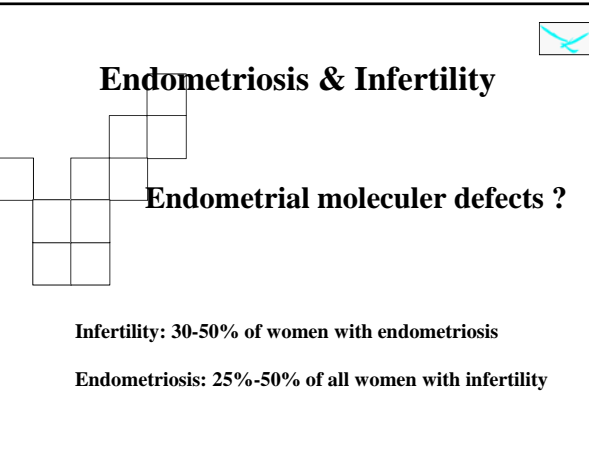


**Eutopic endometrium
among the different types of
endometriosis**

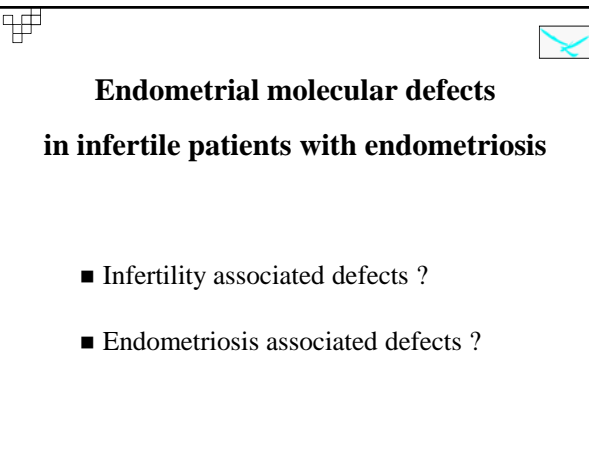
Sachiko MATSUZAKI
CHU Clermont-Ferrand
Clermont-Ferrand, France



Endometriosis & Infertility


Endometrial molecular defects ?

Infertility: 30-50% of women with endometriosis
Endometriosis: 25%-50% of all women with infertility




**Endometrial molecular defects
in infertile patients with endometriosis**

- Infertility associated defects ?
- Endometriosis associated defects ?




Endometrial molecular defects in infertile patients with endometriosis

- Are there any specific genes involved in endometriosis associated endometrial molecular defects?



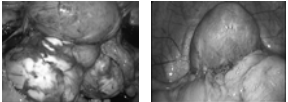
Objective


- To evaluate HOXA-10, E cadherin and β -catenin expression in endometrium from infertile patients with and without endometriosis during the window of implantation

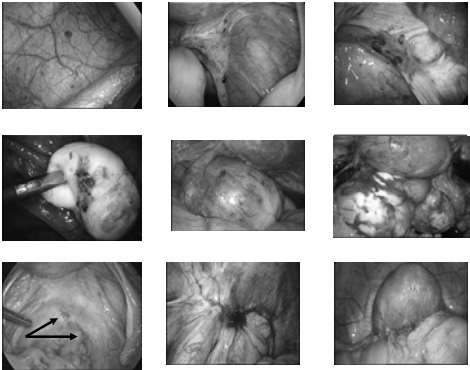



Inclusion & Exclusion criteria

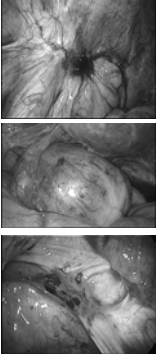
<ul style="list-style-type: none"> ■ Inclusion criteria <input type="checkbox"/> Regular menstrual cycles (between 26 and 32 days) <input type="checkbox"/> Age < 38 y.o. <input type="checkbox"/> No hormonal treatments for at least six months prior to surgery <input type="checkbox"/> Infertility > 2 years 	<ul style="list-style-type: none"> ■ Exclusion criteria <input type="checkbox"/> Bilateral tubal occlusion <input type="checkbox"/> Mechanical distortion of the endometrial cavity by fibroids <input type="checkbox"/> Male factor
---	---



Endometriosis: Multiple different diseases? 




Patients with endometriosis 



Group 1
Deep infiltrating endometriosis
without ovarian endometriosis

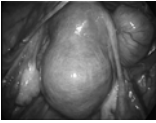
Group 2
Ovarian endometriosis
without deep infiltrating endometriosis

Group 3
Only superficial peritoneal endometriosis

Controls 

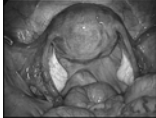
- To assess the specificity of the results (**Infertility associated molecular defects** or **Endometriosis associated molecular defects**), patients with other diseases/disorders that may have clinical, biochemical and metabolic profiles mimicking those of the disease of interest should be included.

Controls



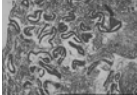
Group 4
Uterine fibromas without endometriosis
Not submucosal fibromas

Group 5
Unexplained infertility

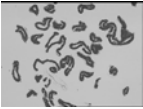
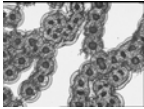


Group 6
Fertile women with normal cavity

Endometrium




Glands **Stroma**

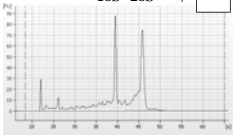
Laser capture microdissection

RNA extraction

RNA integrity



Agilent 2100 Bioanalyzer



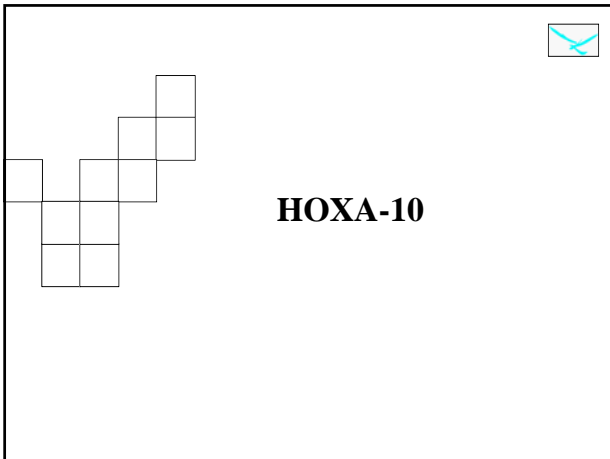
Real-time RT-PCR

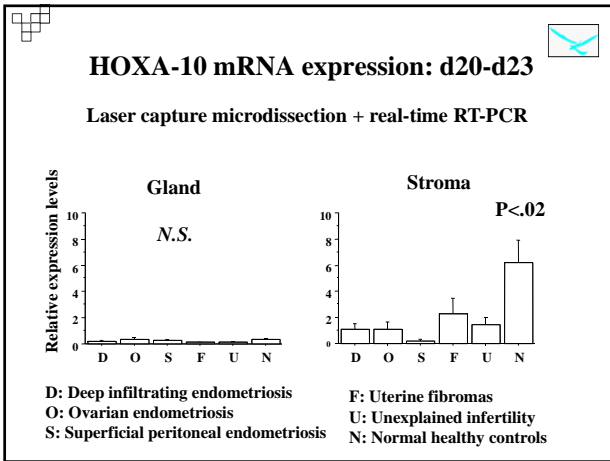
Immunohistochemistry

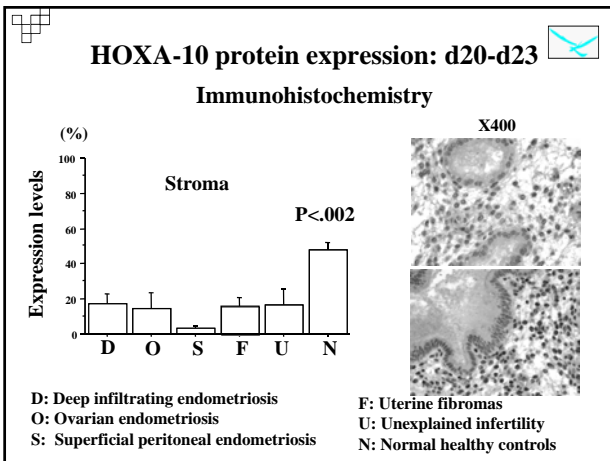
- **Formalin-fixed paraffin embedded section**
- **Primary antibody**
 - HOXA-10 (Santa Cruz, goat polyclonal)
 - E-cadherin (DAKO, mouse monoclonal)
 - β -catenin (DAKO, mouse monoclonal)
- **Quantitation of immunostained cells**

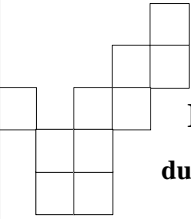

SAMBA 2005 computer analysis system (Alcatel-TITN, France)

- The percentage of immunostained surface (compared with counterstained surface)
- The percentage of immunostained nuclear surface (relative to the total nuclear surface)
- β -catenin-positive microvessel density
(mean β -catenin-positive endothelial cell count per mm² of stroma)









E-cadherin & β -Catenin



during the window of implantation

Wnt, β -catenin and E-Cadherin pathway

E-Cadherin: Cyclic human endometrium

- **mRNA**
 - Increased during mid-secretory phase.**
 - Fujimoto et al., Eur J Obstet Gynecol Reprod Biol. 1996;6:179-83.
- **Protein**
 - Not vary throughout the menstrual cycle.**
 - Van der Linden et al., Fertil Steril. 1995; 63:1210-6.
 - Taylor et al., Am J Obstet Gynecol. 1996;175:411-9
 - Béliard et al. Fertil Steril., 1997; 67:266-72.

β -Catenin: Cyclic human endometrium

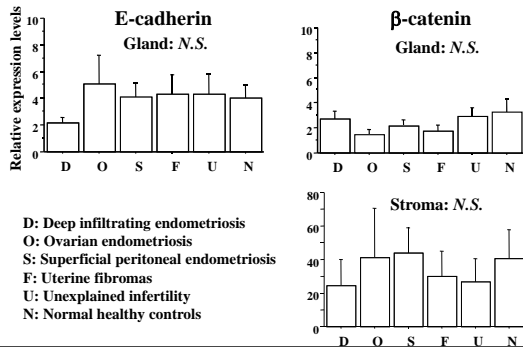
- **mRNA**
 - Increased during the mid-secretory phase.**
 - Fujimoto et al., Eur J Obstet Gynecol Reprod Biol. 1996;6:179-83.
 - Progesterone up-regulates in stromal cells in vitro.**
 - Chen et al., Endocrine. 1998;9:263-7.
 - No cyclical change**
 - Tulac et al., J Clin Endocrinol Metab. 2003;88:3860-6.
- **Protein**
 - Unchanged from the proliferative to the secretory phase.**
Epithelium, stroma, endothelium
 - Tabibzadeh et al., Hum Reprod. 1995;10:776-84.
 - Predominantly observed in the proliferative phase, and decreased in the secretory phase.** *Epithelium*
 - Shih et al., Anticancer Res. 2004;24:3843-50.

E-cadherin & β -Catenin during the window of implantation Animal experiments

- High level of total and active β -Catenin protein: d 3.5, 4.5: mouse
 - Herington et al., J Histochem Cytochem. 2007;55:963-74.
- Up regulation of E-cadherin and β -Catenin protein: mouse
 - Jha et al. FEBS Lett. 2006;580:5653-60.
- Decreased active β -Catenin protein: d 4: mouse
 - Li et al., Biol Reprod 2005; 72:700-6.
- Decreased E-cadherin protein: rat
 - Li et al., J Biol Chem. 2002;277:46447-55.
- Loss of β -Catenin protein in luminal epithelium: d 12-14: ovine
- Decreased E-cadherin protein in luminal epithelium: d 10-14: ovine.
 - Hayashi et al., Endocrinology 2007;148:3496-506.
 - Satterfield et al., Endocrinology 2007;148:3922-931.

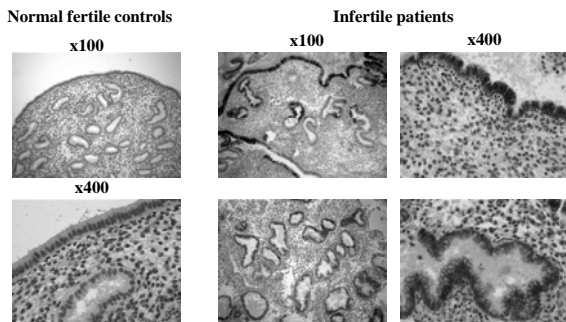
E-cadherin & β -catenin mRNA expression

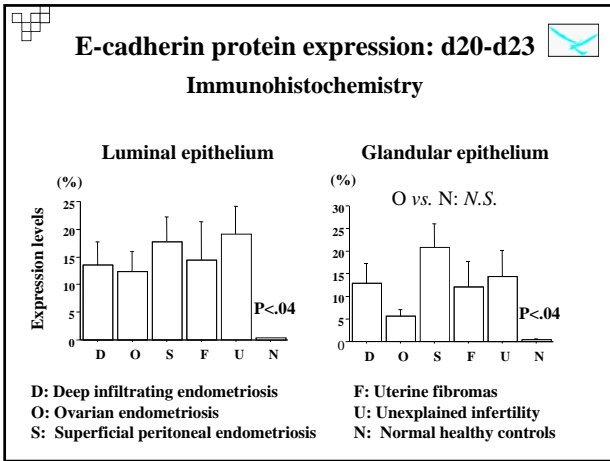
d20-d23
Laser capture microdissection + real-time RT-PCR

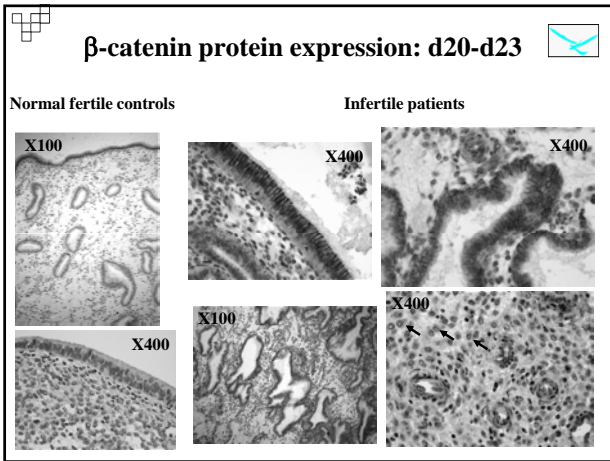


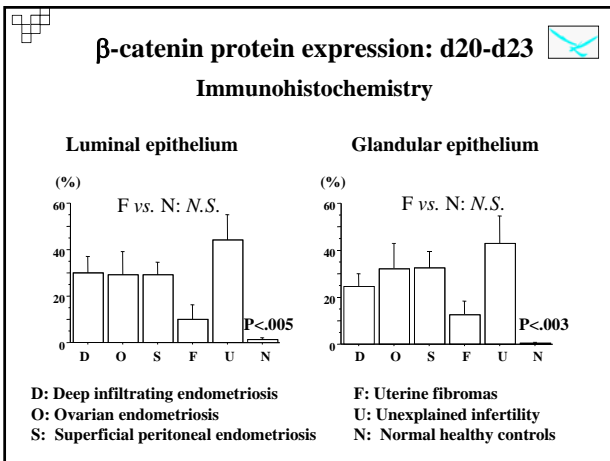
E-cadherin protein expression: d20-d23

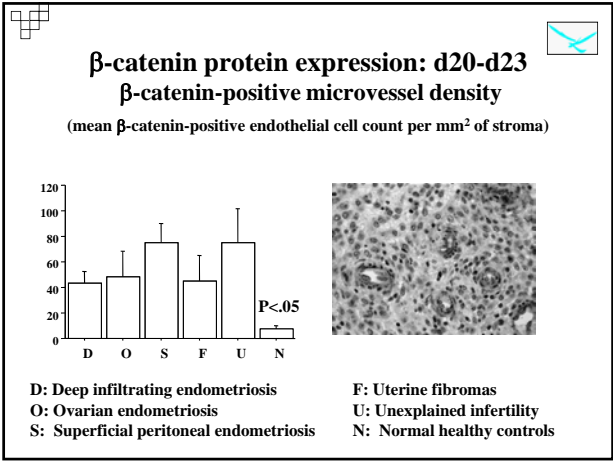
Immunohistochemistry











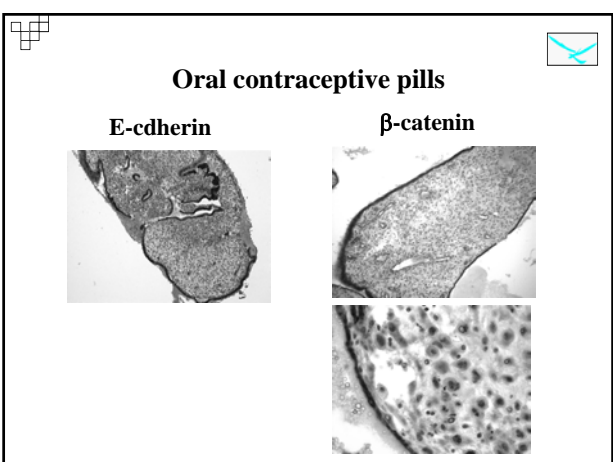
E-cadherin & β-Catenin during the window of implantation

The present study

- Loss of E-cadherin protein in luminal and glandular epithelium
- Loss of β-Catenin protein in luminal and glandular epithelium, endothelium and stroma

Animal experiments

- Decreased active β-Catenin protein: d 4: mouse
 - Li et al., Biol Reprod 2005; 72:700-6.
- Decreased E-cadherin protein: rat
 - Li et al., J Biol Chem. 2002;277:46447-55.
- Loss of β-Catenin protein in luminal epithelium: d 12-14: ovine
- Decreased E-cadherin protein in luminal epithelium: d 10 -14: ovine.
 - Hayashi et al., Endocrinology 2007;148:3496-506.
 - Satterfield et al., Endocrinology 2007;148:3922-931.

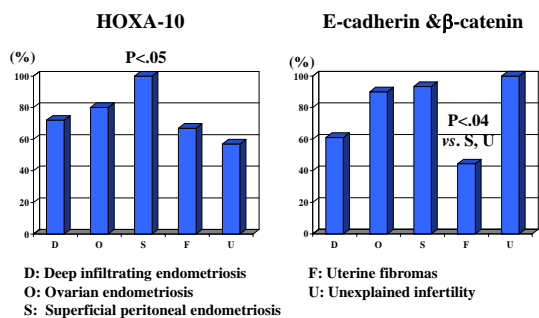


Endometrial molecular defects in infertile patients with endometriosis in the present study

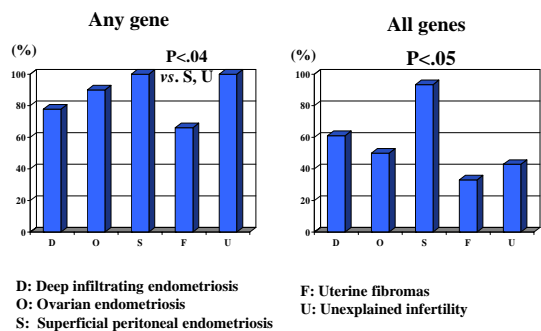
- HOXA-10 mRNA & protein
- E-cadherin & β -catenin protein
 - Altered at post-transcriptional levels

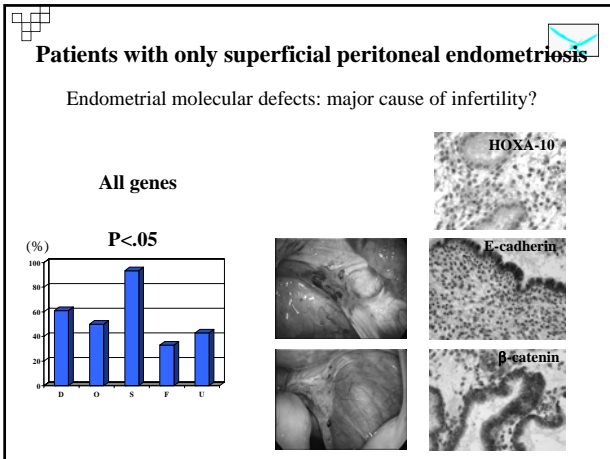
Infertility associated defects in certain patients

Percentage of patients with altered expression



Percentage of patients with altered expression HOXA-10, E-cadherin & β catenin





Acknowledgments

- All the patients who kindly participated in our project and provided invaluable information on endometriosis.
- The staff at the Polyclinique de l'Hôtel Dieu, CHU Clermont-Ferrand, particularly the residents and staff in the operating room.

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- Conseil Régional Auvergne (Recherche et Innovation Technologique) (Clermont-Ferrand, France)
- Karl Storz Endoscopy & GmbH (Tuttlingen, Germany)

**Altered HOXA-10 expression
in infertile patients**

Mid-secretory phase

- **Hydrosalpinx**
 - Daftary et al., Fertil. Steril. 2007;87:367-72.
- **Polycystic ovary syndrome**
 - Cermik et al., J Clin Endocrinol Metab. 2003;88:238-43.
- **Endometriosis**
 - Taylor et al., Hum Reprod. 1999;14:1328-31.

Proliferative phase

- **Submucosal, but not intramural uterine leiomyomas.**
 - Rackow & Taylor, Fertil Steril. 2008, in press

HOXA-10: Infertility associated molecule in certain patients

