Section: Pediatrics

Original Research Article

A COMPARATIVE STUDY OF SERUM PROGESTERONE, SERUM BETA HUMAN CHORIONIC GONADOTROPHIN, SERUM ESTRADIOL AND VASCULAR ENOTHELIAL GROWTH FACTOR IN ECTOPIC PREGNANCY

Asha Makwana[1], Dheeraj Makwana[2]

[1]Associate Professor, Department of Biochemistry, JLN Medical College, Ajmer, Rajasthan, India.
[2]Physiotherapist, Department of Physiotherapy, N, W.R Hospital, Ajmer, Rajasthan, India.

Received : 08/02/2023
Received in revised form : 11/03/2023
Accepted : 03/04/2023

Keywords:
VEGF, β-hCG, Progesterone, Estradiol, Ectopic Pregnancy, Normal Intrauterine Pregnancy

Corresponding Author:
Dr. Asha Makwana,
Email: tvaritaishu10@gmail.com

DOI: 10.47009/jamp.2023.5.3.52

Source of Support: Nil,
Conflict of Interest: None declared

Int J Acad Med Pharm
2023; 5 (3); 239-244

Abstract

Background: Ectopic Pregnancy is a common life threatening complications of early pregnancy refers to a gestation in which the fertilized ovum implants itself outside the uterus, usually in one of the fallopian tubes. Several hormones eg Serum Progesterone, Serum Beta Human Chorionic Gonadotrophin, Serum Estradiol are affected in the body during pregnancy, which plays major role during pregnancy. One of the most important growth and survival factors for endothelium is vascular endothelial growth factor (VEGF). VEGF induces angiogenesis and endothelial cell proliferation and it plays an important role in regulating vasculogenesis. It participates in the process of implantation and placentation, and it is significantly increased in early pregnancy complications. The value of serial measurement of serum β subunit of human chorionic gonadotropin (β-hCG) and ultrasonography in the early diagnosis of ectopic pregnancy has already been established. **Objective:** This study was to evaluate and compare Serum VEGF, Serum Progesterone, Serum Beta Human Chorionic Gonadotrophin, Serum Estradiol in women with ectopic pregnancy and normal intrauterine pregnancy and to correlate the levels of Serum Vascular endothelial growth factor (VEGF), Serum Progesterone, Serum β-hCG and Serum Estradiol in women with ectopic pregnancy and normal intrauterine pregnancy. **Methodology:** It is a prospective, case controlled, hospital based study in which Serum levels of vascular endothelial growth factor, beta-human chorionic gonadotropin , Estradiol were measured by enzyme linked immuno sorbent assay (ELISA) technique and progesterone was measured by Radio immuno Assay (RIA) technique in 100 symptomatic women with ectopic pregnancy and 100 women with normal intrauterine pregnancy in the wards of Rajkiya mahila Chikitsalya, J.L.N. Medical College and Associated Group of Hospitals, Ajmer after taking approval from ethical committee. These values were compared by t-test. By determining cut-off levels of these parameters the sensitivity and specificity of them in prediction of ectopic pregnancy was estimated. **Results:** The mean serum level of VEGF ie 260.16 ± 59.05(pg/ml) was significantly higher in women with EP than in women with normal intrauterine pregnancy ie 26.8 ± 4.23(pg/ml)($p<0.001$). The mean serum levels of Serum progesterone, Serum β-hCG, Serum Estradiol in women with ectopic pregnancy (5.86±1.18 ng/ml ,511.7±245.5mIU/ml and 304.3 ± 118.10 (pg/ml)) were significantly lower than in women with normal intrauterine pregnancy (23.5±7.38 ng/ml, 1903.0±425.5 mIU/ml and 1055.5 ± 413.0 pg/ml respectively) ($p<0.001$). **Conclusions:** The increased serum VEGF levels and low levels of serum β-hCG, serum progesterone and serum estradiol in women with Ectopic Pregnancy in comparison to Normal Intrauterine Pregnancy can serve as an excellent diagnostic tool for the prediction of ectopic pregnancy.

INTRODUCTION

Ectopic pregnancy(EP) is a potentially life threatening condition,as it is still a major cause of maternal morbidity and mortality, resulting for 9-13% of all pregnancy-related deaths.[1] Despite the introduction of highly sensitive assays for the estimation of serum human chorionic gonadotropin

ISSN (O): 2687-5365; ISSN (P): 2753-6556

(hCG) and an increase in the sensitivity of transvaginal sonography(TVS),it is believed 40-50% of cases initially are misdiagnosed.[2] Ectopic pregnancy is the implantation of an embryo outside of the uterine cavity most commonly in the fallopian tube. Smooth muscle contraction and ciliary beat within the fallopian tubes to assists in the transport of an oocyte or embryo. Damage to the fallopian tubes, usually secondary to inflammation, induces tubal dysfunction which can result in retention of an oocyte or embryo. There are several local factors, such as toxic, infectious, immunologic and hormonal that can induce inflammation. There is upregulation of pro-inflammatory cytokines following tubal damage, this subsequently promotes embryo implantation, invasion and angiogenesis within the fallopian tube.[3]

Vascular endothelial growth factor (VEGF) is a most potent angiogenic factor[4,5] which has a important role in establishment of a viable pregnancy, participating in the process of implantation and placentation. This acts as a major modulator of vascular growth, remodelling, and permeability in endometrium, decidua, trophoblast.[6,7,8,9] VEGF is also indispensable for trophoblast development during the vascular development of the embryo.[10] The secretion and expression of the VEGF is dependent on local conditions, such as hypoxia, and it has been observed that the cellular VEGF is increased in hypoxic conditions.[11,12,13] The implantation environment in oviduct is very different from that of well-vascularised endometrium, and the production and secretion of VEGF may be affected in EP.[14,15]

Several hormones levels are affected in the body during pregnancy, with several hormones playing major role during pregnancy. These includes human chorionic gonadotrophin hormone (β-hCG), estrogens, human placental lactogen, progesterone etc. In the very early stage of normal intrauterine pregnancy, the corpus luteum secretes **progesterone**, a C-21 steroid hormone.

Its level in the blood rises rapidly after ovulation and declines sharply when the corpus luteum degenerates. During pregnancy following the development of trophoblast, progesterone is synthesized and secreted in increasing amount from placenta. It stimulates the thickening of the uterine lining in anticipation of implantation of a fertilized ovum.

Estradiol, the steroid hormone is the most important in amongst three major estrogens. It is the most important natural hormone. From the onset of puberty estradiol is secreted cyclically by the granulosa and the maturing follicle cells. After ovulation, estradiol is secreted by the corpus luteum. During pregnancy, estradiol and other estrogens are produced in increasing amounts by the placenta. At term, their secretion is higher than at any other time. Then the levels fall abruptly after delivery. The role of its production is in syncytiotrophoblast. The placenta is an incomplete endocrine organ as it has no capability to have independent steroidogenesis like that of ovary. For steriodogenesis, estrogen in particular depends much on the precursors derived mainly from fetal and partly from maternal sources, specially in the later stages of pregnancy.

Human chorionic gonadotropin (hCG) composed of two dissimilar subunits alpha (α) and beta (β), linked together with hydrogen and disulphide bonds. It is a peptide hormone produced in pregnancy that is made by the embryo soon after conception and later by the syncytiotrophoblast (part of the placenta). Its role is to prevent the disintegration of the corpus luteum of the ovary and thereby maintain progesterone production that is critical for a pregnancy in humans. Early pregnancy testing in general is based on the detection or measurement of β-hCG. Because an early and accurate laboratory diagnosis of tubal EP could assists clinical management, so various laboratories have directed their research toward biochemical markers of tubal EP.[16,17,18,19]

The study was aimed to evaluate serum vascular endothelial growth factor, serum progesterone, serum beta-human chorionic gonadotropin and serum estradiol in women with ectopic pregnancy and normal intrauterine pregnancy, so to evaluate their capacity to serve as novel markers for early detection of ectopic pregnancy. The levels of serum VEGF ,Serum Progesterone, β-hCG and Serum Estradiol were also correlated in EP and normal IUP cases.

MATERIAL AND METHODS

This is a case controlled, prospective study in which the subjects included were 100 of ectopic pregnancy (EP) cases and 100 of normal intrauterine pregnancy(IUP) cases as controls of different age groups (20-40 years) attending the out patient clinics or admitted in the wards of Rajkiya Mahila Chikitsalya, J.L.N. Medical College and Associated Group of Hospitals, Ajmer, after taking approval from ethical committee. Serum levels of VEGF, β-hCG, Estradiol were measured by enzyme linked immuno sorbent assay (ELISA) technique and progesterone was measured by radio immuno assay (RIA) technique in 100 symptomatic women with ectopic pregnancy and 100 women with normal intrauterine pregnancy. SPSS 13/win statistical software was used for analyzing the data. Data were presented as mean±standard deviation. A parametric independent sample t-test was used to compare differences between two groups. Level of statistical significance was set at $p<0.05$. By determining cut-off levels of these parameters the specificity and sensitivity of each in prediction of ectopic pregnancy were estimated.

ISSN (O): 2687-5365; ISSN (P): 2753-6556

RESULTS

Demographic data of IUP and EP are shown in tables.

Table 1: **Mean Serum VEGF, Serum Progesterone, Serum Estradiol and Serum β-hCG values in normal IUP and EP**.

S. No.	Parameters	Normal IUP	EP	t- value	P value
1.	Serum VEGF (pg/ml)	26.8±4.23	260.16±59.05	40.4	<0.001 (HS)
2	S.Progesterone(ng/ml)	23.5±7.38	5.86±1.18	23.6	<0.001 (HS)
3	Serum Estradiol (pg/ml)	1055.5±413.0	304.3±118.10	17.4	<0.001 (HS)
4	Serum β-hCG (mIU/ml)	1903.0±425.5	511.7±245.5	28.3	<0.001 (HS)

P<0.001 = Highly Significant(**HS)**

Table 2: **Sensitivity, Specificity and Cut off values of parameters**

S. No.	Name of Parameter	Sensitivity	Specificity	Cutoff Value
1.	S. VEGF	88%	100%	< 200 pg/ml
2.	S. Progesterone	99%	100%	< 15 ng/ml
3.	S. Estradiol	100%	99%	< 650 pg/ml
4.	S. ß hCG	91%	85%	< 1500 mIU/ml

Table. 3: **Pearson's Correlation Table in Normal Intrauterine Pregnancy Subjects**

	ß-hCG	Serum Estradiol
Serum VEGF	r = 0.113 p > 0.10 (NS)	r = 0.024 p > 0.10 (**NS)**
Serum Progesterone	r = 0.281 p < 0.001 (HS)	-

Table 4: Pearson's Correlation Table in Ectopic Pregnancy Subjects

	Serum ß-hCG
Serum VEGF	r = -0.176 p > 0.05 (NS)
Serum Progesterone	r = 0.007 p > 0.10 (NS)

In the present study the **[Table 1]** mean ± S.D. values of serum VEGF of subjects with ectopic pregnancy and normal intrauterine pregnancy was 260.163 ± 59.05 pg/ml and 26.89 ± 4.23 pg/ml respectively. The serum concentrations of VEGF among women with EP were significantly higher than those with normal intrauterine pregnancy. So, there was statistically highly significant difference in serum VEGF concentration between ectopic pregnancy and normal intrauterine pregnancy (P < 0.001). Similar findings of results has been noticed in the various studies done by Felemban A *et al.*[15], Fasouliotis SJ *et al.* .[20], Muller MD *et al.*[21], Daponate A *et al.*[22] and Cabar FR *et al.*[23]

All the serum concentration of VEGF in women with normal intrauterine pregnancy were < 200 pg/ml. With this cut off point, a normal intrauterine pregnancy could be distinguished from EP with a sensitivity of 88% and specificity of 100% **[Table 2].**

In the present study another biochemical marker evaluated for early diagnosis of ectopic pregnancy was serum progesterone. The mean serum levels of serum progesterone were 23.5±7.38 ng/ml and 5.86±1.18 ng/ml in normal intrauterine pregnancy and ectopic pregnancy respectively. Serum progesterone levels in normal intrauterine pregnancy were significantly higher than those with ectopic pregnancy **[Table 1]** when compared statistically, highly significant difference (p <0.001) was seen between the two subject groups. Using cut off of 15 ng/ml of progesterone level women with an ectopic pregnancy (n=100) when compared to normal intrauterine pregnancy (n=100) could be predicted with sensitivity and specificity of 99% and 100% respectively **[Table 2]**. The difference of serum progesterone level in normal intrauterine pregnancy and ectopic pregnancy was highly significant (p <0.001). The cut off level of serum progesterone value which can differentiate between viable and non viable pregnancies has been found to be varied between 10 ng/ml to 20 ng/ml in various studies. The results of the present study were found similar to Phipps *et al.*[24], Gharabaghi P *et al.*[25] and Cartwright *et al.*[26].

Another biomarker studied was ß-human chorionic gonadotrophin (ß-hCG). The serum ßhCG levels in normal intrauterine pregnancy and ectopic pregnancy were 1903.97 ± 425.5 and 511.7 ± 245.5 mIU/mL and cut off was < 1500 mIU/mL i.e. the discriminatory range between normal and ectopic pregnancy with the sensitivity 91% and specificity

ISSN (O): 2687-5365; ISSN (P): 2753-6556

85% **[Table 2].** The mean serum levels of ßhCG in normal intrauterine pregnancy is statistically very significantly higher than ectopic pregnancy **[Table 1]** In the present study another parameter studied was serum estradiol which is secreted by corpus luteum in response to HCG and it could function as a luteal marker of pregnancy dynamics. The mean ± std deviation serum estradiol levels in normal intrauterine pregnancy is 1055.5±413.0 pg/ml an in ectopic pregnancy is 304.3±118.10 pg/ml. So the significantly elevated levels ($p < 0.001$) of serum estradiol has been observed in normal intrauterine pregnancy when compared with ectopic pregnancy. A highly significant difference has been observed in the serum estradiol values between normal intrauterine and ectopic pregnancy **[Table 1].** The cut off i.e. discriminatory value between normal intrauterine and ectopic pregnancy in the present study was found < 650 pg/ml and sensitivity 100% and specificity 99% **[Table 2].** The results of the present study are in consistent with the results of Kuscu *et al.*[27]; Gharabaghi P *et al.*[25] where serum estradiol levels are significantly higher in normal intrauterine pregnancy than ectopic pregnancy,

Estradiol is the most potent estrogen which is produced primarily by developing follicles in the ovaries, placenta and corpus luteum and secreted into blood. In luteal phase estradiol in conjunction with progesterone, prepares the endometrium for implantation. During pregnancy estradiol increases due to placental production. Due to implantation site of trophoblast. The levels of serum estradiol in ectopic pregnancy were reduced in comparison to normal intrauterine pregnancy and the values plateaued after 6th week and declined after 8th week of gestation.

In the present study in normal intrauterine pregnancy subject group serum VEGF values were correlated with serum progesterone. There was no correlation i.e. r=0 when serum VEGF values were correlated with serum progesterone but when serum VEGF values were correlated with ßhCG and serum estradiol the Pearson's correlation was r=0.113, $p > 0.10$ and r=0.024, $p > 0.10$ **[Table 3]** respectively showing a insignificant positive correlation among them.

The processes of implantation and trophoblast invasion which characterize early normal intrauterine pregnancy are accompanied by major changes in the uterine vasculature by vascularisation of corpus luteum and by the development of the villous vasculature connecting embryo and trophoblast. Evans *et al.*[13] reported that concentration of serum VEGF is increased in normal intrauterine pregnancy. It is also suggested that human chorionic gonadotrophin (HCG) influences VEGF production. Exposure of human granulosa cells to HCG stimulates the expression of VEGF mRNA. According to Driesche SVD *et al.*[28] hypoxia is the primary inducer of VEGF, in the corpus luteum it is up regulated by HCG.

The presence of HCG receptors on trophoblast cells may enable HCG to initiate VEGF transcription in the placenta. HCG has already been shown to have this effect on granulosa cells in the ovary, an action which is likely to promote the vascularisation of the corpus luteum. Serum concentration of both HCG and VEGF increase during the first trimester, so they are inevitably positively correlated.

Estradiol may be involved since this hormone is known to increase nitric oxide synthase (NOS) expression in endothelial cells. Estradiol level is high during normal intrauterine pregnancy suggesting that other mechanisms may operate which amplify its influence. Such factors may include HCG and VEGF. So it was found in this present study that serum VEGF is positively correlated with both ß-hCG and estradiol.

In ectopic pregnancy subject group, serum VEGF was found to have not significant inverse correlation i.e. r =-0.176, $p > 0.05$ with ß-hCG [**Table 3].** To explain correlation the reason is that immediately after implantation of the blastocyst the developing trophoblast secretes ß-hCG into maternal circulation in normal intrauterine pregnancy. Therefore the level ßhCG in blood increases rapidly with maximal level of 50000-100000 mIU/ml attained at about 8-10 weeks of gestation.

So ß-hCG rises so rapidly indicating a supramaximal stimulation of the corpus luteum by the trophoblast in early intrauterine pregnancy, but in ectopic pregnancy it is possibly not the corpus luteum which responds poorly to stimulation by ß-hCG but insufficient synthesis of ß-hCG by the trophoblast. So ß-hCG levels are lower. On the other hand due to implantation milieu in fallopian tube is different than endometrium the production and secretion of VEGF is also affected. VEGF is a potent angiogenic factor and its secretion depends on local condition including hypoxia. [Torry DS *et al.*[11]). In contrast to hCG and progesterone which are trophoblast dependent VEGF is produced both by trophoblast and endometrium [Evans *et al.* [13]]. So due to local condition of hypoxia in fallopian tube there is increased production of VEGF. So due to implantation site ß-hCG synthesis is low and VEGF synthesis is more. So these are negatively correlated in ectopic pregnancy. These results are similar to Muller *et al.*[21]

In normal intrauterine pregnancy subject group when progesterone was correlated with the other three parameters, the Pearson's correlation derived was that, no correlation ie. r = 0 was found with serum VEGF, serum estradiol. But with ß-hCG and highly significant positive correlation was obtained i.e. r=0.281, $p < 0.001$**[Table 3].**

In ectopic pregnancy subject group serum progesterone had non-significant positive correlation only with ß-hCG, r = 0.007, $p > 0.10$

ISSN (O): 2687-5365; ISSN (P): 2753-6556

[Table 4] and with rest of two parameters no correlation was obtained i.e. r = 0 with serum VEGF and serum estradiol. It is clear that in ectopic pregnancy immediately after implantation in fallopian tube it is not that corpus luteum which responds poorly to stimulation of ß-hCG but it is insufficient synthesis of ß-hCG by the trophoblast, so ß-hCG is low.

When ß-hCG was correlated in normal intrauterine pregnancy subject groups there was positive correlation with serum VEGF, serum progesterone where Pearson's correlation is r = 0.113, p > 0.10 (non significant), r = 0.281, p < 0.001 (highly significant), respectively and with serum estradiol, no correlation i.e. r = 0. The reason for positive correlation has been explained earlier where correlation between VEGF and ß-hCG and progesterone, ß-hCG has been determined.

When ß-hCG levels were correlated in ectopic pregnancy group it was found to have only non significant positive correlation with serum progesterone i.e. r = 0.007, p > 0.10 (non significant) and with serum VEGF non-significant inverse correlation i.e. r = - 0.176, p > 0.05 **[Table 4]** is obtained. And with serum estradiol r = 0 i.e. there was no correlation). The cause of positive and negative correlation as stated above has been explained earlier where correlation between progesterone and ß-hCG and between VEGF and ß-hCG respectively has been determined in ectopic pregnancy.

Serum estradiol in normal intrauterine pregnancy had no correlation i.e. r = 0 with serum progesterone, ß-hCG, but it was found to have insignificant positive correlation with serum VEGF i.e. r = 0.024, p > 0.10 **[Table 3].**

In ectopic pregnancy no correlation i.e. r = 0 was found of serum estradiol with any of the parameter i.e. serum VEGF, serum progesterone, ß-hCG.

DISSCUSSION

Ectopic Pregnancy which become life and fertility threatening condition which accounted for 9% of all pregnancy related deaths now can be detected early for the successful and conservative management of women with ectopic pregnancy. Earlier 40-50% of women with ectopic pregnancy were misdiagnosed initially. But now in order to improve diagnosis various serum biomarkers were studied in this present study. These are serum vascular endothelial growth factor (VEGF), serum progesterone, serum ß-human chorionic gonadotrophin (ß-hCG), serum estradiol. Serum vascular endothelial growth factor was found to be significantly higher in ectopic pregnancy (P < 0.001) as compared to normal intrauterine pregnancy. VEGF dependent angiogenesis is essential for normal luteal development. Although hypoxia is the primary inducer of VEGF in the corpus luteum. Because the extrauterine implantation environment is very different from well vascularised endometrium, hypoxia triggers increased VEGF production at this ectopic site i.e. fallopian tube. So, VEGF is a potent angiogenic factor and its secretion depends on local conditions including hypoxia, so it can be selected as best possible serum biomarker for early diagnosis of ectopic pregnancy.

Serum VEGF was found to have positive correlation with serum ß-hCG and serum estradiol in normal intrauterine pregnancy. But in ectopic pregnancy subject groups VEGF was found to have negative correlation only with ß-hCG.

1. Serum Progesterone levels were evaluated in normal intrauterine pregnancy subjects because it is synthesized and secreted in increasing amount from placenta. Its levels are low during pre ovulation phase of menstrual cycle but rises after ovulation and remains elevated during luteal phase. But in ectopic pregnancy subjects serum progesterone levels are low because the synthesis by the corpus luteum is diminished as the trophoblast is implanted in the fallopian tube. In normal intrauterine pregnancy subject group serum progesterone had positive correlation with ß-hCG. But in ectopic pregnancy progesterone had positive correlation with ß-hCG, so serum progesterone levels in ectopic pregnancy subject group are significantly lower than normal intrauterine pregnancy.
2. Serum ß- human chorionic gonadotrophin (ß-hCG) levels are found significantly lower in ectopic pregnancy subjects than in normal intrauterine pregnancy. In normal intrauterine pregnancy ß-hCG rises exponentially for the first 10 weeks and then decline to approximate one fifth of peak value i.e. 1,50,000 mIU/ml and remain around one fifth of peak levels until term. In ectopic pregnancy due to abnormal implantation the prodution of ß-hCG is very low. So it can be used a diagnostic tool for early detection of ectopic pregnancy. In normal intrauterine pregnancy Serum ß-hCG was found to have positive correlation with serum VEGF and serum progesterone. But in ectopic pregnancy and ß-hCG levels was found to have positive correlation with serum progesterone and inverse relation with VEGF.. So serum progesterone can also be added to the diagnostic tools for early detection of ectopic pregnancy.
3. Serum Estradiol levels were significantly elevated in normal intrauterine pregnancy than in ectopic pregnancy. Serum estradiol levels are increased due to placental production and in ectopic pregnancy due to implantation site of trophoblast its levels are lower. In normal intrauterine pregnancy serum estradiol was found to have positive correlation with serum VEGF only and in ectopic pregnancy no correlation was found with any of the parameter.

ISSN (O): 2687-5365; ISSN (P): 2753-6556

Thus, serum estradiol levels are lower in ectopic pregnancy subjects.

CONCLUSION

So the results in the present study indicates that ectopic pregnancy, the threatening condition can be predicted by analysing various serum biomarkers comprising serum vascular endothelial growth factor, serum progesterone, ß-human chorionic gonadotrophin and serum estradiol. Except higher levels of serum vascular endothelial growth factor all the other serum biomarkers were lower in ectopic pregnancy in comparison to normal intrauterine pregnancy. These serum biomarkers can serve as excellent diagnostically accurate, clinically available serum biomarkers for the prediction of ectopic pregnancy, so that 40-50% of initially misdiagnosed cases which resulted earlier in significant morbidity and mortality can be overcomed.

REFERENCES

1. Centers for disease Control and Prevention (CDC). Ectopic Pregnancy-United States 1990-1992.MMWR Morb Mortal Wkly Rep 1995;44(3):46-8.
2. Abbott J Emmans LS Lowenstein SR. Ectopic Pregnancy: ten common pitfalls in diagnosis.Am J Emerg Med 1990;8(6):515-22
3. Panelli DM, Phillips CH, Brady PC. Incidence, diagnosis and management of tubal and nontubal ectopic pregnancies: a review. Fertil Res Pract. 2015;1:1-20.
4. Leung DW,Cachianes G,Kuang WJ,Goeddel DV,Ferrara N. Vascular endothelial growth factor is a secreted angiogenic mitogen.Science 1989:246:1306-9
5. Neufeld G, Cohen T, Gengrinovitch S, Poltorak Z **:** Vascular endothelial growth factor (VEGF) and its receptors. *FASEB J* 1999; 13: 9-22.
6. Torry DS, Torry RJ**.** Angiogenesis and the expression of VEGF in endometrium and placenta. *Am J Reprod Immunol.* 1997; 37: 21-29.
7. Torry DS,Holt VJ, Keeman JA,HarrisG, Caudle MR, Torry RJ.Vascular endothelial growth factor expression in cycling human endometrium.Fertil Steril 1996;66:72-80.
8. Shifren JL,Tseng JF,Zaloudek CJ,Ryan IP,Meng YG,Ferrara N,etal.Ovarian steroid regulation of vascular endothelial growth factor in the human endometrium implications for angiogenesis during the menstrual cycle and in the pathogenesis of endometriosis.J Clin Endocrinol Metab 1996;81:3112-8.
9. Clark DE, Smith SK, Sharkey AM ,Charnock-Jones DS.Localisation of VEGF and Expression of its receptors fit and KDR in human placenta throughout pregnancy.Hum Rep. 1996;11:1090-8.
10. Carmeliet PV,Ferrara G,Brerier S,Polleyfeyt S, Kieckens L, Gertsenstein M etal. Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele.Nature 1996;380:435-9.
11. Torry DS, Torry RJ**.** Angiogenesis and the expression of VEGF in endometrium and placenta. *Am J Reprod Immunol.* 1997; 37: 21-29.
12. Neufeld G, Cohen T, Gengrinovitch S, Poltorak Z **:** Vascular endothelial growth factor (VEGF) and its receptors. *FASEB J* 1999; 13: 9-22.
13. Evans PW, Wheller T, Anthony FW and Osmond C **:** A longitudinal study of maternal serum VEGF in early pregnancy. *Hum Rep.* 1998; 13(4): 1057-1062.
14. Daniel Y, Geva E, Lerner-Geva L, Eshed-Englender T, Gamzu R, Lessing JB, Bar-AMA, Amit A**.** Levels of vascular endothelial growth factor are elevated in patients with ectopic pregnancy : is a novel marker. *Fertil Steril.* 1999; 72(6): 1013-1017.
15. Felemban A, Sammour A and Tulandi T. Serum VEGF as a possible marker for early ectopic pregnancy. *Hum Reprod.* 2002; 17: 490-492.
16. Cabar FR, Fettback PB, Pereira PP, Zugaib M. Serum markers in the diagnosis of tubal pregnancy. Clinics (Sao Paulo) 2008, 63: 701–708.
17. Gerton GL, Fan XJ, Chittams J, Sammel M, Hummel A, Strauss JF, Barnhart K: A serumproteomics approach to the diagnosis of ectopic pregnancy. Ann N Y Acad Sci 2004, 1022: 306–316.
18. Rausch ME, Sammel MD, Takacs P, Chung K, Shaunik A, Barnhart KT: Development of a multiplemarker test for ectopic pregnancy. Obstet Gynecol 2011,117:573–582
19. Segal S,Mercado R, Rivnay B: Ectopic pregnancy earlydiagnosis markers. Minerva Ginecol 2010, 62: 49–62.
20. Fasouliotis SJ, Spandorfer SD, Witkin SS, Liu HC, Roberts JE and Rosenwark Z. Maternal serum VEGF levels in early ectopic and intrauterine pregnancies after in vitro fertilization treatment. *Fertil Steril.* 1004; 82: 309-313.
21. Muller MD, Raio L, Spoerri S, Ghezzi F, Dreher E and Bersinger NA. Novel placental serum markers in ectopic versus normal intrauterine pregnancy. *Fertil Steril*. 2004; 81: 1106-111.
22. Daponte A, Pournaras S, Zintzaras E, Kallitsaris A, Lialios G, Manitis NA and Messinis IE : The value of a single combined measurement of VEGF glycodelin progesterone PAPP-A, HPL and LIF for differentiating between ectopic and abnormal intrauterine pregnancy. *Human Reproduction* 2005; 20(11): 3163-3166.
23. Cabar FR,Pereira PP,Oliveira MA,Pulcinelli R Francisco V. Serum vascular endothelial growth factor for tubal pregnancy.Rev Assoc Med Bras 2022;68(6):860-865
24. Phipps, Maureen Glennon MD, Hogan, Joseph W ScD, Peipert, Jeffrey F, MD, MPH, Lambert-Messerlian, Geralym M Ph.D. Canick Jacob A, Ph.D. Seifer David B. MD. Progesterone Inhibin and HCG multiple marker strategy to differentiate viable from non-viable pregnancies. *Obs and Gynae.* 2000; 95: 227-231.
25. Ghrabaghi P, Seddighe A, Ghrabaghi M, Nourt M. Comparing the leves of β-HCG, progesterone and estradiol between ectopic pregnancy and normal intrauterine pregnancy. Iranian Journal of Reproductive Medicine.2007; 5(4): 187-190.
26. Cartwright, P.S. Diagnosis of ectopic pregnancy. Obstet. Gynecol.Clin North Am.1991;18: 1937.
27. Kuscu E, Vicdan K, Jurhan NO, Oquz S, Zorlu G, Gokmen O. The hormonal profile in ectopic pregnancies. *J Pak Med Assoc.* 1994; 44(2): 45-47.
28. Driesche SVD, Myers M, Gay E, Thong K Joo, Duncan WC: HCG up regulates hypoxia inducible factor-1 alpha in lutenised granulosa cells, implications for the hormonal regulation of VEGF in the human corpus luteum. *Mol Hum Reprod*. 2008; 14(8): 455-464.

ISSN (O): 2687-5365; ISSN (P): 2753-6556