HISTOCHEMICAL STUDY OF THE HUMAN OVARY DURING PREGNANCY

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Introduction

Ovary has attracted the attention of scientists of all fields. Histological and histochemical studies have been carried out to understand its, intricate and complex morphological cyclical variations both in animals and human. The ovary during pregnancy has not been studied to that detail has been the subject of the present histochemical evaluation.

Maternal and Methods

Histochemical methods for glycogen PAS (Mc manus and Mouery 1960) lipids (Bakers Sudan Black B, 1944) and Nile Blue A (Casselman, 1950), Alkaline Phosphatase and acid phosphatase (Burstone, 1958) succinate dehydrogenase (Pearse, 1953), Lipase (Pearse 1961) have been employed.

Ovarian biopsy specimens were obtained from 62 cases. Hysterotomy was done in 8 and caesarean section in 54. These cases were between the age of 20-35 years and 61% were para 1-4.

Analysis of Data

After carefully examining the slides it was found necessary to evolve some method of assessing the quality of glycogen, lipids and activity of enzymes in the specimen, so as to know the functional status. The criteria used are given below.

Criteria

The histochemical findings for glycogen, lipids and enzymes were visually assessed and have been graded in the following manner.

4(++++)	Intensity of colour good. Distribution uniform, coalesced globules. All the cells show activity.
3(+++)	Intensity of colour moderate to good. Distribution uniform with occasional variation. Discrete globules but coalesced at places, only about 75% of cells show activity.
2(++)	Intensity of colour moderate, Distribution variable, Discrete globules or particles only about 50% of the cells active.
1(+)	Intensity of colour poor. Distribution variable. Discrete particles. Only about 25% of the cell active.
	No or very little activity.

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Results

Surface epithelium

The cells showed minimal amount of PAS positive diastase resistant material and some lipids.

Primordial follicle

Very little of glycogen was present in the cytoplasm of the ovum. In the pre-antrum follicle, glycogen was seen in some of the cells of the granulosa layer.

TABLE I

Showing Glycogen and Carbohydrate Protein Complex Lipids and the various Enzymes in the Different Constituents of the Ovary

	PF	GF	AF	CL	CA	IGT	STROMA
1. Glycogen & Carbohydrate protein complex	+	+	++	+	++to +++	+	++
Early pregnancy Term. Pregnancy	+ to ++	+ to ++	+ to ++	+ to ++	+++	+ to ++	++
2. Lipids							
Early pregnancy	+	++	++	+++	++	++	+
Term. Pregnancy	+ to ++	+ to ++	+ to ++	+++ to +++	++ to +++	++ to +++	+
3. Enzymes							
i. Alkaline Phosphatase							
Early pregnancy	—	++	++	++++	—	++	+
Term. Pregnancy	+ to ++	++ to ++	++	++++	+ to ++	++ to +++	+ to ++
ii. Acid phosphatase							
Early pregnancy	—	++	+ to ++	+++	+ —	—	+
Term. Pregnancy	+	++	+ to ++	+++ to ++++	++	++ to +++	+ to ++
iii. Succinate dehydrogenase							
Early pregnancy	—	+ to ++	+ to ++	+++ to ++++	—	—	+ to ++
Term. Pregnancy	+	+ to ++	++ to +++	+++ to ++++	++ +++	++ to +++	+ to ++
iv. Lipase							
Early pregnancy	—	+	—	+++	—	—	+
Term. pregnancy	+	+ to ++	+	+++	++	—	+

PF—Primordial follicle, GF—Graafian follicle, AF—Atretic follicle, CL—Corpus Luteum, CA—Corpus albicantia, IGT—Interstitial gland tissue.

Membrana limitans externa was rich in PAS positive diastase resistant material, lipids were segregated at the juxta nuclear position (site of mitochondria) and the zona pellucida was rich in it. Granulosa cells showed minimal to moderate quantities of alkaline phosphatase, acid phosphatase and lipase. Succinate dehydrogenase activity was minimal in the oocyte and granulosa cells.

Graafian follicle

The granulosa cells of the Graafian follicle showed less of glycogen and the glycoproteins were in one or two cells. Lipids were in moderate amount. Alkaline phosphatase, acid phosphatase, succinate dehydrogenase and lipase activity was also moderate.

The cells of the thecal layer showed glycogen, glycoprotein lipids, alkaline phosphatase, acid phosphatase, succinate dehydrogenase and lipase. The activity in these cells was more than in the granulosa cells. The follicular fluid show only the presence of PAS positive diastase resistant material.

Atretic follicle

The convoluted glass membrane with PAS stain was uniformly pink. The theca cells had more of lipids and less of glycogen. Carbohydrate protein complex was moderate. The activity of alkaline phosphatase, acid phosphatase and succinate dehydrogenase was moderately good but lipase was minimal.

Interstitial gland tissue

The interstitial gland tissue was well developed in specimens of term pregnancy than that of the early pregnancy. Minimal amount of glycogen and glycoproteins was seen in the cells of the gland tissue. They were rich in lipids. The activity of alkaline and acid phosphatase, succinate dehydrogenase and lipase was not consistant.

Corpus luteum and Corpus albicans

The cells showed minimal amount of glycogen. The diastase resistant material was in the intercellular septas. Lipids were in abundance. The activity of alkaline and acid phosphatase, succinate dehydrogenase and lipase in granulosa lutein cells was good. There was no enzymatic activity in theca cells. The corpus albicans had only PAS positive diastase resistant material. Macrophages were rich in lipids, alkaline and acid phosphatase, succinate dehydrogenase and lipase.

Stroma

Stromal cells had less of PAS positive diastase resistant material, lipids, alkaline and acid phosphatase. succinate dehydrogenase and lipase.

Discussion

White *et al* (1961) found that steroid producing tissue or cells of the human ovary were characterised by a common histochemical pattern. These tissues exhibited sudanophilic droplets, alkaline phosphatase, acid phosphatase, non-specific esterase and succinate dehydrogenase activity.

According to him the reactions concerned with localization of steroid material are interpretable as an indication of the presence of the precursors of hormones. During steroid hormone systhesis they observed good alkaline phosphatase activity and vice verse. In the presence of trophic hormones they stated the testis and adrenal were stimulated to secrete steroids. The adrenal cells and the Leydig cells showed intense alkaline phosphatase activity. Following hypophysectomy the enzyme activity repidly dis-

appeared from the adrenal cortex and the interstitial cells of the testis. This was concomitent with disappearance or diminution in steroid production. In the theca lutein cells the presence of the enzyme was seen when large quantities of oestrogen was demonstrated (physiologically). The increased activity of succinate dehydrogenase and alkaline phosphatase in growing and actively functioning corpora lutea has been stated to be an instance of enzyme hormone relationship (Zuckerman, 1961). Meyer and MoShan (1950) have suggested that this enzyme may be involved in the development of the necessary energy for the specific cell function, synthesis and secretion of hormone. This enzyme is mainly, if not exclusively, localized in mitochondria.

Deane (1962) emphasized that distribution of and changes in the cholesterol containing lipids be speaks the capacity to form steroid hormones. Localization of enzymes like alkaline phosphatase and acid phosphatase have been correlated with sites for hormone production, since he found them in the adrenal and placenta of rodents.

In the present study, lipids and enzyme, e.g. alkaline and acid phosphatase, succinate dehydrogenase, beta-glucuronidase and lipase have been found in the various ovarian elements. Results for the sake of discussion are summarized in Table I. Presence of lipase indicates that the enzyme helps in the breakdown of fats and acts upon the long chained fatty acids.

The battery of tests performed suggested that the various constituents of the ovary, as stated above are taking part in steroidgenesis to some extent. The staining intensity exhibited by different components of ovary for lipids or enzymes, denotes their differential capacities to participate in steroid production.

Summary

Sixty two ovarian biopsy specimens obtained at the time of hysterotomy sterilization or caesarean section were subjected to detail histochemical study. The result were suggestive of an active ovary through out gestation. Presence of lipids, alkaline phosphatase succinate dehydrogenase in the various elements were in fevour of steroid production.

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