Clinical Data Supporting the Importance of Vascular LH/ hCG Receptors of Uterine Blood Vessels

Ali Hassan Mohammed, FICMS,CABOG, Department of Obstetrics and Gynecology, College of Medicine, Diyala University

المعلومات السريريه السانده لاهميه مستقبلات هرمون في الشريان الرحمي hCG

علي حسن محمد ــ فرع النسائية والتوليد ــ كليه الطب جامعة ديالى

Abstract

Recent studies revealed functional extragonadal gonadotropin receptors at several sites of the human body. The human chorionic gonadotropin/luteinizing hormone (hCG/LH) receptor messenger RNA and protein were found in the endothelial and vascular smooth muscle layers of the human uterine arteries. In vivo administration of hCG decreased the blood flow resistance in the human uterus and in vitro increased vasodilating eicosanoids in the vascular wall.

الخلاصة

الهدف :ــ أثبات ان اعطاء الحوامل المصابة بالاسقاط المتكرر مادة ال HCG ذو فائدة كبيرة .

الطريقة :ــ اعطاء المريضة مغنيسوم او بروجستيرون او هرمون ال HCG .

النتائج :ــ تبين ان نسبة المرضى الحوامل اللاتي يتقدمن مرحله الحمل الثانية عالية عند استخدام مادة HCG مع ملاحظة قلت الممانعة في ضغط الشريان الرحمي .

تحليل النتائج على المدى الطويل ان نسبة الولادات المبكرة تكون اقل عندما تستخدم مادة HCG في بدايه الحمل .

الاستنتاج :ــ ان المستقبلات الرحمية تلعب دور مهم في فترة قبل التعشيش . و هرمون ال HCG يشارك في تكوين و تنشيط المشيمة على المدى البعيد.

Aim of the Study

Whether the administration of hCG to patients with signs of threatened abortion has any beneficial effect.

Material & Methods

The patients were treated with either magnesium or progesterone and/or hCG.

The results

Showed that the rate of patients reaching second trimester was higher when hCG was included in the treatment protocol, and a parallel significant decrease in uterine blood flow resistance was also found. Analyzing the long-term results, the rate of preterm and growth-retarded deliveries was lower when hCG was administered in the first trimester.

Conclusion

The uterine vascular LH/hCG receptors play a significant role in the peri-implantation period. The hCG might also participate in angiogenesis, enhancing long-term clinical results.

Introduction

For a long time, it was believed that gonadotropin receptors were localized and had an effect only on gonadal tissues. Researchers in different laboratories, especially Professor Rao's group, found and characterized in detail luteinizing hormone/human chorionic gonadotropin (LH/hCG) receptors in several nongonadal tissues including placenta and fetal membranes,[1] myometrium,[2] fallopian tube,[3] breast,[4] lymphocytes,[5] and the central nervous system.[6] Following detection and molecular biological characterization of these receptors, we learned that they are functional, and the next step was to find their functional significance. During characterization of myometrial LH/hCG receptors, it was found by immunohistochemistry that

LH/hCG receptor protein was located in blood vessels of the myometrium as well as the main trunk of the uterine artery.

Subsequent studies were directed toward characterizing in detail the vascular LH/hCG receptors and their functional relevance.[7] These studies showed that the human uterine artery contains LH/hCG receptor protein and messenger RNA (mRNA), and the receptor protein binds ^{125}I-labeled hCG. Also, we learned that both the endothelium and smooth muscle layer of the arteries contain the receptor protein and mRNA and there is a gradient in the concentration of the receptors. Quantitative immunohistochemistry revealed that the receptor protein is more abundant in the small intramyometrial arteries than in the main trunk of the uterine artery. Thus, the receptor is located in most abundant fashion in the part of the uterine circulation that is important in the regulation of vascular resistance.

Regarding function, it was hypothesized that hCG can influence local eicosanoid metabolism in the vascular wall, thus regulating the smooth muscle tone. The basis for this hypothesis was the earlier finding that hCG regulates local eicosanoid production in the ovarium.[8]

Tissue culture studies with isolated uterine artery segments provided evidence for the influence of the vascular wall's eicosanoid metabolism by hCG.[7] Subsequent studies detected that hCG acts on the local eicosanoid synthases and production in different kind of tissues, such as fallopian tube,[3] fetal membranes,[9,10] and brain.[11]

In vivo evidence for the vascular resistance-regulating role of the vascular LH/hCG receptors was obtained by Doppler blood flow measurements. hCG was given to patients in an infertility treatment program and Doppler blood flow measurements were carried out before and after hCG was administered. After 16 hours of hCG treatment, a significant decrease in the peripheral resistance of the uterine circulation was observed. Measurements of estradiol and progesterone did not show significant change in this time frame, so it was supposed that the effect on blood flow was due to a direct vascular tone-regulating mechanism possibly via a local eicosanoid mechanism.

These results initiated a further study presented here. The question raised was whether hCG treatment, in the case of threatened abortion, could be beneficial and, if so, whether it could influence uterine vascular resistance. From a clinical point of view, it is important to find new therapy possibilities for threatened and/or repeated abortion because most pregnancy loss occurs at early gestation.[12] Many pathomechanisms such as immunological, corpus luteum insufficiency, and delayed trophoblast invasion are accepted and treatment trials are focused on these factors. The peri-implantation period is extremely important because more than one third of lost pregnancies are spontaneous abortions during that time.[13]

Methods

The presented study is ongoing, so results summarized here are a preliminary evaluation of the study. The following experiments and measurements were completed:

Participants were recruited from patients admitted to the 2nd Department of Obstetrics and Gynecology, Semmelweis University for signs of threatened abortion. Patients were divided in four treatment groups based on severity of the symptoms and were not selected randomly. Severity was assessed by clinical signs regarding subjective grade of abdominal cramps, light vaginal spotting, or vaginal bleeding.

Patients with signs of threatened abortion were treated with four different protocols.

1. Magnesium 2 X 250 mg/day
2. Magnesium 2 X 250 mg/day + progesterone (Duphaston, Solvay Pharma) 2 X 10 mg/day
3. Magnesium 2 X 250 mg/day + hCG (Choriogonin, Richter Ltd.) 2 X 4500 IU/week
4. Magnesium 2 X 250 mg/day + hCG (Richter Ltd.) 2 X 4500 IU/week + progesterone (Duphaston, Solvay Pharma) 2 X 10 mg/day

The number of treated patients was 154, and their gestational age was 5-11 weeks of pregnancy at the start of therapy. Effectiveness of the combined therapies were compared with

that of the magnesium-only therapy. By definition, therapy was considered effective if pregnancy continued in the second trimester.

Next, blood flow parameters were measured before and 16 hours after hCG addition to magnesium treatment in patients hospitalized for threatened abortion at 6-8 weeks of gestation. The resistance index (RI) and systolic/diastolic (S/D) ratio were measured by using a transvaginal 7.5-MHz pulsed Doppler probe.

Blood for serum progesterone and estradiol levels was drawn and compared at the same time as the Doppler measurements.

The clinical outcome of pregnancy was assessed in a subset of patients (n = 37) treated with magnesium or magnesium and hCG in early gestation and could be followed until delivery. The birth weight of the fetus, length of gestation, relative frequency of intrauterine growth retardation (IUGR), and premature delivery were compared.

The unpaired Student's test and Fisher's test were used in the study for statistical analysis.

Results

Most patients were treated with only magnesium (Fig. 1). They were of 92 and had the slightest signs of threatened abortion. Assignment of patients to magnesium plus Duphaston and/or hCG therapy did not show a significant difference. However, patients with the most serious signs of threatened abortion received combined therapy with magnesium, Duphaston, and hCG.

Patients with threatened abortion were treated with either magnesium or magnesium combined with progesterone and/or hCG. Treatment started between 5 and 11 weeks of gestation and continued for 14 weeks of gestation. It was considered effective when spontaneous abortion did not occur until the beginning of the second trimester.
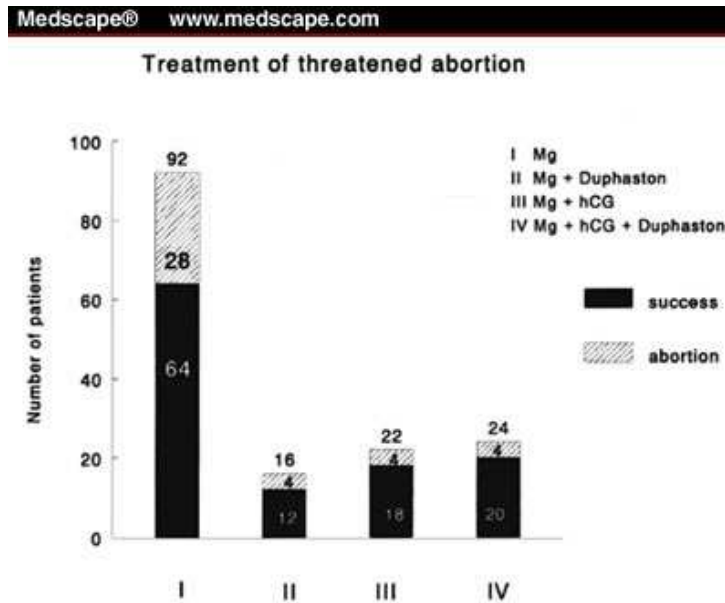
Figure 1.

Patients with threatened abortion were treated with either magnesium or magnesium combined with progesterone and/or hCG. Treatment started between 5 and 11 weeks of gestation and continued for 14 weeks of gestation. It was considered effective when spontaneous abortion did not occur until the beginning of the second trimester.

When only magnesium was applied even with slight signs of threatened abortion, almost one third (30.6 %) of the patients miscarried (Fig. 2). The combination of magnesium with progesterone or hCG yielded better results. The best response, even in the case of the most severe clinical signs, was achieved with the combination of magnesium, progesterone, and hCG. The first-trimester abortion rate was then as low as 16.6%. Regarding the data, the combination of magnesium with only hCG yielded almost the same success rate (18.1% abortion rate). Thus, magnesium and hCG therapy was almost as effective as its further combination with progesterone. Calculating the change in abortion rate as a percentile after hCG addition to therapy, there was a 46.8% decrease compared with magnesium treatment only. Contrary to this, progesterone addition (25% abortion rate) decreased the abortion rate by only 18.4% compared with magnesium treatment only.

The rate of spontaneous abortion in the first trimester after treatment with magnesium and magnesium combined with progesterone and/or hCG because of threatened abortion.
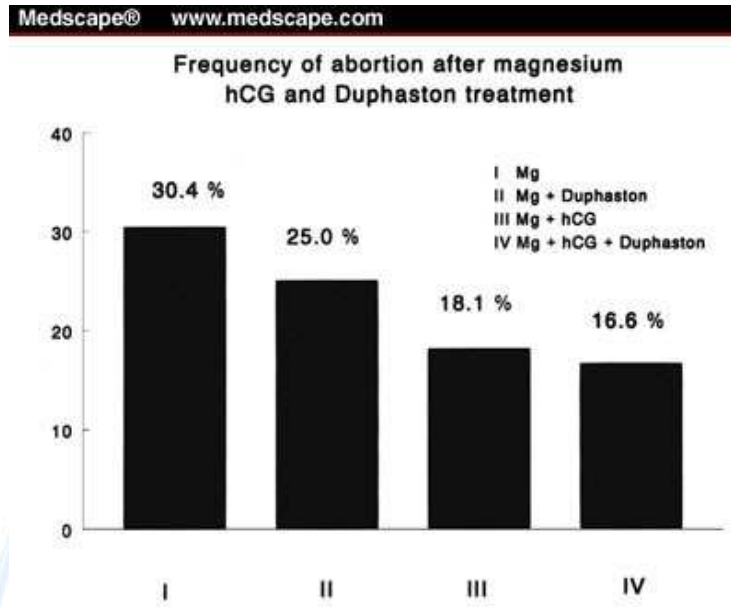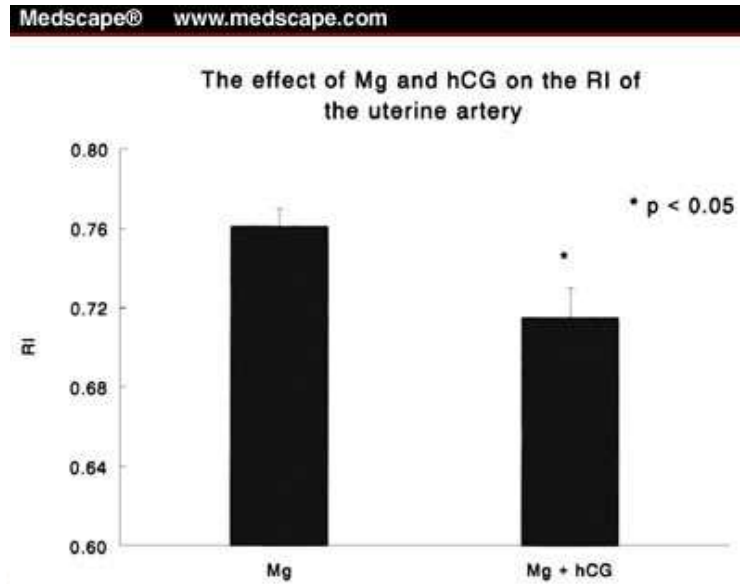

Figure 2.

The rate of spontaneous abortion in the first trimester after treatment with magnesium and magnesium combined with progesterone and/or hCG because of threatened abortion.

The results and previous data raised the possibility that hCG's effect might be explained by two mechanisms: the hormone can act directly on vascular smooth muscle through its own receptor or increase the level of the steroid hormone estradiol and/or progesterone, thus indirectly lowering vascular resistance in the uterine circulation and improving implantation.

When RI was measured (Fig. 3) by intravaginal pulsed Doppler probe in the main uterine artery, it was shown that 16 hours after hCG treatment, RI significantly decreased in the uterine circulation (0.761 ± 0.009 versus 0.715 ± 0.015).

The resistance index (RI) values measured in the uterine artery before and 16 hours after the hCG administration. The hCG significantly decreased the RI in the uterine circulation (0.761

± 0.009 versus 0.715 ± 0.015, n = 8 patients).

Medscape®   www.medscape.com

The effect of Mg and hCG on the RI of the uterine artery

* p < 0.05

Figure 3.

The resistance index (RI) values measured in the uterine artery before and 16 hours after the hCG administration. The hCG significantly decreased the RI in the uterine circulation (0.761 ± 0.009 versus 0.715 ± 0.015, n = 8 patients).

This result suggests a decrease in smooth muscle tone of the vessel wall in the resistance arteries. These changes are similar to those of the decrease when hCG was administered in ovulation induction courses. [7]

Blood was drawn from patients who participated in the Doppler blood flow study at the time of flow measurements to test the hypothesis of whether hCG increases the estradiol and/or progesterone levels. These hormones mediate hCG's effect by acting on the smooth muscle of the vessel wall or by other local effects in the gestational tissues. However, values of estradiol measurements (Fig. 4, 758.4 ± 113.5 versus 758.5 ± 172.6) showed no significant change after hCG administration in the time frame investigated.

The serum estradiol measurements were made at the time of Doppler examinations. hCG

did not change significantly in the frame of investigation of the estradiol level (758.4 ± 113.5 versus 758.5 ± 172.6 pg/mL, n = 8 patients).

Medscape®   www.medscape.com

The effect of Mg and hCG on serum estradiol
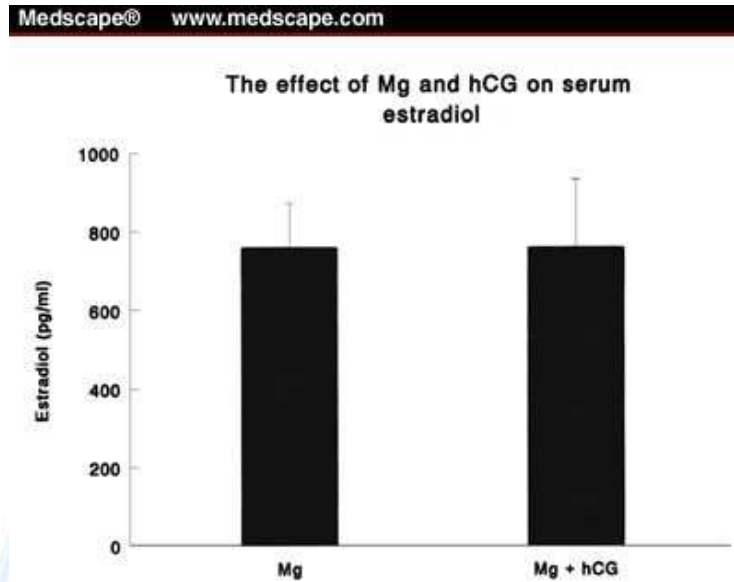
Figure 4.

The serum estradiol measurements were made at the time of Doppler examinations. hCG did not change significantly in the frame of investigation of the estradiol level (758.4 ± 113.5 versus 758.5 ± 172.6 pg/mL, n = 8 patients).

As in the case of estradiol, there was a hypothesis that hCG can increase the progesterone level, thus increasing the success rate in the treatment of threatened abortion. The progesterone level increased slightly 16 hours after hCG administration (Fig. 5); however, it did not reach a significant change in the investigational time frame (22.1 ± 7.68 versus 23.2 ± 4.46). Results of these hormone measurements did not support the hypothesis that steroid hormones might influence the uterine vascular resistance shortly after hCG administration.

For serum progesterone measurements, blood samples were collected at the time of Doppler ultrasound examinations. As with estradiol, besides a slight increase, hCG did not significantly change the progesterone level in the time frame of investigation (22.1 ± 7.68 versus
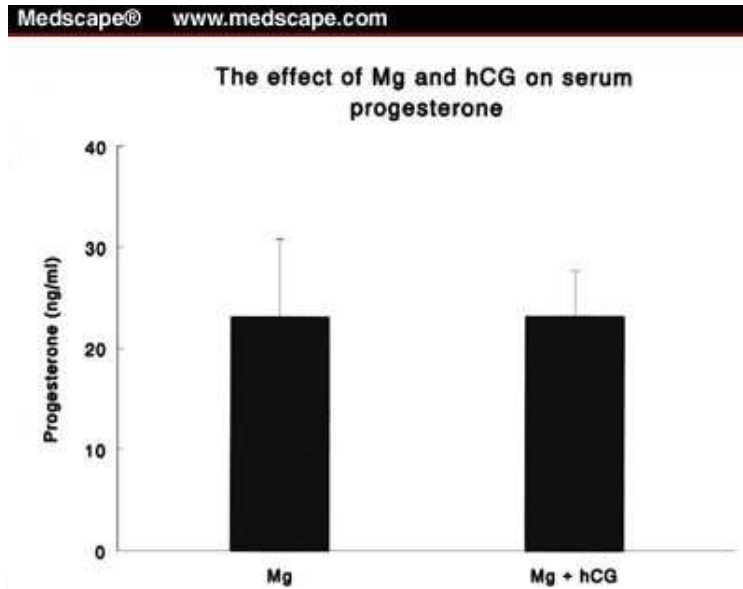
23.2 ± 4.46 ng/mL, n = 8 patients).

Figure 5.

For serum progesterone measurements, blood samples were collected at the time of Doppler ultrasound examinations. As with estradiol, besides a slight increase, hCG did not significantly change the progesterone level in the time frame of investigation (22.1 ± 7.68 versus 23.2 ± 4.46 ng/mL, n = 8 patients).

A subset of 37 patients (28 patients in the magnesium group and 9 patients in the magnesium plus hCG group) treated with either magnesium or magnesium plus hCG at an early gestational age could be followed to delivery. There was no significant difference in the length of gestation until delivery between the two groups (Table 1). However, the magnesium-treated group had a higher premature delivery rate (6/28, 21.42%) than the hCG-treated group (1/9, 11.11%). Whereas the magnesium-treated group had three IUGR fetuses (3/28, 10.71%), we did not find any in the hCG-treated group. However, because of the small number of patients especially in the hCG-treated group, it necessary to collect additional clinical data in this part of the study.

As an extension of the study, patients with recurrent pregnancy loss in the first trimester were treated with hCG according to the protocol mentioned earlier. Prior to a new pregnancy, a detailed laboratory examination was done to rule out immunology or phospholipid metabolism alterations. One of the patients had previously had 10 spontaneous abortions. Until the preparation of this article, four patients were followed. All patients had ovulation induction for their present pregnancy to optimize follicular function. After they became pregnant, regular serum progesterone and ß-hCG level measurements were made with uterine artery Doppler studies. The hCG treatment given was 4500 IU intramuscularly twice a week until the 14th week of gestation. Three patients delivered mature fetuses (one had twins) and one pregnancy is now in the 36th week of gestation. In this small group of patients, there was no fetal loss even in the presence of a poor obstetrical medical history.

Discussion

The results show that hCG added to the therapy protocol in cases of threatened abortion might increase the success rate of therapy in the first trimester of pregnancy. This increase is almost double that seen in the case of magnesium therapy only. Also, it can be concluded that addition of progesterone to the therapy in this study was less effective than that of hCG. Another important observation was that magnesium might not be effective enough in the case of threatened abortion even if the signs are less serious.

To explain the beneficial effect of hCG, there can be many possible mechanisms. hCG can exert its effect by more mechanisms. The gap junctions between myometrial cells are regulated by hCG, [14] which can affect myometrial contractile activity. hCG can affect prostacyclin metabolism in myometrium [15] and eicosanoid synthesis in gestational tissues,[9] which again might contribute to the quiescence of the myometrium. Much less is understood about the embryo-maternal intertalk; however, hCG can be one important signal transducer in this process. hCG plays an important role in implantation. [16] Implantation and vascular resistance of the uterine blood flow may be closely related to each other. For a long time, we have known that the success rate in in vitro fertilization cycles depends on the vascular resistance

measured in uterine arteries. [17] On the other hand, while it is easy to measure Doppler indices in the uterine arteries, it is also not cumbersome for the patients and can easily be repeated. On the basis of previous observations, [7] it was hypothesized that the beneficial effect of hCG observed in the present study might at least be due to the regulation of vascular resistance. Therefore, blood flow in uterine arteries was measured by a pulsed Doppler ultrasound probe before and after hCG treatment. The short-term effect on the vascular resistance was also studied. The finding that hCG decreased resistance raised two hypotheses; it could be mediated either by a change of eicosanoid metabolism in the vascular wall or by stimulation of estradiol and progesterone. Earlier it was shown that hCG in vitro can increase vasodilating eicosanoid synthesis in the vessel wall of the uterine [7] and umbilical [18] artery. It was also shown that sex steroids can influence vascular resistance in uterine arteries.[19]

In this human study, it was not possible to measure local eicosanoid metabolism in vivo. Therefore, we investigated the other hypothesis on the effect of hCG on estradiol and progesterone levels during the time frame when blood flow resistance changes were measurable. In that time frame, hCG did not significantly increase the level of sex steroids, and this required an explanation of the vascular eicosanoid metabolism influence. However, it cannot be ruled out that, in a long-term fashion, sex steroids might increase the blood flow to the gestational tissues as well.

There is a trend for a lower incidence of IUGR and premature delivery when threatened abortion is treated with hCG. This can be seen as a long-term effect. Peripheral resistance can also be influenced by local angiogenesis. However, it belongs to the long-term effects and, if hCG can influence angiogenesis in the field of uterine blood circulation, that could be an explanation for effects such as a lower incidence of IUGR in pregnancy. Our unpublished observations and other results [20] indicate that hCG indeed influences angiogenesis.

Summarizing the results, hCG administration in threatened and repeated abortion can be a part of the treatment. Its effect can be monitored with Doppler blood flow measurements in uterine arteries and serum progesterone and estradiol measurements. To secure more supporting evidence, collecting clinical data is now ongoing.

References

1. Lei ZM, Rao CV. Gonadotropin receptors in human fetoplacental unit: implications for hCG as an intracrine, paracrine, and endocrine regulator of human fetoplacental function. Trophoblast Res. 1992;6:213-224

2. Reshef E, Lei ZM, Rao CV, Pridham DD, Chegini N, Luborsky JL. The presence of gonadotropin receptors in nonpregnant human uterus, human placenta, fetal membranes, and decidua. J Clin Endocrinol Metab 1990;70:421-430

3. Lei ZM, Toth P, Rao CV, Pridham D. Novel coexpression of human chorionic gonadotropin (hCG)/luteinizing hormone receptors and their ligand hCG in human fallopian tubes. J Clin Endocrinol Metab 1993;77:863-872

4. Rao CV. Does full-term pregnancy at young age protect women against breast cancer through hCG? Obstet Gynecol 2000;96:783-786

5. Lin J, Lojun S, Lei ZM, Wu WX, Peiner SC, Rao CV. Lymphocytes from pregnant women express human chorionic gonadotropin/luteinizing hormone receptor gene. Mol Cell Endocrinol 1995;111:R13-R17

6. Lei ZM, Rao CV, Kornyei J, Licht P, Hiatt ES. Novel expression of human chorionic gonadotropin/luteinizing hormone receptor gene in brain. Endocrinology 1993;132:2262-2270

7. Toth P, Li X, Rao CV, et al. Expression of functional human chorionic gonadotropin/human luteinizing hormone receptor gene in human uterine arteries. J Clin Endocrinol Metab 1994;79:307-315

8. Huslig RL, Malik A, Clark MR. Human chorionic gonadotropin stimulation of immunoreactive prostaglandin synthase in the rat ovary. Mol Cell Endocrinol 1987;50:237-246

9. Toth P, Rao CV. Direct novel regulation of cyclooxygenase and prostacyclin synthase by human chorionic gonadotropin in the human amnion. Trophoblast Res 1993;8:503-514

10. Toth P, Li X, Lei ZM, Rao CV. Expression of human chorionic gonadotropin (hCG)/luteinizing hormone receptors and regulation of the cyclooxygenase-1 gene by

exogenous hCG in human fetal membranes. J Clin Endocrinol Metab 1996; 81:1283-1288

11. Toth P, Lukacs H, Hiatt ES, Reid KH, Iyer V, Rao CV. Administration of human chorionic gonadotropin affects sleep-wake phases and other associated behaviors in cycling female rats. Brain Res 1994;654:181-190

12. Edmunds DK, Lindsay KI, Müller JF. Early embryonic mortality in women. Fertil Steril 1982;38:447-453

13. Wilcox AJ, Weinberg CR, O Connor JF, Baird DD, Schlatter JP, Canfield BE. Incidence of early loss of pregnancy. N Engl J Med 1988;319:189-194

14. Ambrus G, Rao CV. Novel regulation of pregnant human myometrial smooth muscle cell gap junctions by human chorionic gonadotropin. J Clin Endocrinol Metab 1994;135: 2772-2779

15. Eta E, Toth P, Rao CV. Direct regulation of human myometrial contractions by human chorionic gonadotropin [abstract 324]. Biol Reprod 1993;48(suppl 1):139

16. Fazleabas A. Uterine receptivity in the primate-modulation by chorionic gonadotropin. Recent Advances in Gonadotropin Research, Minisymposium, Budapest, September 24, 1999

17. Battaglia C, Larocca E, Lanzani A, Valentini M, Genazzani AR. Doppler ultrasound studies of uterine arteries in spontaneous and IVF stimulated cycles. Gynecol Endocrinol 1990; 4:245-250

18. Rao CV, Li X, Toth P, Lei ZM, Cook VD. Novel expression of functional human chorionic gonadotropin/human luteinizing hormone receptor gene in human umbilical cords. J Clin Endocrinol Metab 1993;77:1706-1714

19. deZiegler D, Bessis R, Frydman R. Vascular resistance of uterine arteries: physiological effects of estradiol and progesterone. Fertil Steril 1991;55:775-779

20. Zygmunt M. hCG in the human placentation and angiogenesis of the early pregnancy. Recent Advances in Gonadotropin Research, Minisymposium, Budapest, September 24, 1999