New trisomy screening approach targeting fetal DNA present in maternal blood

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Several recent studies have demonstrated that intact fetal cells, as well as free fetal DNA, circulate in maternal blood. However, fetal cells are in very small quantities and they are difficult to isolate.

Finding baby’s DNA...

Recently, researchers have discovered a technique to isolate free fetal DNA in the blood of pregnant women. Fetal DNA comes mainly from the cells of the placenta and is a product of apoptosis (programmed cell death). The amount of fetal DNA found in the blood can vary from 4% to 20%. The size of the DNA fragments is between 150-300 bp and the fetal genome is present in full.

Contrary to our belief that the placenta is an impermeable barrier between the mother and the baby, circulation does occur in both directions.

Figure 1: The apoptotic trophoblast cells liberate the fetal DNA in the maternal bloodstream.
The half-life of free fetal DNA is brief and the baby’s DNA fragments are no longer detectable 45 minutes after birth. Consequently, the analysis of the mother’s blood cannot be confounded by the presence of fetal DNA from a previous pregnancy.

The majority of the techniques used to analyze fetal DNA are sequencing techniques (DNA reading). These techniques require sophisticated equipment, are costly and consume many hours. Their use is therefore limited.

**ddPCR**

In the Genetics Laboratory at OVO Clinic, a fetal DNA analysis test has been developed with a more economic and faster technology called droplet digital PCR (ddPCR).

This is a non-invasive technique, hence it is not dangerous to the fetus. All that is needed is a blood sample from the pregnant mother as early as the 9th week of pregnancy. Subsequently, the DNA in the maternal blood sample is extracted to be analyzed.

**Trisomies**

Detection of trisomies is performed by amplifying specific sequences from the chromosomes involved in the most common trisomies (trisomy 21, 13 and 18) so they become visible (by fluorescence). We can also use this technique to determine the sex of the baby, and detect sex chromosome related diseases and, as early as the 5th week of pregnancy.

**PCR stands for Polymerase Chain Reaction. It is a technique used in molecular biology to amplify DNA, in other words to make copies of selected regions of the genome. Droplet Digital PCR is performed using a very small amount of DNA. The device, called "DROPLET GENERATOR" will divide the DNA droplet in 20 000 droplets that will be submitted to PCR cycles. Finally, another device, the "DROPLET READER" will read and analyse the amplified sequences.**

**Figure 2: The human genome is stored on 23 chromosome pairs for a total of 46. Trisomy is caused by the presence of a third chromosome on one of these 23 pairs. For example, in trisomy 21, a third chromosome is present on the 21st pair, for a total of 47 chromosomes instead of 46.**

**QX100™ DROPLET READER et DROPLET GENERATOR from the BIO-RAD company.**
The amplified sequences, specific to the chromosomes involved in trisomies, are fluorescently labeled during the PCR process and subsequently detected with an apparatus for detecting fluorescent sequences (the QX100 DROPLET READER). The detection of trisomy in children is done by calculating the positive fluorescent sequences to determine if they are in greater numbers than expected.

Since cells are derived from the placenta and that placental cells divide rapidly, they can divide abnormally and have 47 chromosomes instead of 46. The abnormal cells may be located in a limited area of the placenta and the baby can still be normal. That is why, following this test, if the risk is high, the patient must undertake an amniocentesis to confirm the result (Figure 2).

A step forward for screening

The advantage of this test is that it reduces the number of patients who undergo amniocentesis and detects chromosome abnormalities early in pregnancy.

In conclusion, it must be kept in mind that this is a prenatal screening test and not a prenatal diagnosis test. However, it remains very reliable with its sensitivity and specificity of 99%.

Figure 2:
A) Cells with 47 chromosomes (green) in a restricted area of the placenta: normal fetus
B) Greater number of cells with 47 chromosomes: fetus at risk of low birth weight, malformations and intrauterine death.

A new target for breast cancer treatment highlighted by a RQR team

Dr. Nicolas Gévry, from the Université de Sherbrooke, and Stéphanie Bianco, postdoctoral fellow, demonstrated that the nuclear receptor "LRH-1 plays a critical role in breast cancer by controlling the proliferation of cancer cells, which may lead to resistance to treatment and thus a major obstacle to healing patients. Inhibiting this receptor would produce a novel treatment."

Consult the article (in French only)
RQR Knowledge Transfer Award

During the 7th Symposium in November, the RQR presented awards of $400 to two members who distinguished themselves for their engagement with the end users. The awardees are Dr. Claude Robert from the Université Laval and Clotilde Maurice, PhD student at the Université Laval.

By knowledge transfer, it is implied that the activities are intended for end users and not only to the scientific community. Thus we speak of scientific popularization to make the reproductive biology accessible to end users, including:

- the general public,
- pharmaceutical and biotech companies,
- veterinarians,
- clinicians,
- government
- and other appropriate targets.

Dr. Robert distinguished himself through his many publications in science popularization magazines such as Le producteur de lait québécois. He was also guest speaker at seminars organized for producers, including the Symposium sur les bovins laitiers. He was also involved in the RQR networking workshop with veterinarians in June 2014. Clotilde participated in the Cogito television program organized by graduate students at the Université Laval which is broadcasted on Canal Savoir, in addition to being involved in writing articles for the industry.

The RQR will again offer in 2015 researcher and student awards for knowledge transfer. Feel free to submit your application or to nominate a colleague using the KT award form! Applications can be submitted at any time by email at j.blouin@umontreal.ca.

A new cause of infertility: article published in the journal Le fil

The article, published in the December 4th edition of the newspaper of the University Laval community, reports the research results of Dr. Claude Robert's team, published in the journal "Biology of Reproduction" in October 2014.

"In mammals, it was believed that the oocyte depended solely on its genetic material at the time of its formation to ensure its development. Our study shows the opposite", said Dr. Robert in this article. Consult the article (in french only).

RQR Networking workshop with clinicians

A networking activity between the RQR researchers and the clinicians is scheduled for September. We asked clinicians: «In terms of reproduction, what issue would you like to resolve or what tool/method/protocol would you like to be developed to help your practice and help your patients?»

The networking activity will be held during the annual meeting of the Club de Recherches Cliniques du Québec (CRCQ), from September 24 to 26, 2015 at the Sacacomie Hotel in Saint-Alexis-des-Monts.

To reserve, please contact the RQR Transfer Agent by email at j.blouin@umontreal.ca or by phone at (450) 773-8521 extension 8221. Do not hesitate to contact us to submit your topics!