A circadian clock in inflammation and adhesion formation?

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Just a few questions….

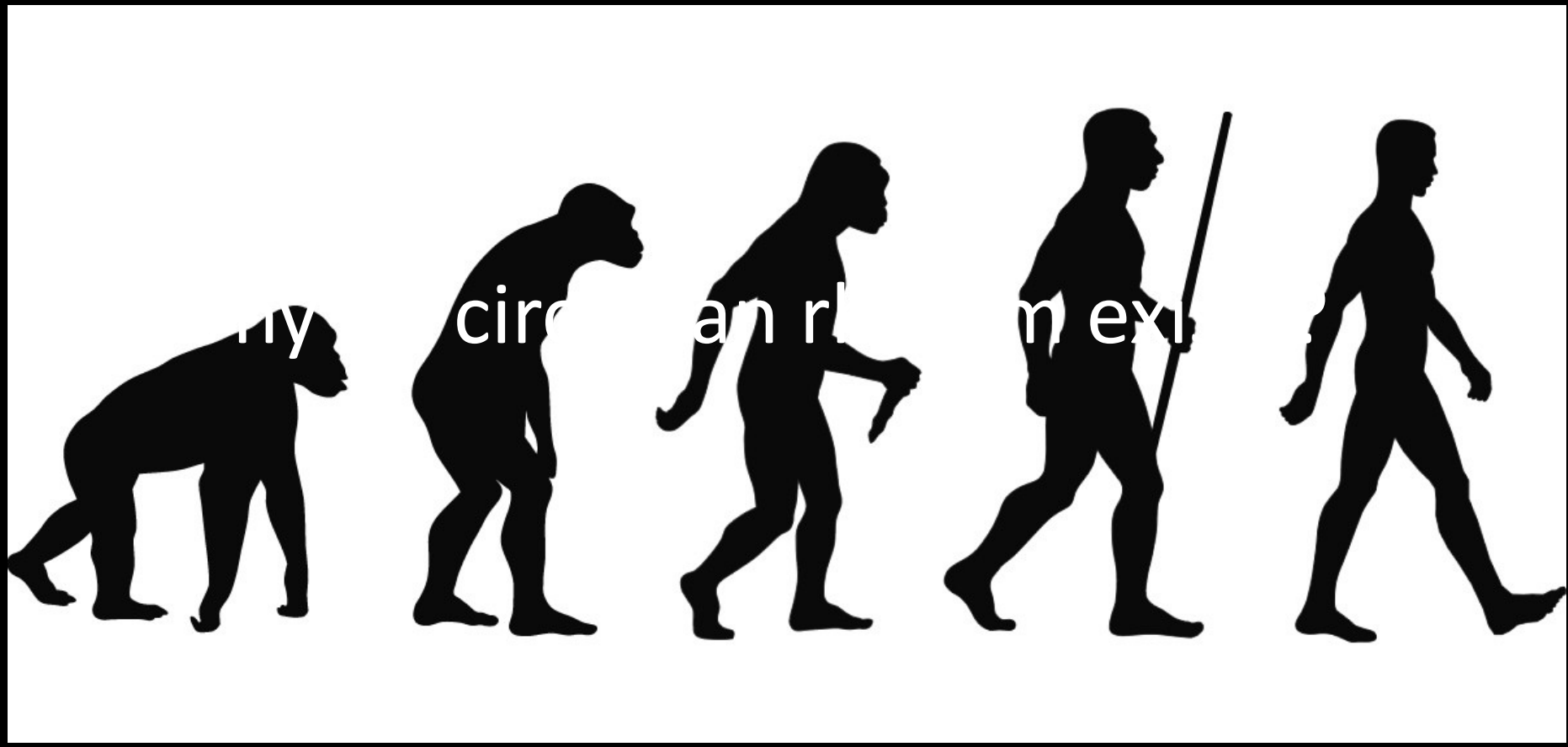
1. What is a circadian rhythm and do we need it?
2. Is adhesion formation a consequence of inflammation?
3. Is inflammation circadian?
4. Is there a circadian clock in adhesion formation?
5. What are the wider implications of the study of circadian clock?

What constitutes a circadian rhythm?

Types of rhythm

Rhythm	Definition
Ultradian	High Frequency – repeats many times in the day
Infradian	Repeats at intervals much longer than 24 hours
Circadian	Approximately a day (24hours)
Circatidal	Approximately every 12.4 hours (with the tide)
Circalunar	Approximately once a month
Circannual	Approximately once a year
Free runnning	Not syncronised to external signals – no rhythm

Body clock - Evolutionary advantage



SCN
+
Peripheral cells

24 hour clock

CLOCK

BMAL

E-Box

Clock genes/
clock controlled genes

CRYs

PERs

AMPK + CKIε

P

Cell nucleus

Other feedback loops

Rev-erb
Ror

Sensory inputs

Endocrine
- Glucocorticoids
- Hormones
- Melatonin

Nervous system
- central
- peripheral (motor & autonomic)

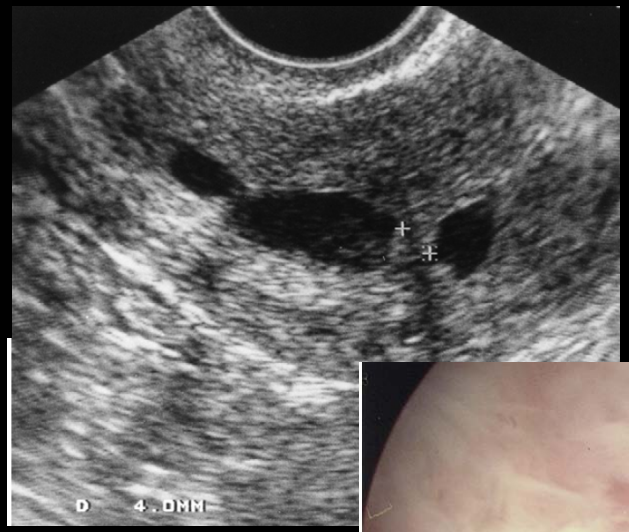
Local signals
- Cellular mediators
- Cells

Vascular systems
Lymphatics?

Some currently identified clock related pathways/components:
-Peroxisome proliferator-activator receptor PPARα (Eckel-Mahan et al, Physio Rev 2013)
-nuclear receptor (hepatic transcription factor, HNF) (Eckel-Mahan et al, Physio Rev 2013)
-Adenosine monophosphate-activated protein kinase AMPK/MTOR (Eckel-Mahan et al, Physio Rev 2013)
-heat shock factor 1 (HSF1) (Tamaru et al, PlusOne 2011)
-cellular redox state (NAD dependent SIRT-1) (Belden et al, Cell 2008)
-Toll-like receptors (Silver et al, Immuni 2011)
-Wnt pathway (Lin et al, Cell Biochemistry & Function, 2012)
-NF-K (Lee et al PNAS 2011; Narasimamurthy et al, 2012)

Adhesion formation is an inflammation dependent process

Salpingo-ovariolysis



Is adhesion formation an inflammatory process?

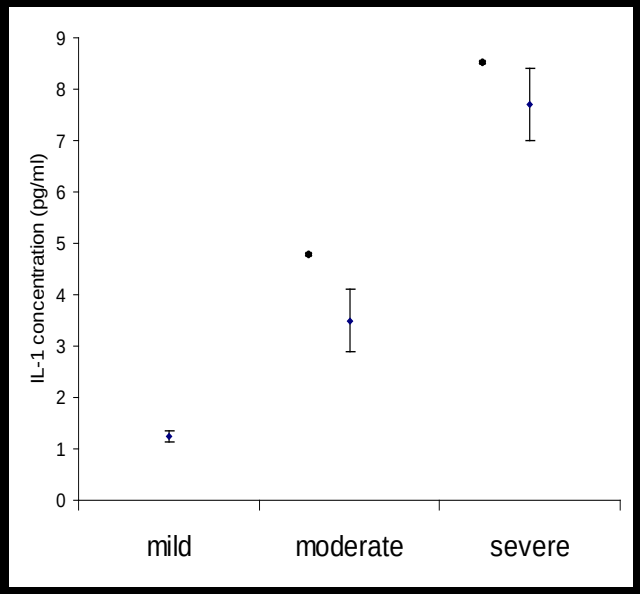
What is the pro-inflammatory cytokine profile of peritoneal fluid in women with adhesions?

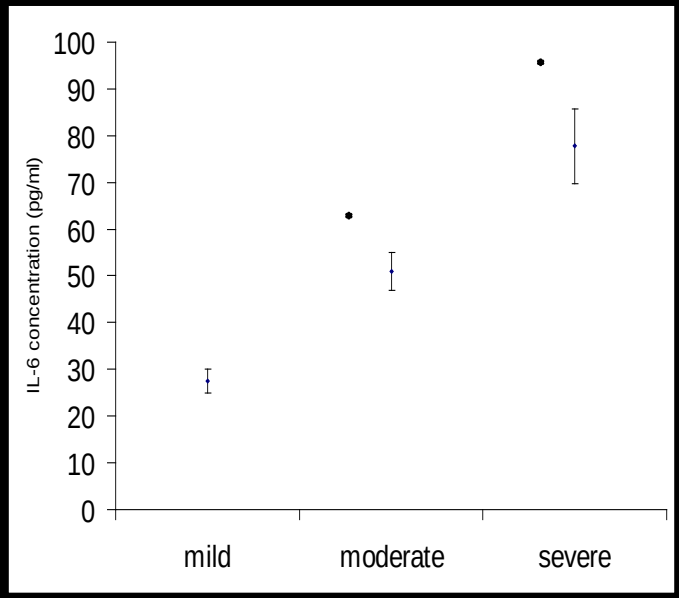
- Peritoneal fluid composition significantly influence the process of adhesion formation
- Hypothesis: pro-inflammatory cytokines concentration (IL-1, IL-6 and TNF-alpha) in the peritoneal fluid of women with adhesions are different to those of women without adhesions

Severity of adhesions

- **153 peritoneal fluid collected**
- **Pelvic adhesions**: 71(46%)
  - mild: 25 (35%)
  - moderate: 25 (35%)
  - severe: 21 (30%)

- **Endometriosis**: 53(35%)
  - grade I: 21(39%)
  - grade II: 10(19%)
  - grade III: 11 (21%)
  - grade IV: 11 (21%)
- **Normal pelvis**: 37(24%)





*** p<0.05**

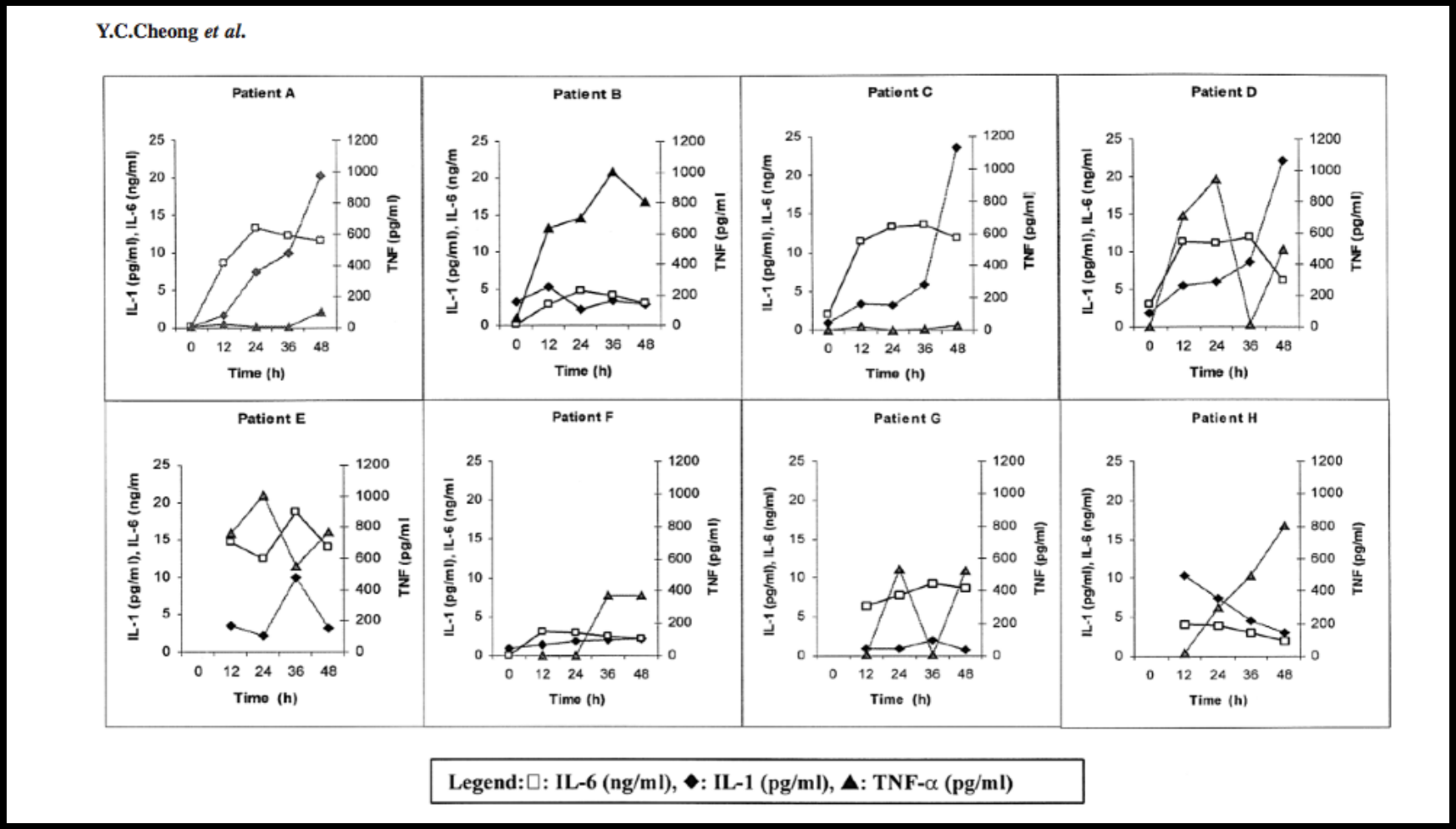
Cheong et al, Hum Reprod 2001

What is the peritoneal fluid pro-inflammatory cytokine profile at the time of adhesion formation/reformation?

- Examine the kinetics of the peritoneal fluid IL-1, IL-6 and TNF-alpha levels within the 48 hour period after adhesiolysis (during the process of adhesion reformation)

- Correlate the results to adhesion reformation

Kinetics of peritoneal fluid concentrations of interleukin (IL)-1, IL-6 and TNF-α 48 hours after adhesiolysis

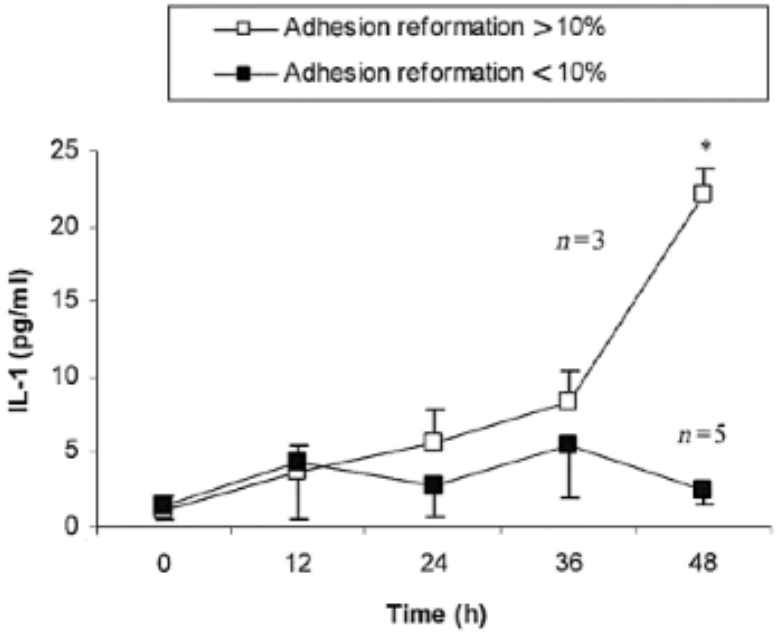


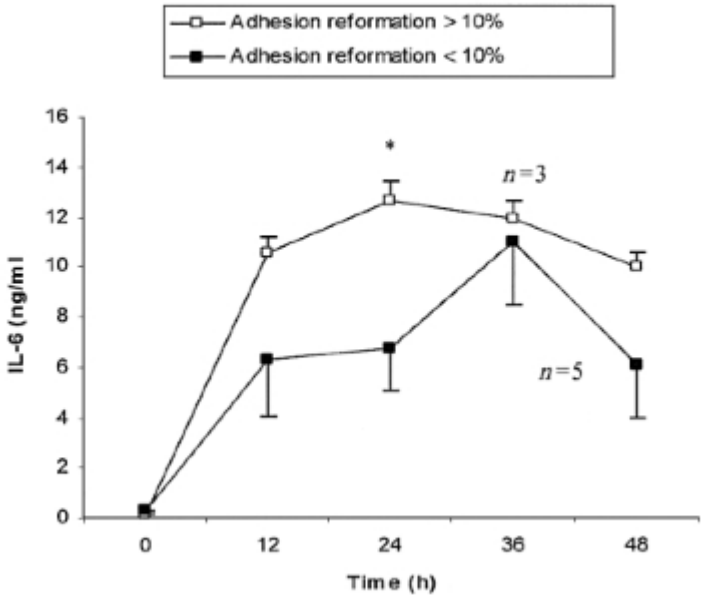
Cheong et al, Hum Reprod, 2002

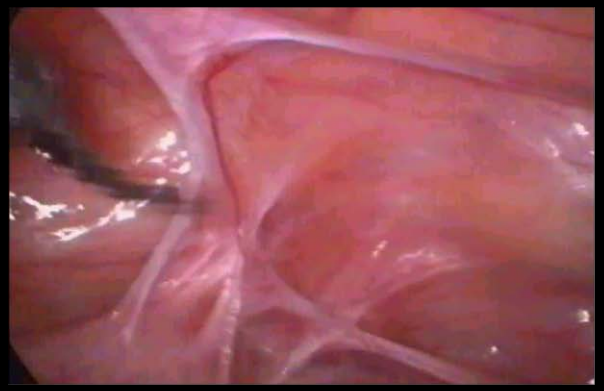
Human Reproduction Vol.17, No.4 pp. 1039–1045, 2002

The correlation of adhesions and peritoneal fluid cytokine concentrations: a pilot study

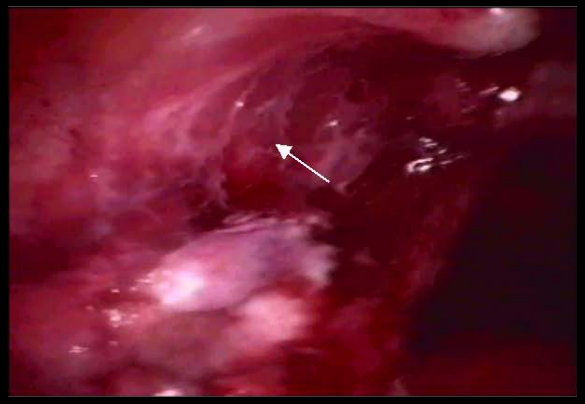
Y.C.Cheong[1], S.M.Laird[2], J.B.Shelton[2], W.L.Ledger[1], T.C.Li[1,3] and I.D.Cooke[1]

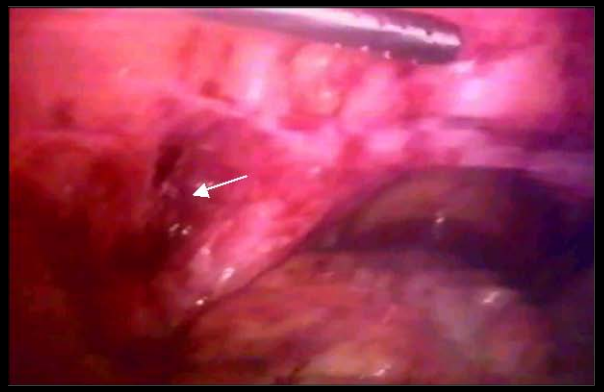




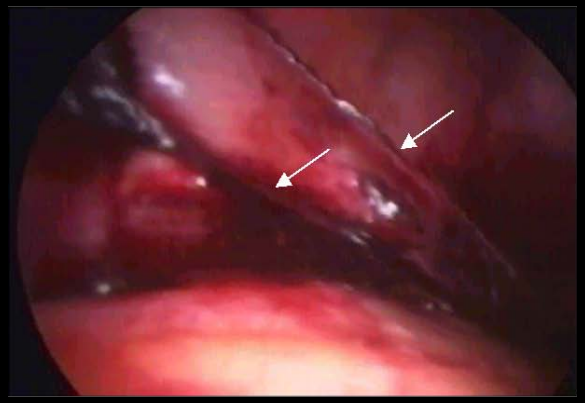


Time
0hrs





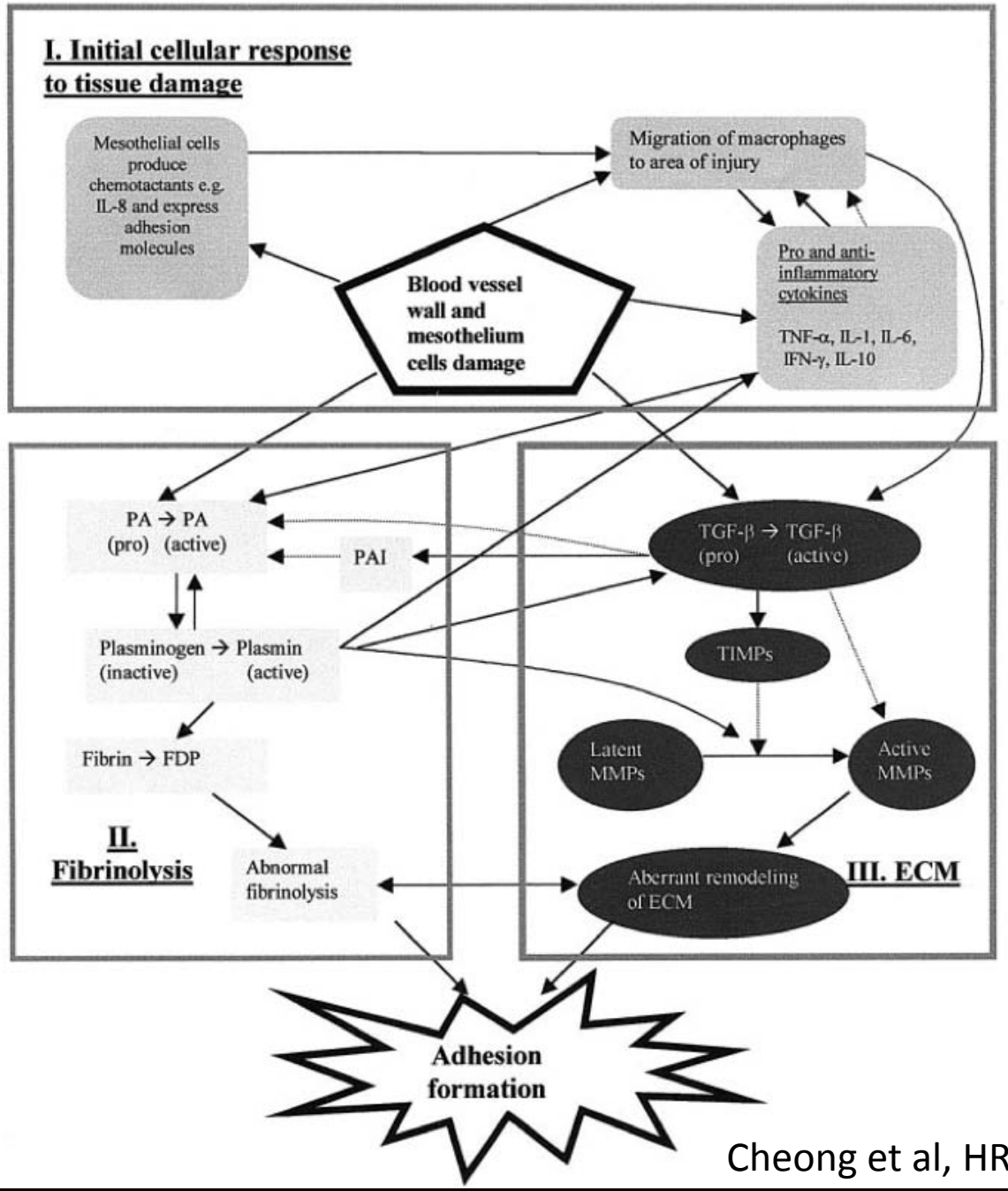
24hrs



48hrs

Patient 1

Patient 2

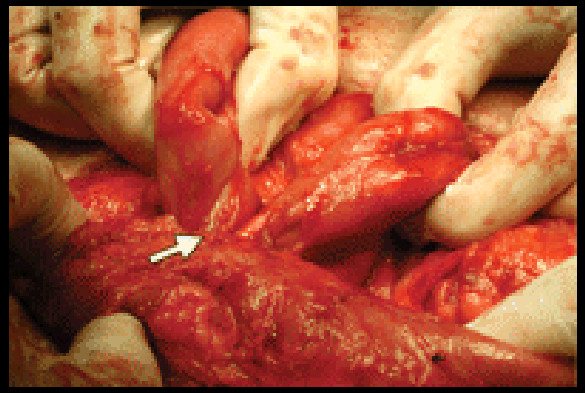


Cheong et al, HRU, 2001

Inflammatory conditions of the peritoneum are adhesiogenic

Pelvic inflammatory disease

Endometriosis



Inflammatory bowel

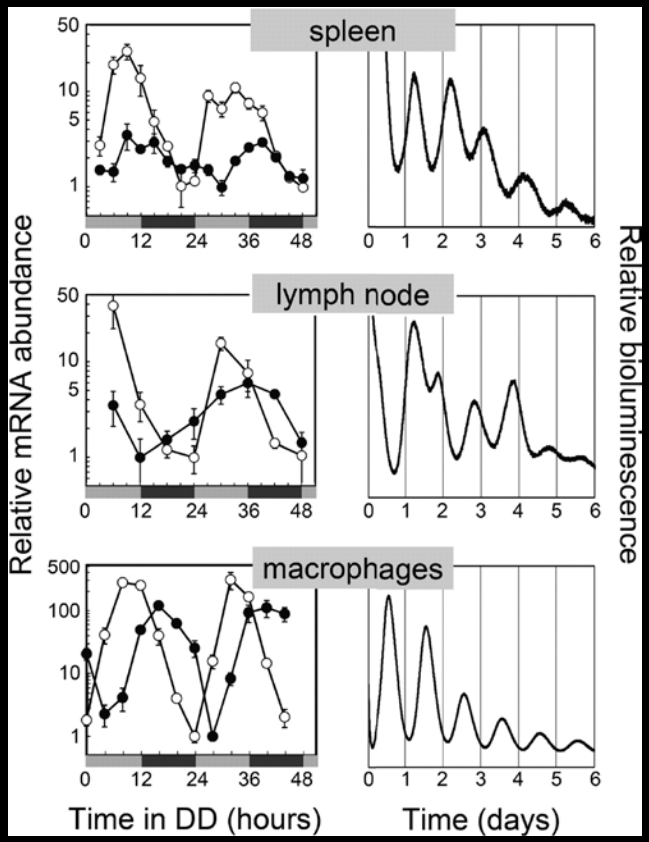
Just a few questions….

1. ✔ What is a circadian rhythm and do we need it?
2. ✔ Is adhesion formation a consequence of inflammation?
3. Is inflammation circadian?
4. Is there a circadian clock in adhesion formation?
5. What are the wider implications of the study of circadian clock?

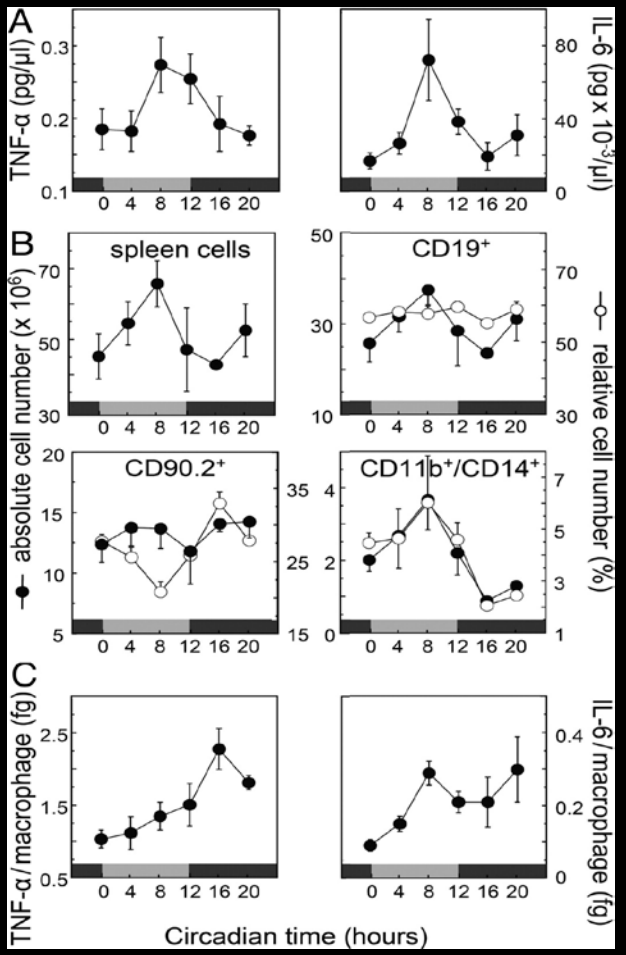
Is inflammation circadian? The laboratory evidence…

Is inflammation circadian?

Fully competent circadian clocks in tissues and cells of the immune system.

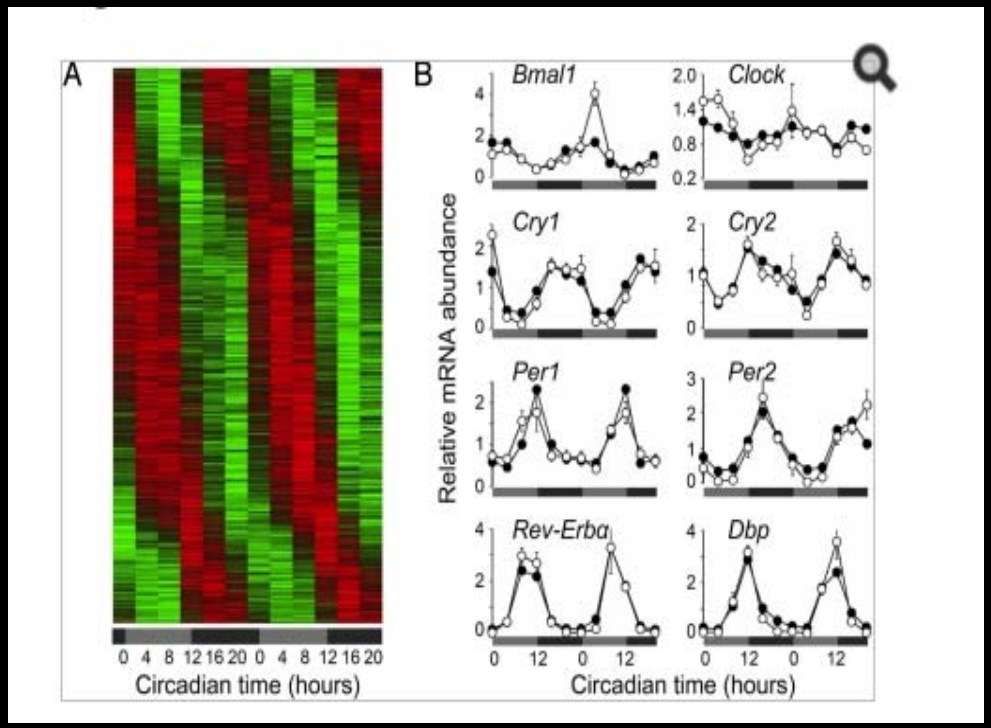


Circadian cytokine secretion upon challenge with bacterial endotoxin.



Keller M et al. PNAS 2009;106:21407-21412

Eight percent of all transcripts in macrophages are expressed with a circadian rhythm.



Inflammation is a circadian process!

Factors key to adhesion formation	Participants	Circadian	References
Cellular components			
Macrophage	Mouse peritoneal macrophagess	✓	Hayashi et al, Biol Pharm Bull, 2006; Keller et al, PNAS 2009;
Fibroblast	Human skin	✓	Sandu et al, Cell Mol Life Sc 2012; Yagita et al, Science 2001;
Lymphocyte	Rat	✓	Esquifino et al, Brain Behav immun, 1996
Leukocyte			
Platelets			al, Platelets, 2012
NK cells			
Erythrocytes			
T & B Cells			
Mediators			
Fibrinolysis PA PAI			ti et al, Chronobiol Int
Cytokines IL-1 IL-6 TNF-α TGF-β/activin VEGF GMCSF	Mouse, human	✓	Young et al, Chronobiol Int, 1995; Motzkus et al, J Mol Neurosci 2002; Cavadini et al, Proc Natl Acad Sci USA 2007; Kon et al, Nat Cell Biol 2008; Koyanagi et al, Cancer Res 2003
Integrins MAC-1 VCAM	Human	✓	Redwine et al, Brain Behav Immun 2004; ten Hacken et al, Clin Exp Allergy 1998.
Matrix remodelling MMP, TIMPs Collagen	Human	✓	Martino et al, J Mol Med 2004;Markoulli et al, Invest Ophthalmol Vis Sci, 2012

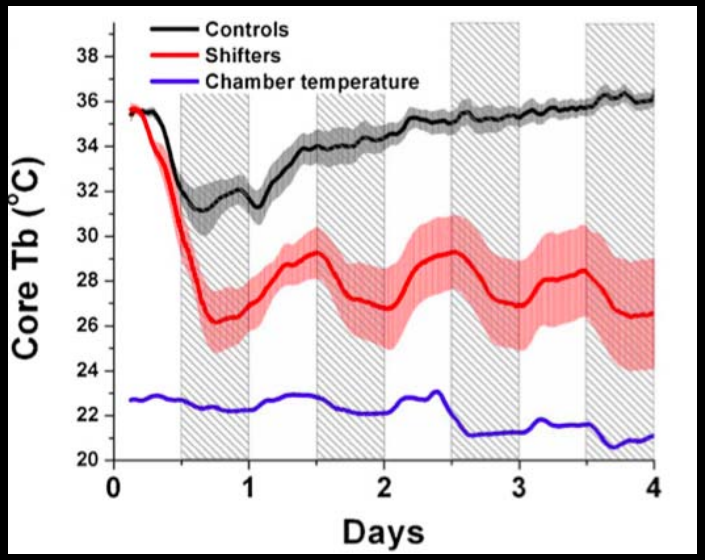
Many key components of adhesion formation process has been shown to be circadian…

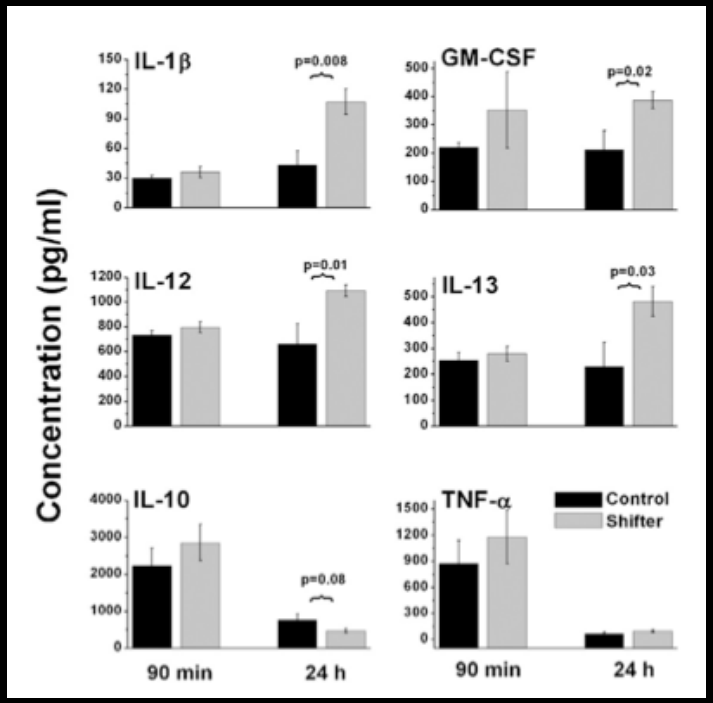
Modified from Sadek et al, Med Hypothesis 2010

When does disrupted circadian rhythm becomes pathological?
Tipping the balance….

If we alter the body clock, what happens to inflammation and healing? Laboratory data…

- Exposure to altered day night rhythm dysregulates inflammatory response and increases susceptibility to endotoxins in mice (Castanon-Cervantes et al, J Immunol 2010)





- Knock out models

Knock out models	Biological effect	References
Bmal1$^{-/-}$ mice NONO^{gt} mice Per1/per2^{mut}	Poor wound healing; disorganised granulation tissue Immature granulation and fibroblast proliferation Defective wound healing	Kowalska et al, PNAS 2012

Circadian rhythm in clinical practice

1. Many inflammation dependent pathologies are predisposed to the time of day

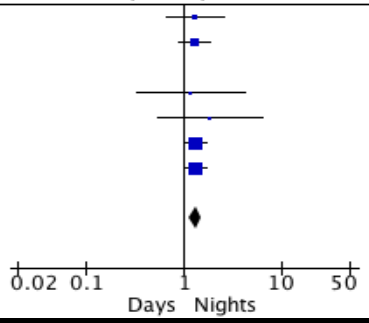
Clinical entity	Circadian manifestation	References
Rheumatoid arthritis	Maximum stiffness, pain ~ 8am and symptoms correlates with higher and more prolonged inflammatory response	Straub et al, Arthritis & Rheumatism, 2007
Myocardial infarction, angina, stent thrombosis	More prone to occur at night/ early morning hours	Isik et al, 2012
Renal colic	Morning peak	Manfredini et al, BMJ 2002
Asthmatic attack	Worst at night	Martin et al, Chronobiol Int 1999

2. Disruption of circadian rhythm is associated with reproductive disorders

Miscarriage

Study or Subgroup	Nights Events	Nights Total	Days Events	Days Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Axelsson 1989	19	163	17	185	5.0%	1.30 [0.65, 2.60]
Axelsson 1996	57	367	71	567	16.6%	1.28 [0.88, 1.87]
Eskenazi 1994	0	0	353	1057		Not estimable
Hemminki 1985	4	12	27	90	1.5%	1.17 [0.32, 4.20]
Infante-Rivard 1993	4	11	165	700	1.1%	1.85 [0.54, 6.41]
Lawson 2012	89	664	536	5109	37.7%	1.32 [1.04, 1.68]
Whelan 2007	91	680	541	5242	38.0%	1.34 [1.06, 1.70]
Total (95% CI)		**1897**		**11893**	**100.0%**	**1.33 [1.14, 1.54]**
Total events	264		1357			

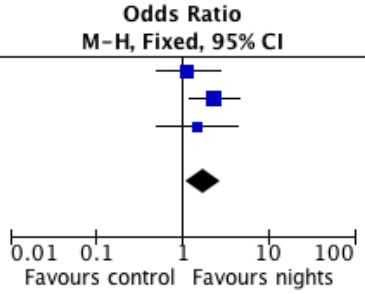
Heterogeneity: $Chi^2 = 0.36$, df = 5 (P = 1.00); $I^2 = 0\%$
Test for overall effect: Z = 3.72 (P = 0.0002)



Menstrual dysfunction

Study or Subgroup	Experimental Events	Experimental Total	Control Events	Control Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Chung 2005	12	41	19	72	35.9%	1.15 [0.49, 2.71]
Hatch 1999	35	79	21	83	42.0%	2.35 [1.21, 4.57]
Su 2008	33	280	4	49	22.1%	1.50 [0.51, 4.45]
Total (95% CI)		**400**		**204**	**100.0%**	**1.73 [1.08, 2.77]**
Total events	80		44			

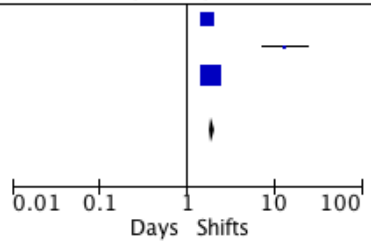
Heterogeneity: $Chi^2 = 1.74$, df = 2 (P = 0.42); $I^2 = 0\%$
Test for overall effect: Z = 2.29 (P = 0.02)



Small for gestational age

Study or Subgroup	Shifts Events	Shifts Total	Days Events	Days Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Croteau 2006	609	1657	900	3613	37.1%	1.75 [1.55, 1.99]
Xu 1994	59	298	14	762	0.7%	13.19 [7.23, 24.05]
Zhu 2004	758	7772	1722	32465	62.2%	1.93 [1.76, 2.11]
Total (95% CI)		**9727**		**36840**	**100.0%**	**1.94 [1.80, 2.08]**
Total events	1426		2636			

Heterogeneity: $Chi^2 = 41.68$, df = 2 (P < 0.00001); $I^2 = 95\%$
Test for overall effect: Z = 18.04 (P < 0.00001)

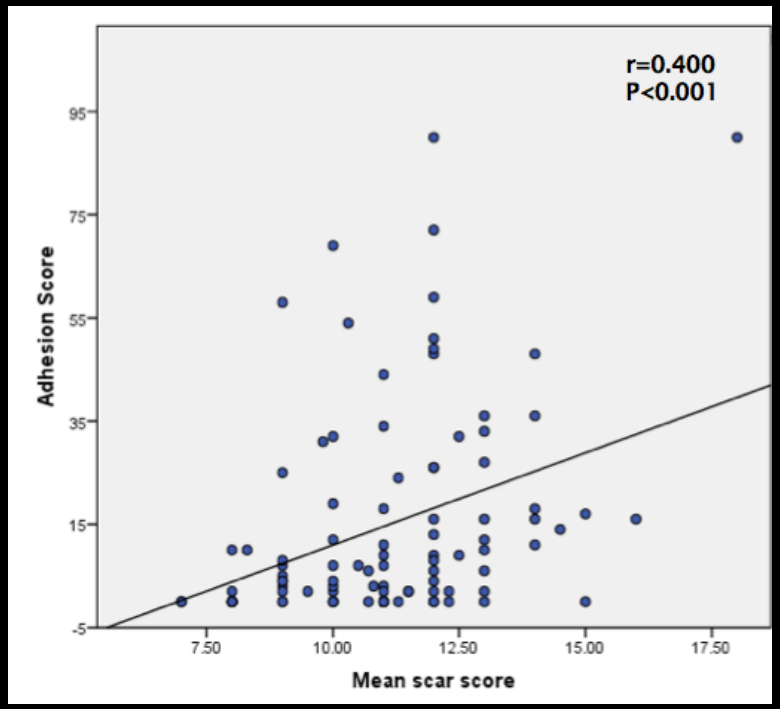


Stocker et al.

2. Disruption of circadian rhythm predispose to many inflammatory disorders

Clinical entities	Risk	References
Cancers (Breast, prostate, colorectal, lymphoma)	↑	Schernhammer et al, J Natl Cancer Inst, 2003; Schernhammer et al, Epidemiology 2006; Lahti et al, Int J Cancer 2008; Kubo et al, Am J Epidemiol, 2006
Metabolic disorders	↑	Morikawa et al, Scand J Work Environ, 2005; Karlsson et al, Occup Environ Med 2001;
Cardiovascular event	↑	Tenkanen et al, Scand J Work Environ 1998; Haupt et al, Artherosclerosis, 2008; Tuchsen et al, Occup Environ Med 2006;
Skin disorders	↑	Ruiz et al, Innate Immunity 2012; Hirotsu et al, PlusOne 2012

Similarities between cutaneous and peritoneal healing

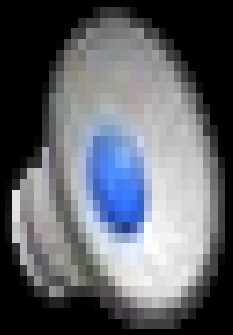


	No Adhesions (n= 29)	Mild	Moderate Adhesions (n= 71)	Severe	p value
Colour					
Perfect	4	6	0	0	P=0.73
Slight mismatch	18	40	6	1	
Obvious mismatch	7	13	3	0	
Gross mismatch	0	1	1	0	
Colour					
Matt	25	43	8	1	P=0.63
Shiny	4	17	2	0	
Contour					
Flush with the surrounding skin	15	26	2	1	p=0.23
Not flush	14	34	8	0	
Texture*					
Normal	17	21	2	1	p=0.01
Palpable	12	39	8	0	
Margins					
Distinct	22	37	7	1	P=0.51
Indistinct	7	23	3	0	
Size*					
<1cm	15	16	0	0	
1-5cm	6	18	3	1	p=0.03
>5cm	8	26	7	0	
Number of incisions*					
Single	27	35	6	1	p=0.01
Multiple	2	25	4	0	

Stocker et al

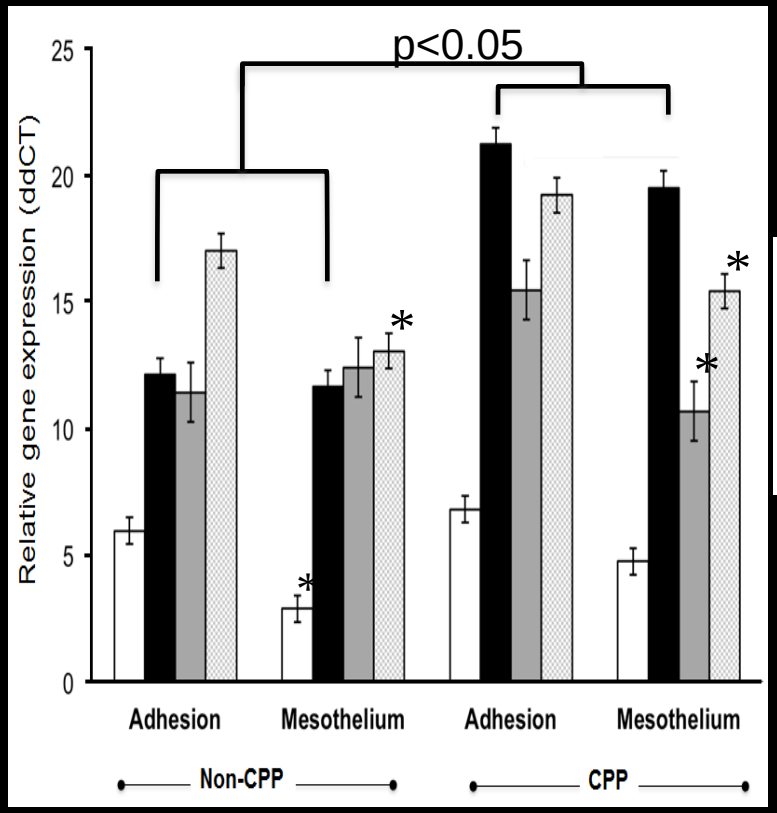
Sadek et al

Do human adhesion tissue have clock genes?

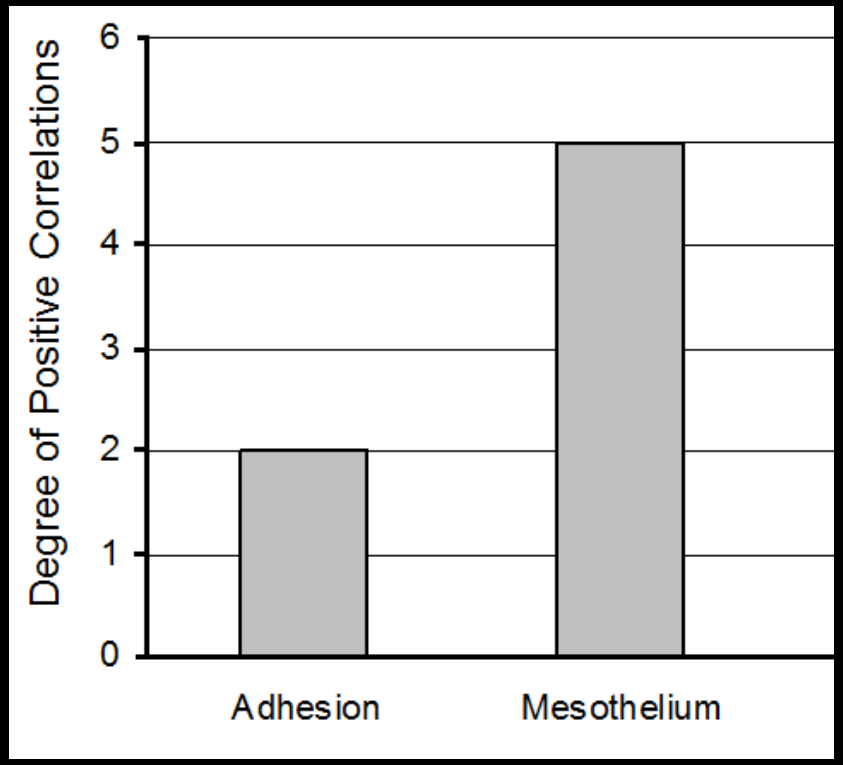


- N=26 paired adhesion and mesothelium
- Recruited women were aged 18-45 years old, had no history of malignancy, endometriosis, recent pregnancy or shift work

Expression level of clock genes in adhesion and mesothelium



Values are means±SEM. *p<0.05 between adhesion and mesothelium in the non-CPP or CPP groups.



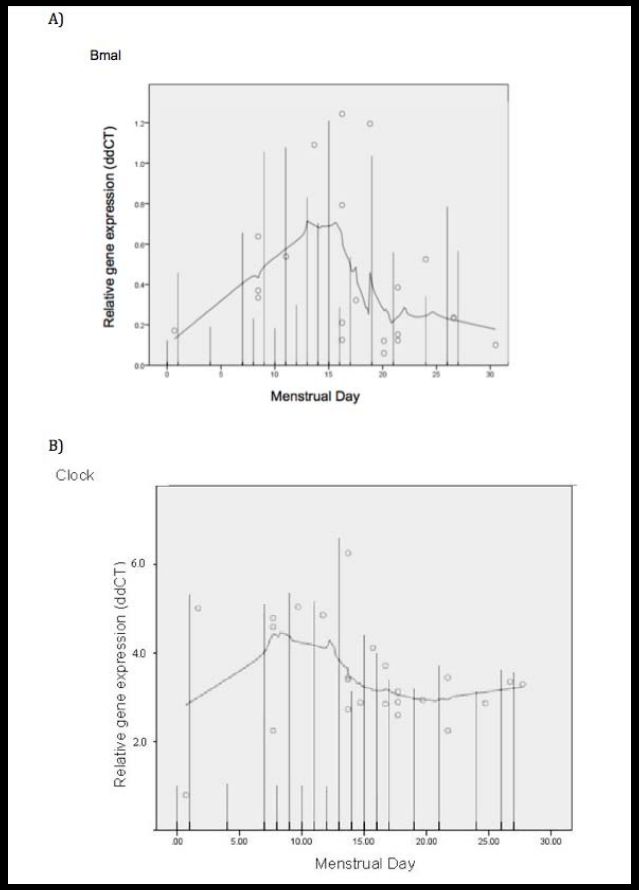
Degree of correlation in gene expressions within normal or pathological tissue, stratified for disease state

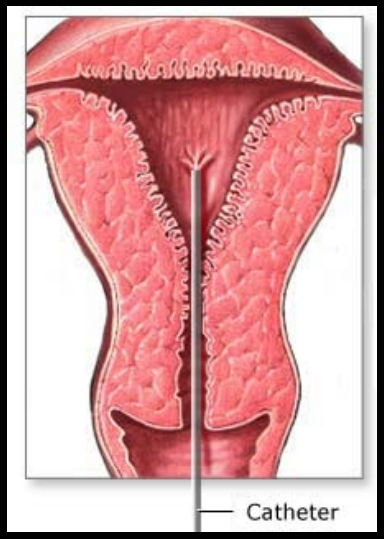
Sadek et al,

Clock genes are also expressed in the endometrium

Sadek et al

Relative gene expression (ddCT) values plotted against date of last menstrual period (A) *Bmal1* expression (B) *Clock* expression





50 endometrial biopsies
Mean age of 34±5.4 years
Mean BMI of 25.8±6.6

Levels of mRNA expression for the clock genes were quantified by qRT-PCR

Why are these clock genes in the adhesion and endometrial tissue?

No one knows

Yet….


OK so what??

Therapeutic implications

Time for chronotherapy? Clock genes dictate sensitivity to cyclophosphamide

Carla B. Green*
Department of Biol

PNAS

Temporal
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Regulation of Circadian Behavior and Metabolism by Synthetic REV-ERB Agonists

Laura A. Solt[1,*], Yongjun Wang[1,*], Subhashis Banerjee[1], Travis Hughes[1], Douglas J. Kojetin[1], Thomas Lundasen[1], Youseung Shin[2], Jin Liu[1], Michael D. Cameron[2], Romain Noel[2], Seung Hee Yoo[3], Joseph S. Takahashi[3], Andrew A. Butler[4], Theodore M.

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Chronotherapy With Low-Dose Aspirin for Prevention of Complications in Pregnancy

Diana E. Ayala,[1] Rafael Ucieda,[2] and

[1]*Bioengineering & Chronobiology Laboratorie*
Physiopathology Service, Obstetrics and Gyne
Coruña, Spain

EXTENDED REPORT

Low-dose prednisone chronotherapy for rheumatoid arthritis: a randomised clinical trial (CAPRA-2)

Frank Buttgereit,[1] Daksha Mehta,[2] John Kirwan,[3] Jacek Szechinski,[4] Maarten Boers,[5] Rieke E Alten,[6] Jerzy Supronik,[7] Istvan Szombati,[8] Ulrike Romer,[9] Stephan Witte,[9] Kenneth G Saag[10]

ABSTRACT
Objective To assess the efficacy and safety of low-dose prednisone chronotherapy using a new

with circadian biological rhythms. The chrono-therapeutic approach has shown promise in sev-eral therapeutic areas, including the management

Is it time for chrono-therapeutics in healing?

Conclusion

- Circadian rhythm is important for many biological / physiological processes
- Disruption of the circadian rhythm is associated with diseases such as cancer, cardiovascular disorders and metabolic diseases
- Inflammation is govern by a circadian rhythm
- Many molecular and cellular pathways of adhesion formation has been shown to be circadian in nature
- Adhesion tissue and endometrium express clock genes although the biological function is yet to be uncovered
- Future research needs to investigate the molecular and clinical importance of the body clock in the pathogenesis of adhesions in order to translate chronobiological mechanisms into possible chronotherapy

Thank you

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Linden Stocker
Jane Glazebrook
Thommai Anthony
Asha Jeganathan
Joanna Radcliff
Kate Gomes

T C Li
Susan Laird
William Ledger
Ian Cooke

Felino Cagampang
Kim Bruce

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Sheffield Charitable Foundation
Complete Fertility Centre Southampton
Research Medical Council Southampton
Moulton Charitable Foundation