



ELSEVIER

Induction of premature labor and delivery by allergic reaction and prevention by histamine H₁ receptor antagonist

Egle Bytautiene, MD,^a Roberto Romero, MD,^c Yuri P. Vedernikov, MD, PhD,^a
Faten El-Zeky, PhD,^b George R. Saade, MD,^a Robert E. Garfield, PhD^{a,*}

Department of Obstetrics and Gynecology, Division of Reproductive Sciences,^a Preventive Medicine and Community Health Office of Biostatistics,^b University of Texas Medical Branch, Galveston, Tex, Perinatology Research Branch, National Institutes of Child Health and Human Development/National Institutes of Health/Department of Health and Human Services, Bethesda, Md, and Detroit, Mich^c

KEY WORDS

Premature labor and delivery
Allergy
Mast cell
Guinea pig
H₁ receptor antagonist
Cromolyn sodium

Objective: The purpose of this study was to determine whether type I hypersensitivity can induce preterm labor and delivery that may be prevented by the administration of an H₁ receptor antagonist or a mast-cell stabilizer.

Study design: At days 40 through 50 of gestation, ovalbumin-sensitized guinea pigs that had not been treated or had been pretreated with an H₁ receptor antagonist (ketotifen) or a mast-cell stabilizer (cromolyn sodium) were challenged with either ovalbumin or saline solution. Nonsensitized guinea pigs were challenged with ovalbumin.

Results: The duration of gestation was significantly shorter in sensitized animals that were challenged with ovalbumin than in the other groups. Pretreatment with ketotifen significantly increased the duration of pregnancy in sensitized animals, compared with untreated animals. Cromolyn sodium had no effect.

Conclusion: An allergic reaction can induce premature labor/delivery, and pretreatment with histamine H₁ receptor antagonist can prevent it. These observations provide evidence of a novel mechanism of disease for premature labor and delivery.

© 2004 Elsevier Inc. All rights reserved.

Prematurity is the leading cause of perinatal morbidity and death worldwide, and approximately 50% of all preterm births are idiopathic. Accumulating evidence

indicates that premature parturition is a syndrome that is characterized by increased uterine contractility and cervical ripening that is produced by multiple causes.¹ We have previously proposed that a subgroup of patients with premature labor may have an allergic-like mechanism of disease, but the evidence for this has been indirect.²

A type I hypersensitivity reaction is common. A positive skin test for allergens has been estimated to be present in 40 to 60 million Americans who have allergic disorders. Type I hypersensitivity reactions may also be

Presented at the Twenty-Fourth Annual Meeting of the Society for Maternal-Fetal Medicine, February 2-7, 2004, New Orleans, La.

* Reprint requests: Robert E. Garfield, PhD, Department of Obstetrics and Gynecology, Division of Reproductive Sciences, University of Texas Medical Branch, 301 University Blvd, Rt J-62, Galveston, TX 77555-1062.

E-mail: rgarfiel@utmb.edu

responsible for gastrointestinal disorders, conjunctivitis, asthma, etc. Yet, little attention has been paid to the possibility that the uterus may be a target.

The major mediator-secreting effector cell in immunoglobulin E (IgE)–mediated anaphylactic responses to antigens (such as grass pollens, house dust, mice feces) is mast cells, basophils, and eosinophils. Environmental allergens enter through mucosal surfaces and are presented to T cells, which in turn secrete cytokines that induce proliferation of B cells and the production of allergen-specific IgE. IgE binds to mast cells through the FcεRI receptor. After re-exposure, the allergen can cross-link with mast cell-bound IgE and induce the release of mediators. Collectively, these mediators cause symptoms of allergy.

In 1910 and 1913, Schultz³ and Dale⁴ demonstrated that smooth muscle (including uterine) that was taken from sensitized animals contracts after challenge with a specific antigen. It has been shown since then that mast cell mediators (such as histamine, serotonin, prostaglandin_{2α}, and thromboxane A₂ induce contractions of isolated human^{5–7} and animal⁸ myometrium and in the guinea pig cervix.⁹

Mast cells release mediators in response to various secretagogues (such as a specific antigen, compound 48/80). Previous studies indicated that compound 48/80 augments contractility of the isolated human¹⁰ and animal^{11,12} myometrium and of the guinea pig cervix⁹ and that supernatant from an established mast cell line treated with compound 48/80 stimulates uterine contractility in vitro.¹³ Moreover, challenge of isolated tissues from ovalbumin-sensitized animals with ovalbumin increased uterine contractility.^{12,14}

This study was conducted to determine whether a type I hypersensitivity reaction can induce preterm labor in pregnant guinea pigs and to examine whether treatment with histamine H₁ receptor antagonist and mast cell stabilizer could prevent allergen-induced preterm labor/delivery.

Material and methods

Animals

Hartley strain guinea pigs (2-month-old virgin females and 3-month-old males) were obtained from Harlan Sprague Dawley, Inc (Indianapolis, Ind). Guinea pigs were selected for the following reasons: (1) They exhibit distinct signs of estrus (vaginal membrane opening); (2) their sex hormone profile is similar to that of humans during the estrus cycle and pregnancy; (3) guinea pigs have a relatively long and stable gestational period (59–72 days; mean, 63 days), which allows sufficient time to breed, sensitize, and challenge animals; and (4) they have been used extensively to study type I hypersensitivity responses. A limitation of using this species is that

guinea pigs are extremely sensitive to histamine and prone to respond with anaphylaxis and asphyxia to antigen challenge.

Guinea pigs were bred in our Animal Care Facility. One female and one male guinea pig were caged together. Day 1 of gestation was assigned when the vaginal membrane opening was first observed.

Guinea pigs were sensitized as described previously.¹⁵ On days 30 to 35 of pregnancy, guinea pigs received injections of a suspension that contained 100 μg ovalbumin and 100 mg Al(OH)₃ per 1 mL of saline solution, 0.7 mL intraperitoneally and another 0.3 mL subcutaneously, in the neck region. Animals that were injected with only Al(OH)₃ (100 mg/mL saline suspension) served as nonsensitized controls. Sensitivity to ovalbumin was tested 2 weeks after sensitization by 2 intracutaneous injections of 0.1 mL of ovalbumin-saline solution and 0.1 mL saline solution (vehicle) on the shaved back of the animal. The development of edema and erythema within 24 hours of the antigen injection was considered a sign of sensitization (edema in sensitized guinea pigs, 5.9 ± 0.3 mm vs 2.8 ± 0.4 mm, in nonsensitized guinea pigs; erythema in sensitized guinea pigs, 24.8 ± 1.1 mm vs 0, in nonsensitized guinea pigs).

Optimal doses and administration routes were established during our preliminary studies. Intraperitoneal injection was chosen because it is the most direct route to deliver an allergen to the uterus and because it provides better control of the amount of antigen to which the animal is exposed, as compared with the use of a nebulizer. We found that if guinea pigs are challenged with 3 mg of ovalbumin in 1 mL of saline solution, the death rate from anaphylactic shock is 22%, although preterm labor is still observed (35%). Guinea pigs that died within 24 hours from antigen challenge were excluded from final analysis, because the effect of exposure to antigen could not be tested in these animals. The study protocol was approved by the Animal Care and Use Committee at the University of Texas Medical Branch at Galveston.

Experimental protocols

Experiment 1

At 40 to 50 days of gestation, ovalbumin-sensitized guinea pigs were divided randomly into 2 groups and challenged intraperitoneally with either ovalbumin (3 mg in 1 mL of saline solution) or saline solution (1 mL) every 48 hours until delivery. Nonsensitized animals received intraperitoneal ovalbumin injections for only 2 weeks to avoid sensitization to antigen.

Experiment 2

Ovalbumin-sensitized guinea pigs at 40 to 50 days of gestation were divided randomly into 3 groups: the first group received no treatment; the second group was injected intramuscularly with histamine H₁ receptor

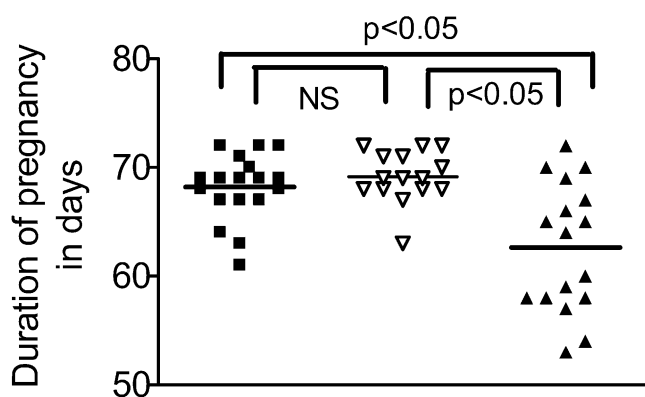


Figure 1 The duration of pregnancy is shown as a scattered dot plot. The *closed rectangles* indicate nonsensitized guinea pigs that were challenged with ovalbumin ($n = 19$); the *open triangles* indicate sensitized guinea pigs that were challenged with saline solution ($n = 15$); the *closed triangles* indicate sensitized guinea pigs that were challenged with ovalbumin ($n = 17$). Each indicator represents a single guinea pig. The *line* is the mean length of gestation for each group. *NS*, No significant difference. A probability value of $<.05$ is statistically significant.

antagonist (ketotifen, 0.01 mg/kg) 20 minutes before challenge,¹⁶ and the third group was treated with a mast cell stabilizer (cromolyn sodium, 50 mg/kg, intraperitoneal) 1 hour before exposure to antigen,¹⁷ as described previously. All guinea pigs were challenged intraperitoneally with ovalbumin.

Data analysis

The duration of pregnancy was analyzed with the Student t test for comparison of the means of 2 groups by the Satterthwaite method, with unequal variances with the SAS software (SAS Institute Inc, Cary, NC). Survival analysis (Kaplan-Meier test) was also used to assess the time interval from the first challenge with antigen to delivery (GraphPad Prism 4.0 software; GraphPad Software, Inc, San Diego, Calif). A probability value of $<.05$ was considered statistically significant.

Chemicals

Aluminum hydroxide, cromolyn sodium, ovalbumin (albumin, chicken egg), saline solution, and ketotifen were obtained from Sigma-Aldrich (St. Louis, Mo).

Results

The duration of pregnancy in nonsensitized guinea pigs that were challenged with ovalbumin (68.2 ± 0.7 days) was not different from that of sensitized animals that were challenged with saline solution (69.1 ± 0.6 days). This demonstrated that exposure to ovalbumin did not reduce the length of gestation (Figure 1). However, the

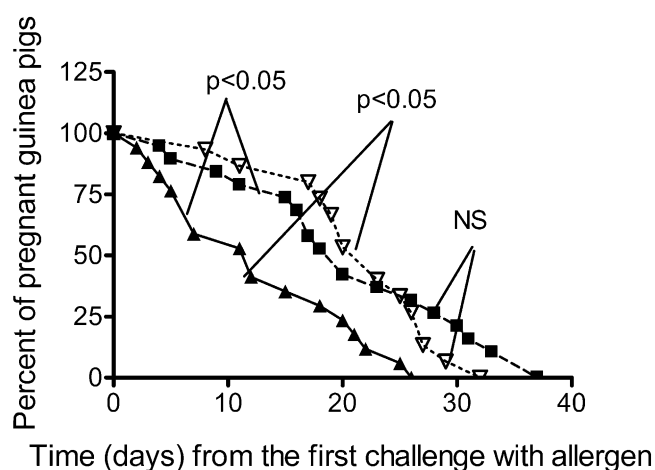


Figure 2 Proportions of undelivered guinea pigs are shown by survival analysis. The *Y-axis* represents the percentage of guinea pigs that were still pregnant; the *X-axis* represents the time in days from the first challenge with antigen to delivery. The *closed rectangles* indicate nonsensitized guinea pigs that were challenged with ovalbumin ($n = 19$); the *open triangles* indicate sensitized guinea pigs that were challenged with saline solution ($n = 15$); the *closed triangles* indicate sensitized guinea pigs that were challenged with ovalbumin ($n = 17$). *NS*, No significant difference. A probability value of $<.05$ is statistically significant.

duration of pregnancy was significantly shorter in sensitized guinea pigs challenged with ovalbumin (62.7 ± 1.4 days) than in those in the other 2 groups. Proportions of undelivered pregnant guinea pigs after the first challenge with ovalbumin/saline solution to delivery in the 3 experimental groups are shown in Figure 2. The proportion of undelivered guinea pigs was lower in sensitized animals that were challenged with allergen than in the other 2 groups (Figure 2). Premature labor and delivery was observed in 35% of sensitized guinea pigs that were challenged with ovalbumin. None of the sensitized guinea pigs that received saline injections and nonsensitized guinea pigs that received an ovalbumin challenge delivered prematurely (Figures 3 and 4).

The second set of experiments was designed to test the hypothesis of whether pretreatment of sensitized animals with cromolyn (mast cell stabilizer) or ketotifen (histamine H_1 receptor antagonist) could prevent allergy-induced preterm labor and delivery. There was no significant difference in the duration of pregnancy between nontreated (62.7 ± 1.4 days) guinea pigs or guinea pigs that were pretreated with cromolyn (63.6 ± 2.4 days; Figure 5), and there was no significant difference in the survival curves from the first challenge between these 2 groups (Figure 6). In contrast, the duration of pregnancy was significantly longer in animals that were pretreated with ketotifen (67 ± 1.4 days vs 62.7 ± 1.4 days, in nontreated animals; Figure 5).



Figure 3 Ovalbumin-sensitized guinea pig challenged with ovalbumin that delivered stillborn pups prematurely at day 57 of gestation (term gestation for guinea pigs, 59-72 days).



Figure 4 Nonsensitized guinea pig that was challenged with ovalbumin and delivered healthy pups at term (day 68).

Proportions of undelivered pregnant guinea pigs were also significantly higher in ketotifen-treated animals (Figure 6).

Comments

Our study demonstrates that preterm labor and delivery can be induced by exposing sensitized animals to an allergen and that ketotifen, an H_1 receptor antagonist, prevents allergy-induced preterm labor and delivery. This represents the first evidence that a type I hypersensitivity reaction in pregnant animals can induce preterm labor and delivery.

The concept that an allergy-like mechanism could be one of the causes of preterm labor was proposed in the early 1990s.^{2,18} Contractility studies of isolated human myometrium demonstrated that a challenge with ragweed enhances the contractility of myometrial strips from ragweed-sensitized patients and the contractility of strips that were incubated with serum from ragweed-sensitive patients. Also, some patients who reported a history of allergies had eosinophils as the predominant cell in their amniotic fluid and delivered prematurely.^{2,18}

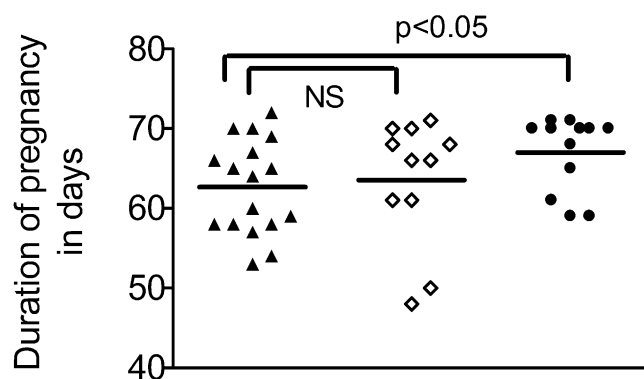


Figure 5 The duration of the pregnancy is shown. The *closed triangles* indicate sensitized guinea pigs challenged with ovalbumin ($n = 17$); the *open rectangles* indicate sensitized guinea pigs that received pretreatment with mast-cell stabilizer (cromolyn) before challenge with ovalbumin ($n = 11$); the *closed circles* indicate sensitized guinea pigs that were pretreated with an antihistamine (ketotifen) before challenge with antigen ($n = 12$). Each indicator represents a single guinea pig. The line is the mean length of gestation for each group. *NS*, No significant difference. A probability value of $< .05$ is statistically significant.

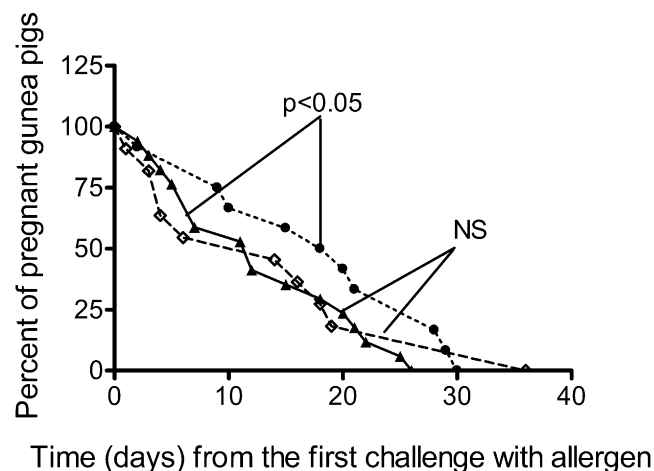


Figure 6 Proportions of undelivered guinea pigs are shown by survival analysis. The *Y-axis* represents the percent of undelivered pregnant guinea pigs; the *X-axis* represents the time in days from the first challenge with antigen to delivery. The *closed triangles* indicate sensitized guinea pigs challenged with ovalbumin ($n = 17$); the *open rectangles* indicate sensitized guinea pigs that received pretreatment with a mast-cell stabilizer (cromolyn) before challenge with ovalbumin ($n = 11$); the *closed circles* indicate sensitized guinea pigs that were pretreated with an antihistamine (ketotifen) before challenge with antigen ($n = 12$). *NS*, No significant difference. A probability value of $< .05$ is statistically significant.

It was proposed that a subset of patients might have uterine allergy as a mechanism of disease. This hypothesis was consistent with the following observations: an allergen challenge increased the contractility of

myometrium from sensitized animals,¹⁴ and a significant increase in the intrauterine pressure and electromyographic uterine activity was observed when sensitized animals were challenged with antigen *in vivo*.¹⁹ Our results provide additional biologic plausibility to the hypothesis that a subset of preterm labor may be induced through an allergic reaction.

Mast cells are associated with type I hypersensitivity reaction, although eosinophils and basophils may also be involved. Uterine mast cells release histamine, leukotriene₄, and prostaglandin D₂ in response to challenge with anti-IgE, the same reaction as lung mast cells.²⁰ Functional studies on isolated uterine tissues have demonstrated that certain products of mast cell degranulation can stimulate uterine contractility.^{5-8,10-14} Therefore, it is clear that mast cells are present in the uterus.

Exposure to an allergen can occur in several ways: inhalation (eg, pollens), contact (eg, plants), orally (eg, foods), injection (eg, stinging insects), and even through the vaginal route. The question of whether allergens could reach the uterus remained open until recently. It has been reported that allergens that are fed orally to pregnant rats can be detected in amniotic and uterine fluids.²¹ A house dust mite allergen has been found in the amniotic fluid and in the blood from the umbilical cord from healthy pregnant women who were exposed to high concentrations of house dust mite antigen in their home dust.²² Thus, the uterine environment can be exposed to allergens.

For the development of type I hypersensitivity reactions, the host also needs an immune response to be biased towards a Th2 type, which favors the production of IgE by B cells. Pregnancy is such a state.²³ Thus, all the factors that are required for a type I hypersensitivity reaction (such as effector cells, allergens, and predisposition of B cells to produce IgE) are present during pregnancy.

Symptoms of allergy could be prevented either by inhibiting the effector cells or blocking the effect of the mediator on the tissues. Cromolyn sodium blocks the chloride channel activity that maintains cells in a normal resting physiologic state (ie, inhibits their degranulation). It is known to be more effective systemically, and this could explain its ineffectiveness in our study. Histamine H₁ receptor antagonists have long been used for the symptomatic treatment of allergies. At the dose used in these experiments, we were able to prevent allergen-induced preterm labor.

The data presented herein represent the first experimental evidence in support of the hypothesis that a type I hypersensitivity reaction can initiate premature labor and delivery. A limitation of the study is the lack of direct evidence that mast cell degranulation stimulates the uterus, which results in premature labor. However, previous studies provide strong evidence that this is the case.^{9,10,12,14}

It has been reported that a history of hives is associated significantly with premature labor.²⁴ Until now, this is the only report available on the epidemiologic condition of preterm labor in allergic subjects. However, this may simply represent that the symptoms caused by allergy, as assessed in other organ systems (eg, respiratory tract), may not be a reliable indicator of "uterine allergy." Women with bronchial asthma have a 2-fold increase in the risk of premature labor and delivery.²⁵ However, the report does not discriminate between different causes of asthma.

Inasmuch as a large proportion of the US population is affected by symptoms of allergic conditions, the possibility that a type I hypersensitivity reaction may be operative in some cases of premature labor must be considered. The data presented also identify interventions that may be effective in some patients, namely those who experience allergies during pregnancy.

References

1. Romero R, Mazor M, Munoz H, Gomez R, Galasso M. Preterm labor syndrome. *Ann N Y Acad Sci* 1994;734:414-29.
2. Romero R, Mazor M, Avila C, Quintero R, Munoz H. Uterine "allergy": a novel mechanism for preterm labor. *Am J Obstet Gynecol* 1991;164:375.
3. Schultz WH. Physiological studies in anaphylaxis: the reaction of smooth muscle of the guinea pig sensitized with horse serum. *J Pharmacol Exp Ther* 1910;1:549-67.
4. Dale HH. The anaphylactic reaction of plain muscle in the guinea pig. *J Pharmacol Exp Ther* 1913;4:167-223.
5. Rudolph MI, Reinicke K, Cruz MA, Gallardo V, Gonzalez C, Bardisa L. Distribution of mast cells and the effect of their mediators on contractility in human myometrium. *BJOG* 1993;100:1125-30.
6. Senchyna M, Crankshaw DJ. Characterization of the prostanoid TP receptor population in human nonpregnant myometrium. *J Pharmacol Exp Ther* 1996;279:262-70.
7. Bytautiene E, Vedernikov YP, Saade GR, Romero R, Garfield RE. Effect of histamine on phasic and tonic contractions of isolated uterine tissue from pregnant women. *Am J Obstet Gynecol* 2003;188:774-8.
8. Goyal RK, Verma SC. Pharmacological investigations into the effects of histamine and histamine analogs on guinea pig and rat uterus. *Agents Actions* 1981;11:312-7.
9. Bytautiene E, Vedernikov YP, Saade GR, Romero R, Garfield RE. Endogenous mast cell degranulation modulates cervical contractility in the guinea pig. *Am J Obstet Gynecol* 2002;186:438-45.
10. Bytautiene E, Vedernikov Y, Saade G, Romero R, Garfield R. Degranulation of uterine mast cell modifies contractility of isolated myometrium from pregnant women. *Am J Obstet Gynecol* [In press].
11. Bytautiene E, Vedernikov YP, Saade G, Romero R, Garfield RE. Effect of endogenous mast cell degranulation on uterine contractility in pregnant guinea pigs. *J Soc Gynecol Investig* 2000;7:273A.
12. Garfield RE, Bytautiene E, Vedernikov YP, Marshall JS, Romero R. Modulation of rat uterine contractility by mast cells and their mediators. *Am J Obstet Gynecol* 2000;183:118-25.
13. Bytautiene E, Vedernikov Y, Ali M, Saade G, Romero R, Garfield RE. Exogenous mast cells induce contraction of isolated

- non-pregnant and pregnant guinea pigs uterus [abstract]. *Am J Obstet Gynecol* 1999;180:S133.
14. Bytautiene E, Vedernikov Y, Saade G, Romero R, Garfield RE. Challenge with ovalbumin increases contractility of uterine and cervical strips from sensitized term pregnant guinea pigs [abstract] *J Soc Gynecol Invest* 2002;9:208A.
15. Van Amsterdam RG, Brouwer F, Zaagsma J. Analysis of the beta-adrenoceptor mediated inhibition of IgG1 and IgE dependent guinea-pig anaphylactic tracheal smooth muscle contraction. *Agents Actions* 1989;26:48-51.
16. Levrier J, Duval D, Prouteau M, Voltz C, Berry CN, Lloyd KG, et al. Anti-anaphylactic activity of the novel selective histamine H1 receptor antagonist mizolastine in the rodent. *Arzneimittelforschung* 1995;45:559-68.
17. Singh R, Nath A, Gupta PP, Shukla M, Khare SK, Kundu B. Antiallergic/antiasthmatic effect of novel antiallergic hexapeptide-95/220 in various experimental models. *Indian J Exp Biol* 2001;39:871-7.
18. Tabb TN, Garfield RE. Ragweed-induced stimulation of uterine contractility [abstract 605]. *Proceedings of the 38th annual meeting of the Society for Gynecologic Investigation*; 1991, March 20-23.
19. Bytautiene E, Vedernikov Y, Maner W, Shi SQ, Mackay L, Saade G, et al. Antigen challenge in sensitized guinea pigs increases uterine EMG and intrauterine pressure [abstract]. *J Soc Gynecol Invest* 2001;8:191A.
20. Massey WA, Guo CB, Dvorak AM, Hubbard WC, Bhagavan BS, Cohan VL, et al. Human uterine mast cells. Isolation, purification, characterization, ultrastructure, and pharmacology. *J Immunol* 1991;5:1621-7.
21. Dahl GMK, Telemo E, Westrom BR, Jakobsson I, Lindberg T, Karlsson BW. The passage of orally fed proteins from mother to foetus in the rat. *Comp Biochem Physiol A Mol Integr Physiol* 1984;77:199-201.
22. Holloway JA, Warner JO, Vance GHS, Diaper ND, Warner JA, Jones CA. Detection of house-dust-mite allergen in amniotic fluid and umbilical-cord blood. *Lancet* 2000;356:1900-2.
23. Marzi M, Trabattini D, Vigano A, Villa ML, Salvaggio A, Clevici E, et al. Characterization of type 1 and type 2 cytokine production profile in physiologic and pathologic human pregnancy. *Clin Exp Immunol* 1996;106:127-33.
24. Kramer MS, Coates AL, Michoud MC, Dagenais S, Moshonas D, Davis GM, et al. Maternal asthma and idiopathic preterm labor. *Am J Epidemiol* 1995;142:1078-88.
25. Sorensen TK, Dempsey JC, Xiao R, Frederick IO, Luthy DA, Williams MA. Maternal asthma and risk of preterm delivery. *Ann Epidemiol* 2003;13:267-72.