The effect of anti-thrombotic therapy on cell-free DNA release from first- and third-trimester placental explants

Pearce BJG, Beard S, De Alwis N, Hannan NJ, Hui L

RESEARCH WEEK
5-9 OCTOBER 2020
INSPIRED RESEARCHERS
Northern Health

Background

- Non-invasive prenatal testing (NIPT) is an increasingly popular means of screening for fetal chromosomal abnormalities
- NIPT analyses cell-free fetal (cff) DNA present in the maternal plasma, which is derived largely from the turnover of trophoblast cells[1]
- Pregnant women receiving low-molecular-weight-heparin such as enoxaparin (Clexane) are more likely to receive a "failed" NIPT result[2]
- Heparin and aspirin modify trophoblast survival *in vitro* [3]
- We hypothesised that enoxaparin increases the risk of NIPT failure by reducing trophoblast turnover and hence, the amount of cffDNA released into maternal plasma
- Other anti-thrombotic therapies such as aspirin and $P2Y_{12}$ inhibitors may also have a similar effect on trophoblast turnover and cffDNA release

Aims & Objectives

- To measure the amount of cffDNA released by placental explants when exposed to enoxaparin and other anti-thrombotic medications *in vitro*
- To compare first and third trimester placental responses to these treatments

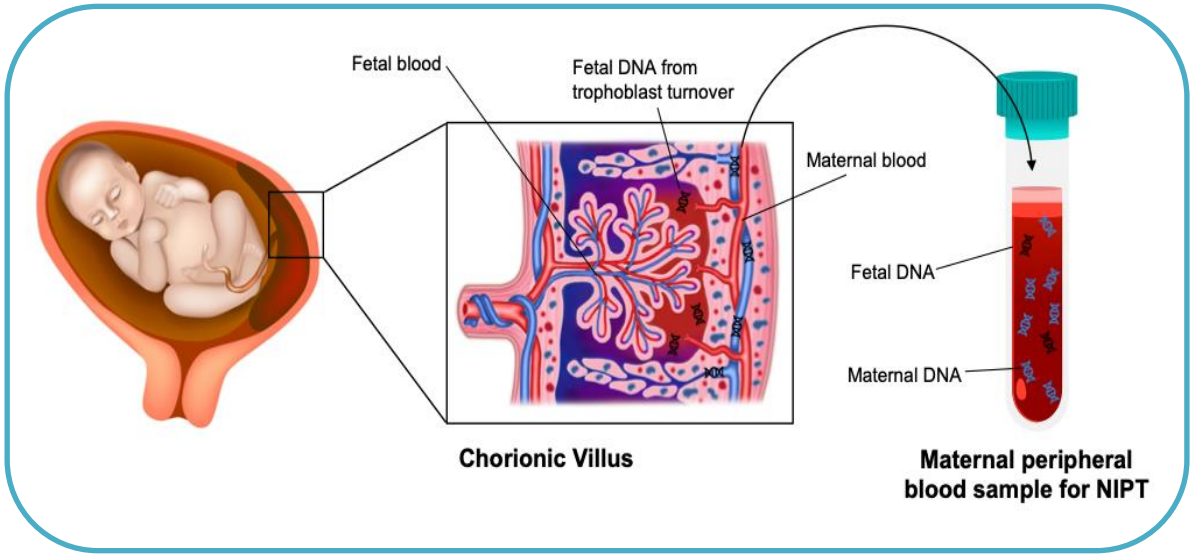

Figure 1. Cell-free fetal DNA is released during trophoblast cell turnover. It is detectable in maternal plasma and is analysed for non-invasive prenatal testing

References

1. Hui L. Noninvasive prenatal testing for aneuploidy using cell-free DNA–New implications for maternal health. Obstet Med. 2016;9(4):148–152.
2. Burns W, Koelper N, Barberio A, et al. The association between anticoagulation therapy, maternal characteristics, and a failed cfDNA test due to a low fetal fraction. Prenatal Diagn. 2017;37(11):1125–1129.
3. Bose P, Black S, Kadyrov M, et al. Heparin and aspirin attenuate placental apoptosis in vitro: implications for early pregnancy failure. Am J Obstet Gynecol. 2005;192(1):23–30.

The effect of anti-thrombotic therapy on cell-free DNA release from first- and third-trimester placental explants

Pearce BJG, Beard S, De Alwis N, Hannan NJ, Hui L

RESEARCH WEEK
5-9 OCTOBER 2020
INSPIRED RESEARCHERS
Northern Health

Methods

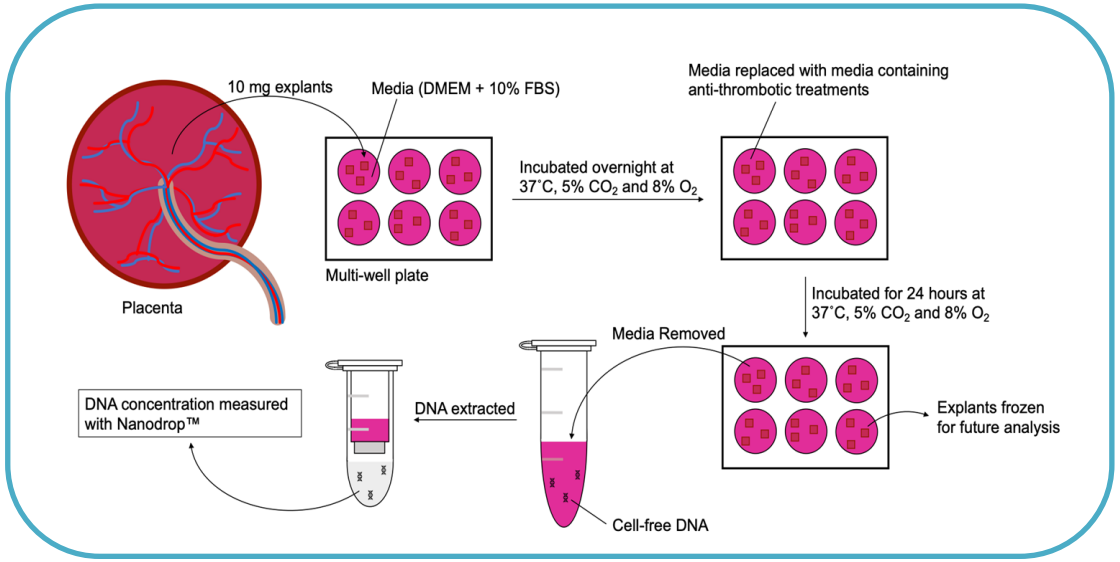

Figure 2. Experimental method. Anti-thrombotic treatments were: enoxaparin (0.5, 2 and 4 IU/mL, aspirin (25 and 50 µg/mL), clopidogrel (12.5 and 50 µg/mL), prasugrel (1.5 and 10 µg/mL) and ticagrelor (15 and 30 µg/mL).

- Placental samples from term elective caesarean births and first trimester surgical terminations of pregnancy were obtained from the Northern Centre for Health Education and Research (NCHER) Reproductive Health Biobank
- ~10 mg placental explants were incubated under physiological conditions with anti-thrombotic medications or control media for 24 hours
- These treatment concentrations are known to be non-cytotoxic[4]
- cffDNA was extracted from the pooled media for each treatment using the QIAamp® Circulating Nucleic Acid Kit
- cffDNA was quantified with the NanoDrop™ spectrophotometer
- The cffDNA concentrations for the treated explants were adjusted for explant mass and expressed as a ratio relative to the untreated controls
- Statistical analysis performed with IBM SPSS® using One Sample T-test

Northern Health

4. Nguyen N. Anti-thrombotic and anti-platelet therapy and their influence on placental release of cell-free DNA. University of Melbourne; 2019 MDRP monograph.

The effect of anti-thrombotic therapy on cell-free DNA release from first- and third-trimester placental explants

Pearce BJG, Beard S, De Alwis N, Hannan NJ, Hui L

RESEARCH WEEK
5-9 OCTOBER 2020
INSPIRED RESEARCHERS
Northern Health

Results

- Three first trimester and 7 third trimester placentas were obtained
- cffDNA release was significantly higher in placental explants treated with enoxaparin, and appeared to increase in a dose-dependent manner (**fig. 3**)
- The effect size of enoxaparin was greater in first than third trimester explants
- Aspirin and $P2Y_{12}$ inhibitors did not appear to have any effect on cell-free DNA release from first or third trimester placental explants

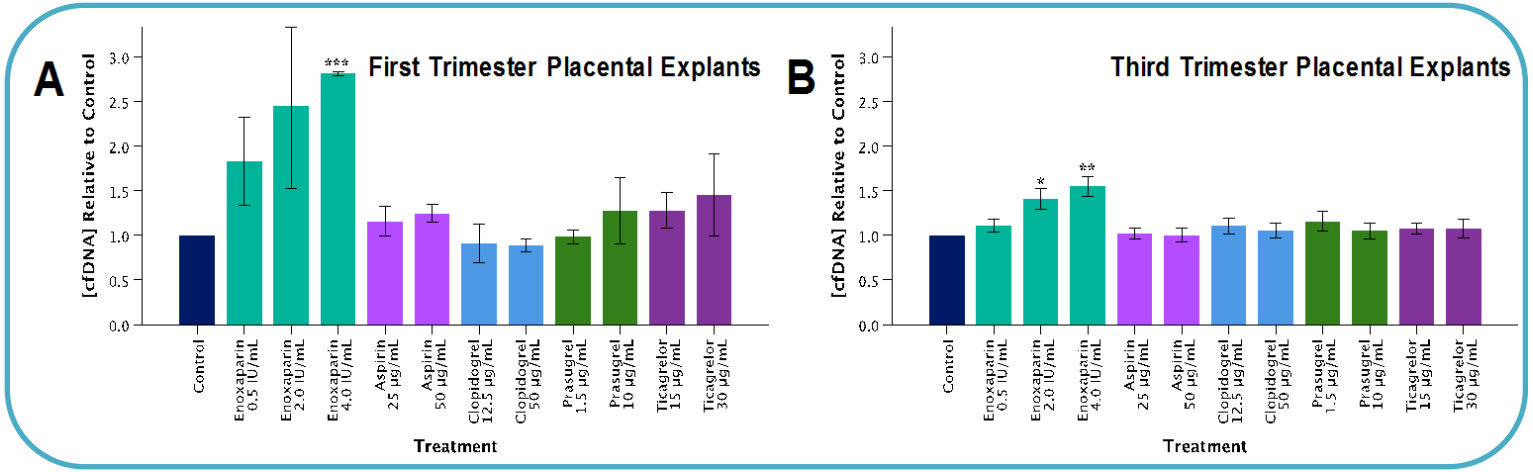

Figure 3. Amount of cell-free DNA produced by **A** first trimester explants or **B** third trimester placental explants after incubation with different treatments. Values represented as mean ± SEM relative to the control group (no anti-thrombotic treatments). Stars indicate significant difference from a value of 1 with $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***)

Conclusions

- **The observed increase in cffDNA release with enoxaparin treatment did not support our hypothesis that enoxaparin reduces the release of cffDNA into the maternal circulation.**
- The increased risk of NIPT test failures in association with heparin treatment appears not be mediated by a direct effect on trophoblast release of cffDNA
- The larger effect size for the first trimester samples suggests that placental tissue is more sensitive to enoxaparin earlier in pregnancy.
- We plan to investigate the mechanism by which enoxaparin increases cell-free DNA release from trophoblast, including histochemical staining and RNA analysis for markers of apoptosis.

Northern Health