Mechanisms for ectopic implantation

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Outline

- Clinical problem
- Appraisal of data to date researching aetiology of ectopic pregnancy
- Limitations of current studies
- New data on risk factors
- Conclusion

Pubmed was searched using the terms 'Fallopian tube' and 'ectopic pregnancy' for studies published from 1999.

For a study to be included, it needed to be primarily focused on elucidating a functional mechanism behind one of the known risk factors for tubal ectopic pregnancy, such as cigarette smoking, chlamydial infection or IVF.

Studies which were solely epidemiological in nature were not included.

Introduction

- Pregnancy implanted outside of the uterine cavity
- (>98% implanting in the Fallopian tube)
- 1–2% of all pregnancies in Europe and the USA are ectopic
- In Western world most common cause of maternal mortality in the first trimester of pregnancy
- In developing world 1 in 10 women admitted with a diagnosis of ectopic pregnancy ultimately die from the condition

Farquhar, Lancet 2005; Walker, Clin Obstet Gynecol 2007; Varma & Gupta, Clin Evid (Online)2009

Hypothesis

Impaired tubal transport causing arrest of the embryo in the Fallopian tube

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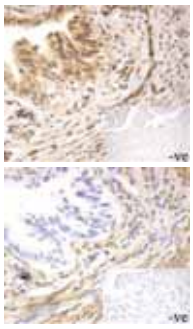
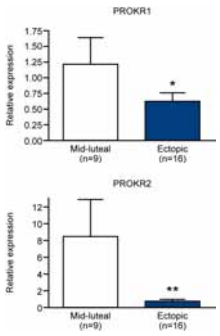
A change in the normal tubal environment encouraging early implantation to occur

Embryo-Tubal Transport

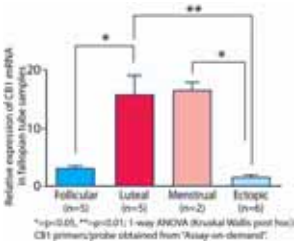
Prokineticins





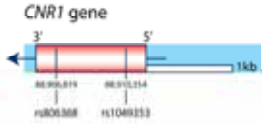
Shaw et al. Fertil Steril 2010

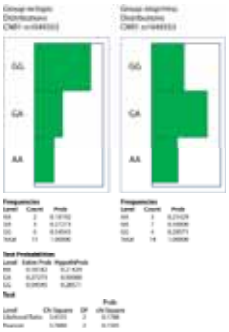
Endocannabinoids



Wang et al. Nat Med 2004; Horne et al. PLoS One 2008

Endocannabinoids





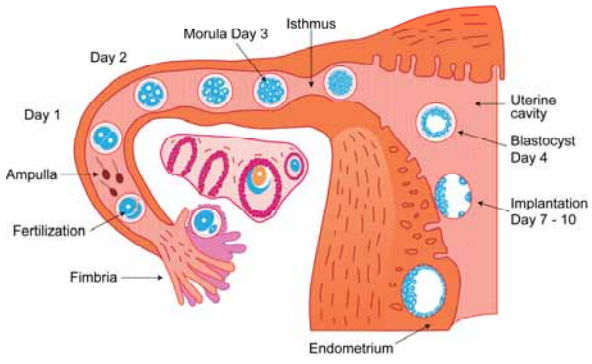
Wang et al. Nat Med 2004; Horne et al. PLoS One 2008

Nitric oxide and nitric oxide synthase

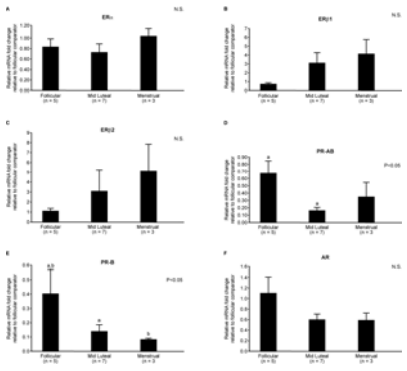
- NO is expressed by Fallopian tube and has relaxing effect on tubal smooth muscle
- Administration of iNOS inhibitors increases tubal smooth muscle contractility in rat
- NO synthesized by iNOS increases ciliary beat frequency in epithelial cells of airway
- iNOS greater in Fallopian tube of women with ectopic pregnancy compared with pseudo-pregnant women

Ekerhovd et al. Gynecol Endocrinol 1997; Perez et al. J Reprod Fertil 2000; Al-Azemi et al. Hum Reprod 2009

Tubal environment



Sex steroid hormone receptors



Horne et al. JCEM 2009

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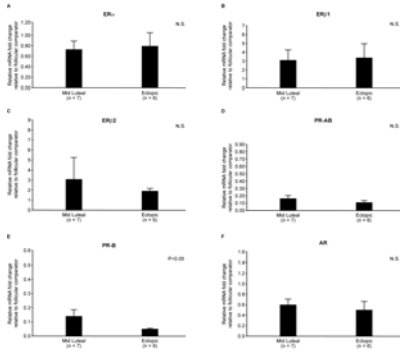

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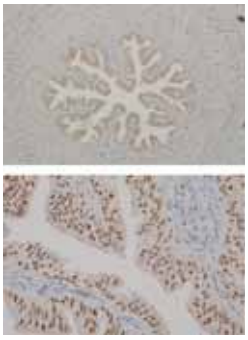
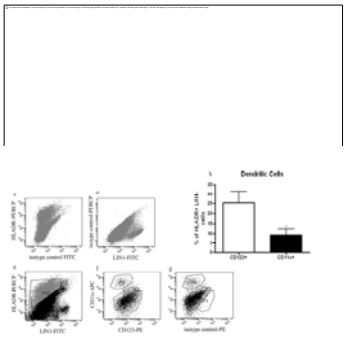


ERα protein is absent from Fallopian tube from women with ectopic pregnancy

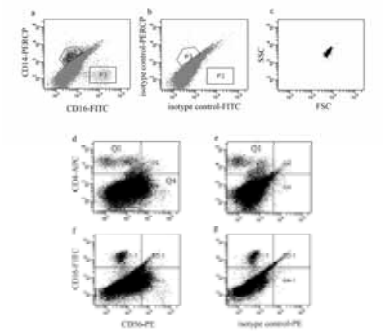
Horne et al. JCEM 2009

Lymphoid and myeloid cells



Shaw et al, in review

Lymphoid and myeloid cells



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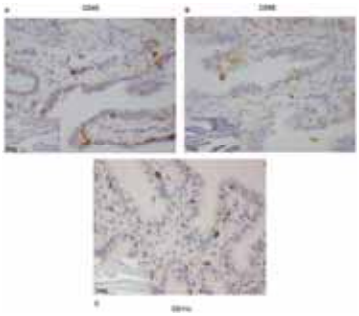

Shaw et al, in review

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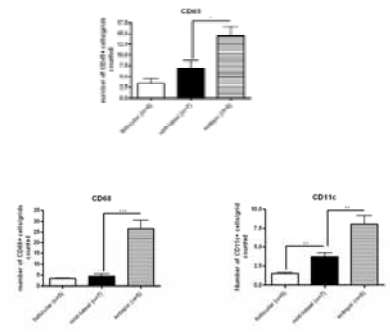
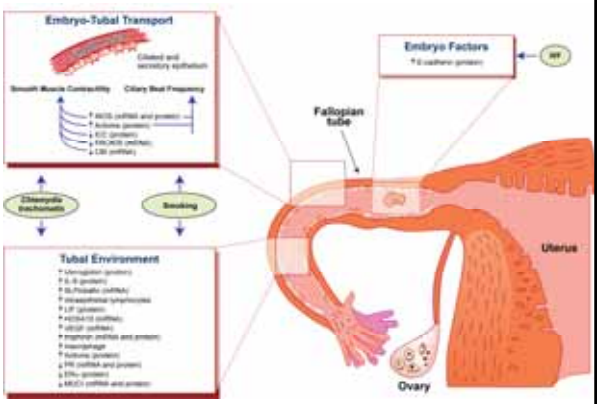


Shaw et al, in review

Gene	Ectopic pregnancy	Implantation site	Explanation	Refs
Uteroglobin	Increased	Decreased	?regulated by embryo	*Quintar et al, 2008*
LIF	Increased	Increased	?chronic salpingitis	*Guney et al, 2008; Ji et al, 2009*
HOXA10	Increased	Increased	?regulated by embro	*Salih & Taylor, 2004*
VEGF	Increased	Increased	?regulated by hCG	*Lam et al, 2004*
MUC1	Reduced Glycosylation altered	Not known	Increased receptivity	*Savaris et al, 2008; Al-Azemi et al, 2009*
Activins	Increased	Not known	?due to chlamydia	*Refaat et al, 2008; Refaat et al, 2009*
Trophinin	Increased	Not known	?regulated by hCG	*Nakayama et al, 2003*
Natural antimicrobials	SLPI and elafin increased	Not known	?due to chlamydia	*Dalgetty et al, 2008*



Shaw et al. Hum Reprod Update 2010

Limitations of studies to date

- Largely descriptive
- Focus on dysregulated gene or protein expression
- Little analysis of functional consequences of observed changes
- Exact mechanism by which risk factors (e.g *Chlamydia trachomatis*, smoking) lead to tubal implantation is unexplained

Animal models

Tubal implantation in animals rare

Abdominal cavity most common site

Embryos degenerate rather than implant in obstructed tubes in non-primates

Only three cases reported in primates

Few epidemiological studies in laboratory animals

From *Corpa, Reprod 2006*

Human *in-vivo* models

- Difficult to collect Fallopian tube from women with healthy intrauterine pregnancies to compare with ectopic pregnancy
- Closest comparator Fallopian tube from non-pregnant women in mid-luteal phase
- 'Pseudopregnant' women

In-vitro models

- Numerous co-culture studies with human embryos and endometrium
- Similar Fallopian tube studies lacking
- Isolation of epithelial cells from tube difficult and they lose morphological features
- Only two immortalised oviductal epithelial cell lines

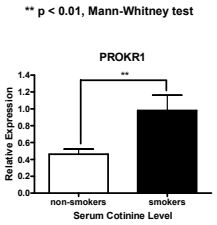
Risk factors for ectopic pregnancy

Chlamydia trachomatis infection, smoking and IVF are most significant risk factors

Farquhar Lancet 2005

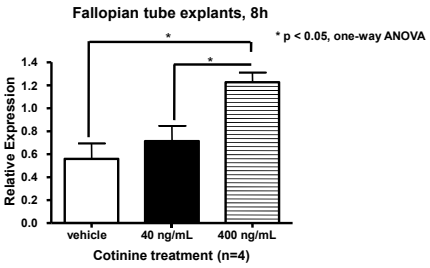
Prokineticins

	Serum cotinine (ng/mL)	Self-reported smoking status
1	< 5	Non-smoker
2	11.3	Non-smoker
3	< 5	Non-smoker
4	8.6	Non-smoker
5	7.4	Non-smoker
6	< 5	Non-smoker
7	< 5	Non-smoker
8	< 5	Non-smoker
9	< 5	Non-smoker
10	< 5	Non-smoker
11	161	Smoker
12	< 5	Non-smoker
13	< 5	Non-smoker
14	> 500	Smoker
15	< 5	Non-smoker
16	> 500	Smoker
17	> 500	Smoker
18	< 5	Non-smoker
19	> 500	Smoker
20	< 5	Non-smoker
21	> 500	Smoker



Shaw et al. Am J Path 2010

Prokineticins



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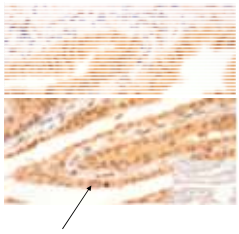
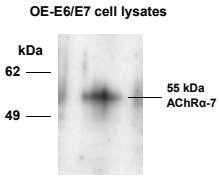
Nicotinic acetylcholine receptor α-7



nAChR⟨-7 expression in the Fallopian tube epithelium



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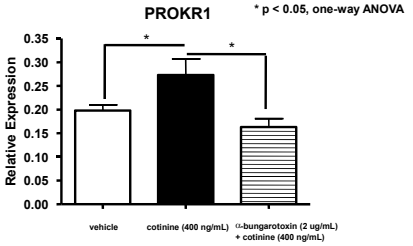
⟨-bungarotoxin (nAChRα-7 antagonist)



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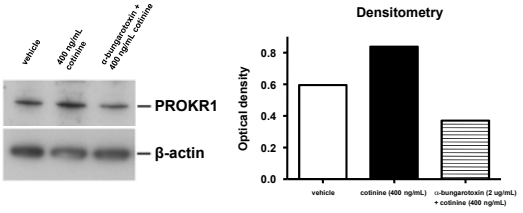
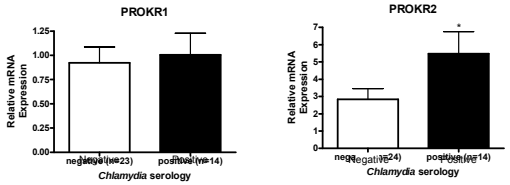


Shaw et al. Am J Path 2010

Gene	Fold change	Function
PDGF-BB	1.5 up	mitogenic, role in wound healing
leptin	1.9 up	in rat oviductual cells, inhibits uterine contractility, role in embryo development
eotaxin-2	1.5 up	chemotactic for lymphocytes / neutrophils, not monocytes
LIGHT	2.0 up	member of TNF family, activates NFKB
eotaxin-3	1.6 up	chemotactic for basophils / eosinophils
SCF	1.5 up	stem cell factor, role in implantation
MCP-2	1.6 up	chemokine for monocytes
TARC	1.9 up	chemokine for immune cells, expressed by cultured endometrial epithelial cells
MIP-3 alpha	1.5 up	chemokine for dendritic cells / lymphocytes
endoglin	1.5 up	anti-angiogenic factor, ↑ in placentas from pre-eclampsia, role in inhibition of trophoblast invasion
MMP-1	1.6 up	important in menstruation and embryo implantation, ↑ mRNA in bovine oviduct at mid-luteal phase, inhibits contraction of bovine SM cells
IL-18 R beta	1.6 up	↓ in secretory endometrium, ↑ in endometrial stromal cells co-cultured with trophoblast cells
Tie-2	1.6 up	localized to cilia of oviductal epithelium in mice
TIMP-4	1.6 up	↑ in mid-secretory phase in endometrium - localized to the stroma

Prokineticins and *Chlamydia*



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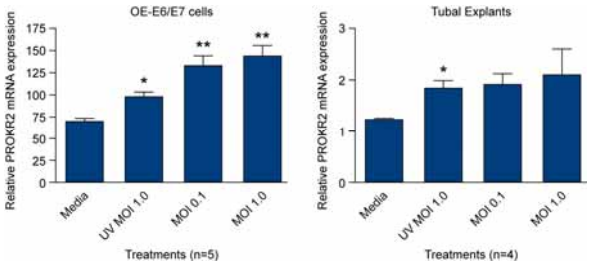
Prokineticins and *Chlamydia*



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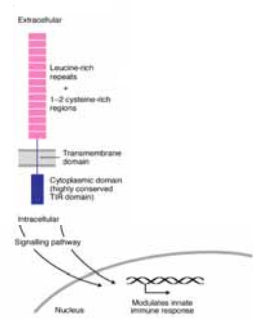
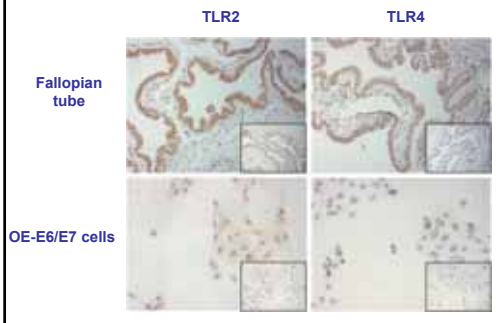
Toll-like receptors

TLRs are pattern recognition receptors

Found mainly on cells of the innate immune system and epithelial cells

Activation and downstream signaling leads to phagocyte activation and production of inflammatory cytokines





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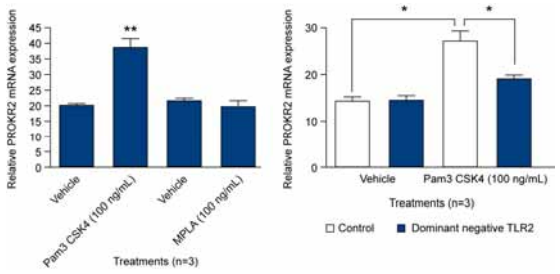
Toll-like receptors



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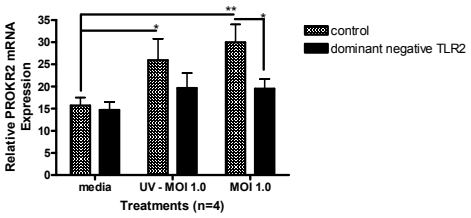
Prokineticins and *Chlamydia*



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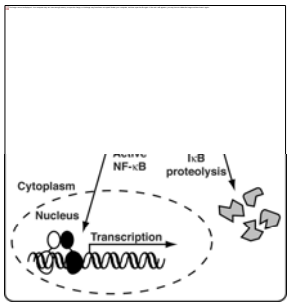
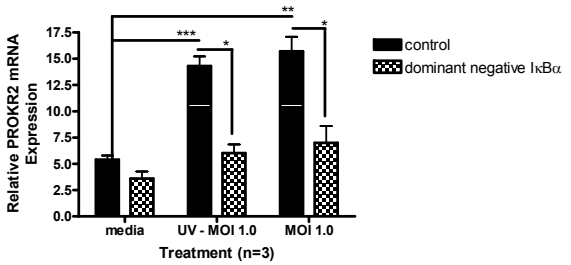
Dominant-negative IκBα



Prokineticins and *Chlamydia*



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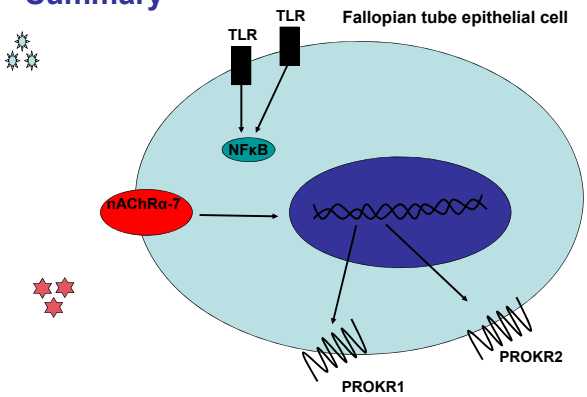
Summary



Conclusions 1

- Ectopic pregnancy caused by Fallopian tube dysfunction causing embryo arrest and changes in tubal environment
- No suitable animal models
- Ethical constraints inhibit the collection of Fallopian tube biopsies from women with healthy pregnancies
- Rely on information derived from Fallopian tube biopsies taken from women with ectopic pregnancies rather than prior to the event
- Exact mechanism by which infection, or smoking, leads to tubal implantation remains unexplained

Conclusions 2

- Data to date largely descriptive and mechanistically speculative
- Require studies on functional consequences of smoking and infection on Fallopian tube pathophysiology
- Greater understanding of aetiology of ectopic pregnancy critical for development of improved preventative measures, advancement of diagnostic screening methods and development of novel treatments

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