A novel method to enrich and characterize circulating fetal cells using the Parsortix™ cell separation system

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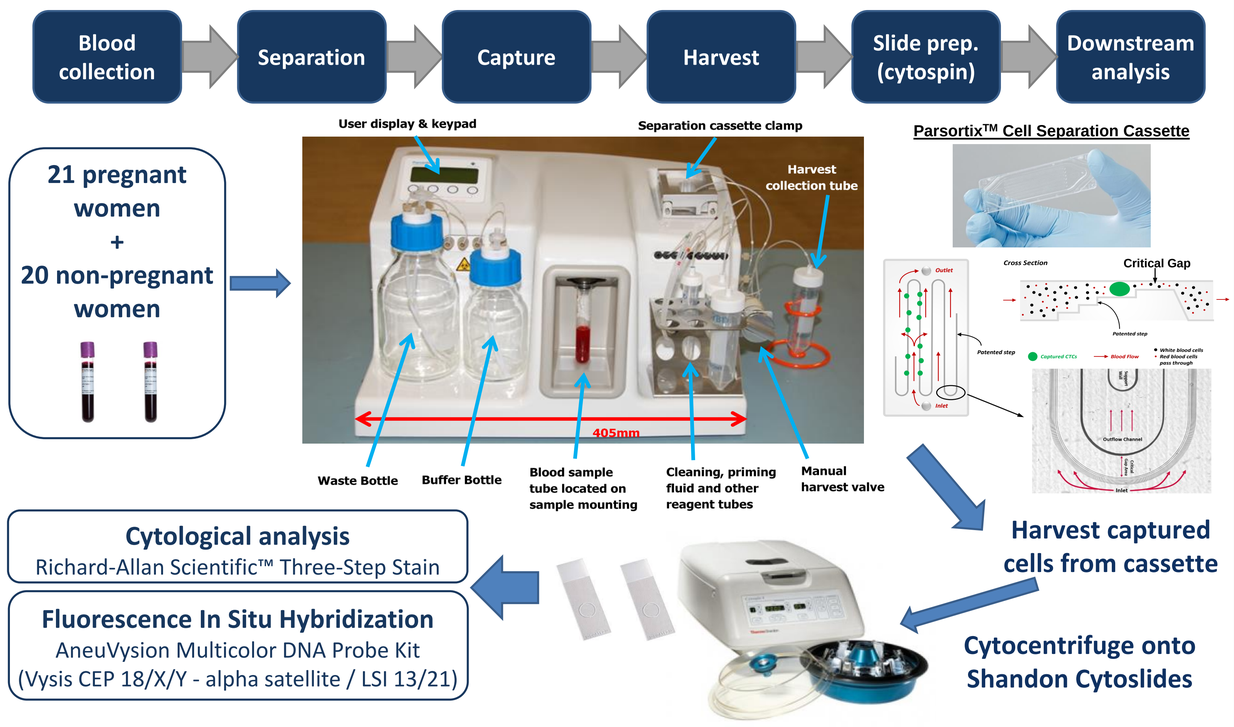
Introduction

Prenatal diagnostics historically involved invasive screening tests, such as amniocentesis, which carry risks to both the fetus and mother. There is increasing interest in non-invasive prenatal diagnosis (NIPD) using cell-free fetal DNA (cffDNA) analysis to screen for genetic abnormalities such as Down syndrome. However, this approach is limited in its application. The direct isolation and characterization of circulating fetal cells may allow more comprehensive prenatal genetic analyses. In this study, we evaluated the capability of the Parsortix™ cell separation system, an epitope independent system, to isolate circulating fetal cells.

Materials & Methods

- 7mL peripheral whole blood samples were collected into two (2) separate 10mL EDTA tubes from 21 pregnant women and from 20 age-matched non-pregnant women as controls. The cohort of pregnant women included covered different stages of pregnancy:
  - 1st trimester = 2
  - 2nd trimester = 10
  - 3rd trimester = 9
- Blood samples were separated using the Parsortix™ system within 2 hours of collection. The Parsortix system enriches for rare cells, epitope-independently, based on cell size and compressibility, using a disposable microscope slide-sized cassette (Figure 1).
- Enriched cells were harvested and deposited onto coated Shandon™ cytoslides using a Cytospin™ 4 cytocentrifuge.
- Slides were analyzed:
  - by fluorescence in-situ hybridisation (FISH); and
  - by cytological staining.
- FISH was performed according to the manufacturer's instructions (AneuVysion Multicolor DNA Probe Kit, Abbott) to allow for the detection of X and Y chromosomes and trisomies 13, 18, and 21 (Down syndrome).
  - Slides were analyzed using a fluorescence microscope and a 40x magnification.
  - Signals for chromosomes X and 13 were visualized in the green channel
  - Signals for chromosomes Y and 18 were visualized in the orange (Y3) channel
  - Signals for chromosome 21 were visualized in the cyan channel.
- Cytologic staining was performed using Richard-Allan Scientific™ Three-Step Stain, according to the manufacturer's instructions.
  - Slides were analyzed in bright field at 20x magnification.

Figure 1: Work flow of the Parsortix System

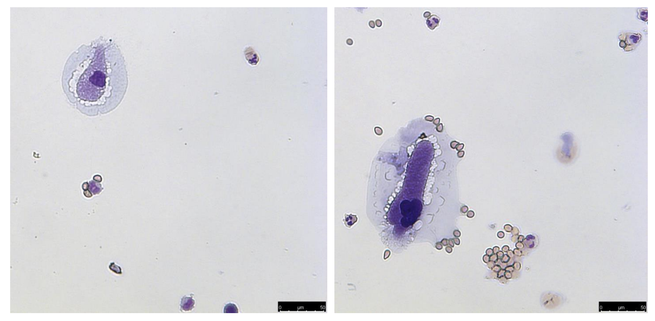


Results

Cytological Demonstration of Fetal Cells Captured from Maternal Blood

Fetal cells were successfully enriched by the Parsortix system from maternal blood. Large putative fetal cells (around 50µm) showing a small nucleus (dark purple) and a large, distortional cytoplasm in lighter purple (**Figure 2**) were observed on the cytospun slides from pregnant women, whatever their stage of pregnancy. No putative fetal cells have been found on the cytospun slides from the non-pregnant controls (0/20).

Figure 2: Representative fetal cell images from cytological slides

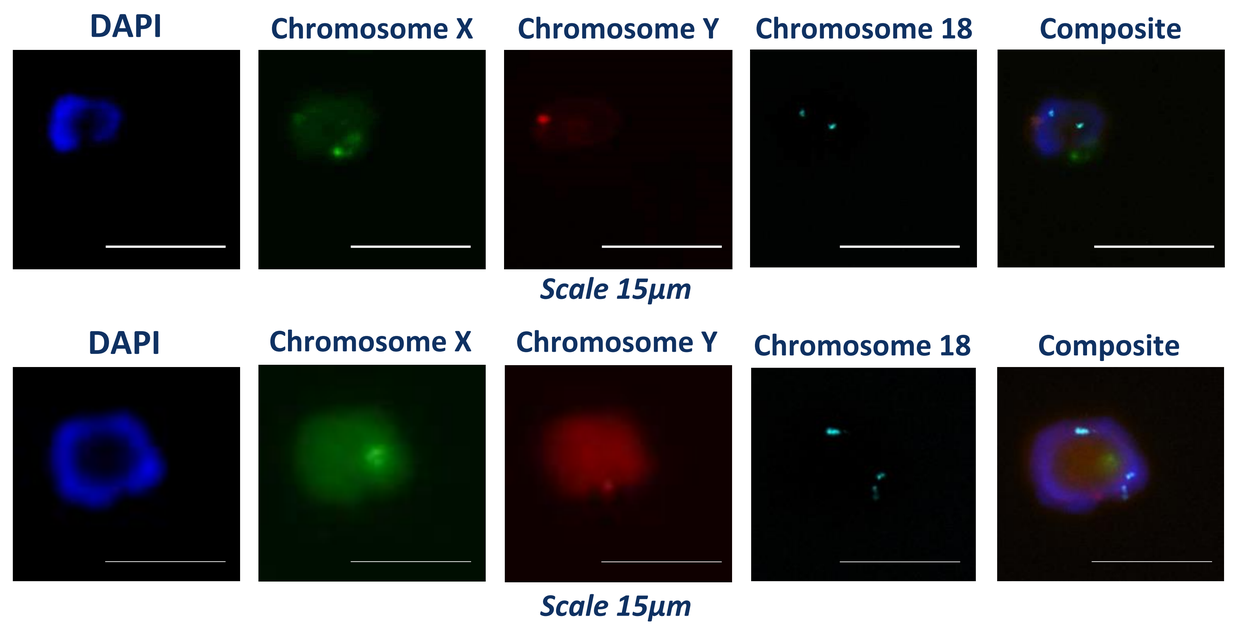


Richard-Allan Scientific™ Three-Step Staining performed on cytospun slides. One large putative fetal cell is visible in each image. Scale bar: 50µm

Demonstration of Male Fetal Cells Captured from Maternal Blood

19 out of 21 pregnant women sample were analysable by FISH. In 8/19 (42%) of the blood samples from the pregnant women, at least one putative fetal cell that showed one X signal and one Y signal (**Figure 3**) was found, independently of stages of pregnancy, indicating that the cell was of male origin as defined by its X/Y FISH profile. No putative fetal cells were found in any of the non-pregnant control blood samples (0/20).

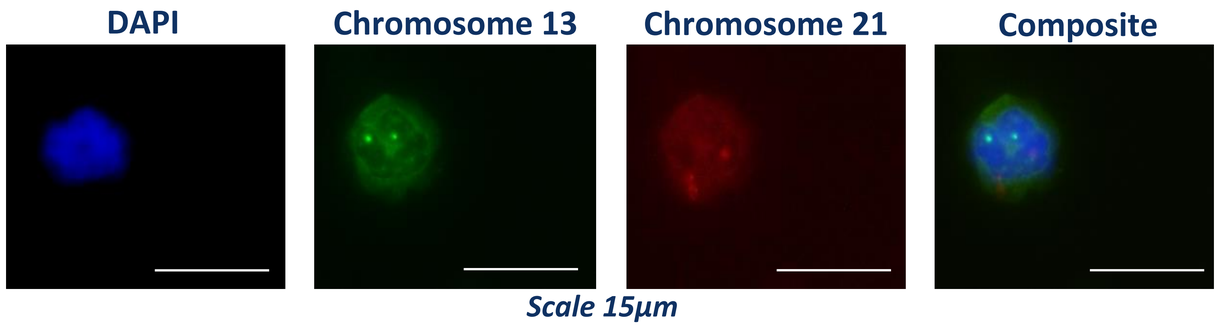
Figure 3: Representative images of 18/X/Y FISH using AneuVysion Multicolor DNA Probe Kit



Demonstration of Trisomy 21 Fetal Cells Captured from Maternal Blood

One blood sample drawn from a pregnant woman diagnosed with a Down syndrome fetus (trisomy 21) was processed and hybridized using Vysis LSI 13/21. Fetal cells with an abnormal number of chromosome 21 (x3) were detected in this sample (**Figure 4**).

Figure 4: Representative image of 13/21 FISH using AneuVysion Multicolor DNA Probe Kit



CONCLUSIONS

- XY cells were identified in 42% of maternal blood samples while no XY cells were identified in the control samples from non-pregnant women.
- The presence of a third copy of chromosome 21 was detected in cells enriched from a pregnant woman diagnosed with a Down syndrome fetus.
- This preliminary work suggests that the Parsortix™ system may have value for the enrichment and characterization of fetal cells from maternal blood.