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SIZE DISTRIBUTION OF LUTEAL CELLS ISOLATED FROM MID-LUTEAL BOVİNE CORPUS LUTEUM

İneklerin Mid-Luteal Korpus Luteumlarından İzole Edilen Luteal Hücrelerin Büyüklük Bakımından Gösterdikleri Dağılım

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SUMMARY

This study was conducted in order to examine the size distribution of luteal cells isolated from mid-luteal bovine corpus luteum. The cells were isolated from heifer ovaries by collagenase digestion. To identify steriodogenic luteal cells, aliquots of cells were fixed in 1% paraformaldehyde and stained for 3β-hydroxysteroid dehydrogenase (3β-HSD) activity. The size distribution of freshly dissociated cells, stained positively and negatively, was determined by measuring 784 cells using a x20 objective on a light microscope. Stained cells ranged from 12 to 42 μm in diameter. 3β-HSD positive cells smaller than 12 μm were never observed, and negative cells larger than 25 μm were rare. All cells bigger than 33 μm were stained positively for 3β-HSD. The percentage of small luteal cells (between 12 μm and 22 μm) and large luteal cells (≥ 23 μm) were 79% and 21%, respectively. The percentage of unstained cells was 31% in small luteal cells and 13% in large luteal cells.

Key Words: HSD, Bovine, Corpus luteum, Luteal cells.

ÖZET

Bu çalışma mid-luteal inek korpus luteumlarından izole edilen luteal hücrelerin, büyüklük bakımından gösterdikleri dağılımı incelemek için yapıldı. Hücreler, düve ovariumlarından kollegenaz enzimi kullanılarak izole edildi. Steroid hormonu üretebilme kapasitesine sahip olup olmadıklarının belirlenmesi için hücreler, önce %1'lik paraformaldehid ile fiksasyona tabi tutulup ve ardından da 3β-hydroxysteroid dehydrogenaz (3β-HSD) enziminin aktivasyonu bakımından test edildi. Pozitif ve negatif olarak boyanan hücrelerin büyüklük bakımından gösterdikleri dağılımın analizi, x20 büyütmeli objektif yardımı ile 784 hücrenin çapı ölçülerek tayin edildi. Pozitif olarak boyanan hücrelerin çapının 12 μm ile 42 μm arasında bir dağılım gösterdiği saptandı. 12 μm'den daha küçük çapa sahip olan hücrelerin tümünün 3β-HSD aktivitesi bakımından negatif olduğu ve çapı 25 μm den daha fazla hücreler arasında ise boyanmayan hücrelerin az olduğu gözlendi. Çapı 33 μm den büyük olan hücrelerin hepsi 3β-HSD aktivitesi bakımından pozitif olarak boya almıştır. İncelenen hücrelerin %79'unu küçük luteal hücreler (çapı 12 ile 22 μm arasında) ve % 21'ini ise büyük luteal hücreler (çapı 23 μm veya daha büyük) oluşturmaktaydı. Boya almayan hücrelerin boya alanlara oranı küçük luteal hücrelerde % 31 iken bu oran büyük luteal hücrelerde ise % 13 olarak tespit edildi.

Anahtar Sözcükler: HSD, İnek, Korpus luteum, Luteal hücre.

INTRODUCTION

The corpus luteum (CL) is a temporary endocrine gland that secretes progesterone during the luteal phase in the oestrous cycle (10) and pregnancy (8). The CL develops after the collapse of the follicle at ovulation. In the bovine, mass and progesterone production of the CL increase rapidly between days 3 and 8 of the cycle and remain relatively constant until day 16, when regression begins (6).

Corpus luteum cells have been classified into 3 groups based on site; non-steroidogenic cells (≥12 μm), small luteal cell (12-22 μm) and large luteal cells (≥23 μm) (12). Steroidogenic cells change to dark blue in colour whereas non-steroidogenic cells stay unchanged. The microscopic characteristics of the small and large bovine luteal cells have also been described in detail by Hansel et al (4). They reported that the small cells have a peripherally located, deeply lobuled, cup-shaped nucleus with densely staining nucleoplasm and mitochondria arranged in the arc opposite the nucleus. However, large luteal cells have round, central

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nuclei with a distinct nucleolus and mitochondria surrounding the nucleus.

The origin of steroidogenic luteal cells in the corpus luteum has been a controversial topic. Large luteal cells are thought to develop from the granulosa layer of the preovulatory follicle, while small luteal cells are believed to develop from the thecal layer (1). It has also been demonstrated that small luteal cells could develop into large luteal cells as the corpus luteum ages.

The size distribution of luteal cells from mid-luteal corpus luteum has been reported previously in sheep (3,11) and monkey (5). The results from their studies demonstrate the presence of cells that are compatible in site with luteal cells but not having steroidogenic activity. The large luteal cells produce several times more progesterone on a per-cell basis than do small luteal cells (12). It is therefore interesting to examine the size distribution of both steroidogenic and non-steroidogenic cells so as to understand whether these differ in quantities of progesterone production which may be partly due to different quantities of non steroidogenic luteal cells in both small and large luteal cell preparations.

MATERIALS and METHODS

All chemicals were obtained from Sigma. Bovine ovaries were collected immediately after slaughter from the local abattoir and transported to the laboratory in ice-cold phosphate-buffered saline. Ovaries were judged to be at mid-cycle by the criteria of Ireland et al. (7). Luteal cells were isolated from mid-luteal heifer ovaries by collagenase digestion as described by O'Shaughnessy and Wathes (9). To identify steriodogenic luteal cells, the cells were stained for 3β-HSD activity according to the method of Bao et al (2). In brief the cell suspension (0.5 ml) was fixed in 1% paraformaldehyde (4 ml) for 20 min. Cells were then centrifuged at 150 g for 5 minutes and resuspended in 1 ml staining solution (0.1 M phosphate buffered saline (PBS) containing 0.1 % BSA, 1.5 mM NAD, 0.25 mM nitro blue tetrazolium and 0.2 μM5β-androstene-3β-ol-17 one, which was prepared from 8 mM stack solution in ethanol). Cells were then incubated in dark in a metabolic shaker at 37 °C for 4 hours. After incubation the cells were centrifuged and then resuspended in PBS.

The size distribution of freshly dissociated cells (stained positively and negatively) was determined by measuring 784 cells using an ocular micrometer and x20 objective on a light microscope (Olympus, CK 2). Measurements were carried out in randomly selected areas. In order to eliminate individual differences between the ovaries, cells isolated form 3 different ovaries were used.

RESULTS

Microscopic examination of cell suspensions stained for 3β-HSD activity provided identification of cell with steroidogenic capacity. The size distribution of freshly dissociated bovine luteal cells stained for 3β-HSD activity is shown in Figure 1. The data is expressed as the percentage of total cells in each site range. 3β-HSD positive cells covered a wide spectrum of sizes ranging from 12 to 42 microns. The small cells (<12 μm) did not stained for 3β-HSD and were generally present in clumps. Most of the 3β-HSD-positive cells were ranged between 12 μm and 18 μm in diameter. 29% of luteal cells in total did not stained for 3β-HSD activity.

The majority of cells not stained for 3β-HSD activity were smaller than 20 μm in diameter. All cells bigger than 33 μm were showed a positive stain for 3β-HSD. The peak of stained cells reached its maximum high at about 15 μm in diameter. The percentage of small and large luteal cells were 79% and 21%, respectively. The percentages of unstained cells were 31% and 13% in small luteal cells and large luteal cells, respectively. Therefore, small luteal cells have twice more unstained cells than do the large luteal cells.

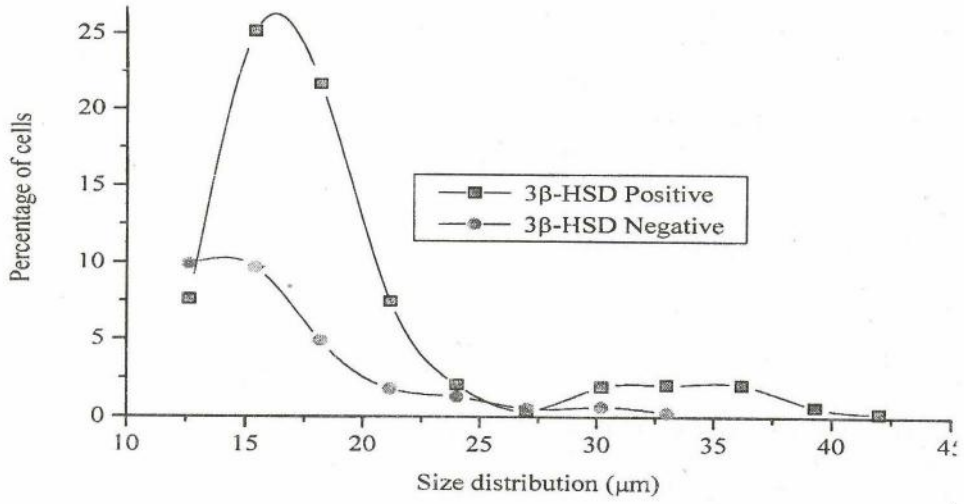

Figure 1. Size distribution of luteal cells stained for 3β-HSD
Grafik 1. 3β-HSD için boyanmış luteal hücrelerin büyüklüklerine göre dağılımı.

DISCUSSION

Results from the present study showed that all cells smaller than 12 μm and some of the cells up to 32 μm in diameter did not stain for 3β-HSD activity. This is in agreement with previous published works carried out using ovaries from other species. For instance, the presence of 3β-HSD-negative cells in the luteal cell size range has been demonstrated in both monkey (5) and sheep (3). In both studies, small luteal cells were containing more unstained cells than large luteal cells. In contrast to the present study, lowest size limit for 3β-HSD-positive cells was 10 μm in both studies. It was found as 12 μm in our study. The difference between the studies in terms of the lowest luteal cell size limits might be due to the differences of the animals which were used in the experiments. All of luteal cells (>12 μm in diameter) supposed to be steroidogenically active. Therefore, the presence of unstained cells which are compatible with luteal cell size in diameter suggests that the mid-luteal corpus luteum may have other cells or cell kind, within the range of luteal cell size but originating and/or functioning differently.

It is also believed that luteal cells originate either from granulosa or thecal cells of ovarian follicles after ovulation. The origin of bovine luleal cells have been studied by Alila and Hansel (1) using a different method in which specific monoclonal antibodies to theca and granulosa cells of the pre-ovulatory follicle were used as markers for the cells in the corpus luteum of the oestrous cycle and pregnancy. They showed that granulosa antibodies were bound mainly to large luteal cells, while the thecal antibody are bound primarily to small luteal cells. However, 10-12 days after oestrous, 28% and 6% of small and large luteal cells, respectively were not labelled by either antibody. Similarly, in our study %31 of small luteal cells and %13 of large luteal cells both dissociated from mid-luteal ovaries were not stained for 3β-HSD. Thus, the similarity between the two studies suggest that the cells were not labelled by either antibody in the work of Alila and Hansel (1) and those cells were not stained for 3β-HSD activity in our study may be the same kind of cells both having similar origins and not having steroidogenic activity. This is another evidence that indicate the presence of other cell types, which are compatible in size with the luteal cells but not having any steroidogenic activity.

Results from the present study have also shown that the small luteal cells contained twice as more unstained cells thando the large

luteal cells. This result could partly explain why the same number of large luteal cells secrete larger amounts of progesterone than do the small luteal cells (12). Thus it can be concluded that at least some of the differences in the quantity of progesterone production between large and small luteal cells might have arisen from the presence of different quantities of non-steroidogenic cells in those cell preparations.

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