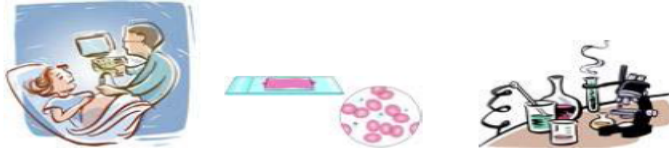


CERVIX



Cervical Immunobiology in Women at
Risk of Preterm Labour

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CERVICAL IMMUNOBIOLOGY IN WOMEN AT RISK OF PRETERM LABOUR

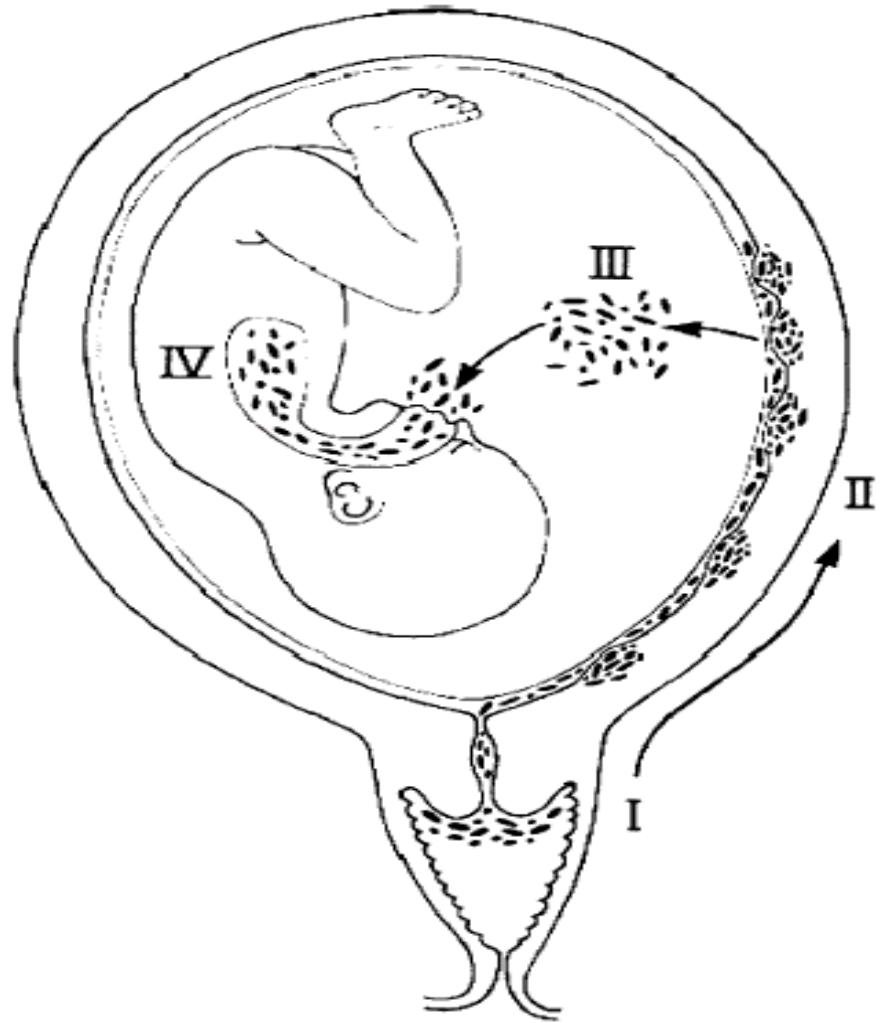
BACKGROUND

- × Preterm birth.
- × Incidence 9% to 11%.
- × Maternal infection is present in 30% to 50% of all preterm births.

(Gonc 2002; Romero 2002)

Stages of Ascending infection:

- I -cervicitis
- II -deciduitis
- III -amnionitis
- IV -fetal bacteraemia and sepsis



Stages of ascending infection Adapted from Romero 1988 with permission

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- ✘ Microbial bioactive substances act directly on cervical collagen and fetal membranes
 - premature cervical ripening and weakening the fetal membranes → PPRM
 - ✘ Microorganisms stimulate the maternal Immune system → specially monocytes and macrophages → phospholipase A2 → prostaglandins E2 and F2α

{Bernal 1993; Hay 2001; Howe 1999; Lamont 2001}

- ✘ **Activated Macrophages produce Cytokines such as Tumour Necrosis Factor (TNF) and Interleukin (IL1a, IL1b, IL6, IL8).**
- ✘ **Recent studies revealed a positive correlation between high levels of some cytokines and the onset of preterm labor.**

{Bernal 1993; Dodson 1988; Howe 1999}

RATIONALE

- ✘ Ascending infection occurs in the presence of a defective cervical barrier (immunologically and morphologically).
- ✘ Only 3 studies conducted to understand cervical macrophages (Bokstrom 1997, Sakamoto 2005, Whitworth 2007).
- ✘ All previous studies used manual counting of macrophages.
- ✘ Strikingly, they found that women with a low macrophage count were more likely to have preterm birth compared with women who had normal cervical macrophage count (Odds Ratio 4.9, 95%CI 1.5 to 18.7; P 0.0037).
- ✘ There is no studies on cervical volume and vascularisation changes.

OBJECTIVE

- ✘ investigate to what extent a defective cervical barrier is a contributory factor to recurrent preterm labour.**

HYPOTHESIS

- × Patients at high risk of preterm labour have lower numbers of macrophages and in turn less vascular cervix.**

IN ORDER TO ACHIEVE OUR OBJECTIVE

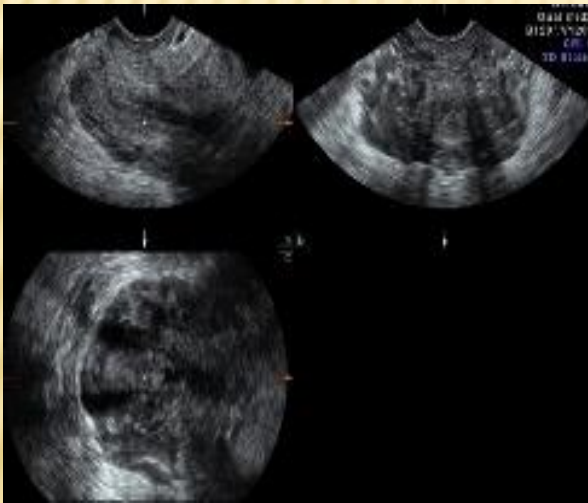
- ✘ Measure cervical volume and vascularity by 3D volume measurement and 3D Power Doppler.**
- ✘ We investigate what proportion of CD14+ cells are macrophages (CD14 is widely used as a macrophage marker; however T cells, dendritic cells and granulocytes may also express CD14).**

PROTOCOL

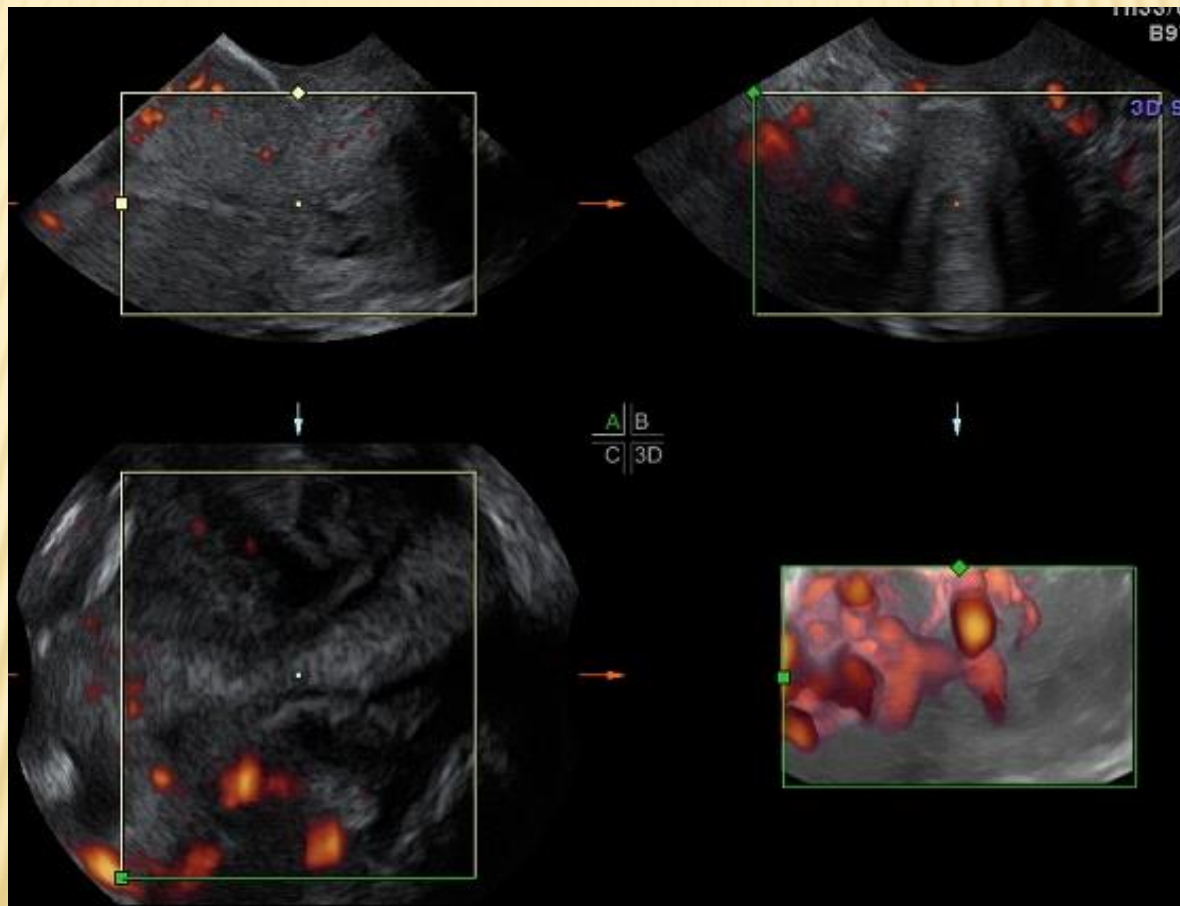
- ✘ Hospital prospective observational cohort non interventional study
- ✘ Conducted for 60 months 10/2007-9/2012
- ✘ Women between 13 and 17 weeks are informed about the study → if they agree to participate → consent → cervical sample & 3D Ultrasound

METHODOLOGY

- ✘ 3 D Ultrasound scan to measure cervical length and volume.
- ✘ 4DView programme for the analysis.



- ✘ 3 D Power Doppler to evaluate cervical vascularisation.



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- ✘ **A cytobrush is used to collect cervical cells then the tip of the brush is soaked in a specimen pack of PBS and PSG, which is processed in the same day.**
 - ✘ **fluorescent labelled monoclonal antibodies are used for the labelling of the samples.**

Leukocyte Type	Monoclonal antibodies
Granulocytes	CD66b (specific), CD49d, CD16
Lymphocytes	CD3 (specific).
T cells	CD19 (specific).
B cells	CD16, CD49d
NK cells	
Dendritic cells	CD1a (specific), CD49d
Monocytes	CD14, CD163, CD49d
Macrophages	CD14, CD163

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- × fluorescence activated cell sorting (FACs) is used for the analysis of the samples.**
 - × WinMDI 2.9 programme is used to extract Data.**

DEMOGRAPHIC RESULTS

Participants N=356	Patients N=156	Controls N=200	P
Age Median (range)	31.5 (20-39)	29 (19-39)	0.09
Smoking N (%)	6 (27)	11 (22)	0.32
Parity Median (range)	1 (1-6)	1 (1-3)	0.166
Previous preterm birth Median (range)	1 (1-6)	0 (0)	0.0001
Gestational age Median (range)	16 (13-16)	15 (13-16)	0.91

LEUKOCYTES RESULTS

Cell count /10 ⁴ cells	PTL <34 ⁺⁰ N= 68	Controls N= 200	P
Macrophages median (range)	14 (10-21)	16 (4-53)	0.11
Monocytes median (range)	0 (0-1)	0 (0-2)	0.19
B cells median (range)	0 (0)	0 (0-2)	0.73
T cells median (range)	0 (0-3)	0 (0-26)	0.12
Granulocytes median (range)	0 (0-23)	0 (0-54)	0.17
Dendritic cells median (range)	0 (0)	0 (0-21)	0.14

Macrophages were the most common leukocytes

OBSTETRIC RESULTS

Participants N= 356	Patients N= 156	Controls N= 200
Preterm delivery < 34 ⁺⁰ N (%)	68 (43.6%)	0 (0%)
Delivery > 34 ⁺⁰ N (%)	88 (56.4%)	200 (100%)
Cesarean section N (%)	4 (2.6%)	24 (12%)
Neonatal death N (%)	13 (8.3%)	0 (0%)
Admission to NICU N (%)	20 (12.8%)	0 (0%)

ULTRASOUND RESULTS

Cervix mean (SD)	PTL <34 ⁺⁰ N= 68	Controls N= 200	P
Length (mm)	40.7 (4.3)	44.4 (7.8)	0.04
Volume (cm ³)	32.5 (10.1)	33.1 (14.7)	0.31

Significant association between cervical length and preterm labour, but not the cervical volume

3D ULTRASOUND WITH POWER DOPPLER

RESULTS

Doppler indices median (SD)	PTL <34 ⁺⁰ N= 68	Controls N= 200	P
Vascular Index (%)	31.3 (8.7)	15.5 (9.6)	0.0006
Flow Index (unit)	36.1 (2.6)	35.2 (2.9)	0.61
Vascular Flow Index (unit)	12 (3.5)	5.3 (3.7)	0.0009

**Inverse association with preterm labour
may be due to preconceptional
hypervascularity**

CERVICAL MACROPHAGES AND CERVICAL 3D ULTRASOUND PARAMETERS CO-RELATIONS

- ✘ No significant correlation was found between cervical macrophages and cervical length, volume, vascular index, flow index, vascular flow index.

CONCLUSIONS

- ✘ Flowcytometry can be used for cervical leukocytes characterisation and beneficial in resolving any concerns about blood contamination**
- ✘ The most prevalent cervical leukocyte was the macrophages**
- ✘ There was a significant association between cervical length and preterm labour**
- ✘ Cervical volume may not be associated with preterm labour**
- ✘ No correlation found between cervical macrophages and any of the cervical 3D ultrasound parameters measured**
- ✘ There is a real requirement for more research on cervical leukocyte population. Adding the 3D ultrasound and power Doppler will provide voluble information about the cervical morphology**



Mohammad Othman

Cervical leukocytes and 3D ultrasound in preterm birth high risk women

Non-interventional study

Thank

you