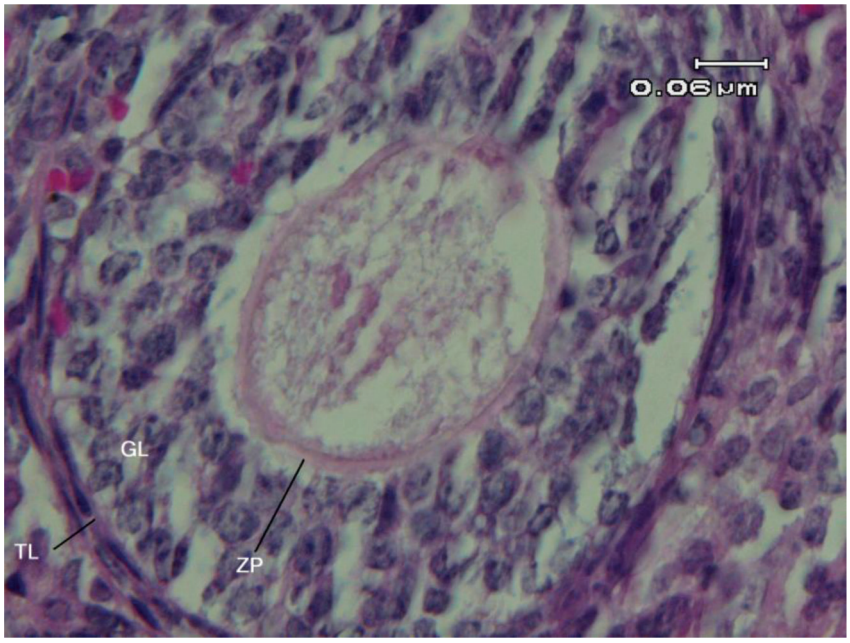

Figure 8. Late secondary follicle, Fuulicular cells are differentiating and two cells layers are developing. ZP, Zona pellucida, TL, Theca layer , GL,Granulosa layer. (PAS staining, ×1000).

PAS staining revealed that zona pellucid was absent in primordial follicles but it can be observed in early secondary follicles (follicles with single cuboidal cell) (Figure 3).

Observations of this study showed that follicular cells were differentiated in late secondary follicle to two layer cells, granulosa and theca layer firstly. Future developing of theca layer continues in tertiary and graafian follicles. Theca layer cells were cells with euchromatin and cigarette-shaped nucleus. Later, it formed two different cells layers as theca interna and theca externa (Figure 4).

4. Discussion

The Syrian or golden hamster "Mesocricetus auratus" (waterhous, 1893) is one of the best established experimental animals and probably among the most popular pets in the Western world. The history of the golden hamsters' capture and domestication is well documented by Murphy (1985) and Gattermann (2000) [6, 9]. Almost, all pets and laboratory colonies of the golden hamster of the world are originated from the wild by I. Aharoni of the Department of Zoology, Herbrew University, Jerusalem, who excavated a mother with 11 pups near Aleppo, Syria on 12 April 1930. From this litter, only three male animals and one female survived and formed the source of a breeding stock [6]. Number of ovarian follicles and condition of oocytes is very important and they can be used for evaluation of animals' fertility. Number of primordial follicles has variation in different species animals. For example, they were reported 2,500 in the mice, 10,500 in the cattle, and 210,000 in the swine [14]. Number of follicles in cyclic animals is more than in uncyclic animals. For example, they were evaluated 12,663 in cyclic river buffalo and 10,132 in uncyclic buffalo [15].

Cushman et al reported that natal follicle count in beef cows and heifer is influenced by weight and age. They believe that number of natal follicle (graafian follicle) increase to 5 years of age and then begins to decline. This may indicate that a decrease in fertility due to decline of ovarian reserve may begin earlier than previously through in beef cows [3].

Ozdemir et al (2005) reported that length, width, and thickness of the ovary of porcupine (Hystrix cristata) were almost the same for right and the left ovary [11].

In the present research, size of ovaries and number of different types of ovarian (left and right) follicles of golden hamster were measured. This research results showed that there is no significant difference ($P<0.05$) between the size of hamster's ovaries (right and left). Also, the evaluations indicated that there is no significant difference ($P<0.05$) between the number of graafian follicles of right and left hamsters ovary. Therefore, right and left ovaries of hamsters have equal activity for ovulation, contrary to many mammals for example cow, horse, and etc [4, 10].

Sadrkhanlo et al reported that primordial follicles had maximum number and graafian follicles had minimum number in buffalo [12]. The present research indicated that primordial follicles were abundant in number and developed follicles were decreasing ordinally. Graafian follicles had the minimum number.

There was no significant difference ($P<0.05$) between the number of graafian follicles and tertiary follicles but there was significant difference ($P<0.05$) between graafian and tertiary follicles with primordial, primary, early secondary, and late secondary follicles. Number of graafian follicles was approximately equal to the number of pups in each parturition [6]. Williams and Erickson reported that primordial follicles are smallest and natal follicles are largest follicles in woman. Graaffian follicles were largest follicles in hamster and no significant difference was observed between the size of tertiary and graafian but there was significant difference between sizes. In addition, they showed that oocytes in secondary oocyte achieved their maximum size because oocyte stored maximum cytoplasm nutrient in secondary follicles size [16]. In this research, oocytes presented in the late secondary follicles had maximum size. There was no significant difference between size of oocytes presented in graafian, tertiary, and late secondary follicles but there was significant difference in size of oocytes presented in grafiian follicles with oocytes presented in primordial and late secondary follicles.

First sign of zona pellucida was observed in primary follicles (follicles with one cuboidal cell layer) which this result is agreement to other reports on other mammals [4, 7].

Theca layer cells were observed in the late secondary follicles firstly and then it was developed as two different cells layers (theca interna and theca externa) in tertiary and graafian follicles.

Generally, the results of this research show that histomorphometry of hamster ovaries has many resemblance to human or other mammalian ovaries, except number of follicles and oocytes. The reason of low number of hamster ovaries in comparison with other mammals is short living period of this animal.

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