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Original Article

COMPARATIVE STUDY OF GONADOTROPIN-RELEASING HORMONE RECEPTOR IN FALLOPIAN TUBE BY IMMUNOHISTOCHEMISTRY AMONG WOMEN WITH ECTOPIC PREGNANCY, HYSTERECTOMY AND TUBAL LIGATION

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ABSTRACT

Objective: Detection of GnRH receptor expression by immunohistochemistry techniques in human fallopian tubes during luteal phase of menstrual cycle among women subjected to tubal ligation with history of normal pregnancy, ectopic tubal pregnancy and hysterectomized women.

Patients and Methods: This comparative study involved 39 females with history of ectopic pregnancy who underwent emergency laproscopy (ages ranged from 15-45 years), 40 women were operated on for elective hysterectomy (ages ranged 25-45 years) due to various benign gynecological reasons. The causes of hysterectomy include 30 patients with multiple uterine fibroid, 7 patients with adenomyosis and 3 patients with vaginal bleeding and not responding to treatment. Further 40 women subjected to cesarean section with bilateral tubal ligation at term pregnancy (ages ranged 26- 45 years) were also included in the study. The exclusion criteria included patients with pelvic inflammatory disease, endometriosis and luteinizing-hormone-releasing hormone analogue users. The study was carried out during the period from September 2014 till June 2015 at Basrah Maternity and Childhood Hospital. Their histopathologic examinations of the endometrium proved to be in luteal/secretory phase.

Fallopian tubes were removed and collected from patients undergoing surgical removal of it due to tubal ectopic pregnancy after hysterectomy as well as women operated on for tubal ligation. Fallopian tubes from ectopic pregnancy cases, hysterectomy patients and women with tubal ligation were preserved in 10% formalin and were taken to the Pathology Laboratory, Al-Saddar Teachiung Hopspital for the purpose of histopathology (Hematoxyline and eosin staining method) and immunohistochemical (avidin-biotin-alkaline phosphatase technique) investigations.

RESULTS

The highest incidence responding rate (49%) of ectopic pregnancy noticed among age group of >25-35 years in comparison to the hysterectomized group (75%) among age group of >35-45 years and to women with tubal ligation group which was >35-45 years. All relationships are statistically significant (P< 0.001). Also, women with history of ectopic pregnancy (28.2%) are more reliable for reoccurrence of ectopic in comparison to the hysterectomized women (7.5%) with significant statistical difference (P<0.05). An interesting result is women with no parity and infertility (53.8%) tend to develop ectopic pregnancy more than others. The relationship is statistically significant (P<0.05).

On histpathological examination, numerous pale chorionic villi were detected in the lumen of the fallopian tube. There were also sheets of trophoblast, lying free in the lumen. A brown precipitate in the cytoplasm of the cells of the fallopian tube indicated positive staining by primary antibody while no staining was detected in negative samples by using immunohistochemistry examination.

Even there is a difference in the distribution positivity of gonadotropin-releasing hormone (GnRH) receptor detection among ectopic pregnant women (58.9%), hysterectomized women (82%) and only 10% among women with tubal ligation in the fallopian tube at the luteal phase of menstrual cycle by using immunohistochemistry technique but statistically is marginally significant (P=0.069). While the negative distribution for GnRH receptor was higher among women with ectopic pregnancy (41.1%) in comparison to women who were operated on for hysterectomy (18%) and to women with tubal ligation (90%).

The immunoreactive GnRH receptor was identified in the fallopian tube samples from patients with ectopic pregnancy, in the mucosa of the tube alone (5 out of 39) or chorionic villi only (6 out of 39) or both of them the mucosa as well as villi (13 out of 39). The differences were statistically insignificant (P>0.85).

Conclusion: Therefore, the interaction between the embryo and the maternal reproductive tract via the GnRH system may play an important role during gamete maturation, fertilization, pre-implantation, implantation and embryonic development.

KEYWORDS: Comparative Study of Gonadotropin-Releasing Hormone, Histpathological, Hysterectomized

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INTRODUCTION

Even the ectopic pregnancy rates have been decreased in many countries such as Sweden, Finland, France and United Kingdom (Jurkovic, 2012), but it is still a serious public health problem with a high morbidity and mortality rates. Also, the danger for reoccurrence of ectopic pregnancy is 12-18% (Bouyer, *et* al., 1996). In Iraq, ectopic pregnancy is a public health matter but unfortunately, there is no published data about its epidemiology, incidence and mortality rates**.**

Many causes might be involved in a such situation as pelvic infection, pelvic surgery, cigarette smoking …. etc. which will mentioned in quite details in the literature review. Those factors may cause an abnormalities in the fallopian tube morphology, function, low smooth muscle activity or varied oestrogen/progesterone ratio. That would lead to block the normal passage of the fertilized ovum into uterine cavity for implantation (Vasquez *et al*., 1983; Peretz *et al*., 1984; Cartwright, 1993). Improvement in surgical operations is essential to prevent tubal damage and retain future fertility. In a cases, in *vitro* fertilization (IVF) is recommended. Most ectopic pregnancies include an early pregnancy failure and the associated symptoms are brown vaginal discharge, bleeding, abdominal pain due to intra-uterine hemorrhage and discharge of a deicidal cast in addition to gastrointestinal disorders. Serum human chorionic gonadotrophin (hCG) measurements have been used for investigation further to ultrasound examination.

In addition, normal reproductive activity needs a hormonal secretion at all levels of the hypothalamic-pituitary-gonadal relationship. GnRH which is produced by the pituitary gland is important in the reproductive function. GnRH attach to its receptor on gonadotrope cells. The outcome would be the secretion of the gonadotropins, luteinizing hormone

(LH) and follicle-stimulating hormone (FSH). LH and FSH activate gametogenesis (formation of mature ova and sperms) and steroidogenesis (production of gonadal hormones, oestrogen, progesterone and androgens) (Conn and Crowley, 1991; Ortmann and Diedrich, 1999).

Therefore, the interaction between the embryo and the maternal reproductive tract via the GnRH system may play an important role during gamete maturation, fertilization, pre-implantation, implantation and embryonic development. Thus, this field of work offers the future of new clinical applications for GnRH analogues including improved reproductive activity and may reduce the chance of ectopic pregnancy. Therefore, the aim of the study is to detect the GnRH receptor expression by immunohistochemistry techniques in human fallopian tubes during luteal phase of menstrual cycle among women subjected to tubal ligation with history of normal pregnancy, ectopic tubal pregnancy and hysterectomized women.

MATERIALS AND METHODS

Subjects

This comparative study involved 39 females with history ectopic pregnancy who underwent emergency labroscopy at Basrah Maternity and Childhood Hospital, during the period from September 2014 till June 2015. Their ages ranged 15-45 years. Another 40 women with age ranged 25-45 years were undergoing elective hysterectomy for various benign gynecological reasons as multiple uterine fibroids, dysfunctional uterine bleeding, ensuring histopathologic examination of the endometrium proved to be in luteal/secretory phase. Furthermore, 40 women were subjected to cesarean section with bilateral tubal ligation at term pregnancy were also included in the study. Their ages ranged 26-45 years. This work has been approved ethically by the Ethical Committee of the College of Medicine, University of Basrah, Iraq. The exclusion criteria included patients with pelvic inflammatory disease, endometriosis and luteinizing-hormone-releasing hormone analogue users.

Sampling

Fallopian tubes were removed and collected from patients undergoing surgical removal of it due to tubal ectopic pregnancy. Similarly, fallopian tubes were collected after hysterectomy as well as women operated on for tubal ligation. Fallopian tubes from ectopic pregnancy cases, hysterectomy patients and women with tubal ligation were preserved in 10% formalin and were taken to the Pathology Laboratory, Al-Saddar Teaching Hospital, Basrah for the purpose of histopathology (Hematoxyline and eosin staining method) (Rosai, 2012) and immunohistochemical (avidin-biotin-alkaline phosphatase technique) (Cuely, 2013) investigations.

Statistical Analysis

It was performed by SPSS version 17.P value of < 0.05 was considered to indicate statistical significance.

RESULTS

The Major Characteristics of the Studied Groups

The demographic features for the ectopic pregnant women and the hysterectomized women and women operated on for tubal ligation are illustrated in Table (1, 2). The highest incidence responding rate (49%) of ectopic pregnancy Table

(1) noticed among age group of >25-35 years in comparison to the hysterectomized group (75%) among age group of >35-45 years and to women with tubal ligation group which was >35-45 years (Table 1). All relationships are statistically significant (P< 0.001). The causes of hysterectomy include 30 patients with multiple uterine fibroid, 7 patients with adenomyosis and 3 patients with vaginal bleeding and not responding to treartment. Also, women with history of ectopic pregnancy (28.2%) are more reliable for reoccurrence of ectopic in comparison to the hysterectomized women (7.5%) with significant statistical difference (P<0.05). An interesting result is women with no parity and infertility (53.8%) tend to develop ectopic pregnancy more than others. The relationship is statistically significant (P<0.05) (Table 2).

Table 1: The Distribution of Cases of Ectopic Pregnancy, Hysterectomy and Tubal Ligation in Relation to Age

Groups	Age (Years)			X^2	Df	P-Value
	15-25	>25-35	>35-45			
Ectopic pregnancy. n=39	16(41%)	19(49%)	4(10%)	19.58	2	0.001
Hysterectomy. n=40	0	10(25%)	30(75%)	19.09	1	0.001
Tubal ligation. n=40	0	16(40%)	24(60%)	4.00	1	0.05
Both Age & Groups				137.67	4	0.001

Table 2: The Distribution of Ectopic Pregnancy, Hysterectomy and Tubal Ligation Cases in Relation to Parity

Groups	Parity			X^2	Df	P-Value
	None	P_1-P_5	>P_5			
Ectopic pregnancy. n=39	21(53.8%)	11(28.2%)	7(18%)	4.65	2	0.132
Hysterectomy. n=40	8(20%)	12(30%)	20(50%)	4.53	2	0.162
Tubal ligation. n=40	0	20(50%)	20(50%)	0	2	-
Both Parity & Groups				81.32	4	0.005

Histopathology

In the lumen of the tube there were numerous pale chorionic villi. There were also sheets of trophoblast, lying free in the lumen (Figure 1, 2, 3)

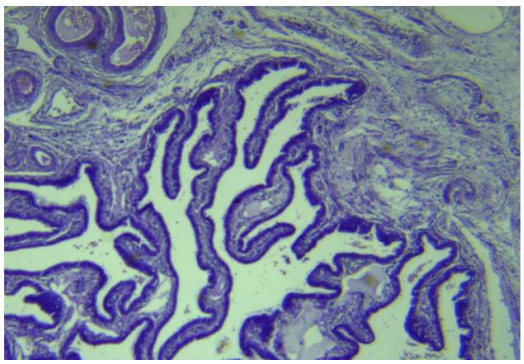

Figure 1: Normal Fallopian Tube Mucosa. (H&E 100X)

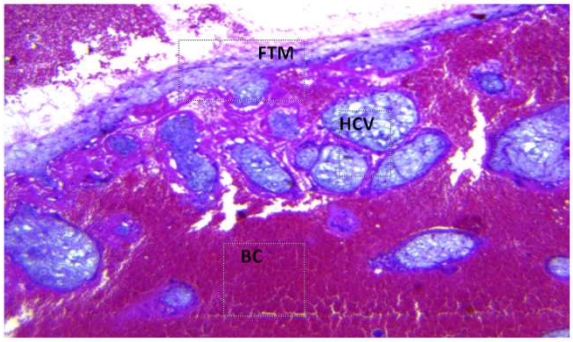

Figure 2: Tubal Ectopic Pregnancy Shows HYDROPIC Chorionic VILLI (HCV) within Blood Clot (BC) and Fallopian Tube Mucosa (FTM) (H&E 100X)

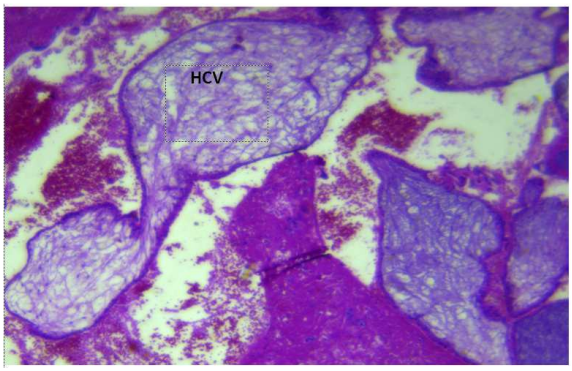

Figure 3: Hydropic Chorionic Villi (HCV) (H & E 400X)

Immunohistochemistry

The presence and distribution of GnRH receptor in the fallopian tube during luteal phase of the menstrual cycle was studied by application of immunohistochemical investigation (avidin-biotin-alkaline phosphatase technique).

A brown precipitate in the cytoplasm of the cells of the fallopian tube indicated positive staining by primary antibody while no staining was detected in negative samples (Figure 4, 5, 6, 7, 8, 9).

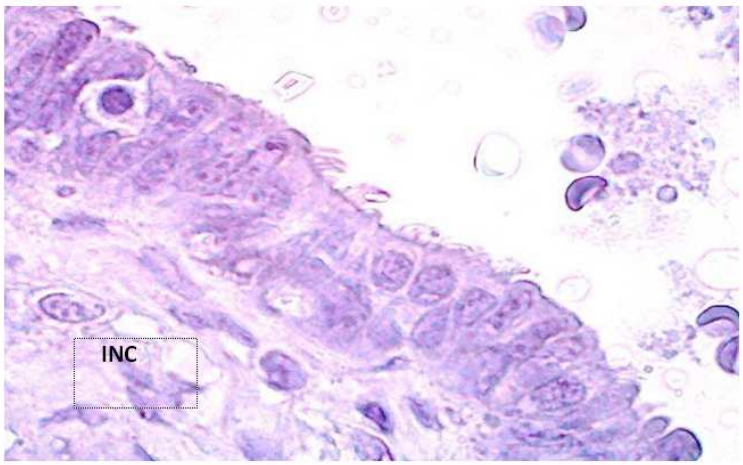

Figure 4: Internal Negative Control (INC) (400X)

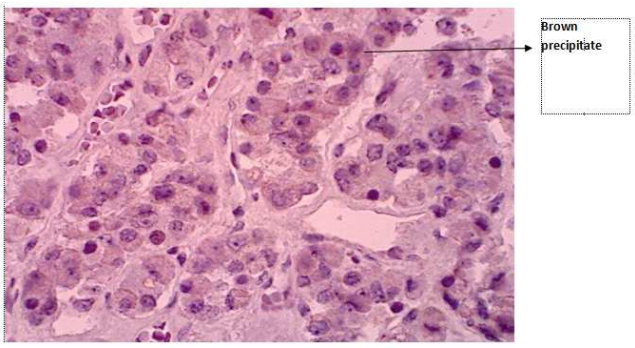

Figure 5: Normal Pituitary Gland Shows Positive Control for GNRH Expression in the Cytoplasm (400X)

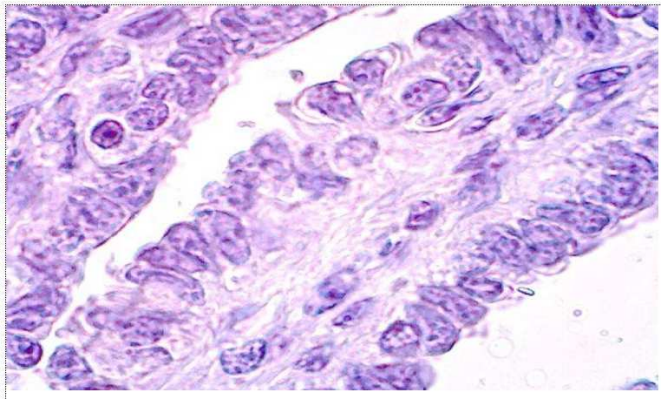

Figure 6: Fallopian Tube Mucosa Shows Negative Expression for GNRH Receptors (400 X)

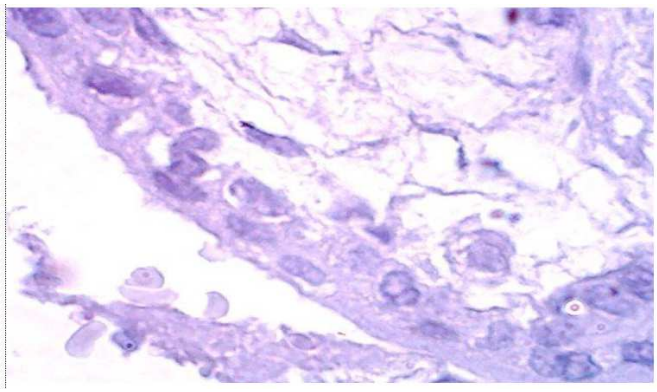

Figure 7: Trophoblasts Show Negative Expression for GNRH Receptors (400 X)

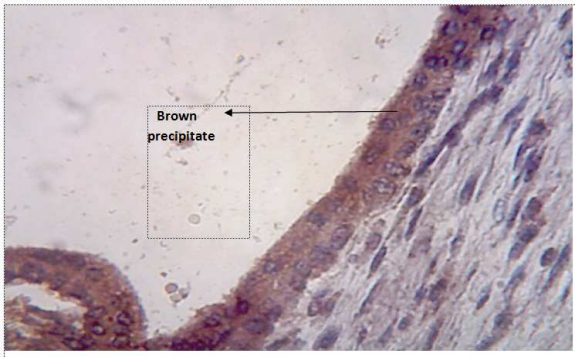

Figure 8: Fallopian Tube Mucosa Shows Positive Cytoplasmic Expression of GNRH (400X)

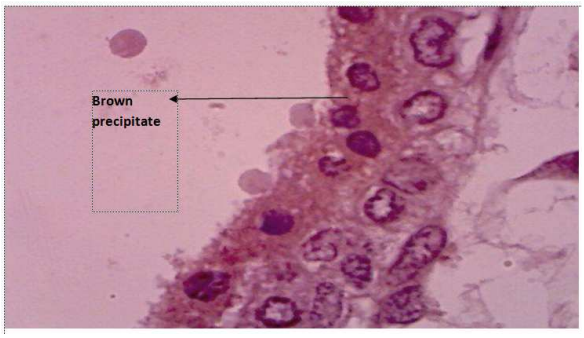

Figure 9: Trophoblasts Show Positive Cytoplasmic Expression for GNRH (400 X)

The Distribution of Gnrh Receptor in the Studied Groups

Even there is a difference in the distribution positivity of GnRH receptor detection among ectopic pregnant women (58.9%) hysterectomized women (82%) and only 10% among women with tubal ligation in the fallopian tube at the luteal phase of menstrual cycle by using immunohistochemistry technique but statistically is marginally significant (P=0.069) (Table 3, Figure 10). While the negative distribution for GnRH was higher among women with ectopic pregnancy (41.1%) in comparison to women who were operated on for hysterectomy (18%) and to women with tubal ligation (90%) (Table 3, Figure 10).

Table 3: The Distribution of GNRH Receptor among Women with Ectopic Pregnancy, Hysterectomy and Tubal Ligation Groups

Group	GNRH Positive	GNRH Negative
Ectopic pregnancy. n=39	23 (58.9%)	16 (41.1%)
Hysterectomy. n=40	32 (82%)	8 (18%)
Tubal ligation. n=40	4 (10%)	36 (90%)

$X^2 = 3.309$ DF = 1 P = 0.069

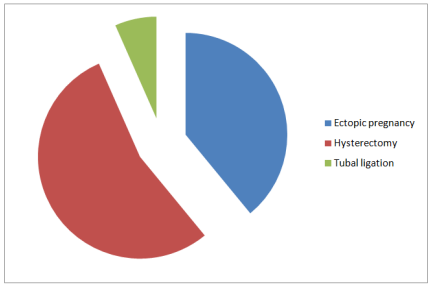

Figure 10: The Distribution of GNRH Receptor among Women with Ectopic Pregnancy, Hysterectomy and Tubal Ligation Groups

The immunoreactive GnRH was identified in the fallopian tube samples from patients with ectopic pregnancy, in the mucosa of the tube alone (5 out of 39) or chorionic villi only (6 out of 39) or both of them the mucosa as well as villi (13 out of 39). The differences were statistically insignificant (P>0.85) (Table 4)

Table 4: The Distribution of GNRH Receptor among Studied Ectopic Pregnancy Group in Relation to the Site

Site	GNRH Positive	GNRH Negative
Tubal mucosa	**5**	**6**
Chorionic villi	**6**	**5**
Both sites	**13**	**15**
X^2 = 0.317	**Df = 2**	**P = 0.854**

DISCUSSIONS

The present study indicates that GnRH receptor is available in the fallopian tube during the luteal phase of the menstrual cycle of the examined women by application of the immunostaining technique. Immunohistochemistry combines anatomical, immunological and biochemical techniques to identify tissue components by the interaction of target antigens with specific antibodies by the aid of a visible label. As noticed in this study, the method is an excellent detection technique and has great advantage of being able to show exactly where a given protein is located within the tissue or even within cells examined including GnRH receptors (Ramos-Vara and Miller, 2014).

It is the second work involving the usage of immunohistochemistry technique in Basrah after a postgraduate study which was done by Talib (2014) concerning the clinical and morphological features in conjunction with immunophenotyping for hematological malignancies in Basrah Province by application of immunohistochemistry and immunocytology**.**

GnRH receptors have been found in a wide variety of normal and tumor human reproductive system. Therefore, there has been a growing interest in the physiology of these peripheral receptors. It has been indicated that GnRH in tissues functions as an autocrine-paracrine regulator by activating peripheral GnRH receptor (Yu *et al*., 2011). However, the effects of GnRH are complicated and appear to be cell basic dependent (Cheung and Wong, 2008). Most studies have only been carried out in cellular models, but *in vivo* approaches will be important to complete the understanding of the specific role of GnRH. Recently, the significant function of extra-hypothalamic GnRH on regulation of reproductive functions has increased due to the specific distribution of its classicals receptor in reproductive tissues (Hapgood *et*

al.,2005; Schirman-Hildesheim *et al.*, 2005). Thus, this is the first step for more effective and perhaps new therapeutic strategies for improving clinical outcome and to reduce the morbidity and maternal mortality caused by this common condition.

The present study provides evidence that GnRH receptor is produced in the human fallopian tube during the luteal phase of the menstrual cycle among groups, women with ectopic pregnancy as well as women subjected to hysterectomy operation by the presence of brown stained precipitate in the cytoplasm of epithelial cells by using qualitative immunohistochemistry technique. Although, this is in agreement with other works involving the hysterectomy cases (Casan *et al.*, 1998; Dong *et al.*, 1998; Raga *et al.*, 1998) but to the best of my knowledge there is no published article concerning the relation of GnRH receptor with ectopic pregnancy**.**

According to the present data in this study, the highest incidence rate (49%) of ectopic pregnancy noticed among age group of >25-35 years which can be explained by the high rate of marriages, high sexual activity and reproduction. Also, women with history of ectopic pregnancy (28.2%) are more reliable for reoccurrence of ectopic. An interesting result is women with no parity and infertility (53.8%) tend to develop ectopic pregnancy more than others.

The demonstration of GnRH receptor and GnRH are synthesized within the same cell and are expressed in both cytotrophoblast and syncytiotrophoblast and exhibit changes paralleling the time course of chorionic gonadotrophic hormone (hCG) secretion during pregnancy, that would provide a better understanding of GnRH paracrine-autocrine regulation of hCG secretion by placenta, through an elevation followed by a low concentration in GnRH receptor gene expression from the first-trimester to term placenta (Lin et al., 1995). These findings indicate an important role for the GnRH receptor in regulating hCG secretion during pregnancy. Nevertheless, the highest GnRH concentrations in the placenta are present during the first term of pregnancy, along with the transient distribution of hCG synthesis (Siler-Khodr et al., 1984)

Even there is a difference in the positivity of GnRH distribution among ectopic pregnant women (58.9%) and hysterectomized women (82%) but statistically nearly significant (P=0.069). The lowest distribution of GnRH receptor (10%) has been noticed among women who were operated on for tubal ligation indicating the absence of the role of GnRH at term pregnancy. It can be explained by that GnRH is suppressed in pregnancy by the elevated corticotropin-releasing hormone, endorphins alfa and cortisol with blunted response of the pituitary to GnRH and low LH and FSH levels by 6-7 weeks of pregnancy (Scheithauer et al., 1990; Foyouzi et al., 2004) and become undetectable in the second trimester on ward. FSH and LH responses to GnRH stimulation are also decreased (Garner and Burrow, 2004;) and the rapidly rising hCG concentration suppresses secretion of both FSH and LH hormones, thus inhibiting ovarian follicle development by blunting response to gonadotropin-releasing hormone (Karaca, 2010; pipkin, 2012).

The results of this study in ectopic pregnancy confirm that by clear observation of the positivity in presence of GnRH receptor in the mucosa of the tube alone is (5 out of 39) or chorionic villi alone (6 out of 39) or both of them (13 out of 39), the fact that GnRH mRNA and protein expression are increased in the hatching blastocyst stage compared to morula stage (Casan *et al*., 1999; Raga *et al*., 1999), as this hormone has been recently implicated as a possible important paracrine factor in the process of embryonic implantation (Casan *et al*., 1998; 1999; 2000; Raga *et al*., 1998; Seshagiri *et*

al., 1994; Yang *et al*., 1995).

Thus, the theory that the embryo communicates with maternal tubal epithelium and endometrium through the GnRH system to stimulate embryonic development and endometrial receptivity has been confirmed (Yang *et al*., 1995; Casan *et al*., 1999; Raga *et al*., 1999). These findings suggest that although embryonic GnRH has an autocrine role in early embryonic formation, the fallopian tube GnRH is likely to lead for enhancement of the embryonic development by a paracrine action. The delay in development of the embryo has been improved when an embryos are kept in medium containing GnRH (Seshagiri *et al*., 1994; Yang *et al*., 1995; Casan *et al*., 1999; Raga *et al*., 1999;) or are cultured with fallopian tube tissue (Bongso *et al*., 1992; 1994; Yeung *et al*., 1996).

Seshagiri *et al.* (1994) showed that immunoreactive GnRH and hCG were produced *in vitro* by cultured rhesus monkey embryos during the entire peri-attachment period, from morula to attached blastocyst stage and found that the GnRH secretion started before that of hCG. GnRH and GnRH receptors have also been shown to be present in pre-implantation human embryos and the fallopian tubes in the luteal phase at both mRNA and protein levels (Casan *et al.*, 1999; Casan *et al*, 2000).

Human implantation is a complicated procedure that under normal circumstances begins after the loss of zona pellucida till the blastocyst reaches the uterine cavity and attaches to the endometrial epithelium. These steps are the result of an embryonic-maternal reaction, in which the embryo and the fallopian tube induce changes in each other to promote receptivity. That is possibly the situation in ectopic pregnancy with fixed problem is the insufficient room for accommodating the embryo in the fallopian tube. Macrophages in turn are a source of prostaglandins which influence the contractibility of the fallopian tubes by acting on the smooth muscle. They are important components for the normal function of the tubes including the fertilization process. This may be an essential factor in the pathogenesis of salpingitis, which result in infertility and ectopic pregnancy (Safwat, 2008).

Therefore, the results of the *in vivo* studies in both human (Raga *et al*., 1998; Uehara *et al*., 1998; Fujiil *et al.* 2001; Dutta and Konar, 2014; Sabin *et al*., 2015) and animals (Seshagiri *et al*., 1994; Yang *et al*., 1995) showing a useful effect of GnRH agonist on fertilization, early embryonic development and implantation which may have outstanding therapeutic outcome.

Moreover, functional as well as structural changes within the fallopian tube have been be associated with infertility and ectopic pregnancy. Since little is known about the fallopian tube function at the cellular level, the area is open for research in hormones, cytokines, growth factors and macrophages for their effects on growth and function of fallopian tube cells. Also, factors that negatively affect the fertilization process and contribute to ectopic pregnancy must be investigated in the future in order to develop therapeutic achievement to treat these disorders.

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