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Localization and Activity of Mouse Endometrial Alkaline Phosphatase after Hyperstimulation and Progesterone Injection at the Implantation Time

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ABSTRACT

The activity of mouse endometrial alkaline phosphatase after hyperstimulation and progesterone injection at the implantation time Alkaline phosphatase (ALP) of endometrium may play a critical function in the development and implantation of embryo. The aim of this study was to determine the localization of endometrial ALP activity after hyperstimulation and progesterone injection. Thirty adult female NMRI mice were hyperstimulated using human menopasual gonadotropic hormone and human chorionic gonadotropic hormones (hCG). Then, daily injections of progesterone (1 mg/mouse) were performed in one hyperstimulated group. Animals were sacrificed by cervical dislocation 4.5 day after hCG injection. Tissues were obtained from 1/3 middle part of uterine horns and used for enzyme histochemistry and morphological studies. The samples were cryosectioned at -30°C and the enzyme histochemistry was carried out by azo-coupling technique using alpha naphtol phosphate as substrate. In the control groups, the ALP activity was localized on the apical and basal border. Cytoplasm of glandular and surface epithelium was stronger than the other hyperstimulated groups on the day four of pseudoprgnancy. Our results showed that the ALP activity was decreased after ovarian hyperstimulation and progesterone injection and the defect of implantation of embryo after hyperstimulation and embryo transfer in animals may be due to the reduction of enzyme activity. *Iran. Biomed. J. 8 (3): 121-126, 2004*

Keywords: Alkaline phosphatase (ALP), Mouse endometrium, Hyperstimulation, Progesterone

INTRODUCTION

Epithelium of mammalian endometrium is the first site of contact between trophectoderm and maternal tissue during the period of attachment and implantation of embryo. Both of these surfaces undergo specific molecular, morphological and ultrastructural changes, which are mediated by ovarian hormones [1, 2]. In preparation of endometrium for blastocyst adhesion and attachment not only the microvilli alters in shape, size and number but also glycocalyx and its enzymes on the surface of this structure has some changes [3-5]. The endometrial surface acts as a site for secretion and maintenance of luminal meleniu which influences on development and implantation of embryo. The endometrium has direct effect on the transport of material across epithelium by some hydrolyze enzyme which catalyzes the transport of material from luminal surface to lamina propria. Alkaline phosphatase (ALP) is a major sialoglycoprotein that is localized on the basal and apical cell membrane and cytoplasm of surface and glandular epithelium [6]. This enzyme is involved in the hydrolysis of extracellular phosphate esters. It may play a role in the attachment of blastocyst to the endometrium. The activity of this enzyme may alter the composition and the volume of luminal secretion, which is essential for development and adhesion of embryo. There were controversially reports about the activity of this enzyme during early pregnancy [7-10]. The data showed that an increase in ALP activity immediately adjacent to the implantation of embryo as the decidual reaction was occurred [8] but the other reports indicated that during the early stage of pregnancy, the activity of these enzymes was high on the microvilli but near the implantation time the enzyme activity was

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decreased [7]. Ovarian hormones controlled endometrial enzyme activity [11].
After ovarian hyperstimulation, the estrogen secretion increased to the supraphysiological levels that may affect the activity of enzyme on the endometrial surface. There is no report about pattern of ALP activity following ovarian hyperstimulation. The aim of this study was to determine the activity of ALP of mouse endometrium during implantation time following ovarian hyperstimulation and progesterone injection using azo-coupling technique.

MATERIALS AND METHODS

Animals. All animals were cared and used according to the Guide for the Care and Use of Laboratory Animals at Tarbiat Modarres University (Iran). Thirty NMRI females mice, aged 6-10 weeks, were housted under 12 h light: 12 h dark condition and were randomly divided into three groups:

Group 1) Hyperstimulated mice. Female mice in this group were superovulated using an intraperitoneal injection of 10 IU human menopasual gonadotropic hormone (HMG), which was followed 48 h later by another injection of 10 IU human chorionic gonadotropic hormones (hCG). On the evening of the second injection, the mice were rendered pseudopregnant by cervical stimulation.

Group 2) Hyperstimulated mice with progesterone administration. After superovulation of mice with the same manner as previuos group, daily subcutaneous injections of progestrone (1 mg/mouse) were performed [12].

Group 3) Control group. Female mice were rendered pseudopregnant.

Tissue preparation. Ten mice in each group were sacrificed by cervical dislocation on 4.5 days after hCG injection or pseudopregnancy. The samples were immediately obtained from the 1/3 middle part of the uterine horns and used for enzyme histochemistry and morphological studies. At the same time, as the uterine horns were removed for positive control of enzyme histochemistry, liver tissue of mouse also was removed.

Morphological staining. For light microscopic study, the tissues were fixed in formaldehyde embedded in paraffin wax, sectioned at 6 micrometer and stained using trichrome technique [13].

Localization of ALP. The tissues were cryosectioned at -30°C with 6-micrometer thickness. The enzyme histochemistry was carried out by azo-coupling technique using alpha naphtol phosphate as substrate. All of these reagents were obtained from Sigma LTD (Cat. 86R, St. Louis, USA). Briefly, sections were fixed in solution of acetone and formaldehyde and then incubated in the substrate solution at 37°C for 1 hour. The counter staining was done by hematoxylin. The intensity of ALP reaction was scored between zero to 4+ according to the colorless to dark pink.

RESULTS

At the light microscopic levels, the morphology of control (Fig. 1a), hyperstimulated (Fig. 1b) and hyperstimulated-progesterone injected (Fig. 1c) groups were simple columnar, pseudostratified columnar and simple columnar epithelium, respectively. The density of collagen fibers just under the epithelium was increased especially in the hyperstimulated group without progesterone injection.

ALP enzyme activity was observed in the surface and glandular epithelium, stroma and muscular layer of endometrium, which was summarized in Table 1.

Table 1. The intensity of ALP activity in the mouse endometrium at the implantation time.

Groups	Surface epithelium	Glandular epithelium	Lamina propria	Muscle
Control	Apical and basal Borders (++++) Cytoplasm: Granular	Apical and basal Borders (++++) Cytoplasm: Granular	Granular	**(++++)**
Hyperstimulated	Apical border (+++), Basal border (++) Cytoplasm: Granular	Apical and basal Borders (++) Cytoplasm: Granular	Granular	**(++++)**
Hyperstimulated+ progesterone	Apical and basal Borders (0) Cytoplasm: Granular	Apical and basal Borders (++) Cytoplasm: Granular	Granular	**(++++)**

The intensity of ALP was scored between 0-4+ according to the color less to dark pink.

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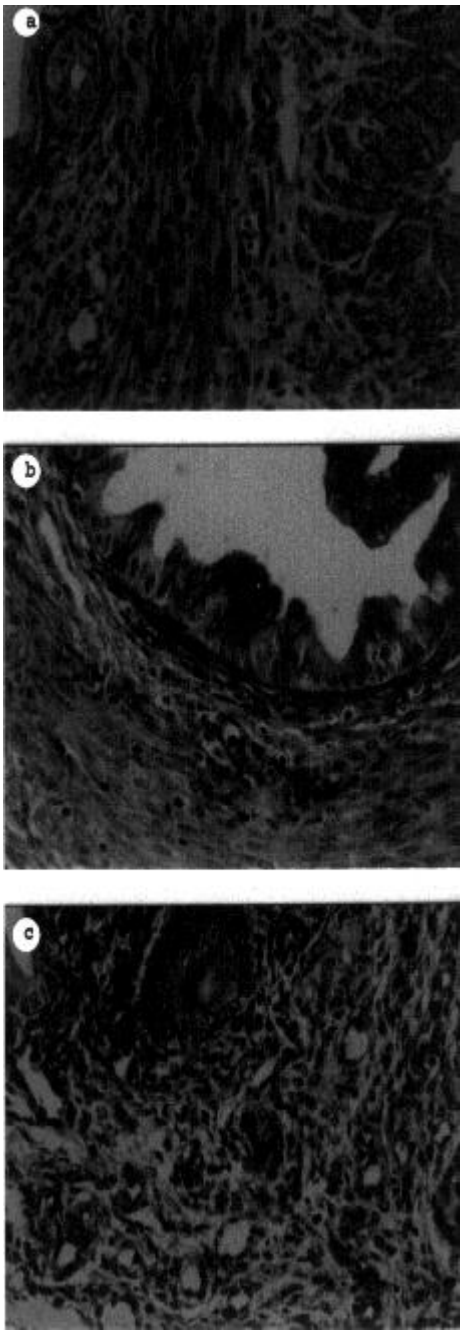

Fig. 1. Light micrographs of mouse uterus thickness with trichrome staining. **(a),** The control group after fourth days of pseudopregnancy; **(b),** Hyperstimulated group on day 4 of hCG injection; **(c)**, Hyperstimulated and progesterone injected group on fourth day of hCG injection (×400).

In the control groups, the ALP activity was stronger than the other hyperstimulated groups on the day four of pseudoprgnancy (Fig. 2a). The enzyme activity was localized on the apical and basal border and cytoplasm of glandular and surface epithelium (Fig. 2b and c). The pattern of enzyme in the cytoplasm of epithelial cells and stroma was granular. On the superovulated groups without progesterone injection the enzyme activity was weaker than the control group (Fig. 2d). It seems that the ALP was mainly localized on the apical border than the basal part.

On the superovulated-progesterone injected groups, the activity of enzyme was decreased. On the surface epithelium, there was no enzyme activity (Figs. 2e and f) whereas on the glandular epithelium very week staining for ALP was observed. In all hyperstimulated groups, the reaction of enzyme on the stroma was week.

The muscular coat of uterine horn in all groups had intense activity of ALP.

DISCUSSION

At the time of implantation in mammals, the cell surface of luminal epithelium of endometrium undergoes changes, which terminologically named ” plasma membrane transformation” [14, 15]. There are a lot of studies about transformation of microvilli to pinopodes-cup like cell projections- at the early stage of embryo attachment due to implantation marker [16-18].

The biochemical analysis of uterine secretion showed that the nature and the composition of its materials alter during the early stages of pregnancy [19]. When embryo started, its journey from fallopian tube l to the lumen of uterus, its development and nutrition were dependent on tubal fluid. Thus, endometrium secrets various amino acids, carbohydrates and specific proteins, which mediate embryo development [20]. However it is shown that, during apical transformation, the morphology and the content of glycocalyx and membrane associated proteins such as enzymes were altered during the implantation time, and where may have important roles in attachment of embryo [4, 21-23].

Our results in the present study showed that the activity of mouse endometrial ALP during implantation time was intense especially on the apical and basal border of surface and glandular epithelium. There are controversially reports about the activity of ALP during the early pregnancy in mammals [7-10, 24, 25]. In agreement with our results, Hall [24] reported an increase in activity of ALP at the light microscopic level in the mouse uterus at blastocyst implantation time. It has been shown an increase in ALP and vascular permeability of stroma of endometrium immediately adjacent to the implantation in the mouse [25] and during the pre-peri implantation period in rhesus monkey

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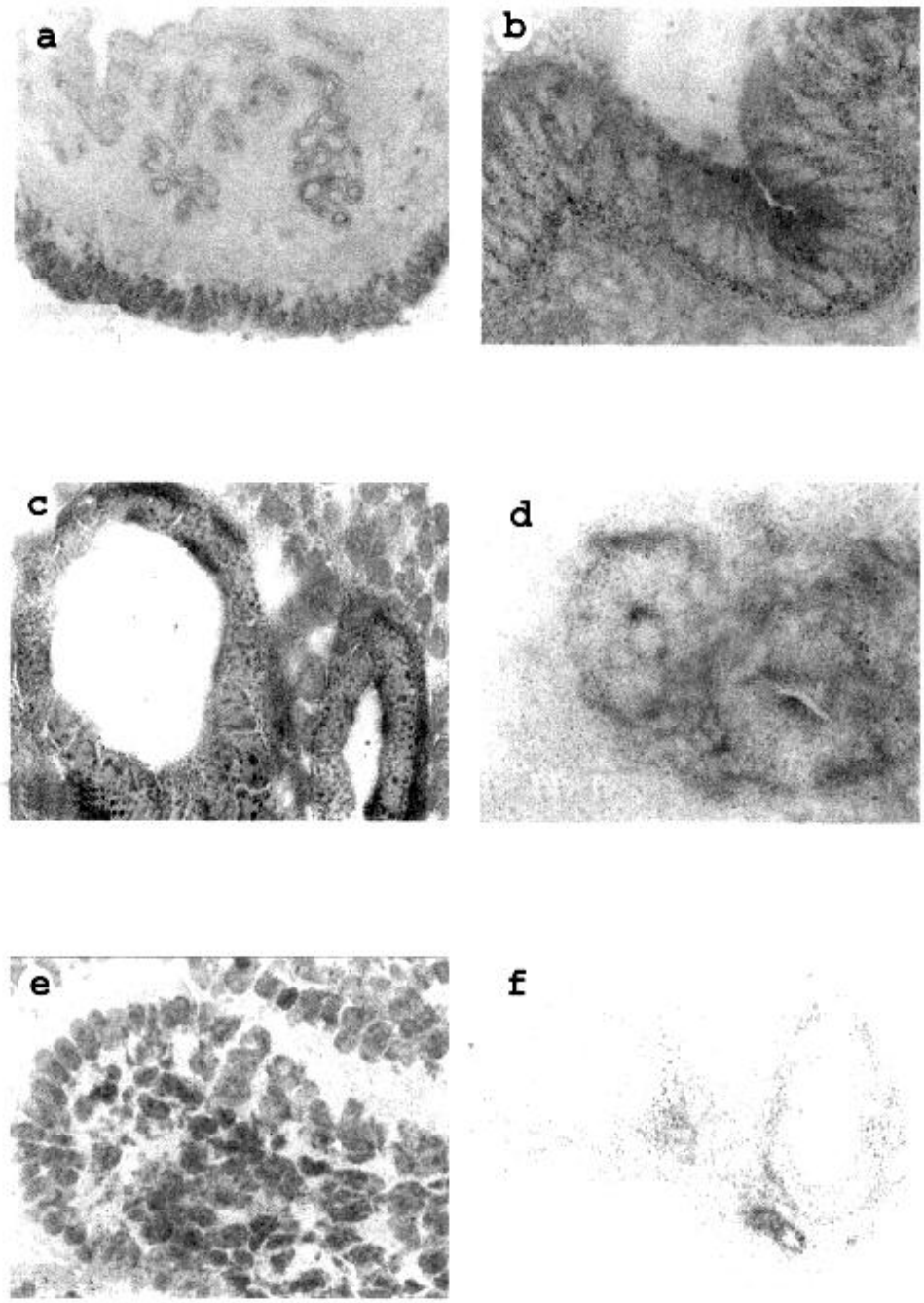

Fig. 2. Light micrographs of mouse uterus on day 4 of pseudopregnancy and or HCG injection. **(a)**, ALP reaction were observed on the surface and glandular epithelium and muscular coat (×100); **(b)**, ALP activity in the surface epithelium of control group which localized on the apical, basal and cytoplasm of these cells (×1000); **(c)**, The same reaction on the glandular epithelium with hematoxylin counterstaining (×1,000); **(d)**, The surface epithelium enzyme activity on the previous group with hematoxylin counterstaining (×1000); **(e)**, The ALP activity on the hyperstimulated-progesterone injected group on the surface epithelium with hematoxylin counterstaining (×1000); **(f)**, The glandular epithelium enzyme activity on the previous group (×1000).

[26]. There were also similar results on ALP activity of bovine [27] and sheep [9] endometrium. Bansode *et al.* [8] also showed that at the maximal endometrial sensitivity in the rat, there was the high ALP activity in the luminal and glandular epithelium and stroma of endometrium. They concluded that there was a correlation between increase in ALP activity and implantation window or endometrial receptivity. In contrast to our results, it shown that a progressive loss of ALP activity to the time of implantation in rabbit [28, 29], human [30], cow [27], rat [7, 10].

It is established that the activity of endometrium enzyme is under control of steroid hormones [11].

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Also, our results showed that after hyper-stimulation using hMG and hCG, the activity of enzyme on the glandular and surface epithelium in comparison with the control group was decreased relatively. Whereas, on hyperstimulated -progesterone injected groups, there was no sign of ALP activity on the surface epithelium but a weak enzyme activity was observed on the glandular epithelium. Thus, we conclude that the ALP activity of endometrium is dependent on the ratio of estrogen and progesterone and its activity may be down regulated by progesterone. In relation to our results, the observation of Bucci and Murphy [11] clearly established that there were marked changes in the distribution of endometrial enzymes, which were coupled with the particular regimes of hormone used. They showed that ALP staining in ovariectomized animals was upregulated by estrogen whereas was reduced in response to progesterone and estrogen treatment. They also concluded that ALP could be differentially regulated by progesterone or progesterone plus estrogen. Suzuki *et al.* [31] also obtained similar results *in vitro* study. They showed that the administration of estradiol to the culture medium of human endometrial cancer cell line, a relative of the ALP activity, was induced but progesterone prohibited the elevation of ALP activity. In contrast to our and previously results, Jelinek *et al.* [32] showed that after progesterone injection to women, the ALP activity was significantly higher than the control group.

These different results in ALP activity of endometrium may be due to the type of technique, which used for determination of ALP activity, however it seems that the complementary technique is necessary. Also, different mammalian species may have different ALP activity patterns during their pregnancy.

Overall, our results showed that the ALP activity was decreased after ovarian hyper-stimulation and progesterone injection and defect on implantation of embryo after hyperstimulation and embryo transfer in animals may be due to this reduction in enzyme activity.

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