as well as in research, we were interested to find out how reproducible the technique was when used in routine clinical practice. We therefore used a commercially available duplex scanner, and measurements were made by the five people who were responsible for the routine ultrasound surveillance of high risk newborn infants on our nursery. Two of these observers were research personnel and three were in clinical service posts; three had less than one year's experience in ultrasound and Doppler techniques.

If the mean pulsatility index for all observers is taken as the most accurate estimate of the 'true' pulsatility index, then this small sample did not contain any infant with a pulsatility index below 0.55. It was therefore not possible to determine the risk of a true low pulsatility index being missed by an individual observer. There was no false positive measurement of a pulsatility index less than 0.55. Incomplete innervation of a vessel can give the false impression of low or absent diastolic flow and therefore a high pulsatility index, whereas the finding of high diastolic flow and low pulsatility index is much less likely to be the result of observer error. There is therefore less chance that a clinically important error would be made in neonatal cerebral artery Doppler studies than in obstetric scanning, where absent or reduced diastolic flow is the main diagnostic feature.

This study showed that interobserver variability did not exceed intraobserver variability in the measurement of pulsatility index using a duplex Doppler scanner. In some infants, however, measurements of pulsatility index varied widely, and it would therefore seem sensible not to make clinical judgments on a single pulsatility index estimate. This small study was confined to preterm infants, none of whom had a pulsatility index in the pathological range. Reproducibility of measurement of pulsatility indices over a wider range of normal and abnormal values should be established if the pulsatility index is to be used as a prognostic indicator.

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Virilisation of female preterm infants

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Abstract

Two cases of hypertrophy of the clitoris in premature girls are reported; this was associated with persistently high concentrations of adrenal fetal zone androgens.

Isolated hypertrophy of the clitoris in neonates is known to be associated with congenital adrenal hyperplasia, administration of androgens to the mother in the second and third trimesters of pregnancy,¹ and maternal androgen secreting tumours. We report two cases of progressive hypertrophy of the clitoris developing after birth, and associated with persistent excretion of high concentrations of 3 β -OH-5-ene steroids, produced by the fetal adrenal zone and which are known to be weak androgens. This association has not previously been reported.

Case reports

CASE 1

A girl, birth weight 640 g, was one of twins delivered at 24 weeks' gestation for antepartum haemorrhage; the mother was aged 24, para 1+1. The second twin died aged 11 hours.

The surviving twin developed hyaline membrane disease, and was ventilated for five weeks, remaining oxygen dependent for a further seven weeks. Apnoea of prematurity was treated with theophylline from 4 to 10 weeks. She had a patent ductus arteriosus, which failed to close with indomethacin, and led to treatment with digoxin and frusemide from 9 weeks until spontaneous closure at 11 weeks. She had recurrent hyponatraemia and required sodium supplements.

Clitoromegaly was noted at 4 weeks of age (fig 1A). She had a left inguinal hernia with a palpable gonad (a macroscopically normal ovary was later found at herniotomy). Ultrasound showed a normal uterus and adrenals. The karyotype was normal female. Plasma 17 α -OH progesterone was 27.2 nmol/l at 7 weeks, 61.8 nmol/l at 9 weeks, and 11.2 nmol/l at 20 weeks. Adrenocorticotrophic hormone (ACTH) was 4.8 pmol/l at 11 weeks and 6.6 pmol/l at 20 weeks (immunoradiometric assay, Eurodiagnostics, Netherlands). The plasma cortisol on five occasions, when not receiving hydrocortisone, ranged from <69 nmol/l to 316 nmol/l and after corticotrophin stimulation increased to >1380 nmol/l at 60 minutes. Plasma dehydroepian-

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Clitoromegaly in case 1(A) and case 2(B).

drosterone sulphate (DHAS) was $6.8 \mu\text{mol/l}$ at 12 weeks and $2.5 \mu\text{mol/l}$ at 20 weeks. At 12 weeks plasma testosterone was 0.5 nmol/l . The results of serial 24 hour urinary steroid analysis using gas chromatography with capillary column² are shown in the table.

Hydrocortisone 1.6 mg/kg/day was given from 13 weeks to 19 weeks on the advice of an endocrinologist because it was felt that congenital adrenal hyperplasia had not been ruled out at the time. At the age of 3 years, when she was admitted for surgical reduction of the clitoris, a 24 hour urinary steroid profile was normal, as were plasma DHAS and $17\alpha\text{-OH}$ progesterone concentrations.

CASE 2

A girl, birth weight 1056 g, was born to a mother aged 36 years, para 2+3, who had had one neonatal death at 26 weeks. The pregnancy was complicated by vaginal blood loss and cervical dilation at 19 weeks. A cervical suture was inserted and labour suppressed with ritodrine and indomethacin. Maternal serology was positive for toxoplasmosis and she was treated with spiramycin. Premature rupture of membranes occurred at 22 weeks and there was spontaneous vaginal delivery at 27 weeks' gestation.

The baby had mild respiratory distress requiring oxygen for three days, and was given benzylpenicillin and gentamicin; theophylline was given for apnoea. She was tube fed with expressed breast milk containing disodium hydrogen phosphate supplements. Additional sodium chloride was given for hyponatraemia. There was no clinical or serological evidence of congenital toxoplasmosis.

24 hour urinary steroid excretion rates. The results from two full term infants and from two female preterm infants who were not virilised are given for comparison

	Postnatal age (weeks)	Total cortisol metabolites ($\mu\text{g/kg/day}$)	$3\beta\text{-OH-5-ene}$ metabolites ($\mu\text{g/kg/day}$)
Case 1	12	<10	1730
	20	40	500
Case 2	9	220	1410
Preterm 1	13		924
Preterm 2	12		1172
Term (n=2)	8	100	130

A large clitoris was noted at 6 weeks of age (fig 1B). Blood pressure was normal. Chromosomes showed a normal female karyotype. Plasma $17\alpha\text{-OH}$ progesterone at 8 weeks was 22 nmol/l . The 24 hour urine steroid excretion rates are shown in the table. Plasma ACTH was 10.3 pmol/l at 8 weeks and 10.1 pmol/l at 10 weeks. Cortisol concentrations ranged from <60 to 137 nmol/l .

She was discharged home at 8 weeks. Clitoromegaly gradually resolved over the next three months.

Discussion

Virilisation of female newborn infants is usually caused by excess androgen production due to an inborn error of steroid metabolism. This is not thought to be the cause in these two infants because the virilisation was not present at birth and urine steroid profiles showed no evidence of 21 or 11β hydroxylase deficiency. Furthermore, plasma $17\alpha\text{-OH}$ progesterone concentrations were not as high as those reported in congenital adrenal hyperplasia and were within the range reported for preterm infants without congenital adrenal hyperplasia.^{3 4}

The plasma and urine measurements (table) show that both of these cases continued to excrete $3\beta\text{-OH-5-ene}$ steroids, which are weak androgens, long after the virilisation was first noticed, at concentrations which in term babies would only be found in the first few days of life.⁵ The persistence of the $3\beta\text{-OH-5-ene}$ steroids (mainly dehydroepiandrosterone (DHA) and its metabolites) in preterm infants has been described,⁴ but the association with virilisation of female infants has not. The cause of the persistent secretion of these fetal zone steroids has never been satisfactorily explained. There is evidence that ACTH regulates the adrenal cortex before and after birth, though other trophic factors may also be involved.⁶ In cell culture ACTH will stimulate fetal zone cells to produce DHA (and cortisol). In utero this DHA would be exported to the placenta and aromatised to oestrogens, a route of disposal not available to the infant after birth. The persistent $3\beta\text{-OH-5-ene}$ steroid excretion might be caused by excess ACTH drive, due for example, to

inadequate cortisol synthesis secondary to diminished activity of 3β hydroxysteroid dehydrogenase. The concentrations of ACTH and of cortisol observed in the plasma, however, do not support this hypothesis. The persistent rise of the 3β -OH-5-ene steroids might therefore be due to the response of an adrenal cortex, consisting predominantly of DHA secreting fetal zone cells, to the normal ACTH drive required to maintain plasma cortisol concentrations.

It seems most likely that the weak fetal zone androgens are responsible for the virilisation. This cannot be the only cause however because both we (see table) and others^{4,5} have observed female preterm infants with raised concentrations of 3β -OH-5-ene steroids, who were not virilised. There may be other factors involved, for example variations in end organ sensitivity, localised conversion of fetal steroids to more potent androgens at the site of action in the genitalia, or high concentrations of circulating free androgens. Another factor may be the degree of immaturity of the preterm infant. Grumbach and Ducharme have pointed out that the virilising effect of an androgen depends on the maturity of the fetus. Androgen exposure between 8–13 weeks' gestation causes labial

fusion and clitoromegaly, whereas after this time only hypertrophy of the clitoris is seen.¹ The fact that the most severely affected infant was 24 weeks' gestation may be a manifestation of the different response of the immature fetus to weak androgens. The increasing survival of such immature infants is a recent phenomenon and this may be the reason why clitoromegaly has not been reported before in preterm babies.

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Topical prostaglandin E₂ gel for cervical ripening and closure of the ductus arteriosus in the newborn

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Abstract

The closure time of the ductus arteriosus was investigated in 29 full term babies born vaginally after induction with prostaglandin E₂ and in 22 controls. Serial Doppler echocardiography studies showed a significantly prolonged closure time in babies induced by prostaglandin E₂. Whether the difference is related to changes in fetal prostaglandin E₂ concentration remains to be established.

Patients and methods

Fifty one babies born at full term by vaginal delivery were studied. Twenty nine babies (mean (SD) gestational age 39·86 (1·66) weeks) were born to mothers who had received a 3 mg prostaglandin E₂ pessary (Upjohn) to ripen the cervix before induction of labour. Twenty two babies (mean (SD) gestational age 39·82 (1·14) weeks) whose mothers did not receive prostaglandin E₂ acted as controls.

In sheep prostaglandin E₂ given intravenously to the ewe can gain access to the fetus and cause fetal hypertension and vasoconstriction of the fetal renal, placental membrane, and umbilical circulations.¹ In pregnant woman local instillation of prostaglandin E₂ is being increasingly used to ripen the cervix before induction of labour. It is desirable therefore to find out whether vaginal administration of prostaglandin E₂ affects haemodynamic function in the fetus during the perinatal period. This study reports maternal and fetal blood concentrations of prostaglandin E₂ after local application, and compares the closure times of the ductus arteriosus in babies born after induction of labour with prostaglandin E₂ with those in a control group born by spontaneous delivery.

DETERMINATION OF DUCTAL CLOSURE TIME

Serial Doppler echocardiography studies were done with an Aloka SSD-280S ultrasound scanner within 12 hours of birth, then twice daily at intervals of about 12 hours until ductal shunting could no longer be detected.²

MEASUREMENT OF MATERNAL AND CORD PROSTAGLANDIN E₂ CONCENTRATIONS

Cord blood was drawn from the umbilical vein immediately after clamping and cutting of the cord, and before placental separation. Paired maternal venous blood samples were collected at the same time. The samples were then transferred into prechilled polypropylene tubes containing EDTA and indomethacin, and cen-

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