

## The Changes in PNA, WGA, and UEA I Lectin Binding Pattern in the Uterine Tube Epithelium by Effects of Estradiol and Progesterone Therapy in Rat

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### Abstract

<b>Background</b>	The carbohydrate histochemistry in the epithelium of the uterine tube was found to be related to the uterine tube physiology and morphology.
<b>Objectives</b>	This study investigated the changing binding pattern of peanut agglutinin, wheat germ agglutinin, and ulexeuropaeus agglutinin I lectin to the epithelial cells of the rat oviduct ampullary region in response to the effect of combined estrogen and progesterone hormonal administration.
<b>Methods</b>	This experimental study was based on injecting the animals with doses of estradiol and Progesterone hormones during the successive estrous cycles. Then, the ampulla of uterine tubes were identified and prepared for paraffin sections. The peanut agglutinin, wheat germ agglutinin, and ulexeuropaeus agglutinin I lectins were used to label the tube section; the sections were examined by fluorescent microscope.
<b>Results</b>	The three lectins used in this study showed analogous pattern of binding behavior. The uterine tube epithelium of the controlled group showed bimodality of binding, while that of the treated group showed a third pattern of lectin binding in some of the epithelial cells.
<b>Conclusion</b>	The ciliated cells that are transformed to display a secretory function are called the transitional cells, the formation of these cells is influenced by hormonal factors and the pattern of the lectins binding to these cells could be considered as a histochemical marking.
<b>Key word</b>	Lectin, uterine tube, carbohydrate histochemistry, hormone.

**List of abbreviation:** PNA = peanut agglutinin, UEA I = ulexeuropaeus agglutinin I, WGA = wheat germ agglutinin, NBF = neutral buffer formalin, FITC = fluorescein isothiocyanate, PBS = phosphate buffered saline.

### Introduction

The uterine tube is responsible for transport of gametes and embryos; in addition it is a physiologically suitable site for both fertilization and early development<sup>(1)</sup>. The uterine tube wall is formed of external tunica serosa, intermediate tunica muscularis, composed of inner circular and outer longitudinal layers and an internal tunica mucosa; however, there is no submucosa<sup>(2)</sup>.

The epithelial cells of the mucosa are of a simple columnar type resting on a basement membrane and a lamina propria. These mucosal epithelia are of four types; the ciliated columnar cells, the non-ciliated (secretory) cells, the intercalary (intercalated) cells, and the basal (reserve or indifferent) cells<sup>(3)</sup>.

The uterine tube epithelium changes in height and thickness during the estrus cycle. In rodents, the secretory cells predominate the ampullary region during the estrous and metestrous phases, while the ciliated columnar cells predominate during the latter part of the diestrous and the proestrous phases<sup>(4)</sup>.

Ciliogenesis and deciliation processes in the lining epithelium are influenced by the level of ovarian hormones<sup>(5)</sup>.

The uterine tube showed much histochemical reactivity indicating the existence of many chemical compounds including; carbohydrates, proteins, lipids and others, these histochemical compounds were found to have certain role to maintain the uterine tube physiological functions. The histochemical carbohydrate constituents are found to be important structural cytoplasmic elements of the uterine tube, these carbohydrates were found mainly in the cytoplasm, glycocalyx, secretory granules and connective tissues and other parts of the epithelium<sup>(6)</sup>.

Lectins are carbohydrate binding proteins of a non-immune origin that agglutinate cells and/or precipitate glycoconjugates. The lectins have no enzymatic activity, may be soluble or membrane bound, and are of bacterial or plant origin<sup>(7)</sup>. There are various biological roles of lectins in animals<sup>(8)</sup>. At the level of the tissues, lectins had been used in many directions, the lectins are considered as specific probes for various cell types<sup>(9)</sup>, the lectins are used to define cells at various stages of differentiation or maturation<sup>(10)</sup>, and are also used as probes to detect microenvironment<sup>(11)</sup> and detecting the functional changes<sup>(8)</sup>.

The aim of this study was to investigate the binding pattern of PNA, WGA, and UEA I lectins to the epithelial cells of the rat oviduct ampullary region, and elaborate the alteration in binding configuration in these epithelial cells affected by a combined estrogen and progesterone hormonal administration.

## Methods

Fourteen adult healthy female albino rats (*Rattus Norvegicus*) weighing between 200-280g, and having an estrous cycle of 4 days period used for this study were obtained from the animal house of the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University.

The following drugs were used in this study:

- B-Estradiol 17-acetate (Sigma-Aldrich) 1g, in a solid form, was dissolved at a concentration of 20µg/ml sesame oil.
- Primolute Depot (Bayer Schering Pharma AG/Germany) in form of Hydroxyprogesterone ecaproate 250mg/1ml in oily solution.
- Purified Sesame oil used as a vehicle for dilution of both hormones. The total quantity of the oil used was adjusted to be fixed in every daily dose or doses to be a net of 2 ml.

The animals were divided into 2 main groups; a Control group (6 rats) and treated group (8 rats) treated with the 10 µg/day of estradiol for a period of two successive estrous cycles (i.e. 8 days).

The Progesterone hormone was given in a dose of 4 mg/kg body weight for the treated group on the third and fourth days of the two successive estrous cycles. The drugs were given daily in the morning, as a subcutaneous injection at the lower back region of the rats.

Vaginal cytology smears were collected from the experimental animals to check the length of the estrous cycle and to identify the estrous phases for the rats depending on the criteria of vaginal cytology.

In this study; 4 days estrous cycle rats have been selected. The vaginal smear done using Ayres Spatula that was placed deeply inside the vagina of the rat, then the spatula rotated through 360 degree maintaining contact with the vaginal wall.

After the swab was removed from the vagina, the cells were transferred to a clean glass slide by rolling the swab along the surface of the slide. Intact cells were obtained during transfer by rolling the swab. Once the cells have been transferred, the slides were fixed immediately using absolute alcohol for 2-6 hours.

The vaginal smear for the control group was done with the rat under anesthesia and just before termination, while for the treated groups it was done two times (for each animal), the first one done before starting treatment, and the second done after finishing the

treatment and just before sacrificing the animal.

The sacrificed animals were terminated 24 hours after the last dose of drugs by cervical dislocation. Then, the right and left uterine tubes of each animal were identified and the ampullary region (0.2-0.3 cm long) was removed on both sides, fixed in 10% Neutral Buffer Formalin (NBF) prepared for paraffin sections according to Bancroft and Stevens (1982) for lectin binding study<sup>(12)</sup>.

#### **Procedure of lectin histochemistry**

Three types of Lectins (Sigma™ USA) were used in this study:

1. Peanut agglutinin (PNA) from Arachis-hypogaea (peanut) which has affinity to bind the D-Galactose sugar.
2. Ulexeuropaeus agglutinin I (UEA-I) from Ulexeuropaeus which is a Fucose binding lectin.
3. Wheat germ agglutinin (WGA) from Triticumvulgaris (wheat) which has the affinity for binding the N- acetylglucosamine and Neuraminic (sialic) acid.

All lectins used were fluorescein isothiocyanate (FITC) labeled.

The Procedure of lectin histochemical staining was as follow<sup>(13)</sup>:

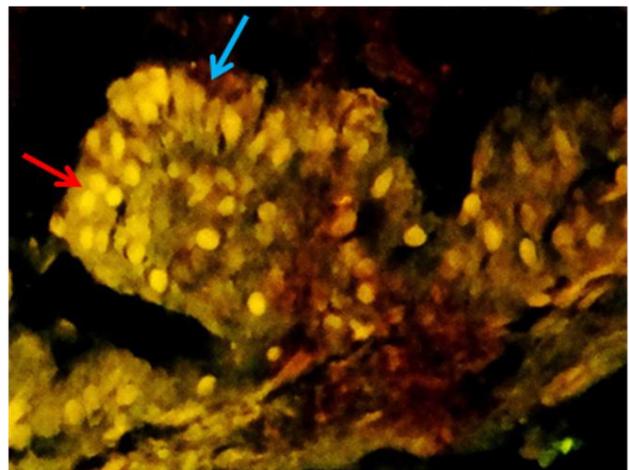
- Dewaxing of the paraffin sections was done by use of xylene for 30 minutes.
- Hydration of the sections through graded concentrations of ethyl alcohol.
- Hydrated paraffin sections were washed in phosphate buffered saline (PBS).
- Keep the slides flooded by the lectin-PBS solution.
- Sections were flooded by lectin-PBS solution and kept for 1.5 hours.
- Sections were washed in PBS and mounted in non-fluorescent fractoilmountant.
- Sections were examined under the ultraviolet light of the fluorescent microscope.
- Digital camera (Sony cyber shot) was used for capturing pictures after microscopic evaluation of the fluorescent reactivity.

## **Results**

### *The PNA binding*

#### **PNA binding to the controlled group**

The PNA lectin binding to the mucosal epithelium of the ampullary region of the uterine tube in the control group (Fig. 1) showed predominate intracellular binding cells given positive fluorescence activity, these cells are low columnar with central round nucleus. The epithelium showed also singly settled narrow columnar cells having weaker fluorescence activity (especially in the supranuclear cytoplasm), these cells show central elliptical nucleus that is arranged parallel to the long axis of these cells.

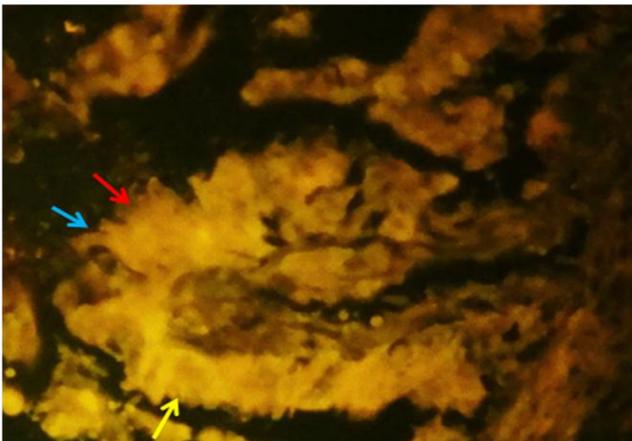


**Fig. 1. PNA binding to the uterine tube mucosa of the control group showing the predominate intracellular binding cells (red arrow); singly settled narrow columnar cells having weaker fluorescence activity (blue arrow). X400.**

#### **PNA binding to the treated group:**

The PNA binding to the mucosal epithelial cells showed 3 levels of fluorescence activity (fig. 2), the predominant cells showed strong intracellular fluorescence, others showed progressive diminishing fluorescence reaching to the least fluorescent cells that have very weak fluorescence activity. The latter showed the same criteria in the epithelium of the controlled group as these cells are singly settled with narrow columnar cell body and central elliptical nucleus arranged parallel to the long axis of these cells. A part from these

columnar cells, the predominant cells of the epithelium have rounded central nuclei, the cellular boundaries of the whole epithelial cells seemed obscure that make the fluorescence of these cells appearing cloudy with 3 different fluorescent activities, even the nuclei were masked compared to those nuclei of the cells in the epithelia of the controlled group. The predominant epithelial cells are lower columnar in shape compared to those seen in the controlled group and have intracellular fluorescence.

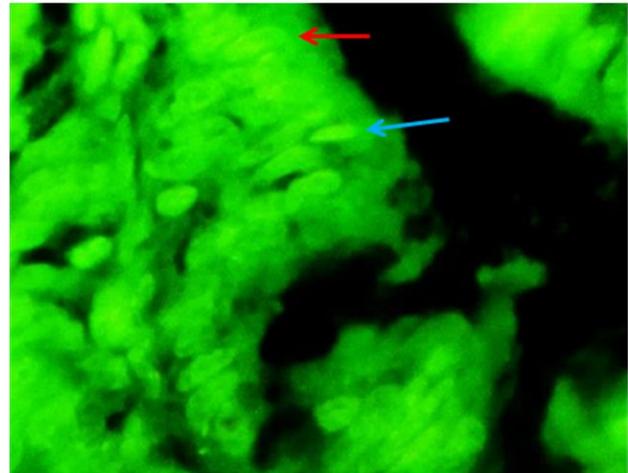


**Fig. 2. PNA binding to the mucosal cells of the treated group showed 3 levels of activity, the least fluorescent cells showed singly settled with narrow columnar cell body (longer blue arrow). X400.**

#### *The WGA binding*

##### **WGA binding to the controlled group:**

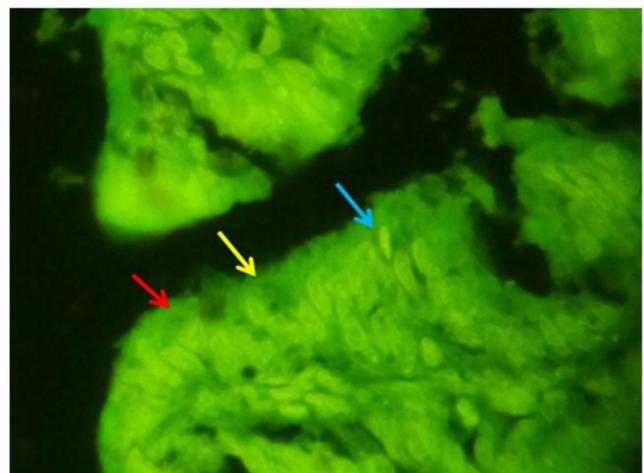
The WGA lectin binding to the mucosal epithelium of the ampullary region of the uterine tube in the control group (Fig. 3) showed predominate intracellular binding cells given positive fluorescence activity, these cells are low columnar with central round nucleus. The singly settled narrow columnar cells having central elliptical nucleus arranged parallel to the long axis of these showed equivalent fluorescence at the supranuclear region, but the perinuclear cytoplasm inferior to the nucleus showed weaker fluorescence activity.



**Fig. 3. WGA lectin binding to the uterine tube mucosal epithelium of the control group. The predominate intracellular binding cells (red arrow) and the singly settled narrow columnar cells (blue arrow) are seen. X400.**

##### **WGA binding to the treated group**

The fluorescence activity of WGA binding to the epithelial cells showed equivalent pattern and intensity in both forms of cells described above (the predominant and the single settled cells), apart from few of the predominant cells have comparable weaker fluorescent activity (fig. 4). The epithelial cellular boundaries are also obscured.

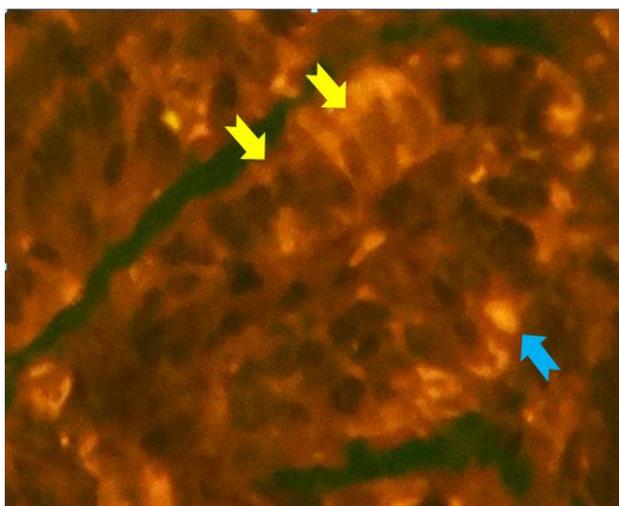


**Fig. 4. WGA binding to the uterine tubal epithelium in the treated group showed predominant (red arrow); single settled cells (blue arrow) with few of predominant cells having comparable weaker (yellow arrow). X400.**

### *The UEA I binding*

#### **UEAI binding to the controlled group**

The predominant cells of the mucosal epithelium showed cell surface fluorescence of UEAI binding with variable intensities; this fluorescence is brighter on the luminal surface of these cells. The single settled narrow cells showed weak intracellular UEAI binding (fig. 5).



**Fig. 5. UEA I binding in the uterine tube mucosal epithelium of the control group. The predominant cells showed cell surface fluorescence (yellow arrows), the single settled narrow cells showed weak intracellular binding (blue arrow). X400.**

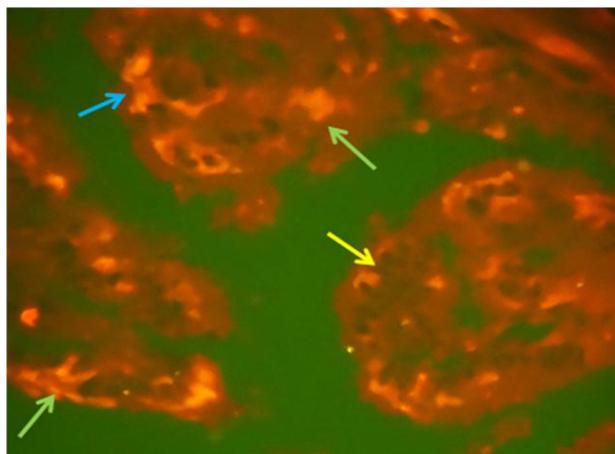
#### **UEAI binding to the treated group**

The UEAI binding to the mucosal epithelial cells showed similar pattern and intensities as that seen in the epithelial cells of the controlled group. In addition, some of the predominant cells showed intracellular reactivity (fig. 6). The cellular boundaries of the whole epithelial cells are obscured resulting in elusive pattern of negative rather than cell surface fluorescence. The nuclei were masked compared to those nuclei of the cells in the epithelia of the controlled group, especially in the intracellular fluorescent predominant.

### **Discussion**

The three lectins used in this study showed analogous pattern of binding behavior. The uterine tube epithelium of the controlled group

showed bimodality of binding, represented in the epithelial predominant cells (low columnar with central nucleus) and the single settled cells (narrow columnar cells).



**Fig. 6. UEAI binding to the mucosal epithelial cells of the treated group showed similar pattern and intensities as that seen in the epithelial cells of the controlled group. Some of the predominant cells showed intracellular reactivity (green arrows). X400.**

The epithelium of the treated group showed a third pattern of lectin cellular binding only in some cells matching in their histological criteria to the predominant epithelial cells, and hence these cells may represent a deviation in the binding pattern of the predominant cells in response to hormonal treatment. These results may suggest that hormonal treatment did not have consequences on the bimodal pattern but it is associated with the appearance of a third mode of lectins binding pattern.

The predominant cellular binding pattern represents the marking pattern of ciliated epithelial cells, and the pattern of singled settled cells is the marking pattern of the secretory epithelial cells. This conclusion has been predicted depending on the illustrious histological descriptions verified previously that traditionally described the predominant ciliated cells and the scattered secretory cells in the tubal epithelium of rat<sup>(14)</sup>.

It was reported that some of the ciliated cells have the ability to be transformed to display a

secretory function, these cells were describe to have the same light microscopic morphological criteria but with a paler cytoplasm. The ultrastructural criteria of these transformed cells differ from the predominant ciliated epithelial cells. These transformed cells were nominated as the transitional cells between ciliated and secretory cells, and the formation of these cells was suggested to be influenced by hormonal factors <sup>(15)</sup>. Hence, the third pattern of the lectins binding may be interpreted as the marking pattern of a transitional cells developing by the effect hormonal treatment applied in this study.

These analyses of the results of this study was congruently reported by Gheril et al (2001) that described no difference in sugar localization and distribution in a study on the lectins binding pattern in the uterine tube epithelium at variable physiological circumstances <sup>(13)</sup>.

In this study, the PNA binding in the controlled group showed intracellular binding to the predominant cells (i.e., the ciliated cells), but weaker supranuclear binding in the singly settled cells (i.e., the secretory cells). This finding is supported by the histochemical elaboration of Schulte et al (1985) that demonstrated apical localization of galactose in the ciliated but not secretory cells. These authors suggested that there is no menstrual phase related variability of the carbohydrate distribution in the epithelial cells <sup>(16)</sup>. This definite analysis may indicate that the iatrogenic hormonal manipulation can cause an alteration that is not seen in normal hormonal variation.

The WGA binding to the controlled group showed intense apical cytoplasmic binding in the secretory cells, the ciliated cells showed diffuse intracellular WGA reactivity. The WGA binding in the treated group showed weaker reactivity in few of the epithelial cells having the histological criteria simulating the predominant ciliated cells. The pattern of WGA binding in ciliated epithelial cells in the treated and control group was supported by the results reported by Gehri et al (2001) <sup>(13)</sup>, therefore,

the lectins PNA and WGA could be considered as the marker of the ciliated epithelium during physiological condition as the hormonal treatment done in this study results in fading of fluorescence WGA reactivity in these cells.

Ingrid and Bavdek (1997) reported that WGA and UEAI showed binding reactivity in the supranuclear Golgi apparatus and in the secretory granules of the epithelial cells of the ampulla of the uterine tube <sup>(17)</sup>. This description supported the result found in this study demonstrating supra-nuclear WGA binding in the single settle secretory cells. Accordingly, this supra-nuclear WGA pattern maybe interpreted as an WGA binding to the Golgi apparatus at the supra-nuclear cytoplasm position between the nucleus and the secretory surface <sup>(18)</sup>.

The UEAI binding to the secretory single settle cells showed intracellular binding in the controlled group, this pattern sustained the interpretation of Ingrid and Bavdeck (1997) considering the UEAI binding to the Golgi apparatus and the secretory granules.

The lectin binding specificity to the carbohydrate has been discussed in consideration to the intracellular machinery and in relation to the intercellular milieu in many literatures <sup>(19)</sup>. The binding pattern of the lectins used in this study could be related to the physiological and pharmacological hormonal adjustments of the uterine mucosal epithelium.

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### Conflict of interest

The author declares no conflict of interest.

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