The Effects of HCG on the Distribution Pattern of Mature Pregnant Rat Uterine Glycoconjugates during Implantation Period

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ABSTRACT

Introduction: One of the most common reasons of infertility is un-ovulatory period. Hormone therapy is the most common treatments of this type of infertility. Gonadotropins may exert toxic effects on the molecular organization of uterine surface, such as glycoconjugates. Glycoconjugates are the most important components of the uterine and trophoblast surface playing an important role in embryo implantation. In this study, the effects of one of these gonadotropin hormones, Human Chorionic Gonadotropin (HCG), on glycoconjugates distribution of uterine epithelium (apical membrane, Golgi zone and basement membrane of rat endometrial cells) and uterine glands studied during implantation period.

Methods: The mature female rats were selected and divided into two groups (Experimental, sham and Control). Experimental rats were injected with 10 I.U HCG intraperitoneally in estrus phase and mated with proven fertile male rats. The rats were sacrificed at 5.5 day of pregnancy (time of implantation) and their uteruses removed. The pregnant uterine tissues prepared histologically. Using WGA, DBA, PNA, ConA, SBA and UEA lectins, Lectin histochemistry was done.

Results: The intensity of the reactions to WGA in apical membrane and Golgi zone of the uterine epithelium were lower in HCG-treated group compared with the control group. After HCG treatment, uptake of DBA and UEA lectins by uterine glands was low.

Conclusion: HCG led to modification of the uterine surface glycoconjugates and affected the content of these critical molecules involved in implantation. Therefore, HCG may also exert adverse impact on the fertility rate.

Key Words:
Glycoconjugates,
Uterine Surface,
HCG,
Implantation.

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1. Introduction

Implantation is a time-dependent event. Implantation is an essential process in viviparous birth allowing mammals to support and supply the nutrient needed for their embryos during early development [1]. Human implantation begins with the blastocyst fixation with the endometrium and then, invades into it. In order to establish an efficient blood-placental circulation, sequential events must be occurred. These events include apposition of the blastocyst to the endometrial epithelium, adhesion to it, penetration through the epithelium and basal lamina and invasion into the lamina propria contained vasculature [2]. Although, the mechanism of implantation is different in various mammalian species, the events occurred between fertilization and initiation of implantation seems to be a conservative process [1]. Also, due to both practical and ethical reasons, it is not possible to study the implantation process in normal humans. Therefore, in order to study both molecular and mechanical events associated with implantation, the animal models are necessary [3]. The better understanding of the molecular signals that regulate uterine receptivity and implantation of the blastocyst is clinically important and the nature of these signals may lead to choose new strategies to improve implantation failure and pregnancy rates [4].

Many factors such as chemokines, cytokines, adhesion molecules, growth factors and glycoconjugates involved in facilitating the process of implantation [5,6]. Glycoconjugates defined as macromolecules contain sugar moieties [7]. Both uterine and blastocyst cell surfaces have glycoconjugates that mediate cell-cell or cell-matrix interaction during implantation [8, 9]. The distribution of some glycoconjugates in non-pregnant uterine epithelial surface was studied before (10). Several factors influence the endometrial glycocalyx. One of these factors is exogenous gonadotropins administrated in In Vitro Fertilization program to induce the ovulation. These hormones may lead to adverse conditions in the endometrium which are inconsistent with those for normal implantation [11, 12]. Since the glycocalyx of the endometrium is the first site of attachment between the blastocyst and uterus, it was shown that carbohydrate moieties in the glycocalyx play key role in the aposition and subsequently implantation of the blastocyst [6].

To investigate carbohydrates composition, lectins are useful tools [13]. Lectins are proteins from non-immune origin that bind carbohydrates with a high degree of specificity [14]. In this study, we studied the toxic effects of Human Chorionic Gonadotropin (HCG) on glycoconjugates Distribution in mature rat uterine surface that normally exist in implantation period using different lectins.

2. Materials & Methods

2.1. Experimental Design

Sixteen mature female Sprague-Dawley rats with 200-250 gram body weight [15] were maintained in a 12h light-12h dark cycle [6] in the standard condition with free access to water and food. The animals were divided into two groups, experimental (n=8), control (n=8) groups, randomly.

Vaginal smears were prepared and the animals in the estrous phase chosen for injecting. Each animal received a single injection of 10 U of HCG (purchase from Daro Pakhsh Co, Iran) intraperitoneally. The mice were mated with proven fertile male rats. Vaginal plug observation mentioned as 0.5 day of gestation. The sham groups received the same volume of vehicle of HCG (distilled water) and control group were not injected at all. In this phase, the control group was mated with male. The animals were kept in the standard condition with ad Labitum. The vaginal plug observation considered as the day 0.5 of gestation. At 5.5 day of gestation, implantation day, the rats were sacrificed and their right uterus horns removed and fixed in Bouin's fixative.

2.2. Verification of Pregnant Animals

In order to verify pregnancy, right horns of uterus prepared histologically and stained with H & E. For lectin histochemistery staining, only animals showing the typical pregnancy signs (i.e. invagination of deciduas area to create pocket for the blastocyst [3], decidual reaction [3] and presence of immune cells [5]) were selected.

2.3. Lectin Histochemistry

Uterine sections were mounted on poly-L-lysine coated slides. The specimens were rinsed in 0.1M phosphate-buffered saline (PBS). In order to block the endogenous peroxidase, sections were treated with %1 H2O2 in methanol for 20 min. After washing with PBS, incubation of the sections with peroxidase-conjugated lectins was carried out at room temperature for 2h. The lectins were used at 10 µg/mL in PBS. WGA(Wheat Germ Agglutinin), PNA (Peanut Agglutinin), DBA (Dolichos Biflorus Agglutinin), ConA (Concanavalin A), SBA (Glycin Max or Soybean) and UEA (Ulex Europaeus Agglutinin) were used and bound to sialic acid/N-acetylglucoseamine (GluNac), galactose/N-acetylgalactoseamine

(Gal/GalNac), N-acetylgalactoseamine, mannose (Man), N-acetylgalactoseamine (GalNac) and fucose (Fuc), respectively [16-19]. The sections were rinsed in PBS for 45 min and then incubated in DAB/H2O2 solution (0.03 g DAB/100mL PBS contained 200µL of H2O2) for 10 min. After washing with tap water for 15 min, counterstaining done with alcian blue. Then, the sections were washed with deionized water, dehydrated and mounted.

In order to prove the specificity of the reaction, the control sections incubated in PBS at room temperature.

2.6. Quantization

To compare the intensity of positive staining, an arbitrary scale from 0 to 4 was used (no reaction to strong) [20]. The reaction of the uterine epithelium, Golgi zone, basement membrane of the uterine epithelium and also uterine glands were evaluated. For each specimen, the evaluation was done twice blindly. The data were analyzed statistically by Kruskal-wallis and non-parametric test of Mann-Whitney. A p-value less than 0.5 considered as significant difference.

3. Results

Lectin histochemistry of the uterine tissue of the control group showed that apical surface of the uterine epithelium could uptake all lectins with maximum intensity for WGA and minimum for PNA. In experimental group, the pattern of the reactions changed, so that the amount of WGA-reacted sugars showed a significant decrease in HCG-treated group compared to the control group (P=0.0001) (Figure 1). The same results obtained for Golgi zone. In experimental group, the reactions of Golgi zone of the uterine epithelium to WGA showed a significant decrease compared with control group (P=0.0001) (Figure 2). However, no significant difference was observed in the uptake of the other lectins between the control and experimental group.

The results obtained from control group indicated that basement membrane could uptake WGA and UEA with "very weak" intensities. No significant difference observed in the lectin reactivity of the basement membrane (figure 3). The basement membrane of the uterine epithelium in experimental group reacted with PNA and SBA reacted glycoconjugates "very weakly " as well.

The uterine glands of the control group reacted with DBA "strongly" and with UEA and WGA " weakly" and with SBA and Con A "very weakly". The reaction with PNA was negative. The HCG administration led to a significant decrease in DBA (P=0.01), WGA (P=0.001) and UEA (P= 0.0001) staining intensity of the glands (Figure 4).

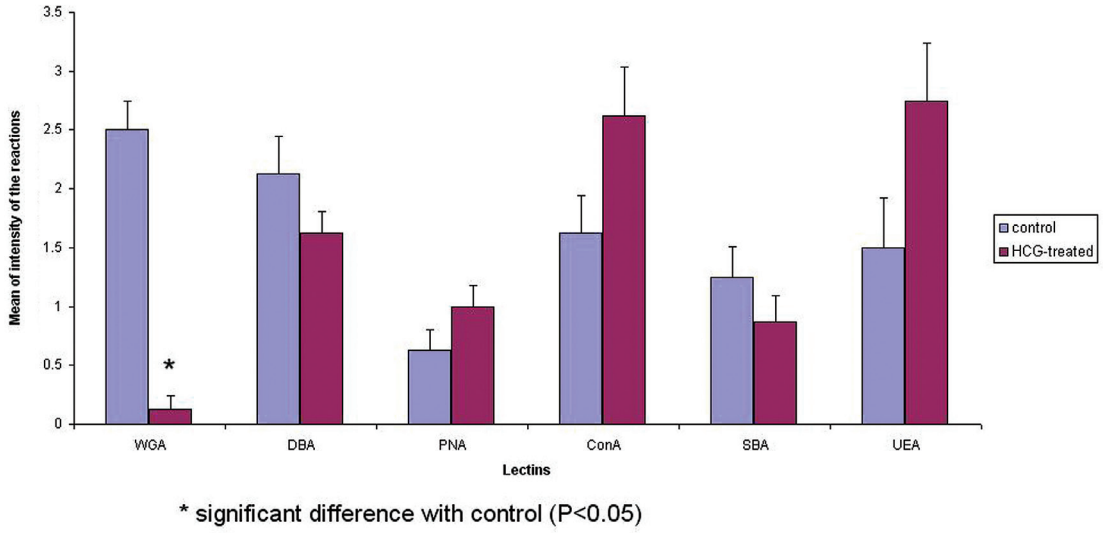

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Figure 1. Comparison of the Mean of lectin reactivity of apical surface of uterine epithelium to various lectins in experimental and control groups.

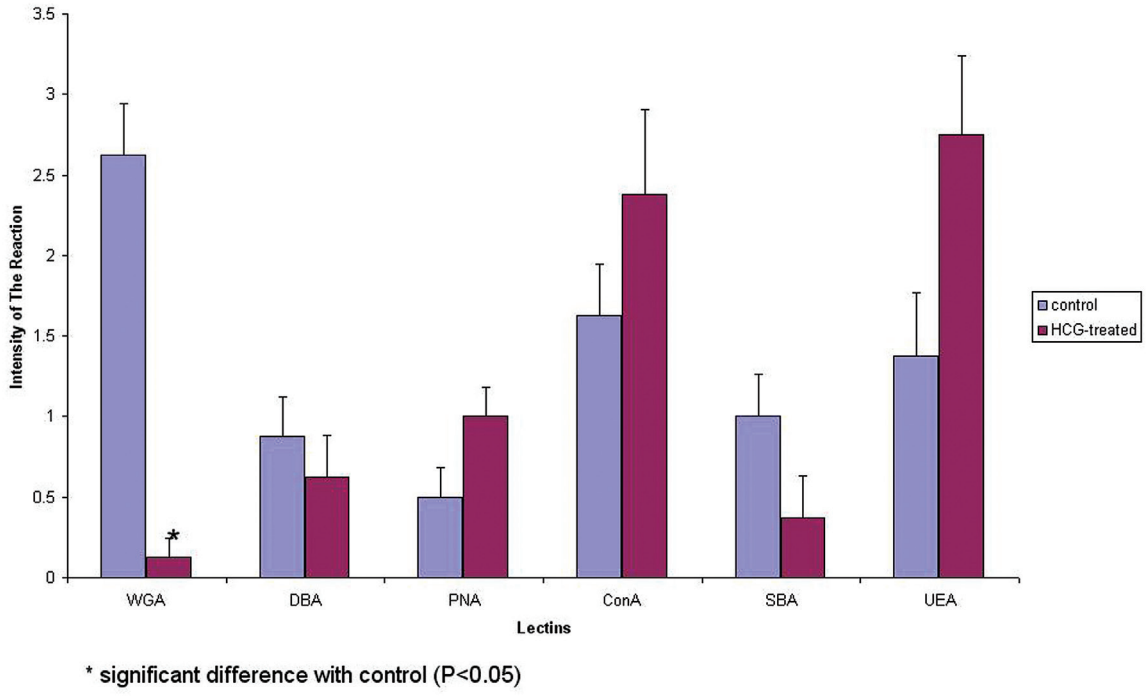

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Figure 2. Comparison of the mean of the lectin reactivity of Golgi zone of uterine epithelium to various lectins in experimental and control groups epithelium.

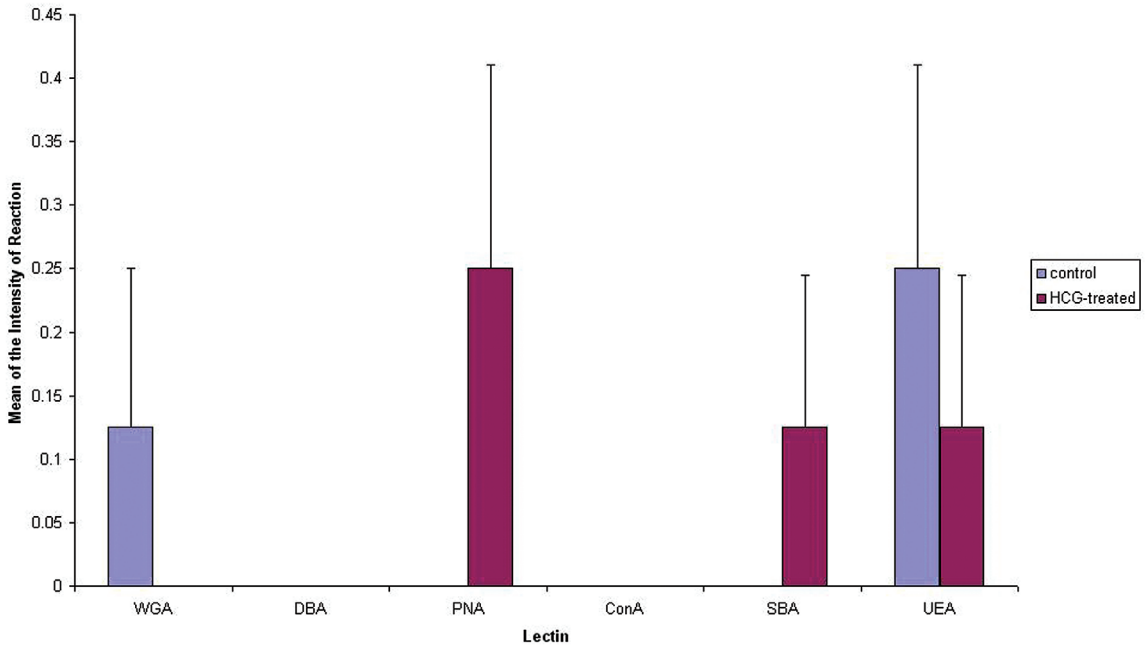

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Figure 3. Comparison of the lectin reactivity of basment membrane of uterine epithelium to various lectins in experimental and control groups.

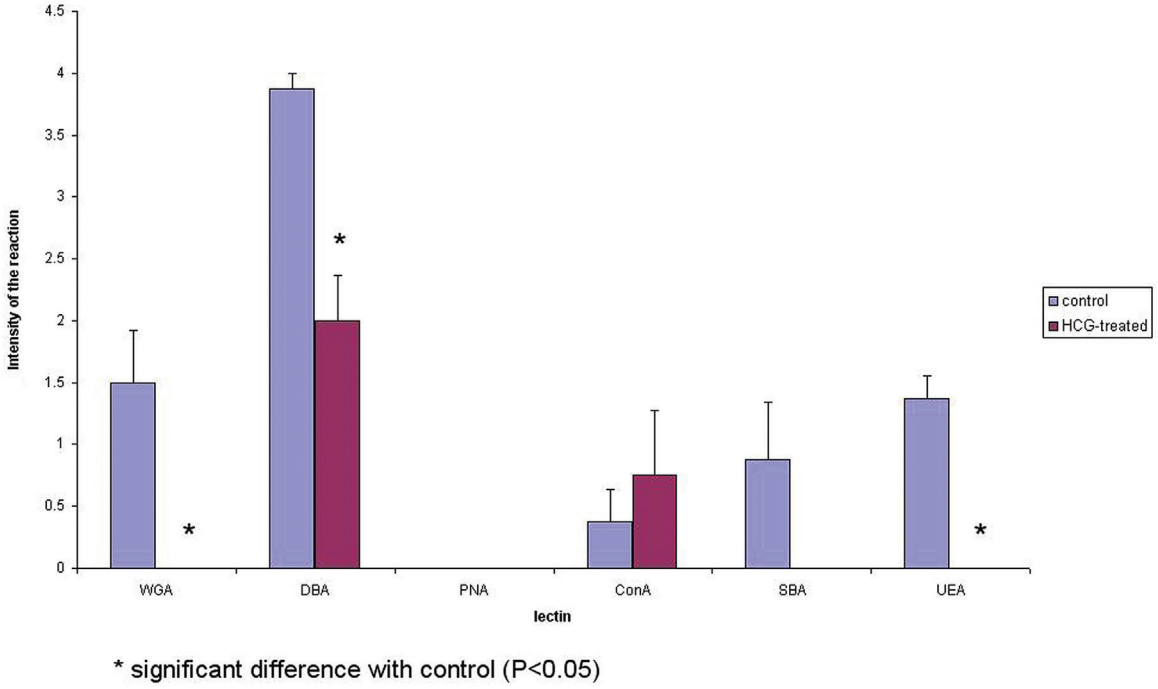

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Figure 4. Comparison of the mean of the lectin reactivity of Glands of uterus to various lectins in experimental and control groups.

4. Discussion

Glycoconjugate production showed a correlation with functional status of the endometrium. These glycoconjugates suggested playing a role in endometrial development necessary for implantation, and alterations in lectin distribution may represent the induction of functional abnormalities not apparent by light microscopic study [21]. General alterations in the cell surface glycocalyx reported in implantation [22, 23].

In control group, apical membrane and Golgi zone of the endometrial epithelium reacted to all used lectins. In control group, the epithelium reacted with WGA during implantation phase. It confirmed the previous studies that revealed WGA reacted residues increase on the rat uterine epithelial surface during implantation. [24]. After HCG administration, the reaction of the apical membrane of the uterine epithelium reduced. GlcNac mediate cell-cell or cell-matrix interaction during embryonic development [9]. Besides, it may represent recognition site for implantation of the embryo [25]. The data also showed an increase in the intensity of the reaction to WGA in sham animals. WGA also bound to sialic acid. It seems that stress of the injection without gonadotropin could also decrease the rate of implantation through increase in negatively charged glycoconjugates, e.g. sialic acid, that reduce the receptivity of uterus for blastocyst.

WGA-reacted residues in Golgi zone was the only glycoconjugates that modified by HCG. GalNac contained components collect in Golgi zone and then transport to the apical membrane where it can interact with embryo. Thus, it seems that HCG can reduce the receptivity of endometrium via reduction of GlcNac during implantation period.

In control group, basement membrane of the uterine epithelium reacted with WGA and UEA. WGA- and UEA-reacted carbohydrates involved in cell-cell interaction and cell invasion, respectively [9, 26].

In the present study, all lectins reacted with uterine glands except PNA. It was reported that rat uterine glands can react with WGA, DBA, UEA, and Con A at the day 10, 12, and 15 of pregnancy [27]. The data indicated that these residues exist from the beginning of the implantation; however, it seems that the expression of the PNA-reacted sugars start after implantation. It seems that the pattern of PNA expression in rat uterine glands is the same as human [28]. Uterine glands secrete the molecules that support the growth and development of fetus at peri-implantation period [28]. Thus, the uterine glands and their secretions can affect the blastocyst implantation indirectly. HCG injection influenced the expression of the WGA and DBA-reacted components. UEA reactivity was also changed with HCG administration. It may lead to modification of the embryo conditions.

Briefly, it can be concluded that failure of IVF program may be related to the changes in the biosynthesis and distribution of the glycoconjugates in endometrium that treat with HCG as inducer of ovulation.

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