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Effects of Acidosis on Fetal and Maternal Blood Coagulation: A Fetal Lamb Model

C. THOMAS KISKER, DAVID P. BOHLKEN, AND WILLIAM R. CLARKE

Departments of Pediatrics and Preventive Medicine, University of Iowa College of Medicine, Iowa City, Iowa 52242

ABSTRACT. The effects of fetal acidosis (mean pH 6.93) on fetal and maternal blood coagulation were measured. Test results from 10 fetal lambs and mother ewes (127 ± 2 days mean gestation) before and after fetal lactic acid infusions were compared to test results from eight control fetal lambs and mother ewes (127 ± 3 days mean gestation) before and after control glucose infusion. Significant changes found in acidotic fetal lambs not seen in control fetuses included an increase in the white blood cell count (mean 2800/mm^3 before to 3600/mm^3 after acidosis; p = 0.0009), a shortening of the thrombin time (mean 17.8 s before to 11.2 s after acidosis; p = 0.0001), and decreases in the activities of factor V (mean 57% before to 37% after acidosis; p = 0.0014) and factor IX (mean 35% before to 29% after acidosis; p = 0.0128). There was also a reduction in the concentration of fibrinogen (mean 147 mg/100 ml before to 125 mg/100 ml after acidosis; p = 0.0492) but no significant changes in the levels of fibrin monomer, fibrinogen/fibrin degradation products, or antithrombin III. *In vitro* exposure of five different fetal whole blood samples to a pH of 6.9 for 2 h at 37° C did not result in significant changes in any of the coagulation factor activities. A significant decrease in the level of factor V was also found in the mother ewes of the acidotic fetuses (mean 141% before to 113% after acidosis; p = 0.006) and a decrease in the level of maternal factor IX approached significance (mean 119% before to 102% after acidosis; p = 0.0564). Two hours of severe fetal lactic acidosis induces changes in blood coagulation, but not the usual findings of disseminated intravascular coagulation. Corresponding decreases in factor V and IX activities in the mothers of acidotic fetal lambs suggest the liberation of a mediator capable of crossing the placenta and influencing maternal coagulation. (*Pediatr Res* 19: 78–82, 1985)

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Address correspondence C. Thomas Kisker, M.D., Department of Pediatrics, University of Iowa Hospitals and Clinics, Iowa City, IA 52242.
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Significant acidosis is likely to occur in the fetus in association with placental infarction, cord compression, or partial abruption. Acidosis may also be present in association with hypoxia in

newborns suffering from severe birth asphyxia or the respiratory distress syndrome. Previous studies in our laboratory indicated that hypoxia in the absence of acidosis did not result in intravascular coagulation in either early gestation or near-term fetal lambs (10, 11). Thus, we concluded that acidosis rather than hypoxia might be the trigger for the intravascular coagulation observed in stressed neonates with acidosis.

Acidosis is known to be associated with a shortening of the whole blood clotting time and disseminated intravascular coagulation (4, 7, 13). Studies by Rivers and Hathaway (17) demonstrated that tissue factor coagulant activity developed in human newborn umbilical cord leukocytes when exposed to severe pH reduction by lactic or hydrochloric acid. Bishop *et al.* (3) observed decreased platelets and fibrinogen in pregnant ewes when thromboplastin was infused into exteriorized fetal lambs although neither thromboplastin nor other blood coagulation factors cross the placenta (2, 3, 5, 14). To examine the effects of fetal acidosis on fetal blood coagulation *in utero* and to assess the possibility of detecting changes in the maternal circulation, 10 chronically catheterized fetal lambs were subjected to 2 h of acidosis by infusing lactic acid intravenously. The studies were conducted in the absence of hypotension or hypoxia so that the isolated effects of acidosis in the intact fetus could be measured. Blood samples were obtained on both the fetuses and mother ewes before and after the 2 h of fetal acidosis. Results of coagulation measurements on the samples from this test group were compared with results on blood samples obtained on eight control fetal lambs and their mothers before and after glucose infusion.

MATERIALS AND METHODS

Pregnant mixed breed Dorset-Suffolk ewes were obtained from existing sources and were housed throughout the study at the University of Iowa Animal Quarters.

The gestational ages of the sheep fetuses were known based on induced ovulation techniques (1). Before surgery, the animals were fasted for 24 h. Anesthesia of the ewe and surgery on the fetus were performed as described previously (9, 18). Under operating room conditions with sterile technique, polyethylene catheters (i.d., 0.86 mm, and o.d., 1.27 mm) to which heparin had been bound to both internal and external surfaces (CORMED, Inc., Middleport, NY) were inserted into the femoral artery and vein of the fetus and advanced to the aorta and inferior vena cava, respectively. A separate but nonheparinized catheter was also fixed to the fetal external abdominal wall to measure amniotic pressure. The catheters were brought out through a skin pouch to the side of the ewe and enclosed in a zip lock bag. After surgery, the ewe was kept in a restricted area and fed a standard diet. A minimum of 5 days for recovery was allowed before studies were done.

During the studies, the ewes with their chronically catheterized fetuses were transferred into small carts restricting them to upright positions. Acute catheters were then placed in the external jugular veins of the mother ewes to obtain maternal blood samples.

Fetal and maternal blood (0.5 ml) was collected anaerobically in heparinized plastic syringes and measurements of pH, P_{CO_2}, P_{O_2} were immediately determined with the appropriate pH, P_{CO_2}, P_{O_2} electrodes at 39° C using a radiometer pH amp 72 NK2 acid base analyzer (Radiometer America Inc., Dallas, TX). Hb, hematocrit, and white blood cell counts were measured using a Coulter Model S on 0.5-ml aliquots of fetal and maternal blood that were anticoagulated with EDTA. Platelet counts were also obtained on these same aliquots using phase microscopy.

Coagulation factor activities were measured on fetal and maternal plasma from 2.7-ml aliquots of blood anticoagulated with 0.3 ml of 0.05 M sodium citrate, pH 5.0. Standard techniques for measuring the prothrombin times (19) and partial thromboplastin times (16) were used. Specific factor activities II, V, VII, VIII, IX, X, XI, and XII were measured using one-stage assays measuring the ability of test plasma to correct plasma known to be deficient in the factor to be tested (15). All activities measured are quoted as percents of a reference standard pool of sheep plasma obtained from 10 nonpregnant ewes. Plasma digestion products of fibrinogen/fibrin were measured in serum from a 0.5-ml aliquot of blood allowed to clot in 5 μl of a solution of 0.05 M EACA, 0.125 M $CaCl_2$, 10 U/ml thrombin. The method of Mersky *et al.* (12) using a rabbit antisheep fibrinogen antibody and sheep fibrinogen coated red blood cells was used. Fibrin monomer was measured according to the method of Kisker (8) on plasma from 2.7-ml aliquots of blood collected in 0.3 ml of anticoagulant containing 2% EDTA, 10 mg/ml soybean trypsin inhibitor, 100 units/ml heparin pH 7.4. Sheep fibrinogen and antisheep fibrinogen antibody were used throughout.

Fetal arterial, venous, and amniotic pressures were recorded during the experiments using Stratham P23Db pressure transducers (Stratham Instruments, Oxnard, CA) and a beckman R-611 recorder. Fetal heart rate was monitored with a cardiotachometer triggered from the fetal arterial pressure pulse.

To establish acidosis (pH 6.9–7.0), the test fetal lambs were initially infused with 1.1 M lactic acid in D5%W at a rate of 0.6 ml/min for 30 min, after which the infusion rate was decreased to 0.42 ml/min for 2 h, a rate which maintained the pH between 6.9 and 7.0. The eight control fetal lambs were infused at the same rates with dextrose 5% water for the same periods of time. Blood samples for measurement of blood coagulation factor activities and the other parameters described were obtained prior to and at the completion of the two hours of infusion.

The *in vitro* effect of exposure of fetal blood to lactic acid-induced acidosis was carried out by collecting blood from five fetal lambs in buffered 0.1 M Na citrate pH 7.4. To 2.5-ml aliquots of this blood, 4 μl of 30% lactic acid or 5% dextrose was added. The addition of the lactic acid resulted in a decrease of the pH to 6.95 ± 0.01. The pH of the dextrose samples was 7.39 ± 0.02. Both aliquots were incubated at 37° C for 2 h prior to centrifuging for 30 min at 4° C to obtain platelet poor plasma. Coagulation tests on these plasmas were then done as described for the *in vivo* experiments.

Analysis of covariance measures were used to test for differences in test results before and after the *in vivo* infusions in the control and acidotic fetuses and ewes. The paired *t* test was used to test for differences in the control and test samples exposed to lactic acid *in vitro*.

RESULTS

Baseline values and the values postinfusion for the acidotic fetal lambs, the control lambs, and their mother ewes for Hb, hematocrit, white blood cell count, P_{O_2}, P_{CO_2}, pH, and serum lactate are presented in Table 1. There were no changes in the heart rate or mean arterial pressure in either control or acidotic fetal lambs. Changes specific to the acidotic fetal lambs included the decrease in pH during the period of acidosis from 7.37 to 6.95; an increase in the blood lactate level from 665–3044 pg/dl; and a slight but significant increase in the white blood cell count from 2800–3600/mm^3. There was a slight although significant decrease in the hematocrit in both the acidotic and the control fetal lambs, thought to be the result of blood sampling during the experiments.

In Table 2 are presented the values pre- and postinfusion for the acidotic fetal lambs, the control lambs, and their mother ewes for the various coagulation parameters measured. Although there was not a significant change in the prothrombin time or the partial thromboplastin time in any of the animals, the thrombin time in the fetal lambs was shortened from 16.6 to 11.2 s. No similar shortening was seen in the mothers of the acidotic fetal lambs. A small although significant decrease in the fibrinogen concentration was also seen in the fetal lambs exposed to acidosis (159 mg/100 ml before acidosis to 137 mg/100 ml after acidosis). The greatest change in activity was seen in factor V

Table 1. *Results of in vivo exposure to lactic acid (mean ± SD)*

	Fetus				Ewe			
	Acidotic ($n = 10$)		Control ($n = 8$)		Acidotic ($n = 10$)		Control ($n = 8$)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Hb (g/dl)	10.7 (±1.2)	10.0 (±1.1)	10.9 (±1.3)	10.2 (±1.4)	9.0 (±1.2)	9.1 (±0.9)	9.4 (±1.0)	9.4 (±1.3)
Hematocrit %	32.7 (±3.5)	31.3 (±3.6)	34.2 (±4.5)	31.6 (±4.8)	26.7 (±3.5)	25.6 (±2.5)	28.1 (±3.1)	27.4 (±3.7)
White blood count ($mm^3 \times 10^3$)	2.8 (±0.8)	3.6* (±1.7)	3.3 (±1.7)	2.6 (±1.6)	7.8 (±2.1)	8.1 (±1.5)	8.6 (±1.2)	8.8 (±1.3)
Po_2 (mm Hg)	21.3 (±4.2)	25.8 (±3.5)	17.4 (±6.2)	21.2 (±7.1)	52.5 (±10.2)	48.4 (±10.9)	48.6 (±10.1)	45.5 (±10.2)
Pco_2 (mm Hg)	48.6 (±7.6)	52.2 (±8.2)	46.4 (±4.0)	47.3 (±5.5)	33.3 (±8.2)	35.5 (±6.7)	33.2 (±3.0)	41.6 (±10.3)
pH	7.37 (±0.04)	6.95* (±0.05)	7.38 (±0.05)	7.36 (±0.06)	7.45 (±0.04)	7.45 (±0.04)	7.52 (±0.04)	7.50 (±0.05)
Lactate (pg/dl)	665 (±308	3044* (±670)	623 (±445)	859 (±780)	363 (±212)	315 (±72)	376 (±94)	284 (±117)
Mean arterial pressure (mm Hg)	49.7 (±5.4)	52.7 (±6.1)	41 (±2.9)	40 (±5.6)				
Heart rate (beats/min)	180 (±12)	188 (±12)	198 (±26)	179 (±18)				

* Acidotic fetuses pre and post significantly different from controls (white blood cells, $p = 0.0009$; pH, $p = 0.0001$; lactate, $p = 0.0001$).

Table 2. *Results of in vivo exposure to lactic acid (mean ± SD)*

	Fetus				Ewe			
	Acidotic ($n = 10$)		Control ($n = 8$)		Acidotic ($n = 10$)		Control ($n = 8$)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
PT (s)	17.1 (±1.7)	18.4 (±2.3)	16.9 (±1.6)	17.3 (±2.0)	13.6 (±1.7)	13.1 (±1.1)	14.0 (±1.1)	13.5 (±0.9)
PTT (s)	47.3 (±11.3)	47.1 (±13.3)	44.5 (±8.3)	45.9 (±9.3)	30.2 (±6.5)	33.1 (±6.3)	42.6 (±9.0)	35.9 (±8.6)
Thrombin time (s)	16.1 (±5.2)	11.2* (±1.7)	18.3 (±5.4)	19.1 (±6.5)	14.7 (±2.9)	13.9 (±3.3)	16.7 (±1.9)	16.1 (±2.9)
Fibrinogen (mg/100 ml)	159 (±81)	137* (±58)	120 (±38)	124 (±41)	305 (±105)	313 (±48)	364 (±64)	367 (±60)
Factor II activity (% of adult)	44 (±9)	39 (±4)	39 (±9)	40 (±7)	148 (±72)	142 (±85)	99 (±23)	107 (±19)
Factor V activity (% of adult)	59 (±33)	38* (±27)	55 (±20)	55 (±23)	137 (±47)	116* (±22)	112 (±44)	126 (±49)
Factor VII activity (% of adult)	48 (±21)	44 (±18)	52 (±21)	55 (±19)	153 (±60)	142 (±35)	125 (±24)	127 (±23)
Factor VIII activity (% of adult)	34 (±13)	28 (±13)	29 (±10)	29 (±12)	129 (±47)	113 (±27)	85 (±25)	103 (±22)
Factor IX activity (% of adult)	33 (±12)	27* (±9)	39 (±23)	37 (±18)	113 (±33)	103 (±22)	111 (±22)	118 (±24)
Factor X activity (% of adult)	36 (±15)	29 (±13)	37 (±23)	38 (±21)	117 (±16)	112 (±21)	107 (±16)	105 (±14)
Factor XI activity (% of adult)	40 (±23)	32 (±19)	51 (±20)	47 (±20)	163 (±80)	138 (±51)	104 (±29)	135 (±46)
Factor XII activity (% of adult)	42 (±14)	39 (±22)	35 (±13)	39 (±18)	119 (±43)	108 (±23)	83 (±19)	107 (±33)
Fibrin monomer (g/ml)	4.4 (±3.2)	6.3 (±5.9)	5.9 (±4.0)	6.8 (±3.3)	9.6 (±5.6)	9.4 (±4.6)	9.1 (±5.0)	15.6* (±8.9)
Antithrombin III (% of adult)	100 (±19)	95 (±21)	103 (±25)	104 (±27)	92 (±21)	92 (±31)	100 (±43)	94 (±33)
Platelets ($mm^3 \times 10^3$)	301 (±144)	259 (±103)	235 (±120)	212 (±107)	424 (±217)	470 (±89)	488 (±169)	486 (±205)

* Acidotic Pre and Post significantly different (fetal thrombin time, $p = 0.0001$; fetal fibrinogen, $p = 0.0492$; fetal factor V, $p = 0.0014$; fetal factor IX, $p = 0.0128$; ewe factor V, $p = 0.0066$; control ewe fibrin monomer, $p = 0.0390$).

levels from acidotic fetal lambs. Factor V decreased from 59% before acidosis to 38% after acidosis. Of interest, the factor V levels in the mother ewes also showed a significant decrease in activity from 137 to 116%. Factor IX activity decreased significantly in the acidotic fetal lambs from a level of 33% to a level of 27%. There was also a decrease in the factor IX activity in the ewes of the acidotic fetal lambs from 129 to 113% which approached significance ($p = 0.0564$). None of the other clotting factor activities including antithrombin III and fibrin monomer showed significant changes in either group of fetal lambs. There was an increase in the level of fibrin monomer in the control mother ewes from 9.1 to 15.6 μg/ml which is unexplained although probably the result of fibrin formation during sample collection from some of these ewes. Fibrinogen/fibrin degradation products were also measured on all animals, but none exceeded the normal 8 μg/dl and are therefore not reported in Table 2.

In Table 3 are presented the results of exposure of fetal blood to lactic acid *in vitro*. The *in vitro* exposure of fetal blood to 2 h of acidosis (pH 6.96) at 37° C did not result in any changes of blood coagulation factor activities.

DISCUSSION

Crowell and Houston (6) first demontrated that lowering the pH of blood to 6.5 *in vitro* and *in vivo* led to a marked shortening of the whole blood clotting time, even in the presence of heparin. They also observed that when lactic acid was injected into an arterial venous shunt, the animals died much more rapidly than when it was given interarterially at the same rate where rapid mixing occurred. Hardaway *et al.* (7) demonstrated a significant shortening of the whole blood clotting time in heparinized blood when the pH was reduced with lactic acid to 7.1 or below. Their results suggested that heparin was not an effective anticoagulant at acid pH. Hardaway's studies further demonstrated that a combination of acidosis and hypotension could initiate disseminated intravascular coagulation in experimental dogs. Broersma *et al.* (4) were also able to initiate intravascular coagulation in dogs by infusing lactic acid. Their studies indicated that a pH of below 7.2 was the "trigger" for intravascular coagulation. As the pH dropped, the heart rate fell, the platelet count progressively decreased, and although the blood clotting time initially shortened it eventually became prolonged. Within 2 h there were significant decreased in the platelet count as well as in the levels of fibrinogen, prothrombin, factor V, and factor VIII.

Table 3. *In vitro exposure of fetal blood to lactic acid (n = 5; mean ± SD)*

	Before lactic acid	After lactic acid
Pro time (s)	17.5 (±1.9)	17.3 (±1.48)
PTT (s)	60.1 (±14.5)	61.8 (±16.1)
Thrombin time (s)	16.2 (±1.9)	14.9 (±1.4)
Fibrinogen (mg/100 ml)	123.1 (±32.8)	118.6 (±28)
Factor II activity (% of adult)	31.0 (±5.2)	30.3 (±2.8)
Factor V activity (% of adult)	46.5 (±6.9)	46.2 (±5.9)
Factor VII activity (% of adult)	44.1 (±6.3)	42.9 (±8.5)
Factor VIII activity (% of adult)	37.4 (±16.5)	37.1 (±14.9)
Factor IX activity (% of adult)	24.7 (±7.2)	25.7 (±7.7)
Factor X activity (% of adult)	27.5 (±5.9)	28.6 (±9.4)
Factor XI activity (% of adult)	36.3 (±6.5)	37.2 (±10.1)
Factor XII activity (% of adult)	42.7 (±9.4)	41.6 (±7.3)

Nagasue *et al.* (13) infused lactic acid into the superior mesenteric artery of dogs to establish a mean pH of 6.9 and were also able to initiate intravascular coagulation as indicated by decreased platelet counts, fibrinogen concentrations, and increased prothrombin times. All of these studies, done in adult animals, clearly established a relationship between acidosis and intravascular coagulation.

Studies by Rivers and Hathaway (17) on the production of tissue factor by leukocytes from the human umbilical cord blood indicated that exposing newborn leukocytes to severe reductions of pH (6.75 or below) resulted in the liberation of tissue factor, a potent initiator of blood coagulation. In order to study the effects of acidosis on the immature hemostatic mechanism, 10 fetal lambs of approximately 127 days gestation were rendered acidotic (pH 6.95) for 2 h by infusing lactic acid intravenously.

Despite the severe reduction of pH in the fetal lambs (mean 6.95), the changes in coagulation factor activities were relatively minor and not in a range that would be clinically significant. The thrombin time shortened from 16.1 to 11.2 s, suggesting a "hypercoagulable state." There was a slight decrease in fibrinogen concentration from 159 to 137 mg/100 ml. Similar decreases in factor V from 59 to 38%, and factor IX from 33 to 27%, were also found. Of interest, evidence for the initiation of intravascular coagulation was not found. Specifically, there was no generation of fibrin monomer or fibrin split products, nor were there significant reductions in antithrombin III levels, platelet counts, or factor VIII activities. In our studies, fetal acidosis was examined without associated hypotension or hypoxia. The initiation of disseminated intravascular coagulation by acidosis in the previous studies by Hardaway, Broersma, and Nagasue differ in that their animals were also hypotensive. It is probable that increased stasis associated with hypotension is necessary in addition to acidosis for the initiation of intravascular coagulation.

The precise mechanism whereby acidosis induces a shortening of the thrombin time, decreases in fibrinogen, factor V, and factor IX activities is not clear. We could not demonstrate similar alterations by reducing the pH of fetal whole blood to 6.9 with the *in vitro* addition of lactic acid. This reduction in pH corresponded to that achieved in our fetal lambs but was not as low as the pH 6.5 used by Crowell and Houston or the pH 6.75 used by Rivers and Hathaway in their *in vitro* studies. The lack of change in our *in vitro* experiments suggest that the changes observed in the intact fetal lambs are not simply related to structural changes in the factors induced by an acid environment. Furthermore, changes in the rates of synthesis and/or degradation of the factors as a direct effect of lactic acidosis are unlikely explanations as decreases in factor V and factor IX activities were also apparent in mother ewes who were not acidotic or had increased blood lactate levels. Rather, the results suggest the liberation of a mediator generated during lactic acidosis which is capable of crossing the placenta.

Bishop *et al.* (3), in experiments on fetal lambs, found administering tissue thromboplastin to either the mother ewe or to the fetus resulted in the development of intravascular coagulation in both mother and fetus. They were not able to identify the factor or factors that crossed the placenta, although they did demonstrate that the transfer was not due to fibrin or fibrinogen breakdown products or to the infused thromboplastin. Their animals showed significant decreased in fibrinogen and factor V. Factor IX activity levels were not presented in their report.

In summary, our studies indicate that a 2-h period of severe acidosis (mean pH 6.93) in the fetal lamb results in a slight although significant change in some of the blood coagulation

factor activities in both the fetal lamb and in the mother ewe. The changes were not consistent with classical disseminated intravascular coagulation. An additional stress such as hypotension is probably required to induce intravascular coagulation in the fetus. The exact mechanism responsible for the observed changes remains unclear. The changes are not related to mere exposure of fetal blood to a lowered pH. The observation that the mother ewes also experience a reduction in factor V and factor IX activity in the absence of lactic acidosis suggests the liberation of a mediator from the fetus capable of crossing the placenta. These observations support the original findings of Bishop *et al.* (3) which also suggested the liberation of a factor or factors capable of crossing the placenta and influencing coagulation activity in both fetal and maternal blood.

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Pituitary-Gonadal Function in Klinefelter Syndrome before and during Puberty

JAMES A. SALBENBLATT, BRUCE G. BENDER, MARY H. PUCK, ARTHUR ROBINSON, CHARLES FAIMAN, AND JEREMY S. D. WINTER

National Jewish Hospital and Research Center/National Asthma Center; Departments of Pediatrics, Psychiatry, and Biochemistry, Biophysics and Genetics, University of Colorado School of Medicine, Denver, Colorado [J.A.S., B.G.B., M.H.P., A.R.]; and the Departments of Paediatrics, Medicine and Physiology, University of Manitoba, Winnipeg, Manitoba, Canada [C.F., J.S.D.W.]

ABSTRACT. Serum concentrations of follicle-stimulating hormone, luteinizing hormone, testosterone, and estradiol were determined at intervals before and during puberty in 40 individuals with Klinefelter syndrome (47,XXY karyotype), of whom 27 had been detected in neonatal cytogenetic screening programs. Prior to the appearance of secondary sexual changes, basal serum hormone concentrations and acute responses to stimulation with gonadotropin-releasing hormone and human chorionic gonadotropin were normal. The timing of the onset of clinical puberty was normal. Early pubertal boys showed initial testicular growth and normal serum testosterone levels, while serum follicle-stimulating hormone and estradiol concentrations were significantly elevated. By midpuberty, the Klinefelter subjects were uniformly hypergonadotropic and their testicular growth had ceased. Serum testosterone concentrations after age 15 remained in the low-normal adult range. Serum estradiol levels remained high, irrespective of the presence or absence of gynecomastia. Exaggerated responses to gonadotropin-releasing hormone are seen in pubertal subjects with elevated basal gonadotropin values. (*Pediatr Res* 19: 82–86, 1985)

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Address for correspondence Arthur Robinson, M.D., National Jewish Hospital and Research Center, 3800 East Colfax Avenue, Denver, CO 80206.
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Following the submission of this manuscript, one pubertal aneuploid subject has been found to have a low percent mosaicism with karyotype 46,XY/46,XX/47,XXY (6:13:175) in blood and fibroblasts.