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Transient Immunologic Effects of Betamethasone in Human Pregnancy After Suppression of Preterm Labor

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ABSTRACT: **Maternal immune suppression is a potentially significant adverse effect when betamethasone is used to hasten lung maturation of the fetus at risk for preterm delivery. However, increased incidence of infection has not been observed consistently after betamethasone treatment of pregnant women. This study was designed to determine if the cellular immune response to steroids may be modified during pregnancy in a way that would diminish the infection risk associated with steroid treatment.**

The effect of betamethasone on immunocytes among patients with preterm labor or in nonpregnant subjects were determined following administration of 12 mg of betamethasone intramuscularly. We measured serially the circulating leukocytes, lymphocytes, T lymphocytes, and their subsets. Measurements were also made of localized leukocyte mobilization to serum-filled skin chambers covering experimental inflammatory sites.

Patients in preterm labor had increased WBC counts prior to treatment with betamethasone but no additional leukocytosis was induced nor was mobilization of leukocytes to the skin chambers decreased. Lymphopenia and depression of T cells was more transient among pregnant patients compared to nonpregnant. Thus, the pregnant patients studied had diminished or more transient potentially adverse immunocyte responses to betamethasone as compared to nonpregnant subjects. (Am J Reprod Immunol. 1983; 4:83-87.)

Key words: Betamethasone, maternal immunity, T lymphocytes, leukocyte mobilization.

INTRODUCTION

Glucocorticoids are often administered to women at risk for preterm delivery in order to prevent respiratory distress in premature infants. Among nonpregnant subjects, glucocorticoids cause a prompt and profound leukocytosis and lymphopenia with selective reduction of thymus derived lymphocytes (T lymphocytes).[1-4] Steroid induced changes have also been described for some leukocyte functions such as migration of granulocytes to an inflammatory site,[5] leukocyte chemotaxis,[6] and granulocyte adherence.[7] When these indices of immune response are persistently disturbed, there is an associated increase in susceptibility to infection.[8] In obstetrical practice these adverse glucocorticoid side effects on immunocytes are of particular concern because preterm labor may sometimes be caused by incipient amnionitis.[9] Other women may be at risk for developing an intrauterine infection due to premature rupture of membranes and use of glucocorticoids could worsen this risk. Despite the theoretical infection risk, most studies have not demonstrated an actual increase of infection rate when steroids are given to mothers with intact membranes.[10-12] Exceptions are isolated reports of increased neonatal infections[11] and increased postpartum endometritis if membranes were ruptured when steroids were given.[13]

In this study we determined serial differential leukocyte counts before and following betamethasone treatment in pregnant women with preterm labor. The effect on immunocytes was studied and compared to effects in nonpregnant volunteers using quantitative determination of circulating lymphocytes, T-lymphocyte subsets, and localized leukocyte mobilization (LLM). Shifts in circulating leukocyte counts and changes in leukocyte migration were evaluated to determine if the potentially adverse immunocyte responses to glucocorticoids were modified by pregnancy.

METHODS

Human Subjects

Betamethasone study patients were women with spontaneous onset of preterm labor. An attempt was made to suppress labor with ritodrine if gestational age was between 28 and 34 weeks and there was no evidence of amnionitis. To avoid labor suppression and betamethasone treatment of infected patients, we excluded patients with initial WBC above 20,000/mm^3 and polymorphonuclear band forms above 20%. Patients with counts exceeding these arbitrary limits nearly always developed clinical signs of infection within a few hours. Magnesium sulfate was used for labor suppression in one patient who experienced adverse side effects from ritodrine. Once labor had been successfully suppressed, ultrasound evaluation was done for documentation of fetal biparietal diameter, abdominal circumference, presence of normal amniotic fluid volume (greater than 1 × 1 cm smallest pool) and placental location. Amniotic fluid was obtained by transabdominal amniocentesis and examined by Gram's stain. If bacteria were present, a diagnosis of amnionitis was assumed and these patients were also excluded from the study. Amniotic fluid samples were also tested for lecithin/sphingomyelin (L/S) ratio; patients with L/S ratio less than 2.0 were given two doses of 12 mg of betamethasone intramuscularly with a 24-hour interval between doses. The immunocyte responses reported here were those which occurred after the first dose of betamethasone. WBC counts were done on all patients but the studies of lymphocytes and T-lymphocyte subsets were performed on different subjects than those of leukocyte mobilization. The pregnant subjects in this report had no recognized infections or other

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medical illnesses. Nonpregnant controls included healthy male and female volunteers. The study protocols were reviewed and approved by the institutional research and human subjects committee.

Leukocytosis, Lymphopenia, and T-Lymphocyte Shifts

Thirty patients in preterm labor had baseline WBC and differential counts performed on admission. After administration of betamethasone, serial WBC counts were repeated every 6 hours for 24 hours. The second dose of betamethasone was given after 24 hours and subsequently WBC and differential counts were repeated every 12 hours. WBC counts were done by an automated technique using a Coulter Counter (model Ssr). Differential leukocyte counts were done by microscopic examination of 100 cells following Wright stain of the blood smear. If the differential count was outside the normal adult range, an additional 100 cells were counted.

In 12 patients who had preterm labor and 5 nonpregnant controls, determinations of the T-cell fraction of total lymphocytes and the percentages of two T-cell subsets were done. T-lymphocyte changes following the second dose of betamethasone showed no apparent differences when compared to the first dose; therefore, we report only the response to initial treatment. After phlebotomy, blood samples were stored in 50% RPMI 1640 culture media at room temperature and lymphocyte staining and analysis was done within 24 hours. Fluoresceinated monoclonal antibodies to T-cell differentiation antigens were kindly provided by Becton Dickinson Monoclonal Center (Mountain View, California) and were used for T-cell labelling. The antibodies used were Leu 1 (total T cells), Leu 2a (suppressor/cytotoxic subpopulation), and Leu 3a (helper/inducer subpopulation).[14] Lymphocytes were separated by Ficol–hypaque gradient, stained by standard direct immunofluorescence techniques, and analyzed using a fluorescence-activated cell sorter (FACS).[15] Statistical tests used for evaluating differences in WBC, lymphocyte increments and increments in T-cell subsets included the Student's t, Duckworth, and Mann–Whitney tests.

Localized Leukocyte Mobilization

The technique used was similar to that described by Peters et al.[5] A template of adhesive tape was placed on the volar forearm after punching out six round holes with diameters of 0.6 cm and areas of 0.28 cm^2. The skin exposed through these holes was then abraded by repeated application and removal of another adhesive tape over the area for about 2 minutes. The abrasion procedure caused a glistening erythema but no bleeding. Small skin chambers were made of ¼-inch inner diameter plexiglass cut in ¼-inch lengths to contain a volume of 0.2 ml of the attractant solution. Six of these were linked together to fit the abrasion template pattern and were applied with tape to the abraded skin areas using K-Y jelly to form a seal at the edges. Rubber caps over the exposed ends of the individual chambers provided access for injecting the attractant solution, which in these studies was autologous serum. Leukocyte mobilization into the skin chamber was determined by removing serum after 24 hours and performing hemocytometer leukocyte counts on the exudate. Mobilization was expressed as WBC per square centimeter of abraded skin surface. Greater than 98% of the white blood cells migrating into the chambers were polymorphonuclear cells. In addition to the 13 patients in preterm labor tested during the 24 hours after the first betamethasone dose (12 mg given intramuscularly), leukocyte mobilization was tested in 49 prenatal patients who were 28 to 35 weeks gestation and 22 patients who were in early labor after 37 weeks gestation.

RESULTS

Patients in preterm labor had increased leukocyte counts at the time of initial evaluation. Prior to betamethasone treatment the mean WBC count was 11,900 ± 3,450 per cu mm (mean ± standard deviation, number = 30). Figure 1 shows the serial determinations of WBC following betamethasone treatment. Betamethasone did not induce a leukocytosis in the 30 patients in preterm labor (Fig. 1). In contrast, the five nonpregnant

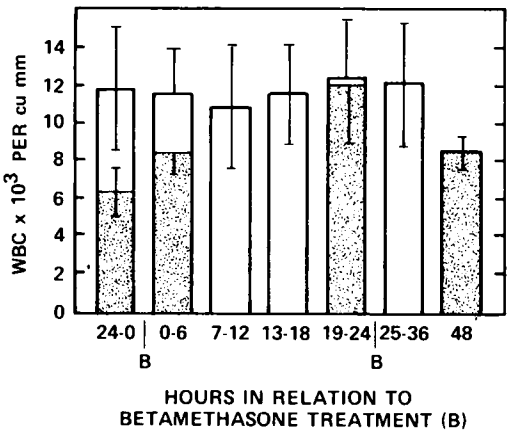

Fig. 1. WBC counts in 30 preterm labor patients before and after two injections of 12 mg betamethasone (B). Shaded columns represent five nonpregnant controls who received betamethasone only at zero hours. Brackets show standard deviations. The increase in WBC from before (6.4 ± 1.3) compared to 19–24 hours after betamethasone (11.7 ± 2.0) was significant ($p < 0.01$) by Student's t test.

subjects had lower initial WBC counts. Following 12 mg of betamethasone, given intramuscularly, leukocytosis occurred within 6 hours. WBC counts of nonpregnant subjects reached levels similar to those of the patients in preterm labor by 19–24 hours after betamethasone. Neither among the pregnant nor the nonpregnant subjects treated with betamethasone did we find a significant change in the percentage of band forms among polymorphonuclear cells.

Significant lymphopenia occurred within 6 hours after betamethasone in patients with preterm labor as well as in nonpregnant control subjects. The lymphocyte response after betamethasone in the two groups was similar initially (Fig. 2) but the lymphopenia was more prolonged in the nonpregnant subjects. At 24 hours, the decrease in lymphocytes in nonpregnant differed significantly from that of pregnant subjects ($p < 0.01$). Not shown in Figure 2 was the observation that the lymphocyte counts of nonpregnant subjects returned to baseline by 48 hours after betamethasone.

Betamethasone induced changes in total T lymphocytes (Leu 1) and helper T lymphocytes (Leu 3a) as

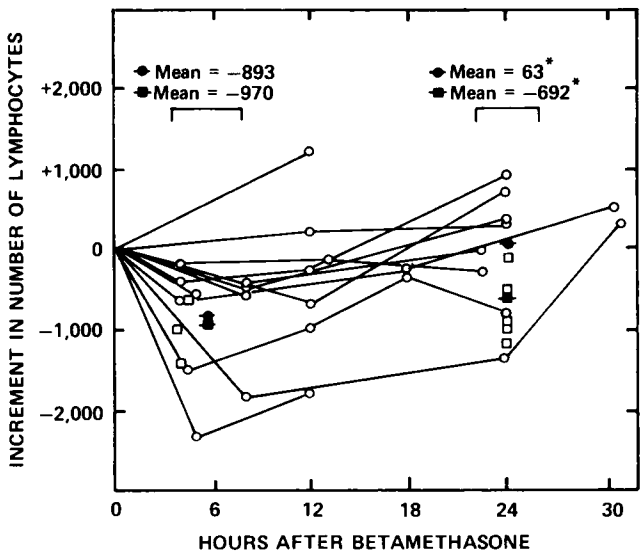

Fig. 2. Increments in the number of lymphocytes per cubic millimeter following 12 mg of intramuscular betamethasone. Initial counts were 1438 ± 731 (mean standard deviation) for 12 preterm labor patients (○), and 1768 ± 483 for 5 nonpregnant subjects ([ ]). Only three controls were tested at the 4-hour time interval. Solid shaded symbols on the figure are mean values and significantly different means ($p < 0.01$) are indicated by asterisks.

shown in Figures 3 and 4. There was considerable individual variation, however, the lymphopenia in the five nonpregnant subjects was more prolonged and was largely accounted for by a depression of helper T cells, which persisted for 24 hours (Fig. 4). Subsequent samples from nonpregnant subjects not plotted in Figure 4, showed that by 48 hours the lymphocyte counts and their subsets had returned to baseline values.

The Leu 2a (suppressor/cytotoxic) fraction of T lymphocytes in patients with preterm labor was 22.4 ± 10.2% (mean ± SD). Betamethasone treatment did not result in significant change in the Leu 2a fraction during the 24-hour observation period. For example, 4–6 hours following betamethasone when the lymphopenia was most pronounced, the mean fraction of Leu 2a cells was 21.1%.

A summary of the betamethasone-mediated effects on lymphocytes and T-cells is shown in Figure 5. Samples from pregnant and nonpregnant subsets at 4–8 hours showed a decrease in lymphocytes and in all the T-cell subsets. The decrease in total T cells (Leu 1) did not account entirely for the decrease in lymphocytes. This implies a decrease in B lymphocytes as well, although tests for B lymphocytes were not done. Twenty-four hours following betamethasone, pregnant subjects had returned to baseline lymphocyte counts but nonpregnant subjects had not.

Localized leukocyte mobilization was 8.9 ± 7.8 (leukocytes × 10^6 cm^2) in 49 third trimester pregnant patients prior to the onset of labor. Table I shows LLM was similar for patients in labor at term but was significantly increased ($p < 0.01$) in patients with preterm labor who were treated with betamethasone.

DISCUSSION

Leukocytosis is a normal phenomenon in late pregnancy. Andrews and Bonsnes[16] showed WBC counts in early labor after 37 weeks gestation of 10,500 ± 2,800 per mm^3. In the present study, leukocytosis (11,900 ± 3,400) was also found in patients who had preterm labor before 36 weeks gestation.

Steroids produce leukocytosis by decreasing the egress of cells from the total blood granulocyte pool as well as by increasing the influx of cells from the bone marrow into the blood.[12] Increases in peripheral leukocyte counts have been consistently observed after single doses of cortisol, prednisone, or dexamethasone.[17] Maximum leukocyte counts have been observed within 4–6 hours of the steroid treatment and the WBC counts have been shown to return to baseline within 24 hours.

Our studies of leukocytosis following betamethasone differ from these previous reports in two ways. First, the nonpregnant subjects had a more prolonged lymphopenia and leukocytosis. WBC's did not return to baseline counts until 48 hours after the dose. This difference is probably attributable to a more sustained effect of betamethasone acetate as compared to other glucocorticoids. Secondly, the pregnant patients in preterm labor already had elevated WBC's and no additional leukocytosis was prompted by betamethasone. In contrast, Edelstone et al have shown dexamethasone to induce a significant leukocytosis in chronically instrumented pregnant ewes despite elevated initial total leukocyte counts of 10,500 per mm^3.[18]

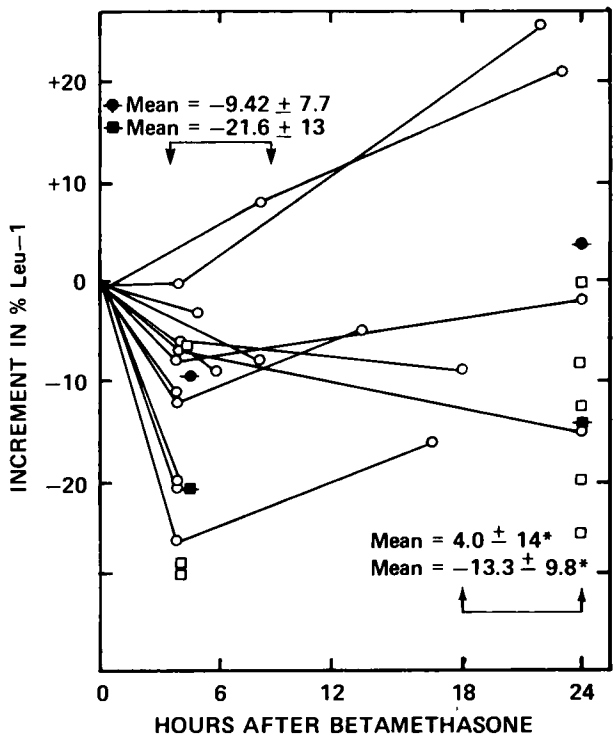

Fig. 3. Changes in the percentage of total T lymphocytes, determined by monoclonal antibody labelling (Leu 1), during the 24 hours following a 12-mg intramuscular dose of betamethasone. Pregnant patients in preterm labor (○) initially had 66 ± 7.7% (mean ± standard deviation, N = 12) of their lymphocytes labelled by Leu 1 and the nonpregnant subjects (□) had a baseline Leu 1 fraction of 61 ± 16% (N = 5). Solid shaded symbols on the figure indicate the means of samples within brackets. Because of recurrence of labor in seven patients, only five were tested in the 18–24-hour period. Significantly different groups by Duckworth test ($p < 0.05$) are indicated by asterisks.

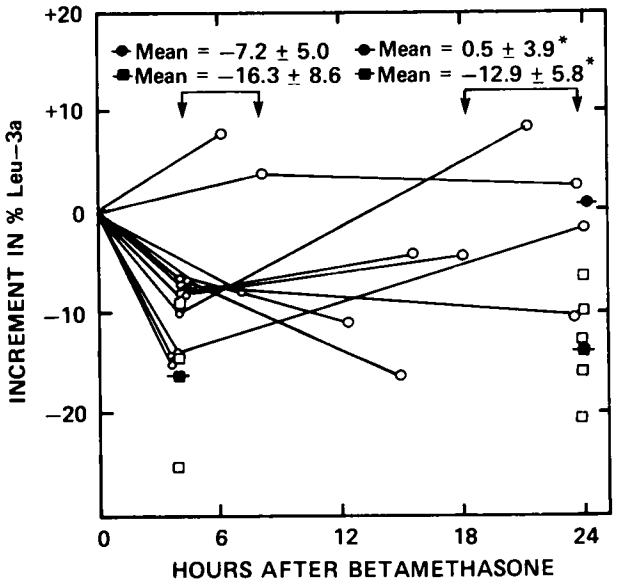

Fig. 4. Changes in the percentage of helper/inducer T lymphocytes, labelled by monoclonal antibody (Leu 3a), during the 24 hours following 12 mg of intramuscular betamethasone. Pregnant patients in preterm labor (○) initially had 38 ± 6% (mean ± standard deviation, N = 12) of lymphocytes in the Leu 3a fraction and nonpregnant subjects (▯) had 33 ± 14% (N = 5). Solid shaded symbols on the figure are mean values and significantly different groups by Mann–Whitney and Duckworth tests ($p < 0.01$) are indicated by asterisks.

Frequently when betamethasone treatment is given to hasten maturation of the fetal lung, there is concern that subclinical amnionitis could be present and that the steroid treatment could mask the signs and symptoms that ultimately lead to diagnosis. Our data show that moderate leukocytosis occurs with preterm labor even without betamethasone treatment and yet this objective test for infection remains valid with marked leukocytosis. In practice, we have found the following guideline to be reliable: a WBC count of greater than 20,000 is indicative of infection regardless of time lapse since betamethasone treatment. This value is approximately two standard deviations above the mean WBC count we found in preterm labor. Lesser shifts are generally of no assistance in diagnosing amnionitis or other maternal infections associated with preterm labor.

Transient lymphopenia is another well known effect of glucocorticoids.[3,4] Previous reports have shown the maximum depression of lymphocyte counts occurs 4–6 hours after treatment and counts return to normal within 24 hours with no cumulative effect after daily doses of 140–400 mg of hydrocortisone or 5–120 mg of prednisone. All types of lymphocytes are effected but the decrease in T cells is more than that in B cells. Using 12 mg of betamethasone, we found lymphopenia in both pregnant and nonpregnant individuals with decreases of about 55% between 4 and 6 hours. In pregnant subjects, the counts normalized by 24 hours but not until 48 hours in the nonpregnant subjects.

Studies of T lymphocytes and their subsets showed decreases in all the subsets without selective depletion of a particular type. The observation that the helper T

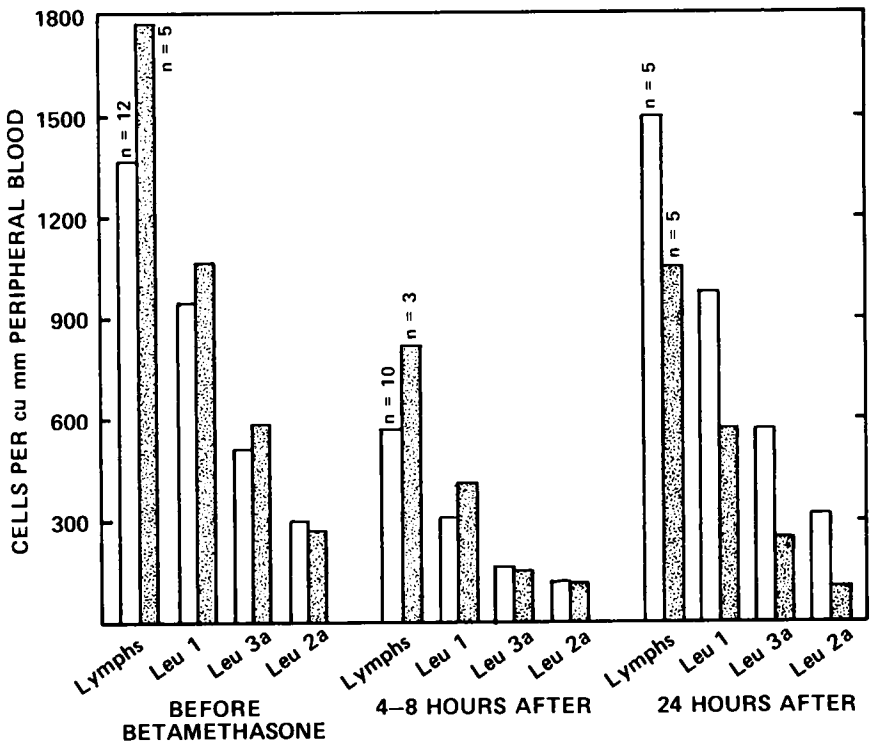

Fig. 5. The absolute numbers of peripheral lymphocytes, T lymphocytes (Leu 1), helper/inducer T lymphocytes (Leu 3a), and suppressor/cytotoxic T lymphocytes (Leu 2a) are shown before and after giving 12 mg of intramuscular betamethasone. Pregnant patients in preterm labor are illustrated in open columns and nonpregnant subjects in shaded columns.

TABLE I. Localized Leukocyte Mobilization (LLM) in Prenatal Patients in the Third Trimester, Patients in Term Labor, and Patients in Preterm Labor Who Received 12 mg Intramuscular Betamethasone

Study group	LLM (leukocytes × 10^6/cm^2)
Prenatal (49)	8.9 ± 7.8
Term labor (22)	10.5 ± 11.1
Preterm labor (13) (betamethasone)	21.0 ± 21.4

LLM is expressed as the number of migrating cells per square centimeter of skin abrasion. After betamethasone, the LLM was significantly increased ($p \leq 0.01$) when compared to prenatal or term labor patients.

cells (Leu 3a fraction) were decreased in the nonpregnant group after 24 hours (Fig. 4) would raise some concern about decreased immunity and possible increased risk for infections. However, the same figure shows that in patients with preterm labor, who are those more often treated with betamethasone, the Leu 3a fraction returned to normal within 12–24 hours. The rapid normalization of immunocytes implies less risk for susceptibility to infections.

One of the possible adverse effects of glucocorticoids on the immune response is the previously mentioned diminution in egress of polymorphonuclear cells from the circulation. Diminished leukocyte mobility to inflammatory sites has been correlated with increased susceptibility to infections. Skin chamber techniques have previously shown leukocyte mobilization to be decreased in humans after prednisone treatment whereas LLM was acutely enhanced following treatment with dexamethasone.[5] In the present study, pregnant patients had leukocyte mobilization values elevated twofold following betamethasone, very similar to the previous reports on dexamethasone in nonpregnant subjects. Finding that mobilization was enhanced rather than inhibited in pregnant, betamethasone-treated patients also provides reassurance that this aspect of the cellular immune response is intact.

Some previous studies have indicated that infections occur more frequently in patients receiving daily high doses of steroids.[19–21] Our studies show that betamethasone effects on immunocytes are favorably modified during pregnancy. Particular assurance of safety is provided by our finding that the potentially adverse betamethasone suppression of T lymphocytes is more transient in pregnant than in nonpregnant subjects. Rapid immunocyte recovery in betamethasone-treated pregnant patients may lower their infection risk in a way comparable to that of alternate day steroids in other subjects.[18]

Betamethasone treatment did not further increase the WBC counts in the preterm labor patients although leukocytosis was already present in pretreatment samples. The diagnosis of amnionitis in these patients remains problematic and depends primarily on alertness to the clinical findings of maternal or fetal tachycardia, maternal fever, uterine tenderness, or purulent cervical discharge. However, we routinely perform transabdominal amniocentesis in the initial evaluation of patients with preterm labor or premature rupture of membranes provided adequate fluid is visualized by ultrasound scan. If the Gram stain of amniotic fluid contains bacteria, a diagnosis of amnionitis is made and betamethasone therapy is withheld. Even though betamethasone effects on immunocytes are transient, they could be clinically important in patients who have subclinical amnionitis. When the amniotic fluid is colonized with bacteria, our approach is not to prolong the pregnancy but rather to treat with antibiotics and manage the patient with an objective of prompt delivery.

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