

10th Anniversary of CIHR-IGH

Dr Yves Tremblay

Professeur Titulaire

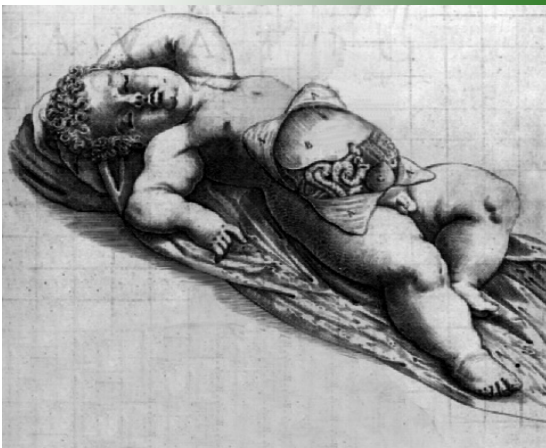
- **Département d'Obstétrique et de Gynécologie / U. Laval**
- **Axis in Reproduction and Child Health, Development and Well-Being at the CRCHUL**
- **Research Center in Reproductive Biology**

Major Interest since the beginning of my PhD:
The Steroid Action in Peripheral Tissues

Pulmonary Development



Research Context:
Steroid Action in
Lung Development



ma Faculté pour la vie



UNIVERSITÉ
LAVAL

Faculté de médecine

Steroids:

ANDROGENS, Estrogens,

Glucocorticoids, and Mineralocorticoids

in the Mechanism of Fetal Lung Development and Lung
Maturation:

How a Sex-Based Approach has been successful to
understand the role of androgens and this for the
benefit of premature infants

Clinical Context: **Respiratory Distress
Syndrome** of the Neonate, Bronchopulmonary
Dysplasia and more generally the
consequences to be born alive with immature
lungs.



Respiratory Distress Syndrome of the Neonate

- The one links with a **deficiency in the surge of surfactant synthesis** that affects
 - 1- infants who were born premature
and
 - 2- Boys who are at higher risk (**1.62:1** Statistic Canada, 2008)

General Information

- Late in gestation, the last pulmonary system of the lung matures allowing for the transition from water to air environment.
 - This process requires the maturation of the specialized cells and the finality results in the production/secretion of the **SURFACTANT** by the alveolar Type II cells.
- A deficiency in this production of surfactant is related to the **Respiratory Distress Syndrome (RDS) of the neonates**

General Information

➤ This production of surfactant does not occur at the same period of pregnancy for male and female fetuses. The production of surfactant by male is DELAYED and this temporal delay is NORMAL

- at comparable developmental time points, sex influences lung maturation, i.e., males exhibit delayed development of the lung and BOYS represent ~65% (1.62 male:1 female) of all RDS cases reported in North America

SUMMARIZE

- The risk of RDS increases in newborn infant with: **1- PREMATURITY** (Extremely-low-birth-weight infant, before 28w); **2- SEX-MALE**

Etiology of the Sex-Difference in RDS

- To which factor(s) RDS is linked: To the presence of ANDROGENS in MALE lung.
- The ANDROGENS are responsible for the DELAY in lung maturation in male vs female babies.

Summarize

- The literature is clear and presents RDS as being the result of a **SEXUAL DIMORPHISM** and the cause is **THE PRESENCE OF ANDROGENS** in male lung.

What are the clinical avenues

- **Antenatal glucocorticoid administration** to mothers at high risk to deliver prematurely was introduced in 1972 to accelerate-enhance fetal lung maturation and surfactant-dependent processes.
- **At birth: Pulmonary surfactant** replacement therapy assisted by new mechanical ventilation strategies that include oxygenation protocol and inhalation of nitric oxide to reduce lung injury

Summarize Therapeutic Action

- While actual therapies have resulted in **SIGNIFICANT** improvements in mortality rates in micro-preemies, **SEX DIFFERENCES** in survival persist and the poorer prognosis for the male is still present.

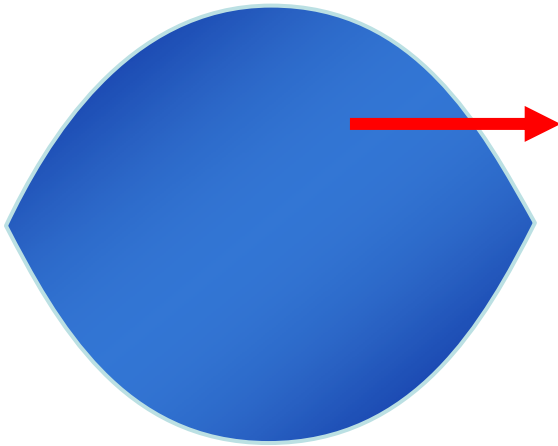
Why the difference male-female persists

Because actual therapies exert **SIMILAR** effects on lung development in **BOTH SEXES** and thus keep the delay unchanged and keep the male **production of surfactant delayed** compared to the female.

- How to track this sex difference: the first reflex is that only the male lung are exposed to androgens since only boys produce androgens during the fetal life if we refer to the testicular differentiation. But Testis differentiation occurs earlier.

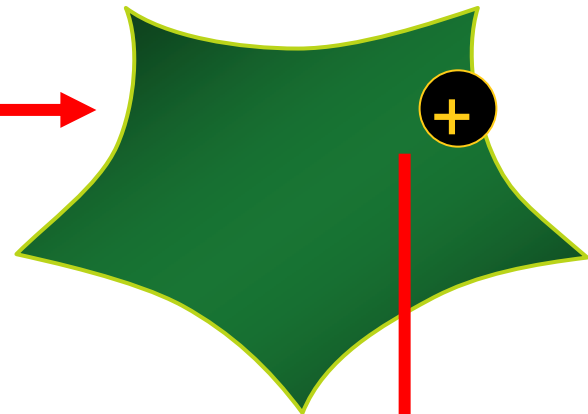
Mechanism

Fibroblasts



Paracrine
Factors

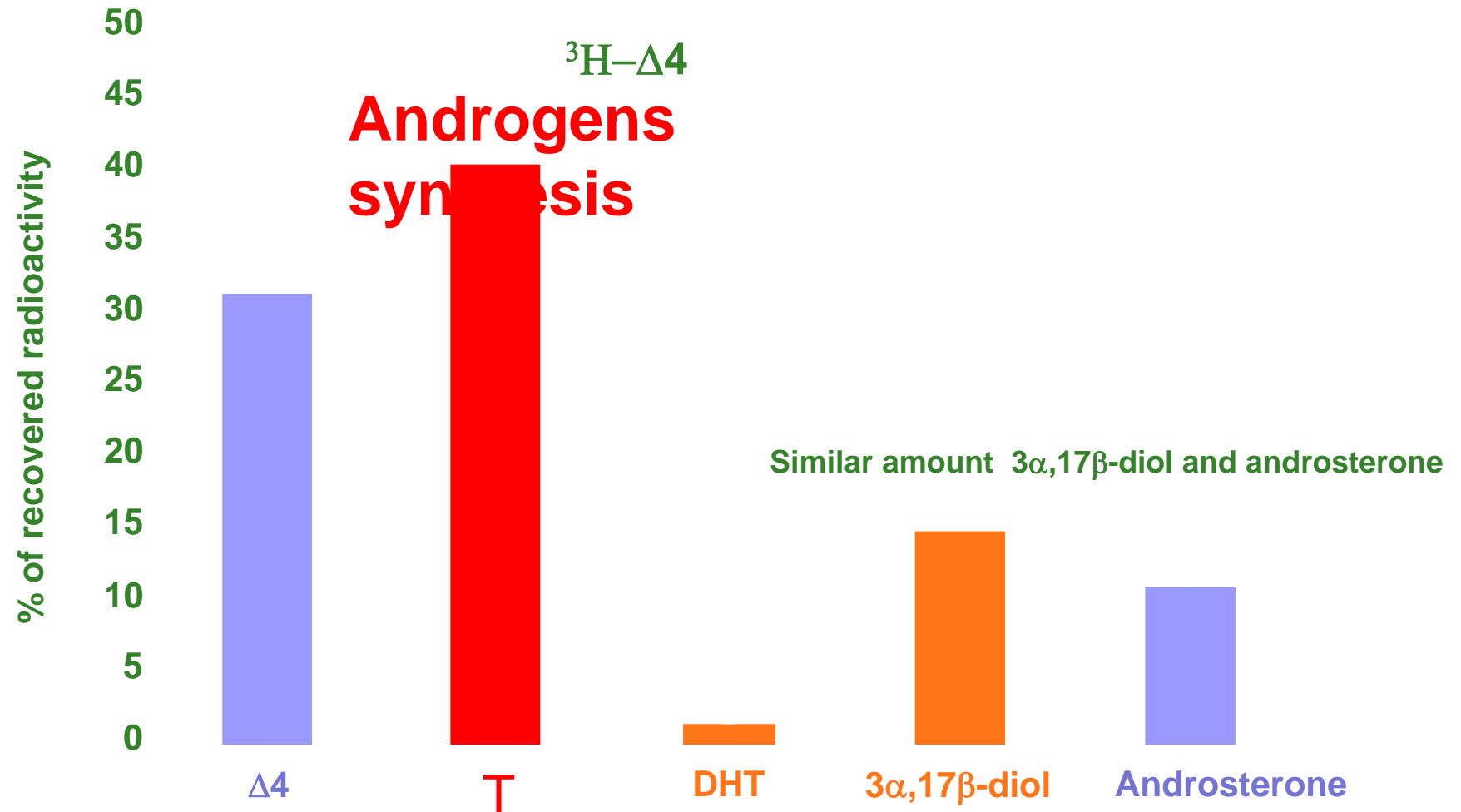
Epitheliales Type II
(Alveolar)



Androgens: -
Glucocorticoids: +

Surfactant

A-549 cells (h, epithelial and male)



Provost et al., Endocrinology (2000) 141, 2786

Inactivating activity of androgens in human fibroblast

cell lines

Fibroblastic Cell lines	Cell origin (1)	Age stage (2)	Sex (3)	17 β -HSD-2 / 5 α -reductase activity ratio (4)	
LL24	L	5 years	M	0.2	
CCD-34Sk (5)	S	N (2,5w)	F	0.2	WEAK
Hs 389(B).Lu	L	F (16w)	F	0.4	
Hs 907.Lu	L	N (7w)	F	0.4	
FHs 738Lu	L	F (sd trim)	M	2.4	OR
CCD-34Lu (5)	L	N (2,5w)	F	2.9	
CCD-32Lu	L	N (1m)	M	3.1	HIGH
IMR-90	L	F (16w)	F	4.4	
MRC-5	L	F (14w)	M	4.9	

1) lung (L), skin (S)

(2) fetal (F), new born (N), week (w), month (m), second trimester (sd trim)

(3) male (M), female (F)

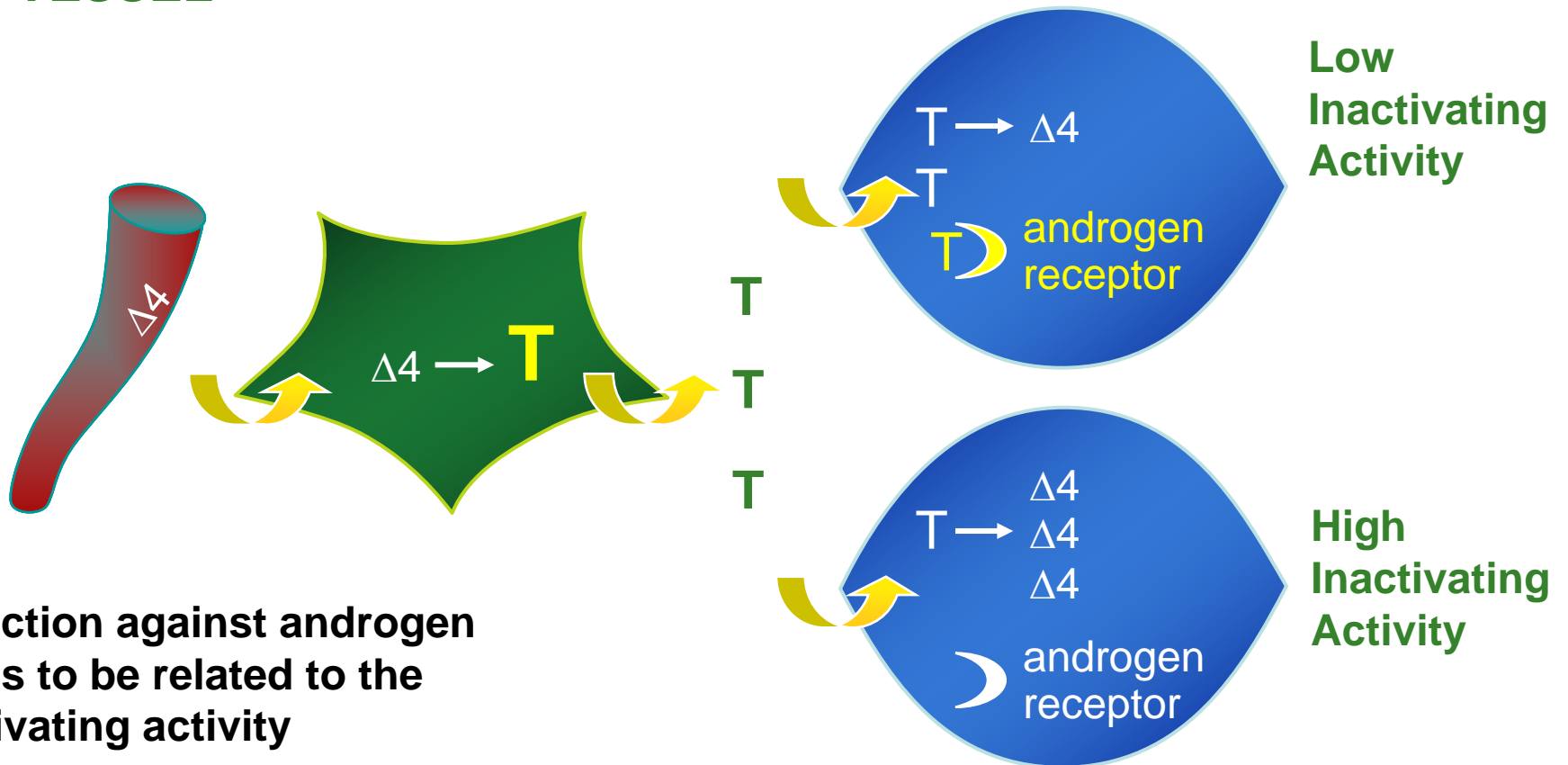
(4) Tritiated testosterone and androstenedione were respectively used for measurement of 17 β -HSD-2 and 5 α -reductase activities

A549 and Fibroblasts

BLOOD
VESSEL

TYPE II
EPITHELIAL CELL

FIBROBLAST



Provost et al Endocrinology 2000 ; J. Clin. Endocrinol. Metab 2002

From these in vitro results: One major question?

1- How to explain that the fibroblasts derived from female can inactivate androgens if only male can produce them?

Do the lung from female can make androgens.

In vivo MODEL

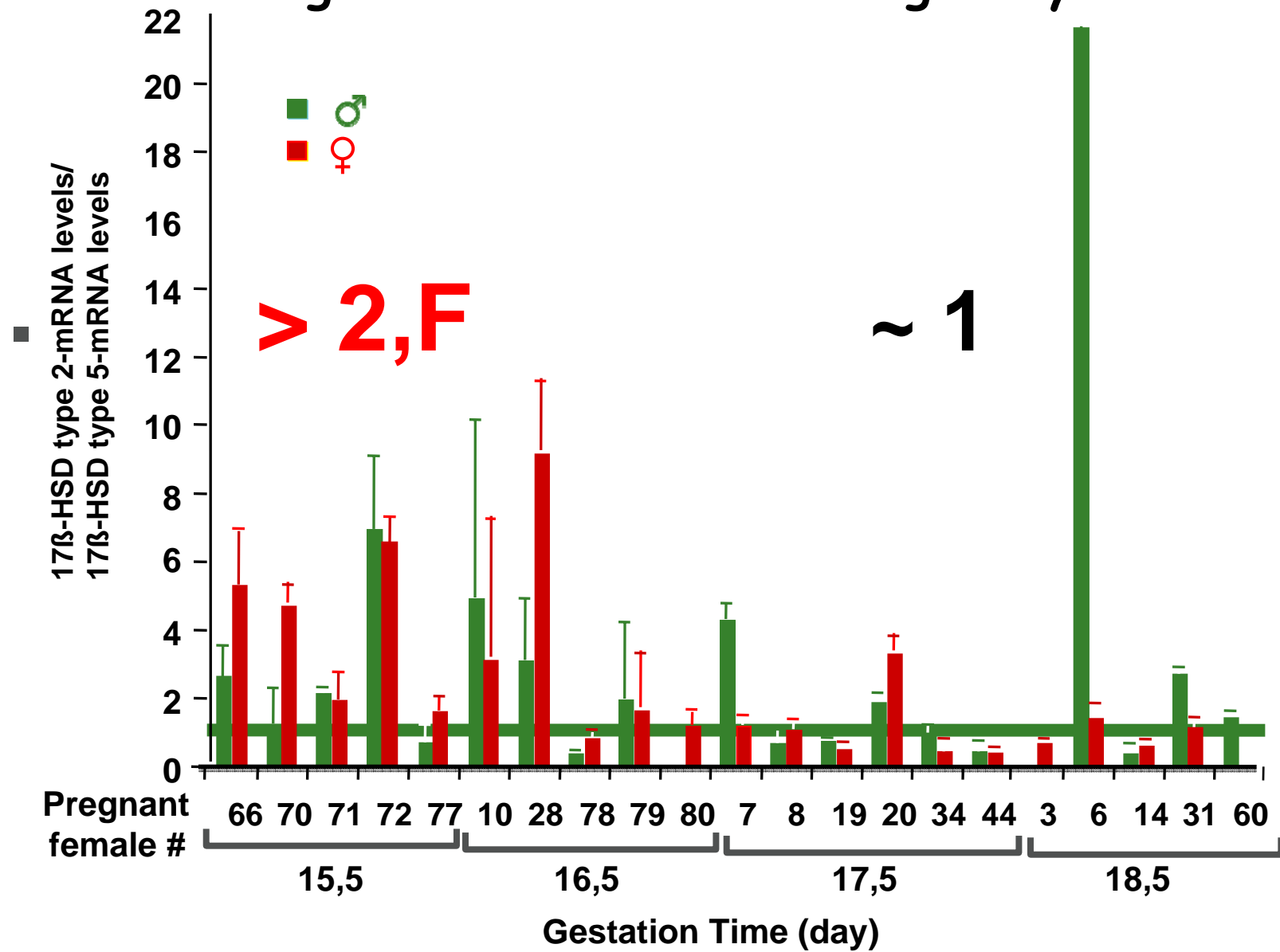
Mouse

- Mouse is a model where **androgens** and **sex** are etiologic factors recognized for RDS.
- Mouse lung contains the same androgen-expressing genes as in human
- **Glucocorticoid receptors** are expressed by the fetal lung tissues.
- **Balb/c**; inbred F-1 strain, genetically similar; less individual variations

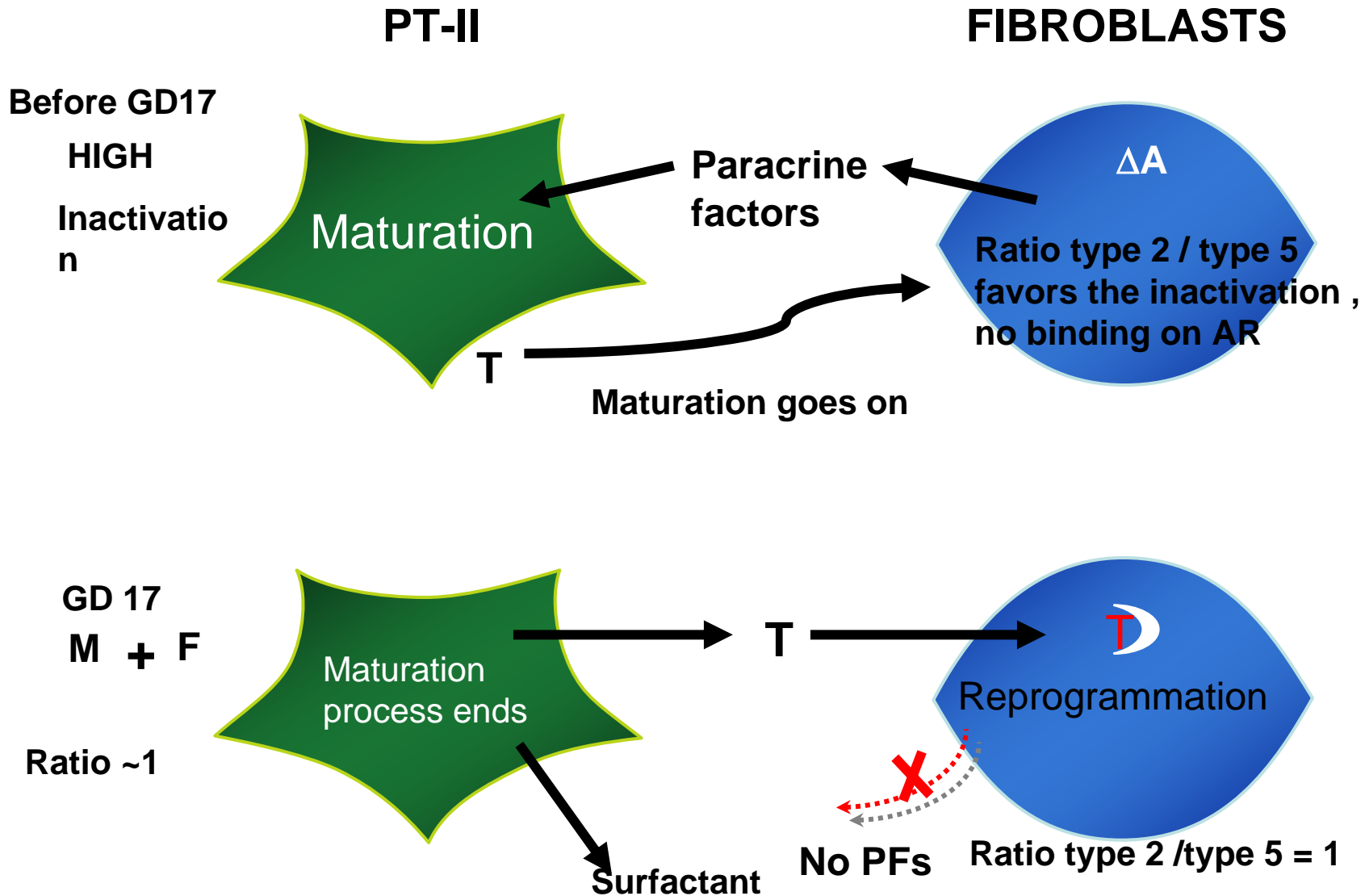
Experimental Design

- Balb/c were mated in our animal core facility
- Pregnant mice were sacrificed at **GD 15, 16, 17, 18** (during the gestational period where expression of the Surfactant Protein-C an indicator of lung maturity is increased; **Term GD21**)
Fetuses were sexed (expression of *Uty* and *Sry* in the male)
- Fetal lungs were collected (one pool / sex / pregnant mother)
- Isolation of total RNA
- **Real time PCR**
 - **Transcriptional analysis of androgen-metabolizing enzyme genes**
 - SP-C
 - Expression were normalized with several housekeeping mRNAs known to be not constant during that period (SDHA, TBP, YWHAZ, GAPDH, HPRT-1)

Ratio Androgen inactivation / Androgen Synthesis



Hypothesis



From in vivo study we conclude that this capacity of the fetal lung to maintain an androgen-dependent pressure was unexpected and new. It is present in male and female.

This capacity of the lung to produce androgens with the emergence of the mature surfactant-producing cells acts as a signal to indicate these are mature and are ready to produce surfactant suggesting that androgen play a role in cell reprogramming.

PRÉMATURÉS. AU SERVICE DE LA RELATION MÈRE-ENFANT



Photo Hubert Fanthomme

Pour respecter les besoins du prématuré, après une observation très précise de ses rythmes naturels de vie, de son niveau de stress, de ses réactions, un programme de soins « sur mesure » est établi par l'infirmière-puéricultrice.

Sabine de la Brosse - Paris Match

sos préma
Une longueur d'avance

**Au service des
prématurés**

Créée en 2004, l'association nationale d'aide aux parents d'enfants prématurés, SOS Préma, soutient les familles confrontées à une naissance avant terme et œuvre pour l'amélioration de leur prise en charge, pendant l'hospitalisation et après le retour à la maison. Site internet, guides de conseils pour les parents, permanences téléphoniques, visites bénévoles dans 41 antennes locales, sensibilisation des pouvoirs publics, dialogue avec les équipes

médicales (collaboration avec les unités de néonatalogie, participation aux réseaux de périnatalité, livret d'information médical), prévention... SOS Préma, reconnue d'intérêt général et agréée association d'usagers par le ministère de la Santé, fonctionne principalement grâce aux contributions privées (dons, sponsoring, mécénat financier et de compétences). Pour en savoir plus, pour soutenir l'action de SOS Préma, www.sosprema.com.

La salle de réanimation : tout est mis en œuvre pour le confort du prématuré



Assise un peu à l'écart dans la salle de réanimation, une jeune femme brune aux formes généreuses ouvre son corsage pour y blottir son « bout de chou » d'une trentaine de centimètres. Elle se penche maintenant tête contre tête sur son petit trésor et, avec une infinie tendresse, lui murmure des mots doux à l'oreille. Durant cette séance « peau à peau », torse nu contre torse nu, baptisée « méthode kangourou » par le corps médical, elle tente de lui transmettre sa propre chaleur, son influx de vie, le rassure, le sécurise... « Pendant et après ces échanges intimes, assure Ingrid, infirmière-puéricultrice, les bébés sont plus calmes et les mères (souvent culpabilisées à tort de n'avoir pu mener leur grossesse à terme) ont réellement l'impression de retrouver l'enfant qui leur a été enlevé sitôt après la naissance. Ce peau à peau est bénéfique pour les deux ! »

Toujours absorbée par son transfert d'amour, la jeune femme oublie le cadre de haute technologie qui l'entoure : une pouponnière-laboratoire où des êtres minuscules, allongés dans des incubateurs transparents et reliés à des sondes, tubes et capteurs lumineux, luttent vingt-quatre heures sur vingt-quatre pour survivre. Leurs petits thorax recouverts d'électrodes telles des armures de combat se soulèvent à intervalles réguliers. Dans la couveuse, miniature d'un vaisseau spatial, la teneur en oxygène est contrôlée par un saturomètre, appareil relié par un fil à un capteur lumineux rouge, fixé sous le pied du bébé (signal très important, car un excès d'oxygène peut entraîner des risques pour la vision ou une lésion cérébrale).

Pour protéger tous ces précieux nids de la lumière froide du plafonnier, et laisser les prématurés jour et nuit sous un éclairage tamisé, on a recouvert les toits des incubateurs d'un petit drap de teinte claire. Les infirmières-puéricultrices, vêtues de blouses vertes, coiffées de charlottes, évoluent dans un silence feutré... Ça et là, des chariots roulants chargés de pompes-seringues et munis d'écrans lumineux achèvent de donner une atmosphère surréaliste à ce centre de survie ultramoderne. De temps à autre, le silence est rompu par une sonnerie d'alarme... très vite arrêtée par une infirmière : un signal déclenché tantôt par une anomalie du taux d'oxygène, tantôt par un problème d'apnée chez un nourrisson qui a oublié de respirer. Une puéricultrice arrive alors aussitôt sur les lieux, et introduit ses mains gantées de blanc dans l'incubateur du prématuré en difficulté. Avec des gestes lents, d'une grande douceur, elle le tapote gentiment pour le stimuler, le caresse pour le rassurer. Ici tout est amour, vigilance et... haute précision.

Cet article a été publié dans le n°3065 paru en février 2008.

The extreme prematurity is still a question of general interest and is still a delicate question. During the last months we are working in the development of an integrate project trying to capture more generally how the **Sex Differences and Gender Influences** the fate of Premature Infants.

Question

How to address such a delicate question of a birth at the limit of the viability in term of medical action, ie the decision to begin and /or to maintain medical assistance to maintain life (ressuscitation) or to stop or do nothing

GENDER DIFFERENCES AND PREMATURE INFANTS:

**A Novel Integrative Approach to
Evaluate the Impact of the Fetal Sex
on Clinical Practice**



➤ By developing **NOVEL INTEGRATIVE APPROACHES** to evaluate the **IMPACT OF THE FETAL SEX ON CLINICAL PERINATAL PRACTICE**. This means **MORE PARTICIPATING DISCIPLINES** to evaluate more precisely how fetal sex impact on pulmonary morbidity when a mother is a higher risk of premature delivery or generally when neonatal complications are predictable.

GOAL of MUST

- Promote **uni-/ multi-/ and trans/-disciplinar** researches to document the question of how the fetal sex should impact on critical medical situations in case of premature infants with the objective to elaborate new evidence-based guidelines in perinatology and public policy.

- MUST groups professors from 3 Faculties and two Universities asking **ONE** question using several approaches **WHERE THE FETAL SEX AND THE FATE OF BABIES BORN EXTREME PREMATURE IS CENTRAL, RDS, BPD**
- Sectors are: biomedical, clinical, public health, and bioethicist
- Biomedical (Molecular physiology and endocrinology; tool: molecular biology
- Clinical (Ob, Neo, Epidemiology) and Populational approaches
- To disseminate, to translate and transfer, and put existing and new knowledges into action for the benefit of these infants we set up a Web-based discussion forum at <http://www.fmed.ulaval.ca/prema/>

On-Going Activities

- 1- We have created the Web-Based Platform**
- 2- The First round of Discussion forum with up-date knowledge in Biomedical, Obstetrical, Pediatric, Ethic, Epidemiology, Psychologic have been published in an Ebook
(<http://www.fmed.ulaval.ca/prema/>)**
- 3- Focus Group with several practioners is on-going.**
- 4- Follow-up of children with recruitment of infants at 5 years-old is on-going. We expect 200 infants in Montréal and Québec**

Acknowledgements

Le lab:

Dr Pierre R. Provost, Res Assistant (1993-)

Dr Tommy Seaborn, *PDF* (2005-2007)

Dr Eva Bresson *PDF*(2008- 04/2009)

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Julie-Alexandra Moulin, *student MSc* (2005-2007)

Julie Plante, *MSc* (2002- 2005)

Mélissa Côté, *student MSc* (2006- 2008

Nouvelles (2008-):

Marie-Christine Gérard Hudon

Audrey Devillers, Geneviève Cormier



Collaborators:

Members of the MUST Group as well as other to different ways

Support:

CHUQ and JLL Foundations, Lung Association from Québec, NSERC, CHIR, **INSTITUTE OF GENDER AND HEALTH** who has believed into this novel approach

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Thank you



GENDER DIFFERENCES AND PREMATURE INFANTS:

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of the Fetal Sex on Clinical Practice**

N-Principal Investigator: Dr Yves Tremblay

STERIODS



CLINICIANS:

BASIC SCIENTISTS :

HEALTH POPULATION :

FORUM'S PARTICIPANTS:

GENDER DIFFERENCES AND PREMATURE INFANTS:

A Novel Integrative Approach to Evaluate the Impact
of the Fetal Sex on Clinical Practice

NPI: Dr Yves Tremblay

STERIODS

CLINICIANS:

PIs: Bruno Piedboeuf, (neo)
Emmanuel Bujold, (Ob)
Francine Lefebvre, (neo)

Project: To document sex differences in long term neurobehavioral development while taking into account neonatal complications and health related difficulties (5 years).

Project: To question the impact of fetal sex on counselling provided by clinicians. We are using small Focus-Group across Canada with obstetricians and neonatologist separately: Québec, McGill, Montréal, Sherbrooke, BC, Edmonton

BASIC SCIENTISTS:

HEALTH POPULATION:

FORUM'S PARTICIPANTS:



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NPI: Dr Yves Tremblay

CLINICIANS:

STERIODS



BASIC SCIENTISTS:

Yves Tremblay, (PhD)
Guy Poirier (PhD)
Pierre Provost (PhD)

Project: To use advanced biomedical technologies to document, at the molecular level, the effect of biological sex on lung development and maturation: genomic, proteomic and metabolomic approaches

HEALTH POPULATION:

FORUM'S PARTICIPANTS:

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CLINICIANS:

STERIODS

BASIC SCIENTISTS

HEALTH POPULATION:

PI: Gina Muckle (psychosocial)

Project: To also document, through access to a population-based cohort (QLSD) of infants born at term, whether the risk of difficulties at school entry for premature infants is similar to that found in the normal population.

FORUM'S PARTICIPANTS:

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NPI: Dr Yves Tremblay

CLINICIANS:

STERIODS

BASIC SCIENTISTS

HEALTH POPULATION:

Pls Raymond Lambert, Yves Tremblay:
Coll: Anne-Marie Gagné (psychologist)
Ginette Mantha (PrémaQuébec)
Mireille Carpentier (Éthique UQAR)
Jean-Pierre Rogel (Journalist and KT)
Caroline Chapraude (Jurist)
Stéphanie Roberge (Epidemiology)

FORUM'S PARTICIPANTS:

To translate existing and new knowledge into realizations for premature infants, we set up a transdisciplinary research-analysis using Web-based discussion forum. Using precise questions, participants evaluate how knowledge gained from research can be integrated to formulate transdisciplinary advice.

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CLINICIANS:

BASIC SCIENTISTS

HEALTH POPULATION:

FORUM'S PARTICIPANTS:



NEXT

To develop new research approach/project
in a near future

Ethic; law; economic prospective related to the cost to be born
prematurely for the person himself but also for Society.

YES

HOW ?

- How to follow-up on the sex difference. There is one way, that consist to target genes under the regulation of androgen early in gestation (microarray)
 - These studies are undergoing and we succeeded to identify cascade that are androgen-dependent.
 - In parallel to the studies on the androgens we also showed that the fetal lung can also act as an adrenals, in that it can produce glucocorticoids and that this production also presents a sex difference (MANY FETUSES DO NOT RESPOND TO ANTENATAL GLUCOCORTICOID EVEN WITH OPTIMAL REGIMEN (Timing))
 - We also identify genes that are involved in the lipid synthesis or transport and therefore could regulate surfactant production. These are also expressed with sex-differences

- In short androgens exert two distinct roles
 - One is deleterious and probably occur as a consequence of the gonad differentiation
 - One is good and occurs in both sexes and is involved in the fibroblasts reprogramming.

- This is great but for the purpose of today these data cannot explain the sex difference.