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Evaluation of Endometrial Urocortin Secretion for Prediction of Pregnancy after Intrauterine Insemination

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BACKGROUND: Urocortin is a neuropeptide produced by the human endometrium and has biological effects putatively important for promoting blastocyst implantation. We measured urocortin concentrations in samples of endometrial wash fluid collected from women with unexplained infertility who underwent intrauterine insemination (IUI).

METHODS: Patients 28–42 years of age (n = 71) were consecutively enrolled after a complete clinical evaluation. Endometrial wash fluid was retrieved before IUI, at the time of ultrasound evaluation of endometrial thickness. Urocortin concentrations were assayed with a specific ELISA.

RESULTS: After IUI, 28 patients (39%) became pregnant. Urocortin concentrations were significantly higher in women who became pregnant than in those who did not (0.38 μg/L vs 0.13 μg/L, $P < 0.0001$). At a cutoff of 0.321 μg/L, urocortin results were positive in 61% [95% confidence interval (CI), 41%–78%] of women who had successful implantation and negative in 98% (95% CI, 88%–99.6%) of those who did not. The pregnancy rate for women with urocortin concentrations >0.32 μg/L was 94%, which differed significantly ($P < 0.05$) from the overall pregnancy rate of 39% in the study population.

CONCLUSIONS: Urocortin is measurable in endometrial wash fluid, and its concentrations before IUI are higher in women who subsequently achieve pregnancy. These data suggest that the probability of having a successful pregnancy-producing IUI may be better estimated by measuring urocortin in endometrial wash fluid.

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The human endometrium undergoes morphologic and functional changes to prepare the local environment for blastocyst implantation and for the fine tuning of trophoblast invasion when pregnancy is achieved. Decidualization of the human endometrium, an essential preparative event to enable and to regulate embryo implantation, involves the differentiation of stromal cells through tissue remodeling and an inflammatory-like response *(1)*. Although the exact molecular pathways involved in the endometrial changes necessary for successful implantation remain to be elucidated, the role played by several locally produced peptides and proteins is emerging. Of these molecules, urocortin has been suggested to function in the processes of endometrial differentiation, trophoblast invasion, and embryo implantation *(2)*.

Urocortin, a peptide of 40 amino acid residues, is closely related to corticotropin-releasing hormone (CRH)[1], not only in sequence homology (45%) but also because it binds both type 1 and type 2 CRH receptors with high affinity *(3)*. In the human endometrium, urocortin is produced by luminal and glandular epithelial cells at both the proliferative and secretory phases of the menstrual cycle *(4)*. Moreover, research with different techniques has demonstrated that (*a*) the endometrial production of urocortin increases throughout the endometrial cycle and is highest in the secretory phase, (*b*) ovarian steroids stimulate urocortin secretion from cultured endometrial cells, and (*c*) urocortin induces endometrial cell decidualization *(5)*.

We investigated whether urocortin can be measured in endometrial wash fluid collected from women undergoing intrauterine insemination (IUI) and whether urocortin concentrations in women who subsequently become pregnant differ from those in women who do not.

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[1] Nonstandard abbreviations: CRH, corticotropin-releasing hormone; IUI, intrauterine insemination; AUC, area under the ROC curve; and CI, confidence interval.

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Materials and Methods

STUDY PARTICIPANTS AND STUDY DESIGN

This prospective controlled study was performed with a group of women who were undergoing IUI as first-line therapy for infertility related to a cervical factor, a male factor, or unexplained infertility. We enrolled 71 women [age range, 28–42 years; mean (SD), 35 (4.1) years] with infertility durations of 3–8 years [mean (SD), 4.6 (2.8) years]. Informed consent was obtained from all study participants before their inclusion in the study, which was approved by the local Human Investigation Committee.

All women underwent endocrine tests (for serum follicle-stimulating hormone, luteinizing hormone, prolactin, progesterone, and testosterone) at the early follicular phase, hysterosalpingographic evaluations for the presence of tubal damage, and pelvic ultrasound examinations for uterine fibroids. A complete medical history was obtained from each woman, and a physical examination was performed. The menstrual cycle day was calculated on the basis of the last menstrual period and confirmed by transvaginal ultrasound scanning (real-time ultrasound scanning equipment; Siemens SONOLINE Elegra® Millenium Edition) with a transvaginal probe at 4.5–7.0 MHz *(6)*.

All partners of the study participants underwent an andrologic evaluation including at least 2 semen analyses and hypo-osmotic swelling tests. Semen samples were collected by masturbation after 48–72 h of sexual abstinence. Semen volume and sperm concentration, motility, and morphology were measured according to standard WHO criteria. The hypo-osmotic swelling test was performed after examination of standard semen variables. Women whose partners were affected by severe infertility (sperm concentration $<10 \times 10^9$/L, progressive motility <15%, total motility <30%, and normal morphology <30%) were excluded from the study.

Sperm samples were prepared for IUI by means of a conventional layering technique. Approximately 1.0 mL of Sperm Preparation Medium (Medi-Cult) was layered onto 1.0 mL of semen, and the specimen was incubated at 37 °C for 45 min. The supernatant was removed and used for treatment.

All women were treated for up to 3 consecutive cycles with controlled gonadotropin-induced ovulation followed by IUI. Only 3 consecutive cycles of the same treatment were evaluated to prevent carryover effects of the ovarian-stimulation treatment from affecting results. Ovarian stimulation was conducted with recombinant follicle-stimulating hormone (follitropin beta, Puregon; Organon), starting at a daily dose of 50–100 IU on the third day of the cycle. Before starting treatment with controlled ovarian stimulation, women underwent transvaginal ultrasound examinations every other day from the fifth day of treatment until the mean diameter of the dominant follicles reached 14 mm. Examinations were then performed daily. We administered 10 000 IU of human chorionic gonadotropin (Profasi; Serono) when the largest follicle had reached a mean diameter of at least 18 mm and conducted IUIs with a Frydman catheter 30–36 h after human chorionic gonadotropin administration. The cervix was exposed, the catheter was passed into the uterus to about 0.5 cm from the top of the uterine cavity, and the semen was expelled.

Pregnancy was assessed 4 weeks after IUI by measuring serum human chorionic gonadotropin and by ultrasonographic detection of fetal heart activity.

COLLECTION OF ENDOMETRIAL WASH FLUID

Endometrial wash fluid was collected during the first IUI cycle, as previously described *(7)*. In brief, before IUI and when the women were in the periovulatory phase, a sonohysterography examination was performed with a transvaginal probe of 5–7.5 MHz. After first evaluating the uterine dimensions and the endometrial lining, a pediatric Foley catheter was inserted into the uterine cavity under transvaginal ultrasound guidance, and the catheter balloon was inflated with 2 mL of sterile saline solution (9 g/L NaCl). The same volume of saline solution was then gradually flushed into the uterine cavity, and endometrial measurements were performed. Afterward, gentle suction was applied via the same catheter to recover the fluid. The wash fluid was centrifuged (3000 rpm for 10 min) to discharge the cellular component and stored at −20 °C until assayed.

UROCORTIN MEASUREMENT

Endometrial wash fluids were assayed for urocortin with a Urocortin (Human) EIA Kit (Phoenix Pharmaceuticals) according to the manufacturer's instructions. In brief, we added calibrators and samples (50 μL) to 96-well immunoplates coated with biotinylated goat antirabbit IgG (capture antibody). We then added 25 μL of rabbit polyclonal antihuman urocortin serum (detection antibody) and an equal volume of biotinylated peptide to each well and incubated the plate for 2 h at room temperature. We washed the wells with assay buffer, added streptavidin-conjugated horseradish peroxidase, and again incubated the immunoplates for 1 h at room temperature. We washed the wells, added substrate solution (3,3′,5,5′-tetramethylbenzidine), and incubated the plates for 1 h at room temperature. We stopped the reaction by adding 2 mol/L HCl and read the absorbance at 450 nm (MR 600; Dynatech Laboratories).

The urocortin concentration range detectable with this reagent set is 0.01–100 μg/L, and the calibra-

tion curve for absorbance vs log urocortin concentration is linear between the second and fourth calibrators (0.1–10 μg/L), according to the manufacturer and as confirmed in our assays. The intraassay and interassay CVs obtained for 9 replicates of culture medium incubated with placental explants (concentration range, 3.2–7.9 μg/L) were 5.2% and 14%, respectively.

ENDOCRINOLOGIC ASSESSMENT

We measured hormones (follicle-stimulating hormone, luteinizing hormone, prolactin, progesterone, and testosterone) with commercially available reagent sets (Diagnostic Systems Laboratories), with intraassay and interassay CVs measured by the manufacturer to be <10% with routine quality-control material.

STATISTICAL ANALYSIS

The Kolmogorov–Smirnov test showed experimental values to have a gaussian distribution; therefore, data are expressed as the mean (SD). Differences between groups were analyzed with the unpaired Student *t*-test, and the Pearson correlation coefficient was calculated to evaluate the linear correlation between urocortin concentration and endometrial thickness. After adjusting for various clinical variables (age, tubal damage, uterine fibroids, male factor), we performed multiple logistic regression analysis with pregnancy as the dependent variable to analyze whether urocortin concentration was associated with successful implantation.

We used ROC curve analysis *(8)* to estimate the probability of a successful implantation according to urocortin concentration or endometrial thickness and compared the results with the pretest probability, which was defined as the overall pregnancy rate *(9)*. Statistical significance was assumed for *P* values <0.05.

Results

CLINICAL FINDINGS

After IUI, 28 patients (39.4%) became pregnant. There were no differences between the pregnant and nonpregnant patient subgroups with respect to age, endometrial thickness, or the prevalence of tubal pathology, uterine fibroids, and male factor (data not shown).

UROCORTIN CONCENTRATIONS

Urocortin was measurable in all of the evaluated samples of endometrial wash fluids. In particular, urocortin concentrations in fluid samples collected from women who became pregnant [0.38 (0.19) μg/L] were significantly higher ($P < 0.0001$) than in women who did not [0.13 (0.11) μg/L; Fig. 1]. Moreover, urocortin concentration was significantly correlated with endometrial thickness in patients who became pregnant (Pearson $r = 0.9544$; $P < 0.0001$), whereas such a correlation was absent in the remaining patients ($r = 0.025$; $P = 0.8733$) (Fig. 2).

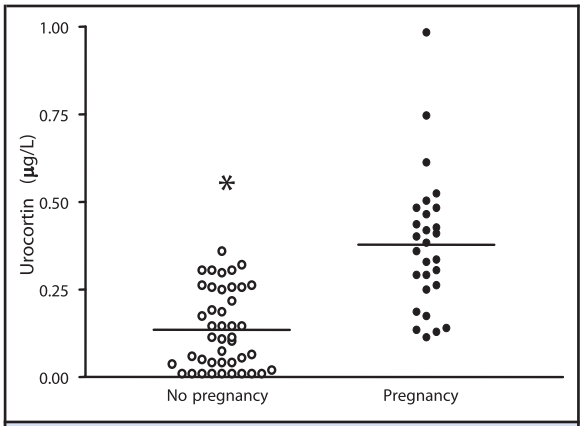

Fig. 1. Urocortin concentrations in endometrial wash fluid.

Urocortin concentrations in endometrial wash fluid collected from women who became pregnant (black circles) and those who did not (open circles). *$P < 0.001$

UROCORTIN CONCENTRATION, ENDOMETRIAL THICKNESS, AND THE PREDICTION OF PREGNANCY

A multiple logistic regression analysis with pregnancy as the dependent variable showed a positive correlation with urocortin concentration (adjusted odds ratio, 31.7; $P < 0.001$), whereas no significant correlations

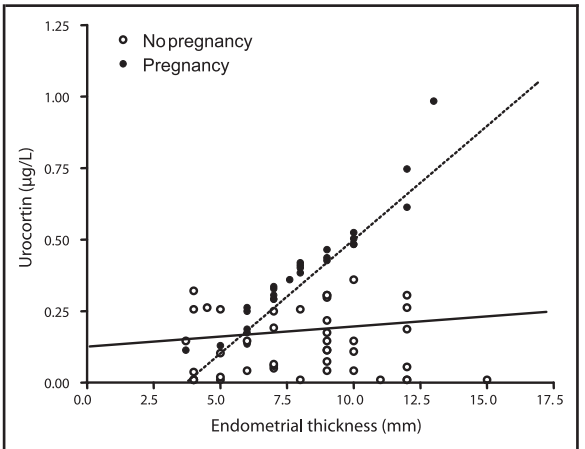

Fig. 2. Correlation analysis of urocortin concentration and endometrial thickness.

Linear correlation between urocortin concentration and endometrial thickness in the women who became pregnant (black circles) ($r = 0.9544$; $P < 0.0001$) and in the remaining patients (open circles) ($r = 0.025$; $P = 0.8733$).

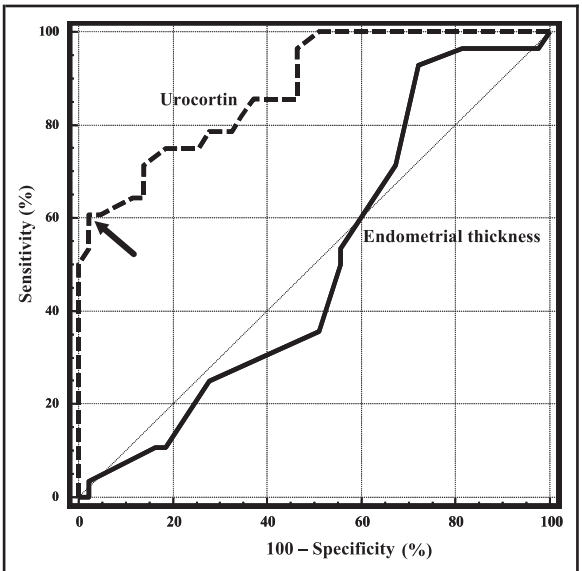

Fig. 3. ROC curve analysis of urocortin concentration and endometrial thickness for predicting successful implantation.

Arrow indicates the best cutoff point for urocortin concentration (0.321 μg/L).

were found with clinical variables (age, presence of tubal obstruction, and male factor).

We evaluated sensitivity and specificity, positive and negative predictive values, positive and negative likelihood ratios, and areas under the ROC curves (AUCs) for urocortin concentration and endometrial thickness as diagnostic tests. Urocortin concentration at a cutoff value of 0.321 μg/L had a sensitivity of 60.7% [95% confidence interval (CI), 40.6%–78.5%] and a specificity of 97.7% (95% CI, 87.7%–99.6%) as a single marker for predicting successful implantation in patients undergoing IUI (AUC, 0.88; 95% CI, 0.78–0.94), and the positive and negative likelihood ratios were 26.11 and 0.40, respectively (Fig. 3).

Endometrial thickness at a cutoff value of 5.0 mm had a sensitivity of 92.9% (95% CI, 76.5%–98.9%) and a specificity of 27.9% (95% CI, 15.3%–42.7%) for predicting successful implantation, an AUC of 0.50 (95% CI, 0.38–0.62), and positive and negative likelihood ratios of 1.29 and 0.26, respectively (Fig. 3). The AUC for urocortin concentration was significantly higher than for endometrial thickness (difference between the AUCs, 0.38; 95% CI, 0.25–0.50; $P < 0.001$).

The probability of implantation after no or 1 positive test result was calculated for the entire group of patients and compared with the pretest probability for the evaluated population. Twenty-eight of 71 patients had a pregnancy, with an overall prevalence in the study population of 39.4% (95% CI, 28.7%–50.9%). This value was the predicted probability of proceeding to pregnancy before measurement of urocortin concentration or endometrial thickness (i.e., the pretest probability).

When urocortin concentrations were greater than the threshold defined by the ROC curve (0.321 μg/L), the probability of having a pregnancy was as high as 94.4% (95% CI, 74.2%–99.0%), whereas the probability of having a pregnancy with urocortin values below this cutoff was 20.1% (95% CI, 12.0%–33.5%) (Fig. 4). On the contrary, if the endometrial thickness was >5.0 mm, the pregnancy rate was 45.6% (95% CI, 33.4%–58.4%), whereas if the endometrial thickness was <5.0 mm, the pregnancy rate was 14.3% (95% CI, 4.0%–39.9%) (Fig. 4).

Discussion

The present study provides the first evidence that urocortin is measurable in endometrial wash fluid, suggesting that the human endometrium is an important source of this neuropeptide. Urocortin is produced by endometrial luminal and glandular epithelial cells *(4)*, the endometrial components that are able to secrete peptides and proteins into the uterine lumen. We previously demonstrated with different techniques that the endometrial production of urocortin changes during the menstrual cycle and is highest in the secretory phase *(5)*.

Urocortin concentrations in the endometrial wash fluid collected from women undergoing IUI were higher in those who became pregnant than in those who did not, and urocortin concentration was correlated with endometrial thickness only in women who proceeded to successful implantation. These findings suggest that urocortin may be secreted in appreciable amounts only by an endometrium that has been primed correctly to receive the embryo, and therefore urocortin may be a marker of endometrial receptivity. Data from in vitro experiments support this hypothesis. Urocortin has been demonstrated to induce endometrial cell decidualization *(5)*, an essential preparative event to enable and regulate embryo placentation *(1)*. Urocortin also induces the secretion of extracellular matrix metalloproteinase 9 by human trophoblasts *(11)*. Therefore, one may infer that when the endometrium has become "ready" for implantation, it secretes urocortin, which would then bind to its locally expressed receptors *(12)* and stimulate the trophoblast to produce matrix metalloproteinase 9. This would enable the trophoblast to invade the maternal decidua *(13)*.

One may hypothesize other important targets for the locally secreted urocortin, however. During implantation, numerous macrophages are present at the implantation site, and their presence was originally thought to represent an immune response against the invading trophoblasts *(14)*. Because macrophages express urocortin receptors *(15)* and because urocortin acts on macrophages to induce their apoptosis *(16)*, urocortin might promote blastocyst implantation and early maternal tolerance primarily by killing activated T cells *(2)*. Finally, because vascular endometrial cells express the CRH type 2 receptor *(17, 18)* and because urocortin is a potent vasodilator *(19–23)*, urocortin may be an additional peptide released by the human endometrium for regulating endometrial angiogenesis and/or the tone of the vascular endothelium, key events during implantation.

These putative activities and the present data indicate that urocortin—along with CRH, interleukin-11, prostaglandin E_2, activin A, and leukemia inhibitory factor—contributes to the implantation process *(24)*. This suggestion is reinforced by measurements of urocortin in the endometrial wash fluid that show higher concentrations in women who achieved pregnancy after IUI.

In a study of the products of conception containing myometrium, fetal membranes (amnion and chorion), and chorionic villi, Madhappan et al. *(25)* found increased CRH and urocortin concentrations in tissues from women with 2 or more spontaneous abortions, suggesting that stressful conditions increase the intrauterine release of CRH and urocortin through a local stress response and activate endometrial mast cells to secrete abortogenic mediators *(25)*. Hence, it is possible that urocortin at the concentrations we measured may be helpful early in pregnancy, whereas these or higher concentrations at later times may be detrimental owing to interactions with local mast cells and inflammation mediators. In addition, because mast cells themselves produce both urocortin *(26)* and matrix metalloproteinase 9 *(27)*, endometrial mast cells might be a contributory source of urocortin and thereby take part in the cross-talk between local immune and inflammatory responses *(28)* and in the paracrine/autocrine control of endometrial remodeling in early pregnancy.

The pregnancy rate in this study (39.4%) was higher than expected, but this result reflects only chance fluctuations and not a particular method of patient selection or treatment. In a study of 566 couples and 1 763 IUI cycles *(29)*, the pregnancy rate per patient was 21.4%. Indeed, the overall pregnancy rate can be reliably calculated only over a longer time and with a larger number of cases than in the present study. This limitation does not compromise the statistical accuracy of our study, however, because the sensitivity, specificity, and likelihood ratios of the test do not depend on the pregnancy rate. Thus, if we consider a more realistic estimation of the pretest probability, such as 21.4% *(29)*, and use the positive likelihood ratio of 26.11, then the posttest probability of pregnancy in women with a positive urocortin test result would still be high (87.7%, as calculated with the Bayes theorem) *(30)*.

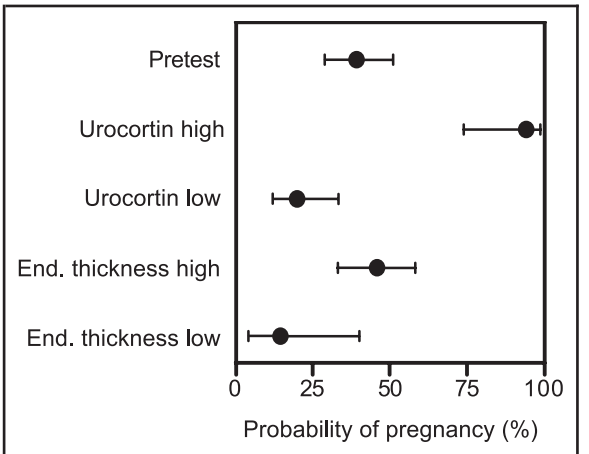

Fig. 4. Pregnancy rates among all patients (pretest probability) and after different test results (posttest probability).

Data are presented as percentages, and the error bars indicate the 95% CI for each probability. End., endometrial.

In clinical practice, the detection of markers of endometrial receptivity in uterine wash fluid may be particularly desirable for patients who undergo complex assisted-reproduction procedures, such as in vitro fertilization and intracytoplasmic sperm injection, and who are already undergoing ultrasound-guided manipulations. With the future development of a rapid format for the urocortin test and the combination of urocortin measurement with new markers to improve its sensitivity, this assay may become a useful tool in support of clinical decisions during the process of oocyte retrieval, in vitro fertilization, and embryo transfer.

In conclusion, urocortin is measurable in endometrial wash fluid, its concentrations are high in women who become pregnant after IUI, and its measurement may add prognostic information for predicting implantation.

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References

1. Strowitzki T, Germeyer A, Popovici R, von Wolff M. The human endometrium as a fertility-determining factor. Hum Reprod Update 2006;12:617–30.
2. Florio P, Vale W, Petraglia F. Urocortins in human reproduction Peptides 2004;25:1751–7.
3. Donaldson CJ, Sutton SW, Perrin MH, Corrigan AZ, Lewis KA, Rivier JE, et al. Cloning and characterization of human urocortin. Endocrinology 1996;137:2167–70.
4. Florio P, Arcuri F, Ciarmela P, Runci Y, Romagnoli R, Cintorino M, et al. Identification of urocortin mRNA and peptide in the human endometrium. J Endocrinol 2002;173:9–14.
5. Torricelli M, De Falco G, Florio P, Rossi M, Leucci E, Vigano P, et al. Secretory endometrium highly expresses urocortin messenger RNA and peptide: possible role in the decidualization process. Hum Reprod 2007;22:92–6.
6. Severi FM, Bocchi C, Florio P, Cobellis L, Ignacchiti E, Petraglia F. Transvaginal ultrasonography in women receiving emergency contraception. Fertil Steril 2003;79:1074–7.
7. Florio P, Severi FM, Luisi S, Ciarmela P, Calonaci G, Cobellis L, Petraglia F. Endometrial expression and secretion of activin A, but not follistatin, increase in the secretory phase of the menstrual cycle. J Soc Gynecol Investig 2003;10:237–43.
8. Stephan C, Wesseling S, Schink T, Jung K. Comparison of eight computer programs for receiver-operating characteristic analysis. Clin Chem 2003;49:433–9.
9. Richardson WS, Wilson MC, Guyatt GH, Cook DJ, Nishikawa J. Users' guides to the medical literature: XV. How to use an article about disease probability for differential diagnosis. Evidence-Based Medicine Working Group. JAMA 1999;281:1214–9.
10. Florio P, De Falco G, Leucci E, Torricelli M, Torres PB, Toti P, et al. Urocortin expression is down-regulated in human endometrial carcinoma. J Endocrinol 2006;190:99–105.
11. Li W, Challis JR. Corticotropin-releasing hormone and urocortin induce secretion of matrix metalloproteinase-9 (MMP-9) without change in tissue inhibitors of MMP-1 by cultured cells from human placenta and fetal membranes. J Clin Endocrinol Metab 2005;90:6569–74.
12. Florio P, Franchini A, Reis FM, Pezzani I, Ottaviani E, Petraglia F. Human placenta, chorion, amnion and decidua express different variants of corticotropin-releasing factor receptor messenger RNA. Placenta 2000;21:32–7.
13. Curry TE Jr, Osteen KG. The matrix metalloproteinase system: changes, regulation, and impact throughout the ovarian and uterine reproductive cycle. Endocr Rev 2003;24:428–65.
14. Abrahams VM, Kim YM, Straszewski SL, Romero R, Mor G. Macrophages and apoptotic cell clearance during pregnancy Am J Reprod Immunol. 2004;51:275–82.
15. Suda T, Kageyama K, Sakihara S, Nigawara T. Physiological roles of urocortins, human homologues of fish urotensin I, and their receptors. Peptides 2004;25:1689–701.
16. Tsatsanis C, Androulidaki A, Dermitzaki E, Charalampopoulos I, Spiess J, Gravanis A, Margioris AN. Urocortin 1 and urocortin 2 induce macrophage apoptosis via CRFR2. FEBS Lett 2005;579:4259–64.
17. Simoncini T, Apa R, Reis FM, Miceli F, Stomati M, Driul L, et al. Human umbilical vein endothelial cells: a new source and potential target for corticotrophin-releasing factor. J Clin Endocrinol Metab 1999;84:2802–6.
18. Jain V, Longo M, Ali M, Saade GR, Chwalisz K, Garfield RE. Expression of receptors for corticotropin-releasing factor in the vasculature of pregnant rats. J Soc Gynecol Investig. 2000;7:153–60.
19. Schilling L, Kanzler C, Schmiedek P, Ehrenreich H. Characterization of the relaxant action of urocortin, a new peptide related to corticotropin-releasing factor in the rat isolated basilar artery. Br J Pharmacol 1998;125:1164–71.
20. Yao X, He GW, Chan FL, Lau CW, Tsang SY, Chen ZY, Huang Y. Endothelium-dependent and -independent coronary relaxation induced by urocortin. J Card Surg 2002;17:347–9.
21. Huang Y, Chan FL, Lau CW, Tsang SY, He GW, Chen ZY, Yao X. Urocortin-induced endothelium-dependent relaxation of rat coronary artery: role of nitric oxide and K^+ channels. Br J Pharmacol 2002;135:1467–76.
22. Huang Y, Chan FL, Lau CW, Tsang SY, Chen ZY, He GW, Yao X. Roles of cyclic AMP and Ca^{2+}-activated K^+ channels in endothelium-independent relaxation by urocortin in the rat coronary artery. Cardiovasc Res 2003;57:824–33.
23. Weisinger RS, Blair-West JR, Burns P, Denton DA, Purcell B, Vale W, et al. Cardiovascular effects of long-term central and peripheral administration of urocortin, corticotropin-releasing factor, and adrenocorticotropin in sheep. Endocrinology 2004; 145:5598–604.
24. Achache H, Revel A. Endometrial receptivity markers, the journey to successful embryo implantation. Hum Reprod Update 2006;12:731–46.
25. Madhappan B, Kempuraj D, Christodoulou S, Tsapikidis S, Boucher W, Karagiannis V, et al. High concentrations of intrauterine corticotropin-releasing hormone, urocortin, tryptase, and interleukin-8 in spontaneous abortions. Endocrinology 2003;144:2285–90.
26. Kempuraj D, Papadopoulou NG, Lytinas M, Huang M, Kandere-Grzybowska K, Madhappan B, et al. Corticotropin-releasing hormone and its structurally related urocortin are synthesized and secreted by human mast cells. Endocrinology 2004;145:43–8.
27. Tanaka A, Matsuda H. IgE crosslinkage of Fcε receptor I induces both production and activation of matrix metalloproteinase-9 in mast cells. Cell Immunol 2004;228:66–75.
28. Arck PC, Rose M, Hertwig K, Hagen E, Hildebrandt M, Klapp BF. Stress and immune mediators in miscarriage. Hum Reprod 2001;16:1505–11.
29. van der Westerlaken LA, Naaktgeboren N, Helmerhorst FM. Evaluation of pregnancy rates after intrauterine insemination according to indication, age, and sperm parameters. J Assist Reprod Genet 1998;15:359–64.
30. Fagan TJ. Letter: nomogram for Bayes theorem. N Engl J Med 1975;293:257. .