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GnRH-I – A SUITABLE BIOMARKER FOR ASSESSMENT OF ENDOMETRIAL RECEPTIVITY

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Abstract: The hypothalamus and the pituitary gland are the main source and target sites of gonadotropin releasing hormone (GnRH), but numerous studies have demonstrated that GnRH-I and GnRH-II exist in different reproductive tissues such as the ovary, endometrium etc. Major effects of GnRH-I in the reproductive tissues are regulation of cell proliferation, activation of apoptosis and remodulation during the process of embryo implantation.

In this retrospective study, endometrial samples were obtained from 30 women with normal menstrual cycles. They were divided into two groups: (1) women with successful implantation (n=15) and (2) women with recurrent implantation failure (RIF) and unsuccessful implantation (n=15). The endometrial biopsies were dated histologically and immunohistochemically by using available criteria in the literature. Using immunohistochemistry, we identified GnRH-I in stromal, luminal and glandular epithelial cells.

Our results show that GnRH-I is expressed in different cell types of the human endometrium during the mid-luteal phase (window of implantation) – luminal epithelial cells, glandular epithelial cells and stromal cells. Considerably higher expression of this hormone in the patients with successful implantation was detected in the gland (Group 1 - 2.07 vs. Group 2 - 1.75, P = 0.042) and luminal epithelial cells (Group 1 - 2.05 vs. Group 2 - 1.73, P = 0.049). While only 30% of the patients with unsuccessful implantation had values for glandular epithelial cells H≥2, in the patients with successful outcome more than 70% exceeded this value.

As a conclusion an insufficient expression of GnRH-I in human endometrium during the mid-luteal phase could be related to embryo implantation failure. The immunohistochemical detection of GnRH-I expression should be considered as a suitable biomarker for assessment of endometrial receptivity during the window of implantation.

INTRODUCTION

GnRH, a hypothalamic neuronal secretory decapeptide, is essential for human reproduction (Wu et al., 2009). Besides its well-known endocrine function, GnRH may directly regulate some extrapituitary reproductive tissues such as endometrium, oviduct, ovary and placenta (Islami et al. 2001, Chou et al. 2003, Casan et al. 2000, Grundker et al. 2004, Kim et al. 2006)

The presence of GnRH and its receptor as messenger RNA (mRNA) and protein in various human endometrium cells have been demonstrated by several studies (Casan et al., 1998, Raga et al., 1998). It has a clear dynamics during the menstrual cycle with significant rise in the midluteal phase (Dong et al. 1998, Raga et al. 1998)

Human gametes usually interact with GnRH during their journey through the male and female reproductive tracts (Aten et al., 1987, Oikawa et al., 1990, Morales, 1998). It is well known, that GnRH is an important modulator of spermatozoa function and spermatozoa-ZP binding (Morales and Llanos, 1996). Moreover, this hormone is involved in the regulation of the cleavage rate of the oocyte (Funston and Seidel, 1995). Additional studies from Dong and colleagues have shown the presence of GnRH not only in the uterus, but also in the embryo, which suggests that GnRH may play a crucial role in the implantation process (Dong et al. 1998, Imai et al., 1994; Ikeda et al., 1996).

Endometrial receptivity is defined as a temporary unique sequence of factors that make the endometrium receptive to the embryonic implantation (Bergh P. and Navot D. 1992). It has been shown that GnRH could participate in this process by remodeling the extra cellular matrix (Goto et al., 1999). The mechanism involves inducement of structural luteolysis through stimulation of matrix metalloproteinase (MMP-2) and membrane type 1-MMP expression, leading to the degradation of collagens types IV, I, and III, respectively (Goto et al., 1999). Here, we are trying to find relation between recurrent implantation failure (RIF) cases and protein levels of GnRH in different cell types in human endometrium. For implementation of this task we compare GnRH immunoreactivity in endometrial biopsy samples obtained from RIF group women with immunoreactivity observed in control group women (with successful implantation).

MATERIALS AND METHODS

Study design

In this retrospective study endometrial samples were obtained from 30 women with normal menstrual cycles. The women are patients of the fertility clinic Nadezhda Women's Health Hospital and their cases are classified as unexplained infertility. They were divided into two groups: (1) women with successful implantation (n=15) and (2) women with recurrent implantation failure (RIF) and unsuccessful implantation (n=15). Recurrent implantation failure in our cases refers to failure to achieve a clinical pregnancy after transfer of at least four good-quality embryos in a minimum of three fresh or frozen cycles in a woman under the age of 40 years. Patients with endometriosis, pelvic inflammatory disease, polycystic ovarian syndrome, age>40, BMI>30 and bad quality embryos were excluded from the study. The stage of menstrual cycle was established by the previous menstrual period, transvaginal ultrasonographic finding and LH surge. The endometrial biopsies were dated histologically and immunohistochemically by using available criteria in the literature (Oluwole and Wenxin, 2005).The level of protein accumulation (H) was scored as 0 (no staining), 1 (weak staining), 2 (moderate staining) and 3 (strong staining).

Tissue collection

Endometrial samples were taken using a Novak (Novak Inc., Palo Alto, CA) curette in the operating room immediately before surgical procedures. The tissue was fixed with 4% paraformaldehyde.

Immunohistochemical analysis

To determine the presence of GnRH in the human endometrium, Novolink™ Polymer Detection System (Leica Biosystems, RE7150-K) and GnRH antibody (Antibodies-online Inc.; Epitope AA 24-92; ABIN1173767) was used by following manufacturer's instructions.

Statistical analysis

Student *t*-test was performed using SPSS version 21.0 (SPSS Inc., Chicago, IL, USA) in order to compare the mean score of GnRH-I. $P < 0.05$ was considered as statistically significant. Results were reported as mean values.

RESULTS AND DISCUSSION

Our results show that GnRH-I is expressed in different cell types of the human endometrium during the mid-luteal phase (window of implantation) – luminal epithelial cells, glandular epithelial cells and stromal cells.

The reaction intensity was significantly different between the studied patient groups (Fig.1).

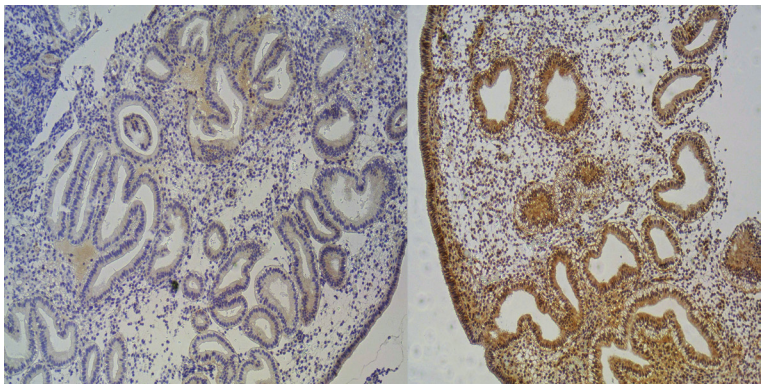

Fig. 1. Immunohistochemical staining for the expression of endometrial gonadotropin releasing hormone I (GnRH-I) during the implantation window in women. (a) low expression (weak staining, H=1) and (b) high expression (strong staining, H=3)

Significantly higher expression of this hormone in the patients with successful implantation was detected in the gland (Group 1 - 2.07 vs. Group 2 - 1.75, P = 0.042) and luminal epithelial cells (Group 1 - 2.05 vs. Group 2 - 1.73, P = 0.049) (Fig. 2). While only 30% of the patients with unsuccessful implantation had values for glandular epithelial cells H≥2, in the patients with successful outcome more than 70% exceeded this value.

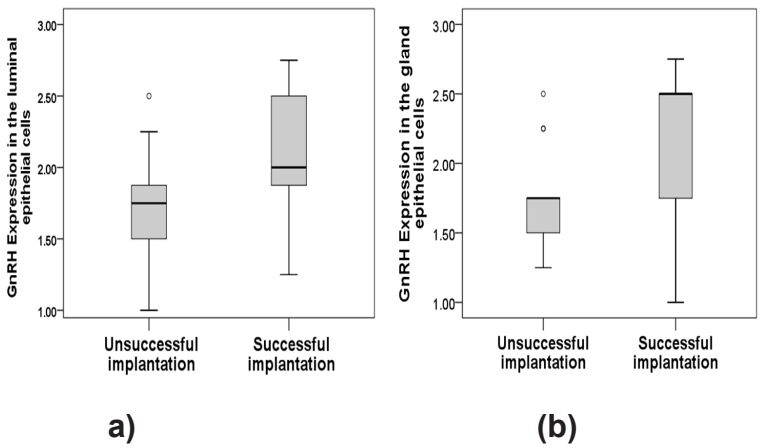

Fig. 2. Boxplots representing HSCORE for the immunohistochemical staining intensity of endometrial GnRH in luminal (a) and gland (b) epithelial cells during the implantation window and its comparison among women with successful and unsuccessful implantation.

The observed lower expression of GnRH-I in the endometrium of patients with RIF could be explained by its crucial role in the modification of specific types of endometrial cells during the implantation window. GnRH-I is already known to trigger urokinase-type plasminogen activator in human extra-villous cytotrophoblasts and decidual stroma cells *in vitro*, indicating that this hormone may have an important role in regulating the proteolytic degradation of the extracellular matrix of the endometrial stroma. This process is directly connected to decidualization and trophoblast invasion through the uterine wall (Paria et al. 2002, Chou et al. 2003).

Several studies have reported a positive effect of administration of GnRHa in the mid-luteal phase. In a prospective study, some authors found a higher implantation rate and live birth rate after a single dose of 0.1 mg GnRHa administered 3 days after embryo transfer in both agonist and antagonist protocols (Tesarik et al. 2006). We could hypothesized that the existed deficit of GnRH-I in the endometrium of women with RIF could be overwhelmed by appropriate application of recombinant GnRH.

CONCLUSIONS

As a conclusion, an insufficient expression of GnRH-I in human endometrium during the mid-luteal phase could be related to embryo implantation failure. The immunohistochemical detection of GnRH-I expression should be considered as a suitable biomarker for assessment of endometrial receptivity during the window of implantation.

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