Experimental
&
Clinical Article

Obstetrics; *Maternal-Fetal Medicine and Perinatology*

The Ultrastructure of the Zona Fasciculata Layer of Suprarenal Gland During Pregnancy

Özgür EROĞLU[1], Neşe LORTLAR[2], Sevtap KILIÇ[1], M Tahir HATİPOĞLU[2] , Suna ÖMEROĞLU[2]

Ankara, Turkey

OBJECTIVE: Increased estrogen levels affect the suprarenal gland cortex, thus raising the adrenocorticotropic hormone (ACTH) during pregnancy. This study aimed to examine possible structural changes in the suprarenal gland cortex in relation to the hormonal fluctuations during pregnancy.
STUDY DESIGN: The suprarenal glands of 6-day, 12-day and 18-day pregnant rats were removed and compared with those of the control group under electron microscope.
RESULTS: Lipid droplets in zona fasciculata cells were observed to increase significantly in number and volume, enlarged mitochondria, active Golgi region in comparison to the control groups on 6th and 12th days of pregnancy. On day 18 were observed to lipid droplets were seen to decrease and similar characteristics with the control group.
CONCLUSION: In conclusion, with the increased estrogen hormone of pregnancy, various structural changes were observed in the cortex of suprarenal glands as related to mutual interaction among secreted hormones from the suprarenal glands, hypophysis and placenta.

Key Words: Pregnancy, Suprarenal gland cortex, Zona fasciculata, Ultrastructure

Gynecol Obstet Reprod Med;14:3 (140 - 144)

Introduction

Pregnancy affects a woman's entire endocrine system. Although this is mainly due to the increased metabolic load that accompanies pregnancy, it is also associated with the effect of the hormones secreted by placenta on basically the hypophysis and other endocrinal organs.[1] As a result, the amounts of estron (E_1) and estradiol (E_2) increase by approximately 100 times. Increased estrogen levels then affect the suprarenal gland cortex through the hypothalamo-hypophysis tract, thus raising the adrenocorticotropic hormone (ACTH). This may cause structural changes in the parenchymal cells on the suprarenal glant cortex and an increase in their dimensions.[2]

The three-fold increase of aldosteron secretion in pregnant women, which reaches the highest level at the end of pregnancy, is a significant result indicating the existence of structural and functional changes in the zona glomerulosa of the suprarenal gland cortex. Similarly, the rise in the level of glucocorticoids secreted in the zona fasiculata layer of the suprarenal gland cortex during pregnancy causes an increase in the maternal amino acid mobilization. This may be a possible route to supply the necessary amino acid for the fetal tissue.[1]

Physiological, histological and biochemical studies mainly indicate that the structural and functional changes in suprarenal glands which occur particularly at the onset of pregnancy disappear at the end. For this reason, this study chose days corresponding to the beginning, middle and end of pregnancy in rats, and aimed to examine changes in suprarenal gland cortex occurring on these days focusing particularly on the functional organelles in steroid hormone synthesis (smooth endoplasmic reticulum, mitochondrion, Golgi complex).

Material and Method

Wistar type mature female rats weighing 200-250 g on average, were used in the present study. Female rats with a vaginal plaque were admitted to the study on their day 0 of pregnancy, and pregnant groups of 6th, 12th and 18th days were performed. Non-pregnant rats were separated to be used as control groups on these days. Rats in all groups were sacrificed at the end of their pregnancy. According to the epoxy resin embedding method [3] suprarenal glands were removed and fixed

[1]*Dr. Zekai Tahir Burak Women Health Resarch Hospital Cebeci, Ankara*

[2]*Department of Histology and Embryology, Gazi University Faculty of Medicine, Ankara*

Address of Correspondence: *Sevtap Kılıç*
Süleymanbey Sok. 29/10 PK:06570
Maltepe, Ankara
sevtapkilic@gmail.com

Submitted for Publication: *02.09.2008*
Accepted for Publication: *29.09.2008*

in 2.5% gluteraldehyde solution in phosphate buffer. Following the primary fixation, suprarenal glands were fixed for a second time with 1% osmium tetroxide with phosphate buffers in 1/15 M. The tissue samples were treated with series of alcohol and buried in a solution which consisted of Araldite CY 212 and dodecenyl succinic acid and benzyl dimethylamine. The semi-thin sections taken from the resultant blocks were stained with toluidine blue and examined under BH2 photo light microscope (Olympus, Germany). The thin sections (0.5) were stained with lead citrate and uranyl acetate and examined under Carl-Zeiss EM-900 electron microscope (Carl Zeiss EM-900, GERMANY).

Results

Light and electron microscopic evaluations were conducted by comparing the zona fasciculata layer of the suprarenal gland cortex in the control and pregnant groups.

The suprarenal glands of the semi-thin cross-sections of the control group were noted to be externally covered and the internal layer encapsulated by a cell-rich capsule. Just below the capsule was observed the zona glomerulosa layer, formed with circular or glomerular lining of pyramid-shaped cells with round nuclei. Just below this was observed the zona fasciculata layer, poligonal in shape and with round nuclei and separated by sinusoidal capillary (Figure 1). In the electron microscopic microphotographs of same group, round shape nucleus of zona fasciculata layer, the existence of varying sizes of lipid droplets was noted. (Figure 2). Cells were noted with oval and/or round shape tubular crista mitocondrions and a widespread smooth endoplasmic reticulum, attached to each other with interdigitations.

Differently from the control group, a considerable increase in lipid droplets in zona fasciculata layer was observed in the semi-thin sections of the suprarenal gland cortex of 6-day pregnant rats (Figure 3). It was noted that the lipid droplets of different densities particularly the cells of the zona fasciculata layer formed vacuoles in irregular dispersed combinations, giving the cells a foamy appearance. In the zona fasciculata, the cells were noted to be filled with irregularly spaced lipid droplets and the lipid contents were observed in the form of cellular structures (lamellar matter). The smooth endoplasmic reticulum tubules were in the form of narrow tubules filled with low density secretion and lysosomes were noted (Figure 4).

In the semi-thin sections of 12-day pregnant rat group, as similar to the 6-day pregnant group, vacuoles were formed enabling the lipid droplet cells in the zona fasciculata layer to appear in light color, as different from the control group (Figure 5). In the electron microscopic examination, in the fasciculata layer, it was noted that the Golgi region was fairly active; the mitochondrions were enlarged and connected. The smooth endoplasmic reticulum was noted to have substantially enlarged (Figure 6).

In the 18-day pregnant rat group, the increasing lipid accumulation observed on the 6th and 12th days in the zona fasciculata had disappeared and smooth endoplasmic reticulum tubules of the cells were seen to be fairly narrow (Figure 7,8)

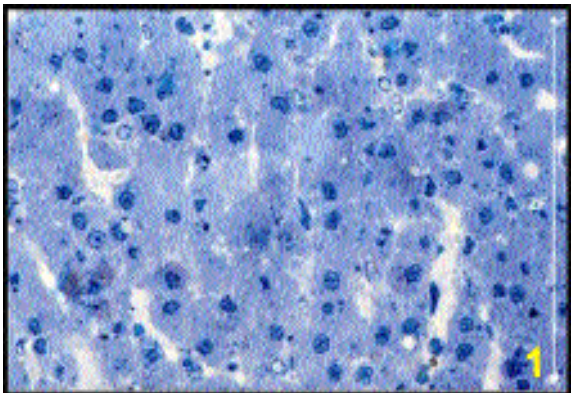

Figure 1: Zona fasciculate (ZF) layer of control group. The cells display a longitudinal beam-shaped organization. Some sinusoidal type capillary(s) are observed. (Toluidin blue, x 400)

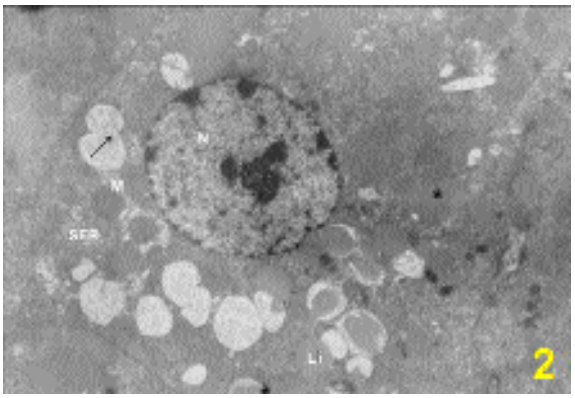

Figure 2: Zona fasciculate layer of the control group. It can be observed that lipid droplet (Li) secretion content in the cells melted in some cells and a membrane-like structure (↑) is formed. Irregularly spaced lipid droplets (*), round, oval or long shaped and mostly tubular-crysta mitocondrions (M), and dispersed widespread smooth endoplasmic reticulum tubuls (SER) are noted (Uranil Acetate-Lead Citrate, x 3000).

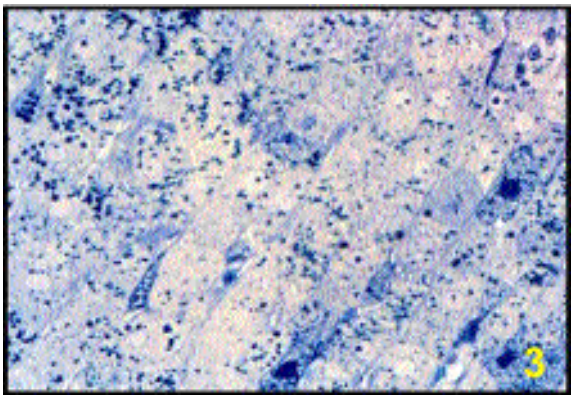

Figure 3: The appearance from the zona fasiculata layer in a 6-day pregnant rat. It can be observed that light colored cytoplasmic cells with round nucleus formed lines which display a beam-like organization. A dispersed bubbly appearance and density difference are found in cells (↑). (Toluidin blue, x 400).

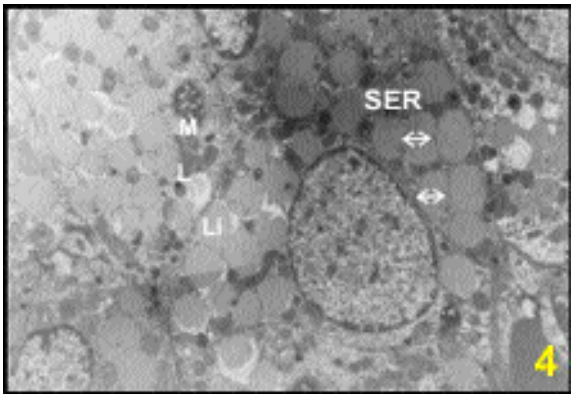

Figure 4: This displays cells filled with lipid droplets (li) in the zona fasciculate layer and lipid content gained cell-like structures (↔). Mitocondrions (M) are seen in varying sizes, round, oval or long in shape. Smooth endoplasmic reticulum tubuls (SER) are seen as narrow with low density secretion. Dispersed lysosomal structures are noted (L) (Uranyl Acetate-Lead Citrate, x3000)

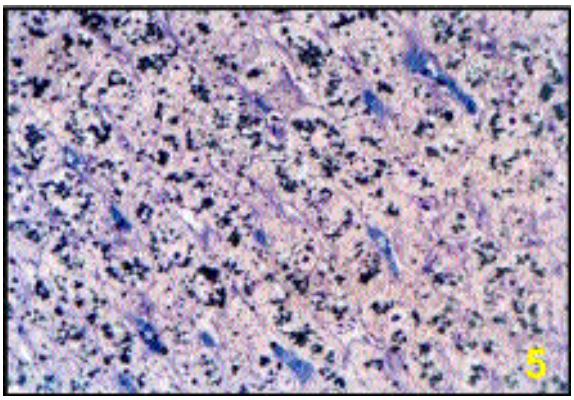

Figure 5: This shows a 12-day pregnant rat's suprarenal gland cortex. Polygonal cells with round nuclei form longitudinally organized cell cordons separated by sinusoidal capillars (S) (Toluidin blue, x 200).

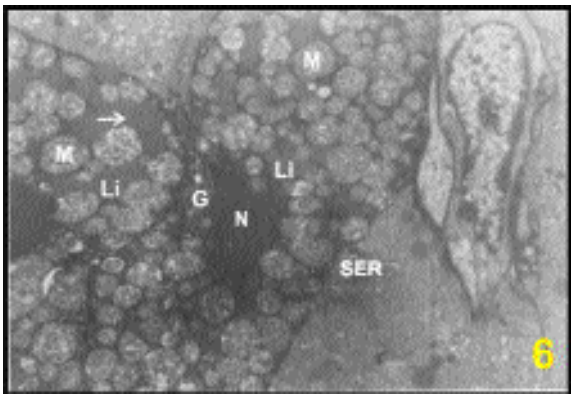

Figure 6: Zona fasciculate layer of the 12-day pregnancy group. Cells can be discerned with light and dark cytoplasm, dense nucleus (N), lower number of lipid droplets (Li), enlarged mytocondria (M) and irregularly spaced combining (↑). Smooth endoplasmic reticulum (SER) is in the form of narrow tubuls in very low number.Golgi region is highly active (G) (Uranyl Acetate-Lead Citrate, x 3000)

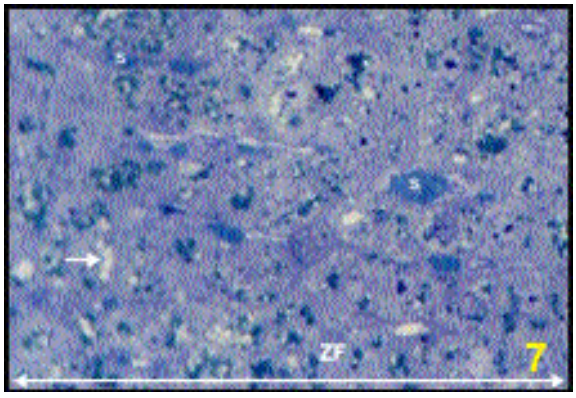

Figure 7: Zona fasciculata layer (ZF) of suprarenal gland cortex of 18-day pregnant rats are noted to form irregularly spaced sinusoidal capillary (S), masses formed by lipid granules (↑) (Toluidin blue, x 100).

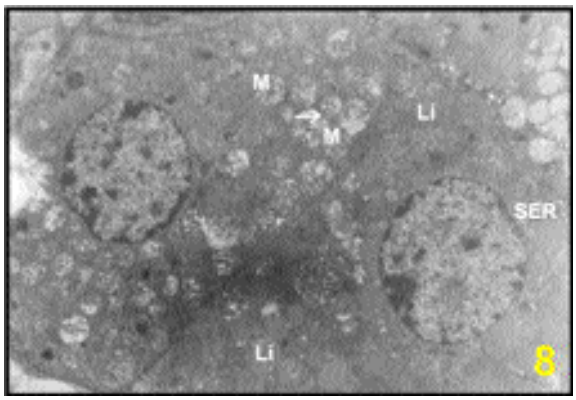

Figure 8: Zona fasciculate layer of the 18-day pregnant group is seen. It is noted that mitocondrions (M) are quite large, combined irregularly spaced (↑) and degenerated, and that lipid droplets (Li) are scarce in some cells and dense in some others, smooth endoplasmic reticulum tubuls (SER) are rather narrow. (Uranyl Acetate, Lead Cytrate x 3000)

Discussion

The weight, dimensions, parenchymal cell number and volume demonstrate diversity based on species, age, gender, and physiological conditions such as pregnancy or stress. The physiological, histological, and biochemical studies in literature indicate that the structural and functional changes in the suprarenal gland disappear at the end of pregnancy. The ACTH levels which interact with increasing estrogen hormones during pregnancy and which control the suprarenal glands may form changes in the weight, volume, and structure of these glands.

The most significant effect of estrogen on suprarenal gland is observed during pregnancy. At the beginning of pregnancy, both plasma level of estron (E_1) and estradiol (E_2) increase 100 times. Increased estrogen levels then affect the suprarenal gland cortex through the hypothalamo-hypophysis tract, thus

raising the adrenocorticotropic hormone (ACTH). This may cause structural changes in the parenchymal cells on the suprarenal gland cortex and an increase in their dimensions[4,5]

The effects of pregnancy and ACTH on suprarenal glands were studied. Distinct lipid accumulation in the layers of suprarenal gland cortex was determined. This increased in the A acyl cholesterol transferase enzyme which performs in the synthesis of cholesterol esters synthesis, stored as lipid droplets but that lipid droplets caused an accumulation in the parenchymal cells of the suprarenal gland cortex.[6]

Long-term ACTH led to hypertrophy in the zona glomerulosa parenchymal cell, and an increase in the number of smooth endoplasmic reticulum tubules.[7]

In our study, particularly distinct in the 6 and 12 days of pregnancy, a lipid accumulation was observed which was noted in the zona fasciculata layer cells of rat suprarenal glands.

In the stereological and functional studies that Nowak et al. conducted on the suprarenal gland cortex in pregnant hamsters, they recorded that the weight of suprarenal glands reached its highest point on the 5th day of pregnancy, and that these values fell significantly towards the end of pregnancy. They stated that there is no remarkable change during the entire pregnancy in the surface density of mitochondrial crista and smooth endoplasmic reticulum. However, they noted a clear increase in the 11-beta hydroxylase, 3-beta-hydroksysteroid dehydrogenase / isomerase activity on the 5 and 10 days of pregnancy. In our study, structural changes in suprarenal glands during the beginning, middle and end of rat pregnancy which lasts 21 days were studied. In some animal species, including humans, functions of suprarenal glands are associated with the Corticosteroid Binding Globulin levels (CBG). The CBG levels of female rats are higher than those of male rats. It is possible that this change, which occurs due to estrogen hormon, may increase to reach its maximum values during pregnancy.[2,8]

The increase in the maternal steroid secretion during pregnancy has a supportive function in late pregnancy for both maternal and fetal systems and that this basically occurs through maternal volume increase and finally fetal homeostasis. Maternal adrenal secretion maintains the normal increase in maternal plasma volume and fetal cortisol and indirectly supports fetal arterial oxygen tension, blood pressure, and adrenal secretion.[9]

Estradiol (E_2) injection can cause significant increase in the activity of the adrenal cortex and medulla. Bilateral ovariectomy can result in a decrease in the activity of the adrenal cortex[10] whereas infusion of IGF-I in late gestation resulted in a marked hypertrophy of the steroidogenic and adrenaline-containing cells of the fetal adrenal in the absence of changes in the mRNA levels of adrenal steroidogenic or catecholamine-synthetic enzymes or in fetal plasma cortisol concentrations.[11]

Adrenal neoplasm often causes to Cushing's syndrome which occurs during pregnancy. Adrenal gland tumors occasionally respond to luteinizing hormone (LH) or human chorionic gonadotropin (hCG).[12]

In conclusion, with the increased estrogen hormone of pregnancy, various structural changes were observed in the cortex layer of suprarenal glands as related to mutual interaction among secreted hormones from the suprarenal glands, hypophysis and placenta.

Surrenal Bez Zona Fasikülata Tabakasının Gebe Sıçanlarda Ultrastruktür Değerlendirmesi

Gebelik boyunca artan östrogen surrenal bez korteksini ve adrenocorticotropic hormon (ACTH) duzeylerini etkiler.Bu çalışmanın amacı gebelik boyunca hormonal dalgalanmalara bağlı olası surrenal bezde oluşabilen olası yapısal değişiklikleri araştırmayı hedeflemiştir.

Surrenal bez gebeliğin 6. 12. 18. günlerinde dokudan ayrıldı electron mikroskopik değerlendirmeler kontrol grubuyla karşılaştırıldı.

Gebe farelerin 6. ve 12. gün değerlendirmelerinde kontrol grubuna göre zona fasikülatalarında sayı ve volümleri artmış lipid dropletleri, mitokondrodionlarında genişleme ve aktive golgi aygıtları izlendi.gebeliğin 18. gününde lipid dropletlerinde azalma ve kontrolle benzer karakteristikler izlendi.

Sonuç olarak gebeliğin artırdığı östrogen hormonu ile hipofiz, plesenta,surrenal bezden salınan hormonlar arasındaki ilişki sebebiyle surrenal bez kortexinde çeşitli yapısal değişklikler izlenmektedir.

Key Words: Gebelik, Surrenal bez korteksi, Zona fasciculata, Ultrastruktür

References

1. Junquira LC, Carniora J. Basic Histology. Tenth Edition. The Mc Grow- Hill Company.2003; 413-9
2. Nowak M., Rebuffat P, Mazzocchi G, Nussdorfer GG, Malendowicz LK: Gestational changes in hamster adrenal cortex: morphometric and ultrastructural stereologic studies. Cell Tissue Res 1989; 256: 241-6
3. Luft JH. Improvements in epoxy resin embedding methods. The Journal of Biophysical and Biochemical Cytology. 1961; 9: 409-14
4. Hill PA, Coghlan JP, McDougall JG, Ryan GB. Structural studies of the adrenal zona glomerulosa and renal juxtaglomerular apparatus in pregnant sheep. Cell Tissue Res. 1983; 232:177-87
5. Hill PA, Coghlan JP, Scoggins BA, Ryan GB. Structural

and functional studies of the adrenal cortex and renal juxtaglomerular apparatus in pregnant sheep subjected to sodium depletion or loading. Pathology 1984; 16: 285-90.

6. Nussdorfer GG. Cytophysiology of the adrenal cortex. Int Rev Cytol 1986; 98: 1-405
7. Malendowicz LK: Sex differences in adrenocortical structure and function. I. The effects of post pubertal gonadectomy and gonadal hormone replacement on nuclear volume of adrenocortical cells in the rat. Cell Tissue Res. 1974;151: 525-36
8. Nowak M, Nussdorfer GG, Nowak KW, Mazzocchi G, Malendowicz LK.Gestational changes in hamster adrenal cortex: stereologic and functional studies. Res. Exp. Med.1990; 190: 163-71
9. Jensen E, Wood C, Keller-Wood M. The normal increase in adrenal secretion during pregnancy contributes to maternal volume expansion and fetal homeostasis. Reproductive Sciences. 2002; 9: 362-71
10. Saruhan BG, Ozdemir N. Effect of ovariectomy and of estrogen treatment on the adrenal gland and body weight in rats. Saudi Med J. 2005;26:1705-9
11. Ross JT, McMillen IC, Lok F, Thiel AG, Owens JA, Coulter CL. Intrafetal insulin-like growth factpr-I infusion stimulates adrenal growth but not steroidogenesis in the sheep fetus during late gestation. Endocrinology. 2007; 148:5424-32
12. Wy LA, Carlson HE, Kane P, Li X, Lei ZM, Rao CV: Pregnancy- associated Cushing's syndrome secondary to a luteinizing hormone/human chorionic gonadotropin receptor-positive adrenal carcinoma. Gynecol Endocrinol. 2002 ; 16(5): 413-7