Review Article

Effects of controlled ovarian hyperstimulation protocols on uterine markers

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Abstract

Controlled ovarian hyperstimulation is based on the development of multiple follicles in the same cycle as achieving the optimal number and quality of oocytes and is a common practice in infertility treatment. In this application, clomiphene citrate and gonadotropins are the most commonly used agents. These agents have some effects on reproductive tissues. Uterine tissue is susceptible to hormonal changes and undergoes a change in hormone action in terms of protein profile, secretory content and molecular structure during the menstrual cycle. In this review, it is aimed that controlled ovarian hyperstimulation has effect on female reproductive organs with different markers and methods, how to gather information about where the change occurs due to the functions of these markers, and to present this information to scientists working in the field by being related to each other. It is seen that controlled ovarian hyperstimulation may have negative effects on many functions such as uterine growth, receptivity, and altered expressions of the markers. Articles in this review, it is generally recognized that the expression of a majority of receptive proteins is decreased, which is thought to have an adverse effect on live birth rates and infertility treatment. More clinical trials are needed for these markers in order to increase live birth rates, implant success and to develop infertility treatment.

Keywords: Controlled Ovarian Hyperstimulation; Uterus; Receptivity Markers.

Controlled ovarianhyperstimulation (COH) is based on the development of a large number of follicles in the same cycle to obtain the optimal number and quality of oocytes from the ovary, within Assisted reproductive technology (ART) which is a common practice in infertility treatment. Many COH protocols have been developed for this purpose (1). The COH technique applied in the framework of ART is largely a hope light for couples who cannot have children in normal conditions, but the long-term effects have not yet been fully clarified. Agents used to stimulate ovulation have been reported to have negative effects on reproductive organs such as ovaries, uterus, and cervix (2).The uterus is a dynamic tissue under the control of ovarian steroids, estrogen and progesterone. The endometrium layer of the uterine undergoes proliferation, secretion and menstrual cycle to reach the receptive state in the implantation of the embryo. When the endometrium carries out its natural functions by stimulation of the hormones, the effects of this stimulation arise as transient secretion of endometrial proteins, changes in cell behavior and in the expression of certain markers. Based on the effects of hormonal changes in the uterine tissue, we aimed to investigate the effect of controlled ovarian hyperstimulation protocols on some uterine markers and to give information about what functions are associated with the molecular alteration.

Effects on Cell Adhesion Molecules

Integrins are adhesion molecules, which function in cell-cell and cell-matrix interactions, cell migration and differentiation. The endometrium has a cyclic change in the expression of the integrin in the epithelial cells, and the maximal expression occurs during the implantation period. The integrins that are expressed in humans during the implantation window and are believed to be indicative of uterine receptivity are α4β1, αvβ3 (3). Experimental animals have shown that α4β1 and αvβ3 integrins play important roles in mice and integrin β3 and avb3 integrins play important roles in implantation in rats (4-7). Mice studies have shown that the expression of integrin β3 subunit in thesurface epithelium and glandular epithelium of the endometrium decreases during the implantation window in the Gonadotropin-releasing hormone agonist (GnRHa), Gonadotropin-releasing hormoneantagonist (GnRHant)

and PMSG(pregnant mare serum gonadotropin) treated groups for ovulation induction (8). In the clomiphene citrate and letrozole ovulation-stimulated rats, the integrin β1 subunit decreased remarkably in the surface and gland epithelium when compared to letrozole and the control group in the clomiphene citrate-treated experimental group (9). In another study conducted with human menopausal gonadotropin (hMG) and recombinant follicle stimulating hormone (rFSH) in rats, it was determined that expression of α3 and β1 integrin in glandular epithelium and stromadecreased during implantation when compared with the control group (10). Some studies in women have shown a decreasing expression of avb3, a4 b1, a1 b1, b3integrins in different compartments such as glandular epithelium, stroma and surface epithelium after stimulation (11-15). There are also studies in which the decrease in integrin expression following COH protocols is related to the delay in gland epithelial development (12,13, 15,16). E-cadherin adhesion molecule, a transmembrane glycoprotein, plays a role in the classification of cells, cell-cell interaction, signal transduction and cell polarization at the same time during embryonic development. (17,18). E-cadherin is expressed in human endometrium and does not change so much during the menstrual cycle (19). However, the level of E-cadherin mRNA was found to be significantly higher during the luteal phase (20). There is a study that shows that ovarian stimulation affects E-cadherin expression in mice as well (21).Selectins are members of the cell adhesion molecule group and mediate cell-cell interactions.

The carbohydrate ligands that bind L-selectin are localized at the time of implantation in the surface epithelium of the human endometrium. L-selectin is most expressed in the surface epithelium during proliferative, early secretory and mid-secretory phase, while L-selectin ligands are most expressed in the glandular epithelium in the mid-secretory phase (22).In a COH protocol study, L-selectin ligand was detected on the endometrial surface and gland epithelium of both experimental groups and control groups during implantation window.However, during the implantation window, the expression of L-Selectin ligand in the endometrial epithelium was reduced in the COH groups compared to the control group.These findings suggest that the COH protocols may adversely affect endometrial receptivity in relation to the L-selectin ligand (23).

Effects on Glycoproteins
MUC-1 is a glycoprotein which expressed on the surface of uterine epithelial cells and believed to have a role in endometrial receptivity. During implantation, MUC-1 forms a uterine barrier for implantation by blocking the interaction between trophoblast and surface epithelial adhesion molecules (24). In a study conducted in females, after COH, MUC-1 mRNA expression in the endometrial gland and surface epithelium was found lower in the high-response patients than in the control group. This result was thought to be related to decrease endometrial receptivity (25).

Effects on Homeobox Proteins
Hoxa11 is expressed in the endometrial epithelium and stroma during the menstrual cycle and this expression reaches a maximum level during the implantation window (26). In a study performed in mice, it was determined that Hoxa11 mRNA and protein expression decreased in the COH groups during peri-implantation period. On this basis; It has been suggested that COH protocols may affect endometrial receptivity, and the impairment of the Hoxa11expression also contributes to this (21).

Effects on Growth Factors
Transforming growth factor beta family members (TGF-β) are multifunctional growth factors that regulate many cellular activities such as cell growth, proliferation, differentiation, tissue remodeling, angiogenesis, apoptosis and embryo-uterine interactions during the peri-implantation and implantation periods (27). In a study with rats, it was found that, hyperstimulation can affect TGFβ1 and TGFβ2 expression during peri-implantation period, and this condition causes the degradation of the endometrial environment required for successful embryo implantation (28).

Effects on Channel Proteins: (7)
Fluid movement has an important role in embryo implantation. There is a link between fluid accumulation in the uterine cavity and impaired IVF (In-vitro fertilisation) outcome. During the implantation, there is a predominant transcellular fluid and ion transport mechanism (29). It is known that fluid transport takes place via aquaporin 5 (AQP5) molecules in rats and aquaporin 2(AQP2) channels in humans AQP2, which is found in human endometrium and is thought to play an important role during implantation, edema development, and menstruation has a menstrual cycle dependent expression. The high levels of AQP2 are observed during proliferative and secretory periods (30, 31). In a study showed that AQP2 protein and mRNA were less expressed in the KOH-administered group. Also the intensity of staining in endometrial epithelial cells was reduced in the same group. It is thought that these diminished expressions of AQP2 may cause endometrial receptivity damage in patients treated with KOH (14). AQP5 is another channel protein that is expressed in human endometrium, which is dependent on the menstrual cycle. AQP5 is expressed mainly in endometrial stroma and gland epithelial cells and high levels of this protein are detected during proliferative and secretory periods of the menstrual cycle(32). In a study conducted in rats, it was found that the expression of AQP5 protein significantly increased during implantation in the ovarian stimulation treated group (33). These results suggest that ovarian hyperstimulation may cause dysregulation of fluid dynamics in uterine glands, which may create a negative environment for implantation and blastocyst.

Effects on Steroid Receptors
Ovarian estrogen and progesterone play an important role in endometrial receptivity and are essential for implantation. The estrogen and progesterone

receptor levels vary throughout the menstrual cycle. Estrogen receptor (ER) and progesterone receptor (PR) concentrations are high particularly in late follicular phase, and reach the highest level at the time of ovulation, begin to falls in the secretory phase and is very low in the late secretion phase (34). İn a study, it was determined that expression of ERα and PR decreased in the uterus stroma and endometrial epithelial cells in the stimulation protocol treated group (35). Likewise in a study conducted in women, the diminish expression of ERα in endometrial gland epithelium has been shown in the stimulated groups (36). In another study, low ER expression and weak nuclear immunoreactivity were shown in uterine endometrial stroma and endometrial glands of women treated ovarian stimulation (37). ER expression after the hyperstimulation protocol was abundant in the endometrial glands despite it decreased in the stroma (38).All these results suggest that ovarian stimulation may lead to decreased ER, PR expressions and thus decreased proliferation in the pre-ovulation period; may alter expression of endometrial receptivity markers and it reveals so could result in low pregnancy rates.

Effects on Extracellular Matrix Enzymes
MMP-9 is an important mediator of cellular invasiveness during embryo implantation and TIMP-3 is the molecular that acts as a regulator in the uterus to limit invasion to the implantation site (39).In a rats study, an increase expression of TIMP-3 was observed in the superovulatory groups, but there was no difference in MMP-9 mRNA expression (40). For this reason, it is thought that stimulation can alter the process of endometrial receptivity by increasing the expression of TIMP-3 and may have harmful effects.

According to the results obtained from all these histological and molecular observations, there is strong evidence that ovarian stimulation may affect the expression of some markers and decreased - increased expression of these proteins may have negative effects on the endometrium. Ovarian stimulation may cause developmental retardation by inhibiting endometrial gland development and the uterus can affect genes that cause dysregulation in fluid dynamics. Stimulation may also increase the risk of cancer through proteins known to increase endometrial cancer risk. Besides these effects, ovarian stimulation may increase the abortion risk by reducing the expression of proteins that are effective in maintaining and protecting the gestation. Decrease gene expressions,in which size that may harm endometrial receptivity, are thought to play a role in embryonic development, functional endometrial differentiation, and formation of a negative environment for optimal conditions of implantation.The further study of the molecules in the implantation mechanism, the clarification of their roles and the understanding of which mechanism they are affected from, is necessary to better understand the link between endometrial development and receptor activity in IVF cycles.These results can provide useful information for increasing live birth rates in female infertility.

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