

Conflict of Interest: none

Endothelial Colony Forming Cells Dysfunction Relates to Cardiovascular Alterations in Preterm Born Adults

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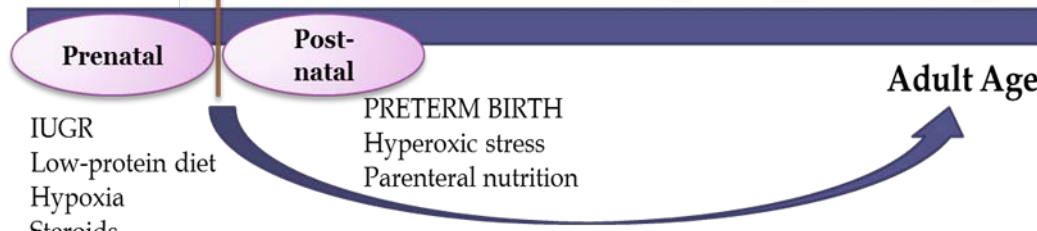
**Endothelial Colony Forming Cells Dysfunction
Relates to Cardiovascular Alterations in
Preterm Born Adults**

Mariane Bertagnolli, PhD

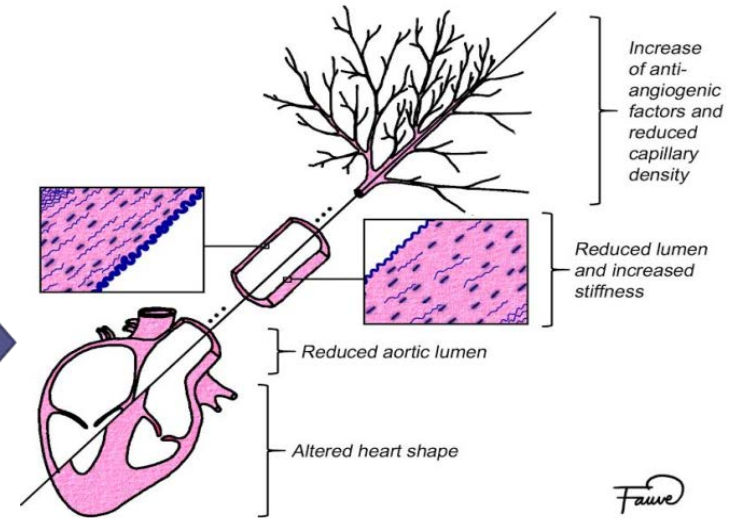
NO CONFLICT OF INTEREST TO DISCLOSE



Preterm birth and hypertension: *is there a link?*



10-11% Worldwide



Luu et al. *CMAJ*, 2015

Lewandowski et al. *Circulation*, 2013

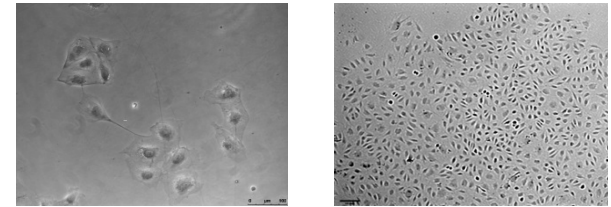
Bertagnolli et al. *Curr Hypert Rep*, 2016

Endothelial colony-forming cells

Origins

EPC subtypes

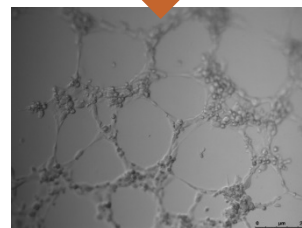
ECFC *in vitro*



CFU-EC
CD45+
CD14+
KDR-

ECFC
CD34+, CD133d
KDR+, CD31+
CD45-, CD14-

CAC
KDR+
CD34
CD133



ECFC *in vivo*



Angiogenesis
Hindlimb ischemia

Tissue repair
Neonatal
hyperoxia-induced
lung injury (BPD)

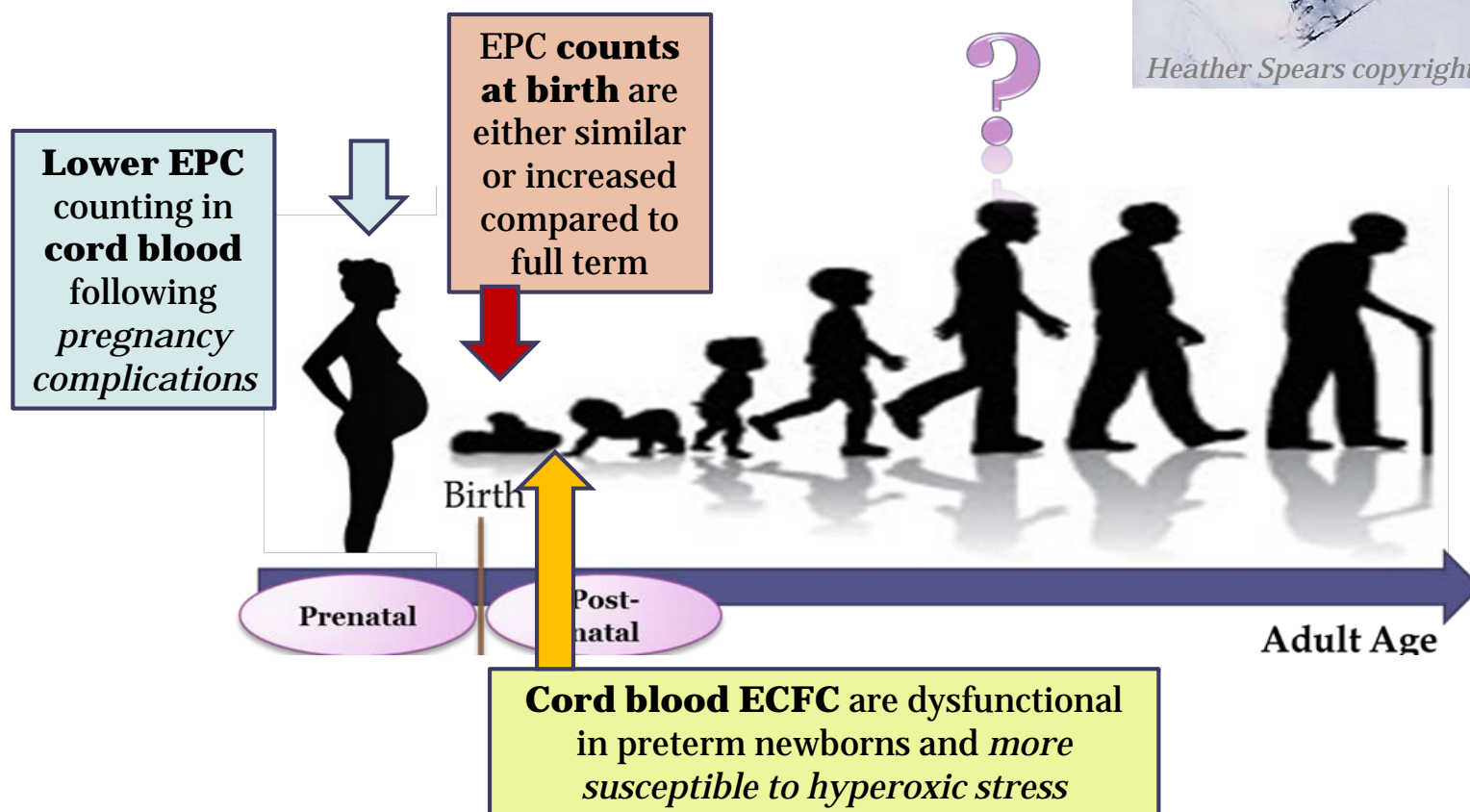
Asahara et al.
AJP Cell Physiol, 2004

Prater et al.
Leukemia, 2007

Schwarz et al. *ATVB*, 2012
Alphonse et al. *Circulation*, 2013

EPC and ECFC in premature birth

- Preterm birth and EPC: 18 eligible studies were systematically reviewed.



Objective

We aim to assess if ECFC function relates to cardiovascular risks in preterm born adults



Health of Adult Preterms Investigation
La santé des adultes nés prématurément

HAPI Project - Health of Adult Preterms Investigation

PI: Dr Anne Monique Nuyt, MD
Dr Thuy Mai Luu, MD, PhD
Sainte-Justine University Hospital



Subjects enrolment and data collection

- ECFCs isolated from peripheral blood of **30 young adults (21-28 years old) born extremely preterm (<29 gestational weeks)** and **30 full term (≥ 37 gestational weeks)**
- Participants were **paired by gender, age and socioeconomic status**
- Birth and neonatal data were obtained by reviewing **birth records**
- **Cardiovascular assessments:** cardiac ultrasound, brachial blood pressure measurements and 24h blood pressure monitoring

Baseline characteristics of subjects

| | Preterm | Term |
|--|---------------------------------|----------------|
| | N=30 | N=30 |
| Mean age \pm SD, years | 24.3 \pm 2.2 | 23.4 \pm 2.3 |
| Male sex, n (%) | 11 (37) | 11 (37) |
| Median height (range), cm | 169 (151-189) | 170 (155-186) |
| Median weight (range), kg | 64 (43-100) | 68 (53-117) |
| Median BMI (range), kg/m ² | 22 (17-35) | 22 (18-35) |
| Smoking, n | 7 (23) | 6 (20) |
| Systolic blood pressure (mean \pm SD), (mmHg) | 125 \pm 10* | 119 \pm 7 |
| Diastolic blood pressure (mean \pm SD), (mmHg) | 74 \pm 6* | 71 \pm 5 |
| Mean VO ₂ max \pm SD, mL/min | 89 \pm 27 | 92 \pm 24 |

SD, standard deviation; BMI, body mass index; VO₂, maximal oxygen consumption

Clinical neonatal characteristics

| | Preterm n=30 | Term n=30 |
|--|-------------------------|----------------------|
| <i>Maternal</i> | | |
| Gestational hypertension | 4 (13) | 0 |
| Gestational diabetes | 0 | 0 |
| PROM (%) | 8 (27) | 0 |
| Antenatal steroids (%) | 6 (21) | 0 |
| <i>Neonatal</i> | | |
| Mean GA \pm SD, weeks | 27 \pm 1 | 39 \pm 1 |
| Mean Birth weight \pm SD, g | 1020 \pm 218 | 3309 \pm 336 |
| Median days of ventilation (range) | 14 (0-53) | - |
| Median days of supplemental O2 (range) | 22 (0-130) | - |
| Median days of hospitalization (range) | 70 (40-139) | - |
| BPD (%) | 6 (21) | - |
| PDA (%) | 9 (30) | - |
| ROP (%) | 3 (10) | - |
| IVH (%) | 5 (18) | - |
| Postnatal steroids (%) | 5 (18) | - |
| Infections (%) | 2 (7) | - |

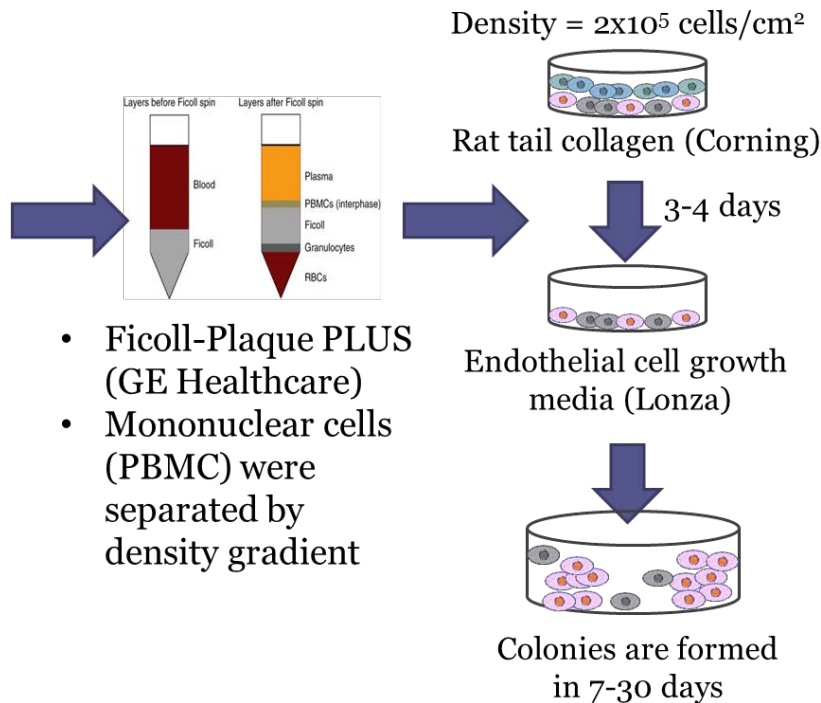
PROM, premature rupture of membranes; GA, gestational age; SD, standard deviation; O2, oxygen; BPD, bronchopulmonary dysplasia; PDA, patent ductus arteriosus; ROP, retinopathy of prematurity; IVH, intraventricular hemorrhage.



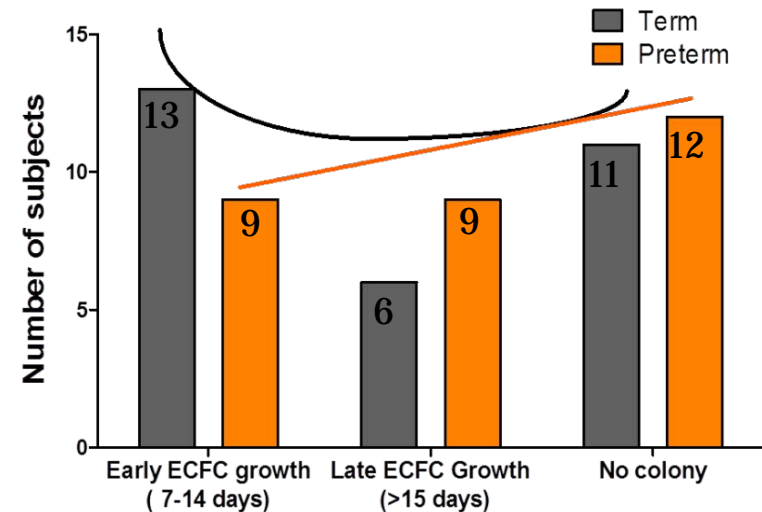
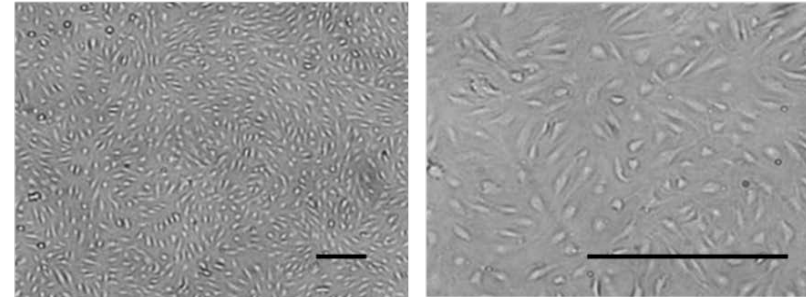
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ECFC isolation from peripheral blood



Peripheral blood ECFC cobblestone-shape colony

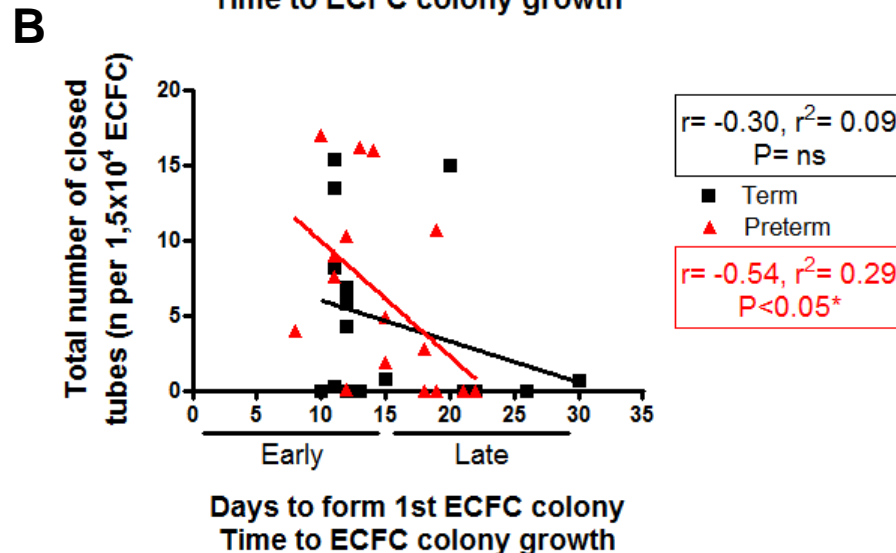
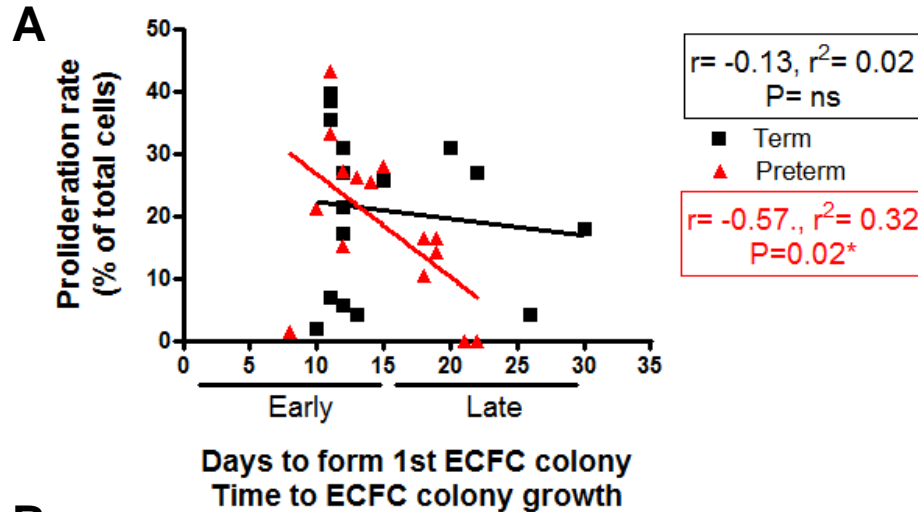


Frequency distribution of ECFC colony formation and growth
(62% of total participants)

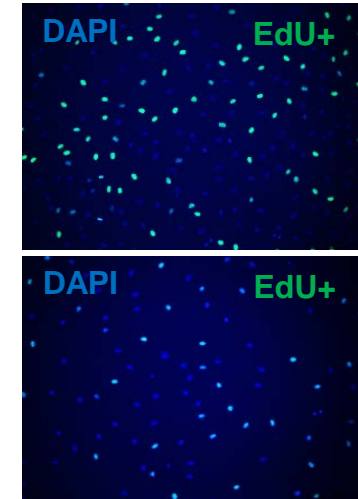
- 25 mL of peripheral blood
- Participants were fasting for 12 hours

- Ficoll-Plaque PLUS (GE Healthcare)
- Mononuclear cells (PBMC) were separated by density gradient

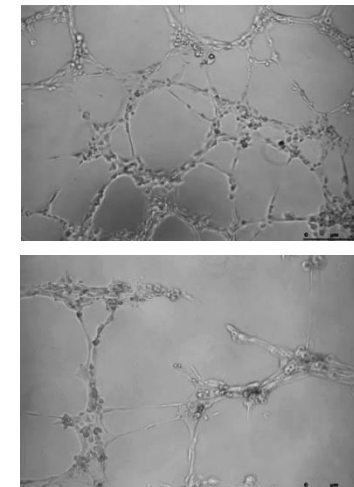
ECFC function in preterm and term born adults



Cell proliferation (Click-it EdU)

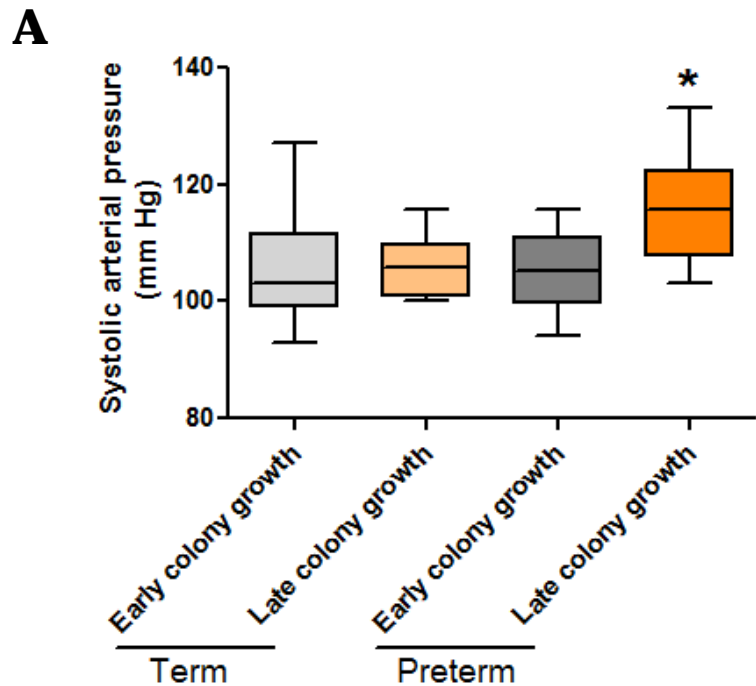


Tube formation Matrigel assay

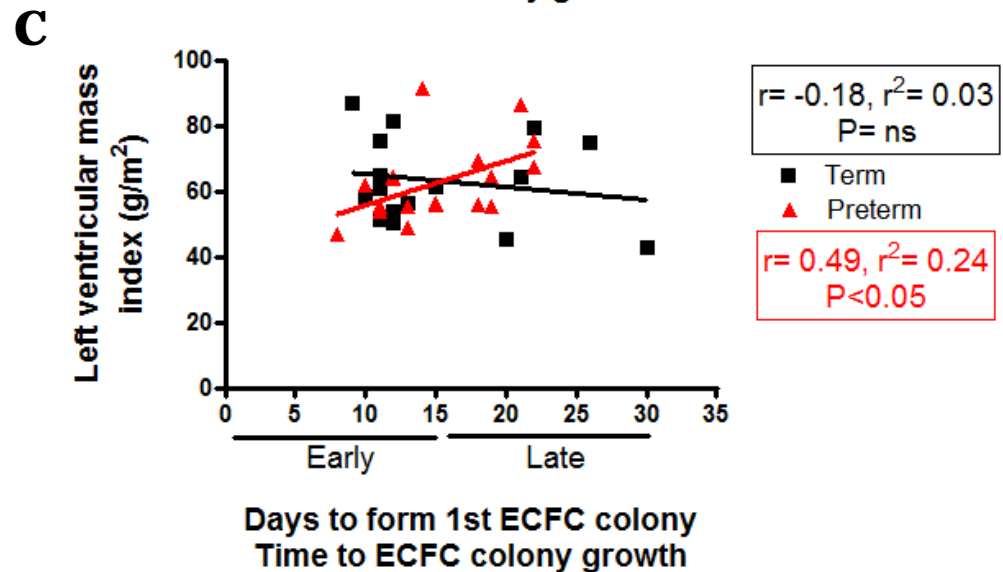
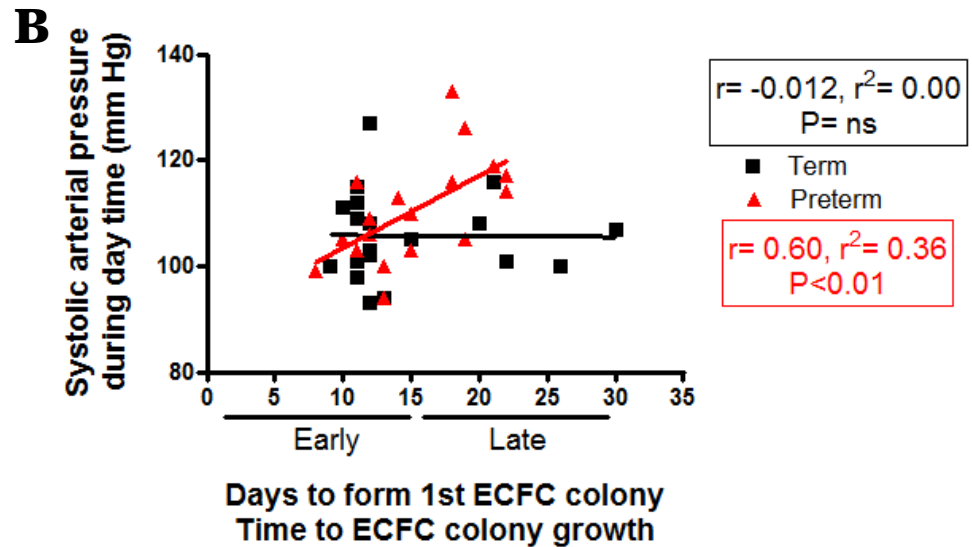


ECFC proliferative (**A**) and tube formation (**B**) properties negatively correlate with ECFC colony growth in preterm-born subjects (preterm=18 vs term=19).

ECFC function relates with cardiovascular clinical characteristics in preterm born adults



ECFC dysfunction relates with higher brachial (A) and day-time (B) systolic arterial pressure, as well as with increased left ventricular mass (C) in individuals born prematurely with **late ECFC colony growth**. Two-way ANOVA, mean \pm SEM.



Preterm born adults exposed to severe neonatal complications have dysfunctional ECFCs

A

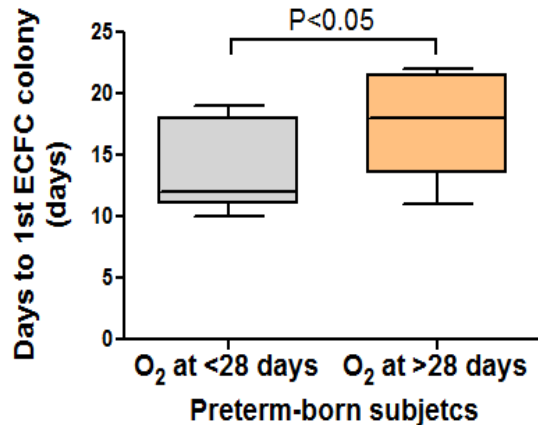


Figure A – Time to ECFC colony growth in preterm-born subjects according to time of exposure to supplemental oxygen (O_2) as newborns. T-test, mean \pm SEM.

B

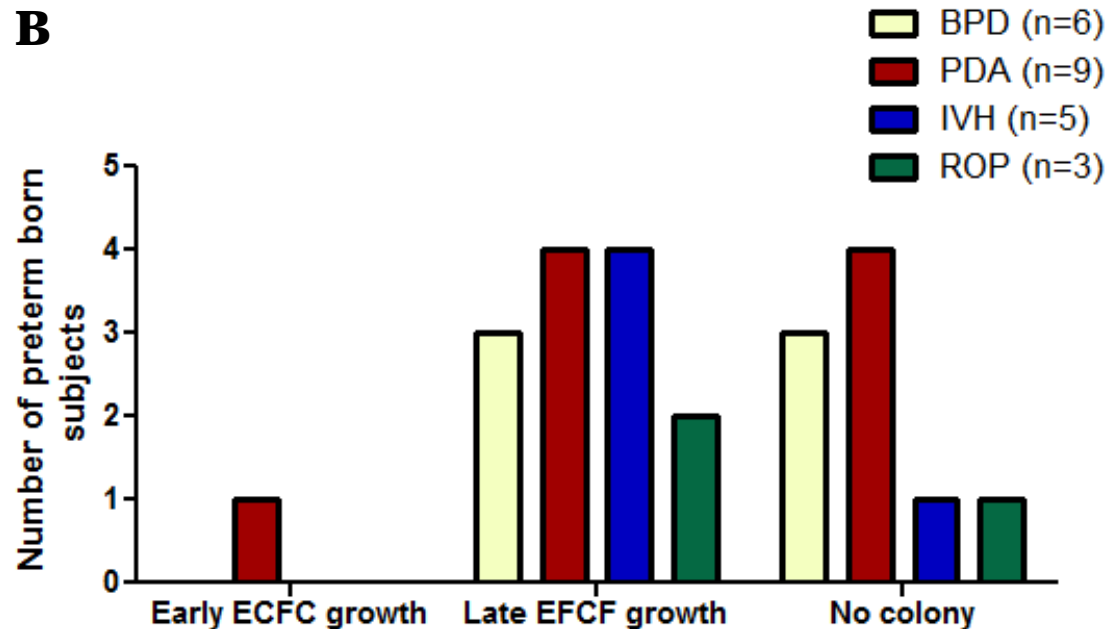


Figure B – Frequency distribution of preterm born subjects that were exposed to more severe neonatal complications as newborns.

BPD, bronchopulmonary dysplasia – O_2 at 36 weeks postmenstrual age; *PDA*, patent ductus arteriosus – treated with indomethacin or ligation; *IVH*, intraventricular hemorrhage; *ROP*, retinopathy of prematurity.

Summary and Clinical Implications

Our findings demonstrate, *for the first time*, that ECFC dysfunction in preterm-born adults significantly relates with important **cardiovascular risk factors**, such as higher blood pressure and increased left ventricular mass.



Exposure to a **proxy of severe neonatal complications** relates with later in life ECFC dysfunction in preterm born adults.

Perspectives

Use of circulating ECFCs for the investigation of:

- Molecular mechanisms related with prematurity.
- The effects of clinical interventions, such as exercise and anti-hypertensive drugs on ECFC function, as well as on its relationship with clinical cardiovascular characteristics.

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Heather Spears, 'Drawing from the Newborn'
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