ORIGINAL ARTICLE

Effect of oestradiol and progesterone replacement on bronchopulmonary dysplasia in extremely preterm infants

A Trotter, L Maier, M Kron, F Pohlandt

Arch Dis Child Fetal Neonatal Ed 2007;**92**:F94–F98. doi: 10.1136/adc.2006.097170

See end of article for authors' affiliations

Correspondence to: A Trotter, Center for Perinatal Medicine, Children's Hospital, University of Bonn, Sigmund-Freudstr 25, 53105 Bonn, Germany; andreas.trotter@web.de

Accepted 4 August 2006
Published Online First
11 August 2006

Objective: To study whether postnatal replacement of oestradiol and progesterone may help to prevent bronchopulmonary dysplasia (BPD).

Methods: This randomised placebo-controlled double-blind study enrolled 83 infants of <29 weeks gestational age and 1000 g birth weight requiring mechanical ventilation within 12 h after birth. Oestradiol (2.5 mg/kg/day) and progesterone (22.5 mg/kg/day) were given by continuous intravenous infusion of a standard lipid emulsion (1.5 ml/kg/day) in the replacement group (ESTRA-PRO). The placebo group received the same lipid emulsion without oestradiol or progesterone. A replacement period of at least 2 weeks but not >4 weeks was strived for and defined as "according to protocol". The primary outcome variable was the incidence of BPD or death.

Results: The median birth weight was 670 g (min–max 400–990 g) and the gestational age 25 weeks (23.1–28.1 weeks). The incidence of BPD or death was 48% in the placebo group and 44% in the ESTRA-PRO group ($p = 0.38$, one-sided testing, intention to treat analysis). In infants treated according to protocol, 32% (9 of 28) in the placebo group and 14% (3 of 21) in the ESTRA-PRO group developed BPD ($p = 0.08$).

Conclusion: Replacement of oestradiol and progesterone was not effective for prevention of BPD or death in extremely preterm born infants. Better-powered trials are needed to evaluate this new approach.

Bronchopulmonary dysplasia (BPD) remains a major factor for morbidity in extremely premature infants. The pathogenesis of BPD is multifactorial (eg the effect of mechanical ventilation, oxygen supply and inflammation on immature lung tissue), but has changed over the years so that the main hallmark of this condition is now reduced alveologenesis,¹ as shown by histopathological studies in preterm infants dying from BPD who show an arrest of lung development.² The concept has evolved that postnatal lung development in extremely preterm infants is arrested by a lack of factors that regulate lung differentiation and maturation in utero.

In a female rat model, it has been shown that ovariectomy reduced the gas exchange surface area by impaired formation of alveoli.³ This was prevented by oestrogen replacement. Moreover, ovariectomy-induced loss of alveoli could be regenerated by treatment with oestrogen.⁴ Oestrogen signalling by both known mammalian oestrogen receptors, ER α and ER β , is required for alveolus formation, as ER α and ER β knockout mice have diminished formation of alveoli during development.⁴ A decisive role of oestrogen and progesterone receptors in prenatal lung development is conceivable, as mRNA expression has been found to be highest in the prenatal mouse lung.⁵ Expression of ER β has also been shown in the lung of the midgestational human fetus.⁶ The administration of oestradiol to pregnant rabbits increased alveolar maturation in the fetuses.⁷ Treatment of pig fetuses with oestrogen and progesterone receptor antagonists considerably impaired alveolar formation.⁸

It is known from umbilical cord blood measurements at different gestational ages that the human fetus is exposed to increasing amounts of both oestradiol and progesterone.⁹ Delivery disrupts the placental supply of these hormones. Within one day, the levels of oestradiol and progesterone decrease 100-fold.¹⁰ No other known biologically active steroid hormone shows these marked changes after birth; this is the physiological condition for the infant delivered at term. Preterm infants, however, are deprived of the placental supply of these

hormones at a much earlier developmental stage. The oestradiol and progesterone withdrawn in preterm infants after delivery may be candidates for regulators lacking in postnatal lung development. Therefore, it is a reasonable approach to replace oestradiol and progesterone postnatally in extremely preterm infants who are most prone to develop BPD.

In a first randomised pilot study which focused on bone mineralisation, a trend towards a reduced incidence of BPD with the replacement was found,¹⁰ but in a Cochrane review of postnatal oestradiol and progesterone replacement, Hunt called for properly powered randomised controlled trials.¹¹

INFANTS AND METHODS

Study infants

Infants admitted to the Section of Neonatology and Pediatric Critical Care, University of Ulm, Ulm, Germany, were enrolled in the study if the following criteria were fulfilled:

1. Gestational age <29 weeks.
2. Birth weight <1000 g.
3. Need for mechanical ventilation within the first 12 h of life.
4. Written informed consent of the parents.

Exclusion criteria were congenital malformations and chromosomal anomalies. This study was approved by the local ethics committee of the University of Ulm. It was based on the Guidelines for Good Clinical Practice and is in accordance with the Declaration of Helsinki.

Study design

This randomised, double-blind study had two parallel groups. Randomisation of the infants to the two treatment groups was carried out in the Department of Biometry and Medical

Abbreviations: ATP, according to protocol; BPD, bronchopulmonary dysplasia; ESTRA-PRO, oestradiol and progesterone replacement; ITT, intention to treat; ROP, retinopathy of prematurity; VEGF, vascular endothelial growth factor

Documentation of the University of Ulm (MK), ensuring that two of four consecutive patients were from each study group. Two study emulsions were supplied by the hospital pharmacy and infused at the same rate of 15 ml/kg/day:

1. ESTRA-PRO emulsion (Lipofundin 20%; B Braun, Melsungen, Germany; diluted with water to 5% (0.75 g/kg/day lipids) and containing 0.165 g/l oestradiol hemihydrate (2.5 mg/kg/day) and 1.5 g/l progesterone (22.5 mg/kg/day)).

2. The placebo emulsion contained the same amount of lipids, but no hormones.

The two study emulsions had identical appearance. We strived for a replacement time of 14–28 days. Infants who met this criterion formed the “according to protocol” (ATP) group. Infusion of the study emulsion was discontinued when there was no longer clinical indication for intravenous access.

Outcome variables

The primary outcome variable was BPD or death. BPD was defined as an oxygen saturation of <90% in room air for more than 30 min at a postmenstrual age of 36 weeks. Table 2 gives the secondary outcome variables.

Maintenance of in utero levels of oestradiol and progesterone by ESTRA-PRO

To document the maintenance of in utero levels of oestradiol and progesterone, these hormones were measured in plasma samples taken 1, 3 and 7 days after birth and, thereafter, once a week as long as the study emulsion was given, using the radioimmunoassay technique ($^{17}\beta$ Oestradiol ^{125}I RIA Kit, Progesterone ^{125}I RIA Kit; ICN Biomedicals, Costa Mesa, California, USA).¹²

Tolerability of the study emulsion and adverse events

Plasma triglyceride levels were determined every 2–3 days during the administration of the study emulsion. Hypertriglyceridaemia was defined as a triglyceride level >3 mmol/l. In the case of hypertriglyceridaemia, all other parenteral lipid administration other than the study emulsion was stopped. If hypertriglyceridaemia persisted for 12 h, administration of the study emulsion was stopped (violation of protocol). The incidence of cholestasis, hepatic cirrhosis and thrombosis was documented as adverse events.

Statistical analysis

In the pilot study,¹⁰ the incidence of BPD according to the definition at that time (requirement for additional oxygen later than the 36th week of postmenstrual age) was 29% in the placebo group. We expected to select a population with a higher risk of developing BPD when using the need for mechanical ventilation within 12 h after birth as an additional inclusion criterion. The Epicure study¹³ reported 74% additional oxygen requirement at 36 weeks postmenstrual age for infants of <26 weeks gestational age. On the basis of this information, we expected the incidence of BPD to be about 60% in our study. In our pilot study,¹⁰ there was no infant with BPD in the ESTRA-PRO group compared with four (29%) infants in the control group. To halve the incidence from 60% to 30% with oestradiol and progesterone replacement seemed an appropriate estimate and a clinically relevant difference. A sample size of 40 infants in each group was calculated ($\alpha = 0.05$ one sided, power 80%). The outcome, incidence of BPD, that was shown to be reduced by the treatment in our pilot study was therefore tested with the one sided χ^2 test to cut down the number of patients needed for confirmatory statistics.

For the descriptive statistical analysis, frequencies and percentages were calculated for qualitative characteristics, and median, minimum and maximum were given for quantitative

characteristics. The primary end point was compared between treatment groups using a one-sided χ^2 test. Secondary outcome variables were tested using either a two-sided χ^2 test (or Fisher's exact test if frequencies were small) or a two-sided Wilcoxon's test for parallel groups. The dose–effect relationship between the occurrence of BPD at 36 weeks postmenstrual age and the duration of administration of the study emulsion was analysed by logistic regression taking into account possible confounders. The results are presented as odds ratios (ORs) with 95% confidence intervals (CIs) and p value of the Wald χ^2 test. A p value of <0.05 was considered significant. Statistical analysis was carried out with SAS V.8.2.

RESULTS

During the study period, from July 1999 through February 2003, 177 infants of <29 weeks gestational age and <1000 g birth weight were admitted. Many of the infants were not eligible for the study, as mechanical ventilation was not initiated within 12 h of birth. Eighty five infants were randomised, but inclusion criteria were violated in two infants with a birth weights of 1040 and 1110 g, respectively, leaving 83 infants (Fig 1). No difference was seen in mortality between the groups (five of 40 (13%) in the placebo group v seven of 43 (16%) in the ESTRA-PRO group). A total of 11 of the 12 infants who died were not treated ATP because they died within the first 14 days. One infant (ESTRA-PRO group) treated ATP died 17 days after birth because of sepsis-associated respiratory failure. During administration of the study emulsion, seven (18%) infants from the placebo group and 16 (37%) infants from the ESTRA-PRO group developed hypertriglyceridaemia ($p = 0.05$, reason for stopping the administration of the study emulsion in six and 14 surviving infants, respectively). Administration of the study emulsion was stopped in one infant from the placebo group accidentally and in one infant from the ESTRA-PRO group because of anuria to minimise the fluid load. This left 28 infants in the placebo group and 21 infants in the ESTRA-PRO group for ATP analysis.

Figure 1 During the enrolment period, 177 patients with a birth weight (BW) of <1000 g and a gestational age (GA) of <29 weeks were admitted. Out of 85 infants, 42 were allocated to the placebo group (two drop-outs because violation of an inclusion criteria) and 43 to the ESTRA-PRO group (intention to treat (ITT) population). Of these 28 and 21, respectively, were treated according to protocol (ATP population). TG ↑, hypertriglyceridaemia.

Table 1 Characteristics of the infants in the intention to treat (ITT) population (placebo group n = 40, ESTRA-PRO group n = 43)

Birth weight (g)	680 (410–990)	660 (400–940)
Gestational age (weeks)	25.1 (23.6–27.9)	24.7 (23.1–28.1)
APGAR scores		
5 min	7 (4–10)	7 (0–10)
10 min	9 (5–10)	8 (1–10)
Female sex	19 (48)	18 (42)
Multiple births	17 (43)	9 (21)
Prolonged rupture of membranes	11 (28)	21 (49)
Positive markers of bacterial infection after birth*	26 (65)	35 (81)
Delivery by caesarean section	27 (68)	28 (65)
Prenatal betamethasone (yes/no)	36 (90)	40 (93)
Completed (two doses)	30 (75)	35 (81)

Two-sided testing showed no differences with $p < 0.05$ between the groups
 *CRP at birth > 10 mg/l or CRP at 24 h > 10 mg/l or interleukin 8 at birth > 70 pg/ml.

CRP, C reactive protein; ESTRA-PRO, oestradiol and progesterone replacement.

The two treatment groups showed similar baseline characteristics (table 1, ITT population). Baseline characteristics were similar for the ATP group (data not shown). The ITT analysis showed no difference for the primary outcome variable BPD or death (44% in the ESTRA-PRO group and 48% in the placebo group, $p = 0.38$, one-sided testing). For surviving infants treated ATP, the incidence of BPD in the ESTRA-PRO group was half that found in the placebo group (14% v 32%, $p = 0.08$). The incidence of BPD in surviving infants not treated ATP was five of seven (71%) infants in the placebo group and nine of 15 (60%) infants in the ESTRA-PRO group.

Table 2 gives a comparison of the secondary outcome variables for surviving infants. None of the infants of the ESTRA-PRO group developed severe retinopathy, whereas some in the placebo group did ($p = 0.13$, two-sided testing). Infants in the ESTRA-PRO group were discharged home earlier ($p = 0.01$) with correspondingly lower body weights ($p = 0.03$).

The dose-response relationship between "duration of administration of the study emulsion" and the outcome BPD was studied by logistic regression in all patients of the ESTRA-PRO

group discharged alive ($n = 36$). Postnatal replacement of oestradiol and progesterone showed a significant dose-response relationship (OR = 0.9, 95% CI 0.8 to 1.0, $p = 0.04$). With every day of replacement, the odds of developing BPD was reduced by 10%. After adjustment for the potential confounders, gestational age ≤ 24 weeks 6 days versus ≥ 25 weeks (OR = 2.6, 95% CI 0.5 to 13.4, $p = 0.24$), and administration of dexamethasone, yes versus no (OR = 1.4, 95% CI 0.3 to 7.4, $p = 0.67$), the dose-response relationship remained nearly the same (OR = 0.9, 95% CI 0.8 to 1.0, $p = 0.07$). Figure 2 shows that the longer the administration of hormone replacement, the lower the risk of developing of BPD, an effect that was not present in the placebo group.

Plasma levels of oestradiol were widely maintained within the in utero ranges in the replacement group (dotted lines, fig 3A). Plasma levels of progesterone were also maintained, but showed a distinct decline after 2 weeks of treatment (fig 3B). The placebo group infants showed plasma oestradiol and progesterone levels 100-fold lower than the ESTRA-PRO group.

The incidence of adverse events was similar in the placebo and ESTRA-PRO group (cholestasis 6 v 3, liver cirrhosis 1 v 0, thrombosis 4 v 4, respectively, ITT population).

Figure 2 Dose-response relationship between the duration of administration of the study emulsion and the incidence of bronchopulmonary dysplasia (BPD) ITT survivors, intention to treat; ESTRA-PRO, oestradiol and progesterone replacement.**Table 2** Secondary outcome variables of surviving infants for the ATP population (placebo group n = 28, ESTRA-PRO group n = 21; two sided testing)

Outcome variable	Placebo	ESTRA-PRO	p Value
Cumulative dose of surfactant (mg/kg)	163 (0–402)	153 (64–330)	0.84
Mechanical ventilation (days)	20 (2–72)	20 (2–51)	0.32
Time at first extubation (days)	9 (0–45)	6 (0–32)	0.41
Length of hospital stay (days)	123 (57–225)	106 (76–156)	0.01
Body weight at discharge (g)	3290 (2000–4230)	2830 (2020–5150)	0.03
Pneumothorax	2 (7)	2 (10)	1.00
Pulmonary interstitial emphysema	5 (18)	4 (19)	1.00
Treatment with dexamethasone	2 (7)	6 (29)	0.05
Persistent ductus arteriosus	4 (14)	6 (29)	0.29
Intracerebral haemorrhage			
All grades	10 (36)	9 (43)	0.61
Severe grades (3/4)	3 (11)	1 (5)	0.63
Periventricular leucomalacia	3 (11)	1 (5)	0.63
Retinopathy			
All grades	18 (64)	10 (48)	0.24
Severe grades (4/5)	4 (11)	0 (0)	0.13
Necrotising enterocolitis	2 (7)	0 (0)	0.50
Supplemental oxygen at discharge	6 (21)	1 (5)	0.21

Values are medians (range) or numbers (%)
 ESTRA-PRO, oestradiol and progesterone replacement.

Figure 3 Plasma levels of oestradiol (A) and progesterone (B) in preterm infants with and without replacement of oestradiol and progesterone at different postnatal ages. Medians, 10th and 90th centiles (conversion factors to SI-units, oestradiol $\times 3.67$ and progesterone $\times 3.18$). The dotted lines indicate the plasma levels of oestradiol and progesterone aimed at, which are derived from umbilical cord blood measurements.¹² ESTRA-PRO, oestradiol and progesterone replacement

DISCUSSION

Maintenance of in utero plasma levels of oestradiol and progesterone was achieved with the ESTRA-PRO emulsion as in our previous study.¹⁰ Surviving infants who received the hormone replacement ATP showed a trend to less BPD ($p = 0.08$), which is in accordance with the result from the pilot study.¹⁰ The effect of the hormone replacement was associated with the number of days with hormone replacement ($p = 0.04$). Altogether, the goal of the study was not reached.

Twenty two infants survived but were not treated ATP. The incidence of BPD was higher in this subgroup (five of seven (71%) in the placebo group and nine of 15 (60%) in the ESTRA-PRO group) compared with 32% and 14%, respectively, in the ATP population. In this subgroup, increased markers of bacterial infection (for definition see table 1) were found postnatally in 91% (71% in the placebo and 100% in the ESTRA-PRO group) compared with 67% in the ATP group. Infection has been recognised as a risk factor for the development of BPD¹⁴ and may have contributed to the high rate of BPD in the non-ATP subgroup.

In the pilot study,¹⁰ there was no difference in plasma triglyceride levels between the replacement (mean 1.3 mmol/l, min-max 0.3–4.8 mmol/l) and the control group (1.47 mmol/l, 0.3–4.9 mmol/l) in the first 28 days after birth, and transient hypertriglyceridaemia (>3.0 mmol/l) was present in 20% of the

infants of both groups (unpublished data). In this study, the incidence of hypertriglyceridaemia in the ESTRA-PRO group was twice as high as in the placebo group ($p = 0.05$). As all other parenteral lipid administration was stopped before withdrawal of the study emulsion, the difference in the incidence of hypertriglyceridaemia between the groups was not explained by a different total lipid supply. For a long time it has been known that plasma triglycerides rise markedly during pregnancy,¹⁵ and this was confirmed by more recent investigations.¹⁶ Removal of triglycerides may be impaired by diminished activity of lipolytic enzymes. The activity of lipoprotein lipase has been shown to be decreased in pregnant women.¹⁷ Oestradiol and progesterone have been shown to decrease the activity of lipoprotein lipase in vitro¹⁸ and in vivo.^{19, 20} Possibly, the response to oestradiol and progesterone is the same in the preterm infant as in the mother, which decreases lipolysis and leads to hypertriglyceridaemia. Sepsis in preterm infants has been associated with reduced ability to use lipids,²¹ and this effect may be explained by downregulation of lipoprotein lipases.²² In the ESTRA-PRO group, there was a trend for higher rates of prolonged rupture of membranes (49% v 28%, $p = 0.07$, table 1) and increased markers of bacterial infections postnatally (81% v 65%, $p = 0.14$, table 1). This may provide an alternative explanation for the higher incidence of hypertriglyceridaemia. In addition, the higher rate of early-onset infections in the ESTRA-PRO group may have diminished the potentially beneficial effects of oestradiol and progesterone replacement on the incidence of BPD.¹⁴

Interestingly, none of the surviving infants of the ESTRA-PRO group treated ATP developed severe retinopathy of prematurity (ROP). This may be an incidental observation and ROP was not a primary outcome variable. However, in vitro and in vivo studies suggest that oestradiol may prevent ROP.^{23, 24} Preterm birth leads to delayed retinal vascular growth with insufficient vascularisation. This results in hypoxia of the retina, with release of factors stimulating new and abnormal blood vessel growth.²⁵ Vascular endothelial growth factor (VEGF) is an important oxygen-regulated factor that, if suppressed, inhibits normal vessel growth, but, if in excess, results in retinal neovascularisation. Recently, oestradiol has been shown to function as a significant stimulator of VEGF mRNA expression in retinal endothelial cells.²³ In a mouse model of ROP, treatment with oestradiol under different oxygen conditions significantly improved retinopathy by acting on VEGF expression.²⁴

Infants of the ESTRA-PRO group were discharged home nearly 2 weeks earlier (ITT and ATP population). Early discharge may be a surrogate for achievement of several conditions of preterm infants not further elaborated in this study. A role of oestradiol and progesterone in the prenatal development of the central nervous system is conceivable.²⁶ The follow-up investigation of the infants of our first pilot study at the corrected age of 15 months showed improved psychomotor development in those infants who had received oestradiol and progesterone replacement after birth.²⁷ Most recently, oestradiol has been recognised as a potentially preventive neuroprotective agent in sick preterm infants who need to be exposed to oxygen or drugs with neurotoxic side effects.²⁸

Several limitations of the study need to be considered. A significant loss of study participants for the ATP analysis was observed because of hypertriglyceridaemia. A major flaw of this study is that we did not compensate for this. The incidence of multiple births in the placebo group was twice as high as found within the verum group (NS), which may have biased the results. On the other hand, no difference was found with regard to birth weight and gestational age between the groups. The protocol violations by hypertriglyceridaemia were seen more

What is already known on this topic

- From in vitro and animal models, it is known that oestradiol and progesterone may have a major effect on lung development and function.

What this study adds

- This study adds clinical data that preterm infants may benefit in lung development from replacement of oestradiol and progesterone after birth
- A larger better-powered study will be required to determine this reliably

often in the replacement group, and this may have biased the results for the ATP analysis. However, the outcome BPD within the surviving dropouts was the same for both groups. Within the ATP population, postnatal dexamethasone was more often used in the ESTRA-PRO group. During the study period, criteria for use of dexamethasone were not strictly defined. Because the adjusted dose–response relationship between “duration of administration of the study emulsion” and the outcome BPD was virtually unaffected using dexamethasone as a potential confounder, we consider this difference to have occurred by chance. As in our pilot study,¹⁰ a trend towards reduced incidence of BPD was seen with the replacement if infants were treated ATP. No obvious serious adverse side effects were observed with the replacement, but lipid metabolism needs to be monitored carefully. Further basic research and sufficiently powered clinical trials are needed to evaluate this new approach in the management of preterm infants.

ACKNOWLEDGEMENTS

We thank the “Deutsche Forschungsgesellschaft” for supporting this study. We also thank Mrs M Schulz (Endocrinological Laboratory, Department of Obstetrics and Gynecology, University of Ulm) for excellent technical assistance.

Authors' affiliations

A Trotter, F Pohlandt, Section of Neonatology and Pediatric Critical Care Medicine, Children's Hospital, University of Ulm, Ulm, Germany

L Maier, Pharmacy, University of Ulm

M Kron, Department of Biometry and Medical Documentation, University of Ulm

Funding: This work was supported by the “Deutsche Forschungsgesellschaft”, DFG 395/2-1, 2-2.

Competing interests: None.

REFERENCES

- 1 **Jobe AJ**. The new BPD: an arrest of lung development. *Pediatr Res* 1999;**46**:641–3.

- 2 **Husain AN**, Siddiqui NH, Stocker JT. Pathology of arrested acinar development in postsurfactant bronchopulmonary dysplasia. *Hum Pathol* 1998;**29**:710–17.
- 3 **Massaro GD**, Mortola JP, Massaro D. Estrogen modulates the dimensions of the lung's gas-exchange surface area and alveoli in female rats. *Am J Physiol* 1996;**270**(Pt 1):L110–14.
- 4 **Massaro D**, Massaro GD. Estrogen regulates pulmonary alveolar formation, loss, and regeneration in mice. *Am J Physiol Lung Cell Mol Physiol* 2004;**287**:L1154–9.
- 5 **Beyer C**, Küppers E, Karolczak M, et al. Ontogenetic expression of estrogen and progesterone receptors in the mouse lung. *Biol Neonat* 2003;**84**:59–63.
- 6 **Brandenberger AW**, Tee MK, Lee JY, et al. Tissue distribution of estrogen receptors alpha (ER-alpha) and beta (ER-beta) mRNA in the midgestational human fetus. *J Clin Endocrinol Metab* 1997;**82**:3509–12.
- 7 **Khosla S**, Smith G, Parks P, et al. Effects of estrogen on fetal rabbit lung maturation: morphological and biochemical studies. *Pediatr Res* 1981;**15**:1274–81.
- 8 **Trotter A**, Ebsen M, Kiess E, et al. Prenatal estrogen and progesterone deprivation impairs alveolar formation and fluid clearance in newborn piglets. *Pediatr Res* 2006;**60**:60–4.
- 9 **Tulchinsky D**, Hobel CJ, Yeager E, et al. Plasma estrone, estradiol, estril, progesterone, and 17-hydroxyprogesterone in human pregnancy. I. Normal pregnancy. *Am J Obstet Gynecol* 1972;**112**:1095–100.
- 10 **Trotter A**, Maier L, Grill HJ, et al. Effects of postnatal estradiol and progesterone replacement in extremely preterm infants. *J Clin Endocrinol Metab* 1999;**84**:4531–5.
- 11 **Hunt R**, Davis P, Inder T. Replacement of estrogens and progestins to prevent morbidity and mortality in preterm infants. *Cochrane Database Syst Rev* 2004;(4):CD003848.
- 12 **Trotter A**, Maier L, Grill HJ, et al. 17β-estradiol and progesterone supplementation in extremely low-birth-weight infants. *Pediatr Res* 1999;**45**:489–93.
- 13 **Costeloe K**, Hennessy E, Gibson AT, et al. The EPICure study: outcomes to discharge from hospital for infants born at the threshold of viability. *Pediatrics* 2000;**106**:659–71.
- 14 **Rojas MA**, Gonzalez A, Bancalari E, et al. Changing trends in the epidemiology and pathogenesis of neonatal chronic lung disease. *J Pediatr* 1995;**126**:605–10.
- 15 **Boyd EM**. The lipemia of pregnancy. *J Clin Invest* 1934;**13**:347–63.
- 16 **Alvarez JJ**, Montelongo A, Iglesias A, et al. Longitudinal study on lipoprotein profile, high density lipoprotein subclass, and postheparin lipases during gestation in women. *J Lipid Res* 1996;**37**:299–308.
- 17 **Kinnunen PK**, Unnerus HA, Ranta T, et al. Activities of post-heparin plasma lipoprotein lipase and hepatic lipase during pregnancy and lactation. *Eur J Clin Invest* 1980;**10**:469–74.
- 18 **Julve J**, Robert MQ, Llobera M, et al. Hormonal regulation of lipoprotein lipase activity from 5-day-old rat hepatocytes. *Mol Cell Endocrinol* 1996;**116**:97–104.
- 19 **Applebaum DM**, Goldberg AP, Pykalisto OJ, et al. Effect of estrogen on post-heparin lipolytic activity. Selective decline in hepatic triglyceride lipase. *J Clin Invest* 1977;**59**:601–8.
- 20 **Tikkanen MJ**, Kuusi T, Nikkila EA, et al. Variation of postheparin plasma hepatic lipase by menstrual cycle. *Metabolism* 1986;**35**:99–104.
- 21 **Park W**, Paust H, Brosicke H, et al. Impaired fat utilization in parenterally fed low-birth-weight infants suffering from sepsis. *J Parenter Enteral Nutr* 1986;**10**:627–30.
- 22 **Lanza-Jacoby S**, Sedkova N, Phetteplace H, et al. Sepsis-induced regulation of lipoprotein lipase expression in rat adipose tissue and soleus muscle. *J Lipid Res* 1997;**38**:701–10.
- 23 **Suzuma I**, Mandai M, Takagi H, et al. 17 Beta-estradiol increases VEGF receptor-2 and promotes DNA synthesis in retinal microvascular endothelial cells. *Invest Ophthalmol Vis Sci* 1999;**40**:2122–9.
- 24 **Miyamoto N**, Mandai M, Takagi H, et al. Contrasting effect of estrogen on VEGF induction under different oxygen status and its role in murine ROP. *Invest Ophthalmol Vis Sci* 2002;**43**:2007–14.
- 25 **Smith LE**. Pathogenesis of retinopathy of prematurity. *Semin Neonatal* 2003;**8**:469–73.
- 26 **Trotter A**, Pohlandt F. The replacement of oestradiol and progesterone in very premature infants. *Ann Med* 2000;**32**:608–14.
- 27 **Trotter A**, Bokelmann B, Sorgo W, et al. Follow-up examination at the age of 15 months of extremely preterm infants after postnatal estradiol and progesterone replacement. *J Clin Endocrinol Metab* 2001;**86**:601–3.
- 28 **Asimadous S**, Bittigau P, Felderhoff-Mueser U, et al. Protection with estradiol in developmental models of apoptotic neurodegeneration. *Ann Neurol* 2005;**58**:266–76.