Université de Montréal

Dépistage prénatal de la trisomie 21 et autres aneuploïdies au premier trimestre

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Cette thèse intitulée :

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Résumé

La présente thèse par articles aborde différentes facettes du dépistage prénatal de certaines aneuploïdies au premier trimestre de la grossesse. L'introduction retrace l'historique du dépistage prénatal et énonce les différents marqueurs biochimiques et échographiques associés aux aneuploïdies. La première publication démontre que le tabagisme maternel abaisse significativement les niveaux sanguins maternels de PAPP-A et de la fraction libre de la β-hCG et augmente significativement la clarté nucale, confirmant la nécessité de contrôler cette co-variable dans le calcul de risque final, du moins pour la trisomie 18. Le deuxième article identifie des seuils de clarté nucale au-delà desquels la biochimie génétique n'apporte aucune valeur additionnelle au dépistage prénatal de la trisomie 21 et de la trisomie 18. Pour les fœtus avec clarté nucale supérieure aux seuils établis, un diagnostic prénatal intrusif devrait être offert sans délai. Le troisième et dernier article porte sur la première détermination des niveaux plasmatiques maternels de la protéine FLRG (*follistatin-related gene*) au premier trimestre de grossesse et sur son rôle potentiel à titre de marqueur biochimique dans le dépistage prénatal de la trisomie 21. Bien que détectables, les niveaux plasmatiques maternels de FLRG ne sont pas significativement altérés en présence de fœtus avec syndrome de Down. Dans la discussion générale, les trois articles sont abordés sous un angle plus spécifique au Québec. Des données complémentaires et originales y sont présentées. Une discussion sur l'évolution future du dépistage prénatal est entamée et des axes de recherche sont proposés.

Mots-clés : aneuploïdie; clarté nucale ; grossesse ; hormone choriogonadotropique, sous-unité bêta ; prééclampsie ; premier trimestre ; protéine A plasmatique associée à la grossesse ; protéines liées à la follistatine ; syndrome de Down ; syndrome d'Edwards ; syndrome de Patau ; tabagisme ; trisomie

Abstract

In this thesis by articles, we explore different facets of first trimester prenatal screening of aneuploidy. Introduction retraces the origin of prenatal screening and enunciates current biochemical and ultrasound markers associated with aneuploidy. In the first article, impact of maternal smoking on first-trimester prenatal screening results is assessed for Down syndrome and trisomy 18. Both maternal blood levels of PAPP-A and free β-hCG are significantly decreased by maternal smoking while fetal nuchal translucency (NT) thickness is significantly increased. Without adjustment, this results in an increase of false positives, at least for trisomy 18. Based on these results, adjustment for smoking should be mandatory in first-trimester prenatal screening. In the second article, we identify NT threshold values above which biochemical screening provides no additional benefit. In pregnancies in which NT is above the proposed upper cut-offs, invasive prenatal screening should be offered without undue delay. In the third and last article, maternal plasma levels of follistatin-related gene protein (FLRG) are determined for the first time in first trimester of pregnancy. Its potential role as a new marker for Down syndrome is assessed. Although FLRG can be successfully detected in maternal plasma, its levels are not significantly altered by the presence of Down syndrome fetuses. In the general discussion, articles are mainly addressed under a Quebec standpoint. Additional and complementary original data are presented and different clinical research avenues are proposed.

Keywords : aneuploidy; chorionic gonadotropin, beta subunit, human; Down syndrome ; Edwards syndrome ; first trimester; follistatin-related proteins; nuchal translucency measurement ; Patau syndrome ; preeclampsia ; pregnancy ; pregnancy-associated plasma protein-A; smoking ; trisomy

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Liste des sigles et abréviations

ACOG: *American Congress of Obstetricians and Gynecologists*

ADAM-12-S : Une désintégrine et métalloprotéase soluble

AETMIS : Agence d'évaluation des technologies et des modes d'intervention en santé

AFP : Alphafœtoprotéine

AG : Âge de la grossesse

AOTN : Anomalies ouvertes du tube neural

B19 : Parvovirus B19, communément appelé la cinquième maladie

β-hCG : Unité bêta de l'hormone choriogonadotrophique

CCMG: Collège canadien des généticiens médicaux

CETS : Conseil d'évaluation des technologies en santé

CN : Clarté nucale

CRL : Longueur céphalocaudale *(crown-rump length*)

CVS : Biopsie des villosités choriales ou biopsie du trophoblaste ou du chorion

D : Détection

DS : *Down syndrome*

DDM : Date des dernières menstruations

ELISA: Dosage immunoenzymatique sur support solide (*Enzyme-linked Immunosorbent Assay*)

FASTER trial: *First and second trimester evaluation of risk trial*

fβ-hCG : Fraction libre de la β-hCG

FBI: *Federal Bureau of Investigation*

FLRG : Gène relié à la follistatine (*Follistatin-related gene*)

FMF: *Fetal Medicine Foundation* (Londres, Angleterre)

FP: Faux positifs

GA : Âge de la grossesse (*gestational age*)

hCG : Hormone choriogonadotrophique

HEC: École des hautes études commerciales

ISUOG: *International Society of Ultrasound in Obstetrics and Gynecology*

LCC : Longueur céphalocaudale

Mab : Anticorps monoclonal

MoM : Multiple de médiane

NIH: *National Institutes of Health*

NT : Clarté nucale (*Nuchal translucency*)

OMIM: *Online Mendelian Inheritance in Man*

PAPP-A : Protéine A plasmatique associée à a grossesse

PlGF : Facteur de croissance placentaire

SD : Écart-type (*Standard deviation*)

SOGC : Société des Obstétriciens et Gynécologues du Canada

SNP : Polymorphisme d'un seul nucléotide (*Single nucleotide polymorphism*)

SURUSS: *Serum Urine and Ultrasound Screening Study*

T1 : Premier trimestre

T2 : Deuxième trimestre

T13 : Trisomie 13 ou syndrome de Patau

T18 : Trisomie 18 ou syndrome d'Edwards

T21 : Trisomie 21 ou syndrome de Down

TIS: Test intégré sérique

TORCH : Toxoplasmose, rubéole, cytomégalovirus, herpès

uE3 : Estriol non conjugué ou libre

VEGF : Facteur de croissance de l'endothélium vasculaire (*Vascular endothelial growth factor*)

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Introduction

Deux à trois pour cent des enfants naissent avec une anomalie congénitale sérieuse (Harper, 1998). Parmi les causes génétiques, la trisomie 21 (syndrome de Down), due à la présence d'un chromosome 21 surnuméraire, paraît être la plus fréquente des aneuploïdies viables. Elle est observée dans environ une naissance sur 700 et le risque d'être porteuse d'un foetus trisomique 21 augmente avec l'âge maternel (Janerich and Bracken, 1986, Yoon et al., 1996). Dans un laboratoire de cytogénétique montréalais, la trisomie 21 est identifiée dans 0,77 % des amniocentèses effectuées pour une indication d'âge maternel avancé, suivi du syndrome d'Edwards (trisomie 18) (0,18 %), d'aneuploïdies des chromosomes sexuels (0,23 %) et du syndrome de Patau (trisomie 13) (0,03 %) (Caron et al., 1999).

La trisomie 21 se traduit par un phénotype particulier (face plate, fente palpébrale mongoloïde, nez plat, cou court, oreille ronde, protusion de la langue, pli palmaire, bouche entre-ouverte en permanence, etc.), un retard mental plus ou moins sévère, de multiples malformations (cardiopathie congénitale, microdontie, malformations de l'intestin grêle et du colon, imperforation anale, agénésie ou hypoplasie rénale, etc.) et une hypotonie musculaire. Le phénotype est décrit pour la première fois de façon convaincante par Jean Étienne Dominique Esquirol (1772-1840), médecin aliéniste à l'hôpital de la Salpêtrière de Paris, puis par Édouard Séguin (1812-1880), psychiatre au même endroit, sous le vocable d'idiotie furfuracée, en le distinguant particulièrement du crétinisme (hypothyroïdie congénitale). John Langdon Haydon Down (1828-1896) donnera également en 1866 une définition clinique de la trisomie 21, qu'il nommera idiotie mongoloïde (Verloes, 2003).

Le coût socio-économique à vie de la trisomie 21 est estimé à environ 612,150 dollars américains [400,000-800,000 $] (Beazoglou et al., 1998, Vintzileos et al., 2000, Caughey et al., 2002, Biggio et al., 2004, Odibo et al., 2005). À lui seul, ce coût social, qui ne tient pas compte du fardeau familial financier, devrait justifier la mise sur pied au Québec d'un programme systématique et universel de dépistage prénatal.

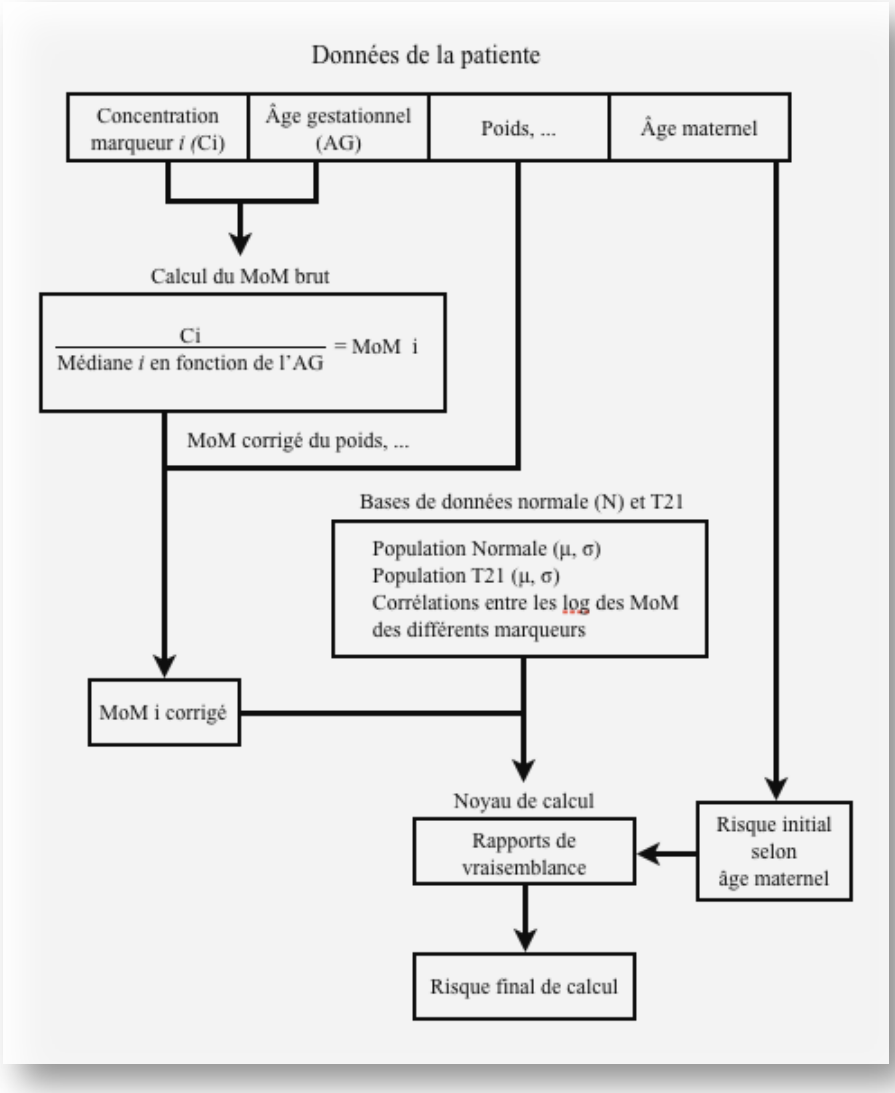
Depuis une quarantaine d'années, le diagnostic prénatal par amniocentèse, fondé sur un caryotype fœtal à partir d'un prélèvement de cellules fœtales, était pratiquement la seule méthode au Québec servant à identifier avant leur naissance les fœtus atteints de trisomie 21. En raison des risques associés à l'amniocentèse, essentiellement la perte fœtale, et de son accessibilité réduite, le besoin de nouvelles approches non effractives s'est fait sentir et a mené au développement du dépistage prénatal.

Le dépistage prénatal de la trisomie 21 et d'autres aneuploïdies comme la trisomie 18 et 13, se singularise en médecine par son principe de faire appel à différentes mesures biologiques, biochimiques, échographiques et cliniques, qui conjointement avec l'âge maternel et l'âge de la grossesse permettent un calcul de risque a posteriori, plus précis que celui se basant uniquement sur l'âge maternel.

La façon dont on procède pour calculer un risque final de trisomie utilise le principe général suivant (Figure 1) :

- on identifie d'abord le risque a priori lié à l'âge maternel ; pour ce besoin, des tables standardisées de risques sont disponibles dans la littérature (Cuckle et al., 1987);
- on détermine le multiple de médiane (MoM) pour de chacun des marqueurs biologiques pertinents, corrigés par les différentes co-variables qui l'influencent significativement, tels que l'âge de la grossesse, le poids, l'âge de la patiente, etc. ;
- on calcule, selon de bases de données disponibles provenant de populations saines et atteintes, le rapport de vraisemblance pour chacun des marqueurs biologiques étudiés ;
- un noyau de calcul est finalement utilisé afin de produire un risque a posteriori, tenant compte non seulement de l'âge maternel, mais également des multiples de médiane corrigés de différents marqueurs, des rapports de vraisemblance provenant des tables de données de populations normales et pathologiques et des coefficients de corrélation entre les logarithmes des multiples de médiane des différents marqueurs (Morin et al., 1999).

Figure 1. Principe général du dépistage prénatal de la trisomie 21.



Adapté de (Morin et al., 2003)

Afin de répondre à un besoin exprimé par la population générale et la communauté médicale et en l'absence d'intérêt du Ministère de la Santé et Services sociaux du Québec, nous avons procédé en 1998, à Montréal, à la mise sur pied d'un tel programme de dépistage prénatal d'abord au second trimestre de grossesse, puis au premier trimestre, en combinant l'échographie fœtale (mesure de la clarté nucale) à des marqueurs maternels sanguins (fraction libre de la β-hCG et PAPP-A).

L'établissement d'un tel programme de dépistage prénatal suivait un courant déjà bien établi dans plusieurs pays occidentaux. Son objectif visait non seulement la détection de la trisomie 21, mais également celle d'autres aneuploïdies tels le syndrome d'Edwards et le syndrome de Patau, et la détection précoce d'anomalies fœtales structurales majeures.

Ce programme vit le jour grâce à une collaboration multidisciplinaire impliquant plusieurs de mes collègues qui ont tout mon respect, dont entre autres, le Pr Jean Lambert (Ph. D. en biostatistiques), Dr Serge Melançon (généticien) et Dr Sylvie Mercier (Ph. D.).

L'expertise acquise au cours des dernières années dans le domaine du dépistage prénatal m'a donc permis d'amorcer la présente thèse de doctorat en Sciences biomédicales (option générale) à l'Université de Montréal.

Afin de compléter ma formation, j'ai pu compléter les cours suivants à l'Université de Montréal et à l'Université McGill :

- Introduction à la biostatistique
- Concepts de base en épidémiologie
- Analyse de données catégorielles
- Recherche clinique biomédicale
- Carrière de chercheur en santé

Mon examen général de synthèse a été complété avec succès en 2008. J'en suis maintenant à l'étape finale, soit la soutenance de ma thèse de doctorat.

L'objectif général de ma thèse était d'améliorer le dépistage prénatal d'aneuploïdies, au premier trimestre de la grossesse. Plus spécifiquement, j'ai étudié l'effet du tabagisme sur les résultats finaux du test de dépistage et démontré l'importance de contrôler cette variable confondante afin de réduire le taux de faux positifs, principalement pour la trisomie 18. Jusqu'à cet article, l'effet du tabagisme sur les marqueurs biochimiques n'était aucunement contrôlé lors d'un dépistage sanguin sur papier buvard (sang séché). J'ai également

identifié des seuils plus précis de clarté nucale qui permettent d'offrir sans délai aux patientes à risque élevé un test diagnostique, sans la nécessité de recourir à une biochimie génétique. Finalement, j'ai étudié le potentiel d'un marqueur biochimique dans le sang maternel, la protéine FLRG, avec l'espoir d'améliorer le taux de détection d'aneuploïdies.

Ma thèse est principalement composée de trois articles qui ont été acceptés et publiés dans des revues avec comité de pairs. Les trois articles dont je suis l'auteur principal ont été publiés en anglais et portent donc sur les sujets suivants :

1. Effet du tabagisme maternel sur le dépistage prénatal de la trisomie 21 (syndrome de Down) et de la trisomie 18 (syndrome d'Edwards) au premier trimestre de grossesse (Miron et al., 2008);

2. Seuils de clarté nucale dans le dépistage prénatal de la trisomie 21 (syndrome de Down) et de la trisomie 18 (syndrome d'Edwards) (Miron et al., 2009);

3. Niveaux plasmatiques de la protéine FLRG (*follistatin-related gene*) au premier trimestre de grossesses avec une trisomie 21 (syndrome de Down) (Miron et al., 2010).

Bien qu'utile cliniquement, le dépistage prénatal d'aneuploïdies en présence de grossesses multiples n'a pas été abordé volontairement. Leur nombre restreint n'aurait pas permis des comparaisons satisfaisantes et leur intégration dans les populations étudiées aurait pu biaiser les résultats.

Historique du dépistage prénatal de la trisomie 21 et autres aneuploïdies

L'amniocentèse et le diagnostic prénatal

Le dépistage prénatal de la trisomie 21 comme on le connaît aujourd'hui prend ses origines du diagnostic prénatal qui se développa grâce à l'amniocentèse. Les premiers prélèvements publiés de liquide amniotique furent réalisés il y a déjà plus de 130 ans. L'amniocentèse transabdominale au troisième trimestre de grossesse est en effet rapportée pour la première fois dans la littérature par Prochownick, Von Schatz et Lambl en 1877 et par Schatz dans les années 1890. Hinkel décrit en 1919 son utilisation pour soulager une patiente souffrant de polyhydramnios (Woo, 2002). En 1933, sa pratique chez 25 femmes normales, puis chez 50 femmes en 1946 pour polyhydramnios, apparaît sécuritaire. Son innocuité tant pour le fœtus que pour la mère, son caractère expérimental sans consentement éclairé et son éthique sont toutefois rapidement questionnés et débattus publiquement (Morowitz, 1957, Parrish et al., 1957a, Parrish et al., 1957b). Malgré tout, sa popularité grandira dans les années 1960 avec les besoins de mieux prédire les risques alors fréquents d'érythroblastose fœtale causée par l'allo-immunisation Rhésus. Les travaux originaux de Liley, permettant de mesurer les taux de bilirubine dans le liquide amniotique et le développement de la transfusion fœtale *in utero*, favoriseront son essor (Liley, 1961, Barton and Stander, 1963, Duggan and Taylor, 1964, Keller, 1965).

Parallèlement au développement de l'amniocentèse, les généticiens médicaux sont dans les années 1940-1950 désespérément à la recherche de méthodes fiables qui leur permettraient d'identifier *in utero* le sexe fœtal en présence d'histoire familiale de maladies liées au X, comme l'hémophilie. Rosa et Fanard démontrent en 1949 que les cellules fœtales obtenues à la suite d'une centrifugation de liquide amniotique diffèrent histologiquement, selon le sexe d'un fœtus, par la présence ou non de l'hétérochromatine sexuelle appliquée contre la face interne de l'enveloppe nucléaire de la plupart des cellules somatiques, le corpuscule de Barr (Rosa and Fanard, 1949). Leur technique est raffinée et en 1955-56, quatre groupes

différents de chercheurs peuvent finalement être crédités de la prédiction du sexe du fœtus humain par analyse de cellules fœtales dans le liquide amniotique et donc, de son utilisation à des fins de diagnostic génétique prénatal (Serr et al., 1955, Fuchs and Riis, 1956, Makowski et al., 1956, Shettles, 1956). La technique ne fait toutefois pas l'unanimité. Ainsi, au Canada, son bénéfice médical réel est initialement questionné par celui-là même qui identifia le corpuscule de Barr (Barr, 1956).

L'utilisation clinique de l'amniocentèse à des fins de diagnostic prénatal génétique présentera un intérêt pratique accru grâce à trois évènements scientifiques cruciaux, soient : (1) l'identification du nombre exact de chromosomes chez l'humain (2) l'association d'une anomalie chromosomique spécifique (un chromosome 21 supplémentaire) au syndrome de Down et (3) la démonstration qu'une analyse chromosomique peut être réalisée avec succès sur des cellules amniotiques mises en culture (Ford and Hamerton, 1956, Tjio and Levan, 1956, Lejeune et al., 1959, Steele and Breg, 1966). On confirme ainsi, peu après ces découvertes, la possibilité d'établir un diagnostic prénatal de la trisomie 21 par amniocentèse (Valenti et al., 1968).

Au début des années 1970, les indications génétiques d'offrir l'amniocentèse sont alors basées sur des paramètres cliniques, comme un antécédent d'enfant trisomique, être porteur d'une malformation ouverte du tube neural ou la présence d'une maladie héréditaire ou d'une aberration chromosomique déjà connue chez l'un des parents (maladies autosomiques, liées au X ou translocations). Grâce aux publications confirmant chez les femmes plus âgées un risque accru de donner naissance à un enfant trisomique, l'âge maternel avancé deviendra rapidement l'indication principale de proposer une amniocentèse (Shuttleworth, 1909, Penrose, 1933, Lindsjo, 1974, Ferguson-Smith and Ferguson-Smith, 1976).

Au Québec, peu de données existent quant à la prévalence réelle de la trisomie 21. Entre 1958 et 1967, on note une prévalence de 1.86 par 1,000 naissances, comparable à celle retrouvée dans la plupart des pays occidentaux. Bien qu'entre 1948 et 1957, la prévalence semble plus faible (0.9/1,000 naissances), elle est attribuable principalement à un taux plus

élevé de mortalité périnatale (McDonald, 1972). Le premier diagnostic prénatal aurait été effectué au Québec dès 1968 (Dallaire et al., 1971). L'expérience de l'hôpital Sainte-Justine, sur plus de 20 ans (de 1972 à 1995) et basée sur 35,131 amniocentèses, confirme que les indications principales à des fins de diagnostic prénatal sont alors : (1) un âge maternel avancé (71 %) (2) une anomalie chromosomique antérieure (3 %) (3) un remaniement de la structure chromosomique chez l'un des parents (0.5 %) et (4) une histoire familiale d'aneuploïdie chez la parenté (6 %). La trisomie 21 représente de loin l'aneuploïdie la plus fréquente (67 %), suivie de la trisomie 18 (12 %) et de la trisomie 13 (7 %) (Caron et al., 1999).

L'amniocentèse connaîtra son apogée au cours des années 1970-80. Grâce à elle, l'identification d'une multitude d'anomalies génétiques et de maladies métaboliques sera ainsi réalisée et publiée (Benson, 1971, Nadler and Gerbie, 1971, Davidson and Rattazzi, 1972). La biochimie jouera alors un rôle grandissant dans le diagnostic prénatal.

L'alphafœtoprotéine et le début du dépistage prénatal biochimique

Au début des années 1970, la communauté scientifique s'intéresse particulièrement à l' alphafœtoprotéine (AFP) découverte plusieurs années auparavant dans le sérum de fœtus humains et dont la particularité est d'être uniquement d'origine fœtale (Bergstrand and Czar, 1956). Des études physiologiques confirment que l'AFP est présente dans le sérum fœtal dès la quatrième semaine de grossesse. Ses concentrations augmentent rapidement pour atteindre un sommet à la fin du premier trimestre de grossesse ; elles demeurent par la suite constantes jusqu'à la 30^{e} semaine de grossesse puis déclinent progressivement jusqu'à la naissance (Gitlin and Boesman, 1966, Gitlin and Boesman, 1967). La découverte que les niveaux sériques fœtaux d'AFP se reflètent également dans le liquide amniotique incite alors les chercheurs à comparer les concentrations d'alphafœtoprotéine dans le liquide amniotique de grossesses normales à celles compliquées par des anomalies ouvertes du tube neural (AOTN). C'est ainsi qu'on note au deuxième trimestre de grossesse, dès 1972, une

augmentation significative des concentrations d'AFP dans le liquide amniotique en présence d'anencéphalie et de spina bifida (Brock and Sutcliffe, 1972, Coltart et al., 1974). Le dosage d'AFP dans le liquide amniotique apparaît toutefois peu pratique cliniquement comme méthode de dépistage. Vu les risques associés à l'amniocentèse, il n'est alors proposé qu'aux femmes enceintes ayant une histoire personnelle ou familiale d'AOTN, offrant ainsi un taux de détection de seulement 5 % de toutes les AOTN. L'approche se doit donc d'être réévaluée.

L'observation que les concentrations d'AFP sont élevées dans le sérum maternel en présence de détresse fœtale ou de mort *in utero* au troisième trimestre de grossesse suscite dès 1973 l'intérêt des scientifiques quant à son potentiel dans la détection non effractive d'AOTN (Seppala and Ruoslahti, 1973). La première publication vérifiant son utilité décrit un cas d'anencéphalie avec des niveaux d'AFP sérique maternelle marginalement élevés à la 16^{e} semaine de grossesse et fortement élevés à la 21^{e} semaine (Brock et al., 1973). Plusieurs études confirmeront par la suite la validité du dosage de l'AFP dans le sérum maternel comme méthode de dépistage des AOTN (Leek et al., 1973, Seller et al., 1974, Wald et al., 1974, Wald et al., 1977). Ainsi, en établissant un seuil supérieur de 2.5 multiples de la médiane (MoM), le dépistage par AFP dans le sérum maternel, effectué idéalement entre les 16^{e}-18^{e} semaines de grossesse, permettra de détecter ≥ 95 % des anencéphalies et entre 65 et 80 % des *spina bifida* ouverts avec un taux de faux positifs variant entre 1 et 3 % (Bradley et al., 2005).

La trisomie 21 et le triple test du 2^{e} trimestre

Une fois la valeur clinique démontrée de l'AFP maternelle sérique dans le dépistage des AOTN, ce n'est qu'une question de temps avant qu'elle ne soit vérifiée dans les cas de syndrome de Down. La première publication sur le sujet, dont l'objet principal était d'étudier au troisième trimestre les niveaux d'AFP dans le liquide amniotique de grossesses avec anencéphalie, rapporte de façon isolée un cas de syndrome de Down avec des concentrations basses d'AFP (Norgaard-Pedersen et al., 1976). Ce n'est pourtant qu'en

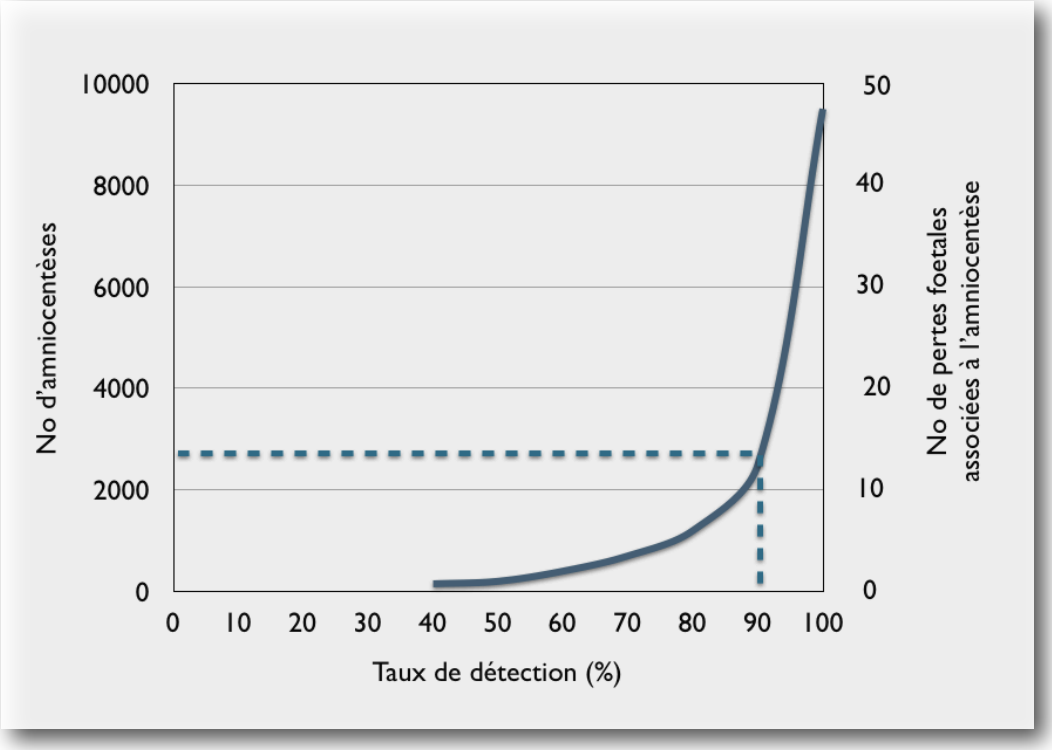
1984 que deux équipes démontreront presque simultanément une relation significative entre les niveaux diminués d'AFP dans le sérum maternel et la trisomie 21 (Cuckle et al., 1984, Merkatz et al., 1984).

C'est le début du dépistage biochimique de la trisomie 21! On construit initialement des tables prédictives calculant le risque de la trisomie 21 en combinant l'âge maternel à des niveaux sériques maternels d'AFP convertis en multiples de médiane (Hershey et al., 1986). S'ajouteront peu après d'autres marqueurs biochimiques qui mèneront au développement de la première génération de tests de dépistage prénatal de la trisomie 21 au second trimestre de grossesse combinant à l'âge maternel, l'AFP (↓), l'hormone choriogonadotrophique (hCG) (↑) et l'oestriol (↓) dans le sérum maternel, communément appelé le « triple test » (Bogart et al., 1987, Canick et al., 1988, Wald et al., 1988a, Wald et al., 1988b, Norgaard-Pedersen et al., 1990). Il est intéressant de noter ici que des niveaux diminués d'oestriol urinaire maternel avaient, en présence de syndrome de Down, été observés bien avant, soit près de vingt ans avant son application clinique ! (Macafee et al., 1970).

La popularité du triple test au second trimestre de grossesse grandira au cours des années 1990. Son application de routine, non seulement chez les jeunes femmes, mais également chez celles de 35 ans et plus, confirmera son efficacité à réduire le nombre d'amniocentèses. Aux États-Unis, une étude démontrera ainsi dès 1994, le potentiel du triple test à réduire de 75 % le nombre d'amniocentèses. Pour une population hypothétique de 10,000 femmes âgées de 35 ans ou plus, la prévalence de la trisomie 21 est estimée à 1 : 100 et le taux de pertes fœtales associées à l'amniocentèse, à 5 par 1,000 procédures. Dans cet exemple proposé par les auteurs, si un tel groupe hypothétique subit d'emblée une amniocentèse sans le dépistage biochimique, 50 pertes fœtales se produiront et tous les cas de syndrome de Down seront identifiés. Si le triple test est plutôt offert en première ligne avec un seuil de détection de 1:200 et un taux de faux positifs de 25 %, seulement 2 500 amniocentèses auraient été effectuées, 13 pertes fœtales associées à l'amniocentèse se seraient produites et 89 % des cas de syndrome de Down auraient été identifiés. Détecter les 11 cas de syndrome de Down non identifiés par la biochimie génétique nécessiterait

donc 7,500 amniocentèses additionnelles et résulterait en une perte fœtale additionnelle de 37 fœtus sains (Haddow et al., 1994) (Figure 2).

Figure 2. Impact du triple test sur le nombre d'amniocentèses et la réduction de pertes fœtales.



Adapté de (Haddow et al., 1994)

En extrapolant ces données aux 380,000 grossesses se produisant chaque année chez les femmes de 35 ans et plus aux États-Unis, les auteurs démontrèrent ainsi, par l'utilisation du triple test, une réduction approximative en coûts diagnostiques de 250 millions $.

La supériorité du dépistage prénatal biochimique universel de la trisomie 21 au second trimestre à l'amniocentèse sera confirmée par une autre étude s'étendant de 1974 à 1997 et portant sur la population américaine. On y note, avec les années, un accroissement

substantiel aux États-Unis de la prévalence de syndromes de Down identifiés au second trimestre, soit 1 dans 740 en 1974 à 1 dans 504 en 1997. La proportion de fœtus atteints identifiés à 16 semaines de grossesse chez les femmes de 35-49 ans passe de 28.5 % en 1974 à 47.3 % en 1997. Pour la population de 1997, il est déduit qu'avec un triple test systématique offert à toutes les femmes enceintes aux États-Unis, le nombre d'amniocentèses serait réduit de 154,756, un nombre supplémentaire de 1,556 fœtus avec syndrome de Down aurait été détecté et 773 pertes fœtales associées à l'amniocentèse auraient été prévenues comparativement à la politique d'offrir de routine le recours à l'amniocentèse chez les femmes de 35 ans et plus. Les auteurs recommanderont d'ailleurs en conclusion l'abandon de cette politique d'amniocentèse telle qu'établie par consensus en 1978 par les Instituts nationaux américains de la santé (NIH, 1979, Egan et al., 2000).

En 1992, un quatrième marqueur sanguin servant à la détection de la trisomie 21 au second trimestre de la grossesse est proposé : l'inhibine. Comparés à la population générale, les niveaux d'inhibine immunoréactive dans le sérum maternel paraissent être, en présence de fœtus trisomiques 21, 1.9 fois plus élevés que la médiane normale. Bien que d'autres confirmeront cette information, certains suggéreront que son ajout au triple test apporte peu au taux de détection déjà obtenu (Van Lith et al., 1992, Spencer et al., 1993). L'utilisation d'un immuno-essai plus spécifique à l'inhibine A dimérique ouvre toutefois la porte à un rôle possible pour ce marqueur dans le dépistage de la trisomie 21 tant au premier qu'au deuxième trimestre de grossesse (Cuckle et al., 1995, Wallace et al., 1995). On suggérera ainsi son ajout aux trois autres marqueurs sériques du second trimestre (β-hCG, AFP, oestriol) en démontrant que le dosage de l'inhibine A augmente, à un taux fixe de faux positifs de 5 %, le taux de détection de la trisomie 21 de 53 % à 75 % (Aitken et al., 1996). Le « Quad test » est né (Wald et al., 1996, Lambert-Messerlian and Canick, 2004).

Tous ces marqueurs s'avéreront également assez efficaces pour dépister d'autres aneuploïdies, leurs concentrations variant selon la pathologie (Tableau I) (Spencer, 2007).

Tableau I. Concentrations des marqueurs biochimiques selon l'aneuploïdie

Aneuploïdie	AFP	hCG	Inhibine A	Estriol libre
T21	↓	↑	↑	↓
T18	↓	↓	↓ légère	↓
T13	↑ légère	N	N	N
Turner	↓ légère	↑/↓ ± anasarque	↑/↓ ± anasarque	↓ légère
Sexuelle	N ou ↑	N ou ↑		N
Triploïdie I	↑	↑		
Triploïdie II	N	↓		

N= normale ; ↑= augmentée ; ↓= diminuée ; Source : adapté de (Spencer, 2007)

Avec les avancées scientifiques, l'utilisation en première ligne des triple et quadruple tests sera toutefois rapidement dépassée par des approches plus précoces de dépistage prénatal au premier trimestre de grossesse.

Évolution du dépistage prénatal vers le premier trimestre

Au cours des années 1990, de nouveaux marqueurs de la trisomie 21 au premier trimestre de grossesse seront identifiés. Ils seront rapidement préférés aux tests de dépistage prénatal du deuxième trimestre. Ce phénomène s'explique aisément par la préférence chez la grande majorité des femmes d'obtenir tôt en grossesse, dès le premier trimestre, l'information la plus complète possible sur la santé de leur enfant en devenir (de Graaf et al., 2002, Spencer and Aitken, 2004). Le dépistage prénatal du premier trimestre présentera aussi l'originalité de combiner deux outils de dépistage, soit l'échographie fœtale avec la mesure entre autres de la clarté nucale et la biochimie génétique (dosage de la protéine-A plasmatique associée à la grossesse (PAPP-A) et la fraction libre de la β-hCG).

La PAPP-A, une métalloprotéinase de haut poids moléculaire principalement d'origine placentaire, fut caractérisée en 1974 (Lin et al., 1974). En 1990, on rapporte pour la première fois, en présence de trisomie 21 et au premier trimestre de la grossesse, des

concentrations basses de PAPP-A dans le sérum maternel. Sur 13 grossesses avec un syndrome de Down, sept ont des niveaux inférieurs ou égaux au 5e percentile (Brambati et al., 1990). Le lien entre des niveaux sériques élevés de la fraction libre de la β-hCG et la trisomie 21 sera, quant à lui, identifié en 1992 (Spencer et al., 1992). La combinaison originale de ces deux marqueurs permettra d'identifier près de 79 % des trisomies 21 entre 8 et 12 semaines et leur incorporation avec la clarté nucale (CN) sera proposée peu après (Brambati et al., 1994, Zimmermann et al., 1996, Forest et al., 1997, Wald and Hackshaw, 1997). Depuis, la validité de cette approche, combinant la clarté nucale, la PAPP-A et la fraction libre de la β-hCG, a été confirmée prospectivement sur plus de 200,000 dépistages au premier trimestre de grossesse, offrant globalement des taux de détection de la trisomie 21 de 88 % avec 5 % de faux positifs (Spencer, 2007).

Le test combiné du premier trimestre servira également à dépister d'autres types d'aneuploïdies, la PAPP-A et la fraction libre de la β-hCG étant diminuées et la clarté nucale augmentée en présence de trisomies 18 et 13 (Zimmermann et al., 1996, Spencer et al., 2000a, Miron et al., 2008).

Une variété de combinaisons entre des marqueurs du premier trimestre et des marqueurs biochimiques du second trimestre sera ensuite suggérée, tels les tests intégré, séquentiel et de contingence, complexifiant le processus et n'améliorant pas nécessairement l'efficience du dépistage prénatal de la trisomie 21 (Cuckle, 2002, Wald et al., 2003). À de telles approches, une étude récente opposera la supériorité du test de dépistage du premier trimestre combiné tout simplement à l'échographie fœtale de routine réalisée à la 20-22e semaine de grossesse (Rozenberg et al., 2007). Par ailleurs, il était prévisible que de nouveaux marqueurs s'ajouteraient sur une base régulière aux marqueurs actuels du premier trimestre, comme l'os nasal et l'angle maxillofrontal fœtaux (Tableau II) (Cicero et al., 2001, Sonek et al., 2007).

Tableau II. Différentes stratégies de dépistage prénatal de la trisomie 21

Stratégies de dépistage	Marqueurs	Moment du test	Moment de la divulgation des résultats
Dépistage combiné du premier trimestre	Clarté nucale ± autres marqueurs échographiques + PAPP-A et βhCG libre	1er trimestre	1er trimestre
Dépistage sérique du deuxième trimestre			
- Triple test	AFP, oestriol et hCG	2e trimestre	2e trimestre
- Test quadruple (QUAD)	AFP, oestriol, hCG et Inhibine A	2e trimestre	2e trimestre
Dépistages en deux étapes			
- Intégré complet	Clarté nucale et PAPP-A (1er trimestre) + AFP, oestriol, hCG et Inhibine A (2e trimestre)	1er et 2e trimestres	Les résultats du premier trimestre ne sont pas communiqués. Les résultats ne sont divulgués qu'au 2e trimestre
- Intégré sérique	PAPP-A (1er trimestre) et AFP, oestriol, hCG et Inhibine A (2e trimestre)	1er et 2e trimestres	Les résultats du premier trimestre ne sont pas communiqués. Les résultats ne sont divulgués qu'au 2e trimestre
- Séquentiel indépendant	Clarté nucale, PAPP-A et βhCG libre (1er trimestre) ± AFP, oestriol, hCG et Inhibine A (2e trimestre)	1er et 2e trimestres	Interprétation indépendante des tests du 1er et du 2e trimestres
- Séquentiel en deux étapes (*stepwise*)	Clarté nucale, PAPP-A et βhCG libre (1er trimestre) ± AFP, oestriol, hCG et Inhibine A (2e trimestre)	1er et 2e trimestres	Pour les patientes à risque élevé (≥ 1:50), les résultats sont communiqués immédiatement au 1er trimestre; pour celles en dessous du seuil établi (<1:50), une évaluation des marqueurs biochimiques du 2e trimestre est réalisée et les résultats combinés sont produits au 2e trimestre
- Contingent (ou conditionnel) avec biochimie du 2e trimestre	Clarté nucale, PAPP-A et βhCG libre (1er trimestre) ± AFP, oestriol, hCG et Inhibine A (2e trimestre)	1er trimestre ± 2e trimestre	Pour les patientes à risque élevé (≥ 1:50) et à risque faible (≤ 1:1000), les résultats sont communiqués immédiatement au 1er trimestre; pour celles à risque intermédiaire (1:50-1:1000), une évaluation des marqueurs biochimiques du 2e trimestre est réalisée et les résultats combinés sont produits au 2e trimestre
- Contingent (ou conditionnel) avec sonogramme génétique du 2e trimestre	Clarté nucale, PAPP-A et βhCG libre (1er trimestre) ± sonogramme génétique (2e trimestre)	1er trimestre ± 2e trimestre	Pour les patientes à risque élevé (≥ 1:250) et à risque faible (≤ 1:1000), les résultats sont communiqués immédiatement au 1er trimestre; pour celles à risque intermédiaire (1:250-1:1000), une évaluation des marqueurs du 2e trimestre est réalisée et les résultats finaux sont produits au 2e trimestre

Échographie foetale du premier trimestre de grossesse dans le dépistage d'aneuploïdies

Outre les marqueurs biochimiques, plusieurs signes d'appel échographiques entre les 11^{e} et 14^{e} semaines de grossesse ont été récemment associés à l'aneuploïdie foetale, dont la trisomie 21, et suggèrent de nos jours une indication de diagnostic prénatal génétique. Déjà, plusieurs anomalies structurales foetales avaient été clairement associées au second trimestre de grossesse à certaines aneuploïdies (Tableau III) (Eydoux et al., 1989, Daniel et al., 2003, Breathnach et al., 2007).

La Société des Obstétriciens et Gynécologues du Canada de concert avec le Collège canadien des Généticiens médicaux et l'*American Congress of Obstetricians and Gynecologists*, ont d'ailleurs identifié dans leur guide de pratique respectif certaines de ces anomalies structurales majeures pour lesquelles un diagnostic prénatal génétique est recommandé (Tableau IV) (Chodirker et al., 2001, ACOG, 2007). Avec le raffinement constant de l'imagerie échographique, de 50 % à 70 % de ces anomalies structurales fœtales majeures seraient maintenant détectables dès les 11^{e}-14^{e} semaines de grossesse, d'où le rôle grandissant de l'échographie fœtale au premier trimestre de grossesse (Flood and Malone, 2008).

Tableau III. Signes échographiques d'aneuploïdies fœtales au 2e trimestre entre les 15e et 20e semaines de grossesse

	Anomalies structurales	Marqueurs subtils	RCIU
Trisomie 21	Cardiopathies Atrésie duodénale Brachycéphalie Hydrocéphalie Clinodactylie Hygroma kystique Anasarque	Pli nucal épaissi Ventriculomégalie Humérus ou fémur court Nez hypoplasique Intestins échogènes Pyélectasie Espace entre 1er-2e orteils	√
Trisomie 18	Cardiopathies Atrésie œsophagienne Tête en forme de fraise Hernie diaphragmatique Omphalocèle Agénésie du corps calleux Fente faciale Pieds bots Pieds en piolet Aplasie radiale Chevauchement des doigts Kystes du cordon ombilical Hygroma kystique Anasarque	Kystes des plexus choroïdes Méga grande citerne Ventriculomégalie Humérus ou fémur court Hypoplasie nasale Intestins échogènes Pyélectasie Artère ombilicale unique	√
Trisomie 13	Cardiopathies Hernie diaphragmatique Omphalocèle Holoprosencéphalie Fente faciale Cyclopie Agénésie du corps calleux Pieds en piolet Polydactylie Pieds bots Hygroma kystique Anasarque	Foyer intracardiaque échogène Mega grande citerne Ventriculomégalie Pyélectasie Artère ombilicale unique	√

Adapté de : The second trimester genetic sonogram (Breathnach et al., 2007)

Tableau IV. Risque d'aneuploïdies en présence d'anomalies structurales majeures

Défaut structural échographique	Incidence populationnelle	Risque d'aneuploïdie	Aneuploïdies les plus communes
Hygroma kystique	1/120 EP/-1/6000 NV	60-75%	45,X ; T21, T18, T13, XXY
Anasarque	1/1,500-4,000 NV	30-80%	T13, T21,T18, 45,X
Hydrocéphalie	3-8/10,000 NV	3-8%	T13, T18, triploïdie
Holoprosencéphalie	1/16,000 NV	40-60%	T13, T18, 18p-
Cardiopathies congénitales	7-9/1,000 NV	5-30%	T21, T18, T13, T22, T8, T9
Cardiopathies congénitales conotroncales		40-70%	T21
Hernie diaphragmatique	1/3,500-4,000 NV	20-25%	T13, T18, T21, 45,X
Omphalocèle	1/5,800 NV	30-40%	T13, T18
Atrésie duodénale	1/10,000 NV	20-30%	T21
Obstruction distale à la vessie	1-2/1,000 NV	20-25%	T13, T18
Fente faciale	1/700 NV	1%	T13, T18, délétions
Anomalies réductionnelles des membres	4-6/10,000 NV	8%	T18
Pieds bots	1.2/1,000 NV	6%	T18, T13, 4p-, 18q-
Hydranencéphalie	2/1,000 AI	Minime	
Gastroschisis	1/10,000-15,000	Minime	
Artère ombilicale unique	1.2/1,000	Minime	

Abréviations : EP, Échographie précoce ; NV, naissance vivante ; AI, autopsie infantile

Adapté de l'ACOG Practice Bulletin. Invasive prenatal testing for aneuploidy (ACOG, 2007)

Parmi les signes échographiques les plus fréquemment rencontrés et identifiables au premier trimestre et qui présentent selon la littérature une indication de diagnostic prénatal, on note, en ordre décroissant de fréquence:

1. Clarté nucale (> 4 mm ou selon l'âge de la grossesse et maternel)
2. Intestin échogène
3. Absence d'ossification des os propres du nez (avec risque *a priori* intermédiaire 1:100 - 1:1000)
4. Cardiopathies congénitales
5. Hygroma kystique
6. Mégavessie
7. Anasarque fœtale non immune

8. Hydrocéphalie
9. Anomalies ouvertes du tube neural
10. Hernie diaphragmatique
11. Omphalocèle
12. Agénésie du rayon radial
13. Atrésie duodénale
14. Holoprosencéphalie

Pour chacun de ces signes échographiques, une brève description est faite ci-dessous incluant entre autres sa prévalence et les anomalies génétiques et chromosomiques les plus souvent associées.

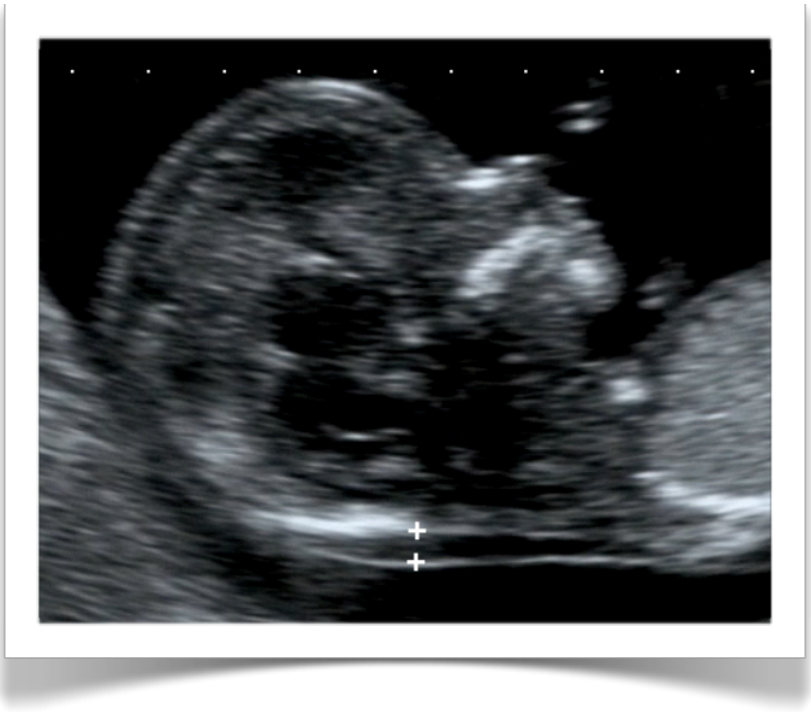
Clarté nucale

Brève description : accumulation de fluide sous la peau nucale fœtale entre les 11e et 14e semaines de grossesse.

Prévalence de clarté nucale augmentée : 5 % des foetus ont à une clarté nucale ≥ 1.9 mm ou ≥ 2.7 mm à la 11e et 14e semaine, respectivement (≥ 95e percentile).

Anomalies chromosomiques principalement associées : syndrome de Down, trisomie 18, trisomie 13, syndrome de Turner et une variété de syndromes génétiques.

Figure 3. Clarté nucale



(Source P. Miron)

En 1866, Langdon Down observe pour la première fois que la peau des enfants avec syndrome de Down manque d'élasticité et qu'elle est épaissie comparativement au reste du corps (Down, 1866). On réalise au milieu des années 1980 que cet excès de peau peut être

visualisé par échographie au second trimestre de grossesse sous la forme d'un épaississement de la nuque, le pli nucal (Benacerraf et al., 1985). On observera par la suite que tous les fœtus présentent entre les 11e et 14e semaines de grossesse une accumulation normale de fluide sous la peau nucale que l'on désigne par la clarté nucale. Ce n'est plus qu'une question de temps avant que l'on associe à la trisomie 21 et à d'autres aneuploïdies, une accumulation excessive de ce fluide sous la nuque.

Ainsi, en 1992, on propose pour la première fois la clarté nucale comme marqueur échographique d'anomalies chromosomiques fœtales au premier trimestre de la grossesse, principalement pour la trisomie 21 (Nicolaides et al., 1992). Dans cette étude portant sur 827 fœtus, 35 % de ceux qui avaient une clarté nucale de 3 à 8 mm d'épaisseur présentaient une anomalie chromosomique confirmée par amniocentèse ou par biopsie du chorion, alors qu'on ne retrouvait que 1 % d'anomalies chromosomiques chez les fœtus avec clarté nucale de moins de 3 mm. Suite à cette publication charnière de Nicolaides, plusieurs études avec un nombre important de sujets confirmeront que le dépistage prénatal de la trisomie 21 par mesure de la clarté nucale présente, combiné à l'âge maternel et à l'âge de la grossesse, un taux de détection variant entre 69 % et 75 % avec un taux de faux positifs de 5 % à 8.1 % (Wald et al., 2003, Wapner et al., 2003, Malone et al., 2005b). De nos jours, la clarté nucale est le marqueur échographique d'aneuploïdies fœtales le plus puissant et donc, le plus populaire.

En 2007, la Société des Obstétriciens et Gynécologues du Canada et le Collège canadien des Généticiens Médicaux recommandent toutefois conjointement que tout dépistage prénatal de la trisomie 21 offert aux Canadiennes devrait permettre au minimum, l'obtention d'un taux de détection de 75 % et d'un taux de faux positif d'au plus 3 %. Ils recommandent également que la clarté nucale « ne doit pas être offerte, à titre de dépistage, sans évaluation de marqueurs biochimiques, sauf dans les cas de grossesses multifœtales » (Summers et al., 2007).

Cette directive clinique peut cependant être contestée avec la venue de nouveaux marqueurs échographiques (tels que les os propres du nez, l'angle fronto-maxillaire, etc.) qui, en

absence d'associations statistiques significatives entre eux, pourraient être combinés à la clarté nucale, et augmenter ainsi les taux de détection de la trisomie 21 à plus de 85 %.

De plus, une variété de syndromes génétiques et de malformations congénitales ont été associés à une clarté nucale augmentée (Tableau V).

Tableau V. Conditions associées à une clarté nucale augmentée

Malformations cardiaques	Syndrome de Jarcho-Levin
Hernie diaphragmatique	Syndrome de Joubert
Omphalocèle	Syndrome de Meckel-Gruber
Achondrogénèse type II	Syndrome de Nance-Sweeney
Achondroplasie	Syndrome de Noonan
Dystrophie thoracique asphyxiante	Ostéogénèse imparfaite
Syndrome de Beckwith-Wiedemann	Syndrome de Perlman
Ostéochondrodysplasie de Blomstrand	Syndrome de Roberts
Syndrome du cordon court (« Body Stalk »)	Syndrome Courtes côtes -Polydactylie
Dysplasie campomélique	Syndrome de Smith-Lemli-Opitz
Syndrome EEC	Atrophie musculaire spinale type 1
Séquence d'akinésie foetale	Dysplasie thanatophore
Syndrome de Fryns	Trigonocéphalie C d'Opitz
Gangliosidose à GM1	Association VACTERL
Syndrome Hydrolethalus	Syndrome de Zellweger

Adapté de la Fetal Medicine Foundation (www.fetalmedicine.com)

Intestin échogène

Brève description : intestin foetal présentant une échogénicité égale ou supérieure à celles des os environnants, généralement l'os iliaque.

Prévalence : 2.4 % des grossesses au premier trimestre et 0.1-1.8 % des grossesses au deuxième et troisième trimestre (Simon-Bouy and Muller, 2003, Dagklis et al., 2008)

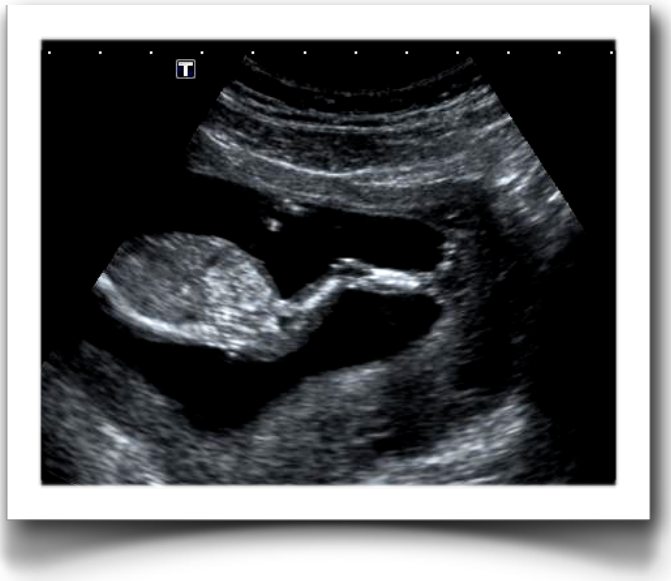
Anomalies chromosomiques principalement associées : syndrome de Down, trisomie 13, trisomie 18 et aneuploïdies des chromosomes sexuels.

La sensibilité de l'intestin échogène pour le syndrome de Down fluctue beaucoup dans la littérature, et se situerait entre 3.3 % et 27 % (Benacerraf, 2010). Cela s'explique par un diagnostic échographique potentiellement subjectif, qui varie selon la fréquence de sonde utilisée et autres réglages de l'appareil échographique (gain, harmonique, etc.). Ainsi, un foetus a 10 fois plus de probabilité de se voir attribuer une diagnostic d'intestin échogène si une sonde 8 Méga-Hertz (MHz) est utilisée plutôt qu'une sonde de 5 MHz (Vincoff et al., 1999). Afin de réduire les faux positifs, une classification a donc été proposée. Un score de 0 est attribué à un intestin foetal normal, isoéchogène au foie; un score de 1 est assigné à un intestin échogène qui, avant l'os iliaque, ne l'est plus lors la réduction progressive du gain; un score de 2 est décerné lorsque l'échogénicité des deux structures disparait simultanément; et un score de 3 est alloué lorsque l'échogénicité de l'os iliaque est perdue avant celle de l'intestin (Slotnick and Abuhamad, 1996).

L'intestin échogène a également été associé chez le foetus à des risques accrus de fibrose kystique, d'infection congénitale (cytomégalovirus, herpès, parvovirus, rubéole, toxoplasmose et varicelle), de saignement intra-amniotique, d'anomalies structurales, de complications intestinales, d'alpha-thalassémie et d'évènements adverses, comme le retard de croissance intra-utérin (Sepulveda et al., 1996, Lam et al., 1999, Strocker et al., 2000, Kesrouani et al., 2003). En plus d'offrir un conseil génétique et le cas échéant, un caryotype foetal, il est donc recommandé de procéder en présence d'intestin échogène de score 2 ou 3

(1) à une analyse génétique parentale pour la fibrose kystique (2) à des tests pour les infections congénitales (3) à une évaluation de l'abdomen foetal afin d'exclure une perforation ou une obstruction intestinale (4) à une évaluation anatomique détaillée du placenta et du foetus (5) et possiblement à une analyse parentale pour l'anémie héréditaire, selon l'origine ethnique (Van den Hof and Wilson, 2005).

Figure 4. Intestin échogène au premier trimestre



(Source : P. Miron)

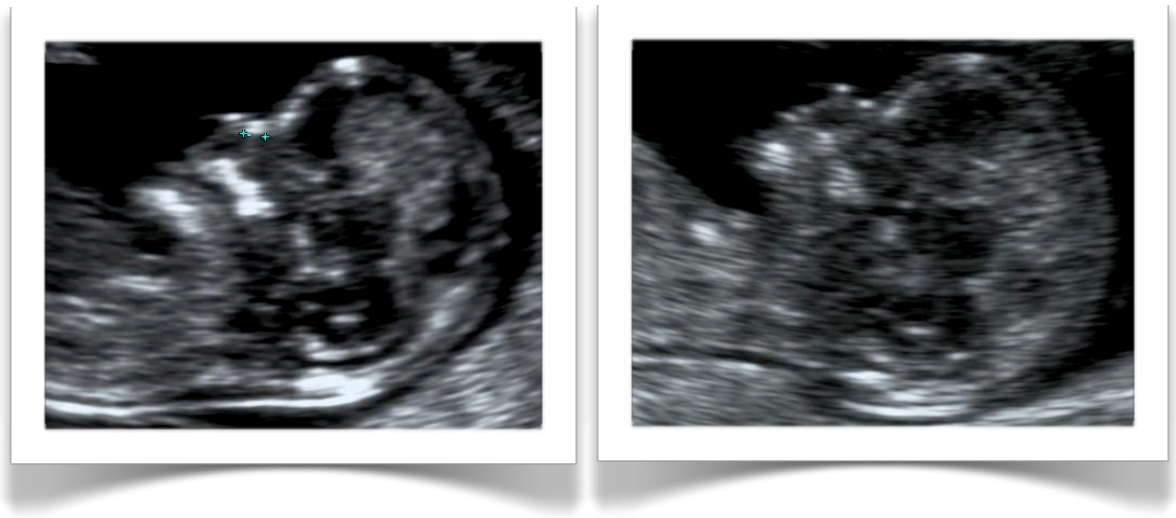
Ossification des os propres du nez

Brève description : identification échographique de l'ossification des os propres du nez fœtal.

Prévalence : absence d'identification de l'ossification des os propres du nez chez 0.6-1 % des fœtus normaux et chez 62 % des fœtus trisomiques 21, entre les 11^{e} et 14^{e} semaines.

Anomalies chromosomiques principalement associées : syndrome de Down, trisomie 18, trisomie 13, syndrome de Turner.

Figure 5. Os propres du nez



Os nasal présent (normal) Os nasal absent

(Source: P.Miron)

Au même titre que l'épaississement cutané, l'hypoplasie nasale chez les enfants trisomiques 21 avait déjà été décrite par Down en 1866 (Down, 1866). Ce n'est pourtant qu'en 1995 qu'une première étude, évaluant par échographie la longueur des os propres du nez fœtal au second trimestre, émet l'hypothèse d'utiliser cette mesure afin de mieux détecter les anomalies chromosomiques fœtales, incluant la trisomie 21 (Guis et al., 1995). Une deuxième étude en 1997, portant sur les *patterns* radiologiques de malformations squelettiques axiales, confirme chez 19 des 31 fœtus humains étudiés trisomiques 21 et âgés entre 12 et 24 semaines, une malformation ou une agénésie des os propres du nez (Keeling et al., 1997). En plus de corroborer l'absence d'ossification des os propres du nez chez 23 % des fœtus, une étude confirmera par radiographie post-mortem que d'autres anomalies squelettiques fœtales peuvent également être associées au syndrome de Down, telles la brachycéphalie et l'hypoplasie de la phalangine (phalange moyenne) du 5^{e} doigt (Stempfle et al., 1999).

Ces données sur l'absence d'ossification des os propres du nez chez les fœtus trisomiques au deuxième trimestre auront sûrement incité les chercheurs à étudier le phénomène plus tôt, au premier trimestre de grossesse. Ainsi, Cicero et ses collaborateurs analyseront rétrospectivement, en 2001, 701 échographies fœtales effectuées chez des femmes adressées pour un diagnostic prénatal à la suite d'un dépistage positif par clarté nucale. Parmi les fœtus subséquemment identifiés d'un syndrome de Down, 73 % avaient une absence d'ossification des os propres du nez, comparativement à 0.5 % chez les fœtus sains. Les auteurs concluront que l'absence d'os nasal augmente le risque de syndrome de Down de 146 fois (95 % IC 50-434), alors que sa présence le réduit d'environ trois fois (95 % IC 0.18-0.40)(Cicero et al., 2001). On prédit alors par modélisation, en absence de corrélation statistique entre l'os nasal, la clarté nucale et les différents marqueurs biochimiques, qu'un taux de détection de plus de 97 % pourrait être atteint avec l'os nasal en le combinant avec tous ces marqueurs (Tableau VI) (Cuckle, 2001).

L'identification des os propres du nez à titre de marqueur échographique de la trisomie 21 au premier trimestre suscitera initialement beaucoup de réactions et de controverses, certains allant jusqu'à questionner son utilisation de routine dans la population générale (De

Biasio and Venturini, 2002, Hutchon, 2002, Monni et al., 2002, Reynolds, 2002, Malone et al., 2004, Ramos-Corpas et al., 2006, Weingertner et al., 2006). On réalise que sa mise en application requiert, comme pour la clarté nucale, le contrôle de variables confondantes, une bonne formation clinique et une courbe d'apprentissage (Cicero et al., 2003b, Senat et al., 2003).

Tableau VI. Taux prédits de détection par modélisation selon différentes stratégies de dépistage

Stratégie	Grossesse (semaines)	Marqueurs	Taux de Détection (%)
1	15–19	β-hCG libre et AFP	63·2
2	15–19	β-hCG libre, AFP et uE_3	66·8
3	15–19	β-hCG libre, AFP, uE_3 et inhibine A	72·1
4	10	β-hCG libre, AFP, uE_3 et PAPP-A	77·4
5	11–13	NT	72·9
6	11—13	β-hCG libre, PAPP-A et CN	88·9
7	11–13	CN et ON	92·4
8	11–13	β-hCG libre, PAPP-A, CN et ON	97·1

* Lorsque 5 % sont adressées pour diagnostic prénatal. CN= clarté nucale ; ON= os nasal.

Adapté de (Cuckle, 2001) et (Cicero et al., 2003a))

En moyenne, 80 mesures (variant de 40 à 120) doivent être effectuées avant d'atteindre un certain seuil de compétence (Cicero et al., 2003b). Une étude prospective portant sur 21,074 grossesses simples confirmera et précisera l'utilité de l'identification de l'os nasal entre les 11^{e} et 14^{e} semaines qui, lorsque combinée à la clarté nucale et aux marqueurs biochimiques du premier trimestre, maintient un taux de détection de la trisomie 21 de 90 % tout en réduisant de moitié le taux de faux positifs de 5 % à 2.5 % (Cicero et al., 2006). La combinaison de données sur 35 213 fœtus, ayant eu entre les 11^{e} et 14^{e} semaines

une évaluation standardisée des os propres du nez, confirme leur absence chez 66.8 % des fœtus trisomiques 21 et chez 0.9 % des fœtus euploïdes (Sonek, 2007b).

Des recherches plus étendues confirmeront également que l'absence des os propres du nez fœtal est également observée chez 55 % des fœtus trisomiques 18, 34 % des fœtus trisomiques 13 et 11 % des syndromes de Turner (45,X) (Cicero et al., 2004).

L'identification des os propres du nez fœtal est habituellement effectuée uniquement dans le cadre d'un dépistage prénatal la combinant à la clarté nucale et aux marqueurs biochimiques. Le processus décisionnel peut toutefois s'avérer plus difficile en absence d'identification des os propres du nez avec clarté nucale normale. Il est généralement recommandé de répéter l'examen échographique une semaine plus tard. La confirmation de cette absence d'ossification nasale deviendrait alors une indication d'offrir un diagnostic prénatal, particulièrement chez les Caucasiennes. Une controverse internationale subsiste cependant toujours à ce sujet. Certains croient que l'évaluation de l'os nasal performe plus ou moins bien pour les populations à faible risque et qu'elle ne devrait être limitée qu'à des populations à haut risque. D'autres ont proposé qu'elle soit utilisée comme test de contingence en présence de risque intermédiaire (entre 1:100 - 1:1000) déterminé par un dépistage prénatal classique du premier trimestre combinant la clarté nucale à la PAPP-A et la fraction libre de β-hGC (Nicolaides et al., 2005, Rosen et al., 2007). Dans un tel cadre, l'absence d'identification des os propres du nez devrait automatiquement suggérer une indication de diagnostic prénatal génétique (Sonek et al., 2006).

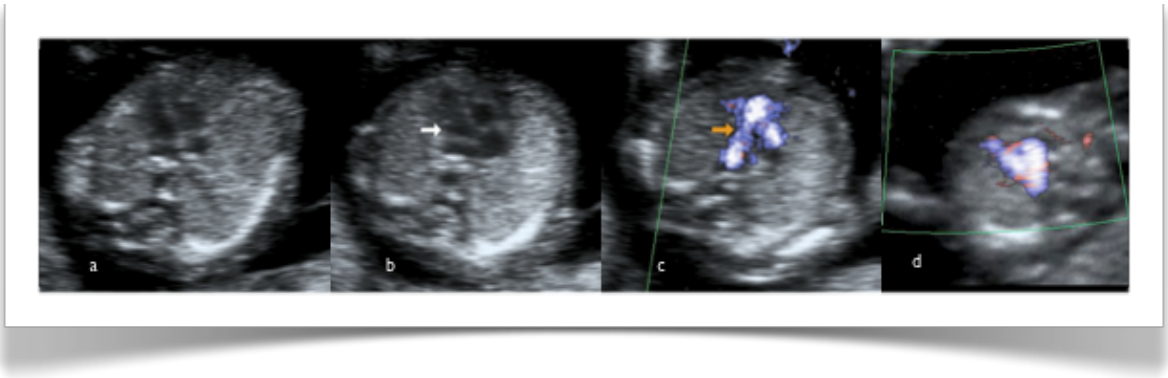
Cardiopathies congénitales

Brève description : inclus une multitude de malformations cardiaques dont, entre autres, les défauts de septum atrioventriculaire, les anomalies conotroncales (tétralogie de Fallot, ventricule droit à double issue, tronc artériel commun, transposition des gros vaisseaux), les hypoplasies du ventricule gauche ou du ventricule droit.

Prévalence : 7-9:1000 naissances vivantes.

Anomalies chromosomiques principalement associées : syndrome de Down, trisomie 18, trisomie 13, syndrome de Turner, tétrasomie 12p, délétion 22q11.

Figure 6. Cœur fœtal normal au premier trimestre (12e semaine) : (a) 4 chambres (b) voie d'éjection de l'aorte (c) voie d'éjection de l'artère pulmonaire et croisement des vaisseaux (d) canal artériel



(Source : P. Miron)

Les cardiopathies congénitales sont les anomalies structurales les plus communes à la naissance et la cause principale de mortalité en périodes néonatale et infantile. La majorité d'entre elles sont d'origine multifactorielle. L'exposition à des agents tératogènes, une maladie maternelle et une histoire familiale se doivent d'être exclues (Welch and Brown, 2000).

Le cœur fœtal et ses quatre chambres sont habituellement complètement formés 56 jours après la fécondation (Moore and Persaud, 1998). Les nouvelles sondes échographiques à haute fréquence et haute résolution, de concert avec les progrès substantiels obtenus dans l'agrandissement et le traitement de l'image, permettent de nos jours une exploration détaillée de l'anatomie cardiaque fœtale dès le premier trimestre de grossesse.

Bien qu'elle ne remplace pas entièrement l'échographie de routine du 2^{e} trimestre, l'échocardiographie fœtale précoce, entre les 12^{e} et 15^{e} semaines, présenterait plusieurs bénéfices dont la confirmation très tôt en grossesse d'une anatomie cardiaque fœtale normale chez les patientes à haut risque et un diagnostic précoce menant rapidement à un caryotype fœtal et à un conseil génétique aux parents avec un fœtus atteint.

L'échocardiographie fœtale de base étendue, telle que recommandée par l'*International Society of Ultrasound in Obstetrics and Gynecology*, avec les cinq plans transverses, peut être réalisée en présence d'une position fœtale favorable dans 95 % des cas à 11-12 semaines et dans 100 % des cas à 13-15 semaines (Yagel et al., 2001, ISUOG, 2006, Yagel et al., 2007). Une étude portant sur 3094 fœtus évalués entre les 11^{e}-14^{e} semaines confirme, avec une prévalence d'anomalies cardiaques majeures de 1.2 %, un taux de détection de 84 %. Ce taux augmente à plus de 96 % en présence d'une clarté nucale augmentée $\geq$ 2.5 mm (Becker and Wegner, 2006).

Jusqu'à 39 % des cardiopathies congénitales isolées sont associées à des anomalies chromosomiques. Combiné à d'autres anomalies extracardiaques, ce taux augmenterait jusqu'à 98 %. Certaines malformations présentent un risque accru d'aberrations chromosomiques spécifiques. À titre d'exemple, la trisomie 21 est associée plus souvent à des défauts de septum auriculaire, ventriculaire ou atrioventriculaire et à la tétralogie de Fallot ; la trisomie 13, à des défauts de septum auriculaire, ventriculaire ou atrioventriculaire, à une persistance du canal artériel ou à une dextrocardie ; le syndrome de Turner à une coarctation de l'aorte. En présence d'une cardiopathie de type conotroncal, un syndrome de DiGeorge (monosomie 22q11.2) ou un syndrome vélocardiofacial se doivent

d’être exclus. Un conseil génétique et un caryotype fœtal sont indiqués (Welch and Brown, 2000, Wimalasundera and Gardiner, 2004).

Hygroma kystique

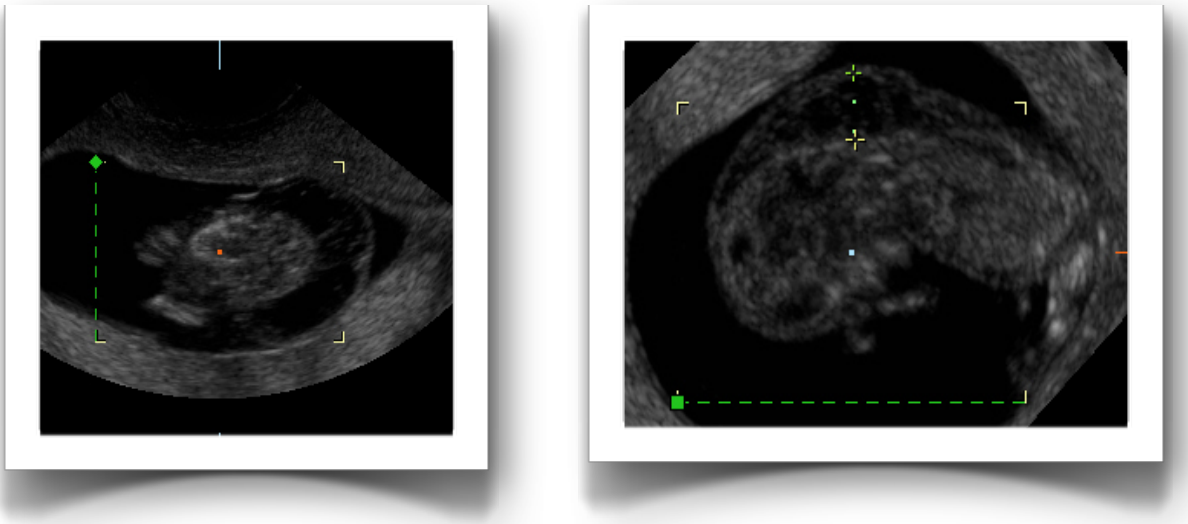
Brève description : malformation congénitale du système lymphatique localisée principalement au niveau du cou fœtal et déterminée par un défaut de communication entre les canaux lymphatiques et la veine jugulaire interne. En échographie, elle apparaît sous la forme de décollements rétro et latérocervicaux qui peuvent parfois être volumineux.

Prévalence : 1:333 des foetus entre les 11e-14e semaines.

Anomalies chromosomiques principalement associées : syndrome de Down, syndrome de Turner, trisomie 18, trisomie 13.

Figure 7. Hygroma kystique vers la 11e semaine (A et B)

A. B.



(Source : P. Miron)

La clarté nucale augmentée et l'hygroma kystique sont deux entités anatomiques différentes. La première, discutée plus haut, est le meilleur marqueur échographique de dépistage prénatal au premier trimestre. L'hygroma kystique, quant à lui, est associé très

fréquemment à l'aneuploïdie fœtale. Il se distingue de la clarté nucale par des cloisons antéro-postérieures séparant des zones plus transsoniques. En présence d'un caryotype normal, il demeure un marqueur d'une multitude de syndromes génétiques. Son association avec une anasarque fœtale dans 40 % des cas résulte d'une séquence obstructive jugulaire lymphatique. La compression du retour veineux au cœur fœtal par les sacs lymphatiques jugulaires mène ainsi souvent, dès le premier trimestre, à une insuffisance cardiaque et offre alors en général un pronostic très réservé (Descamps et al., 1997).

En présence d'hygroma kystique, la clarté nucale n'est pas toujours augmentée. Elle ne l'est pas chez 4 % et 35 % des cas avec ou sans anomalie chromosomique, respectivement. Bien que son association avec une clarté nucale normale offre un meilleur pronostic, l'hygroma kystique est tout de même associé, dans de telles situations, à 57 % de malformations fœtales en faisant ainsi un marqueur distinctif de la clarté nucale (Kharrat et al., 2006).

Globalement, plus de 50 % des fœtus avec hygroma kystique ont une anomalie chromosomique et seulement 17 % résulteront en une naissance en bonne santé. Vu la puissance unique de ce marqueur au premier trimestre, la biochimie génétique apparaît donc futile et un diagnostic prénatal génétique doit être offert sans délai (Malone et al., 2005a).

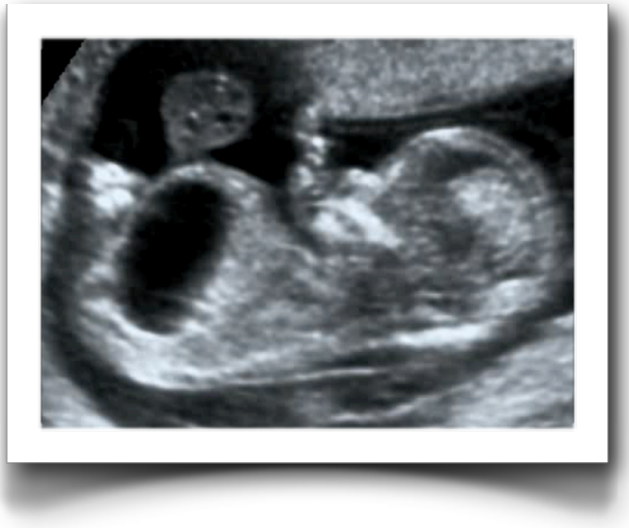
Mégavessie

Brève description : vessie fœtale avec un diamètre de 7 millimètres ou plus, entre les 11e et 14e semaines de grossesse.

Prévalence : 1:1632 des foetus entre les 11e-14e semaines.

Anomalies chromosomiques principalement associées : trisomie 13, trisomie 18, trisomie 15, triploïdies, translocations non équilibrées.

Figure 8. Mégavessie vers la 13e semaine



(Source : P. Miron)

La vessie fœtale peut être identifiée très précocement par échographie. On la détecte chez environ 50 % des fœtus dès la 10e semaine de grossesse et chez plus de 94 % à la 13e semaine de grossesse (Braithwaite et al., 1996, Rosati and Guariglia, 1996, Sebire et al., 1996).

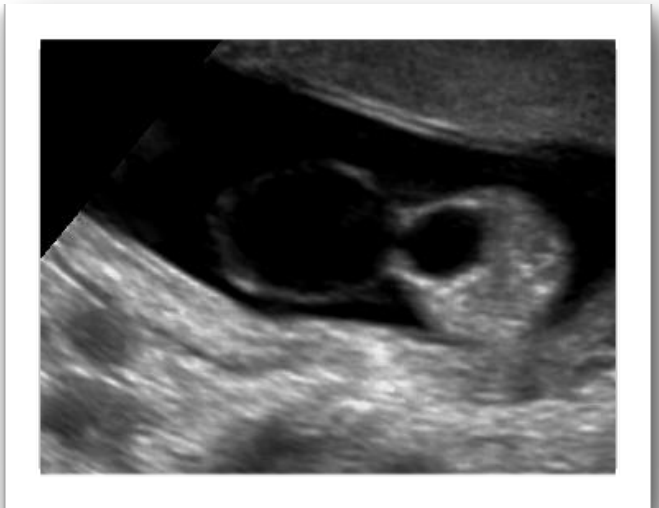
Une mégavessie (diamètre longitudinal de 7 mm ou plus au premier trimestre de grossesse) est notée dans environ 1 grossesse sur 1500 (Sebire et al., 1996). Elle est associée à un

risque accru d'une grande variété d'anomalies chromosomiques et donc, est une indication de diagnostic prénatal.

Plus spécifiquement, une vessie fœtale mesurant au premier trimestre de grossesse de 7 à 15 mm est associée à 24 % de probabilité d'anomalies chromosomiques, principalement des trisomies 13 ou 18. Des cas moins fréquents de trisomies 21, 4 et 15, de triploïdies et de translocations non équilibrées ont également été rapportés. Pour les fœtus avec caryotype normal, une résolution spontanée de la mégavessie se produit dans 90 % des cas. Bien qu'une mégavessie de plus de 15 mm soit le plus souvent causée par une uropathie obstructive progressive grave, elle est malgré tout associée à 11 % d'anomalies chromosomiques (Liao et al., 2003).

Une mégavessie peut parfois être observée en présence d'une persistance du canal de l'ouraque avec kyste vésico-allantoïque. Cette condition est rare (1:50 000 - 1:100 000) et se doit d'être distinguée de l'omphalocèle, du gastroschisis ou d'un kyste ombilical. Elle n'a pas été associée à d'autres anomalies congénitales ou chromosomiques et ne nécessiterait donc pas de caryotype fœtal (Tolaymat et al., 1997, Lugo et al., 2006).

Figure 9. Persistance du canal de l'ouraque avec kyste vésico-allantoïque vers la 13e semaine



(Source : P. Miron)

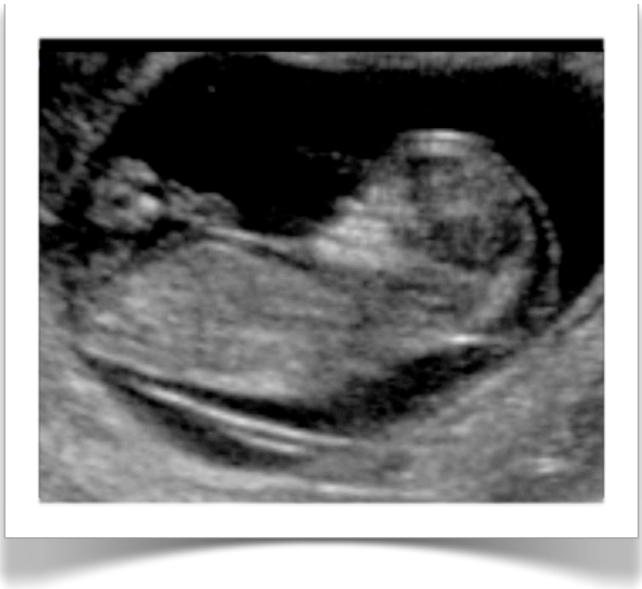
Anasarque fœtale non immune

Brève description : syndrome œdémateux généralisé caractérisé par une augmentation pathologique d'eau corporelle totale fœtale apparaissant initialement au niveau des tissus mous et des cavités séreuses. Puisque non immune, elle n'est pas liée à une incompatibilité sanguine.

Prévalence : 1:1500 - 1:4000 naissances vivantes.

Anomalies chromosomiques principalement associées : syndrome de Down, syndrome de Turner, trisomie 18, trisomie 13, trisomie 16, triploïdie.

Figure 10. Anasarque fœtale non immune vers la 12e semaine



(Source : P. Miron)

Au premier trimestre, l'anasarque fœtale serait toujours d'origine non immune. Le mécanisme étiologique sous-jacent s'explique principalement par une insuffisance cardiaque intra-utérine primaire ou secondaire ou par une hypoprotéinémie (Sonek, 2007a). En plus d'un œdème fœtal généralisé, le placenta est souvent œdématié (28 %) et le site de changements molaires partiels en présence de triploïdie ou de tétraploïdie. Une cavité exo-

cœlomique (dysmorphisme amniotique) est notée vers les 13e-16e semaines de grossesse dans plus de deux tiers des cas (Jauniaux, 1997).

L'anasarque fœtale non immune diagnostiquée très précocement en grossesse, entre 10 et 17 semaines, offre en général un très mauvais pronostic. Elle est souvent associée à d'autres malformations fœtales (83 %). De plus, 47 % à 78 % des fœtus sont porteurs d'une aberration chromosomique. Des causes plus rares d'anasarque au premier trimestre comprennent une infection primaire au parvovirus B19, des syndromes génétiques et des lésions thoraciques (Van Dorpe et al., 1996, Jauniaux et al., 2000, Sohan et al., 2000). Une clarté nucale augmentée (93 %) et un hygroma kystique (73 %) sont communs. Toutes ces grossesses résultent généralement en un avortement spontané, une mort *in utero* ou une interruption de grossesse. Le taux de mortalité est élevé même en présence de chromosomes normaux (Iskaros et al., 1997, Has, 2001).

La biopsie de chorion apparaît la méthode de choix pour un diagnostic génétique avant la 15e semaine de grossesse. Un dépistage maternel de TORCH/B19 est également recommandé.

Hydrocéphalie

Brève description : Élargissement anormal des ventricules cérébraux latéraux avec mesures auriculaires > 15 millimètres entre les 16e et 22e semaines de grossesse. On parle alors d'hydrocéphalie. Au second trimestre, on définit la ventriculomégalie légère par des mesures ≥ 10 mm et ≤ 15 mm.

Prévalence : 3-8:10 000 naissances vivantes.

Anomalies chromosomiques principalement associées : syndrome de Down, trisomie 18, syndrome de Turner, triploïdie.

La ventriculomégalie est l'anomalie cérébrale la plus couramment dépistée en échographie fœtale du 2e trimestre. Elle découle le plus souvent d'une sténose de l'aqueduc de Sylvius à la suite d'une infection, un saignement ou une compression (30-40 %), d'une malformation d'Arnold Chiari (20-30 %), d'une malformation de Dandy-Walker (7-10 %) ou d'une agénésie du corps calleux (20-30 %) (D'Addario et al., 2007).

La ventriculomégalie significative (hydrocéphalie) isolée présente un risque d'environ 6 % d'anomalies chromosomiques. Ce taux augmente à 25 % en présence de malformations additionnelles (Schwanitz et al., 1993).

La ventriculomégalie isolée et légère serait associée à une issue neurologique non favorable en présence de trois critères : (1) une mesure de l'atrium de > 12 mm (2) une progression de l'élargissement avec le temps et (3) une asymétrie et une bilatéralité. Des échographies de contrôle apparaissent donc essentielles d'autant plus que des anomalies associées seront subséquemment identifiées dans 8.6 % des cas (Ouahba et al., 2006).

Le management d'une ventriculomégalie légère et isolée demeure incertain. Le risque d'aneuploïdie associé de 4 % ne peut être ignoré et selon certains auteurs, un caryotype fœtal et une recherche de causes infectieuses dans le liquide amniotique se doivent d'être

considérés et discutés avec la patiente (Pilu et al., 1999, Kelly et al., 2001, Goldstein et al., 2005). Une autre alternative consisterait à multiplier par un facteur de vraisemblance de 9 le risque *a priori* afin de mieux estimer la probabilité d'aneuploïdie fœtale (Annexe 1) (Van den Hof and Wilson, 2005). Plus de 80 % des enfants avec ventriculomégalie légère et isolée n'auront aucune séquelle clinique (Pilu et al., 1999). L'hydrocéphalie présente, quant à elle, une indication claire de caryotype fœtal.

Habituellement diagnostiquée uniquement à l'échographie du second ou du troisième trimestre, la ventriculomégalie cérébrale peut parfois être suspectée très précocement dès le premier trimestre de grossesse (entre les 11^{e} et 14^{e} semaines) en présence de plexus choroïdes qui n'occupent pas entièrement les espaces ventriculaires (Cedergren and Selbing, 2006, Weiner et al., 2007).

Anomalies ouvertes du tube neural

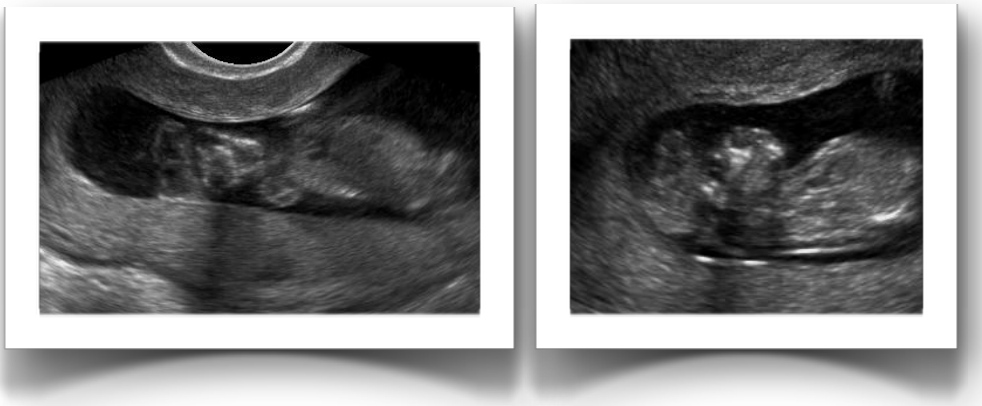
Brève description : anomalies qui résultent d'un défaut de fermeture du tube neural (crâne ou colonne vertébrale) pendant l'embryogenèse, soit vers les 25e-27e jours après la conception.

Prévalence : 5-6:10 000 naissances (au Canada).

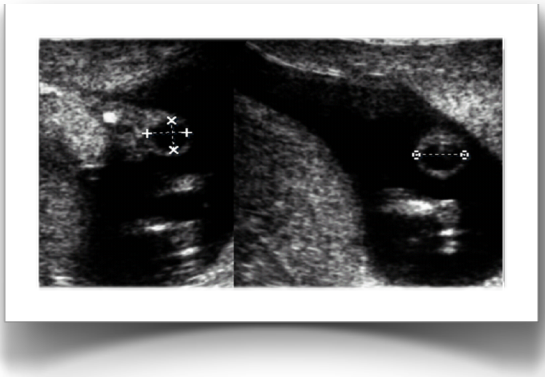
Anomalies chromosomiques principalement associées : trisomie 18, trisomie 13, triploïdie, translocations équilibrées ou non.

Figure 11. Acranie avec exencéphalie (A (signe *Mickey mouse*) et B) et méningomyélocèle sacro-coccygien (C) vers la 12e semaine

A. B.



C.



(Source : P. Miron)

Les anomalies ouvertes du tube neural (AOTN) peuvent être subdivisées principalement en trois types : l'anencéphalie/exencéphalie/acranie, la spina bifida et l'encéphalocèle. Un autre type beaucoup moins commun est l'iniencéphalie.

L'étiologie des AOTN semble être le plus souvent multifactorielle. Certaines formes ont toutefois été associées à des agents iatrogéniques (valproates), à des mutations génétiques (syndrome Meckel-Gruber) et à des maladies maternelles (carence en acide folique et en B12, diabète insulinodépendant, obésité) (Holmes et al., 1976, Main and Mennuti, 1986, Papp, 1992, Frey and Hauser, 2003). Plusieurs études épidémiologiques ont confirmé l'effet préventif de l'acide folique et son utilisation combinée à des suppléments multivitaminés. Il est fortement recommandé de les débuter deux à trois mois avant la conception puis de les poursuivre en grossesse et pendant l'allaitement (Wilson et al., 2007).

L'association des AOTN avec l'aneuploïdie a été clairement documentée (Flannery and Kahler, 1986, Moore et al., 1988, Rodriguez et al., 1990). Pourtant, l'indication de procéder ou non à un diagnostic prénatal cytogénétique pour une anomalie ouverte isolée du tube neural demeure un sujet de controverse (Harmon et al., 1995, O'Reilly and Shields, 2000, Daniel et al., 2003, Sepulveda et al., 2004a, Joo et al., 2007). La prévalence d'aberrations chromosomiques chez les fœtus avec AOTN serait globalement d'environ 2 à 10 %. Plus spécifiquement et selon différentes séries, elle serait de 8 à 39 % en présence de spina bifida, de 14-17% en présence d'encéphalocèle et de seulement 2 % en présence d'acranie, d'anencéphalie et d'iniencéphalie (Sepulveda et al., 2004a). Au Canada, il est recommandé de faire une évaluation génétique lorsqu'une anomalie du tube neural est détectée à l'échographie (Chodirker et al., 2001).

Hernie diaphragmatique

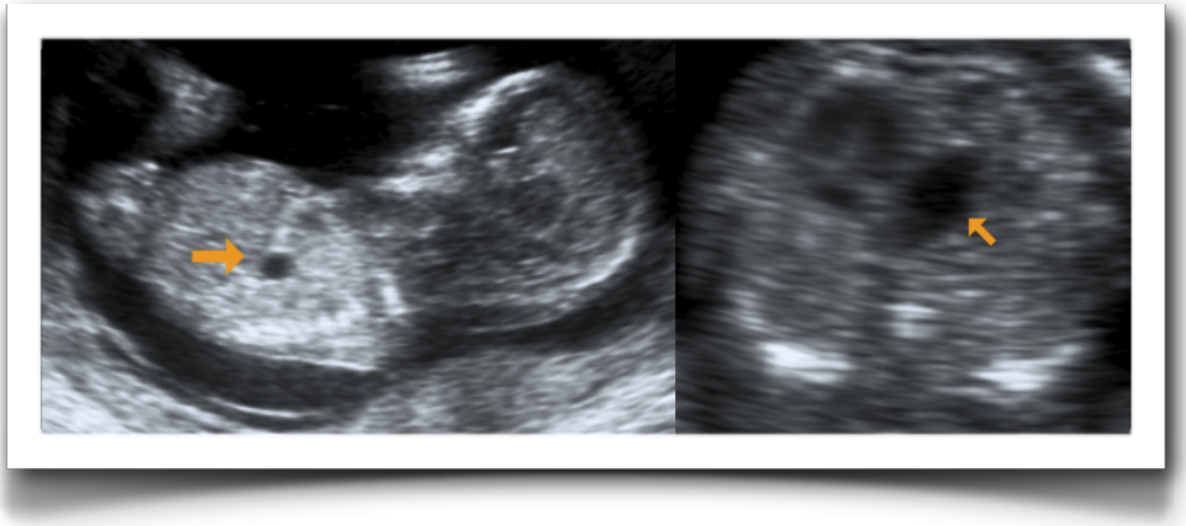
Brève description : absence de développement de tout ou d'une partie de la coupole diaphragmatique avec présence de viscères abdominaux dans le thorax. Souvent accompagnée d'une hypoplasie pulmonaire létale et d'hypertension pulmonaire.

Prévalence : 1:3500 - 1:4000 naissances vivantes.

Anomalies chromosomiques principalement associées : trisomie 18, trisomie 13, syndrome de Down, syndrome de Turner, délétions 15q26.1-q26.2.

Deux publications dans la littérature rapportent un diagnostic de hernie diaphragmatique à 12 semaines de grossesse avec une clarté nucale normale (Souka et al., 2006, Daskalakis et al., 2007). Il s'agit toutefois d'une anomalie structurale plus facilement identifiable au second trimestre de grossesse et une indication d'offrir un diagnostic prénatal génétique.

Figure 12. Hernie diaphragmatique, déviant le coeur (4 chambres) vers la droite



(Source : P. Miron)

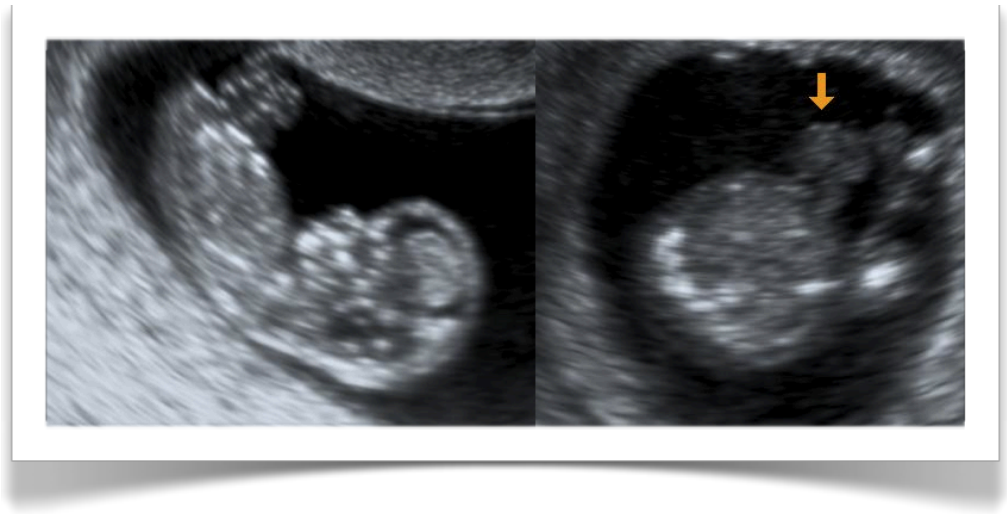
Omphalocèle

Brève description : Absence de fermeture de la paroi ventrale du fœtus avant la 9e semaine de grossesse qui mène à l'extériorisation des viscères par hernie ombilicale, et dont le contenu est protégé par une membrane.

Prévalence : 1:5800 naissances vivantes (1:381 foetus entre les 11e-14e semaines).

Anomalies chromosomiques principalement associées : trisomie 18, trisomie 13, syndrome de Down, syndrome de Turner, triploïdie, délétions/duplications.

Figure 13. Omphalocèle



(Source : P. Miron)

L'omphalocèle est le défaut le plus commun de la paroi ventrale chez le fœtus. Les malformations associées sont fréquentes et atteignent presque un foetus sur deux. Selon une étude récente, on note environ 15 % de malformations associées non chromosomiques, 30 % d'anomalies congénitales multiples et 29 % d'aberrations chromosomiques. Cette embryopathie n'est isolée que dans 26 % des cas. Chez 196 fœtus avec omphalocèle, les

anomalies chromosomiques les plus communes sont la trisomie 18 (66.6 %), la trisomie 13 (25 %), la trisomie 21 (3.6 %), le syndrome de Turner (2.0 %), la triploïdie (1.0 %) et autres délétions ou duplications partielles (1.5 %). Obtenir un caryotype fœtal apparaît donc essentiel (Stoll et al., 2008). De plus, selon la base de données *Online Mendelian Inheritance in Man*, l'omphalocèle est décrite dans cinquante-sept syndromes polymalformatifs, l'un des plus fréquents étant le syndrome de Wiedemann-Beckwith. Il est donc primordial d'assurer une prise en charge immédiate en milieu spécialisé afin de procéder à une échographie détaillée au deuxième trimestre de grossesse et d'offrir un conseil génétique approprié (OMIM, 2008).

En ce qui concerne les fœtus avec omphalocèle isolée et caryotype normal, ils présentent généralement un pronostic assez favorable après chirurgie correctrice. Certains de ces cas ont parfois même une résolution spontanée à l'échographie après la 20^{e} semaine de grossesse (Blazer et al., 2004).

Il ne faut pas confondre ici l'omphalocèle du gastroschisis (laparoschisis). Ce dernier consiste plutôt en une fente de la paroi abdominale, par laquelle s'extériorise uniquement les anses intestinales. De plus, il n'existe aucune membrane les entourant.

Agénésie du rayon radial

Brève description : anomalie réductionnelle associant l'absence du radius à l'absence de la colonne du pouce (scaphoïde, 1er métacarpien et les deux phalanges du pouce). Fais partie d'un groupe d'anomalies réductionnelles des membres, caractérisées par l'absence totale ou partielle de structures squelettiques ou par leur hypoplasie sévère.

Prévalence : 1:10 000 naissances.

Anomalies chromosomiques principalement associées : trisomie 18, délétion partielle du chromosome 13, chromosome 4 en anneau ou délétion 4q, anémie de Fanconi.

L'agénésie du rayon radial peut être totale ou partielle, isolée ou associée à d'autres malformations. Elle s'accompagne en général d'une main botte et d'une angulation du cubitus. Elle peut être un signe de plusieurs syndromes génétiques, dont le syndrome TAR (thrombocytopénie-absence de radius), le syndrome Holt-Oram, le syndrome Aase, le syndrome VACTERL, l'anémie de Fanconi et de certaines anomalies chromosomiques (Tableau VII) (Pfeiffer and Santelmann, 1977, Sepulveda et al., 1995, Kennelly and Moran, 2007).

Les anomalies des extrémités sont identifiées chez un tiers des trisomies 18 et l'agénésie radiale est habituellement la découverte la plus proéminente à l'examen pathologique ou néonatal (Voorhess et al., 1964, Pfeiffer and Santelmann, 1977).

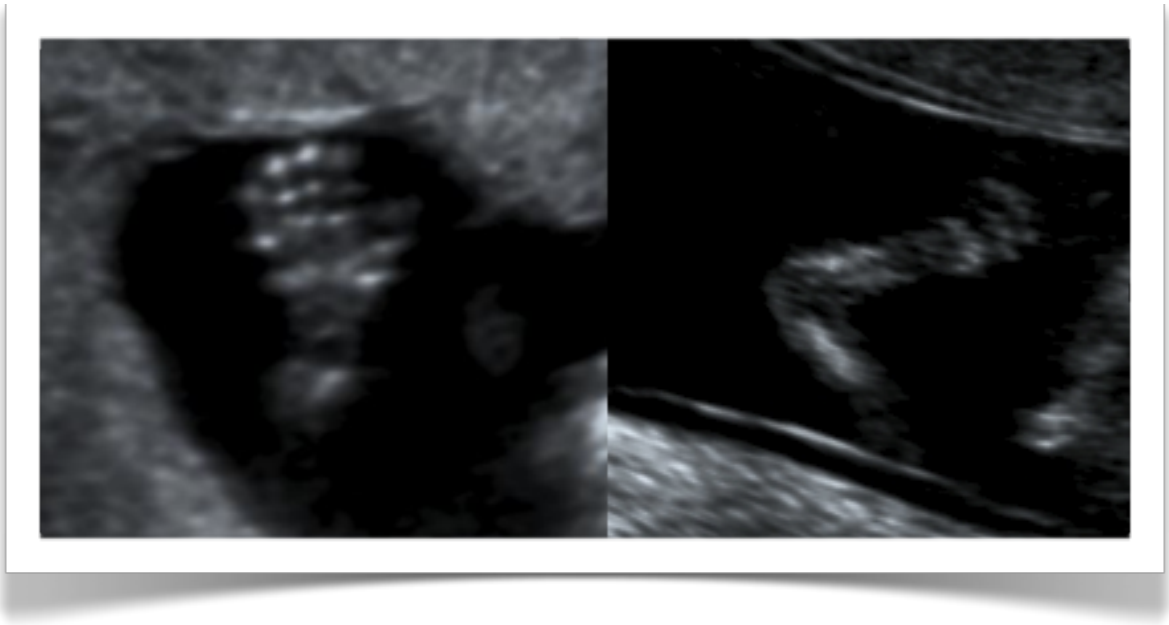
Bien qu'habituellement identifiée au second trimestre de grossesse, l'agénésie du rayon radial peut être diagnostiquée précocement dès la 12e semaine (Bellver et al., 2005). Un caryotype fœtal est toujours recommandé.

Tableau VII. Syndromes associés à l'agénésie du rayon radial

Syndrome	Caractéristiques
Aneuploïdies	Trisomie 18, délétion partielle du chromosome 13, chromosome 4 en anneau ou délétion 4q
Anémie de Fanconi	Transmission autosomique récessive, pancytopénie, hypoplasie radiale, main botte radiale, pouce absent
Syndrome TAR	Agénésie radiale, pouce et métacarpe présents, humérus et cubitus parfois absents, cardiopathies (33%)
Syndrome Aase	Transmission autosomique récessive, anémie hypoplasique, main botte radiale, pouce à trois phalanges, radius distal hypoplasique, cardiopathie
Syndrome Holt-Oram	Transmission autosomique dominante, cardiopathie, aplasie ou hypoplasie radiale, anomalies réductionnelles des membres souvent symétriques
Association VACTERL	Combinaison variable de malformations vertébrale, anale, cardiaque, trachéenne, œsophagienne, rénale et des membres (agénésie radiale)

Adapté de (Rypens et al., 2006)

Figure 14. Main et avant-bras (cubitus, radius)



(Source : P. Miron)

Atrésie duodénale

Brève description : anomalie de développement avec absence complète de la lumière duodénale ; se présente à l'échographie par un signe en double bulle.

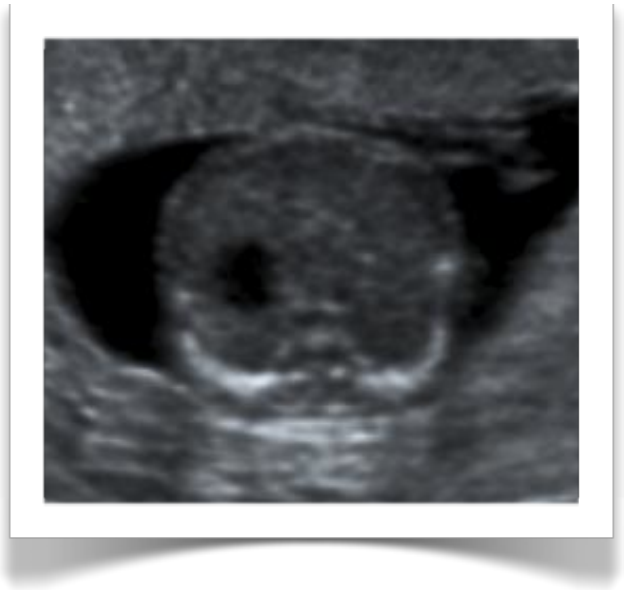
Prévalence : 1:10 000 naissances vivantes.

Anomalies chromosomiques principalement associées : syndrome de Down.

Plus de 50 % des fœtus avec une atrésie duodénale ont des anomalies congénitales associées incluant des anomalies pancréatiques, une malrotation intestinale, une atrésie œsophagienne, un diverticule de Meckel, un anus imperforé, une cardiopathie congénitale et des lésions neurologiques ou rénales. Approximativement 30 % auront un syndrome de Down et 33 à 45 %, un polyhydramnios (Escobar et al., 2004).

L'estomac fœtal est généralement bien visualisé à l'échographie du premier trimestre. Quelques publications dans la littérature ont démontré la possibilité d'identifier dès le premier trimestre une atrésie duodénale (Tsukerman et al., 1993, Dundas et al., 2001). Puisque le signe de la double bulle est parfois noté chez des fœtus normaux, un diagnostic final ne peut toutefois être émis qu'après une confirmation plus tardive au second trimestre (Zimmer and Bronshtein, 1996). Un diagnostic prénatal génétique doit alors être offert.

Figure 15. Estomac normal



(Source : P. Miron)

Holoprosencéphalie

Brève description : malformation cérébrale complexe qui résulterait d'une division incomplète et d'un échec de diverticulation du prosencéphale survenant habituellement entre les 18^{e} et 28^{e} jours de vie embryonnaire et qui se présente sous forme lobaire ou alobaire.

Prévalence : 1:16 000 naissances vivantes (1:1298 foetus entre les 11^{e} et 14^{e} semaines).

Anomalies chromosomiques principalement associées : trisomie 13, trisomie 18, triploïdies, délétion 2(q37.1-q37.3), délétion 21(q22.3).

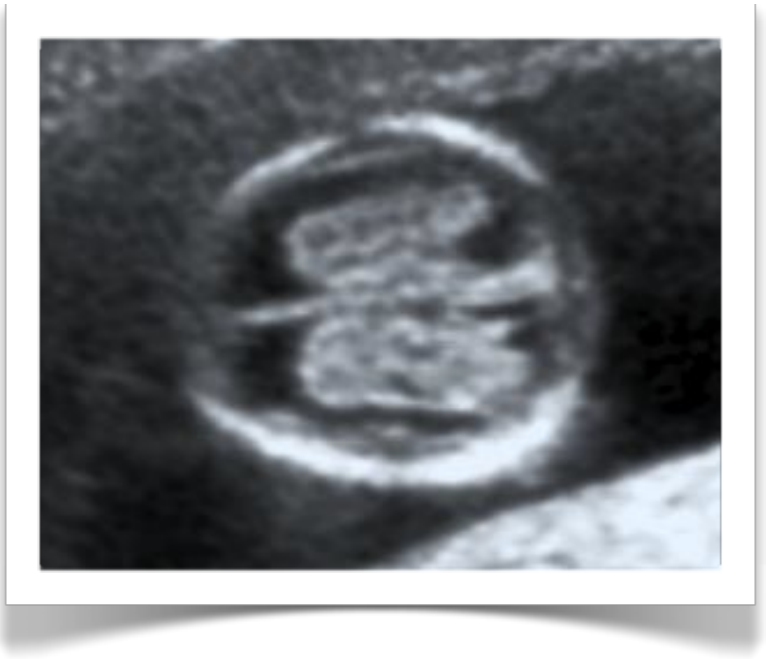
L'holoprosencéphalie est caractérisée principalement par un clivage anormal des hémisphères cérébraux et des structures diencéphaliques (prosencéphale médiobasal) (Shiota et al., 2007). La classification la plus acceptée définit trois degrés de sévérité (DeMyer, 1977) : (1) la forme alobaire ou complète avec un ventricule cérébral unique et (2) la forme semi-lobaire avec scissure interhémisphérique incomplète, qui est presque toujours associée à des anomalies faciales typiques, telles que la cyclopie, l'hypotélorisme et une fente faciale, labiale et/ou palatine ; (3) la forme lobaire qui mime un cerveau normal avec une continuité du cortex frontal sur la ligne médiane. Une forme plus légère, la variante interhémisphérique moyenne ou syntélencéphalie a également été reconnue.

L'étiologie de l'holoprosencéphalie est hétérogène et dans la plupart des cas, l'anomalie est isolée. Des facteurs tératogènes ont été rapportés de façon sporadique comme le diabète insulinodépendant, l'alcool et l'acide rétinoïque. Pour d'autres cas, elle est associée à des aberrations chromosomiques, à d'autres malformations structurales telles les anomalies du tube neural (anencéphalie, Dandy-Walker, encéphalocèle) ou à une multitude de syndromes dont ceux de DiGeorge, de Smith-Lemli-Opitz et de Meckel. Bien qu'une transmission autosomique dominante ait été observée dans certains cas, des données récentes suggèrent une origine multigénique et multicible (Cohen, 2006, Dubourg et al., 2007).

L'holoprosencéphalie peut être diagnostiquée précocement en grossesse (Bronshtein and Wiener, 1991, van Zalen-Sprock et al., 1995, Nicolaides and Wegrzyn, 2005). Entre les 11e et 14e semaines, une attention particulière doit être portée au signe échographique du « papillon » identifiant les plexus choroïdes, qui en son absence, doit automatiquement faire suspecter une holoprosencéphalie (Sepulveda et al., 2004b). De plus, en présence de la forme alobaire (un seul ventricule et un thalamus fusionné), le diagnostic au premier trimestre est relativement facile et fiable (Sonek, 2007a).

Puisqu'elle est associée à un haut risque d'anomalies chromosomiques (30 %), un caryotype fœtal doit être offert dans tous les cas (Peebles, 1998).

Figure 16. Signe du papillon (plexus choroïdes)



(Source: P. Miron)

Articles de thèse

Les trois articles de thèse abordent des éléments essentiels du dépistage prénatal au premier trimestre, soit: (1) l'importance de contrôler adéquatement dans le calcul de risque les variables confondantes, dont le tabagisme (2) le rôle prépondérant de la clarté nucale et (3) celui des marqueurs biochimiques. Les trois articles ont été acceptés et publiés dans des revues avec comité de pairs. Ils portent plus précisément sur les sujets suivants :

1. Effet du tabagisme maternel sur le dépistage prénatal de la trisomie 21 et de la trisomie 21 au premier trimestre de grossesse (Miron et al., 2008);

2. Seuils de clarté nucale dans le dépistage prénatal de la trisomie 21 et de la trisomie 18 (Miron et al., 2009);

3. Niveaux plasmatiques de la protéine FLRG (*follistatin-related gene*) au premier trimestre de grossesses avec un syndrome de Down (Miron et al., 2010).

Article I. Effet du tabagisme maternel sur le dépistage prénatal de la trisomie 21 et de la trisomie 18 au premier trimestre de grossesse

Objectif

Déterminer l'impact du tabagisme maternel sur les résultats du dépistage prénatal de la trisomie 21 et de la trisomie 18.

Méthodologie

Les données suivantes provenant d'une cohorte de 53,114 femmes enceintes au premier trimestre de leur grossesse ont été analysées : statut de tabagisme, âge maternel, âge de la grossesse, niveaux sanguins maternels de la fraction libre de la ß-hCG et de la protéine A associée à la grossesse (PAPP-A) et épaisseur de la clarté nucale fœtale. Une comparaison brute et ajustée des données a été réalisée statistiquement entre les fumeuses et non-fumeuses.

Résultats

Les niveaux de PAPP-A et de la fraction libre ß-hCG dans le sang maternel séché étaient significativement plus bas ($p<0.001$) et la clarté nucale fœtale significativement plus épaisse ($p<0.001$) chez les femmes fumant durant le premier trimestre de leur grossesse. En ce qui concerne l'estimation globale du risque produite pour la trisomie 21 en combinant l'âge maternel, les marqueurs échographiques et biochimiques, aucune différence significative n'a été notée entre les fumeuses et non-fumeuses. Par contre, le risque et le taux de faux positifs étaient significativement plus élevés, en présence de tabagisme maternel, pour la trisomie 18 ($p<0.001$). Une association biologique réelle entre le tabagisme maternel et la trisomie 18 reste à clarifier.

Conclusion

L'ajustement statistique du tabagisme maternel est recommandé pour le dépistage prénatal de la trisomie 18 et est probablement inutile pour la trisomie 21 puisque les effets combinés du tabagisme sur la diminution de la fraction libre de la ß-hCG et sur l'augmentation de la

clarté nucale s'annulent statistiquement. Une recherche plus poussée serait toutefois requise afin de vérifier une association biologique possible entre le tabagisme maternel et la trisomie 18.

Cet article a été publié dans la revue *Prenatal Diagnosis* (Miron et al., 2008).

Contribution

L'auteur principal de l'article a eu l'idée et conçu le devis de l'étude. Les données de l'étude furent extraites d'une base de données, conçue par l'auteur principal. L'entrée de données fut réalisée par différents assistants techniques, au cours des années. L'auteur principal a analysé les données et interprété les résultats, sous la supervision du Pr Jean Lambert. Il a écrit le manuscrit. Les analyses statistiques furent réalisées par l'auteur principal et le Pr Jean Lambert. La révision critique de l'article fut principalement effectuée par Pr Jean Lambert, Dr Yvan Côté et un comité de pairs.

Effect of maternal smoking on prenatal screening for Down syndrome and trisomy 18 in the first trimester of pregnancy

Running head: Dried blood levels of PAPP-A and free β-hCG are significantly decreased and fetal NT significantly increased in maternal smokers. For overall risk calculation of Down syndrome, no significant changes were found with smoking. However, a significant increase in positive rates was found for trisomy 18. Adjustment for smoking is recommended.

Keywords: Nuchal Translucency Measurement, Pregnancy-Associated Plasma Protein-A, Chorionic Gonadotropin, beta Subunit, Human , Smoking, Down Syndrome, Trisomy

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Abstract

Objectives

To assess the impact of maternal smoking on first-trimester prenatal screening results for Down syndrome and trisomy 18.

Methods

Data on maternal smoking status, maternal age, gestational dating, levels of free beta-human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein A (PAPP-A) in maternal blood and fetal nuchal translucency (NT) thickness were analysed from a cohort of 53 114 women. Statistical analyses were carried out for crude and adjusted comparisons between smoking and non-smoking groups.

Results

In women who smoked during the first trimester of pregnancy, PAPP-A and free β-hCG levels from dried blood were significantly decreased ($p<0.001$) and fetal NT thickness was significantly increased ($p < 0.001$). For an overall risk assessment combining maternal age and biochemical and ultrasound markers, no significant changes were found for Down syndrome with smoking, but significant increases in average risk as well as in positive rates were found for trisomy 18 ($p < 0.001$). A potential association between maternal smoking and trisomy 18 remains to be clarified.

Conclusion

Adjustment for smoking is recommended in first-trimester prenatal screening for trisomy 18 and probably not warranted for Down syndrome because of the cancelling effects of decreased free β-hCG and increased NT. Further research is required to demonstrate a biological association between maternal smoking and trisomy 18.

Introduction

Cigarette smoking in the second trimester has been shown to influence maternal serum levels of human chorionic gonadotropin (hCG), unconjugated estriol (uE_3), alpha-fetoprotein (AFP) and more importantly, inhibin-A ans impacts therefore, on second-trimester screening results for Down syndrome (Palomaki et al., 1993, Bartels et al., 1993, Spencer, 1998, Ferriman et al., 1999, Rudnicka et al., 2002). Yet, only a few studies have been published regarding its impact on prenatal screening in the first trimester of pregnancy. Most of these studies have confirmed reduced serum levels of free beta-human chorionic gonadotropin (free β-hCG) and pregnancy associated plasma protein A (PAPP-A) in maternal smokers. Contradictory results have been published regarding nuchal translucency (NT) thickness. While in two cohorts no difference in NT thickness was found between smokers and non-smokers, a significant increase in fetal NT multiples of the median (MoM) was observed by others in smoking populations (Yigiter et al., 2006, Spencer et al., 2004, Niemimaa et al., 2003, Ardawi et al., 2007b).

The impact of maternal smoking on positive rates of prenatal screening has also been poorly studied, mainly with respect to the risk assessment of Down syndrome. Although correcting first-trimester biochemical markers for maternal smoking status appears to have little impact on the detection rates, it allows for a reduction in positive rates for Down syndrome (Spencer et al., 2004). No data has been published yet regarding effect of smoking on the risk assessment of trisomy 18.

The objective of our study was to further define, in a large cohort of women, the influence of maternal smoking on the results of first-trimester prenatal screening for Down syndrome and trisomy 18.

Materials and Methods

Data were extracted from a historical cohort of 53 114 women in Quebec who, between the years 1999 and 2005, underwent prenatal screening for Down syndrome and trisomy 18 in their first trimester of pregnancy (i.e. between the 11^{th} and 14^{th} weeks). Patients signed a consent form indicating that their results could be used anonymously for research purposes. Pregnancy outcomes were not studied in the present analysis, since this information was not obtained from all women.

For all subjects, request forms containing demographic and clinical variables were filled by physicians or their assistants with patient collaboration. For cigarette smoking, a specific box with a "yes" or "no" answer had to be checked. Exact number of cigarettes smoked daily was not determined. Blood samples were collected on filter paper attached to the request form. PAPP-A and free β-hCG levels were analyzed using in-house enzyme-linked immunosorbant assays (ELISAs), as described previously (Krantz et al., 2005).

Sonographers performing NT measurements were sensitized to the importance of following the Fetal Medicine Foundation guidelines (www.fetalmedicine.com/nuchal) (Snijders et al., 1998). They were all recognized by their respective licensing authority to perform first trimester fetal sonography (College of physicians of Quebec and Order of Radiology

technologists of Quebec). Risk assessments of Down syndrome and trisomy 18 were produced by combining both the biochemical and the ultrasound markers. Fetal nasal bone measurement, initiated in 2003, was assessed in 9268 pregnant women but not included in the calculation of risks.

The software program for risk assessment of Down syndrome and trisomy 18 was provided by NTD Laboratories (New York, USA). Risks were determined by multiplying the likelihood ratio by the women's risk for Down syndrome and trisomy 18 before screening, which was based on maternal age, gestational age and prior history of trisomy. Women with risks greater than that of a 35-year-old at the same GA were considered to be at increased risk for Down syndrome (Krantz et al., 2000). Cut-off levels used for positive results were set at 1:210 to 1:266 for Down syndrome, depending on GA, and at 1:150 for trisomy 18.

Statistical analysis

Comparisons of demographic variables and GAs between groups were based on the two-sample Student *t*-test for quantitative characteristics and on the Pearson's $\chi 2$ for qualitative (or categorical) characteristics. *T*-test p-values were adjusted for unequal variances when necessary.

Comparisons of biochemical and ultrasound markers as well as of risks for Down syndrome and trisomy 18 were performed using multiple linear regressions adjusting for demographic variables (maternal age, weight and ethnic origin) and GAs. Other variables such as prior history of Down syndrome, insulin-dependent diabetes, experience and FMF certification

of ultrasonographers (n=467) were considered for adjustment but not included in the models due to lack of statistical significance. Residuals were studied for outliers, non-normal distributions and heteroscedasticities. Logarithmic transformations were performed on all biochemical markers owing to skewed distributions. Comparisons of positive rates for Down syndrome and trisomy 18 were performed using multiple logistic regressions adjusting for demographic variables and GAs. Residuals were studied for outliers and the Hosmer-Lemeshow statistic was used to verify the overall quality of adjustment.

Due to the very large sample size (N = 53 114), the significance level was set to 0.001, and caution must be taken when interpreting the results.

Results

Women participating in this prenatal screening program were significantly older ($\overline{X}$=31.4 years) than those in the general pregnant population of Quebec ($\overline{X}$=29.1 years; p<0.001) (ISQ, 2006).

In our studied population, the proportion of women who smoked during pregnancy was 6.7%. There were more Caucasians and less of other ethnic groups among smokers (p<0.001), as shown in Table 1. All other demographic characteristics and gestational ages were similar between smokers and non-smokers (p>0.064).

When adjusting for demographic variables and GA, significant reduction in dried blood levels of PAPP-A and free β-hCG was observed (p < 0.001), as depicted in Table 2. When

based on maternal age and biochemical markers alone, the risk of Down syndrome was significantly reduced ($p<0.001$) and the risk of trisomy 18 was significantly increased ($p < 0.001$) among smokers (Table 2).

For ultrasound markers, smoking had a significant influence on NT thickness by increasing its mean from 1.59 mm for non-smokers to 1.62 mm for smokers ($p < 0.001$), as described in Table 3. However, smoking had no impact on nasal bone length ($p = 0.646$). When based on maternal age and NT alone, observed risks for Down syndrome and for trisomy 18 were significantly increased among smokers ($p < 0.001$).

Effects of smoking in the first trimester of pregnancy on overall prenatal screening results for Down syndrome and trisomy 18 are summarized in Table 4. No significant changes were found in average calculations of risks and in positive rates for Down syndrome between smokers and non-smokers. However, for trisomy 18, both the calculations of risk and the positive rates were significantly increased in smokers. Positive rates went up from 0.7 % in non-smokers to 2.3 % in smokers ($p < 0.001$), an important rise with significant clinical implication.

Discussion

As previously demonstrated in maternal serum, this study confirmed that levels of PAPP-A and free β-hCG in dried blood samples are also reduced by smoking. In fact, in our study, MoM medians of PAPP-A and free β-hCG were reduced by 17% and 13%, respectively.

The decrease in free β-hCG levels caused by smoking appears to be more important in dried blood samples than the decrease reported in serum in previous large cohort studies (Spencer et al., 2004, de Graaf et al., 2000, Kagan et al., 2007) (Table 5). However, in other smaller studies using also serum, the decrease seems similar to our results with dried blood (de Graaf et al., 2000, Niemimaa et al., 2003, Ardawi et al., 2007a, Ardawi et al., 2007b). Several confounding factors, such as ELISA kit used, could explain this potential difference between dried blood and serum levels of free β-hCG and, therefore, its significance remains to be clarified.

This study also indicated a significant increase in NT thickness caused by maternal smoking. Over the last few years, contradictory results have been published regarding the effect of smoking on NT measurements. Although a large cohort study (n=32 854) suggested that NT thickness was not increased in maternal smokers, two studies with smaller sample sizes of 1 275, 4 436 and 1778 women, respectively, found an increase in NT thickness (Spencer et al., 2004, Niemimaa et al., 2003, Yigiter et al., 2006, Ardawi et al., 2007a). After controlling confounding variables, our large cohort study with 53 114 women confirmed a significant impact of smoking on NT thickness.

By reducing maternal blood levels of PAPP-A and free β-hCG and by increasing NT thickness, maternal smoking significantly influences prenatal screening results for trisomy 18 and increases, by at least three times, the probability for a woman to be offered, on the basis of a positive screening, an invasive diagnostic procedure such as amniocentesis or chorionic villi sampling.

The absence of any significant impact of smoking on the overall risk assessment of Down syndrome may be explained by the cancelling effect of both the decrease of free β-hCG and the increase of NT thickness.

In our study, only 6,7% of women smoked at the time of first-trimester screening. While overall, there appears to be fairly good correlation between maternal report of smoking during pregnancy and actual smoking, the rates of nonreporting and under-reporting are most probably still substantial. One has to assume that most women who state that they are smoking during pregnancy, are, in fact, smoking. But an important percentage of the women who state that they do not smoke are, in fact, smokers who aren't reporting it (Lindqvist et al., 2002, George et al., 2006). Furthermore, many women quit smoking once they discover that they are pregnant, but they may not discover this until late in their first trimester, at which point they stop (Grange et al., 2006). These women will be classified as non-smokers at time of screening, but the smoking they did up to week 6, 8, or 10 is likely to affect their PAPP-A, free β-hCG, and NT measurements. The combination of these factors could actually lead to an underestimate of the effect of smoking.

Number of cigarettes smoked daily was not registered in our database and therefore, the dose relationship of smoking with biochemical and ultrasound variables could not be assessed. In a recent article, a significant inverse relationship of the number of cigarettes per day was found with the level of PAPP-A, but not with free β-hCG. Yet, the impact of correcting for the dose dependant rather than the all or nil effect of smoking appears to be marginal (Kagan et al., 2007).

Physiological mechanism(s) responsible for the effect of smoking on prenatal screening markers remain to be elucidated. Since reduced levels of various serum analytes have been demonstrated with smoking in non-pregnant status, direct interference of cigarette residues in the blood on the measurement of serum analytes cannot be excluded (Rodger et al., 1985, Goodman et al., 1996). However, this hypothesis does not explain the increased NT thickness of fetuses in maternal smokers.

Smoking is known to substantially increase a woman's risk to serious pregnancy complications, including intrauterine fetal growth restriction, placenta abruptio and preterm delivery. Abnormal placentation is a unifying theme of such late complications, suggesting that smoking could also cause adverse events earlier in pregnancy. In fact, maternal cigarette smoking has been shown to impair placental functions very early in gestation (6-8 weeks) by inhibiting cytotrophoblast proliferation, differentiation and invasion, and by increasing production of angiogenic factors, such as vascular endothelial growth factors (VEGFs) (Zdravkovic et al., 2005). In a recent article, mechanisms involved in increased NT thickness have been related to a disturbance of embryonic lymphangiogenesis with alterations in the extracellular matrix composition and hemodynamic disorders (L'Hermine-Coulomb, 2005). One of the proposed biochemical mechanisms involves an increased expression of VEGF-A, as observed by immunohistochemistry in distended jugular lymphatic sacs of increased NT, showing blood vessel characteristics (Bekker et al., 2006). To further substantiate this hypothesis, an involvement of VEGF-A in the development of cystic hygroma of Turner syndrome has also been recently suggested (Brandenburg et al., 2005) . Therefore, our findings of increased NT thickness in maternal smokers may be the result of an up-regulation of VEGFs.

Smoking causes a wide variety of reproductive problems, including DNA damage to spermatozoa and oocytes, transmissible to embryos (Zenzes, 2000, DeMarini, 2004). Although no association has been found with the live birth prevalence of Down syndrome, cigarette smoking may increase the risk of aneuploidy for certain chromosomes, such as 1, 13, and YY disomies (Cuckle et al., 1990, Rudnicka et al., 2002, Harkonen et al., 1999, Rubes et al., 1998, Shi et al., 2001). To date, no effect of smoking on the birth incidence of trisomy 18 has been documented. The significant increase of screen positive rates of trisomy 18 found in maternal smokers raises the possibility of a real cause-to-effect relationship between smoking and trisomy 18. This can only be clarified by further research looking specifically at pregnancy outcomes.

Conclusion

Based on our results, correction for smoking status is highly recommended in first-trimester prenatal screening for trisomy 18, and is probably not warranted for Down syndrome because of the cancelling effects of the decrease of free β-hCG and the increase of NT with smoking. Corrections could be achieved by dividing the weight-corrected MoM in smokers by 0.86 for PAPP-A, 0.87 for free β-hCG and 1.07 for NT. Further research is recommended to clarify a potential biological association between maternal smoking and trisomy 18.

Disclosure

Dr. Miron reports being owner of Prenagen Inc. and consultant through this corporation for Warnex Inc.

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Table 1. Demographic characteristics and gestational ages of smoking and non smoking pregnant women undergoing prenatal screening between 11 and14 weeks (n=53 114)

		Non-smoking	**Smoking**	**P-value**
		N = 49 531	**N = 3 583**	
A.	**Quantitative variables**	**$\overline{X}$ ± SD**	**$\overline{X}$ ± SD**	
	Women's age (years)	31.4 ± 3.88	31.2 ± 4.50	0.064
	Gestational age (GA) at time of echo (weeks)	12.5 ± 0.64	12.5 ± 0.66	0.222
	Corrected GA at time of sampling	12.6 ± 0.59	12.6 ± 0.60	0.261
	Weight (kg)	65.1 ± 12.4	65.4 ± 12.8	0.074
B.	**Qualitative variables**	**%**	**%**	
	Ethnic origin			
	White	95.9 %	98.7 %	
	Asian	2.0 %	0.3 %	
	Afro-Caribbean	0.7 %	0.3 %	0.000
	Native	0.1 %	0.1 %	
	Other	1.2 %	0.6 %	
	Active vaginal bleeding	9.2 %	9.3 %	0.743

Table 2. Biochemical data of smoking and nonsmoking pregnant women undergoing prenatal screening between 11 and 14 weeks (n=53 114)

	Non smoking $\overline{X}$ **± SD**	**Smoking** $\overline{X}$ **± SD**	**P-value**
PAPP-A (UI/L)	5.49 ± 7.43	4.43 ± 3.76	0.000
Free β-hCG (ng/ml)	54.2 ± 35.0	47.4 ± 33.7	0.000
Average risk of Down syndrome (based on age and biochemistry)	1:2 566 ± 1:2 073	1:2 720 ± 1:2 211	0.000
Average risk of trisomy 18 (based on age and biochemistry)	1:8 618 ± 1:2 934	1:7 522 ± 1:3 709	0.000
	Median (MoM)	**Median (MoM)**	
PAPP-A (MoM)	1.03	0.86	0.000*
Free β-hCG (MoM)	1.00	0.87	0.000*

"*" : Groups were compared using multiple linear regressions.

Table 3. Clinical data of smoking and nonsmoking pregnant women undergoing prenatal screening between 11 and 14 weeks (n=53 114)

	Non smoking $\overline{X} \pm SD$	**Smoking** $\overline{X} \pm SD$	**P-value**
CRL (mm)	63.3 ± 8.39	63.6 ± 8.60	0.016
Nuchal thickness (mm)	1.59 ± 0.49	1.62 ± 0.50	0.000
Nasal bone length (mm)	1.79 ± 0.41	1.78 ± 0.44	0.646
Average risk of Down syndrome (based on age and NT)	1:3 222 ± 1:2 163	1:3 170 ± 1 : 2 323	0.000
Average risk of trisomy 18 (based on age and NT)	1:6 425 ± 1:3 337	1:6 158 ± 1:3 429	0.000
	Median (MoM)	**Median (MoM)**	
NT	1.00	1.07	0.001*

"*" : Groups were compared using multiple linear regressions.

Table 4. Effect of smoking on overall prenatal screening results for Down syndrome and trisomy 18 in the first trimester (n=53 114)

		Non-smoking $\overline{X} \pm SD$	Smoking $\overline{X} \pm SD$	P-value
A.	**Quantitative variable**			
	Average risk of Down syndrome (based on age, ultrasound and biochemistry)	1:4 428 ± 1:2 821	1:4 459 ± 1:3 024	0.088
	Average risk of trisomy 18 (based on age, ultrasound and biochemistry)	1:9 433 ± 1:1 996	1:8 795 ± 1:2 868	0.000
B.	**Qualitative variables**			
	Positive rate for Down syndrome (%)	3.8 %	4.3 %	0.210
	Positive rate for trisomy 18 (%)	0.7 %	2.3 %	0.000

Table 5. Published articles on the effect of smoking on free β-hCG levels (serum and dried blood)

Study	Serum (S) vs Dried blood (DB)	ELISA Kit	Smoking	N	MoM free β-hCG
De Graaf et al. (2000)	S	Delfia	Y	117	0,89
			N	1 247	1,00
Niemimaa et al. (2003)	S	Delfia	Y	454	1,06
			N	3 825	1,07
Spencer et al. (2004)	S	Brahms	Y	3 779	0,97
			N	32 730	1,00
Ardawi et al. (2007a)	S	Delfia	Y	304	0,88
			N	1 616	1,03
Ardawi et al. (2007 b)	S	Delfia	Y	420	0,87
			N	1 736	1,00
Kagan et al. (2007)	S	Brahms	Y	13 976	1,003
			N	95 287	1,035
Miron et al. (2007)	DB	NTD	Y	3 583	0,87
			N	49 531	1,00

Article II : Seuils de clarté nucale dans le dépistage prénatal de la trisomie 21 et de la trisomie 18

Objectif

Déterminer si la clarté nucale (CN) peut être utilisée à titre de marqueur de triage du premier trimestre dans le cadre du dépistage prénatal du syndrome de Down et de la trisomie 18.

Méthodologie

Les données issues du dépistage prénatal du premier trimestre mené chez 77 443 femmes ont été stratifiées en fonction des âges maternel et gestationnel. Elles ont par la suite été analysées en vue d'identifier les seuils de CN au-dessus ou en-deçà desquels seuls des résultats positifs (risque élevé) ou négatifs (risque faible) ont été signalés par un test de dépistage prénatal du premier trimestre combinant la PAPP-A, la β-hCG libre et la CN.

Résultats

Le dépistage prénatal combiné était toujours positif en ce qui concerne le syndrome de Down lorsque l'épaisseur de la CN excédait 4.0 mm. Au fur et à mesure du vieillissement chez les femmes, ce seuil supérieur de CN a évolué en fonction de l'âge gestationnel. Chez les femmes âgées de 35 à 37 ans, le dépistage prénatal combiné était toujours positif lorsque la CN dépassait 2.8 mm, 3.0 mm et 3.4 mm à la 11^{e}, 12^{e} et 13^{e} semaine de gestation, respectivement. Chez les femmes de plus de 42 ans, le seuil supérieur de la CN était de 1.8 mm, de 2.4 mm et de 2.7 mm à 11^{e}, 12^{e} et 13^{e} semaine de gestation, respectivement. Chez les femmes de moins de 35 ans, nous avons identifié des seuils inférieurs de CN en deçà desquels le dépistage prénatal combiné était toujours négatif.

Conclusion

Dans le cadre d dépistage prénatal du syndrome de Down et de la trisomie 18, il est possible d'identifier des seuils de CN au-dessus desquels le dépistage biochimique n'offre aucun avantage additionnel. En ce qui concerne les grossesses dans le cadre desquelles la

CN se situe au-delà des seuils supérieurs, un dépistage prénatal effractif pourrait être offert sans délai.

Cet article a été publié dans le Journal d'obstétrique et gynécologie du Canada (Miron et al., 2009).

Contribution

L'auteur principal de l'article a eu l'idée et conçu le devis de l'étude. Les données de l'étude furent extraites d'une base de données, conçue par l'auteur principal. L'entrée de données fut réalisée par différents assistants techniques, au cours des années. L'auteur principal a analysé les données et interprété les résultats, sous la supervision du Pr Jean Lambert. Il a écrit le manuscrit. Les analyses statistiques furent réalisées par l'auteur principal et le Pr Jean Lambert. La révision critique de l'article fut principalement effectuée par Pr Jean Lambert, Dr Yvan Côté et un comité de pairs.

Nuchal Translucency Thresholds in Prenatal Screening for Down Syndrome and Trisomy 18

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Abstract

Objective : To determine if nuchal translucency (NT) can be used as a first trimester triage marker in prenatal screening for Down syndrome and trisomy 18.

Methods: Data from first trimester prenatal screening in 77 443 women were stratified by maternal and gestational ages. They were then analyzed to identify NT thresholds above or below which only positive (high-risk) or negative (low-risk) results were reported by a first trimester prenatal screening test combining PAPP-A, free β-hCG and NT.

Results: Combined prenatal screening was always positive for Down syndrome when NT thickness exceeded 4.0 mm. As women aged, this upper NT threshold value changed according to gestational age. In women aged 35 to 37 years, combined prenatal screening was always positive when NT exceeded 2.8 mm, 3.0 mm, and 3.4 mm at 11, 12, and 13 weeks of gestation, respectively. In women over 42 years of age, the upper threshold value for NT was 1.8 mm, 2.4 mm, and 2.7 mm at 11, 12, and 13 weeks of gestation, respectively. In women less than 35 years of age, we identified lower threshold values below which combined prenatal screening for Down syndrome was always negative.

Conclusion : In prenatal screening for Down syndrome and trisomy 18, it is possible to identify NT threshold values above which biochemical screening provides no additional benefit. In pregnancies in which NT is above the established upper cut-offs, invasive prenatal screening can be offered without delay.

Key Words: Nuchal translucency measurement, pregnancy-associated plasma protein-A, chorionic gonadotropin, beta subunit, human, Down syndrome, trisomy

Competing Interests: see Acknowledgements.

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ABBREVIATIONS

AFP alpha fetoprotein

CRL crown–rump length

CVS chorionic villus sampling

GA gestational age

hCG human chorionic gonadotropin

NT nuchal translucency

PAPP-A pregnancy-associated plasma protein-A

Introduction

Ultrasound measurement of fetal nuchal translucency was proposed for the first time in 1992 to detect fetal chromosomal anomalies in the first trimester of pregnancy.[1] Since then, use of NT has gained in popularity, and it is now the most widely used ultrasound marker for aneuploidy. Because of its highly associated false-positive rate, its use as a stand-alone screening test to identify aneuploidy in single pregnancies was considered by many to be inappropriate.[2] In fact, only a few studies have suggested that first trimester maternal blood screening provides no additional benefit when the NT measurement is above 4.0 mm.[3,4]

To improve the accuracy of prenatal screening in the first trimester, combining NT with biochemical markers such as pregnancy-associated plasma protein-A and free β-subunit of human chorionic gonadotropin was successfully proposed in the 1990s.[5–7] This approach has been prospectively validated in more than 200 000 screens, with a detection rate of 88% for Down syndrome at a fixed false-positive rate of 5%.[8] In order to study more precisely the NT levels that, when combined with serum levels of PAPP-A and free β-hCG, would mark the threshold between positive or negative screening results, we analyzed fetal NT measurements in a large cohort of patients in the context of maternal and gestational ages. The main objective of this analysis was to determine if NT could, in some instances, be used alone as a first trimester triage marker in screening for Down syndrome and trisomy 18.

Material and Methods

Data were extracted from a historical cohort of 77 443 women in Quebec who underwent prenatal screening for Down syndrome and trisomy 18 in their first trimester of pregnancy (i.e., between the 11th and 14th weeks) between 1999 and 2007. These women had given consent for their results to be used anonymously for research purposes. Since pregnancy outcomes could not be obtained from all women, this information was not included in the analysis.

Fetal crown–rump length and nuchal translucency measurements were performed by ultrasonographers who were instructed to follow the Fetal Medicine Foundation guideline.[9] Fetal nasal bone assessments (n = 16 918) were initiated in 2003 but were not included in the calculation of risks.

Overall risk assessments for Down syndrome and trisomy 18 were produced by combining NT with maternal blood levels of PAPP-A and free β-hCG (referred to as combined first trimester prenatal screening). In all subjects, blood samples were collected and transported to the laboratory using dried blood filter paper. Maternal blood levels of PAPP-A and free β-hCG were determined using in-house enzyme-linked immunosorbant assays, as described previously.[10] The software program used for risk assessment of Down syndrome and trisomy 18 was provided by NTD Laboratories (New York, NY). Individual risk was determined by multiplying the likelihood ratio by the woman's risk of having a fetus with Down syndrome and trisomy 18 before screening, based on maternal age, gestational age and prior history of trisomy. Women with a risk greater than that of a 35-year-old at the

same gestational age were considered to be at an increased risk of having a fetus with Down syndrome.[11] Cut-off levels used for positive results were set at 1:210 to 1:266 for Down syndrome (depending on gestational age) and at 1:150 for trisomy 18.

Only women with no missing data related to maternal age, gestational age (as defined by crown–rump length) and combined prenatal screening results were included (N = 77 443).

Gestational age was categorized as follows:

- CRL 45 mm to < 55 mm = 11+1 to 11+6 weeks for GA
- CRL 55 mm to < 69 mm = 12 to 12+6 weeks for GA
- CRL from 69 mm to 84 mm = 13 to 13+6 weeks for GA

Overall positive (high-risk) and negative (low-risk) prenatal screening results for Down syndrome and trisomy 18 were stratified by categorized maternal and gestational ages. For each combination, minimum and maximum NT values were compared between positive and negative overall results to find NT levels above or below which only positive or negative values were observed in a given combination. Finally, NT and maternal age cut-offs were chosen to identify women for whom CVS or amniocentesis would have been offered with or without the biochemical analysis, or for whom no further testing was required because of perceived low risk.

Results

A total of 77 443 prenatal screenings performed in the first trimester of pregnancy (i.e., with fetal CRL from 45 to 84 mm) were analyzed. Mean maternal age was 31.2 ± 3.9 years, significantly older than the Quebec population of pregnant women at the time of screening (29.1 years; $P < 0.001$).[12] The average gestational age on ultrasound was 12.4 ± 0.6 weeks. Most of the women included in this study were of Caucasian origin (96%), followed by Asian (1.9%), and Afro-Caribbean (0.8%) (Table 1).

For the whole cohort, 3.6% of women screened positive for the combined test (n = 2800). As shown in Figure 1, this percentage increased progressively with maternal age. No women under 19 years of age had a positive screening (n = 21), while almost all women over 43 years of age screened positive (86/90 or 96%).

The distribution of NT (mean ± SD, minimum and maximum values), stratified by categorized maternal age, categorized CRL, and overall screen results for Down syndrome, with upper and lower cut-off levels, is summarized in Tables 2, 3, and 4.

Combined prenatal screening was always positive when NT was found to be above 4.0 mm (n = 197), representing 7.0 % of the total positive screen population. Nevertheless, this NT cut-off level, above which combined prenatal screening was always positive, could vary from 1.8 mm to 4.0 mm or more, depending on categorized maternal and gestational ages. For women less than 35 years of age, upper NT cut-off levels were 3.3 mm, 3.8 mm, and 4.0 mm at 11, 12, and 13 weeks of gestation, respectively. NT upper threshold values

progressively decreased with maternal aging, reaching 2.8 mm, 3.0 mm, and 3.4 mm in women aged 35 to 37, and 1.8 mm, 2.4 mm, and 2.7 mm in women over 42 years of age at 11, 12, and 13 weeks of gestation, respectively. With stratification by maternal and gestational ages, an increased number of women (n = 323) had an NT above which combined prenatal screening was always positive, representing 11.5% of the total screen positive population. For each category of maternal and gestational ages, the same upper cut-off values of NT set for Down syndrome could also be used for trisomy 18.

The NT cut-off levels below which combined first trimester prenatal screening for Down syndrome was always negative varied from less than 0.6 mm to 2.2 mm, depending on maternal and gestational ages (Table 4). For women less than 23 years of age, lower NT cut-off levels were found to be 1.2 mm, 1.4 mm, and 2.2 mm at 11, 12, and 13 weeks of gestation, respectively. The NT lower threshold values also progressively decreased with maternal aging, reaching 1.0 mm in women aged 23 to 26, and 0.6 mm, 0.8 mm, and 0.9 mm in women aged 27 to 34 at 11, 12, and 13 weeks of gestation, respectively. Women with NT values below their specific lower cut-off levels represented only a very small proportion of the total screen population (0.08%). No useful lower NT cut-off levels for combined negative screen could be found in women older than 34 years of age.

The same cut-off levels of NT thickness below which combined first trimester prenatal screening for Down syndrome was always negative could also be applied for trisomy 18 results, with the exception of one woman aged 23 with a fetal CRL of 59 mm. In this individual's case, PAPP-A and free β-hCG multiples of the median were found to be very

low at 0.07 and 0.10, respectively. An increased risk for trisomy 18 or 13 was initially suggested. Several major structural abnormalities were found on ultrasound in the second trimester (club hands, cardiac defect, cerebral ventriculomegaly, and asymmetrical intrauterine growth restriction) and a triploidy (69,XXX) was finally diagnosed by fetal karyotyping.

Discussion

Several, often complex, strategies have been proposed to screen for Down syndrome and other common aneuploïdies.[13,14] Over the last decade, the popularity of second trimester prenatal screening (AFP, estriol, AFP ± inhibin A) has declined and first trimester screening using a combination of maternal blood biochemical markers (free β-hCG and PAPP-A) with fetal nuchal translucency measurement has increased. Although more recent approaches combining first and second trimester markers have been proposed, such as integrated, sequential, and contingent screenings, there is a clear trend towards early disclosure of results to a maximum number of patients.[15–17] This trend is confirmed by (a) women's preference for first trimester screening of pregnancy,[18,19] (b) ethical principles of informed consent and respect for patient autonomy, beneficence, and justice,[20] and (c) the recent joint statement of the National Institute of Child Health and Human Development, the Society for Maternal-Fetal Medicine, and the American College of Obstetricians and Gynecologists.[21]

In 1992, the first-trimester NT was reported to be increased in 35% of aneuploid fetuses, compared with only 1% of euploid fetuses.[1] Since then, several large studies have confirmed that NT screening, which takes into account maternal and gestational ages, has a detection rate for Down syndrome ranging from 69% to 75% with a false positive rate of 5% to 8.1%.[22–24] The Society of Obstetricians and Gynaecologists of Canada and the Canadian College of Medical Geneticists jointly stated in 2007 that any prenatal screen for Down syndrome should, as a minimum standard, have a detection rate of at least 75%, with a false positive rate of no more than 5%. Their recommendation was that NT alone without

biochemical markers should never be offered, except in the context of multiple pregnancy.[2] We believe this is not entirely true and that there are some exceptions to this guideline.

With our analysis of the findings in 77 443 women in the first trimester, we confirmed that with a NT value greater than 4.0 mm there is no added value in delaying offering immediate invasive diagnostic testing. On the basis of categorized gestational and maternal ages, our study also demonstrated that better and lower NT cut-offs values can be used by clinicians at the time of NT sonography to disclose positive screening to their patients immediately.

The FASTER Research Consortium has recommended that, in the presence of a NT value ≥ 3.0 mm, immediate invasive testing should be offered to all patients without obtaining serum markers.[4] With this strategy, 31.1% of the women in our study with a NT value ≥ 3.0 mm would have been needlessly offered invasive testing because their final result in combined screening was actually negative. As demonstrated in Figure 2, we believe that upper cut-off NT limits for screen positivity should be adapted instead to maternal and gestational ages at the time of screening. In women older than 37 years of age, this upper cut-off limit of 3.0 mm can be further decreased, while in younger women it most often needs to be raised. With this new strategy, all women with NT values greater than the suggested upper cut-off levels would screen positive with or without biochemical markers. To decrease further the risk of missing chromosomal abnormalities, specifically in women ≤ 35 years old at 12 or 13 weeks of pregnancy, a combination of our strategy with that of

the Fetal Medicine Foundation could also be used (i.e., to offer fetal karyotyping by CVS in this specific group when the NT value is above 3.5 mm).[25]

Prenatal screening is based on respect for a patient's autonomy. It could therefore be argued that communicating a result as being "at risk" or "not at risk" is inappropriate and that communicating a result as a probability is more suitable. In order to fully respect an informed consent process, we believe that, in the presence of a NT value above the suggested thresholds, all of the following options are ethically acceptable and should be thoroughly discussed with the patient: (1) no further screening or diagnostic testing; (2) immediate disclosure of a probability, using NT with maternal and gestational ages; (3) completion of the screening process with biochemical markers; (4) proceeding directly with diagnostic testing such as a transabdominal CVS or an amniocentesis after 15 weeks. Additional assessment of fetal nasal bone, frontomaxillary facial angle and other early soft markers of aneuploidy could also be used eventually to refine the probability of aneuploidy at the time of first trimester ultrasound.[26–28]

NT threshold values below which no further screening is required, because of negative results regardless of biochemical markers, are presented in Figure 3. In this case, however, prediction by NT of a negative screen result pertains to a very limited proportion of women and to very thin NT. We therefore recommend that if such cut-off levels are ever used, they should be restricted mainly to women younger than 27 years of age. With this strategy, one case of triploidy in 74 643 negative screenings would have been missed. However, a high proportion of fetuses with triploidy have major structural abnormalities often detectable by

second trimester ultrasound, and almost all of these affected fetuses will die in utero or within the first year of life.[29–31]

Official reports of first trimester risk assessment, combining biochemical and ultrasound markers, are usually produced by routine diagnostic laboratories within 7 to 10 days after the medical visit. In an attempt to accelerate early disclosure, another concept of point of care service has been suggested, by which combined first trimester results can be provided to the patient within one hour.[32] With the advent of rapid immunoassays, this service provided by one-stop clinics for risk assessment involves serum measurements of free β-hCG and PAPP-A within 30 minutes of obtaining the blood sample with concomitant ultrasound assessment of CRL and NT, allowing rapid production of a combined risk report.[33] However, to be successful and financially viable, the one-stop clinics for risk assessment usually require a large volume of patients and are therefore limited to a very few busy ultrasound clinics. This approach does not preclude use of NT alone in selected cases, as proposed by our results. Taking blood at 8 to 9 weeks of gestation, prior to the NT scan, has also been suggested, but this remains a limited option, particularly in Canada where early access to a first obstetrical consultation has become a matter of concern.[34,35]

Until recently, the recommended maternal age for directly offering amniocentesis or CVS varied from 35 years of age to 38 years of age and over in most developed countries.[36,37] In the context of enhanced prenatal non-invasive screening, this approach is now considered by many to be obsolete. The American College of Obstetricians and Gynecologists recommended in its recent Practice Bulletin that all women regardless of age should have

the option of invasive testing. In Canada, the low risk of fetal loss following mid-trimester amniocentesis recently reported by a secondary analysis of data from the FASTER trial (1/1600) was questioned and quoted as misleading, with an estimate of procedural fetal loss of 0.6% to 1.0% (1/175–1/100) seen as more realistic.[38,39] (Eddleman et al., 2006, Wilson et al., 2007b) Regardless, in Canada it has now been recommended to raise the maternal age for offering CVS or amniocentesis directly from 35 years of age to 40 years of age or over at the time of delivery.[2,39] However, in our cohort, women aged 43 had a probability of at least 69% of having a negative prenatal screening result. In younger women, this proportion of negative screening results increased rapidly. Given that the vast majority of younger women in our study had a negative combined first trimester prenatal screening, and if our results can be confirmed by studies with pregnancy outcomes, it will be reasonable to consider raising the maternal age for directly offering invasive testing to 44 years of age or over (Figure 1).

Until routine non-invasive prenatal diagnosis (using fetal cells or plasma free nucleic acids in maternal blood circulation[40–42]) becomes a reality, first trimester prenatal screening will continue to gain in popularity in centres that are qualified to provide it.

Conclusion

In this study we have defined NT threshold levels above and below which women will always have high- or low-risk results for Down syndrome screening regardless of biochemical findings in maternal blood. Prenatal screening for aneuploidy can be offered as a first step to all pregnant women up to the age of 43, rather than CVS or amniocentesis. Newer approaches to facilitate rapid results to the patient in the first trimester of pregnancy need to be explored. Specific cut-off NT values can sometimes be used alone in a selected population without increasing false positive rates.

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Tables and Legends

Table 1. Demographic and clinical characteristics of the studied population (n = 77 443)

A. Quantitative variables	**N**	**Mean ± SD**
Women's age (years)	77 443	31.2 ± 3.9
Gestational age at time of sonography (weeks)	77 443	12.4 ± 0.6
Weight (kg)	77 424	65.1 ± 12.6
Crown–rump length (mm)	77 443	63.8 ± 8.3
Nuchal thickness (mm)	77 443	1.60 ± 0.48
Nasal bone (mm)	16 918	1.88 ± 0.42
B. Qualitative variables		**%**
Ethnic origin	77 443	
White	74 561	96.3
Asian	1 453	1.9
Afro-Caribbean	633	0.8
Native	60	0.1
Other	736	0.9
Smoking	4 995	6.4
Active vaginal bleeding	7 044	9.1

Table 2. Nuchal translucency distribution stratified by categorized maternal age, categorized crown–rump length and overall results for Down syndrome

Maternal age (years)	CRL (mm)	Screen results	n	Nuchal translucency (mm)		
				Mean ± SD	Minimum	Maximum
< 23	(45–54)	-	134	1.32 ± 0.37	0.7	2.8
		+	2	1.95 ± 1.06	1.2	2.7
	(55–68)	-	419	1.57 ± 0.37	0.4	3.4
		+	11	3.26 ± 1.23	1.4	6.1
	(69–84)	-	219	1.74 ± 0.44	0.8	3.4
		+	2	3.10 ± 1.27	2.2	4.0
23–26	(45–54)	-	1268	1.28 ± 0.35	0.5	3.3
		+	26	3.54 ± 2.06	1.0	8.8
	(55–68)	-	4487	1.54 ± 0.37	0.3	3.8
		+	60	3.11 ± 2.05	1.0	11.4
	(69–84)	-	2138	1.74 ± 0.40	0.6	4.0
		+	32	2.92 ± 1.47	1.0	9.0
27–34	(45–54)	-	7500	1.28 ± 0.33	0.4	3.2
		+	202	2.59 ± 1.51	0.6	10.0
	(55–68)	-	29 477	1.55 ± 0.36	0.2	3.8
		+	624	2.61 ± 1.48	0.8	10.0
	(69–84)	-	15039	1.75 ± 0.39	0.5	3.9
		+	399	2.58 ± 1.02	0.9	10.0
35–37	(45–54)	-	1363	1.29 ± 0.33	0.6	2.8
		+	80	2.11 ± 1.52	0.6	9.1
	(55–68)	-	5759	1.57 ± 0.36	0.5	3.0
		+	345	2.11 ± 1.04	0.9	10.0
	(69–84)	-	3126	1.77 ± 0.38	0.6	3.4
		+	248	2.25 ± 0.71	0.7	5.1
38–41	(45–54)	-	454	1.28 ± 0.29	0.4	2.3
		+	65	2.04 ± 1,49	0.7	8.6
	(55–68)	-	1983	1.56 ± 0.35	0.6	2.8
		+	316	2.06 ± 1.16	0.6	14.0
	(69–84)	-	1014	1.76 ± 0.37	0.7	3.0
		+	219	2.21 ± 1.02	0.9	10.0
≥ 42	(45–54)	-	28	1.35 ± 0.27	0.8	1.8
		+	19	1.56 ± 0.42	0.8	2.4
	(55–68)	-	136	1.55 ± 0.32	0.8	2.4
		+	100	1.89 ± 0.79	0.8	8.0
	(69–84)	-	99	1.80 ± 0.32	1.0	2.7
		+	50	2.07 ± 0.79	0.8	5.7

Table 3. Cut-off levels of nuchal translucency in relation to gestational and maternal age above which there are N combined first trimester prenatal screenings always positive for Down syndrome

Gestational age	CRL (mm)	N/total pop. (%)	N/Positive screen population (%)	NT Cut-off
A. All women				
11+1–11+6 weeks	45–54	77/11 141 (0.7%)	77/394 (20%)	> 3.3 mm
12–12+6 weeks	55–68	115/43 717 (0.3 %)	115/1456 (8%)	> 3.8 mm
13–13+6 weeks	69–84	43/22 585 (0.2 %)	43/950 (5%)	> 4.0 mm
B. Women < 35 years old				
11+1–11+6 weeks	45–54	61/9132 (0.7%)	61/230 (27%)	> 3.3 mm
12–12+6 weeks	55–68	86/35 078 (0.2%)	86/695 (12%)	> 3.8 mm
13–13+6 weeks	69–84	29/17 829 (0.2%)	29/433 (7%)	> 4.0 mm
C. Women 35–37 years old				
11+1–11+6 weeks	45–54	14/1443 (1%)	14/80 (18%)	> 2.8 mm
12–12+6 weeks	55–68	31/6104 (0.5%)	31/345 (9%)	> 3.0 mm
13–13+6 weeks	69–84	16/3374 (0.5%)	16/248 (6%)	> 3.4 mm
C. Women 38–41 years old				
11+1–11+6 weeks	45–54	11/519 (2%)	11/65 (15%)	> 2.3 mm
12–12+6 weeks	55–68	34/2299 (1%)	34/316 (11%)	> 2.8 mm
13–13+6 weeks	69–84	17/1233 (1%)	17/219 (8%)	> 3.0 mm
Women ≥ 42 years old				
11+1–11+6 weeks	45–54	5/47 (11%)	5/19 (26%)	> 1.8 mm
12–12+6 weeks	55–68	12/236 (5%)	12/100 (12%)	> 2.4 mm
13–13+6 weeks	69–84	7/149 (5%)	7/50 (14%)	> 2.7 mm

Table 4. Cut-off levels of NT thickness in relation to gestational and maternal ages below which there are N combined first trimester prenatal screenings always negative for Down syndrome

Gestational age	CRL (mm)	N/total pop. (%)	N/ Negative screen population (%)	NT Cut-off
A. All women				
11+1–11+6 weeks	45–54	22/11 141 (0.2%)	22/10 747 (0.2%)	< 0.6 mm
12–12+6 weeks	55–68	20/43 717 (0.05%)	20/42 261 (0.05%)	< 0.6 mm
13–13+6 weeks	69–84	9/22 585 (0.04%)	9/21 635 (0.04%)	< 0.7 mm
B. Women < 23 years old				
11+1–11+6 weeks	45–54	45/136 (33%)	45/134 (34%)	< 1.2 mm
12–12+6 weeks	55–68	109/430 (25%)	109/419 (26%)	< 1.4 mm
13–13+6 weeks	69–84	185/221 (84%)	185/219 (84%)	< 2.2 mm
C. Women 23–26 years old				
11+1–11+6 weeks	45–54	168/1294 (13%)	168/1268 (13%)	< 1.0 mm
12–12+6 weeks*	55–68	136/4547 (3%)	136/4487 (3%)	< 1.0 mm
13–13+6 weeks	69–84	15/2170 (0.7%)	15/2138 (0.7%)	< 1.0 mm
D. Women 27–34 years old				
11+1–11+6 weeks	45–54	18/7702 (0.2%)	18/7500 (0.2%)	< 0.6 mm
12–12+6 weeks	55–68	126/30 101 (0.4%)	126/29 477 (0.4%)	< 0.8 mm
13–13+6 weeks	69–84	59/15 438 (0.4%)	59/15 039 (0.4%)	< 0.9 mm

* one positive outlier screen test for T18 at 0.8mm

Figure 1. Combined prenatal screening positive according to maternal age

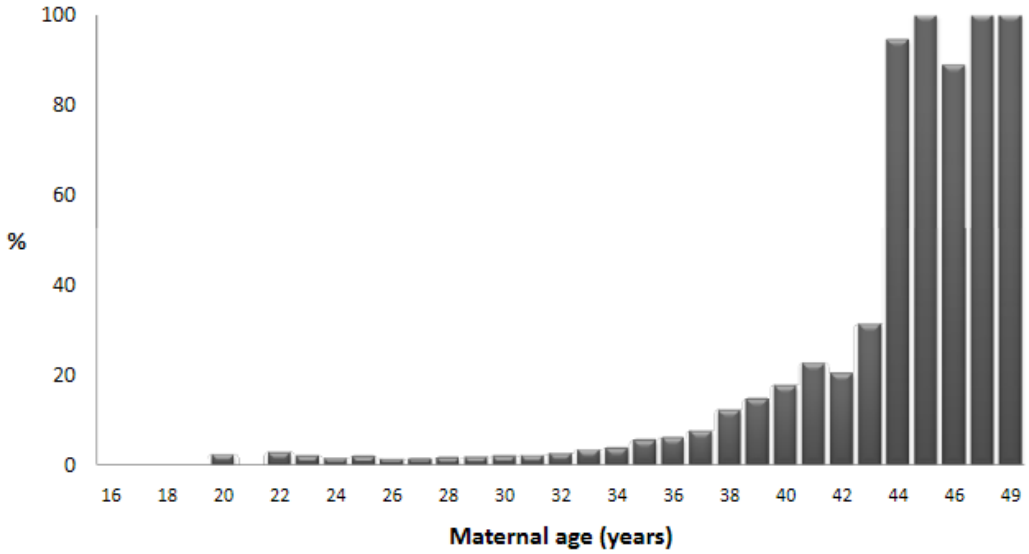
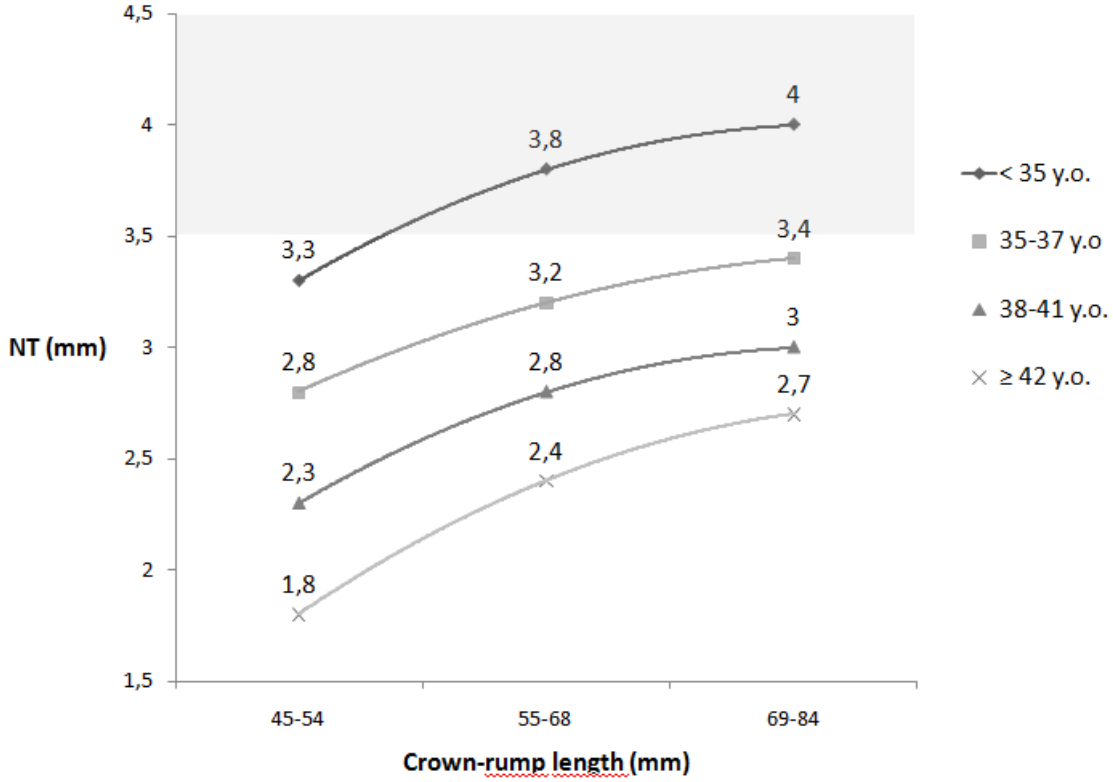
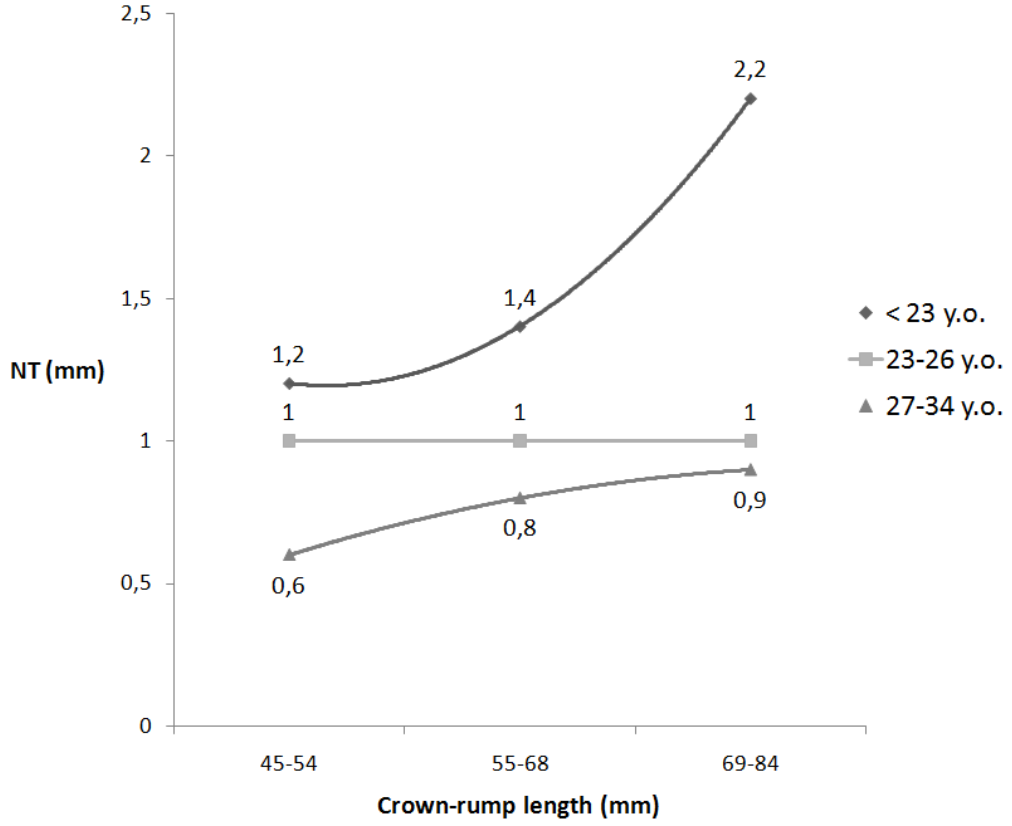


Figure 2. Proposed NT threshold levels above which prenatal diagnosis should be offered, regardless of biochemical results (polynomial curves), compared to FMF strategy in grey area. [25]



The NT threshold level of 3.0mm for the (35–37y.o.) x (55–68mm CRL) combination given in Table 3 was set to 3.2mm in figure 2 to better agree with other functions which seem logarithmic or quadratic. This modification introduces a more conservative threshold value.

Figure 3. Proposed NT threshold levels below which no further screening would be required (polynomial curves)



Article III : Niveaux plasmatiques de la protéine FLRG (*follistatin-related gene*) au premier trimestre de grossesses avec trisomie 21

Objectif

Déterminer les niveaux plasmatiques maternels de la protéine FLRG (*follistatin-related gene*) dans le premier trimestre de grossesse et évaluer son potentiel comme marqueur dans le dépistage prénatal de la trisomie 21.

Méthodologie

Les niveaux plasmatiques maternels de la protéine FLRG ont été déterminés chez cent (100) femmes enceintes avec fœtus normaux au premier trimestre de leur grossesse (c.-à-d. entre les 11^{e} et 15^{e} semaines). Ces résultats ont été comparés à vingt (20) cas avec syndrome de Down, tenant en considération les variables cliniques et démographiques comme l'âge maternel, le poids maternel, l'âge de la grossesse, le tabagisme et l'origine ethnique.

Résultats

La médiane plasmatique maternelle de FLRG dans la population normale est de 1.41 ng/ml entre les 11^{e} et 15^{e} semaines de grossesse avec un intervalle de confiance 95 % de 1.37 à 1.70 et un intervalle interquartile de 0.88. L'âge et le poids maternels sont les seules variables significativement corrélées aux niveaux de FLRG ($p = 0.030$ et 0.020, respectivement). Seuls les âges maternel et de la grossesse sont significativement associés au syndrome de Down ($p=0.039$ et 0.006, respectivement). En présence de fœtus avec syndrome de Down, les niveaux plasmatiques maternels de FLRG ne sont pas significativement différents comparativement à la population normale ($p = 0.63$).

Conclusion

La protéine FLRG est détectable dans le plasma maternel au premier trimestre de la grossesse. Ces niveaux ne sont toutefois pas significativement altérés en présence de fœtus avec syndrome de Down.

Cet article a été accepté et publié dans la revue *Prenatal Diagnosis* (Miron et al., 2010).

Contribution

L'auteur principal de l'article a eu l'idée et conçu le devis de l'étude. Les données de l'étude furent extraites d'une base de données, conçue par l'auteur principal. Les mesures biochimiques et la mise au point de l'ELISA pour la FLRG ont été réalisées par Anne Marcil et son équipe à l'Institut de recherches en biotechnologie du Conseil national de recherche du Canada. L'entrée de données fut réalisée par l'auteur principal. Il a analysé les données et interprété les résultats, sous la supervision du Pr Jean Lambert. Il a écrit le manuscrit. Les analyses statistiques furent réalisées par l'auteur principal et le Pr Jean Lambert. La révision critique de l'article fut principalement effectuée par les Pr Jean Lambert et Kevin Spencer ainsi que par un comité de pairs.

Maternal plasma levels of follistatin-related gene protein in the first trimester of pregnancies with Down syndrome

Running head: Maternal plasma follistatin-related gene protein in pregnancies with Down syndrome

Keywords: Follistatin-related proteins, Pregnancy, First trimester, Down syndrome, Aneuploidy

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Abstract

Objective : To determine maternal plasma levels of follistatin-related gene protein (FLRG) in the first trimester of pregnancy and assess its potential role as a marker for prenatal screening of Down syndrome.

Method : Maternal plasma levels of FLRG were determined in one hundred (100) pregnant women with normal fetuses in their first trimester of pregnancy (i.e. 11^{th} to 15^{th} weeks). These results were compared to twenty (20) cases with Down syndrome fetuses, taking into consideration clinical and demographic variables such as maternal age, maternal weight, gestational age, smoking status and ethnicity,.

Results : Maternal plasma median of FLRG in the normal population was 1.41 ng/ml with a 95% confidence interval (CI) of 1.37 to 1.70 and interquartile range (IQR) of 0.88, during the 11^{th} to 15^{th} weeks of pregnancy. Maternal age and weight were the only variables significantly related to FLRG levels ($p = 0.030$ and 0.020, respectively). Only maternal and gestational ages were related to Down syndrome ($p = 0.039$ and 0.006, respectively). Maternal plasma levels of FLRG were not significantly different in the presence of Down syndrome fetuses compared to normal population ($p = 0.63$).

Conclusion : FLRG can be successfully detected in maternal plasma in the first trimester of pregnancy. However, its levels are not significantly altered in the presence of Down syndrome fetuses.

Introduction

In first trimester of pregnancy, maternal blood levels of pregnancy-associated plasma protein-A (PAPP-A) and free-beta-human chorio-gonadotropic hormone (fβ-hCG) are used routinely in combination with nuchal translucency (NT) to detect an estimated 88% of Down syndrome (DS), at a fixed false positive rate of 5% (Spencer, 2007b). Ultrasound markers, such as fetal nasal bone and facial angle, have been proposed to improve detection and reduce false-positive rates, but both required extensive experience in scanning and can therefore be hardly offered on a large scale (Sonek et al., 2006, Borenstein et al., 2008, Staboulidou et al., 2009).

Additional maternal blood biochemical markers, which are easier to standardize and control, would be welcomed to improve early detection of aneuploidies. In this constant search for new biochemical markers, follistatin-related gene protein (FLRG), also known as FSTL3 or FSRP, could present some interest. Follistatin-related gene protein is produced in abundance by the placenta. Its most intense stain is found in the wall of decidual and placental blood vessels and, as a secreted glycoprotein, should be measurable in maternal blood very early into pregnancy (Ciarmela et al., 2003). Furthermore, FLRG interacts directly, with a known marker of aneuploidies, ADAM12-S, through its cystein-rich domain (Laigaard et al., 2003, Bartholin et al., 2005, Spencer et al., 2008). Also FLGR binds Activin A with high affinity modultating its effect on target tissues, although the value of Activin A as a marker of aneuploidy is quite weak (Spencer et al., 2001a, Spencer et al., 2001b).

The main objectives of this study were to determine maternal plasma concentrations of FLRG in the late first and early second trimester of normal human pregnancy (i.e from 11th to 15th weeks) and assess its potential role as a marker for Down syndrome.

Methods

This study was based on a sample of 120 women, including 100 women with normal fetuses and 20 women with Down syndrome fetuses. The women were from a cohort who had been identified at increased risk for Down syndrome based upon first trimester screening using fβ-hCG, PAPP-A and ultrasound nuchal translucency and who had elected to have CVS to confirm the diagnosis. Plasma samples were collected from these women prior to CVS at the Prenatal Screening Research Department of King George Hospital (UK). Control group consisted of pregnant women who had screened positive but had fetuses with normal karyotype. All frozen plasma samples were randomly selected and came from time period November 2006 to February 2008. They were shipped to Montreal on dry ice where they were kept at -80°C until analysis. To use samples for research, all subjects had to sign beforehand a consent form duly approved by an Institutional Ethics Review Board. Demographic data were gathered including women's age, pre-pregnancy weight (maternal weight), ethnic origin, smoking status and pregnancy outcome. Gestational ages were determined by fetal crown-rump length (CRL).

To measure FLRG, an enzyme-linked immunosorbent assay (ELISA) was set up and performed as follows. One plasma sample from each patient was analyzed in duplicates. Samples were used undiluted. Standard curve was done in duplicate (in bovien serum

albumin (BSA) 1% in phosphate buffered saline (PBS) on each plate. One internal positive control (high FLRG) and one internal control (low FLRG) were included in each plate. On a total of four 96-well microplates, all samples were processed on the same day. Microplates (Corning 9018) were coated overnight at 4°C with 100 µl/well of rat monoclonal antibodies (Mab) anti-mouse FLRG (MAB1255; R&D Systems, Minneapolis, MN, USA) at a concentration of 4 µg/ml in PBS. Microplates were then washed 3 times in PBS. Following a 30-minute blocking step with 200 µl/well of 1% BSA (Sigma-Aldrich, Oakville, ON, Canada) in PBS, microplates were incubated for 2 hours at room temperature with 100 µl/well of serial dilutions of recombinant hFLRG (1288-F3/CF ; R&D Systems, Minneapolis, MN, USA) diluted in BSA 1% in PBS (standard curve) or 100 µl/well of undiluted patient plasma in duplicate. Microplates were washed four times in PBS-Tween 20 0.05% and then incubated with 100 µl/well of biotinylated goat polyclonal antibodies raised against the human FLRG (BAF1288; R&D Systems, Minneapolis, MN, USA) at 0.4 µg/ml in BSA 1%, for 1 hour at room temperature. After washes, microplates were incubated with 100 µl/well of alkaline phosphatase-conjugated streptavidin (Rockland Immunochemicals, Gilbertsville, PA, USA) diluted 1/6000 in BSA 1%, for 1 hour at room temperature. Following washes, 100 µl/well of substrate (pNPP; Sigma-Aldrich, Oakville, Ontario, Canada), 100 µl/well) was added and further incubated at room temperature. Optical density (O.D.) was read at 405 nm after 60 and 90 minutes. Intra and inter-assay coefficients of variation were estimated at 1.8% and 2.8%, respectively.

Statistical Analyses and Power of Testing

Comparisons of demographic variables and gestational ages between women with normal fetuses and women with Down syndrome fetuses were based on the two sample Student *t*-test for quantitative characteristics and on the Pearson's χ^2 for qualitative (or categorical) characteristics. To study to what extent demographic and clinical data were predictors of FLRG (ng/ml), a multiple linear regression was used. To study to what extent FLRG (ng/ml) was a predictor of the pregnancy outcome (unaffected vs Down syndrome) when controlling for demographic and clinical data, a multiple logistic regression was used.

The normality of the FRLG (ng/ml) distribution was verified using the Kolmogorov-Smirnov's test and the hypothesis of normality was not rejected ($p = 0.15$). Therefore, the concept of Multiple of the Median (MoM) was found not to be useful in the present article. The homoscedasticity of the variance was verified with the Levene's test before using the two-sample *t*-test and the homoscedasticity of the variance was confirmed ($p = 0.78$). Goodness of fit was studied for the multiple linear regression and there was no problem of multicollinearity (maximum Variance Inflation Factor = 1.36) and no cases were influential (maximum Cook's distance = 0.16, $p = 0.99$). Goodness of fit was also studied for the simple and multiple logistic regressions with the Hosmer-Lemeshow's test and the fits were good (simple regression: $p = 0.26$; multiple regression : $p = 0.49$).

Based on sample sizes of 100 and 20 women respectively and a 0.05 two-sided significance level, (A) a two sample *t*-test will have 80% power to detect a standardized effect size of

0.7; (B) a linear regression will have 80% power to detect a correlation of 0.25; (C) and a logistic regression will have 70% power to detect an odds ratio of 2.00.

Results

Maternal plasma concentrations of FLRG were determined by ELISA in 120 pregnant women, including 100 women with normal fetuses and 20 with Down syndrome. Demographic and clinical characteristics between the two groups are described in Table 1. Data for smoking status and ethnicity were available in only 91% and 74% of patients, respectively. Maternal age was older ($p = 0.025$) and gestational age younger ($p < 0.005$) in the Down syndrome group.

In the normal pregnancies, maternal plasma levels of FLRG varied from 0.16 to 5.34 ng/ml, during the 11^{th} to 15^{th} weeks, with a median of 1.41 ng/ml (95%CI: 1.37-1.70 ng/ml) and an interquartile range (IQR) of 0.88 . In the Down syndrome group, FLRG levels varied from 0.15 to 2.39 ng/ml (95%CI : 1.15-1.74 ng/ml) and an IQR of 1.19. Mean maternal plasma level of FLRG for the normal population was 1.54 ± 0.83 ng/ml (mean ± SD) compared to 1.45 ± 0.63 ng/ml for Down syndrome ($p=0.64$) (Table 1). No relationship ($p=0.58$) was found between FLRG levels and gestational age across the range of samples (80-105 days) (Figure 1).

Based on a multivariate analysis on the control group ($n = 100$), there were five potential predictors of FLRG (ng/ml) levels as shown in Table 2. However, only maternal age ($p = 0.030$) and maternal weight ($p = 0.020$) were significantly associated with FLRG levels

(Figures 2 and 3). Maternal age was positively associated with FLRG levels (b = 0.037) but maternal weight was negatively associated with FLRG levels (b = -0.017)

When looking at the FLRG alone as a potential predictor of the risk of Down syndrome, its association with Down syndrome is not statistically significant (p = 0.63, Table 3). Even when controlling potential confounders its association remains statistically non-significant (p = 0.27). The only statistically significant predictors of the probability of Down syndrome were maternal age (OR = 1.16, p = 0.039) and gestational age (odds ratio (OR) = 0.25, p = 0.006, Table 3).

Discussion

Until this study, FLRG was poorly studied in human maternal blood. No data have yet been published in regards to its concentrations in the first trimester of human pregnancy and its potential as a marker for aneuploidies. This study confirms that FLRG can be successfully measured in maternal plasma, from 11^{th} to 15^{th} weeks of pregnancy. However, it did not demonstrate its role as a marker for Down syndrome screening.

Unfortunately, we did not have access to PAPP-A, fβhCG and NT results in the database provided to us. The question whether or not there is a relationship between these analytes remains to be clarified. As found with other biochemical markers, FLRG levels are significantly influenced by maternal weight (Spencer et al., 2003). Why they significantly increase with maternal age remains, however, an enigma to be clarified and confirmed in a larger series of patients.

As for maternal and gestational ages that both significantly predict Down syndrome, this was not a surprise. Meiotic nondisjunction of chromosome 21 is well known to be associated with advanced maternal age (Hassold and Chiu, 1985, Ghosh et al., 2009). A high proportion of affected pregnancies with Down syndrome also abort spontaneously if they are allowed to continue. Thus, the earlier antenatal screening is carried out, the higher the proportion of Down syndrome (Morris et al., 1999).

FLRG was first identified as playing a role in leukemogenesis with wide range effects on cell differentiation, proliferation and organization, suggesting a participation in cell transformation and growth regulation (Hayette et al., 1998). Although FLRG shares significant structural and functional homology with follistatin (FS), further characterization confirmed some major differences between the two proteins, suggesting that FLRG is differentially regulated both spatially and temporally and performs distinct functions (Schneyer et al., 2004). FLRG mRNA expression is exceptionally high in the placenta (Tortoriello et al., 2001). In rats, expression of FLRG in the placenta continuously increased during the second half of pregnancy (Arai et al., 2003). In humans, FLRG mRNA is also expressed by various gestational tissues both at early gestation and at term pregnancy, and immunoreactive protein was found in the trophoblast cells, epithelial amniotic and chorionic cells and maternal deciduas (Ciarmela et al., 2003).

Using a home-brewed ELISA, one study has demonstrated that, between 28-37 weeks, maternal serum concentrations of FLRG are significantly elevated in preeclampsia, in a similar manner to activin A and inhibin A (Pryor-Koishi et al., 2007). FLRG has also been

shown to be highly up-regulated in the placenta of pathological pregnancy conditions such as intrauterine fetal growth restriction, suggesting that it is a critical regulator of fetal growth and differentiation (Okamoto et al., 2006). It remains to be clarified if maternal blood FLRG could be used in the first trimester to predict the development of preeclampsia and/or intrauterine foetal growth restriction.

Conclusion

FLRG can be detected in maternal blood in the first trimester of pregnancy. However, our data suggest it is not a useful marker for the prenatal screening of Down syndrome from 11^{th} to 15^{th} weeks of pregnancy.

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Table 1. Demographic and clinical characteristics of studied populations

Demographic and clinical data	**Normal (N=100)**		**Down syndrome (N=20)**		**P value**
A. Quantitative variables					
	$\bar{x}$ ± SD	(Min-Max)	**$\bar{x}$ ± SD**	(Min-Max)	
Women's age (years)	34.4 ± 6.3	(16.0-45.0)	37.9 ± 4.5	(27.0-44.0)	0.025
Gestational age (weeks)	13.1 ± 0.72	(11.4-15.0)	12.5 ± 0.71	(11.0-13.7)	0.002
Women's weight (kg)	67.6 ± 11.9	(46.8-102.0)	64.2 ± 13.3	(46.8-95.3)	0.280
FLRG plasma concentrations					
Nanogram/ml ($\bar{X}$ ± SD)	1.54 ± 0.83		1.45 ± 0.63		0.637
Nanogram/ml (median)	1.41		1.64		
MoM	1.00		1.16		0.637
Weight corrected MoM	0.99		1.12		0.447
B. Qualitative variables	N (%)		N (%)		
Smoking					0.164
No	81 (89.0%)		16 (100%)		
Yes	10 (11.0%)		0 (0%)		
Ethnicity					0.522
Caucasian	63 (90.0%)		18 (94.7%)		
Other	7 (10.0%)		1 (5.3%)		

Table 2. Linear regression model predicting FLRG (ng/ml)

Model	Unstandardized Coefficients		t	Significance
	B	Standard Error		
(Constant)	2.854	1.828	1.562	.123
Maternal age	.037	.017	2.209	.030
Maternal weight (kg)	-.017	.007	-2.373	.020
Gestational age	-.113	.112	-1.011	.315
Ethnicity	-.193	.360	-.535	.594
Smoking	-.071	.345	-.207	.837

Table 3. Logistic regression models predicting diagnosis (Normal vs Down Syndrome)

		B	S.E.	Wald	df	Sig.	Odds ratio	95.0% C.I.for OR	
								Lower	Upper
FLRG as only predictor									
Step 1	FLRG	-.158	.331	.226	1	.634	.854	.446	1.635
	Constant	-1.375	.542	6.443	1	.011	.253		
Controlling for demographic and clinical variables									
Step 1	FLRG	-.549	.494	1.233	1	.267	.578	.219	1.522
	Maternal age	.146	.071	4.275	1	.039	1.157	1.008	1.329
	Maternal weight	-.031	.027	1.284	1	.257	.969	.919	1.023
	Gestational age	-1.396	.507	7.579	1	.006	.248	.092	.669
	Constant	13.709	7.016	3.818	1	.051			

Dependant variable: Diagnosis

Figure 1. Variation of maternal plasma FLRG (ng/ml) with gestational age (weeks) in the control (●) and Down syndrome (◊) populations. Lines represent linear fit and 95%CI of the global population.

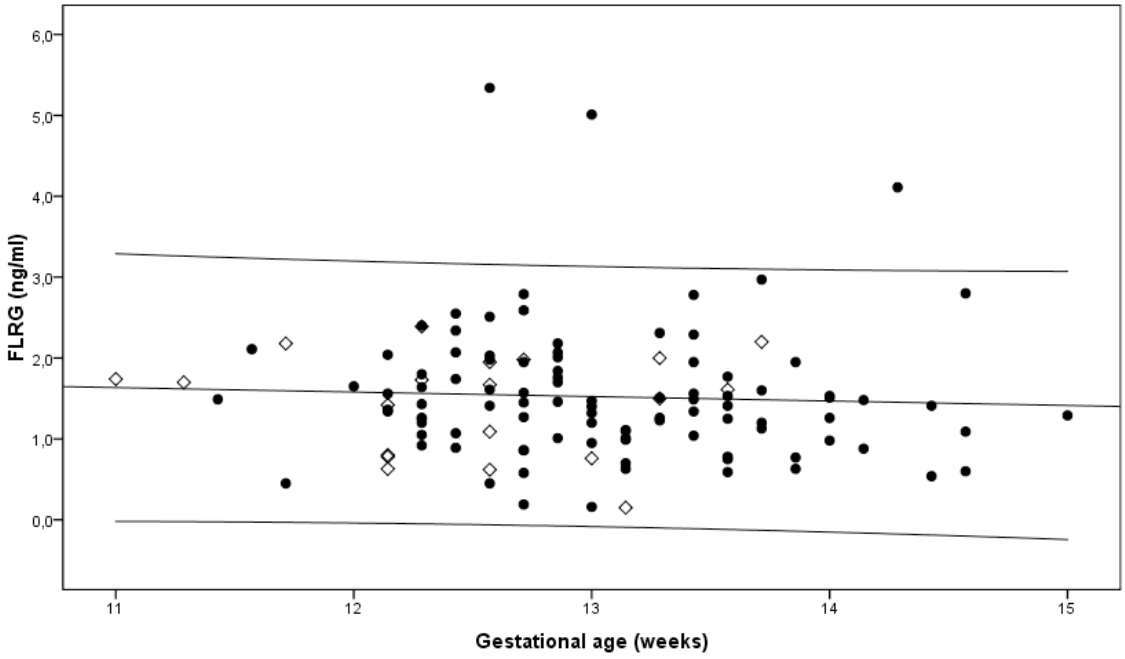


Figure 2. Variation of maternal plasma FLRG (ng/ml) with maternal age (years) in the control (●) and Down syndrome (◊) populations. Lines represent linear fit and 95%CI of the global population.

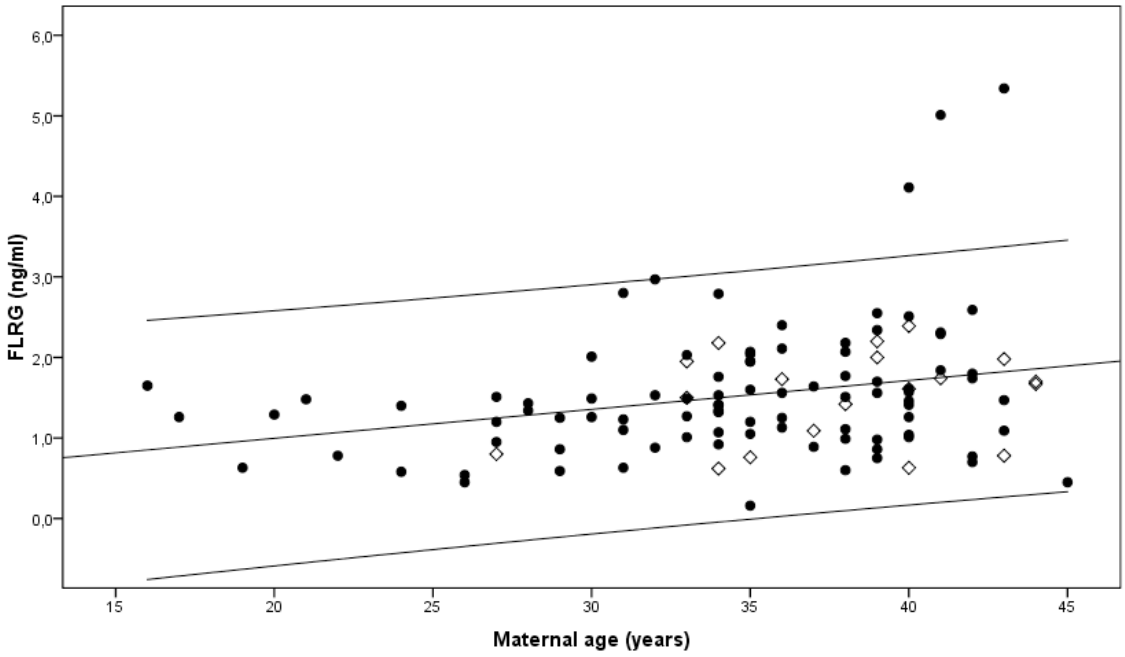
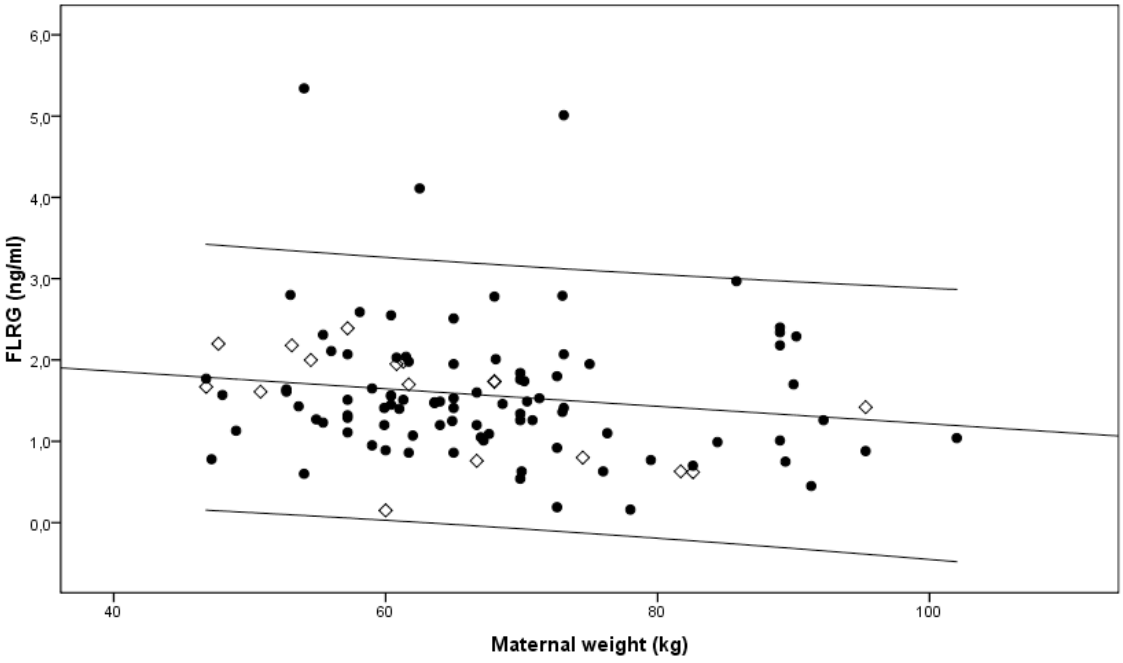


Figure 3. Variation of maternal plasma FLRG (ng/ml) with maternal weight in the control (●) and Down syndrome (◊) populations. Lines represent linear fit and 95%CI of the global population.



Discussion générale

Ce chapitre aborde les articles sous un angle plus spécifique au Québec. Des données complémentaires et originales sont présentées. Une discussion sur l'évolution future du dépistage prénatal est entamée et des axes de recherche sont proposés.

Discussion sur les articles avec une perspective québécoise

Cette thèse écrite sous la forme d'articles scientifiques a porté sur le dépistage prénatal de la trisomie 21 et autres aneuploïdies, au premier trimestre de la grossesse. Les sujets abordés dans chacun des articles ne sont pas un pur hasard. Ils représentent en fait des éléments essentiels du dépistage prénatal non intrusif des aneuploïdies au premier trimestre de grossesse, soit : (1) l'importance d'un contrôle adéquat des variables confondantes dans le calcul du risque foetal (2) le rôle primordial de la clarté nucale et (3) celui des marqueurs biochimiques.

Tabagisme

Le premier article explore l'effet du tabagisme maternel sur les résultats de dépistage prénatal de la trisomie 21 et 18 (Miron et al., 2008). Déjà, dans la pratique actuelle, une multitude de co-variables sont contrôlées et des facteurs de correction sont disponibles entre autres pour le poids maternel, l'origine ethnique, les grossesses gémellaires et celles issues d'assistance médicale à la procréation (Westergaard et al., 1983, de Graaf et al., 2000, Spencer et al., 2000b, Niemimaa et al., 2002, Orlandi et al., 2002). Jusqu'à notre article, des centaines de milliers de tests de dépistage étaient effectués annuellement sur sang séché maternel au niveau international, sans tenir compte de l'effet du tabagisme sur les concentrations maternelles de la fraction libre de la β-hCG et de la PAPP-A et sur la clarté nucale. Or, comme dans le sérum maternel, notre étude démontre que les concentrations de la fraction libre de la β-hCG et de la PAPP-A sur sang séché sont

artificiellement réduites de 13 % et 17 %, respectivement. La clarté nucale, quant à elle, est significativement augmentée par le tabagisme. L'effet combiné de ces trois marqueurs est particulièrement significatif, d'un point de vue clinique, pour le calcul de risque de la trisomie 18. Sans correction, il augmente d'environ trois fois la probabilité pour une patiente d'avoir un résultat de dépistage faussement positif (0.7% chez les non fumeuses vs 2.3% chez les fumeuses) et donc, de se voir offrir inutilement un test diagnostique, telles l'amniocentèse ou la biopsie de trophoblaste. Ces données, originales à propos de la trisomie 18 et du sang séché, confirment l'importance de corriger le facteur du tabagisme dans le calcul final du risque foetal.

D'un point de vue méthodologique, l'impact de facteurs de confusion potentiels a été à notre avis limité par un contrôle statistique adéquat des co-variables confondantes. La procédure d'adresser une patiente pour un dépistage prénatal était systématique, diminuant par le fait même les risques de biais de sélection dans notre étude. Enfin, considérant l'importance de la population étudiée (n=53114) et la faible prévalence de la trisomie 18 à la naissance (1:7907), l'effet identifié du tabagisme sur le calcul de risque foetal pour la trisomie 18 représente vraisemblablement une simple association statistique (Parker et al., 2003). Une association biologique réelle, de cause à effet, entre le tabagisme et le développement d'une trisomie 18 ne pourra toutefois être clarifiée que par une étude dont l'issue de toutes les grossesses est connue.

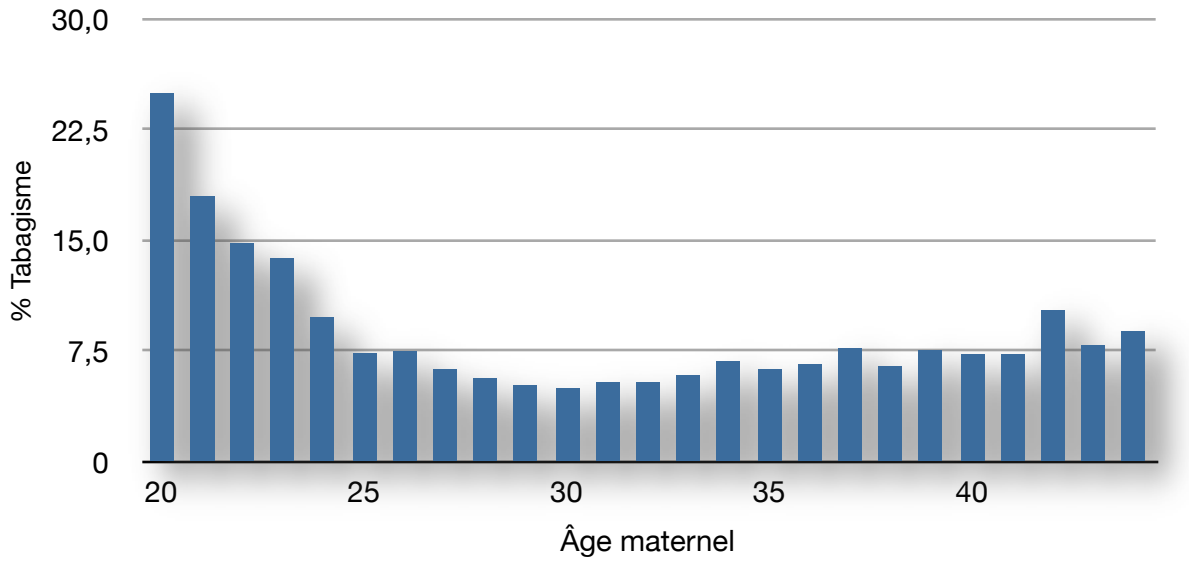
Seulement 6,7 % des femmes enceintes de notre cohorte, se situant entre les années 1999 et 2005, avouaient fumer au moment du test de dépistage (Figure 17). En général, il semble exister une assez bonne corrélation entre avouer fumer et fumer réellement en grossesse. L'image négative de fumer en grossesse inciterait toutefois certaines femmes à cacher cette information (Lindqvist et al., 2002, George et al., 2006). De plus, il est probable que dans notre étude des femmes aient indiqué ne pas fumer au moment du dépistage alors qu'elles le faisaient jusqu'à la sixième, huitième ou même dixième semaine de grossesse. Une telle erreur de classification n'aurait pu toutefois mener qu'à une sous-estimation de l'effet du tabagisme sur les variables étudiées. Pour la même raison, il nous apparaît donc improbable

que la participation à l'étude ait été liée à la fois à l'exposition au tabagisme et à l'issue d'aneuploïdie.

Le faible taux de tabagisme dans notre étude tranche toutefois de façon assez étonnante avec celui rapporté pendant la même période chez les femmes enceintes québécoises par l'Institut national de santé publique du Québec (INSPQ). Il ne peut s'expliquer par une seule erreur de classification.

Selon l'INSPQ, 27.6 % des Québécoises, âgées de 20 à 44 ans, fumaient pendant leur grossesse en 2005. Cette proportion était de 37 % en 2003 (Guyon et al., 2008).

Figure 17. Pourcentage de femmes enceintes dans notre cohorte avouant fumer pendant leur grossesse, selon l'âge maternel (n= 53114)



Cette différence entre notre cohorte et la population générale de femmes enceintes confirme fort possiblement de façon indirecte un écart marqué d'accessibilité au dépistage prénatal entre les différents niveaux socio-économiques du Québec. En absence de programme universel supporté par l'État, le dépistage prénatal du premier trimestre est en effet majoritairement effectué au Québec par les cliniques privées et seules les patientes les mieux nanties peuvent y avoir accès. Ces données sont d'ailleurs appuyées par l'*Enquête*

sur la santé dans les collectivités canadiennes qui indique qu'en 2003 seulement 8,3 % des Québécoises les plus fortunées affirmaient fumer pendant leur grossesse alors que parmi les plus pauvres, plus du tiers avouaient la même chose (ESCC, 2003).

Plus ou moins sensibilisé à ces problèmes d'accessibilité, le Ministère de la Santé et des Services sociaux du Québec réfléchit toujours, depuis plus de 10 ans, à la possibilité d'offrir au Québec un programme universel de dépistage prénatal de la trisomie 21. Pourtant, déjà en 1999, l'agence d'évaluation des technologies et des modes d'intervention en santé, l'AETMIS (anciennement le CETS), lui recommandait la mise en oeuvre d'un programme de dépistage prénatal biochimique de la trisomie 21 au second trimestre de grossesse, accessible à toutes les femmes enceintes peu importe leur âge (Framarin, 1999). Un second rapport, produit en 2003 par le même organisme, arrivera essentiellement aux mêmes conclusions, explorant toutefois de façon plus approfondie la possibilité d'offrir le dépistage prénatal dès le premier trimestre de la grossesse (Framarin, 2003).

Le Ministère confiera en 2007 au Commissaire à la santé et au bien-être le mandat de mener une consultation sur les enjeux éthiques soulevés par le dépistage prénatal de la trisomie 21. Cette consultation confirmera que 88 % des femmes enceintes et 100 % de leur conjoint sont entièrement ou plutôt en accord avec le principe d'offre gratuite du test de dépistage prénatal de la trisomie 21. Pour la mise en oeuvre éventuelle d'un programme de dépistage prénatal de la trisomie 21, le Commissaire recommande toutefois au gouvernement : (1) d'assurer un consentement libre et éclairé des parents ; (2) un soutien psychologique si requis ; (3) un accès équitable et en temps opportun pour toutes les femmes enceintes du Québec, quelle que soit la région ; (4) un mécanisme de suivi du respect des normes de pratique et de bonne conduite du programme ; (5) l'allocation de ressources financières et logistiques adéquates ; (6) la transmission d'une information utile, compréhensive et standardisée pour les parents et la population générale (Salois, 2008).

Or, en avril 2010, rien n'est encore concrétisé et malgré les bénéfices économiques de la prévention en santé, exprimés de nouveau dans le rapport du Groupe de travail sur le financement du système de santé, nous sommes probablement encore bien loin de la mise

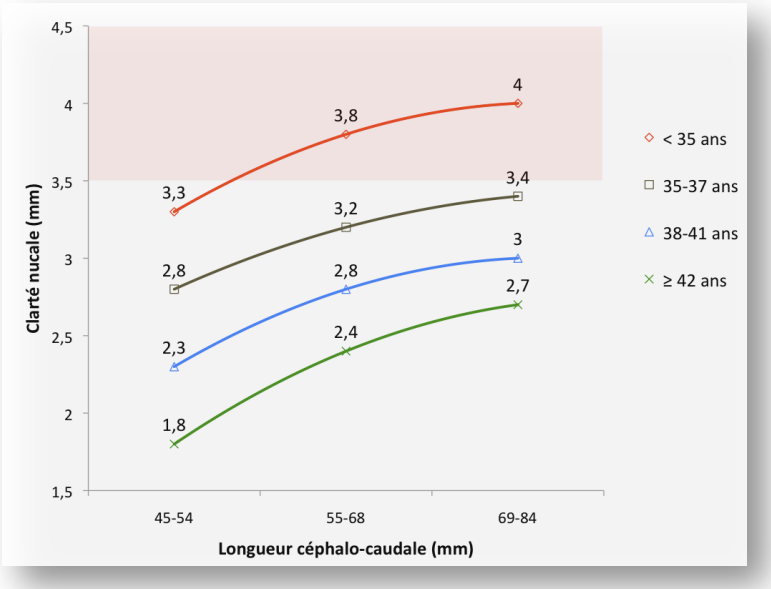
en oeuvre d'un programme universel efficace de dépistage prénatal des aneuploïdies au Québec (Castonguay et al., 2008).

Clarté nucale

Le deuxième article de ma thèse porte sur le rôle de la clarté nucale comme marqueur de triage dans le dépistage prénatal de la trisomie 21 et de la trisomie 18. Étant donné son impact direct sur la pratique clinique, l'élément le plus important de l'article m'apparaît être la figure reproduite ci-dessous (Figure 18). Elle définit, selon l'âge maternel et l'âge de la grossesse, des seuils fiables de clarté nucale au-delà desquels la biochimie génétique n'apporte aucune valeur additionnelle au dépistage prénatal de la trisomie 21 et de la trisomie 18. En présence d'une clarté nucale supérieure aux seuils proposés, un test diagnostique devrait ainsi être offert d'emblée à la patiente, selon les catégories d'âge dans lesquelles elle se situe. Avec une installation adéquate et un conseil génétique approprié, une biopsie du trophoblaste pourrait ainsi être réalisée la journée même du dépistage, entre les 11^{e}-14^{e} semaines.

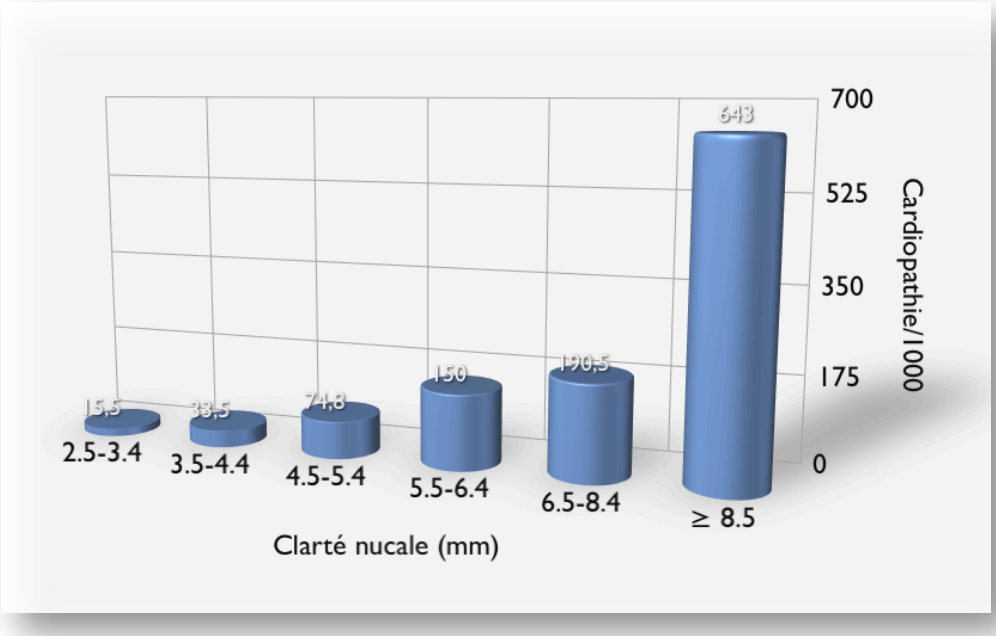
Jusqu'à cet article, un test diagnostique était d'emblée recommandé uniquement en présence d'une clarté nucale au-delà de 3.5 ou 4 mm, indépendamment de l'âge maternel et de l'avancement de la grossesse. Cette approche sous-estime les indications. Pour les cliniciens utilisant un test de dépistage prénatal combinant la clarté nucale, la PAPP-A et la fraction libre de la β-hCG, l'approche que nous proposons est tout à fait originale et apparaît mieux adaptée à la situation clinique.

Figure 18. Seuils de clarté nucale au-dessus desquels un diagnostic devrait être offert d'emblée, indépendamment de la biochimie génétique



Comme mentionnée précédemment, une clarté nucale augmentée est clairement associée à un risque plus élevé d'aneuploïdies (Nicolaides et al., 1992). Même en présence d'un caryotype foetal normal, une clarté nucale augmentée demeure pertinente cliniquement puisqu'associée également à des risques accrus d'une variété de syndromes génétiques, d'évènements adverses en grossesse et de malformations foetales structurales (Bilardo et al., 2010). Cela est particulièrement véridique pour les cardiopathies congénitales. Le risque de malformations cardiaques foetales augmente progressivement avec l'épaisseur de la clarté nucale (Figure 19). Il serait 6 fois plus élevé en présence d'une clarté nucale au-delà du 99^{e} centile. Selon de récentes recommandations internationales, une échocardiographie foetale devrait, dans de tels cas, toujours être proposée avant la 16^{e} semaine. En présence d'une clarté nucale se situant entre les 95^{e} et 99^{e} centiles, une attention particulière devrait être portée aux structures cardiaques lors de l'échographie de routine pour anatomie foetale, généralement effectuée au deuxième trimestre entre les 18^{e}-23^{e} semaines (Carvalho et al., 2004, Lee et al., 2008, Clur et al., 2009).

Figure 19. Probabilité de cardiopathie congénitale selon la clarté nucale (mm). Adapté de (Clur et al., 2009)

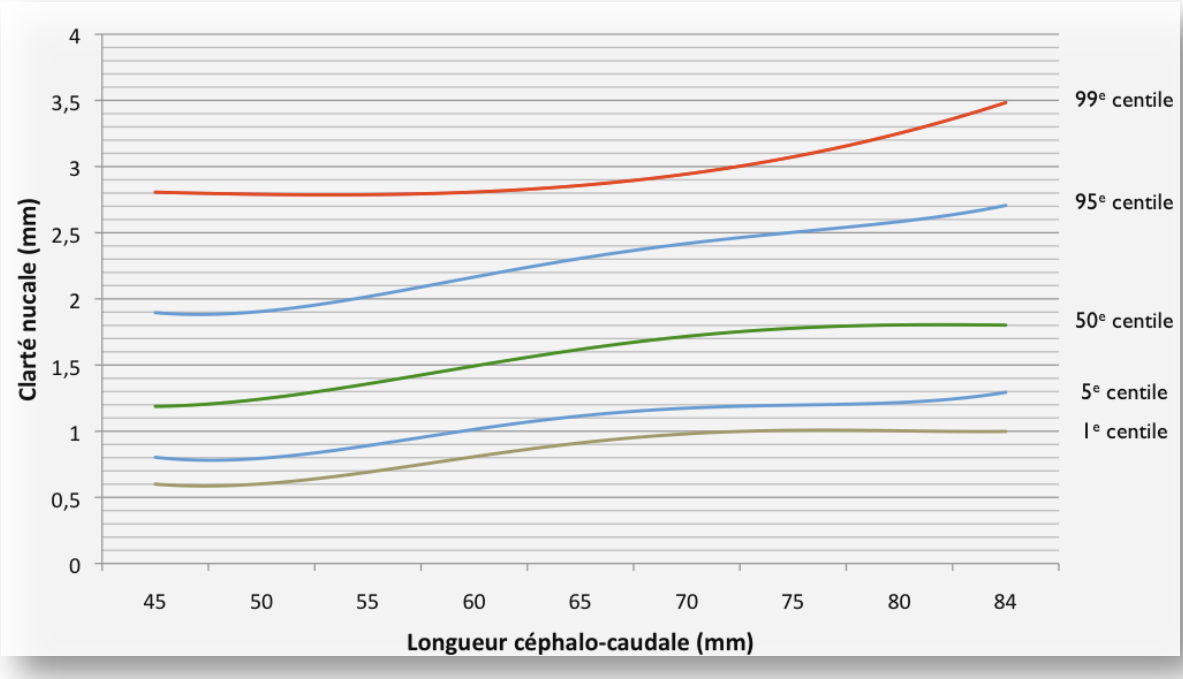


À cet égard, une figure additionnelle, basée sur notre cohorte de patientes, aurait pu être ajoutée à la publication puisqu'elle nous semble aujourd'hui particulièrement utile à la décision clinique de procéder ou non à une échocardiographie foetale. Nous jugeons donc avantageux de la reproduire ci-dessous (Figure 20). Il s'agit de la distribution de la clarté nucale en centiles (1er, 5e, 50e, 95e et 99e), selon la longueur céphalocaudale du foetus, soit de la 11e à la 14e semaine de grossesse (45-84 mm). Ces données sont d'autant plus pertinentes qu'elles portent sur un très grand nombre de patientes (n=77443), et qu'elles sont représentatives de la population québécoise.

Plusieurs auteurs citent une valeur-seuil de clarté nucale de 3.5 mm afin de définir le 99e centile entre les 11e et 14e semaines. Or, les valeurs seuils pour le 99e centile se situeraient plutôt, du moins pour la population québécoise, à 2.8 mm pour les longueurs

céphalocaudales de 45 à 65 mm, et à 3.0, 3.1, 3.2 et 3.5 mm pour celles de 70, 75, 80 et 84 mm, respectivement [1].

Figure 20. Centiles de la clarté nucale selon longueur céphalocaudale (n= 77443)



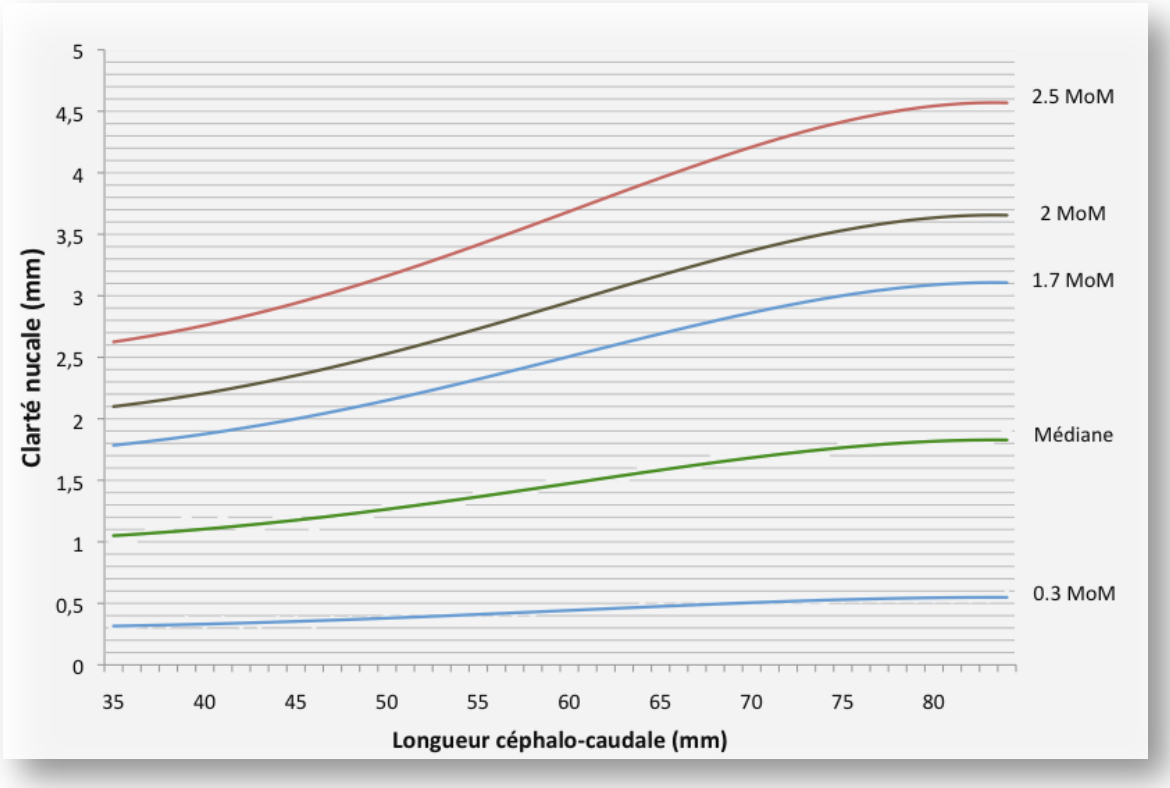
Nous croyons donc que la décision médicale de recommander une échocardiographie foetale avant la 16e semaine serait mieux adaptée à la situation clinique si les valeurs-seuil (99e centile) de nos résultats étaient utilisées, selon l'âge de la grossesse. Pour les cas se situant selon nos courbes entre les 95e et 99e centiles, en absence de flot inversé du ductus veineux et/ou de régurgitation tricuspidienne comme le recommande certains, une attention particulière aux structures cardiaques pourrait être portée plus tardivement lors de l'échographie de routine entre les 18e-23e semaines (Clur et al., 2009).

Une autre alternative serait d'utiliser un multiple de médiane (MoM) de 1.7 ou de 2.0, tel que recommandé par des études récentes, ce qui permettrait de détecter jusqu'à 52 % des

[1] La valeur de 3,9 mm pour (99e centiles) x (45 mm LCC) a été fixée à 2,8 mm pour mieux se conformer aux autres fonctions qui semblent quadratiques ou cubiques. Cette modification introduit une valeur-seuil plus conservatrice.

cardiopathies congénitales avec 5 % de faux positifs (D'Alton and Cleary-Goldman, 2005, Wald et al., 2008). La figure 21 ci-dessous provenant également de notre propre population pourrait alors être utilisée (n= 77775).

Figure 21. Multiples de médiane de la clarté nucale selon la longueur céphalocaudale (n=77775)



FLRG

Le dernier article porte sur les niveaux plasmatiques de la protéine *follistatin related-gene* (FLRG) chez les femmes enceintes ayant un foetus trisomique 21. Notre étude visait essentiellement à déterminer le rôle potentiel de la FLRG dans le dépistage prénatal d'aneuploïdies communes, au premier trimestre de la grossesse.

Il existe en effet un réel besoin d'identifier de nouveaux biomarqueurs qui permettraient d'améliorer les taux de détection et de diminuer les faux positifs au premier trimestre. Pour une population comme le Québec, l'expertise médicale est principalement concentrée dans les grandes villes et, du moins pour l'instant, il est difficile d'avoir accès en régions aux marqueurs échographiques, comme la clarté nucale. Un dépistage limité à des marqueurs sériques a donc été envisagé par certains. Au premier trimestre de la grossesse, la PAPP-A et la fraction libre de la β-hCG sont les deux seuls marqueurs sanguins maternels couramment utilisés de routine dans le dépistage d'aneuploïdies. Combinés à l'âge maternel, ils ne permettent toutefois la détection que de 75 % des foetus trisomiques 21 avec 6 % de faux positifs (Wald et al., 2003). L'ajout de nouveaux marqueurs biochimiques, tels le facteur de croissance placentaire (PlGF) et ADAM12-S semble peu ou pas améliorer la performance du dépistage sérique de la trisomie 21 au premier trimestre (Poon et al., 2009, Valinen et al., 2009, Zaragoza et al., 2009, Christiansen et al., 2010). Une combinaison de la PAPP-A, de la fraction libre de la β-hCG et de l'hCG total offrirait un taux intéressant de détection de 76 % avec 3 % de faux positifs, mais nécessiterait pour atteindre de tels résultats un prélèvement sanguin très tôt en grossesse, vers la 8e-9e semaine. De plus, les auteurs sont très prudents sur la reproductibilité de leurs résultats, l'échantillon de cas anormaux étant petit (Wright et al., 2007). Une étude est actuellement en cours au Danemark afin de valider ces résultats et de déterminer la possibilité d'utiliser deux échantillons - l'un entre les 8e et 10e semaines et le second entre les 12e et 14e semaines (Spencer, 2010).

D'autres alternatives ont été proposées, tel un dépistage intégré uniquement sérique combinant un marqueur biochimique du premier trimestre (PAPP-A) à quatre autres du deuxième trimestre (fraction libre de la β-hCG (ou hCG totale), oestriol, AFP et inhibine A). Cette approche nécessiterait également deux prélèvements sanguins, l'un entre les 9e et 14e semaines et l'autre beaucoup plus tardif, entre les 14e et 20e semaines (Wald et al., 1999). Le Ministère de la Santé et des Services sociaux du gouvernement du Québec envisage d'ailleurs, suite à la recommandation de son « Comité québécois d'experts sur le dépistage du syndrome de Down et autres aneuploïdies », ci-après le « Comité », la mise en oeuvre d'un programme québécois de dépistage prénatal utilisant un test intégré uniquement sérique, mais qui exclurait l'inhibine A et qui remplacerait la fraction libre de la β-hCG par l'hCG totale (Forest, 2004, Forest, 2008). Sans expliquer sa démarche scientifique, ce comité prédisait dans son rapport ministériel de 2008 un taux de détection de la trisomie 21 de 75 % avec ≤ 5 % de faux positifs. Étrangement, cette cible ne respecte pas d'emblée les normes minimales canadiennes de qualité du dépistage prénatal de la trisomie 21, telles que dictées en 2007 par la Société des obstétriciens et gynécologues du Canada (SOGC) et le Collège canadien des généticiens médicaux (CCGM) (Summers et al., 2007, Forest, 2008) :

> *« D'ici 2008, les programmes de dépistage devraient viser à offrir un dépistage qui, au minimum, assure aux femmes qui en sont au premier trimestre de la grossesse un taux de détection de 75 % et un taux de faux positif d'au plus 3 %, en ce qui concerne le syndrome de Down. »*

La recommandation du Comité au Ministère se base principalement sur les données de l'étude SURUSS (Wald et al., 2003). Cette étude cas-témoins nichée, effectuée en Angleterre, confronte la performance de différentes combinaisons de marqueurs par modélisation mathématique, en comparant 98 cas de trisomie 21 à 490 contrôles. Basé sur ces données tout de même assez sommaires, le test intégré sérique (TIS) proposé par l'étude SURUSS, qui combine quant à lui la PAPP-A à la 10e semaine à quatre autres du deuxième trimestre (hCG totale, oestriol, AFP et inhibine A), offre au mieux un taux de détection de 75 % des trisomies avec 2 % de faux positifs.

En utilisant les données brutes de SURUSS avec l'assistance du Pr Howard Cuckle, nous avons pu prédire plus précisément, par modélisation, les taux de détection de la trisomie 21 pour un TIS tel qu'envisagé par le gouvernement du Québec (PAPP-A entre les 10e et 14e semaines et hCG totale, oestriol et AFP entre les 14e-20e semaines), excluant donc l'utilisation de l'inhibine A et la fraction libre de la β-hCG, et selon des taux fixes de faux positifs de 3 %, 4 %, 5 % et 6 %, respectivement. De façon conservatrice, nous avons tenu compte qu'une échographie de datation était effectuée au premier trimestre chez toutes les participantes, alors que le programme québécois prévoit n'en effectuer la première année que chez 40 % et la deuxième année, que chez 60 %. Les résultats obtenus par notre modèle sont désastreux (Tableau VIII). Ils ne sont aucunement ceux prédits approximativement par le Comité. Même avec une échographie de datation, ils sont très éloignés des normes canadiennes minimales, telles qu'énoncées plus haut par la SOGC et le CCGM pour un test acceptable de dépistage prénatal de la trisomie 21 et encore plus de celles instituées en Angleterre depuis avril 2010, soit un taux de détection de la trisomie 21 de plus de 90% avec un taux de faux positifs d'au plus 2% (UK National Screening Commitee, 2008).

Tableau VIII. Taux de détection de la trisomie 21 (%) pour un TIS incluant PAPP-A (T1) et hCG totale, uE3, AFP (T2) selon différents taux fixes de faux positifs

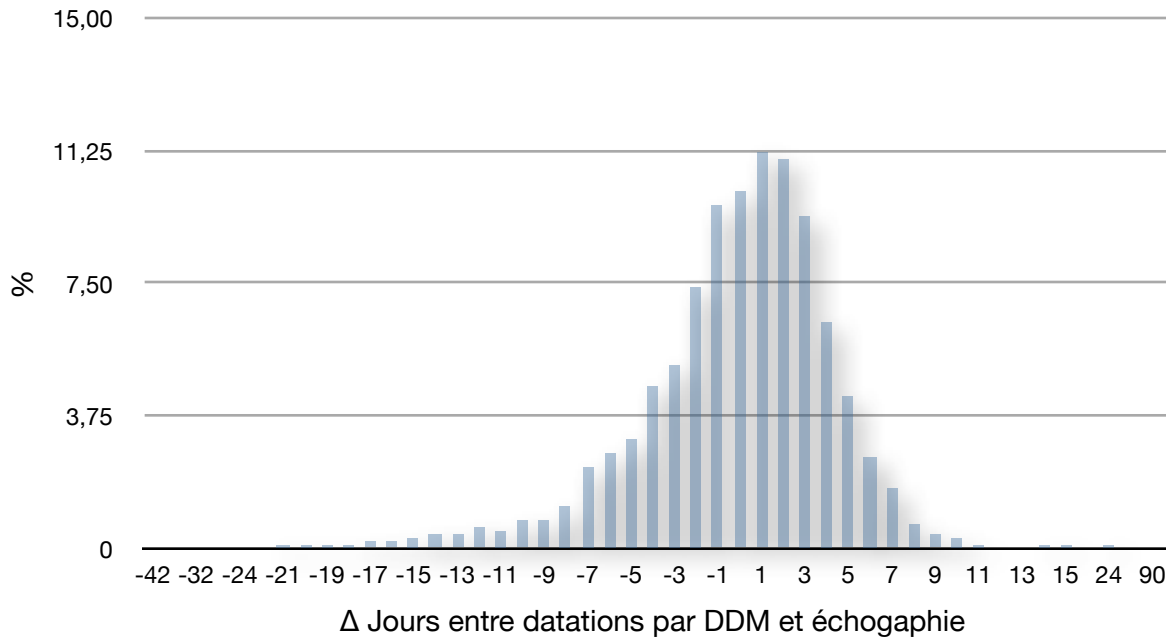
Âge gestationel lors du prélèvement pour PAPP-A (semaines complétées)	Taux fixe de faux positifs			
	3%	4%	5%	6%
10	66%	69%	72%	74%
11	63%	67%	69%	72%
12	59%	63%	66%	69%
13	56%	60%	63%	65%

À part sa performance inacceptable d'un point de vue éthique, le test intégré sérique recommandé par le Comité ministériel, excluant entre autres la mesure de la clarté nucale et une échographie de datation, présenterait de sérieuses lacunes: (1) environ 21 % des femmes ne complèteront pas adéquatement le test intégré, soit en effectuant trop tôt le

premier prélèvement sanguin ou en ne se présentant tout simplement pas au deuxième prélèvement (Knight et al., 2005); (2) en plus de créer un délai d'incertitude chez les parents, l'accès tardif aux résultats (le plus souvent au-delà de la 18e semaine de grossesse[2]) exposera les femmes à des risques accrus de complications chirurgicales lors d'une interruption médicale tardive de grossesse (van Lith et al., 1984, Vanasse and Collin, 2008, Vargas and Diedrich, 2009); (3) sans échographie au premier trimestre, ce type de test ne permettra pas, entre autres, le dépistage précoce de cardiopathies congénitales, d'anomalies structurales foetales et d'évènements adverses maternels.

De plus, ne pas rendre obligatoire une échographie de datation au premier trimestre aggravera inévitablement les résultats du tableau VIII. Nos propres données, basées sur 2437 Québécoises, démontrent à quel point une variation existe entre l'âge de la grossesse déterminé par la date des dernières menstruations et celui déterminé par une échographie de datation du premier trimestre (Figure 22).

Figure 22. Différence de jours dans la datation de grossesse entre calculs par date des dernières menstruations (DDM) et par échographie du premier trimestre (n= 2 437)



[2] Pour qu'un tel modèle fonctionne, les résultats ne peuvent être dévoilés à la patiente et au médecin traitant qu'après l'analyse du deuxième prélèvement sanguin

Sans échographie au premier trimestre, l'efficience du dépistage en sera ainsi grandement affectée, par datation erronée. Les figures 23 et 24, provenant d'une population québécoise importante (n= 77424), démontrent la grande variation qui existe dans les niveaux de PAPP-A et de fraction libre de la β-hCG selon l'âge de la grossesse. Une étude récente confirme d'ailleurs que, sans échographie de datation, le taux de faux positifs double (van Heesch et al., 2010). Tout en détectant trop peu de cas de trisomie 21, l'approche considérée par le Ministère exposerait inutilement, par ses taux élevés de faux positifs, les femmes québécoises à un test diagnostique et donc, à des pertes iatrogéniques accrues de foetus sains.

Figure 23. Niveaux sanguins maternels de la PAPP-A entre les 11e-14e semaines

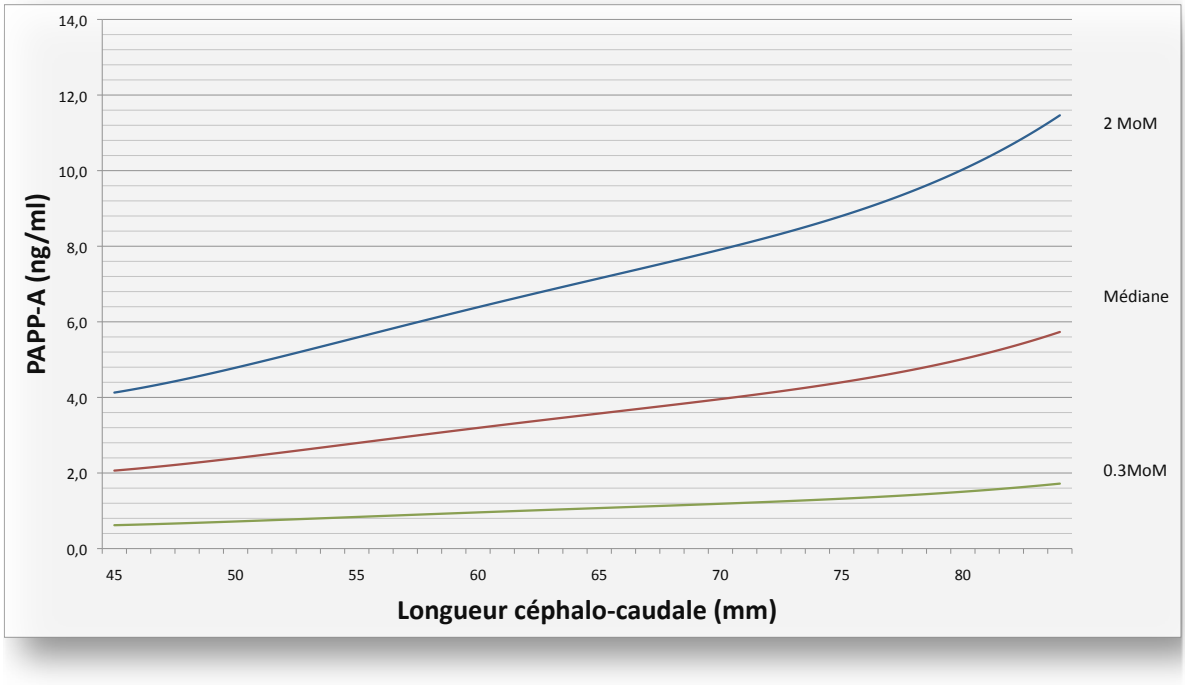
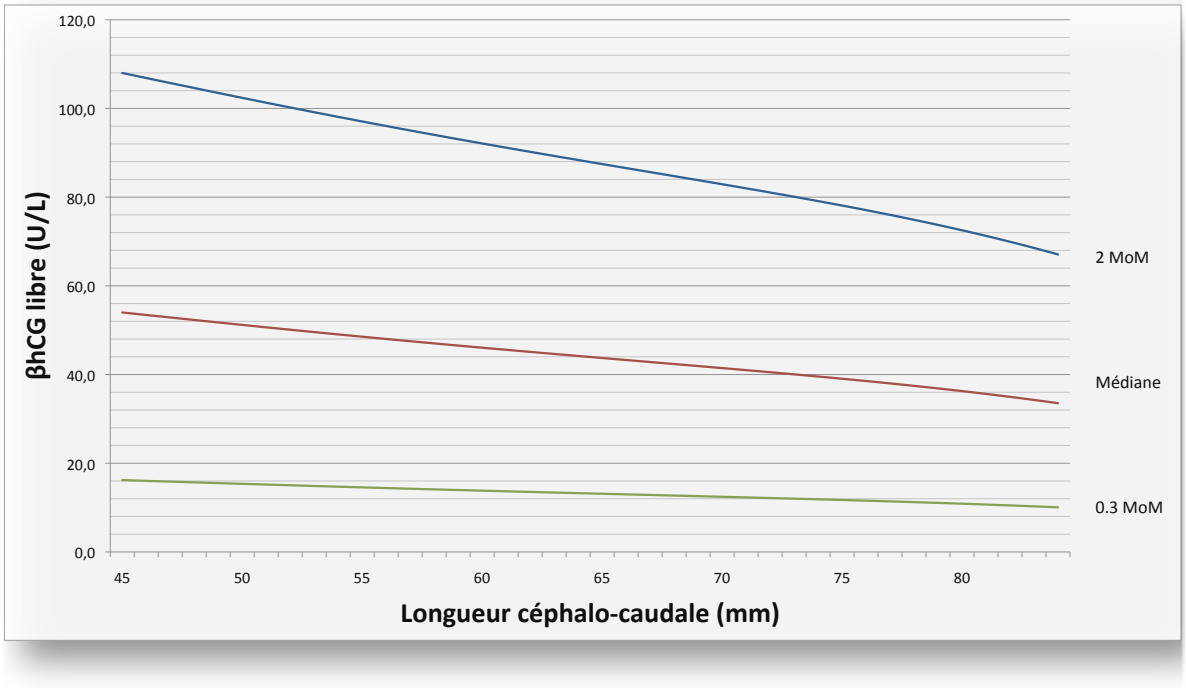


Figure 24. Niveaux sanguins maternels de la fraction libre de la β-hCG entre les 11e-14e semaines



Afin de déterminer le potentiel d'autres approches, nous avons prédit par modélisation les taux de détection de la trisomie 21 selon différents types de test intégré, avec l'assistance du Pr Nicholas Wald, auteur de l'étude SURUSS (Wald et al., 2003, Wald et al., 2004). Les résultats sont présentés et comparés selon que l'âge de la grossesse ait été déterminé par échographie ou par date des dernières menstruations (DDM) (Tableaux IX et X).

Tableau IX. Taux de détection et de faux positifs de T21 pour un TIS incluant PAPP-A (T1) et hCG totale, uE3, AFP et Inhibine A (T2)

Âge gestationel lors du prélèvement pour PAPP-A (semaines complétées)	Estimation de l'âge gestationnel par:			
	Échographie		DDM	
	Détection @ 3% FP	FP @ 75% D	Détection @ 3% FP	FP @ 75% D
10	84%	1,20%	80%	2,00%
11	81%	1,60%	77%	2,50%
12	79%	2,10%	75%	3,00%
13	77%	2,50%	73%	3,40%

Tableau X. Taux de détection et de faux positifs de T21 pour un test intégré complet incluant clarté nucale et PAPP-A (T1) et hCG totale, uE3, AFP et Inhibine A (T2)

Âge gestationel lors du prélèvement pour PAPP-A (semaines complétées)				
	Échographie		DDM	
	Détection @ 3% FP	FP @ 75% D	Détection @ 3% FP	FP @ 75% D
10	94%	0,14%	92%	0,23%
11	93%	0,15%	91%	0,24%
12	91%	0,19%	89%	0,30%
13	89%	0,40%	87%	0,60%

Parmi les tests intégrés, seuls les modèles avec une échographie de datation et l'ajout de l'inhibine A (T2) ou de l'inhibine A avec une clarté nucale permettent de rencontrer ou d'excéder les normes canadiennes.

Même le test QUAD, effectué en une seule visite au deuxième trimestre et qui combine l'hCG totale, l'oestriol, l'AFP et l'inhibine A, ne rencontre pas les critères canadiens, sauf de justesse s'il est combiné à une échographie de datation (Tableau XI).

Tableau XI. Test QUAD incluant hCG totale, uE3, AFP et Inhibine A

Taux de détection @ 3% FP avec âge gestationnel estimé par:	
Échographie	DDM
75%	72%

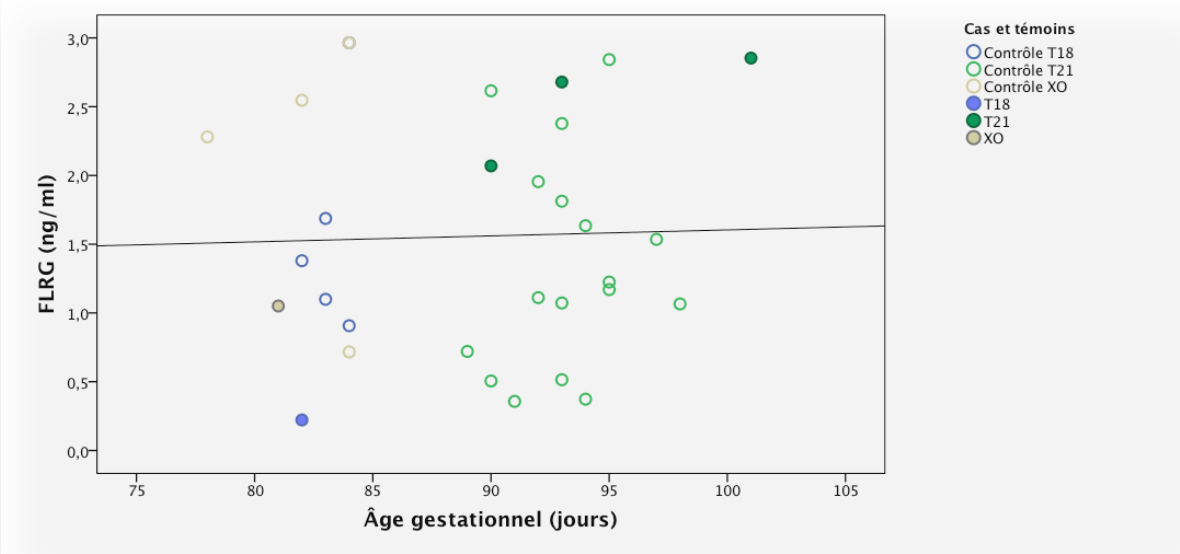
À défaut d'avoir accès à une mesure de la clarté nucale, un dépistage précoce sérique en une seule visite, combiné simultanément à une échographie de datation au premier trimestre de la grossesse, nous semblerait plus performant et mieux adapté aux besoins réels des femmes enceintes qui souhaitent en grande majorité [3] obtenir tôt en grossesse une réponse sur la santé de leur futur enfant (de Graaf et al., 2002). Dans cette optique, la protéine FLRG nous apparaissait être un sujet intéressant d'étude pour les raisons suivantes : (1) elle est principalement sécrétée par le placenta et en dehors de la grossesse, ses niveaux sériques sont bas ; (2) la FLRG n'avait jamais été étudiée jusqu'ici au premier trimestre de grossesse chez l'humain ; (3) ses concentrations maternelles sont significativement élevées entre les 28^{e} et 37^{e} semaines en présence d'une autre pathologie de grossesse, la prééclampsie (Pryor-Koishi et al., 2007); (4) son interaction physico-chimique directe avec un marqueur connue d'aneuploïdie, ADAM12-S (Bartholin et al., 2005).

Une étude pilote cas-témoins fut d'abord réalisée, portant sur trois cas de trisomie 21, un cas de trisomie 18 et un syndrome de Turner, comparés à 25 contrôles appariés. Les échantillons plasmatiques provenaient de notre propre biobanque, composée essentiellement de patientes à faible risque. On notait des concentrations plasmatiques maternelles médianes de FLRG de 2.6 ng/ml pour les cas de trisomie 21, soit significativement plus élevées que celles de 1.3 ng/ml pour les contrôles (analyses de variance paramétrique et non paramétrique de Sheirer-Ray-Hare : p= 0,04 et 0,02,

[3] 86 à 95% des femmes à risque faible ou élevée, respectivement

respectivement). Ces données préliminaires laissaient présager initialement un rôle potentiel de la FLRG dans le dépistage de la trisomie 21 (Figure 25). Cela ne fut toutefois pas confirmé par notre publication finale portant sur un plus grand nombre d'échantillons.

Figure 25. Concentrations plasmatiques maternelles de la FLRG (étude pilote cas-témoins)



La population étudiée dans notre article diffère toutefois de celles de notre étude pilote. Les échantillons utilisés pour la publication provenaient uniquement de patientes à haut risque, recrutées en Angleterre, leur prélèvement sanguin ayant été récolté juste avant de procéder à une biopsie du trophoblaste.

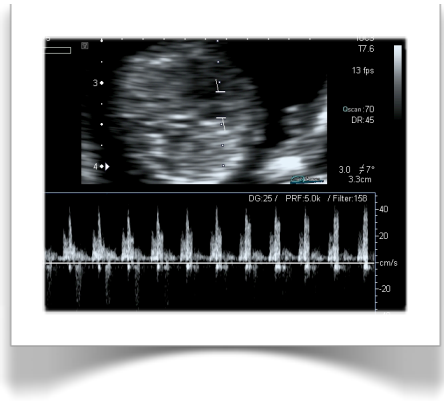
La différence de résultats entre notre étude pilote et l'article pourrait s'expliquer par un biais de sélection. Les moyennes d'âge maternel pour les cas de trisomie 21 et les contrôles étaient particulièrement avancées dans notre publication finale, soit de 38 et 34 ans, respectivement. Il est déjà connu que les concentrations moyennes de PAPP-A, entre les cas et les contrôles, ne sont pas si différentes chez les femmes âgées. Dans de tels cas, l'âge maternel avancé est en soi le plus souvent l'explication principale d'un résultat positif.

Nous avons noté dans notre article un effet significatif de l'âge maternel sur la FLRG. Il aurait donc été utile de déterminer la corrélation entre la FLRG et les autres marqueurs comme la PAPP-A, la fraction libre de la β-hCG et la clarté nucale. Ces données n'ont malheureusement pu nous être fournies par la biobanque anglaise. Une forte corrélation avec la PAPP-A aurait pu expliquer l'absence de différence entre les cas de trisomie 21 et les contrôles, notée dans notre étude finale. Il serait donc intéressant d'étudier éventuellement le potentiel de la FLRG dans une population plus jeune, à faible risque. De plus, en présence d'une forte corrélation entre la FLRG et la PAPP-A, la FLRG pourrait toujours présenter un certain intérêt dans le dépistage prénatal d'aneuploïdies en tant que « mesures répétées » simultanées (Cuckle, 2009).

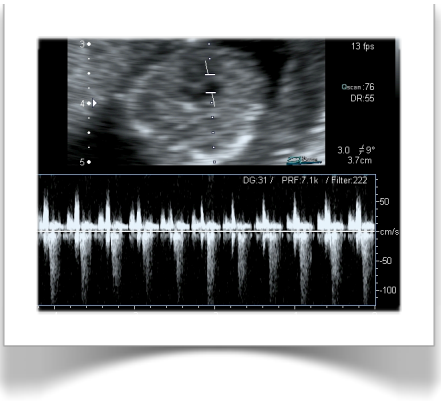
Évolution future du dépistage prénatal

Quelle sera l'évolution du dépistage prénatal du premier trimestre au cours des prochaines années ? Évidemment, cela dépendra des innovations technologiques et des découvertes scientifiques. Au cours des dernières années, ce sont les marqueurs échographiques qui ont pris l'avantage sur les marqueurs biochimiques. Les données qui suivent devraient d'ailleurs susciter une réflexion approfondie pour tout programme qui décide de ne pas intégrer les marqueurs échographiques dans le calcul de risque. Depuis peu, les aneuploïdies ont été significativement associées à une multitude de marqueurs échographiques foetaux complémentaires à la clarté nucale et l'os nasal, tels que (1) le doppler de la valve tricuspidienne ; (2) le doppler du ductus veineux (canal d'Arantius) et (3) l'angle facial maxillofrontal (Figure 26). En fait, lorsque l'on compare individuellement les marqueurs sanguins et échographiques actuels du premier et du deuxième trimestres, les marqueurs échographiques du premier trimestre confirment leur supériorité. Ces derniers sont en effet les seuls à offrir individuellement des taux de détection supérieurs à 60 % pour un taux fixe de faux positifs de 5 % (Tableau XII).

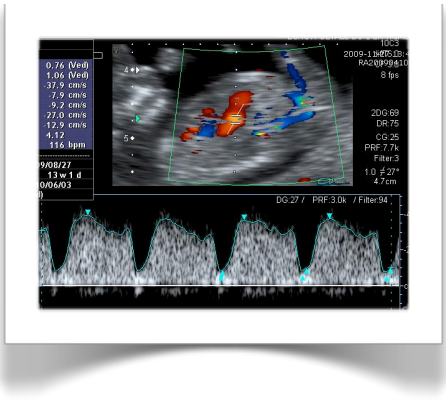
Figure 26. Nouveaux marqueurs échographiques du premier trimestre



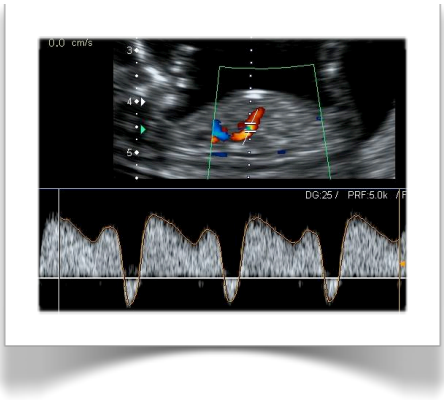
Flot normal de la valve tricuspidienne



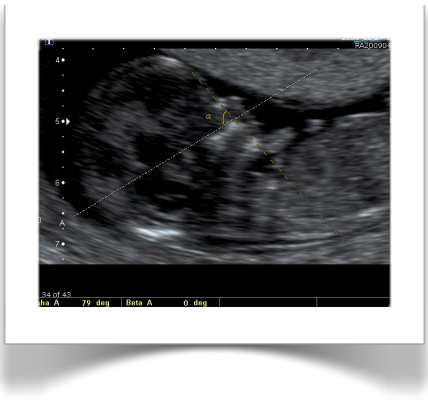
Régurgitation tricuspidienne (≥ 60 cm/sec)



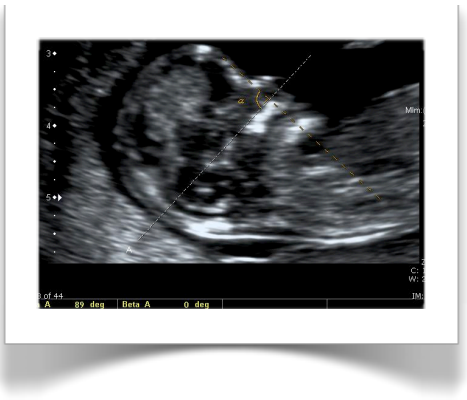
Ductus veineux normal



Ductus veineux avec flot inversé



Angle facial normal



Angle facial augmenté

(Source : P. Miron)

Tableau XII. Performance des marqueurs biochimiques et échographiques pris isolément, aux premier et deuxième trimestres (pour la trisomie 21). Adapté de (Maymon et al., 2005, Borrell, 2009).

Marqueur (sans âge maternel)	Taux de détection (%)	Faux positifs (%)
Marqueurs sanguins du 2e trimestre		
AFP	24	5
hCG	40	5
Estriol	39	5
Inhibine A	49	5
Marqueurs sanguins du 1er trimestre		
PAPP-A	44	5
Fraction libre de la βhCG	28	5
Marqueurs échographiques du 2e trimestre		
Pli nucal	33	0.6
Foyer échogène intra-cardiaque	28	4.4
Intestin hyperéchogène	13	0.6
Pyélectasie	18	2.6
Fémur court	42	5.2
Os nasal	43	5.0
Épaisseur prénasale	58	5.0
Marqueurs échographiques du 1er trimestre		
Clarté nucale	72	5
Os nasal	69	2.5
Ductus veineux	71	5
Régurgitation tricuspidienne	67	4.4
Angle facial maxillo-frontal	69	5

En fait, en combinant au premier trimestre la PAPP-A, la fraction libre de la β-hCG à la clarté nucale, l'os nasal, le ductus veineux, la régurgitation tricuspidienne et l'angle facial, 96 % des foetus trisomiques seraient identifiés avec seulement 2 % de faux positifs ; ceci en ferait à l'heure actuelle le test de dépistage le plus performant (Nicolaides, 2009). S'ajouteront potentiellement à ces résultats, de nouveaux marqueurs d'aneuploïdies au

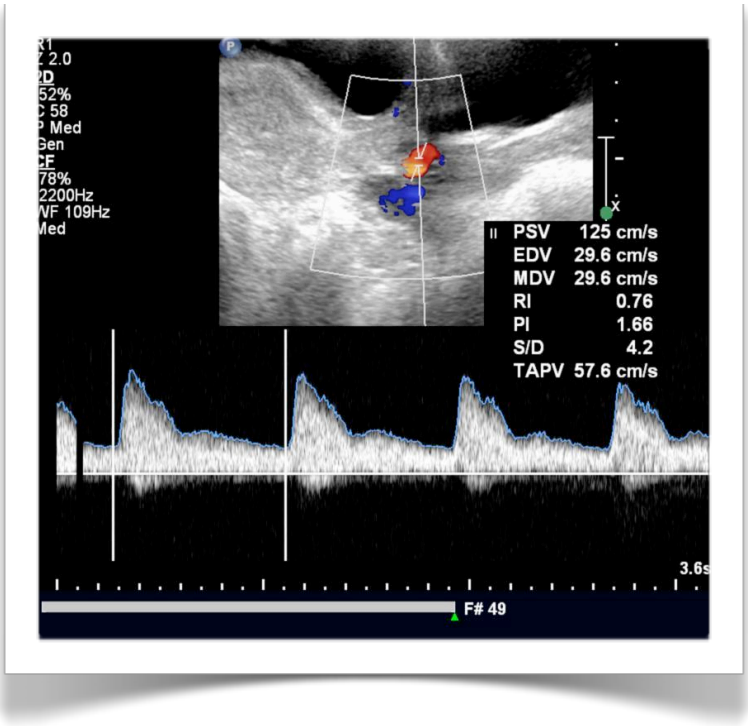
premier trimestre tels le rythme cardiaque foetal, significativement élevé en présence d'une trisomie 21 et d'une trisomie 13 et diminué en présence d'une trisomie 18, ainsi que des marqueurs dits « mineurs » : kyste des plexus choroïdes, foyer échogène intracardiaque, pyélectasie et intestins hyperéchogènes (Dagklis et al., 2008, Kagan et al., 2008). Puisqu'une échographie de datation devrait de toute façon être réalisée dans le cadre d'un dépistage, il y aurait donc avantage à former au Québec les échographistes pour de telles mesures, quel que soit le programme retenu par l'État.

Certains ont proposé que le dépistage prénatal de la trisomie 21, comme on le connaît aujourd'hui, pourrait un jour être entièrement substitué par un diagnostic prénatal non effractif à partir d'ADN fœtal ou d'ARN placentaire, présents dans la circulation maternelle sanguine (Dhallan et al., 2007, Lo et al., 2007, Sekizawa et al., 2007). Deux firmes américaines avaient été particulièrement actives dans la promotion d'un tel test au cours des dernières années. L'une, Ravgen, est complètement silencieuse depuis 2007. L'autre, Sequenom, a avoué en 2009 une manipulation des données scientifiques, ce qui a mené à des enquêtes du FBI et de la *Securities and Exchange Commission* et à la destitution de personnes clés, dont le président, le vice-président aux finances et le vice-président R&D (Edwards, 2009). Nous sommes donc probablement encore bien loin d'une application clinique. Une approche procédant plutôt à une isolation de cellules fœtales en circulation maternelle pourrait toutefois s'avérer plus prometteuse (Krabchi et al., 2001, Krabchi et al., 2006a, Krabchi et al., 2006b, Krabchi et al., 2006c, Gotherstrom et al., 2010).

Par ailleurs, le dépistage prénatal du premier trimestre connaît depuis peu une métamorphose substantielle en étendant ses champs d'activités à la détection non seulement d'aneuploïdies, mais également au dépistage précoce d'autres pathologies en grossesse. Ainsi, le dépistage de la prééclampsie précoce, se développant avant la 34e semaine, peut maintenant être réalisé au premier trimestre en combinant un index de pulsatilité des artères utérines, la PAPP-A, la tension artérielle moyenne et certaines autres données cliniques (origine ethnique, poids maternel, tabagisme et longueur céphalocaudale, antécédents personnels et familiaux) (Figure 27). Cette combinaison de facteurs permettrait de détecter 84 % des prééclampsies précoces avec 5 % de faux positifs. De plus, le remplacement de la

PAPP-A par le PlGF augmenterait ce taux de détection à 90 %. Ces approches ouvrent la voie à des interventions préventives précoces.

Figure 27. Doppler des artères utérines au premier trimestre (index de pulsatilité)



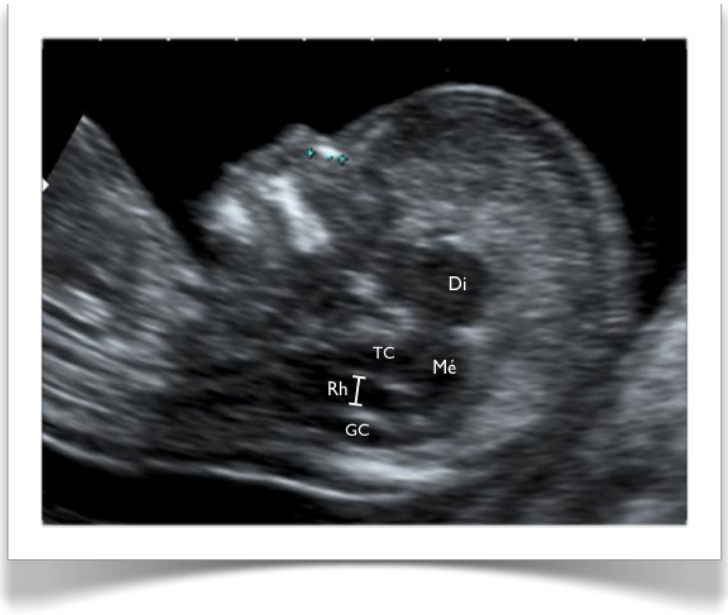
(Source : P. Miron)

D''autres études confirment également qu'un risque élevé de trisomie 21 identifié au dépistage prénatal du premier trimestre (PAPP-A, fraction libre de la β-hCG et clarté nucale) est associé entre autres à des risques significativement élevés de travail préterme, de retard de croissance intra-utérin et de mortalité foetale *in utero* (Krantz et al., 2004, Spencer et al., 2006, Brameld et al., 2008).

Les études confirment aussi de plus en plus qu'une multitude d'anomalies structurelles peuvent maintenant être décelées très tôt en grossesse, dès le premier trimestre. En fait, une étude récente suggère que 84 % des anomalies foetales majeures seraient détectables, ce qui se compare avantageusement à l'échographie effectuée entre les 18e-22e semaines pour l'anatomie foetale (Becker and Wegner, 2006). Même le *spina bifida*, que l'on croyait

jusqu'à récemment très difficile à détecter au premier trimestre, pourrait être dépisté indirectement par la mesure d'une clarté dite intracrânienne, correspondant possiblement au 4e ventricule cérébral, le rhombencéphale, visible dans le plan sagittal médian déjà utilisé de routine pour les mesures de la clarté nucale, de l'os nasal et de l'angle facial (Figure 28). Cet espace (ou celui de la grande citerne) serait en effet affaissé (c.-à-d. absent) en présence d'anomalie ouverte de la colonne vertébrale (Chaoui et al., 2009).

Figure 28. Clarté intracrânienne (Di : diencéphale ; GC : grande citerne; Mé : mésencéphale ; Rh : rhombencéphale (4e ventricule); TC : tronc cérébral)



(Source : P. Miron)

Une étude longitudinale comparant l'efficience des échographies du premier et du deuxième trimestre dans la détection d'anomalies foetales structurelles majeures serait donc de mise.

Axes de recherche proposés

Si le gouvernement du Québec décide malgré tout de procéder à la mise en oeuvre d'un programme de dépistage prénatal d'aneuploïdies sous la forme d'un test intégré uniquement sérique, il lui serait sage de combiner à la PAPP-A du premier trimestre, la fraction libre de la β-hCG, l'oestriol, l'AFP et l'inhibine A au second trimestre et de rendre obligatoire une échographie de datation au premier trimestre. Par prudence, le Ministère devrait toutefois réaliser au préalable une étude-pilote afin de valider prospectivement l'efficacité réelle de ce test intégré sérique. Il devrait également comparer en parallèle les résultats d'un tel test à ceux obtenus prospectivement par des programmes ne nécessitant qu'une seule visite, comme :

- un programme de dépistage prénatal du premier trimestre (11-14 semaines) combinant des marqueurs échographiques (clarté nucale, os nasal, angle facial, ductus veineux, valve tricuspidienne) et biochimiques (PAPP-A, fraction libre de la β-hCG et PlGF) ;

- un programme de dépistage du deuxième trimestre qui combinerait les marqueurs échographiques (pli nucal, os nasal, épaisseur prénasale, doppler du ductus veineux et des artères utérines) et biochimiques (AFP, hCG, oestriol, etc.) à des fins de dépistage prénatal d'aneuploïdies, de cardiopathies foetales et de complications obstétricales [4] (Maymon et al., 2005, Borrell et al., 2007, Gagnon et al., 2008, Hung et al., 2008, Jelliffe-Pawlowski et al., 2009, Maymon et al., 2009).

La comparaison devrait également tenir compte de la préférence des patientes et des avantages que procurent l'échographie foetale pour la datation, le dépistage d'anomalies foetales structurales et de maladies maternelles, comme la prééclampsie.

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[4] Un tel programme serait facile à adapter au Québec, l'échographie foetale du 2e trimestre étant gratuite et accessible à toutes les femmes enceintes.

Pour tous les bénéfices que rapporte un dépistage précoce, la recherche de nouveaux marqueurs biochimiques d'aneuploïdies, au premier trimestre de la grossesse, est toujours de mise et devrait être encouragée.

Avec l'amélioration de l'imagerie médicale, une étude longitudinale pourrait être réalisée afin de comparer le coût-bénéfice des échographies du premier et du deuxième trimestre dans la détection d'anomalies foetales structurelles majeures.

Il serait également intéressant de déterminer le taux de détection de cardiopathies congénitales au premier trimestre selon les seuils proposés par les figures 20 ou 21 de la thèse, combinés ou non au doppler pulsé du ductus veineux, de la valve tricuspidienne et de l'isthme aortique.

Il devient urgent aussi d'identifier les seuils de risque produits pour la prééclampsie précoce par un dépistage prénatal et de déterminer si l'administration d'aspirine à faibles doses et de calcium, débutée avant la 16e semaine, permettrait de prévenir ou de retarder le développement de cette maladie, comme cela est actuellement recommandé chez les patientes ayant un antécédent de prééclampsie précoce (Magee et al., 2008).

En présence d'une clarté nucale augmentée menant à un diagnostic prénatal, étudier le rôle potentiel des micropuces SNP pangénomiques et du séquençage à haut débit d'ADN deviendra inévitablement une nécessité afin d'identifier plus efficacement et plus rapidement les anomalies génétiques associées.

Conclusion

Nous avons démontré par cette thèse l'importance de contrôler, dans le dépistage prénatal du premier trimestre, les variables confondantes comme le tabagisme. Nous avons également confirmé le rôle grandissant de la clarté nucale et celui des autres marqueurs échographiques au premier trimestre de la grossesse qui, dans un futur proche, modifieront inévitablement la pratique obstétricale. Bien que la FLRG ne semble pas utile pour le dépistage de la trisomie 21, il nous apparaît prévisible que d'autres marqueurs biochimiques seront identifiés et amélioreront la performance du dépistage au premier trimestre. Ce dernier ne se limite d'ailleurs plus au dépistage d'aneuploïdies mais s'étend plus largement au dépistage de différentes maladies foeto-maternelles.

Annexe I. Articles tels que publiés

1. Effect of maternal smoking on prenatal screening for Down syndrome and trisomy 18 in the first trimester of pregnancy (Miron et al., 2008);

2. Nuchal translucency thresholds in prenatal screening for Down syndrome and trisomy 18 (Miron et al., 2009);

3. Maternal plasma levels of follistatin-related gene protein in the first trimester of pregnancies with Down syndrome (Miron et al., 2010).

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Effect of maternal smoking on prenatal screening for Down syndrome and trisomy 18 in the first trimester of pregnancy

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Objectives To assess the impact of maternal smoking on first-trimester prenatal screening results for Down syndrome and trisomy 18.

Methods Data on maternal smoking status, maternal age, gestational dating, levels of free beta-human chorionic gonadotrophin (β-hCG) and pregnancy-associated plasma protein A (PAPP-A) in maternal blood and fetal nuchal translucency (NT) thickness were analyzed from a cohort of 53 114 women. Statistical analyses were carried out for crude and adjusted comparisons between smoking and nonsmoking groups.

Results In women who smoked during the first trimester of pregnancy, PAPP-A and free β-hCG levels from dried blood were significantly decreased ($p < 0.001$) and fetal NT thickness was significantly increased ($p < 0.001$). For an overall risk assessment combining maternal age and biochemical and ultrasound markers, no significant changes for Down syndrome were found with smoking, but significant increases in average risk as well as in positive rates were found for trisomy 18 ($p < 0.001$). A potential association between maternal smoking and trisomy 18 remains to be clarified.

Conclusion Adjustment for smoking is recommended in first-trimester prenatal screening for trisomy 18 and probably not warranted for Down syndrome because of the cancelling effects of decreased free β-hCG and increased NT. Further research is required to demonstrate a biological association between maternal smoking and trisomy 18. Copyright © 2008 John Wiley & Sons, Ltd.

KEY WORDS: nuchal translucency measurement; pregnancy-associated plasma protein A; chorionic gonadotrophin,; beta subunit; human; smoking; Down syndrome; trisomy

INTRODUCTION

Cigarette smoking in the second trimester has been shown to influence maternal serum of human chorionic gonadotrophin, (hCG), unconjugated estriol (uE_3), alpha-fetoprotein (AFP) and more importantly, inhibin-A, and impacts therefore, on second-trimester screening results for Down syndrome (Bartels *et al*., 1993; Palomaki *et al*., 1993; Spencer, 1998; Ferriman *et al*., 1999; Rudnicka *et al*., 2002). Yet, only a few studies have been published regarding its impact on prenatal screening in the first trimester of pregnancy. Most of these studies have confirmed reduced serum levels of free beta-human chorionic gonadotrophin, (free β-hCG) and pregnancy-associated plasma protein A (PAPP-A) in maternal smokers. Contradictory results have been published regarding nuchal translucency (NT) thickness. While in two cohorts no difference in NT thickness was found between smokers and nonsmokers, a significant increase in fetal NT multiples of the median (MoM) was observed by others in smoking populations (Niemimaa *et al*., 2003; Spencer *et al*., 2004; Yigiter *et al*., 2006; Ardawi *et al*., 2007b).

The impact of maternal smoking on positive rates of prenatal screening has also been poorly studied, mainly with respect to the risk assessment of Down syndrome. Although correcting first-trimester biochemical markers for maternal smoking status appears to have little impact on the detection rates, it allows for a reduction in positive rates for Down syndrome (Spencer *et al*., 2004). No data has been published yet regarding effect of smoking on the risk assessment of trisomy 18.

The objective of our study was to further define, in a large cohort of women, the influence of maternal smoking on the results of first-trimester prenatal screening for Down syndrome and trisomy 18.

MATERIALS AND METHODS

Data were extracted from a historical cohort of 53 114 women in Quebec who, between the years 1999 and 2005, underwent prenatal screening for Down syndrome and trisomy 18 in their first trimester of pregnancy (i.e. between the 11th and 14th weeks). Patients signed a consent form indicating that their results could be used anonymously for research purposes. Pregnancy

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outcomes were not studied in the present analysis, since this information was not obtained from all women.

For all subjects, request forms containing demographic and clinical variables were filled by physicians or their assistants with patient collaboration. For cigarette smoking, a specific box with a 'yes' or 'no' answer had to be checked. Exact number of cigarettes smoked daily was not determined. Blood samples were collected on filter paper attached to the request form. PAPP-A and free-β hCG levels were analyzed using in-house enzyme-linked immunosorbent assays (ELISAs), as described previously (Krantz *et al*., 2005).

Sonographers performing NT measurements were sensitized to the importance of following the Fetal Medicine Foundation (FMF) guidelines (www.fetalmedicine.com/nuchal) (Snijders *et al*., 1998). They were all recognized by their respective licensing authority to perform first-trimester fetal sonography (College of Physicians of Quebec and Order of Radiology Technologists of Quebec). Risk assessments of Down syndrome and trisomy 18 were produced by combining both the biochemical and the ultrasound markers. Fetal nasal bone measurement, initiated in 2003, was assessed in 9268 pregnant women but was not included in the calculation of risks.

The software program for risk assessment of Down syndrome and trisomy 18 was provided by NTD Laboratories (New York, USA). Risks were determined by multiplying the likelihood ratio by the women's risk for Down syndrome and trisomy 18 before screening, which was based on maternal age, gestational age (GA) and prior history of trisomy. Women with risks greater than that of a 35-year-old at the same GA were considered to be at increased risk for Down syndrome (Krantz *et al*., 2000). Cut-off levels used for positive results were set at 1 : 210 to 1 : 266 for Down syndrome, depending on GA, and at 1 : 150 for trisomy 18.

STATISTICAL ANALYSIS

Comparisons of demographic variables and GAs between groups were based on the two-sample Student *t*-test for quantitative characteristics, and on the Pearson's χ^2 for qualitative (or categorical) characteristics. *T*-test *p*-values were adjusted for unequal variances when necessary.

Comparisons of biochemical and ultrasound markers as well as of risks for Down syndrome and trisomy 18 were performed using multiple linear regressions adjusting for demographic variables (maternal age, weight and ethnic origin) and GAs. Other variables, such as prior history of Down syndrome, insulin-dependent diabetes, experience and FMF certification of ultrasonographers ($n = 467$) were considered for adjustment but not included in the models due to lack of statistical significance. Residuals were studied for outliers, non-normal distributions and heteroscedasticities. Logarithmic transformations were performed on all biochemical markers owing to skewed distributions. Comparisons of positive rates for Down syndrome and trisomy 18 were performed using multiple logistic regressions adjusting for demographic variables and GAs. Residuals were studied for outliers and the Hosmer–Lemeshow statistic was used to verify the overall quality of adjustment.

Due to the very large sample size ($n = 53\,114$), the significance level was set to 0.001, and caution must be taken when interpreting the results.

RESULTS

Women participating in this prenatal screening program were significantly older ($\overline{X} = 31.4$ years) than those in the general pregnant population of Quebec ($\overline{X} = 29.1$ years; $p < 0.001$) (ISQ, 2006).

In our studied population, the proportion of women who smoked during pregnancy was 6.7%. There were more Caucasians and less of other ethnic groups among smokers ($p < 0.001$), as shown in Table 1. All other demographic characteristics and GAs were similar between smokers and nonsmokers ($p > 0.064$).

When adjusting for demographic variables and GA, significant reduction in dried blood levels of PAPP-A and free β-hCG was observed ($p < 0.001$), as depicted in Table 2. When based on maternal age and biochemical

Table 1—Demographic characteristics and gestational ages of smoking and nonsmoking pregnant women undergoing prenatal screening between 11 and 14 weeks ($n = 53\,114$)

	Nonsmoking $n = 49\,531$	Smoking $n = 3\,583$	*p*-value
A. Quantitative variables	$\overline{X} \pm$ SD	$\overline{X} \pm$ SD	
Women's age (years)	31.4 ± 3.88	31.2 ± 4.50	0.064
Gestational age at time of echo (weeks)	12.5 ± 0.64	12.5 ± 0.66	0.222
Corrected GA at time of sampling	12.6 ± 0.59	12.6 ± 0.60	0.261
Weight (kg)	65.1 ± 12.4	65.4 ± 12.8	0.074
B. Qualitative variables	%	%	
Ethnic origin			
White	95.9	98.7	
Asian	2.0	0.3	
Afro-Caribbean	0.7	0.3	0.000
Native	0.1	0.1	
Other	1.2	0.6	
Active vaginal bleeding	9.2	9.3	0.743

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markers alone, the risk of Down syndrome was significantly reduced ($p < 0.001$) and the risk of trisomy 18 was significantly increased ($p < 0.001$) among smokers (Table 2).

For ultrasound markers, smoking had a significant influence on NT thickness by increasing its mean from 1.59 mm for nonsmokers to 1.62 mm for smokers ($p < 0.001$), as described in Table 3. However, smoking had no impact on nasal bone length ($p = 0.646$). When based on maternal age and NT alone, observed risks for Down syndrome and for trisomy 18 were significantly increased among smokers ($p < 0.001$).

Effects of smoking in the first trimester of pregnancy on overall prenatal screening results for Down syndrome and trisomy 18 are summarized in Table 4. No significant changes were found in average calculations of risks and in positive rates for Down syndrome between smokers and nonsmokers. However, for trisomy 18, both the calculations of risk and the positive rates were significantly increased in smokers. Positive rates went up from 0.7% in nonsmokers to 2.3% in smokers ($p < 0.001$), an important rise with significant clinical implication.

DISCUSSION

As previously demonstrated in maternal serum, this study confirmed that levels of PAPP-A and free-βhCG

Table 2—Biochemical data of smoking and nonsmoking pregnant women undergoing prenatal screening between 11 and 14 weeks ($n = 53\,114$)

	Nonsmoking $\overline{X} \pm SD$	Smoking $\overline{X} \pm SD$	p-value
PAPP-A (UI/L)	5.49 ± 7.43	4.43 ± 3.76	0.000
Free β-hCG (ng/mL)	54.2 ± 35.0	47.4 ± 33.7	0.000
Average risk of Down syndrome (based on age and biochemistry)	$1 : 2\,566 \pm 1 : 2\,073$	$1 : 2\,720 \pm 1 : 2\,211$	0.000
Average risk of trisomy 18 (based on age and biochemistry)	$1 : 8\,618 \pm 1 : 2\,934$	$1 : 7\,522 \pm 1 : 3\,709$	0.000
	Median (MoM)	Median (MoM)	
PAPP-A (MoM)	1.03	0.86	0.000[a]
Free β-hCG (MoM)	1.00	0.87	0.000[a]

[a] Groups were compared using multiple linear regressions.

Table 3—Clinical data of smoking and nonsmoking pregnant women undergoing prenatal screening between 11 and 14 weeks ($n = 53\,114$)

	Nonsmoking $\overline{X} \pm SD$	Smoking $\overline{X} \pm SD$	p-value
CRL (mm)	63.3 ± 8.39	63.6 ± 8.60	0.016
Nuchal thickness (mm)	1.59 ± 0.49	1.62 ± 0.50	0.000
Nasal bone length (mm)	1.79 ± 0.41	1.78 ± 0.44	0.646
Average risk of Down syndrome (based on age and NT)	$1 : 3\,222 \pm 1 : 2\,163$	$1 : 3\,170 \pm 1 : 2\,323$	0.000
Average risk of trisomy 18 (based on age and NT)	$1 : 6\,425 \pm 1 : 3\,337$	$1 : 6\,158 \pm 1 : 3\,429$	0.000
	Median (MoM)	Median (MoM)	
NT	1.00	1.07	0.001[a]

[a] Groups were compared using multiple linear regressions.

Table 4—Effect of smoking on overall prenatal screening results for Down syndrome and trisomy 18 in the first trimester ($n = 53\,114$)

	Nonsmoking $\overline{X} \pm SD$	Smoking $\overline{X} \pm SD$	p-value
A. Quantitative variable			
Average risk of Down syndrome (based on age, ultrasound and biochemistry)	$1 : 4\,428 \pm 1 : 2\,821$	$1 : 4\,459 \pm 1 : 3\,024$	0.088
Average risk of trisomy 18 (based on age, ultrasound and biochemistry)	$1 : 9\,433 \pm 1 : 1\,996$	$1 : 8\,795 \pm 1 : 2\,868$	0.000
B. Qualitative variables			
Positive rate for Down syndrome (%)	3.8	4.3	0.210
Positive rate for trisomy 18 (%)	0.7	2.3	0.000

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in dried blood samples are also reduced by smoking. In fact, in our study, MoM medians of PAPP-A and free-βhCG were reduced by 17 and 13%, respectively.

The decrease in free-βhCG levels caused by smoking appears to be more important in dried blood samples than the decrease reported in serum in previous large cohort studies (Niemimaa *et al*., 2003; Spencer *et al*., 2004; Kagan *et al*., 2007) (Table 5). However, in other smaller studies using also serum, the decrease seems similar to our results with dried blood (de Graaf *et al*., 2000; Ardawi *et al*., 2007a,b). Several confounding factors, such as ELISA kit used, could explain this potential difference between dried blood and serum levels of free-βhCG and, therefore, its significance remains to be clarified.

This study also indicated a significant increase in NT thickness caused by maternal smoking. Over the last few years, contradictory results have been published regarding the effect of smoking on NT measurements. Although a large cohort study ($n = 32\,854$) suggested that NT thickness was not increased in maternal smokers, two studies with smaller sample sizes of 1275, 4436 and 1778 women found an increase in NT thickness (Niemimaa *et al*., 2003; Spencer *et al*., 2004; Yigiter *et al*., 2006; Ardawi *et al*., 2007a). After controlling confounding variables, our large cohort study with 53 114 women confirmed a significant impact of smoking on NT thickness.

By reducing maternal blood levels of PAPP-A and free-βhCG, and by increasing NT thickness, maternal smoking significantly influences prenatal screening results for trisomy 18 and increases, by at least three times, the probability for a woman to be offered, on the basis of a positive screening, an invasive diagnostic procedure such as amniocentesis or chorionic villi sampling.

The absence of any significant impact of smoking on the overall risk assessment of Down syndrome may be explained by the cancelling effect of both the decrease of free-β hCG and the increase of NT thickness.

In our study, only 6.7% of women smoked at the time of first-trimester screening. While overall, there appears to be fairly good correlation between maternal reports on smoking during pregnancy and actual smoking, the rates of nonreporting and under-reporting are most probably still substantial. One has to assume that most women who state that they are smoking during pregnancy, are, in fact, smoking. But an important percentage of the women who state that they do not smoke are, in fact, smokers who are not reporting it (Lindqvist *et al*., 2002; George *et al*., 2006). Furthermore, many women quit smoking once they discover that they are pregnant, but they may not discover this until late in their first trimester, at which point they stop (Grange *et al*., 2006). These women will be classified as nonsmokers at time of screening, but the smoking they did up to week 6, 8, or 10 is likely to affect their PAPP-A, free-βhCG, and NT measurements. The combination of these factors could actually lead to an underestimate of the effect of smoking.

The number of cigarettes smoked daily was not registered in our database and therefore, the dose relationship of smoking with biochemical and ultrasound variables could not be assessed. In a recent article, a significant inverse relationship of the number of cigarettes per day was found with the level of PAPP-A, but not with free β-hCG. Yet, the impact of correcting for the dose-dependant rather than the all or nil effect of smoking appears to be marginal (Kagan *et al*., 2007).

Physiological mechanism(s) responsible for the effect of smoking on prenatal screening markers remain to be elucidated. Since reduced levels of various serum analytes have been demonstrated with smoking in a nonpregnant status, direct interference of cigarette residues in the blood on the measurement of serum analytes cannot be excluded (Rodger *et al*., 1985; Goodman *et al*., 1996). However, this hypothesis does not explain the increased NT thickness of fetuses in maternal smokers.

Smoking is known to substantially increase a woman's risk to serious pregnancy complications, including intrauterine fetal growth restriction, placenta abruptio and preterm delivery. Abnormal placentation is a unifying theme of such late complications, suggesting that smoking could also cause adverse events earlier in pregnancy. In fact, maternal cigarette smoking has been

Table 5—Published articles on the effect of smoking on free β-hCG levels (serum and dried blood)

Study	Serum (S) versus dried blood (DB)	ELISA kit	Smoking	*n*	MoM free β-hCG
de Graaf *et al*. (2000)	S	Delfia	Y	117	0.89
			N	1247	1.00
Niemimaa *et al*. (2003)	S	Delfia	Y	454	1.06
			N	3825	1.07
Spencer *et al*. (2004)	S	Brahms	Y	3779	0.97
			N	32 730	1.00
Ardawi *et al*. (2007a)	S	Delfia	Y	304	0.88
			N	1616	1.03
Ardawi *et al*. (2007b)	S	Delfia	Y	420	0.87
			N	1736	1.00
Kagan *et al*. (2007)	S	Brahms	Y	13 976	1.003
			N	95 287	1.035
Current study (2008)	DB	NTD	Y	3583	0.87
			N	49 531	1.00

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shown to impair placental functions very early in gestation (6–8 weeks) by inhibiting cytotrophoblast proliferation, differentiation and invasion, and by increasing production of angiogenic factors, such as vascular endothelial growth factors (VEGFs) (Zdravkovic *et al*., 2005). In a recent article, mechanisms involved in increased NT thickness have been related to a disturbance of embryonic lymphangiogenesis with alterations in the extracellular matrix composition and hemodynamic disorders (L'Hermine-Coulomb, 2005). One of the proposed biochemical mechanisms involves an increased expression of VEGF-A, as observed by immunohistochemistry in distended jugular lymphatic sacs of increased NT, showing blood vessel characteristics (Bekker *et al*., 2006). To further substantiate this hypothesis, an involvement of VEGF-A in the development of cystic hygroma of Turner syndrome has also been recently suggested (Brandenburg *et al*., 2005). Therefore, our findings of increased NT thickness in maternal smokers may be the result of an up-regulation of VEGFs.

Smoking causes a wide variety of reproductive problems, including DNA damage to spermatozoa and oocytes, transmissible to embryos (Zenzes, 2000; DeMarini, 2004). Although no association has been found with the live birth prevalence of Down syndrome, cigarette smoking may increase the risk of aneuploidy for certain chromosomes, such as 1, 13, and YY disomies (Cuckle *et al*., 1990; Rubes *et al*., 1998; Harkonen *et al*., 1999; Shi *et al*., 2001; Rudnicka *et al*., 2002). To date, no effect of smoking on the birth incidence of trisomy 18 has been documented. The significant increase of screen-positive rates of trisomy 18 found in maternal smokers raises the possibility of a real cause-to-effect relationship between smoking and trisomy 18. This can only be clarified by further research, which looks specifically at pregnancy outcomes.

CONCLUSION

Based on our results, correction for smoking status is highly recommended in first-trimester prenatal screening for trisomy 18, and is probably not warranted for Down syndrome because of the cancelling effects of the decrease of free β-hCG and the increase of NT with smoking. Corrections could be achieved by dividing the weight-corrected MoM in smokers by 0.86 for PAPP-A, by 0.87 for free-βhCG and by 1.07 for NT. Further research is recommended to clarify a potential biological association between maternal smoking and trisomy 18.

DISCLOSURE

Dr. Miron reports being owner of Prenagen Inc. and consultant through this corporation for Warnex Inc.

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Nuchal Translucency Thresholds in Prenatal Screening for Down Syndrome and Trisomy 18

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Abstract

Objective: To determine if nuchal translucency (NT) can be used as a first trimester triage marker in prenatal screening for Down syndrome and trisomy 18.

Methods: Data from first trimester prenatal screening in 77 443 women were stratified by maternal and gestational ages. They were then analyzed to identify NT thresholds above or below which only positive (high-risk) or negative (low-risk) results were reported by a first trimester prenatal screening test combining PAPP-A, free β-hCG and NT.

Results: Combined prenatal screening was always positive for Down syndrome when NT thickness exceeded 4.0 mm. As women aged, this upper NT threshold value changed according to gestational age. In women aged 35 to 37 years, combined prenatal screening was always positive when NT exceeded 2.8 mm, 3.0 mm, and 3.4 mm at 11, 12, and 13 weeks of gestation, respectively. In women over 42 years of age, the upper threshold value for NT was 1.8 mm, 2.4 mm, and 2.7 mm at 11, 12, and 13 weeks of gestation, respectively. In women less than 35 years of age, we identified lower threshold values below which combined prenatal screening for Down syndrome was always negative.

Conclusion: In prenatal screening for Down syndrome and trisomy 18, it is possible to identify NT threshold values above which biochemical screening provides no additional benefit. In pregnancies in which NT is above the established upper cut-offs, invasive prenatal screening can be offered without delay.

Résumé

Objectif : Déterminer si la clarté nucale (CN) peut être utilisée à titre de marqueur de triage du premier trimestre dans le cadre du dépistage prénatal du syndrome de Down et de la trisomie 18.

Méthodes : Les données issues du dépistage prénatal du premier trimestre mené chez 77 443 femmes ont été stratifiées en fonction des âges maternel et gestationnel. Elles ont par la suite été analysées en vue d'identifier les seuils de CN au-dessus ou en deçà desquels seuls des résultats positifs (risque élevé) ou négatifs (faible risque) ont été signalés par un test de dépistage prénatal du premier trimestre combinant la PAPP-A, la β-hCG libre et la CN.

Résultats : Le dépistage prénatal combiné était toujours positif en ce qui concerne le syndrome de Down lorsque l'épaisseur de la CN dépassait 4,0 mm. Au fur et à mesure du vieillissement chez les femmes, ce seuil supérieur de la CN a évolué en fonction de l'âge gestationnel. Chez les femmes âgées de 35 à 37 ans, le dépistage prénatal combiné était toujours positif lorsque la CN dépassait 2,8 mm, 3,0 mm et 3,4 mm à la 11e, à la 12e et à la 13e semaine de gestation, respectivement. Chez les femmes de plus de 42 ans, le seuil supérieur de la CN était de 1,8 mm, de 2,4 mm et de 2,7 mm à la 11e, à la 12e et à la 13e semaine de gestation, respectivement. Chez les femmes de moins de 35 ans, nous avons identifié des seuils inférieurs en deçà desquels le dépistage prénatal combiné était toujours négatif.

Conclusion : Dans le cadre du dépistage prénatal du syndrome de Down et de la trisomie 18, il est possible d'identifier des seuils de CN au-dessus desquels le dépistage biochimique n'offre aucun avantage additionnel. En ce qui concerne les grossesses dans le cadre desquelles la CN se situe au-delà des seuils supérieurs établis, un dépistage prénatal effractif peut être offert sans délai.

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Key Words: Nuchal translucency measurement, pregnancy-associated plasma protein-A, chorionic gonadotropin, beta subunit, human, Down syndrome, trisomy

Competing Interests: see Acknowledgements.

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INTRODUCTION

Ultrasound measurement of fetal nuchal translucency was proposed for the first time in 1992 to detect fetal chromosomal anomalies in the first trimester of pregnancy.[1] Since then, use of NT has gained in popularity, and it is now the most widely used ultrasound marker for aneuploidy. Because of its highly associated false-positive rate, its use as a stand-alone screening test to identify aneuploidy in single pregnancies was considered by many to be inappropriate.[2] In fact, only a few studies have suggested that first trimester maternal blood screening provides no additional benefit when the NT measurement is above 4.0 mm.[3,4]

To improve the accuracy of prenatal screening in the first trimester, combining NT with biochemical markers such as pregnancy-associated plasma protein-A and free β-subunit of human chorionic gonadotropin was successfully

proposed in the 1990s.[5–7] This approach has been prospectively validated in more than 200 000 screens, with a detection rate of 88% for Down syndrome at a fixed false-positive rate of 5%.[8] In order to study more precisely the NT levels that, when combined with serum levels of PAPP-A and free β-hCG, would mark the threshold between positive or negative screening results, we analyzed fetal NT measurements in a large cohort of patients in the context of maternal and gestational ages. The main objective of this analysis was to determine if NT could, in some instances, be used alone as a first trimester triage marker in screening for Down syndrome and trisomy 18.

MATERIALS AND METHODS

Data were extracted from a historical cohort of 77 443 women in Quebec who underwent prenatal screening for Down syndrome and trisomy 18 in their first trimester of pregnancy (i.e., between the 11th and 14th weeks) between 1999 and 2007. These women had given consent for their results to be used anonymously for research purposes. Since pregnancy outcomes could not be obtained from all women, this information was not included in the analysis.

Fetal crown–rump length and nuchal translucency measurements were performed by ultrasonographers who were instructed to follow the Fetal Medicine Foundation guideline.[9] Fetal nasal bone assessments (n = 16 918) were initiated in 2003 but were not included in the calculation of risks.

Overall risk assessments for Down syndrome and trisomy 18 were produced by combining NT with maternal blood levels of PAPP-A and free β-hCG (referred to as combined first trimester prenatal screening). In all subjects, blood samples were collected and transported to the laboratory using dried blood filter paper. Maternal blood levels of PAPP-A and free β-hCG were determined using in-house enzyme-linked immunosorbant assays, as described previously.[10] The software program used for risk assessment of Down syndrome and trisomy 18 was provided by NTD Laboratories (New York, NY). Individual risk was determined by multiplying the likelihood ratio by the woman's risk of having a fetus with Down syndrome and trisomy 18 before screening, based on maternal age, gestational age and prior history of trisomy. Women with a risk greater than that of a 35-year-old at the same gestational age were considered to be at an increased risk of having a fetus with Down syndrome.[11] Cut-off levels used for positive results were set at 1:210 to 1:266 for Down syndrome (depending on gestational age) and at 1:150 for trisomy 18.

Only women with no missing data related to maternal age, gestational age (as defined by crown–rump length) and combined prenatal screening results were included (N = 77 443).

Gestational age was categorized as follows:

CRL 45 mm to < 55 mm = 11+1 to 11+6 weeks for GA

CRL 55 mm to < 69 mm = 12 to 12+6 weeks for GA

CRL from 69 mm to 84 mm = 13 to 13+6 weeks for GA

Overall positive (high-risk) and negative (low-risk) prenatal screening results for Down syndrome and trisomy 18 were stratified by categorized maternal and gestational ages. For each combination, minimum and maximum NT values were compared between positive and negative overall results to find NT levels above or below which only positive or negative values were observed in a given combination. Finally, NT and maternal age cut-offs were chosen to identify women for whom CVS or amniocentesis would have

Table 1. Demographic and clinical characteristics of the studied population (n = 77 443)

A. Quantitative variables	N	Mean ± SD
Women's age (years)	77 443	31.2 ± 3.9
Gestational age at time of sonography (weeks)	77 443	12.4 ± 0.6
Weight (kg)	77 424	65.1 ± 12.6
Crown–rump length (mm)	77 443	63.8 ± 8.3
Nuchal thickness (mm)	77 443	1.60 ± 0.48
Nasal bone (mm)	16 918	1.88 ± 0.42
B. Qualitative variables		%
Ethnic origin	77 443	
Caucasian	74 561	96.3
Asian	1 453	1.9
Afro-Caribbean	633	0.8
Native	60	0.1
Other	736	0.9
Smoking	4995	6.4
Active vaginal bleeding	7044	9.1

ABBREVIATIONS

AFP	alpha fetoprotein
CRL	crown–rump length
CVS	chorionic villus sampling
GA	gestational age
hCG	human chorionic gonadotropin
NT	nuchal translucency
PAPP-A	pregnancy-associated plasma protein-A

Table 2. Nuchal translucency distribution stratified by categorized maternal age, categorized crown–rump length, and overall results for Down syndrome

				Nuchal translucency (mm)		
Maternal age (years)	CRL (mm)	Screen results	n	Mean ± SD	Minimum	Maximum
< 23	45–54	–	134	1.32 ± 0.37	0.7	2.8
		+	2	1.95 ± 1.06	1.2	2.7
	55–68	–	419	1.57 ± 0.37	0.4	3.4
		+	11	3.26 ± 1.23	1.4	6.1
	69–84	–	219	1.74 ± 0.44	0.8	3.4
		+	2	3.10 ± 1.27	2.2	4.0
23–26	45–54	–	1268	1.28 ± 0.35	0.5	3.3
		+	26	3.54 ± 2.06	1.0	8.8
	55–68	–	4487	1.54 ± 0.37	0.3	3.8
		+	60	3.11 ± 2.05	1.0	11.4
	69–84	–	2138	1.74 ± 0.40	0.6	4.0
		+	32	2.92 ± 1.47	1.0	9.0
27–34	45–54	–	7500	1.28 ± 0.33	0.4	3.2
		+	202	2.59 ± 1.51	0.6	10.0
	55–68	–	29 477	1.55 ± 0.36	0.2	3.8
		+	624	2.61 ± 1.48	0.8	10.0
	69–84	–	15039	1.75 ± 0.39	0.5	3.9
		+	399	2.58 ± 1.02	0.9	10.0
35–37	45–54	–	1363	1.29 ± 0.33	0.6	2.8
		+	80	2.11 ± 1.52	0.6	9.1
	55–68	–	5759	1.57 ± 0.36	0.5	3.0
		+	345	2.11 ± 1.04	0.9	10.0
	69–84	–	3126	1.77 ± 0.38	0.6	3.4
		+	248	2.25 ± 0.71	0.7	5.1
38–41	45–54	–	454	1.28 ± 0.29	0.4	2.3
		+	65	2.04 ± 1,49	0.7	8.6
	55–68	–	1983	1.56 ± 0.35	0.6	2.8
		+	316	2.06 ± 1.16	0.6	14.0
	69–84	–	1014	1.76 ± 0.37	0.7	3.0
		+	219	2.21 ± 1.02	0.9	10.0
≥ 42	45–54	–	28	1.35 ± 0.27	0.8	1.8
		+	19	1.56 ± 0.42	0.8	2.4
	55–68	–	136	1.55 ± 0.32	0.8	2.4
		+	100	1.89 ± 0.79	0.8	8.0
	69–84	–	99	1.80 ± 0.32	1.0	2.7
		+	50	2.07 ± 0.79	0.8	5.7

been offered with or without the biochemical analysis, or for whom no further testing was required because of perceived low risk.

RESULTS

A total of 77 443 prenatal screenings performed in the first trimester of pregnancy (i.e., with fetal CRL from 45 to 84 mm) were analyzed. Mean maternal age was 31.2 ± 3.9 years, significantly older than the Quebec population of pregnant women at the time of screening (29.1 years; $P < 0.001$).[12] The average gestational age on ultrasound was 12.4 ± 0.6 weeks. Most of the women included in this study were of Caucasian origin (96%), followed by Asian (1.9%), and Afro-Caribbean (0.8%) (Table 1).

For the whole cohort, 3.6% of women screened positive for the combined test (n = 2800). As shown in Figure 1, this

Table 3. Cut-off levels of nuchal translucency in relation to gestational and maternal age above which there are N combined first trimester prenatal screenings always positive for Down syndrome

Gestational age	CRL, (mm)	n/total pop. (%)	n/Positive screen population (%)	NT Cut-off (mm)
A. All women				
11+1 to 11+6 weeks	45–54	77/11 141 (0.7)	77/394 (20)	> 3.3
12 to 12+6 weeks	55–68	115/43 717 (0.3)	115/1456 (8)	> 3.8
13 to 13+6 weeks	69–84	43/22 585 (0.2)	43/950 (5)	> 4.0
B. Women < 35 years old				
11+1 to 11+6 weeks	45–54	61/9132 (0.7)	61/230 (27)	> 3.3
12 to 12+6 weeks	55–68	86/35 078 (0.2)	86/695 (12)	> 3.8
13 to 13+6 weeks	69–84	29/17 829 (0.2)	29/433 (7)	> 4.0
C. Women 35–37 years old				
11+1 to 11+6 weeks	45–54	14/1443 (1)	14/80 (18)	> 2.8
12 to 12+6 weeks	55–68	31/6104 (0.5)	31/345 (9)	> 3.0
13 to 13+6 weeks	69–84	16/3374 (0.5)	16/248 (6)	> 3.4
C. Women 38–41 years old				
11+1 to 11+6 weeks	45–54	11/519 (2)	11/65 (15)	> 2.3
12 to 12+6 weeks	55–68	34/2299 (1)	34/316 (11)	> 2.8
13 to 13+6 weeks	69–84	17/1233 (1)	17/219 (8)	> 3.0
Women ≥ 42 years old				
11+1 to 11+6 weeks	45–54	5/47 (11)	5/19 (26)	> 1.8
12 to 12+6 weeks	55–68	12/236 (5)	12/100 (12)	> 2.4
13 to 13+6 weeks	69–84	7/149 (5)	7/50 (14)	> 2.7

percentage increased progressively with maternal age. No women under 19 years of age had a positive screening (n = 21), while almost all women over 43 years of age screened positive (86/90 or 96%).

The distribution of NT (mean ± SD, minimum and maximum values), stratified by categorized maternal age, categorized CRL, and overall screen results for Down syndrome, with upper and lower cut-off levels, is summarized in Tables 2, 3, and 4.

Combined prenatal screening was always positive when NT was found to be above 4.0 mm (n = 197), representing 7.0% of the total positive screen population. Nevertheless, this NT cut-off level, above which combined prenatal screening was always positive, could vary from 1.8 mm to 4.0 mm or more, depending on categorized maternal and gestational ages. For women less than 35 years of age, upper NT cut-off levels were 3.3 mm, 3.8 mm, and 4.0 mm at 11, 12, and 13 weeks of gestation, respectively. NT upper threshold values progressively decreased with maternal aging, reaching 2.8 mm, 3.0 mm, and 3.4 mm in women aged 35 to 37, and 1.8 mm, 2.4 mm, and 2.7 mm in women over 42 years of age at 11, 12, and 13 weeks of gestation, respectively. With stratification by maternal and gestational ages, an increased number of women (n = 323) had an NT above which combined prenatal screening was always positive, representing 11.5% of the total screen positive population. For each category of maternal and gestational ages, the same upper cut-off values of NT set for Down syndrome could also be used for trisomy 18.

The NT cut-off levels below which combined first trimester prenatal screening for Down syndrome was always negative varied from less than 0.6 mm to 2.2 mm, depending on maternal and gestational ages (Table 4). For women less than 23 years of age, lower NT cut-off levels were found to be 1.2 mm, 1.4 mm, and 2.2 mm at 11, 12, and 13 weeks of gestation, respectively. The NT lower threshold values also progressively decreased with maternal aging, reaching 1.0 mm in women aged 23 to 26, and 0.6 mm, 0.8 mm, and 0.9 mm in women aged 27 to 34 at 11, 12, and 13 weeks of gestation, respectively. Women with NT values below their specific lower cut-off levels represented only a very small proportion of the total screen population (0.08%). No useful lower NT cut-off levels for combined negative screen could be found in women older than 34 years of age.

The same cut-off levels of NT thickness below which combined first trimester prenatal screening for Down syndrome

Table 4. Cut-off levels of NT thickness in relation to gestational and maternal ages below which there are N combined first trimester prenatal screenings always negative for Down syndrome

Gestational age	CRL (mm)	n/Total pop. (%)	n/Negative screen population (%)	NT. Cut-off (mm)
A. All women				
11+1 to 11+6 weeks	45–54	22/11 141 (0.2)	22/10 747 (0.2)	< 0.6
12 to 12+6 weeks	55–68	20/43 717 (0.05)	20/42 261 (0.05)	< 0.6
13 to 13+6 weeks	69–84	9/22 585 (0.04)	9/21 635 (0.04)	< 0.7
B. Women < 23 years old				
11+1 to 11+6 weeks	45–54	45/136 (33)	45/134 (34)	< 1.2
12 to 12+6 weeks	55–68	109/430 (25)	109/419 (26)	< 1.4
13 to 13+6 weeks	69–84	185/221 (84)	185/219 (84)	< 2.2
C. Women 23–26 years old				
11+1 to 11+6 weeks	45–54	168/1294 (13)	168/1268 (13)	< 1.0
12 to 12+6 weeks*	55–68	136/4547 (3)	136/4487 (3)	< 1.0
13 to 13+6 weeks	69–84	15/2170 (0.7)	15/2138 (0.7)	< 1.0
D. Women 27–34 years old				
11+1 to 11+6 weeks	45–54	18/7702 (0.2)	18/7500 (0.2)	< 0.6
12 to 12+6 weeks	55–68	126/30 101 (0.4)	126/29 477 (0.4)	< 0.8
13 to 13+6 weeks	69–84	59/15 438 (0.4)	59/15 039 (0.4)	< 0.9

*One positive outlier screen test for T18 at 0.8 mm.

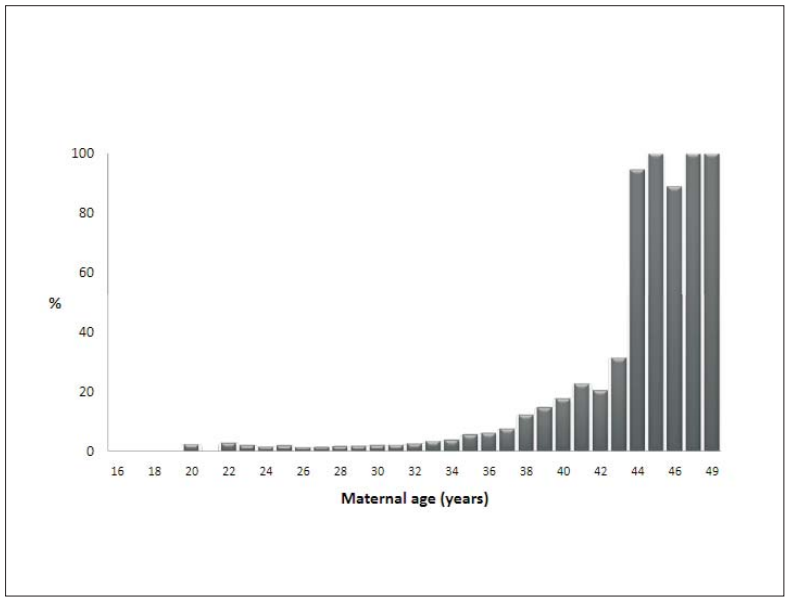
was always negative could also be applied for trisomy 18 results, with the exception of one woman aged 23 with a fetal CRL of 59 mm. In this individual's case, PAPP-A and free-βhCG multiples of the median were found to be very low at 0.07 and 0.10, respectively. An increased risk for trisomy 18 or 13 was initially suggested. Several major structural abnormalities were found on ultrasound in the second trimester (club hands, cardiac defect, cerebral ventriculomegaly, and asymmetrical intrauterine growth restriction) and a triploidy (69,XXX) was finally diagnosed by fetal karyotyping.

DISCUSSION

Several, often complex, strategies have been proposed to screen for Down syndrome and other common aneuploidies.[13,14] Over the last decade, the popularity of second trimester prenatal screening (AFP, estriol, AFP ± inhibin A) has declined and first trimester screening using a combination of maternal blood biochemical markers (free β-hCG and PAPP-A) with fetal nuchal translucency measurement has increased. Although more recent approaches combining first and second trimester markers have been proposed, such as integrated, sequential, and contingent screenings, there is a clear trend towards early disclosure of results to a maximum number of patients.[15–17] This trend is confirmed by (a) women's preference for first trimester screening of pregnancy,[18,19] (b) ethical principles of informed consent and respect for patient autonomy, beneficence, and justice,[20] and (c) the recent joint statement of the National Institute of Child Health and Human Development, the Society for Maternal-Fetal Medicine, and the American College of Obstetricians and Gynecologists.[21]

In 1992, the first-trimester NT was reported to be increased in 35% of aneuploid fetuses, compared with only 1% of euploid fetuses.[1] Since then, several large studies have confirmed that NT screening, which takes into account maternal and gestational ages, has a detection rate for Down syndrome ranging from 69% to 75% with a false positive rate of 5% to 8.1%.[22–24] The Society of Obstetricians and Gynaecologists of Canada and the Canadian College of Medical Geneticists jointly stated in 2007 that any prenatal screen for Down syndrome should, as a minimum standard, have a detection rate of at least 75%, with a false positive rate of no more than 5%. Their recommendation was that NT alone without biochemical markers should never be offered, except in the context of multiple pregnancy.[2] We believe this is not entirely true and that there are some exceptions to this guideline.

Figure 1. Combined prenatal screening positive according to maternal age

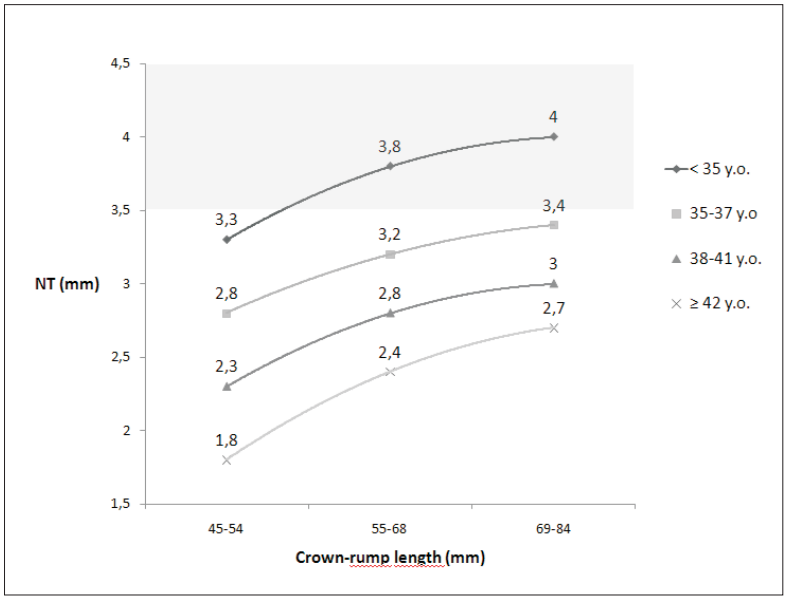


With our analysis of the findings in 77 443 women in the first trimester, we confirmed that with a NT value greater than 4.0 mm there is no added value in delaying offering immediate invasive diagnostic testing. On the basis of categorized gestational and maternal ages, our study also demonstrated that better and lower NT cut-offs values can be used by clinicians at the time of NT sonography to disclose positive screening to their patients immediately.

The FASTER Research Consortium has recommended that, in the presence of a NT value ≥ 3.0 mm, immediate invasive testing should be offered to all patients without obtaining serum markers.[4] With this strategy, 31.1% of the women in our study with a NT value ≥ 3.0 mm would have been needlessly offered invasive testing because their final result in combined screening was actually negative. As demonstrated in Figure 2, we believe that upper cut-off NT limits for screen positivity should be adapted instead to maternal and gestational ages at the time of screening. In women older than 37 years of age, this upper cut-off limit of 3.0 mm can be further decreased, while in younger women it most often needs to be raised. With this new strategy, all women with NT values greater than the suggested upper cut-off levels would screen positive with or without biochemical markers. To decrease further the risk of missing chromosomal abnormalities, specifically in women ≤ 35 years old at 12 or 13 weeks of pregnancy, a combination of our strategy with that of the Fetal Medicine Foundation could also be used (i.e., to offer fetal karyotyping by CVS in this specific group when the NT value is above 3.5 mm).[25]

Prenatal screening is based on respect for a patient's autonomy. It could therefore be argued that communicating a result as being "at risk" or "not at risk" is inappropriate and that communicating a result as a probability is more suitable. In order to fully respect an informed consent process, we believe that, in the presence of a NT value above the suggested thresholds, all of the following options are ethically acceptable and should be thoroughly discussed with the patient: (1) no further screening or diagnostic testing; (2) immediate disclosure of a probability, using NT with maternal and gestational ages; (3) completion of the screening process with biochemical markers; (4) proceeding directly with diagnostic testing such as a transabdominal CVS or an amniocentesis after 15 weeks. Additional assessment of fetal nasal bone, frontomaxillary facial angle and other early soft markers of aneuploidy could also be used eventually to refine the probability of aneuploidy at the time of first trimester ultrasound.[26–28]

Figure 2. Proposed NT threshold levels above which prenatal diagnosis should be offered, regardless of biochemical results (polynomial curves), compared to FMF strategy in grey area[25]



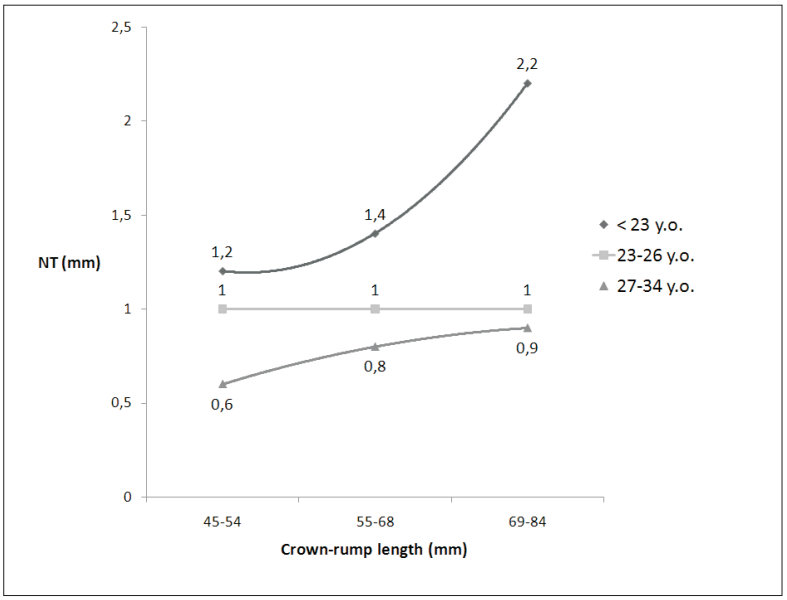
The NT threshold level of 3.0 mm for the (35–37 y.o.) x (55–68 mm CRL) combination given in Table 3 was set to 3.2 mm in Figure 2 to better agree with other functions which seem logarithmic or quadratic. This modification introduces a more conservative threshold value.

NT threshold values below which no further screening is required, because of negative results regardless of biochemical markers, are presented in Figure 3. In this case, however, prediction by NT of a negative screen result pertains to a very limited proportion of women and to very thin NT. We therefore recommend that if such cut-off levels are ever used, they should be restricted mainly to women younger than 27 years of age. With this strategy, one case of triploidy in 74 643 negative screenings would have been missed. However, a high proportion of fetuses with triploidy have major structural abnormalities often detectable by second trimester ultrasound, and almost all of these affected fetuses will die in utero or within the first year of life.[29–31]

Official reports of first trimester risk assessment, combining biochemical and ultrasound markers, are usually produced by routine diagnostic laboratories within 7 to 10 days after the medical visit. In an attempt to accelerate early disclosure, another concept of point of care service has been suggested, by which combined first trimester results can be provided to the patient within one hour.[32] With the advent of rapid immunoassays, this service provided by one-stop clinics for risk assessment involves serum measurements of free β-hCG and PAPP-A within 30 minutes of obtaining the blood sample with concomitant ultrasound assessment of CRL and NT, allowing rapid production of a combined risk report.[33] However, to be successful and financially viable, the one-stop clinics for risk assessment usually require a large volume of patients and are therefore limited to a very few busy ultrasound clinics. This approach does not preclude use of NT alone in selected cases, as proposed by our results. Taking blood at 8 to 9 weeks of gestation, prior to the NT scan, has also been suggested, but this remains a limited option, particularly in Canada where early access to a first obstetrical consultation has become a matter of concern.[34,35]

Until recently, the recommended maternal age for directly offering amniocentesis or CVS varied from 35 years of age to 38 years of age and over in most developed countries.[36,37] In the context of enhanced prenatal non-invasive screening, this approach is now considered by many to be obsolete. The American College of Obstetricians and Gynecologists recommended in its recent Practice Bulletin that all women regardless of age should have the option of invasive testing. In Canada, the low risk of fetal loss following mid-trimester amniocentesis recently reported by a secondary analysis of

Figure 3. Proposed NT threshold levels below which no further screening would be required (polynomial curves)



data from the FASTER trial (1/1600) was questioned and quoted as misleading, with an estimate of procedural fetal loss of 0.6% to 1.0% (1/175–1/100) seen as more realistic.[38,39] Regardless, in Canada it has now been recommended to raise the maternal age for offering CVS or amniocentesis directly from 35 years of age to 40 years of age or over at the time of delivery.[2,39] However, in our cohort, women aged 43 years had a probability of at least 69% of having a negative prenatal screening result. In younger women, this proportion of negative screening results increased rapidly. Given that the vast majority of younger women in our study had a negative combined first trimester prenatal screening, and if our results can be confirmed by studies with pregnancy outcomes, it will be reasonable to consider raising the maternal age for directly offering invasive testing to 44 years of age or over (Figure 1).

Until routine non-invasive prenatal diagnosis (using fetal cells or plasma free nucleic acids in maternal blood circulation[40–42]) becomes a reality, first trimester prenatal screening will continue to gain in popularity in centres that are qualified to provide it.

CONCLUSION

In this study we have defined NT threshold levels above and below which women will always have high- or low-risk results for Down syndrome screening regardless of biochemical findings in maternal blood. Prenatal screening for aneuploidy can be offered as a first step to all pregnant women up to the age of 43, rather than CVS or amniocentesis. Newer approaches to facilitate rapid results to the patient in the first trimester of pregnancy need to be explored. Specific cut-off NT values can sometimes be used alone in a selected population without increasing false positive rates.

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Maternal plasma levels of follistatin-related gene protein in the first trimester of pregnancies with Down syndrome†

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Objective To determine maternal plasma levels of follistatin-related gene protein (FLRG) in the first trimester of pregnancy and assess its potential role as a marker for prenatal screening of Down syndrome.

Methods Maternal plasma levels of FLRG were determined in 100 pregnant women with normal fetuses in their first trimester of pregnancy (i.e. 11th to 15th weeks). These results were compared with 20 cases with Down syndrome fetuses, taking into consideration clinical and demographic variables, such as maternal age, maternal weight, gestational age, smoking status and ethnicity.

Results Maternal plasma median of FLRG in the normal population was 1.41 ng/mL with 95% confidence interval (CI) of 1.37–1.70 and interquartile range (IQR) of 0.88, during the 11th to 15th weeks of pregnancy. Maternal age and weight were the only variables significantly related to FLRG levels ($p = 0.030$ and 0.020, respectively). Only maternal and gestational ages were related to Down syndrome ($p = 0.039$ and 0.006, respectively). Maternal plasma levels of FLRG were not significantly different in the presence of Down syndrome fetuses compared to normal population ($p = 0.63$).

Conclusion FLRG can be successfully detected in maternal plasma in the first trimester of pregnancy. However, its levels are not significantly altered in the presence of Down syndrome fetuses. Copyright © 2010 John Wiley & Sons, Ltd.

KEY WORDS: follistatin-related proteins; pregnancy; first trimester; Down syndrome; aneuploidy

INTRODUCTION

In the first trimester of pregnancy, maternal blood levels of pregnancy-associated plasma protein-A (PAPP-A) and free-beta-human chorio-gonadotropic hormone (fβhCG) are used routinely in combination with nuchal translucency (NT) to detect an estimated 88% of Down syndrome (DS), at a fixed false-positive rate of 5% (Spencer, 2007). Ultrasound markers, such as fetal nasal bone and facial angle, have been proposed to improve detection and reduce false-positive rates, but both required extensive experience in scanning and can therefore be hardly offered on a large scale (Sonek *et al*., 2006; Borenstein *et al*., 2008; Staboulidou *et al*., 2009).

Additional maternal blood biochemical markers, which are easier to standardize and control, would be welcomed to improve early detection of aneuploidies. In this constant search for new biochemical markers, follistatin-related gene protein (FLRG), also known as FSTL3 or FSRP, could present some interest. FLRG is produced in abundance by the placenta. Its most intense stain is found in the wall of decidual and placental blood vessels and, as a secreted glycoprotein, should be measurable in maternal blood very early into pregnancy (Ciarmela *et al*., 2003). Furthermore, FLRG interacts directly with a known marker of aneuploidies, ADAM12-S, through its cystein-rich domain (Laigaard *et al*., 2003; Bartholin *et al*., 2005; Spencer *et al*., 2008). Also, FLGR binds Activin A with high affinity modultating its effect on target tissues, although the value of activin A as a marker of aneuploidy is quite weak (Spencer *et al*., 2001a,b).

The main objectives of this study are to determine maternal plasma concentrations of FLRG in the late first and early second trimester of normal human pregnancy (i.e. from 11th to 15th weeks) and assess its potential role as a marker for Down syndrome.

METHODS

This study was based on a sample of 120 women, including 100 women with normal fetuses (control group) and 20 women with Down syndrome fetuses. The women

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were from a cohort who had been identified at increased risk of Down syndrome based on first trimester screening using fβ-hCG, PAPP-A and ultrasound NT and who had elected to have chorionic villus sampling (CVS) to confirm the diagnosis. Plasma samples were collected from these women prior to CVS at the Prenatal Screening Research Department of King George Hospital (UK). Control group consisted of pregnant women who had screened positive but had fetuses with normal karyotype. All frozen plasma samples were randomly selected and came from time period November 2006 to February 2008. They were shipped to Montreal on dry ice where they were kept at $-80\,^{\circ}$C until analysis. To use samples for research, all subjects had to sign beforehand a consent form duly approved by an Institutional Ethics Review Board. Demographic data were gathered including women's age, pre-pregnancy weight (maternal weight), ethnic origin, smoking status and pregnancy outcome. Gestational ages were determined by fetal crown-rump length (CRL).

To measure FLRG, an enzyme-linked immunosorbent assay (ELISA) was set up and performed as follows. One plasma sample from each patient was analysed in duplicates. Samples were used undiluted. Standard curve was done in duplicate (in bovine serum albumin (BSA) 1% in phosphate buffered saline (PBS)) on each plate. One internal positive control (high FLRG) and one internal control (low FLRG) were included in each plate. On a total of four 96-well microplates, all samples were processed on the same day. Microplates (Corning 9018) were coated overnight at 4 °C with 100 µL/well of rat monoclonal antibodies (Mab) anti-mouse FLRG (MAB1255; R&D Systems, Minneapolis, MN, USA) at a concentration of 4 µg/mL in PBS. Microplates were then washed three times in PBS. Following a 30-min blocking step with 200 µL/well of 1% BSA (Sigma-Aldrich, Oakville, ON, Canada) in PBS, microplates were incubated for 2 h at room temperature with 100 µL/well of serial dilutions of recombinant hFLRG (1288-F3/CF; R&D Systems, Minneapolis, MN, USA) diluted in BSA 1% in PBS (standard curve) or 100 µL/well of undiluted patient plasma in duplicate. Microplates were washed four times in PBS–Tween 20 0.05% and then incubated with 100 µL/well of biotinylated goat polyclonal antibodies raised against the human FLRG (BAF1288; R&D Systems, Minneapolis, MN, USA) at 0.4 µg/mL in BSA 1%, for 1 h at room temperature. After washes, microplates were incubated with 100 µL/well of alkaline phosphatase-conjugated streptavidin (Rockland Immunochemicals, Gilbertsville, PA, USA) diluted 1/6000 in BSA 1%, for 1 h at room temperature. Following washes, 100 µL/well of substrate (pNPP; Sigma-Aldrich, Oakville, ON, Canada), 100 µL/well) was added and then incubated at room temperature. Optical density (O.D.) was read at 405 nm after 60 and 90 min. Intra and inter-assay coefficients of variation were estimated at 1.8% and 2.8%, respectively.

Statistical analyses and power of testing

Comparisons of demographic variables and gestational ages between women with normal fetuses and women with Down syndrome fetuses were based on the two-sample Student's t-test for quantitative characteristics and on the Pearson's χ^2 for qualitative (or categorical) characteristics. To study to what extent demographic and clinical data were predictors of FLRG (ng/mL), a multiple linear regression was used. To study to what extent FLRG (ng/mL) was a predictor of the pregnancy outcome (unaffected vs Down syndrome) when controlling for demographic and clinical data, a multiple logistic regression was used.

The normality of the FRLG (ng/mL) distribution was verified using the Kolmogorov–Smirnov's test and the hypothesis of normality was not rejected ($p = 0.15$). Therefore, the concept of Multiple of the Median (MoM) was found not to be useful in the present article. The homoscedasticity of the variance was also verified with the Levene's test before using the two-sample t-test and the homoscedasticity of the variance was confirmed ($p = 0.78$). Goodness of fit was studied for the multiple linear regression and there was no problem of multicollinearity (maximum Variance Inflation Factor = 1.36) and no cases were influential (maximum Cook's distance = 0.16, $p = 0.99$). Goodness of fit was also studied for the simple and multiple logistic regressions with the Hosmer–Lemeshow's test and the fits were good (simple regression: $p = 0.26$; multiple regression: $p = 0.49$).

Based on sample sizes of 100 and 20 women, respectively, and a 0.05 two-sided significance level, (A) a two-sample t-test will have 80% power to detect a standardized effect size of 0.7; (B) a linear regression will have 80% power to detect a correlation of 0.25; (C) and a logistic regression will have 70% power to detect an odds ratio of 2.00.

RESULTS

Maternal plasma concentrations of FLRG were determined by ELISA in 120 pregnant women, including 100 women with normal fetuses and 20 with Down syndrome. Demographic and clinical characteristics between the two groups are described in Table 1. Data for smoking status and ethnicity were available in only 91% and 74% of patients, respectively. Maternal age was older ($p = 0.025$) and gestational age was younger ($p < 0.005$) in the Down syndrome group.

In the normal pregnancies, maternal plasma levels of FLRG varied from 0.16 to 5.34 ng/mL, during the 11th to 15th weeks (95% CI: 1.37–1.70 ng/mL) with an interquartile range (IQR) of 0.88. In the Down syndrome group, FLRG levels varied from 0.15 to 2.39 ng/mL (95% CI: 1.15–1.74 ng/mL) with an IQR of 1.19. Mean maternal plasma level of FLRG for the normal population was 1.54 ± 0.83 ng/mL (mean $\pm$ SD) compared with 1.45 ± 0.63 ng/mL for Down syndrome ($p = 0.64$) (Table 1). No relationship ($p = 0.58$) was found between FLRG levels and gestational age across the range of samples (80–105 days) (Figure 1).

Based on a multivariate analysis on the control group ($n = 100$), there were five potential predictors of FLRG (ng/mL) levels as shown in Table 2. However,

Table 1—Demographic and clinical characteristics of studied populations

Demographic and clinical data	Normal ($n = 100$)		Down syndrome ($n = 20$)		p Value
Quantitative variables	$\overline{X} \pm$ SD	(Min–Max)	$\overline{X} \pm$ SD	(Min–Max)	
Women's age (years)	34.4 ± 6.3	(16.0–45.0)	37.9 ± 4.5	(27.0–44.0)	0.025
Gestational age (weeks)	13.1 ± 0.72	(11.4–15.0)	12.5 ± 0.71	(11.0–13.7)	0.002
Women's weight (kg)	67.6 ± 11.9	(46.8–102.0)	64.2 ± 13.3	(46.8–95.3)	0.280
FLRG plasma concentrations					
Nanogram/mL ($\overline{X} \pm$ SD)	1.54 ± 0.83		1.45 ± 0.63		0.637
Qualitative variables	n (%)		n (%)		
Smoking					0.164
No	81 (89.0)		16 (100)		
Yes	10 (11.0)		0 (0)		
Ethnicity					0.522
Caucasian	63 (90.0)		18 (94.7)		
Other	7 (10.0)		1 (5.3)		

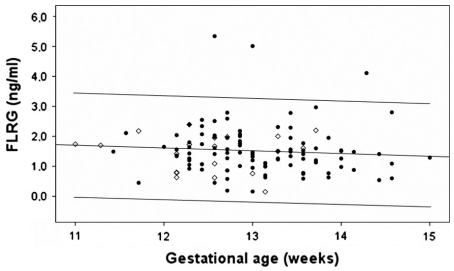

Figure 1—Variation of maternal plasma FLRG (ng/mL) with gestational age (weeks) in the control (●) and Down syndrome (◊) populations. Lines represent linear fit and 95% CI of the control population

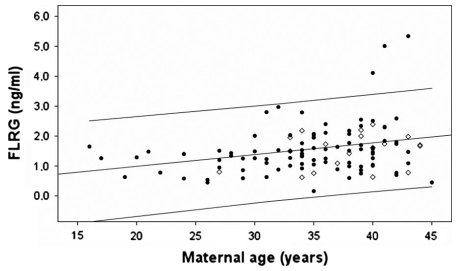

Figure 2—Variation of maternal plasma FLRG (ng/mL) with maternal age (years) in the control (●) and Down syndrome (◊) populations. Lines represent linear fit and 95% CI of the control population

Table 2—Linear regression model predicting FLRG (ng/mL)

Model	Unstandardized coefficients		t	Significance
	B	SE		
Constant	2.854	1.828	1.562	0.123
Maternal age	0.037	0.017	2.209	0.030
Maternal weight (kg)	−0.017	0.007	−2.373	0.020
Gestational age	−0.113	0.112	−1.011	0.315
Ethnicity	−0.193	0.360	−0.535	0.594
Smoking	−0.071	0.345	−0.207	0.837

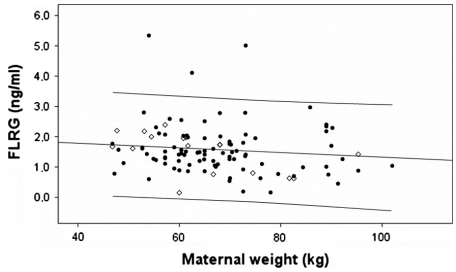

Figure 3—Variation of maternal plasma FLRG (ng/mL) with maternal weight in the control (●) and Down syndrome (◊) populations. Lines represent linear fit and 95% CI of the control population

only maternal age ($p = 0.030$) and maternal weight ($p = 0.020$) were significantly associated with FLRG levels (Figures 2 and 3). Maternal age was positively associated with FLRG levels ($b = 0.037$) but maternal weight was negatively associated with FLRG levels ($b = -0.017$).

When looking at the FLRG alone as a potential predictor of the risk of Down syndrome, its association with Down syndrome is not statistically significant ($p = 0.63$, Table 3). Even when controlling potential confounders its association remains statistically non-significant ($p = 0.27$). The only statistically significant predictors of the probability of Down syndrome were maternal age (OR = 1.16, $p = 0.039$) and gestational age [odds ratio (OR) = 0.25, $p = 0.006$, Table 3].

DISCUSSION

Until this study, FLRG was poorly studied in human maternal blood. No data have yet been published in

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Table 3—Logistic regression models predicting diagnosis (normal vs Down syndrome)

								95% CI for OR	
		B	SE	Wald	df	Significance	Odd ratio	Lower	Upper
FLRG as only predictor									
Step 1	FLRG	−0.158	0.331	0.226	1	0.634	0.854	0.446	1.635
	Constant	−1.375	0.542	6.443	1	0.011	0.253		
Controlling for demographic and clinical variables									
Step 1	FLRG	−0.549	0.494	1.233	1	0.267	0.578	0.219	1.522
	Maternal age	0.146	0.071	4.275	1	0.039	1.157	1.008	1.329
	Maternal weight	−0.031	0.027	1.284	1	0.257	0.969	0.919	1.023
	Gestational age	−1.396	0.507	7.579	1	0.006	0.248	0.092	0.669
	Constant	13.709	7.016	3.818	1	0.051			

Dependant variable, diagnosis.

regards to its concentrations in the first trimester of human pregnancy and its potential as a marker for aneuploidies. This study confirms that FLRG can be successfully measured in maternal plasma, from 11th to 15th weeks of pregnancy. However, it did not demonstrate its role as a marker for Down syndrome screening.

Unfortunately, we did not have access to PAPP-A, fβhCG and NT results in the database provided to us. The question whether or not there is a relationship between these analytes remains to be clarified. As found with other biochemical markers, FLRG levels are significantly influenced by maternal weight (Spencer *et al*., 2003). Why they significantly increase with maternal age remains, however, an enigma to be clarified and confirmed in a larger series of patients.

As for maternal and gestational ages that both significantly predict Down syndrome, this was not a surprise. Meiotic nondisjunction of chromosome 21 is well known to be associated with advanced maternal age (Hassold and Chiu, 1985; Ghosh *et al*., 2009). A high proportion of affected pregnancies with Down syndrome also abort spontaneously if they are allowed to continue. Thus, the earlier prenatal screening is carried out, the higher the proportion of Down syndrome (Morris *et al*., 1999).

FLGR was first identified as playing a role in leukemogenesis with wide-range effects on cell differentiation, proliferation and organization, suggesting a participation in cell transformation and growth regulation (Hayette *et al*., 1998). Although FLRG shares significant structural and functional homology with follistatin (FS), further characterization confirmed some major differences between the two proteins, suggesting that FLRG is differentially regulated both spatially and temporally and performs distinct functions (Schneyer *et al*., 2004). FLRG mRNA expression is exceptionally high in the placenta (Tortoriello *et al*., 2001). In rats, expression of FLRG in the placenta continuously increased during the second half of pregnancy (Arai *et al*., 2003). In humans, FLRG mRNA is also expressed by various gestational tissues both at early gestation and at term pregnancy, and immunoreactive protein was found in the trophoblast cells, epithelial amniotic and chorionic cells and maternal deciduas (Ciarmela *et al*., 2003).

Using a home-brewed ELISA, one study has demonstrated that, between 28 and 37 weeks, maternal serum concentrations of FLRG are significantly elevated in preeclampsia, in a similar manner to activin A and inhibin A (Pryor-Koishi *et al*., 2007). FLRG has also been shown to be highly up-regulated in the placenta of pathological pregnancy conditions such as intrauterine fetal growth restriction, suggesting that it is a critical regulator of fetal growth and differentiation (Okamoto *et al*., 2006). It remains to be clarified if maternal blood FLRG could be used in the first trimester to predict the development of preeclampsia and/or intrauterine foetal growth restriction.

CONCLUSION

FLRG can be detected in maternal blood in the first trimester of pregnancy. However, our data suggest that it is not a useful marker for the prenatal screening of Down syndrome from 11th to 15th weeks of pregnancy.

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Type or print name and title JEAN LAMBERT, Ph.D., PROFESSOR U OF MONTREAL

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A) Déclaration des coauteurs d'un article

1. Identification de l'étudiant et du programme

Pierre Miron

Programme de sciences biomédicales, Option générale

2. Description de l'article

MIRON, P., COTE, Y. P. & LAMBERT, J. 2008. Effect of maternal smoking on prenatal screening for Down syndrome and trisomy 18 in the first trimester of pregnancy. *Prenat Diagn,* 28**,** 180-5.

3. Déclaration de tous les coauteurs autres que l'étudiant

À titre de coauteur de l'article identifié ci-dessus, je suis d'accord pour que **Pierre Miron** inclue cet article dans sa thèse de doctorat à l'Université de Montréal qui a pour titre Dépistage prénatal de la trisomie 21 et autres aneuploïdies.

Coauteur Yvan Coté, PhD		Date JUIN 2008
Coauteur Jean Lambert, PhD	Signature	Date

Journal of Obstetrics and Gynaecology Canada

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Title of article: Nuchal translucency thresholds in prenatal ...

A signature below certifies compliance with the following two statements. When there is more than one author, each must sign this agreement.

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Author(s):

Yvan R. Côté — CPMAC1

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   I have substantially contributed to the conception, composition, and revision of this article, and approved the final version to be published.

   I acknowledge that I have met the terms outlined above.

Author(s): JEAN LAMBERT — APRIL 28 2008

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A) Déclaration des coauteurs d'un article

1. Identification de l'étudiant et du programme

Pierre Miron

Programme de sciences biomédicales, Option générale

2. Description de l'article

MIRON, P., COTE, Y. P. & LAMBERT, J. 2009. Nuchal translucency thresholds in prenatal screening for Down syndrome and trisomy 18. *J Obstet Gynaecol Can,* 31**,** 227-35.

3. Déclaration de tous les coauteurs autres que l'étudiant

À titre de coauteur de l'article identifié ci-dessus, je suis d'accord pour que **Pierre Miron** inclue cet article dans sa thèse de doctorat à l'Université de Montréal qui a pour titre Dépistage prénatal de la trisomie 21 et autres aneuploïdies.

		2010 AL 28
Coauteur Yvan Coté, PhD	Signature	Date
Coauteur Jean Lambert, PhD	Signature	Date

ACQUIESCEMENT OF CO-AUTHORS

A) Declaration of co-authors for an article

1. Identification of the student and the programme

Pierre Miron

Programme of Biomedical sciences, General Option (for a PhD grade)

2. Description of article

MIRON, P., LAMBERT, J., MARCIL, A., COWANS, N. J., STAMATOPOULOU, A. & SPENCER, K. 2010b. Maternal plasma levels of follistatin-related gene protein in the first trimester of pregnancies with Down syndrome. *Prenat Diagn,* 30, 224-8.

3. Declaration all co-authors other than the student

As co-authors of the article cited above, we agree that **Pierre Miron** includes it in his PhD thesis at University of Montréal entitled Prenatal screening of Down syndrome and other aneuploïdies.

Co-author
Kevin Spencer — Signature — 28/4/2010 Date

Coauteur
Nicholas J. Cowans — Signature — 28/04/2010. Date

Co-author
Anastasia Stamatopoulou — Signature — 29/4/2010 Date

Maternal plasma levels of follistatin-related gene protein in the first trimester of pregnancies with Down syndrome. *Prenat Diagn,* 30, 224-8

Co-author	Signature	Date
Jean Lambert		
Anne Marcil		27 avril 2010

Co-author	Signature	Date
Anne Marcil		

Maternal plasma levels of follistatin-related gene protein in the first trimester of pregnancies with Down syndrome. *Prenat Diagn*, 30, 224-8

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Titre : Nuchal translucency thresholds in prenatal screening for Down syndrome and trisomy 18

N° de la revue, page initiale et finale et date de publication :
31, 227-35 (2009)

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2. Identification of editor

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3. Identification of two articles

MIRON, P., COTE, Y. P. & LAMBERT, J. 2008. Effect of maternal smoking on prenatal screening for Down syndrome and trisomy 18 in the first trimester of pregnancy. *Prenat Diagn*, 28, 180-5.

MIRON, P., LAMBERT, J., MARCIL, A., COWANS, N. J., STAMATOPOULOU, A. & SPENCER, K. 2010. Maternal plasma levels of follistatin-related gene protein in the first trimester of pregnancies with Down syndrome. *Prenat Diagn*, 30, 224-8.

The student, Pierre Miron, is authorised to include both articles cited above in his PhD thesis (University of Montreal) entitled First trimester prenatal screening of Down syndrome and other aneuploïdies.

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Annexe II. Curriculum vitae

CURRICULUM VITAE

(en anglais)

Pierre Miron, M.D., F.R.C.S.(c)

Avril 2010

Section I –

IDENTIFICATION AND PERSONAL INFORMATION

Miron, Pierre

Sex :	Male
Work address :	Centre de fertilité et de procréation FERTILYS 1000, montée de Pionniers, 3e étage Terrebonne, Quebec J6V 1S8 Phone number : (450) 657-1313 ext 2266
Place of birth:	Montreal, Quebec, Canada
Citizenship:	Canadian
Languages (spoken, read and written):	French and English
Speciality:	Obstetrics and gynecology (1985-)

Section II –

DIPLOMAS, TRAINING AND MAINTENANCE OF COMPETENCE

Studies and diplomas

1976	Collegial studies' diploma Option in Health Sciences Collège Marie-Victorin, Montreal, Quebec
1980	M.D., Medicine Université de Sherbrooke, Sherbrooke, Quebec.
1980	M.D., License College of physicians of Quebec
1981	Licentiate of the Medical Council of Canada (LMCC), License 51534
1985	Obstetrics and Gynecology, Certificate number 10346 College of physicians of Quebec
1985	Obstetrics and Gynecology, D.E.S. Université de Montréal, Montreal, Quebec
1985	Obstetrics and Gynecology, F.R.C.S.(c), Fellow Royal College of Physicians and Surgeons of Canada
1985	American Board in Obstetrics and Gynecology eligible (written examination)
1986	Fellowship in Reproductive Endocrinology and Infertility Medical Board of Victoria, Certificate number T845 Royal Women's Hospital, Carlton, University of Melbourne, Australia
2001	Diploma from the Fetal Medicine Foundation for First trimester screening

Complementary education

1996-98 Registration at Université de Montréal

Introduction to biostatistics (Université de Montréal, fall session, 1996)
Introduction to epidemiology (Université de Montréal, spring session, 1997)
Clinical Trials (Université McGill, fall session, 1997)
Categorical analyses (Université de Montréal, spring session, 1998)

2006- Registration at Université de Montréal as candidate for a **PhD in Biomedical Sciences**

Carrière de chercheur en santé, Course MMD6100R (Summer 2006, 2 full time weeks ; 3,0 credits, note A)

General exam of synthesis (May 2008), passed with success (PhD(c))

Maintenance of competence

1986 Course on Microsurgery. Continuing Education, American Fertility Society, Toronto, September 27-28, 1986. Credits : 15

1987 The Fifth World Congress in Vitro Fertilization and Embryo Transfer. Continuing Education, American Fertility and Sterility Society, Norfolk, Virginia, USA. Crédits : 26 hours. April 6-10, 1987.

1988 Novel Substances in Reproduction and Endocrinology. Continuing Education, American Fertility and Sterility Society, Atlanta, Georgia, USA. Credits : 15 hours. October 8-9, 1988.

1989 One week spell in IVF-ET: Dept. of Obstetrics and Gynecology, Centre hospitalier universitaire Bretonneau, with Professors Dominique Royère and Jacques Lansac, Tours, France. April 8-15, 1989.

1989 Active participation : Infertility and IVF. Bourn Hall Clinic (Cambridge) and Hallam Medical Centre (London), England. December 3-9, 1989.

1990 Canadian Fertility and Andrology Society and Serono Symposia USA, Estérel, Quebec, October 1990

1991 World Congress on in vitro fertilization and assisted procreation/ESHRE joined meeting, Paris, June 1991.

1992 Canadian Fertility and Andrology Society Annual Meeting, Kananaskis, Alberta, November 1992

1993 39th American Fertility Society and Canadian Fertility and Andrology Society Annual Meeting, Montreal, Quebec, October 1993.

1994 European Society of Human Reproduction and Embryology Annual Meeting, Brussells, Belgium, June 1994

1995 IX World Congress on IVF and Alternate Reproduction, Vienna, Austria, April 8-14, 1995.

1996 Vth World Congress on Endometriosis, Yokohama, Japan, October 21-24, 1996.

1996 Biotechnology Industry Organization Annual Meeting, Philadelphia, 1996

1997 10 th World Congress in Vitro Fertilization and Assisted Reproduction, Vancouver, Canada, May 24-28, 1997.

1997 Biotechnology Industry Organization Annual Meeting, Houston, Texas, June 8-12, 1997

1997 43rd Annual Meeting of the Canadian Fertility & Andrology Society in Niagara on the Lake, Ontario, September 24-27, 1997.

1998 Biotechnology Industry Organization Annual meeting, New York, USA, June 14-18, 1998

1998 Theoretical course: « The 10-14 Week Nuchal Translucency Scan ». Continuing

Eduction, Fetal Diagnosis and Treatment Centre, University of Toronto, Toronto, Octobre 24,1998.

1999 Joined American Society for Reproductive medicine and Canadian Fertility and Andrology Society Annual Meeting, Toronto, Canada, September 25-30, 1999.

1999 International Symposium « Advances in prenatal screening and diagnosis » organised by PROCREA, Novembre 20-21, 1999.

2000 56th Annual Meeting of the American Society for Reproductive Medicine, San Diego, California, October 21-26, 2000.

2000 Biotechnology Industry Organization Annual Meeting, Boston, NH, March 26-29, 2000.

2001 Theoretical course: "Obstetric Ultrasound: "Setting The Standard for 2001". Continuing Education, Faculty of Medicine, University of Toronto; Toronto, February 23-25, 2001. (20 hours)

2001 Theoretical course : "Biology Mammalian Oocyte", American Board of Bioanalysis, Miami, Florida, March 2-3, 2001. (16 hours)

2001 Intensive training : Trans-abdominal Chorionic villi sampling, Medical Clinic of Prof. Bruno Brambati, Milano (Italy), April 30 to May 6, 2001.

2001 Review course 22nd Annual Obstetrics and Gynecology Review Course Chicago, June 4-9, 2001

2001 Practical training :August-December 2001, weekly training (½ day/week) in obstetrical ultrasonography of 2nd and 3rd trimesters at Jewish General Hospital, McGill University (40 hours)

2002 Review Course : Update in gynecology (Congrès de mise à jour en gynécologie), Hôtel Palace Royal, Quebec City, February 1-3, 2002 (8 hours CME)

2001 Practical training: Endoscopic surgery (Laparoscopic hysterectomy): Hands on (Dr Pierre Choquette, Hôpital Cité de la Santé): October 24th and November 14th (16 hours)

2003 Symposium : Measuring Human Embryo Quality: Form, Function and Developmental Genetics, May 7-9, Montreal, Quebec, Canada

2002 Training stage : reintroduction to general obstetrics and gynaecology from May 3rd to August 16th, at Pierre-Boucher Hospital under the guidance and supervision of the College of Physicians of Quebec and Dr Bernard Couturier

2002 Course : SOGC Alarm Course (Advances in Labour and Risk Management, Toronto, December 8-9th (14,75 MOC credits)

2003 Symposium : Prenatal screening for Down Syndrome: Introducing the integrated test into medical practice, March 28-29, Brown University, Providence, RH, USA (11 AMA PRA category 1 credits)

2003 Pre-congress course: The developing fetus: first and second trimester (WFUMB/ AIUM), 10 hours CME

2003 International meeting:10th Congress of the World Federation for Ultrasound in Medicine and Biology, May 31-June 4 (Montreal), 23 hours CME

2003 Symposium : Update in ovulation induction, November 26 th 2003, Montreal (2.5 credits, certificate of maintenance)

2003 Symposium : Research in reproduction R^3, Trois-Rivières, November 20th- 21st (8 hours)

2004 International meeting : International Federation of Fertility & Sterility, Montreal, May 23-28, 2004. (24 credits) ;

2004 National meeting Annual meeting of the Association des gynécologues et obstétriciens du Québec, May 28-30, 2004

2004 National meeting : 2004 Canadian Symposium on Prenatal Screening, Toronto, June 4th 2004. Certification Program of the Royal College and Surgeons of Canada (5.5 credits)

2005 Symposium : Échographie de 11-14 semaines, March 12th . Certification of the Royal College of Physicians and Surgeons of Canada (6 credits)

2005 Annual meeting : Congrès annuel de gynécologie-périnatalité, May 11-13 (10 credits) ;

2005 Postgraduate course: ART 2005: Currents and controversies. American Society for Reproductive Medicine. Montreal, October 15-16, 2005. (14 ACOG credits)

2005 Annual meeting: Joint annual meeting ASRM & CFAS. October 15-19, 2005. (27 ACOG credits)

2005 CME : Nouvelle FMC du Québec en obstétrique « de la conception au postpartum » November 17-18, 2005 (13,25 credits MAINPRO-M1, CRMCC)

2006 Annual meeting: AOGQ Annual meeting, Lac Leamy, Gatineau (Qc) May 2006

2006 Symposium : Canadian Symposium on Prenatal Screening of the 11-13th Week Scan Theoretical Course, October 27-28, 2006 Pointe-Claire, Quebec (12 hours, 12 credits; Section 1 of the Framework of CPD options for the Maintenance of Certification Program of RCPSC)

2007 International meeting : 6th World Congress in Fetal Medicine, 17-21 June 2007, Cavtat, Croatia

2007 Internatioanl meeting: 14th World Congress on In Vitro Fertilization & 3rd World Congress on In Vitro Maturation, September 15-9, 2007, Montreal, Quebec

2008 International meeting : ISPD 14th International Conference on Prenatal Diagnosis and Therapy, 1-4 June 2008, Vancouver, Canada (25.1 credits, Canadian Association of Genetic Counsellors)

2008 International meeting : Royal College of Obstetricians & Gynaecologists, 7th International Scientific Meeting, 17-20 September 2008, Montreal, Canada

2009 Symposium : Obstetric Ultrasound: Setting the standard for 2009. 13-15 February 2009, Toronto, Canada (18 credits, Mainpro), section 1)

2009 Symposium : Journée de développement professionnel continu (AOGQ and AAQ), November 7, 2009, Boucherville, Canada (6.75 credits, category 1, Mainpro-M1,section 1)

2010 International meeting : Ultrasound 2010. March 18-20, 2010. Harvard Medical School, Boston, USA

2010 International meeting: 9th World Congress in Fetal Medicine. June 20-24, 2010. Fetal Medicine Foundation. Rhodes, Greece.

2010 National meeting: 2010 Fetal Medicine Update, October 22-23, 2010. Mount Sinai Hospital, University of Toronto, Toronto, Ontario, Canada.

Section III –

ACADEMIC CARRIER

1985 - 2010	**Hôpital Maisonneuve-Rosemont, Université de Montréal**
1985 - 2010	Active member
1986 – 1988	Clinical Instructor, Obstetrics and gynecology
1986 – 1990	Director and founder of hospital's IVF programme
1989 - present	Assistant professor
1988 - present	Director, Division of Reproductive endocrinology and Infertility
1996 - 1998	**Centre Hospitalier de l'Université de Montréal, campus Notre-Dame,** Associate member
1988 - présent	**Faculty of medicine, Université de Montréal**
1988 - present	Assistant professor
1988 – 1989	In charge of woman's reproduction course for undergraduate medical student
1996	**Faculty of medicine, McGill University** Adjunct professor, Department of Obstetrics and Gynecology
1990 - 2001	**PROCREA Clinical Services inc.** Official affiliation with Hôpital Maisonneuve-Rosemont for teaching and training of medical students and residents in Obstetrics and Gynecology
2001	**PROCREA Clinical Services inc.** Official affiliation with the Faculty of medicine of Université de Montréal for training of residents in Endocrinolgy (Department in medicine)

ACADEMIC & PROFESSIONNAL HONORS

1986	Scholarship recipient of Samuel McLaughlin's grant for a fellowship in RE&I Royal Women's Hospital, University of Melbourne, Melbourne, Australia
1988	Member of the Working Committee on Ovum Donation Donation, CFAS Working Committee.
1990-1992	Founding member of the Canadian Voluntary Regulatory Association for assisted reproductive technologies
1995-1998	President, Committee of Reproductive endocrinology and Infertility Association of Obstetricians and Gynaecologists of Québec
1994-1999	Member of the Committee on Reproductive endocrinology and infertility Society of Obstetricians and Gynaecologists of Canada (SOGC)
1994	Member of Canadian Advisory Board for Reproductive Advancement (Endometriosis consensus) Society of Obstetricians and Gynaecologists of Canada (SOGC)
1994-1998	Member of the Editorial Advisory Board of the Journal Society of Obstetricians and Gynaecologists of Canada (SOGC)
1994-1999	Member of the Joined Committee on Ethics Society of Obstetricians and Gynaecologists of Canada (SOGC) and CFAS
1996	Member of the Assemblée des chercheurs du Centre de recherche Louis-Charles Simard, Hôpital Notre-Dame (CHUM)
1996-1998	Member of the Board of Directors Association of Obstetricians and Gynecologists of Quebec
2000-2001	Spokesperson for Assisted reproductive technologies and Prenatal screening Society of Obstetricians and Gynaecologists of Canada (SOGC)
2001-	Member of the Peer Reviewers for reseach proposals of the Canadian Foundation for Women's Health
2001- 2006	Nucleus Member, Specialty Commitee in Gynecological Reproductive Endocrinology and Infertility of The Royal College of Physicians and Surgeons of Canada
2001-2003	Member, Canadian Expert Advisory Board, Serono Canada Pre-ESRHE meeting, June 29th - July 1st, 2001

PREVIOUS AND/OR PRESENT MEDICAL AFFILIATIONS

Association of Obstetricians and Gynaecologists of Quebec (AOGQ)

Canadian Fertility & Andrology Society

Demeter, Québec Association (of infertiles couples) promoting Fertility

American Society for Reproductive Medicine

Society of Obstetricians & Gynaecologist of Canada (SOGC)

European Society for Human Reproduction and Embryology (ESHRE)

International Society of Untrasound in Obstetrics & Gynecology

Association des couples infertiles du Québec

International Society for Prenatal Diagnosis

Section IV –

ACHIEVEMENTS

In medicine

Establishment of :

- three IVF programmes	1986, 1990 and 1996
- a oocyte donation programme	1988
- a frozen embryo programme	1990
- a ICSI and PESA/TESE programme	1995
- a classical and molecular cytogenetic laboratory first specialized private laboratory in such field in Canada	1995
- a fully integrated prenatal screening and diagnosis programme first private programme in the province of Quebec and in Canada combining nuchal transluscency, biochemical markers and genetic counselling	1998
- a blastocyst-stage embryo transfer programme	1999
- a preimplantation genetic diagnosis programme	2001
- a oocyte's in vitro maturation programme	2001
- a public prenatal screening programme, Maisonneuve-Rosemont Hospital (a teaching hospital affiliated with University of Montreal)	2002

Supervision and coaching of : 1985-

- Several medical students, residents, fellows and scientists in clinical research including directions and co-directions of Master and Ph.D students.

Founder of an infertile couple support group:

- Demeter, Quebec Association for fertility (an infertile couple support group in Quebec since 1988**)**

Lobbying of Ministers (government of Quebec):

Successful direct lobbying with Ministers of Health and Finance of the province of Quebec that resulted in 2000 in a new and original fiscal measure, a refundable tax credit for infertile couples for expenses relating to artificial insemination or *in vitro* fertilization (a first in North America). Such expenses include, in particular, amounts paid to a physician or a licensed private hospital and amounts paid for medication prescribed by a physician and recorded by a pharmacist. In 2001's provincial budget, in order to improve the tax assistance thus granted, the rate of the tax credit for the treatment of infertility was raised from 25% to 30%. Moreover, the amount of expenses giving entitlement to tax credit was increased by $5 000, thereby raising the ceiling on eligible expenses to $20 000.

In Business Administration

Founder of:

Montreal Institute for reproductive medicine inc. in 1990 (known today as PROCREA Clinical Services);

Bio-IMRM R&D inc. in 1994 (known today as PROCREA BioSciences inc.) ;

PROCREA BioSciences inc. in 1995, a biotechnology company focused mainly in the field of functionnal genomics;

PROCREA Clinicals services inc., based in Montreal and involves in the field of human reproductive biology including assisted reproductive technologies and reproductive genetics;

PROCREA Diagnostic Laboratories inc., mainly involves in diagnostic testings such as classical and molecular cytogenetics and molecular biology;

PROCREA Quebec inc. in 1998, based in Quebec city and involves in the field of human reproductive biology including assisted reproductive technologies and reproductive genetics;

PROCREA Cryopreservation centre inc. since 1990, a sperm and embryo bank;

President and CEO of PROCREA BioSciences (until december 2000) and President and CEO of PROCREA Clinical Services inc. and PROCREA Diagnostic Laboratoires inc. until may 2001, which involved:

The setting up, the supervision and the management of more than 100 employees and consultants, including executives (business development, finances, human ressources, marketing and communication), approximately 20 physicians (obstetricians /gynecologists, urologists/andrologists, geneticists, radiologists /sonographists, etc…) and 12 PhDs;

Raising from private investors 550 000$ in 1990 for the creation of IMRM inc.;

Raising from private investors and venture cap institutions 4.0 M$ in 1994 for the creation of Bio-IMRM inc.;

Creation of a Québec Business Investment Company (QBIC) “SPEQ PROCREA inc.”, raising in 1999 4.2 M$ from approximately 350 private investors;

Raising 2 M$ in 2000 from a Quebec’s venture cap institutions (PROCREA BioSciences inc.) ;

Both PROCREA Clinical Services inc (including PROCREA Quebec inc.) and PROCREA Diagnostic Laboratories were highly profitable businesses as of May 2001;

Member, Board of directors 2000-2001
Fonds régional de solidarité de l'île de Montréal

A development capital fund that calls on the solidarity and savings of Quebecers to help create and maintain jobs in Québec by investing in small and medium-sized businesses.

Principal investigator and consultant, Genizon Biosciences 2003-2009

Subject of the research projects:

Identification of genes in endometriosis using single nucleotide polymorphism (2003-);
Identification of genes in polycystic ovary syndrome (to start in april 2004);

Galileo Genomics is a genetics research company dedicated to the discovery of genes unequivocally implicated in the cause of common and complex human diseases. Galileo's research relies on the study of genetic data collected from the Quebec Founder Population, a population of 6 million distantly related individuals descended from a small group of roughly 2,600 common effective ancestors that arrived from France between 1608 and 1760. Founder Populations contain a relatively homogeneous gene pool, a distinct advantage that is being pursued by Galileo to link genes with the diseases they cause. Galileo is building a Biobank of DNA and other tissue samples from the Quebec population to discover true disease genes.

Medical Director, Adaltis 2004-2005

Clinical supervison of Adaltis prenatal screening program;

Active implication in Adaltis Research & Development Unit, based in Centre québécois d'innovation en biotechnolgie (CQIB), Cité de la biotech, Laval ;

Consultant, Warnex 2003-2009

Clinical supervison of a prenatal screening program;

Fouder, President and CEO of Prenagen inc

A bioteh company in the field of prenatal screening and diagnosis 2003-

Founder of Fertilys

Centre de fertilité et de reproduction www.fertilys.org 2007

Section V –

PUBLICATIONS (PEER REVIEWED)

Miron P, Lemieux MC, Granger L et al. Association of ureaplasma urealyticum and sperm antibodies in cervical mucus. **Fertil Steril** 42:169, 1984.

Taylor P, **Miron P**, Livingston R, Jarrell J. In vitro fertilization: concepts and controversies. Symposium. **Contemporary Ob/Gyn** 5:30, 1988.

Miron P, Hay DL, Johnston I, McKenna M, McBain JD. Midcycle changes of 17-OH-Progesterone levels in women superovulated with Clomiphene Citrate and hMG for in vitro fertilization. **Fertil Steril** 49:843, 1988.

Royere D, Hamamah S, **Miron P**, Nicolle JC, Lansac J. Relations entre les altérations chromatiniennes lors de la congélation du sperme humain et les événements précoces de la fécondation in vitro. **Fertil Contracep Sexualité**, 18:532, 1990.

Letendre ED, Roberts KD, **Miron P**, Langlais J. Platelet-activating factor acetylhydrolase activity in human semen. **Fertil Steril** 47:193, 1992.

Hemmings R, Langlais J, Falcone T, Bourque J, Granger L, **Miron P**, Guyda H. Human embryos produce transforming growth factors ? and insulin like growth factors II. **Fertil Steril** 58:101, 1992.

Miron P, Dizazzo A, Auger E, St-Michel P, Faucher G, Langlais J, Granger L. Évaluation de la phase lutéale selon quatre méthodes diagnostiques de l'ovulation. **SOGC Journal**, 1992.

Tulandi T, **Miron P**, Leung P, Jarrel J. Oocyte donation : a proposed guideline of the Canadian fertility and Andrology Society. **SOGC Journal**, 1992.

Hamanah S, Royere D, **Miron P**, Nicolle JC, Lansac J. Human sperm chromatin alterations after freezing-thawing and in vitro fertilization. In : Comparative spermatology 20 years after. **Ed. B. Bacette, Serono Symposia Publications, Ravin Press** 75, 1101 pp 899-903, 1992.

Hemmings R, **Miron P**, Falcone T, Bourque J, Lepage N, Langlais J. Platelet activating factor acetylhydrolase activity and peritoneal fluid of women with endometriosis. **Obstet Gynecol** 1993, 81;276-9.

Falcone T, **Miron P**, Granger L, Bourque J, Hemmings R. Polycystic ovarian syndrome. **Current problems in Obstet and Gynecol** 1993, XVI:65-95.

Lepage N, **Miron P**, Hemmings R, Roberts KD, Langlais J. Distribution of lysophospholipids and metabolism of platelet-activating factor in human follicular and peritoneal fluids. **J Reprod Fertil** 1993, 98:349-56.

Lachapelle MH, **Miron P**, Hemmings R, Falcone T, Granger L, Bourque J, Perreault C, Langlais J. Embryonic resistance to tumour necrosis factor-? mediated cytotoxicity: novel mechanism underlying maternal immunological tolerance to the fetal allograft. **Human Reprod** 1993, 8:1032-38.

Lachapelle MH, Bouzayen R, Langlais J, Jarvi K., Bourque J, and **Miron P.** Effect of lysoplatelet-activating factor on human sperm fertilizing hability. **Fertil Steril** 1993, 59:863-68.

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Hemmings R, and **Miron P.** Release of Growth Factors by Human Embyros. **SOGC Journal**, October 1995.

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Lachapelle MH, **Miron P**, Hemmings R, Baron C, Roy DC. Flowcytometric characterization of hematopoietic cells in non pregnant human endometrium. **Am J Reprod Immunol** 1996, 35:5-13.

Lachapelle MH, **Miron P**, Hemmings R, Roy DC. Endometrial T, B and NK cells in patients with repetitive abortions: Altered profile and pregnancy outcome. **J. Immunol** 1996, 156:4027-34.

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Lachapelle MH, Hemmings R, Roy DC, Falcone T, and **Miron P**. Flow-Cytometric Evaluation of Leukocyte Subpopulations in the Folicular Fluids of Infertile Patients. **Fertil Steril** 1996, 65:1135-40.

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Marcoux S, Maheux R, Bérubé S and The Canadian Collaborative Group on Endometriosis (for one **Miron P**). "Laparoscopic Surgery in infertile women with minimal or mild endometriosis". **The New England Journal of Medicine** 1997, volume 337, 4:217-222.

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Yang Z., **Miron P**, Gu Z, Smitz J, Cortvrindt R, Van Steirteghem A and Goff A. "Two-dimensional analysis of protein synthesis during meiotic maturation of bovine and human oocytes". Proceedings of the 10th World Congress of In Vitro Fertilization And Assisted Reproduction, Monduzzi Editor 1997, p.349-352.

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Miron P, Casper R. "Comparison between HP-FSH and hMG for superovulation in women undergoing in vitro fertilization". Journal SOGC, March 1998, Volume 20, Number 3, pp 283-288.

Miron P and Lambert J. Letter to the Editor concerning the article published in the Journal SOGC (volume 20 : 139-147, 1998) by Vilos & al « Economic evaluation of *in vitro* fertilization-embryo transfert (IVF-ET) and neosalpingostomy for bilateral tubal obstruction », **Journal SOGC**, September 1998, Volume 20, Number 10, pp 927-928.

Martin R, Nisker J, Daya S, **Miron P** and Parish B, Joint Report Canadian Fertility and Andrologie

Society / Society of Obstetricians and Gynaecologists of Canada, presented to the members of the those comittees on January 1998 and adopted in April 1998. **Journal SOGC**, November 1998, Volume 20, Number 13, pp 1235-1241.

Martin R, Nisker J, Daya S, **Miron P** and Parish B, Joint Report Canadian Fertility and Andrologie Society / Society of Obstetricians and Gynaecologists of Canada, Policy Statement Ethical Issues in Assisted Reproduction – Sperm sorting for Medical and Non-medical Reasons. **Journal SOGC**, January 1999, Volume 21, Number 1, pp 67-74.

Martin R, Nisker J, Daya S, **Miron P** and Parish B, Joint Report Canadian Fertility and Andrologie Society / Society of Obstetricians and Gynaecologists of Canada, Policy Statement Ethical Issues in Assisted Reproduction – Oocyte Transfer : Sources of Oocytes and the Nature of the Exchange. **Journal SOGC**, February 1999, Volume 21, Number 2, pp 175-182.

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Pinheiro R, Bénard F, Mauffette F, Lambert J and **Miron P**. Effectiveness of ICSI/MESA/PESA for severe male infertility compared to conventional IVF for complete tubal occlusion in women . **Canadian Medical Association Journal**, November 1999, Volume 161 (11), pp. 1397-1412.

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Miron P, Coté YP, Lambert J. Effect of maternal smoking on prenatal screening for Down syndrome and trisomy 18 in the first trimester of pregnancy. Prenat Diagn. 2008 Feb 8 ;28(3): 180-185

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PATENTS

Issued patents

6,777,182	Methods for determining the likelihood of endometriosis in a female subject (2001)
6,743,595	Method and diagnostic kit for diagnosis of endometriosis (2000)
6,376,201	Use of ligands specific to major histocompatibility complex-class I antigens for diagnosing endometriosis (1999)
5,618,680	Use of ligands specific to major histocompatibility complex-class I antigens for diagnosing endometriosis (1994)

Pending Patent

2008 June	Use of follistatin related gene protein FLRG as a marker for identifying a fetal abnormality in maternal fluid (international publication WO 2009/152601, December 2009)

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Miron P. Fécondation in vitro - démystification d'un traitement révolutionnaire. **L'Actualité médicale**, 1992.

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Section VI –

ORAL PRESENTATIONS AND POSTERS

Bernazzani O, Saucier JF, **Miron P**, Granger L, Hudon M, Faucher G, St-Michel P. Réflexion sur le secret entourant le don de sperme. Conference at the Canadian Society of Bio-Ethics, April 1990.

Langlais J, Lepage N, Roberts K, Ward L, Granger L, & **Miron P**, 1990. High pregnancy rate in ivf using culture media with low paf-acetylhydrolase activity. Abstract. II Joint ESCO-ESHRE (European Society of Human Reproduction and Embryology) Meeting, Milan, June 1990.

Lepage N, **Miron P**, Lambert RD, & Langlais J, 1990. Potential role of lysophospholipids in cell fusion during implantation in the rabbit. Abstract. II Joint ESCO-ESHRE (European Society of Human Reproduction and Embryology) Meeting, Milan, June 1990.

Langlais J, Lepage N, & **Miron P**, 1990. Transient rise in lysophospholipids in rabbit blastocoelic fluid: a mechanism for cell fusion occurring during implantation. Abstract. Canadian Fertility and Andrology Society and Serono Symposia USA, Estérel, Quebec, October 1990. **Prized communication. (Best communication in basic research).**

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Lachapelle MH, Raymond L, Ward L, Bourque J, Falcone T, Hemmings R, Granger L, **Miron P**, & Langlais J. Résistance embryonnaire face a la cytotoxicité du TNF?: Un mécanisme de la tolérance immunologique maternelle. Abstract. "Colloque de l'Université de Montréal, département d'obstétrique-gynécologie", Montreal, Québec, April 24, 1991.

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Langlais J, Letendre ED, **Miron P**, & Roberts KD. Platelet-activating factor acetylhydrolase in human semen. Abstract. Society for the Study of Reproduction, Vancouver, British Columbia, July 1991.

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Hemmings R, Falcone T, **Miron P**, Granger L, Bourque J & Guyda H. Growth factors alterations in endometriosis. Abstract. Annual Meeting of Canadian Fertility and Andrology Society, Quebec, Quebec, September 1991.

Hemmings R, **Miron P**, Falcone T, Granger L, Bourque J, & Guyda H. Human embryos produce transforming growth factor ?. Abstract. Canadian Fertility and Andrology Society Annual Meeting, Quebec, Quebec, September 1991.

Lachapelle MH, **Miron P**, Granger L, Falcone T, Hemmings R, Bourque J, & Langlais J. Embryonic resistance to TNF? Mediated cytotoxicity. Abstract. Canadian Fertility and Andrology Society Annual Meeting, Quebec, Quebec, September 1991.

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Jarvi K, Gagnon C, **Miron P**, & Langlais J. Platelet activating factor (PAF) metabolism by spermatozoa; role of metabolites in paf action on sperm motility. Abstract. American Society of Cell Biology Annual Meeting, December 1991.

Miron, P. Endométriosis - consensus report. Conference at the Westin Hotel in Ottawa, February 1992.

Lachapelle MH, Miron P, Langlais J. Potentiel embryonnaire à résister et à secréter du TNF?: mécanisme de la tolérance immunologique maternelle. Poster, Congress of the Associaiton of Graduate Studies Students, Faculty of Medicine, University of Montreal, Montreal, Quebec, March 1992. **Second prize for posters.**

Miron, P. Endométriose, infertilité et immunologie. Conference for the North Shore Doctors Symposium, Sacré-Coeur Hospital, April 1992.

Miron, P. Fiv - impact dans les traitements en infertilité. Conference given at the Ste-Jeanne d'Arc Hospital, April 1992.

Bouzayen R, Lachapelle MH, Jarvi K, Langlais J, Granger L, Hemmings R, Falcone T, Bourque J, **Miron P**. Effet du lyso-PAF sur le pouvoir fécondant des spermatozoïdes humains. Abstract, Colloque d'Obstétrique-gynécologie de l'Université de Montréal, April 24, 1992.

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Miron, P. Infertility treatment - review. Conference given at the Georges Dumont Hospital, Moncton, New Brunswick, June 1992.

Miron, P. FIV, impact sur les traitements en fertilité. Conference given at the University of Sherbrooke, Sherbrooke, Quebec, October 1992.

Miron P, Lachapelle MH, Bouzayen R, Jarvi K, Langlais J, Granger L, Hemmings R, Bourque J, Falcone T. Effect of lyso-paf on human spermatozoa in zona free hamster test. Abstract, Canadian Fertility and Andrology Society Annual Meeting, Kananaskis, Alberta, November 1992.

Lachapelle MH, **Miron P**, Granger L, Bourque J, Hemmings R, Falcone T, Langlais J. Human embryo potential to secrete TNF-alpha during preimplantation stage. Abstract, Canadian Fertility and Andrology Society Annual Meeting, Kananaskis, Alberta, November 1992.

Falcone T, Desjardins C, Bourque J, Granger L, Hemmings R, **Miron P**, Quiros E. Dysfunctional uterine bleeding in adolescents. Abstract, Canadian Fertility and Andrology Society Annual Meeting, Kananaskis, Alberta, November 1992.

Hemmings R, **Miron P**, Falcone T, Bourque J, Granger L. In vitro culture of human embryos: a predictor of successful pregnancy. Abstract. Canadian Fertility and Andrology Society Annual Meeting, Kananaskis, Alberta, November 1992.
Falcone T, Hemmings R, Bourque J, Granger L, **Miron P**. Recurrent pancreatitis associated with in vitro fertilization. Abstract. Canadian Fertility and Andrology Society Annual Meeting, Kananaskis, Alberta, November 1992.

Hemmings R, Lachapelle MH, Bourque J, Granger L, Falcone T, **Miron P**. Estradiol testosterone concentration in human embryos culture media. Abstract. Canadian Fertility and Andrology Society Annual Meeting, Kananaskis, Alberta, November 1992.

Pham A-M, **Miron P**, Roy D-C. Isolation des cellules endométriales humaines et évaluation de leurs molécules d'adhésion. Abstract. "31ième Congrès des stagiaires d'été", Université de Montréal. Montreal, Quebec, January 1993.

Hemmings R, **Miron P**, Lachapelle MH, Falcone T. Effect of follicular fluid supplementation on the in vitro development of human embryos. Abstract. European Society of Human Reproduction and Embryology Annual Meeting, Thessaloniki, Greece, June 1993.

Lachapelle MH, **Miron P**, Roy DC. Mise au point d'une méthode d'isolation des cellules stromales de l'endomètre humain. Oral presentation. "Journée de la recherche de l'Hôpital Maisonneuve-Rosemont", Montreal, Quebec, June 1993.

Lachapelle MH, Hemmings R, Falcone T, **Miron P**. Evaluation by flow cytometry of leukocyte subpopulations in the follicular fluid of patients with idiopathic infertility. Abstract. 39th American Fertility Society and Canadian Fertility and Andrology Society Annual Meeting, Montreal, Quebec, October 1993.

Miron, P. FIV - Résultats. Conference given at the St-François D'Assise Hospital, January 1994.

Miron, P. Réponse a la Commission Baird : FIV - indications et taux de succes. Conference given at the SOGC Congress, Mont Ste-Anne, Quebec, February 1994.

Miron, P. Fertility - new challenges. Symposium for the Weizmann Institute, February 1994.

Miron, P. Cheminement de carrière. Conference given at the CEGEP Marie-Victorin, February 1994. .

Miron, P. Évolution des traitements en infertilité. Conference given at the Sacré-Coeur Hospital, February 1994.

Miron, P. Endometriosis associated infertility. Colloque du CABRA (Canadian Advisory Board for Reproductive Advancement) , Geneva, Switzerland, April 1994.

Miron, P. Pfizer Advisory Board meeting: new techniques for the assessment of the embryo quality following induction of ovulation. Miami, Florida, April 1994.

Miron, P. Salon de la paternité et de la maternité de montréal. Mise a jour sur les nouvelles techniques de reproduction. April 1994 - May 1994.

Miron, P. Chirurgie endoscopique et utilisation des agonistes de la GnRH. Conference given at the Lakeshore Hospital, May 1994.

Miron, P. L'utilisation des agonistes de la LHRH en fécondation in vitro. Conference given at the L'Ancestral, Laval, Quebec , May 1994.

M.H. Lachapelle, R. Hemmings, D.C. Roy, C. Baron, and **P. Miron**. A new cause of repetitive abortions: increased levels of highly cytotoxic cd16+cd56+ natural killer cells in endometrial stroma. Abstract. European Society of Human Reproduction and Embryology Annual Meeting, Brussells, Belgium, June 1994.

L. Lapensée, R. Hemmings, L. Granger, T. Falcone, **P. Miron**, E. de Lamirande, C. Gagnon. Computer assisted sperm analyzers: can they help predict the in vitro fertilization outcome? Abstract. XIVth World Congress in Gynecology and Obstetrics, Montreal, Quebec, Canada, September 1994.

M.H. Lachapelle, R. Hemmings, C. Baron, D.C. Roy, **P. Miron**. Immunologic characterization of stromal cells from human endometrium by flow cytometry. Abstract. American Fertility Society Annual Meeting, San Antonio, Texas, November 1994.

M.H. Lachapelle, R. Hemmings, T. Falcone, C. Baron, **P. Miron**. Different subpopulations of follicular fluid leukocytes are present in unexplained infertility and endometriosis. Abstract. American Fertility Society Annual Meeting, San Antonio, Texas, November 1994.

L. Lapensée, R. Hemmings, L. Granger, T. Falcone, **P. Miron**, E. de Lamirande, C. Gagnon. Computer assisted sperm analyzers: can they help predict the in vitro fertilization outcome? Abstract. American Fertility Society Annual Meeting, San Antonio, Texas, November 1994.

Miron, P. Release of growth factors by human embryos. Published in the October 1994 issue of the SOGC Journal.

K. Pagidas, T. Falcone, R. Hemmings, **P. Miron**. Comparison of reoperation for moderate (stage III) and severe (stage IV) endometriosis-related infertility with in vitro fertilization and embryo transfer (IVF-ET). Abstract. IX World Congress on IVF and Alternate Reproduction, Vienna, Austria, April 8-14, 1995.

C. Gascon, M.H. Lachapelle, A. Hébert, **P. Miron**. Avortements à répétition : augmentation de la population nk cd 56+cd16+ dans l'endomètre. Abstract. Journée d'éducation médicale continue de l'Association des obstétriciens et gynécologues du Québec, April 8, 1995.

R. Bouzayen, M.H. Lachapelle, A. Hébert, **P. Miron**. Modification des populations leucocytaires endométriales chez les patientes souffrant d'endométriose. Abstract. Journée d'éducation médicale continue de l'Association des obstétriciens et gynécologues du Québec, April 8, 1995.

M.H. Lachapelle, A. Hébert, **P. Miron**. Avortements à répétition : augmentation de la population nk cd 56+cd16+ dans l'endomètre. Abstract. Colloque des obstétriciens-gynécologues à l'Université de Montréal, April 27, 1995. **Berlex Award winner**.

M.H. Lachapelle, A. Hébert, **P. Miron**. Modification des populations leucocytaires endométriales chez les patientes souffrant d'endométriose. Abstract. Colloque des obstétriciens-gynécologues à l'Université de Montréal, April 27, 1995.

Miron, P. Investigation et traitement de l'infertilité. Conference at the Sheraton, Laval, Quebec , May 18, 1995.

Miron, P. Conference at the Complexe Desjardins at the exhibition. Regards sur l'infertilité, May 25, 1995.

Miron, P. Investigation and treatment of infertility: a comprehensive approach.Conference at the René-Laënnec Clinic, Montreal, Quebec, May 30, 1995.

Miron, P. Le role de l'omnipraticien dans l'investigation de l'infertilité. Conference for physicians at the Le Gardeur Hospital, Montreal, Quebec, June 14, 1995.

Miron, P. Le role de l'omnipraticien dans l'investigation de l'infertilité. Conference for physicians of Hull-Gatineau, Quebec, June 15, 1995.

Miron, P. Update on endometriosis / infertility. Royal college speaker for the Society of Obstetricians and Gynaecologists of Canada, 51st Annual Clinical Meeting, Calgary, Alberta, June 23-28, 1995.

Miron, P. Conference for the "Mise à jour en gynécologIE" at the Inter-Continental Hotel in Montreal, September 15, 1995, regarding the investigation of infertility.

M.-H. Lachapelle, **P. Miron**, A. Hébert, D.-C. Roy. Distinct immunophenotypic profile of leukocytes in patients with endometriosis. Abstract. Canadian Fertility and Andrology Society Annual Meeting, Montebello, Quebec, September 20-23, 1995.

M.-H. Lachapelle, D.-C. Roy, A. Hébert et **P. Miron**. Distinct immunophenotypic profile of leukocytes in patients with endometriosis: Diagnosis potential. Abstract. Research day for the Montreal area reproductive and developmental biologists, University of Montreal, November 16, 1995.

Miron P. Technical aspects of in vitro fertilization. Oral presentation at the 1996 Edition of the International CME "What's New in OB/GYN" of the Society of Obstetricians and Gynaecologists of Canada, Cancun, Mexico, March 4-8, 1996.

Miron P. Nouvelles techniques de reproduction. Conference at the Inter Hospital Meeting, Department of Obstetrics and Gynecology, University of Montreal, Ste. Justine Hospital, Montreal, Quebec, April 16, 1996.

Miron P. Endocrinologie de la reproduction et infertilité. Guest speaker. Presentation at the Institut de médecine de la reproduction et infertilité, Montreal, Quebec, May 10, 1996.

Miron P. Le point sur les nouvelles techniques de reproduction. Guest speaker at tje 52th Annual Meeting of the SOGC jointly held with the 30th Meeting of the AOGQ. Quebec City, June 22-27, 1996.

Miron P. Expression of endometrial cell surface antigens in endometriosis. Vth World Congress on Endometriosis, Yokohama, Japan, October 21-24, 1996.

Miron P. Presentation on ART at a symposium organized by the Department of Obstetric Gynecology of the Notre-Dame Hospital, on November 1, 1996.

Miron P. Lecture on In Vitro Fertilization, given to the students of the Medicine Faculty of the Sherbrooke University, on November 19, 1996.

Miron P. Presentation at a meeting organized by The Amercian College of Obstretricians and Gynecologists, on January 29, 1997 at Portland (Maine Section, District 1).

Miron P. Presentation to the Obstetricians and Gynecologists of Abitibi: LaSarre, on February 18, 1997 and Val d'Or, on February 29, 1997.

Miron P. Presentation to the gynecologists of Manchester, Maine, on March 11, 1997.

Miron P. Speaker and moderator invited to the Serono Canada Symposium, on April 23, 1997 for the presentation of their "Continuing Medical Education Programs".

Yang Z. **Miron P** et Goff A. "Maturation in vitro of bovine and human occytes in a chemically defined medium supplemented with EGF", poster presented at the 10th World Congress in Vitro Fertilization and Assisted Reproduction (May 24-28, 1997) in Vancouver, Canada.

Yang Z, St-Jean D, Ward L, and **Miron P**. "Viable pregnancies following transfer of one, two, or three embryos produced by intracytoplasmic sperm injection (ICSI)". Poster presented at the 10th World Congress in Vitro Fertilization and Assisted Reproduction (May 24-28, 1997) in Vancouver, Canada.

Yang Z, **Miron P**, Gu Z, Smitz J., Cortvrindth R, Van Steirteghem A and Goff A. "Two-dimensional analysis of protein synthesis during maturation of human and bovine oocytes".
Poster presented at the 10th World Congress in Vitro Fertilization and Assisted Reproduction (May 24-28, 1997) in Vancouver, Canada.

Miron P, Yang Z, Ward L and St-Jean D. "Introcytoplasmic sperm injection: A reliable treatment for male factor infertility" Poster presented at the Annual Meeting of the SOGC (June 20-25, 1997) in Halifax, Canada.

Miron P, Yang Z and Goff A. "In vitro maturation of bovine and human occytes in a chemically defined medium supplemented with EGF". Abstract presented at the Annual Meeting of the SOGC (June 20-25, 1997) in Halifax, Canada. **(Winner of the Best Clinical Investigation in Reproduction)**

Yang Z. **Miron P**., Gu Z., Cortvrindt R., Smitz J., Van Steirteghem A., and Goff A. "Changes in protein synthesis during in vitro maturation of human and bovine occytes, oral presentation at the 13th Annual Meeting of the European Society of Human Reproduction and Embryology (June 22-25, 1997) in Edinburgh, Scotland.

Yang Z. **Miron P**., Gu Z., Cortvrindt R., Smitz J., Van Steirteghem A., and Goff A. "Maturation In vitro of bovine and human oocytes in a chemically defined medium supplemented with EGF". Poster presented at the 13th Annual Meeting of the European Society of Human Reproduction and Embryology (june 22-25, 1997) in Edinburgh, Scotland.

Yang Z, **Miron P**, Gu Z, Smith J, Cortvrindt R, Van Steirteghem A et Goff A. "Two-Dimensional analysis of protein synthesis during maturation of human and bovine oocytes". Poster presented at the 43rd Annual Meeting of the Canadian Fertility & Andrology Society in Niagara on the Lake, Ontario(September 24-27, 1997).

Miron P, Yang Z. et Goff A. Maturation in vitro of bovine and human oocytes in a chemically defined medium supplemented with EGF". Poster presented at the 43rd Annual Meeting of the Canadian Fertility & Andrology Society in Niagara on the Lake, Ontario(September 24-27, 1997).

Miron P. Rôle thérapeutique de l'hormonothérapie en gynécologie et nouvelles applications. Presentation at a meeting held at Mont Tremblant, on March 20, 21 and 22, 1998 and organized by the gynaecologists of Hôpital du Sacré-Coeur de Montréal with Schering Canada Inc.

Miron P. Up-date on the Assisted Reproductive Technologies. Presentation at the meeting of L'Association des Endocrinologues du Québec at Magog, on May 21, 1999.

Miron P. Speech concerning on the draft for By-Law C-247, *Loi modifiant le Code criminel* (manipulation génétique) to the members of the Comité permanent de la santé de la Chambre des communes in Ottawa, on June 8, 1999.

Miron P. Participation to a rountable concerning the « cadre réglementaire sur les techniques de reproduction et de génétique », set out by Santé Canada, on July 5, 1999.

Gosselin D, Gagné D, **Miron P**, Hugo P. « Modulation of T Lymphocyte Subsets and $CD14^+$, $CD36^+$ or Aminopeptidase $(CD13)^+$ Macrophages Coexpressing HLADR or CD44 Molecules in Peripheral Blood of Patients with Endometriosis. Abstract presented at the Joint Annual Meeting of ASRM/CFAS, at Toronto, September 25-30 1999.

Miron P. « Assisted Reproductive Technology : Embryo Transfer », moderator, Joint Annual Meeting of ASRM/CFAS, at Toronto, September 28,1999.

Miron P. Investigator at the meeting of the « Multricentric Invesigation with Cetrorelix in patients undergoing COS/ART (Study no. D-20761-3161) held in Fort Lauderdale (Florida), October 15, 16 1999.

Miron P. Participation to a roundtable of experts within the context of the Faculty of Law of the University of Sherbrooke to create a program in Law specialized in molecular biotechnology, held in Longueuil, on December 9, 1999.

Goff A, Yang Z,Cortvrindt R, Smitz J, Nogueria D, Everaerdt B, **Miron P**. Talk entitled « Protein synthesis during oocyte maturation », presented at Ghend in Belgium, January 2000.

Gosselin D., Gagné D, Shazand K, **Miron P** et Hugo P. Abstract « Modulation of leukocyte subsets in the endometrium of patients with endometriosis », presented at the 47th Annual Meeting of the SGI : A Millenial Milestone for the Reproductive Sciences : Celebrating the Promise, in Chicago, March 2000.

Miron P. Presentation at the « Colloque d'Obstétrique-gynécologie de l'Université de Montréal, on April 28, 2000 and being also a member of the pannel of judges of that symposium.

Gosselin D, Gagné D, Pagé M, Shazand K, **Miron P** et Hugo P. Abstract« Altered leukocyte subsets in the blood and endometrium of patients with endometriosis : Promising candidates for the development of a screening test », presented in London at the 7th Biennal World Congress of Endometriosis, du 14 au 17 mai 2000.

Cherry E, Bernard M, Malette B, Baban S, Gosselin D, **Miron P**, Hugo P and Shazand K. "Identification of Novel Genetic Markers as Candidates for the Diagnostic of Endometriosis". Poster presented at the SOGC Annuel Meeting in Montreal, June 17-21, 2000.

Gosselin D, Gagné D, Shazand K, **Miron P** and Hugo P. "Several Endometrial Leukocyte Subsets defined as Promising Markers in a Screening Test for Endometriosis". Poster presented at the SOGC Annual Meeting in Montreal, June 17-21, 2000.

Miron, P. Conference given to a group of doctors of the Rimouski area. « Investigation de base en infertilité ». Le Bic, July 11, 2000.

Miron P. "Investigation de base en infertilité". Conference addressed to the residents of the University of Sherbrooke, Sherbrooke, October 31, 2000.

Miron P. "Investigation de base en infertilité". Presentation to a group of doctors at La Cité de la Santé Hospital, Laval, November 2, 2000.

Miron P. "Procréations médicalement assistées". Conference given at the PROCREA Symposium, Montreal, November 17, 2000.

Miron P. "Investigation de base en infertilité". Presentation to a group of doctors at Hôpital du Sacré-Cœur, Montreal, November 30, 2000.

Miron P. "Investigation de base en infertilité". Presentation to a group of doctors at Hôpital Anna-Laberge, Châteauguay, December 11, 2000.

Miron P. "Investigation de base en infertilité". Presentation to a group of doctors at Centre hospitalier des outaouais, Gatineau, January 24, 2001.

Miron P. "L'apport des PMA au bien-être et santé biologique de l'enfant". Conference given at the Colloque sur l'assistance médicale à la procréation by the CHUL, Longueuil, January 26, 2001.

Miron P. "Investigation de base en infertilité". Presentation to a group of doctors of the Joliette area. Joliette, March 13, 2001.

Miron P. "Dilemmes et défis éthiques en médecine de la reproduction". Presentation of a case of surrogate mother to the students of bio-ethic, University of Montreal, Dr. Chantal Rivard's course, Hôpital Maisonneuve-Rosemont, March 26, 2001.

Miron P. "Investiguation de base en infertilité". Presentation to a group of doctors of Basses Laurentides area. Sainte-Adèle, March 27, 2001.

Blondin P, Rivard M, Raymond L and **Miron P**, "Reducing multiple pregnancies in IVF with

blastocyst-stage embryo transfers", Annual symposium of the Department of Obstetrics and Gynecology, Université de Montréal, April 27, 2001.

Lavoie H, Rivard M, Wilhelmy A and **Miron P**, Does metformin improve pregnancy rates in women with polycystic ovarian syndrome (PCOS) presenting high insulin levels ?, Endocrine Society Meeting, Denver, Colorado, June 19-23, 2001.

Miron P. Trans-abdominal chorionic villi sampling : indications and technique, Hôpital Pierre-Boucher. September 13th, 2001

Miron P. Prenatal screening : an update, Maisonneuve-Rosemont, May 31st 2003

Miron P, Buckett W, Baillargeon JP, Casper R. Mise à jour et nouveautés dans l'induction de l'ovulation. November 26th, 2003 ; Preparation and organization of the Symposium, involving 3 other speakers from Universities of Sherbrooke, McGill and Toronto with a maintenance certificate (section 4 of the programme) for 2h30;

Miron P. Induction de l'ovulation et inséminations intra-utérines. Département d'obstétrique-gynécologie. Cité de la Santé. February 19th, 2004.

Miron P. Dépistage prénatal. Presentation to Ministère de la Santé et des Services sociaux (Québec), October 22nd 2004 ;

Miron P. L'échographie de 11 à 14 semaines : Cours théorique. L'expérience québécoise en dépistage prénatal (Hôpital Ste-Justine, Montréal). March 12 th, 2005 ;

Miron P. Dépistage prénatal. Congrès annuel de gynécologie-périnatalité (St-Alexis-des-Monts), May 12, 2005 ;

Miron P. Endometriosis. Organon-Genizon BioSciences meeting. The Netherlands (Organon). February 15th, 2006 ;

Miron P, Update in first-trimester prenatal screening, Centre hospitalier LaSalle, May 11, 2006

Miron P, Update in first-trimester prenatal screening: Nasal bone and other ultrasound markers, Saguenay, October, 2006;

Miron P, Update in first-trimester prenatal screening: Nasal bone and other ultrasound markers, Hôpital du Sacré Coeur, Montréal, December 7th, 2006;

Miron P, Update in first-trimester prenatal screening: Nasal bone and other ultrasound markers, Quebec City, December 18th, 2006;

Miron P. Conference on prenatal screening. Department of familial medicine. Maisonneuve-Rosemont Hospital. December 18th, 2007.

Miron P. Conference on prenatal screening. Department of Obstetrics & gynecology. CHUQ February, 19th, 2008.

Miron P. Statement on assisted reproductive technologies. Parlementary Commission on social affairs, Ministry of Health ans social services, Québec, Québec. June 10th, 2008.
http://www.assnat.qc.ca/FRA/travaux/Debats/banquevideo/cas/procreation2.html

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BENACERRAF, B. R. 2010. The history of the second-trimester sonographic markers for detecting fetal Down syndrome, and their current role in obstetric practice. *Prenat Diagn,* 30**,** 644-52.

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