Preclinical animal models for adhesion research.

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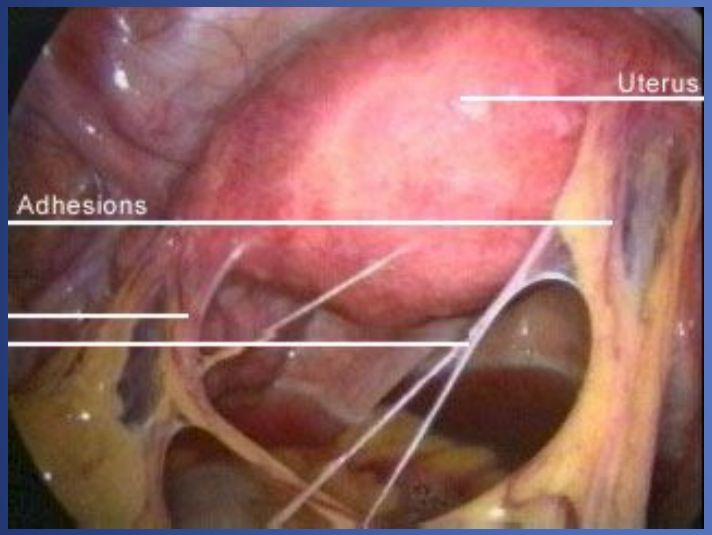
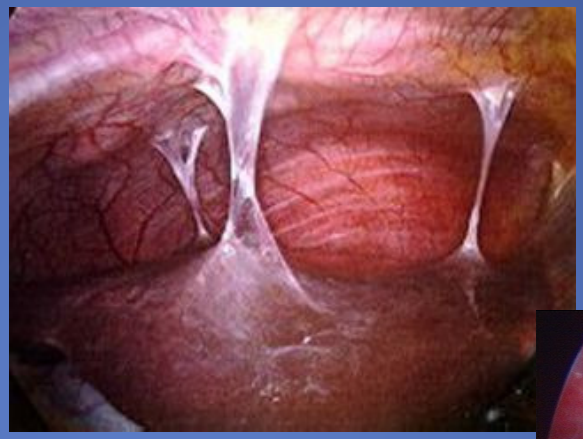
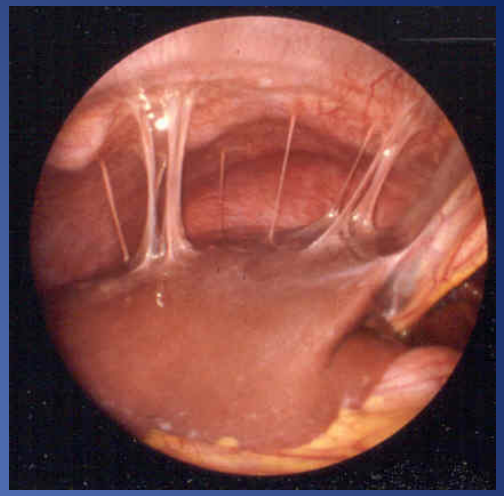
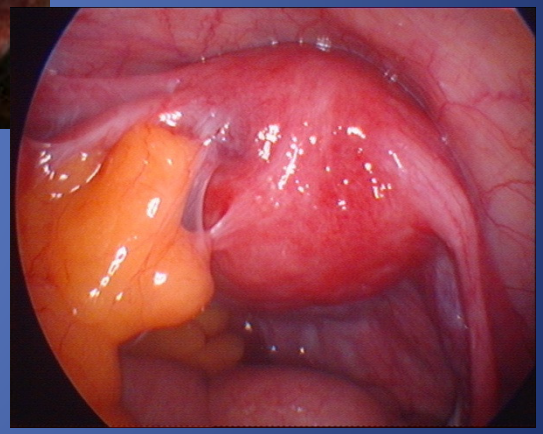
ESHRE symposium course "System biology tools and preclinical models for translational research in endometriosis and adhesion formation: lessons from cancer and inflammation biology". Leuven, Belgium, September 4-5, 2009

Intraperitoneal Adhesions



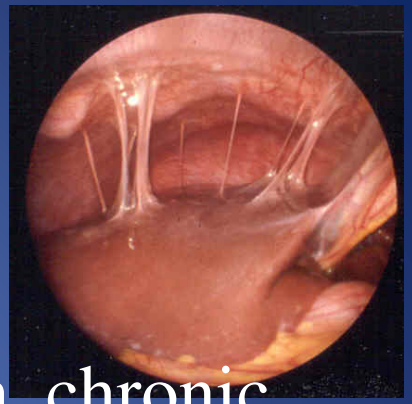






Abnormal fibrous connections between surfaces in the abdominal cavity.

Important Clinical Problem



Associated morbidity: intestinal obstruction, chronic pelvic pain, female infertility

Difficulties at the time of reoperation: difficult access, organs injury, bleeding.

Large utilisation of healthcare resources: increased operating, anaesthesia and recovery time, need of blood transfusion, and use of surgical material.

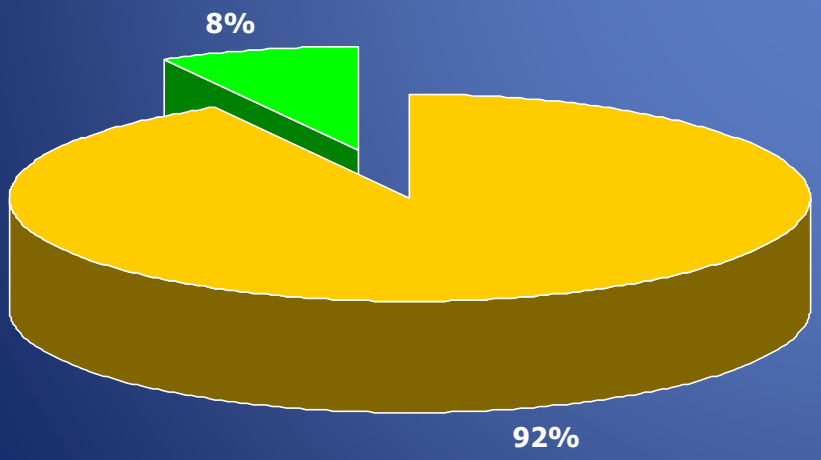
Huge economic impact: e.g. 1.3 billon US$ per year in USA.

Incidence

Menzies D, Ellis H. Ann R Coll Surg Engl 1990

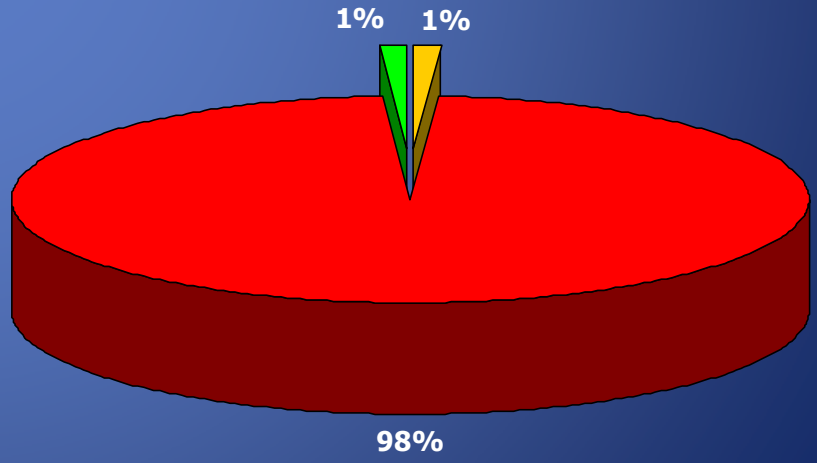
Patients without previous surgery

12/115: 10.4%



Patients with previous surgery

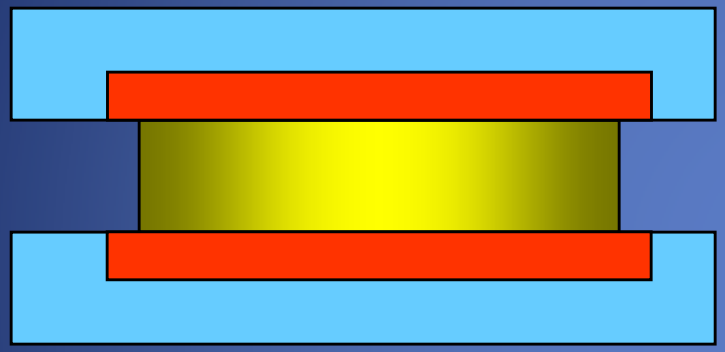
198/210: 94.3%



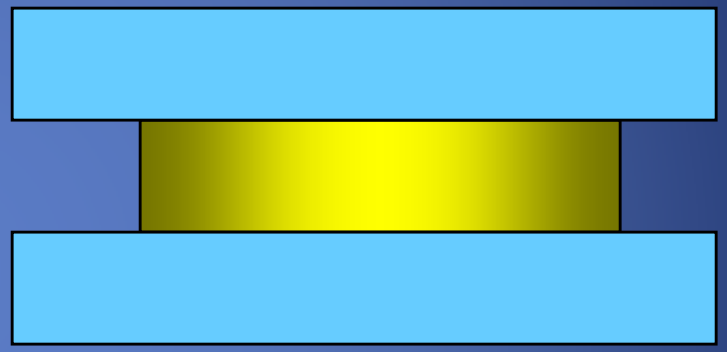
Post-inflammatory Congenital Post-operative

Postoperative adhesions

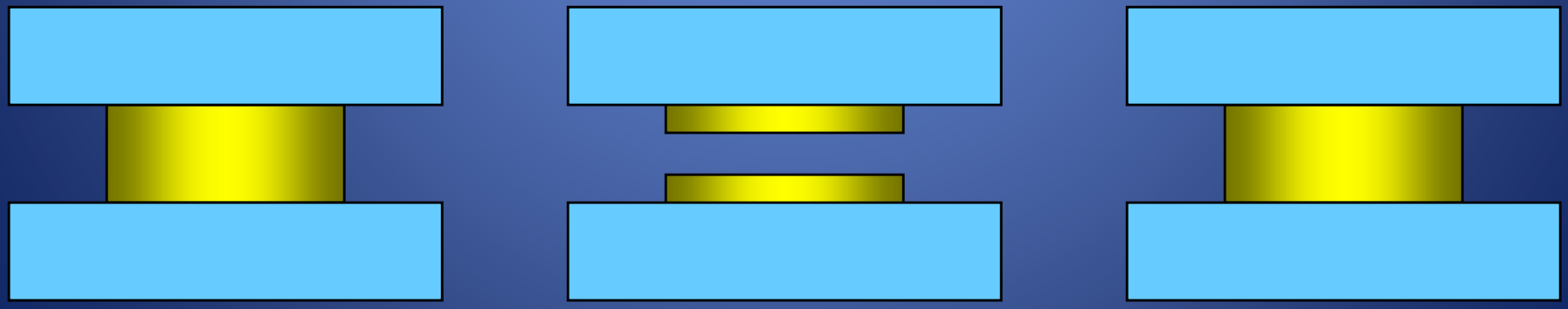
Adhesion formation



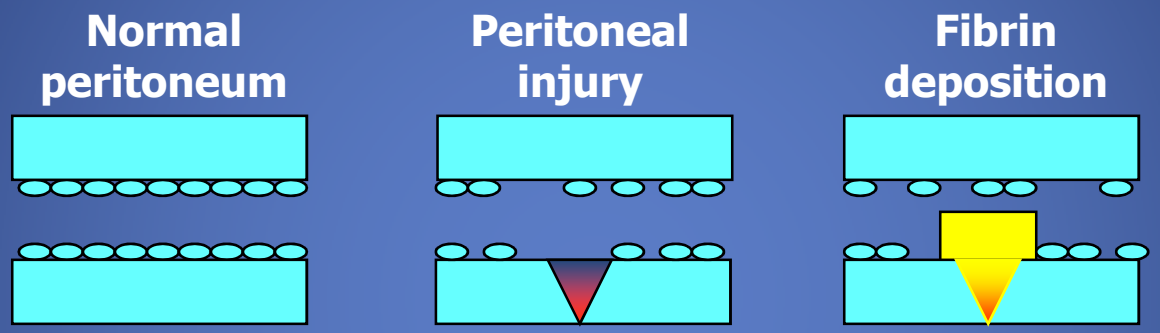
***De novo* adhesion formation**

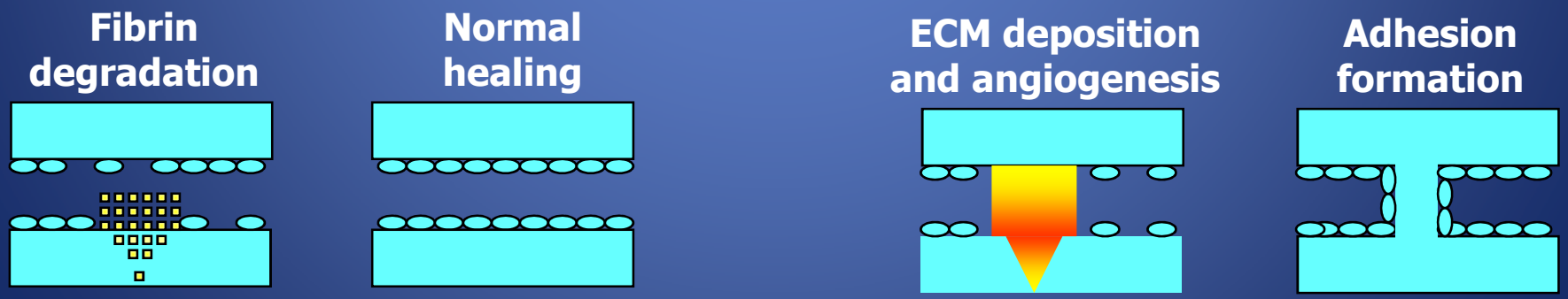


Adhesion reformation



Pathogenesis





Models for adhesion research

1) *Consistency, Reliability and Reproducibility*
2) *Type of animal model: small vs big animals*
3) *Type of adhesiogenic stimulus*
4) *Additional variables*
5) *Statistics and assessment*

Models for adhesion research

1. CONSISTENCY, RELIABILITY AND REPRODUCIBILITY

Consistency

✓The consistency of the model must be established before any testing.

✓A surgeon must be thoroughly familiar with the dissection technique. It should be practiced in animals euthanised and then in live animals to ensure that sufficiently extensive and severe adhesions are obtained consistently from one experiment to the next.

✓ The method of assessing adhesions must be also well defined before an agent is tested.

* Wiseman DM. Animal adhesion models: design, variables, and relevance. In: diZerega G, ed Peritoneal surgery. New York: Springer-Verlag, 2000:459-76.

Models for adhesion research

1. CONSISTENCY, RELIABILITY AND REPRODUCIBILITY

Reliability

✓ This refers to the ability to rely on the results obtained from a model to make correlations with clinical outcomes, under specific surgical situations.

✓A model should not be so severe that no agent reduces adhesions (“challenging” model), nor too permissive such that all agents are efficacious (“permissive” models).

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Models for adhesion research

1. CONSISTENCY, RELIABILITY AND REPRODUCIBILITY

Reproducibility

✓This refers to the ability of one investigator to replicate the results of another. The consistency and reliability of the model must be reproduced for each surgeon in a lab. This should also be checked periodically as models may "drift" even with the same surgeon.

✓Similar precautions must be taken when a model is established in a new lab. If possible a visit to the originating lab and direct observation of every procedure is recommended. Each detail can give variability, variation between laboratories can be considerable, although trends are essentially similar.

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Models for adhesion research

2. TYPE OF ANIMAL MODEL: Small animals

Advantages	Limitations
-they are being readily available -relatively cheap -easy to handle	-size and weigh -thickness of the entire abdominal wall -ratio of the peritoneal surface area relative to body weight -Transport of molecules from the peritoneum.

(Mouse, rat, rabbit) vs (dog, sheep, pig, horse, monkey)

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Models for adhesion research

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-they are being readily available -relatively cheap -easy to handle	-size and weigh -thickness of the entire abdominal wall -ratio of the peritoneal surface area relative to body weight -Transport of molecules highly dependent on the surface area.

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Models for adhesion research

2. TYPE OF ANIMAL MODEL: Small vs Big animals

Thickness of the abdominal wall

- ✓ Thickness of the entire abdominal wall in mice is equivalent to that of the first muscle layer is in rats

- ✓Same relationship exists between rats and rabbits and between rabbits and dogs.

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Models for adhesion research

2. TYPE OF ANIMAL MODEL: Small vs Big animals

*Ratio of the peritoneal surface area relative to body weight**

Animal	Body weight	Peritoneal surface	Peritoneal surface area/body weight	Peritoneal fluid
Mouse	0.025 kg	0.005 m^2	0.195 m^2/kg	0.45 ml (18.1 ml/kg)
Rat	0.25 kg	0.029 m^2	0.115 m^2/kg	2.6 ml (10.7 ml/kg)
Rabbit	2.5 kg	0.209 m^2	0.084 m^2/kg	19.4 ml (7.7 ml/kg)
Dog	25 kg	0.745 m^2	0.030 m^2/kg	69.1 (2.8 ml/kg)
Human	70 kg	1.81 m^2	0.026 m^2/kg	167 ml (2.4 ml/kg)

Higher ratio in small animals than in large animals.

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Higher ratio determines disproportionately higher volumen of peritoneal fluid required to coat the entire peritoneal surface.

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Models for adhesion research

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Models for adhesion research

2. TYPE OF ANIMAL MODEL: Small vs Big animals

*Transport of molecules from the peritoneum**

✓ The ability of the peritoneum to transport molecules is similar in all mammals

✓Highly dependent on the surface area

✓Higher in small animals, thus elimination of molecules will be faster in smaller animals.

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Models for adhesion research

2. TYPE OF ANIMAL MODEL: Small animals

✓Mice and rats, in contrast with rabbits, do not require steril conditions for surgery.

Models for adhesion research

2. TYPE OF ANIMAL MODEL:

Mice: Advantages

- ✓One of the best-developed animal models
- ✓Availability of inbred animals
- ✓Availability of genetically manipulated animals, e.g. knockout mice, mice with under or over expression of specific genes.
- ✓Animals with altered inmune system, e.g. *nude* and SCID mice (human cells in mice).
- ✓Availability of many specific assays and monoclonal antibodies
- ✓Drug screening

Models for adhesion research

2. TYPE OF ANIMAL MODEL:

Inbred Animal Models: *Definition*

•Inbred strains are animals which are nearly identical to each other in genotype due to long inbreeding. Mating of brother-sister pairs for 20 generations will result in lines that are roughly 98% genetically identical, usually sufficient to be considered an inbred strain (compare to identical twins or clones which are 100% genetically identical, or fraternal twins or normal siblings, which are roughly 50% identical).

Models for adhesion research

2. TYPE OF ANIMAL MODEL:

Inbred Animal Models: *Advantages*

- Highly consistent
- Essentially genetically identical -isogenicity
- Highly reproducible across individuals and generations
- Test different chemicals and doses on essentially the same genotype
- Minimize phenotypic variances
- Use multiple strains to ensure that one of the strains is sensitive to a given toxicant

Models for adhesion research

2. TYPE OF ANIMAL MODEL:

Inbred Animal Models: *Limitations**

- Using a single strain of inbred mice cannot reflect the natural variation of the human patient population.
- Indeed, marked strain differences exist in the susceptibly of mice to atherosclerosis, autoimmune diseases, stroke, asthma and adhesion formation (Molinas 2005).
- <10% of new drugs tested in clinical trials receive Food and Drug Administration (FDA) approval.
- Example “A study of drug efficacy using a disease model in a single inbred mouse strain could be compared with a clinical drug trial performed in an isolated South Pacific island population”.

*Gurwitz D, Weizman A. Animal models and human genome diversity: the pitfalls of inbred mice. Drug Discov Today. 2001 1;6(15):766-768.

Models for adhesion research

2. TYPE OF ANIMAL MODEL:

- Different animal models:

  rat (~200)

  rabbit (~140)

  mouse/pig/dog (~30)

  horse (4: model?)

  sheep/monkey (2: model?).

www.pubmed.org

Models for adhesion formation

3. TYPE OF ADHESIOGENIC STIMULUS

Abrasion, crushing, desiccation, incision, excision, electrocautery, laser injury, thermal injury, chemical injury, radiation injury, foreign body-tissue irritation *.

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Models for adhesion formation

4. ADDITIONAL VARIABLES

Bleeding, ischaemia, contamination/infection, anastomosis, other pathology (endometriosis, cancer), formation vs reformation, laparoscopy vs laparotomy. *

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Models for adhesion formation

5.STATISTICS AND ASSESSMENT

- Study size: it will depend on whether data are used for screening or definitive purposes.
- Screening studies: several candidates, further studies; smaller sample size (n=3-8), higher p value (e.g. 0.1-0.2) and lower power (e.g.60%).
- Definitive studies: end of one research phase; number of animals should provide sufficient power (e.g. 80%).

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Models for adhesion formation

5.STATISTICS AND ASSESSMENT

Block Randomization

- If our experiment has 6 groups and 8 animal/group:
- NO: Day 1: 8x group 1; Day 2: 8x group 2, etc.
- YES: Day 1: group 1,3,6,5,4,2

  Day 2: group 2,5,1,4,3,6.... Etc
- To avoid day to day variability, learning curve, climatic conditions, surgeon fatigue

Models for adhesion formation

The laparoscopic mouse model

- ✓Easier to handle
- ✓Cheaper
- ✓Available quickly
- ✓Inbred mice
- ✓Knock out mice
- ✓Not need of steril conditions
- ✓ m Antibodies

The laparoscopic mouse model

Previous work of:

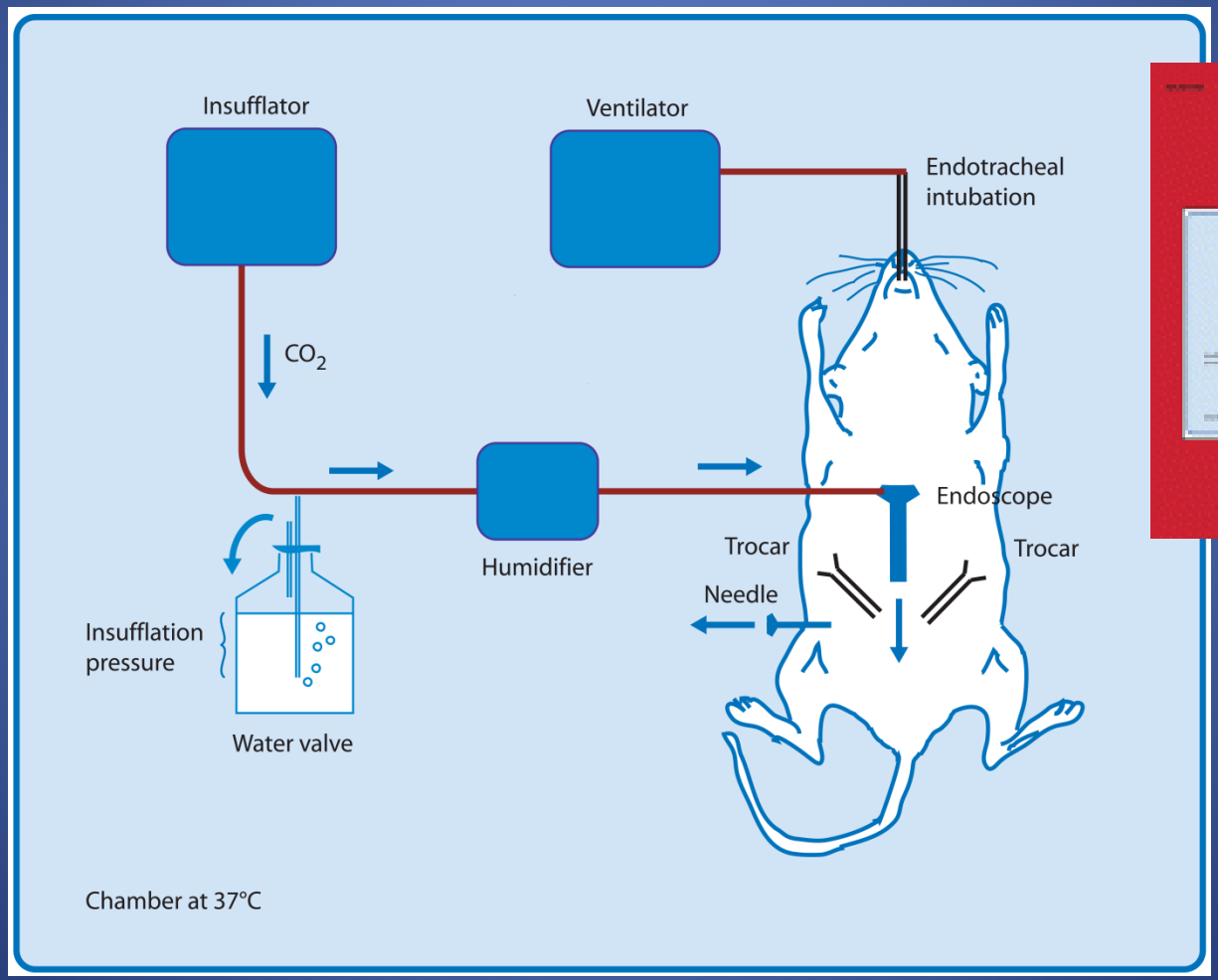
Drs Yesildaglar,Ordonez, Molinas, Elkilani, Mynbaev, Binda, Schonman

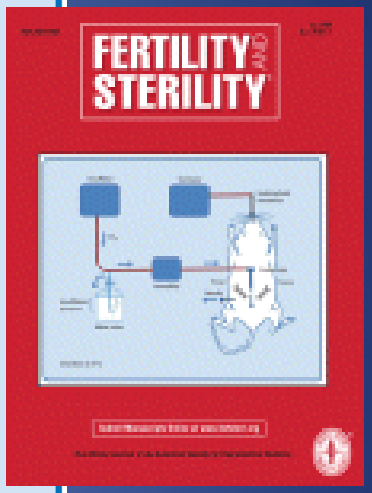
Actual work of: Drs Corona, Verguts

Supervision of Professor Philippe Koninckx

The laparoscopic mouse model

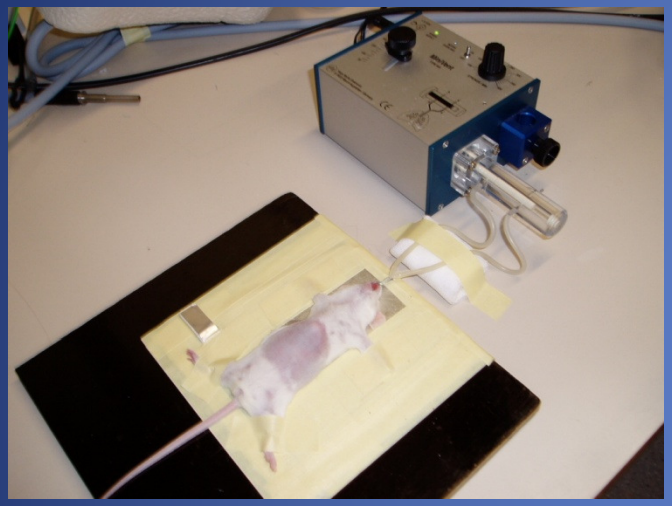
Set up

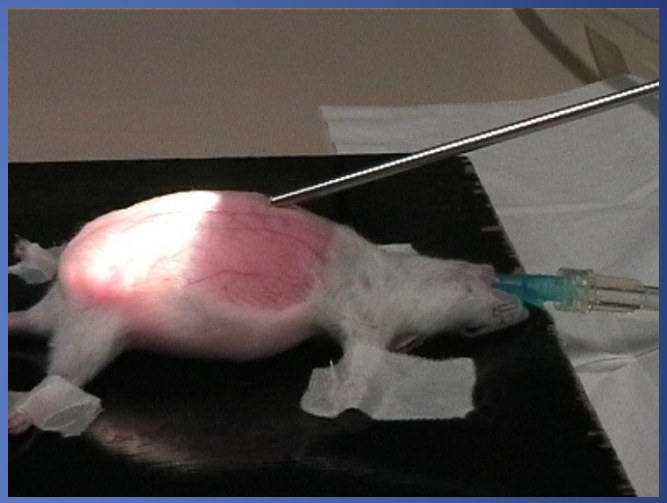


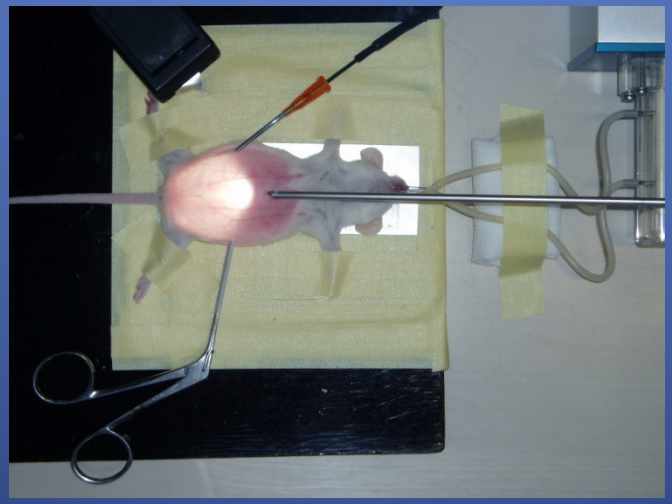


The laparoscopic mouse model

Set up



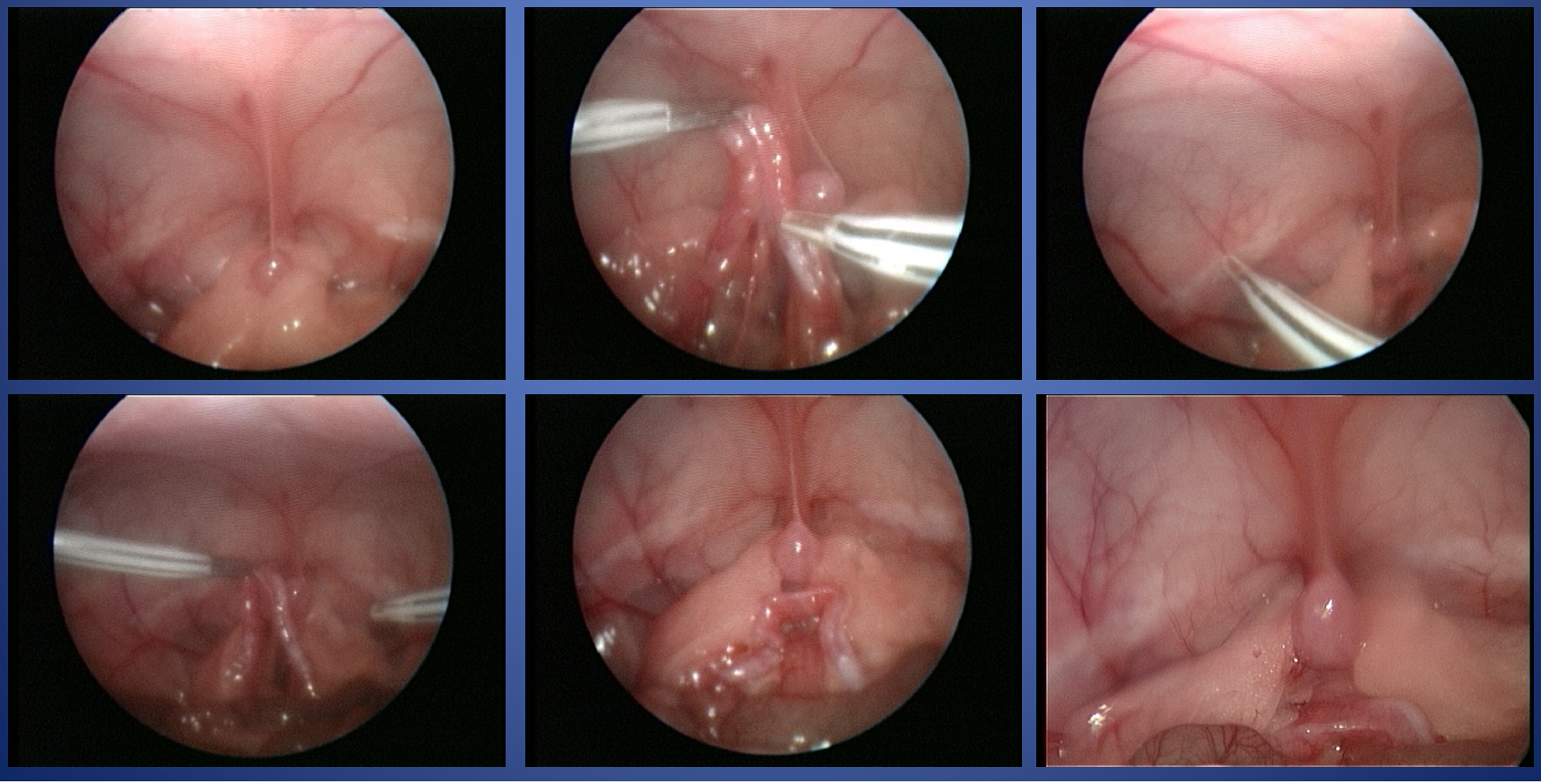




The laparoscopic mouse model

Induction of adhesions

Standardised bipolar lesions in
uterine horns and pelvic sidewalls during laparoscopy



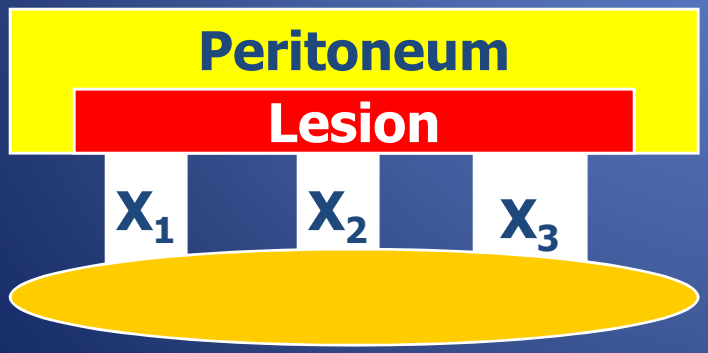
The laparoscopic mouse model

Scoring of adhesions

After 7 days, blindly, under
microscopic vision, during laparotomy

Quantitative scoring

$$\text{Adhesions (\%)} = \frac{\sum \text{length of the individual attachments}}{\text{Length of the lesion}}$$



Qualitative scoring

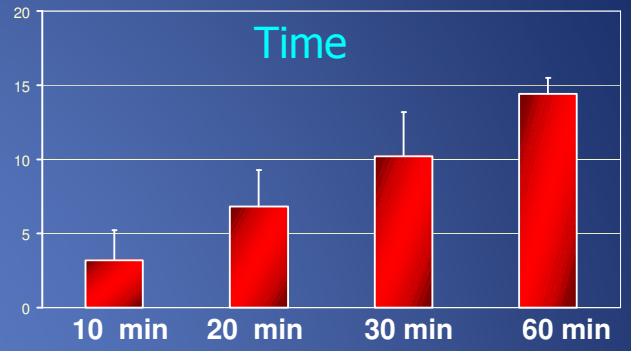
- Extent: 0 - 4
- Type: 0 - 3
- Tenacity: 0 - 3
- Total: 0 - 10

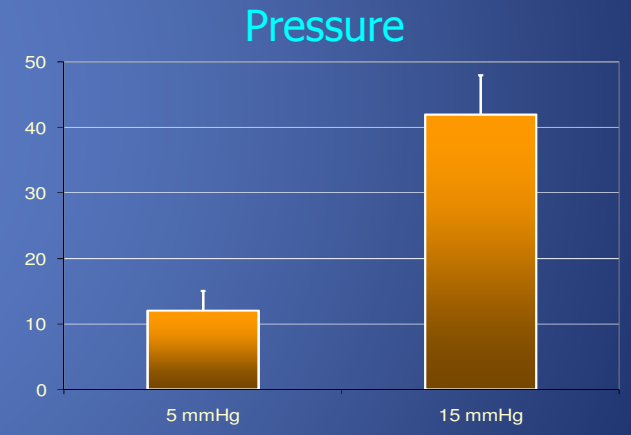
The laparoscopic mouse model

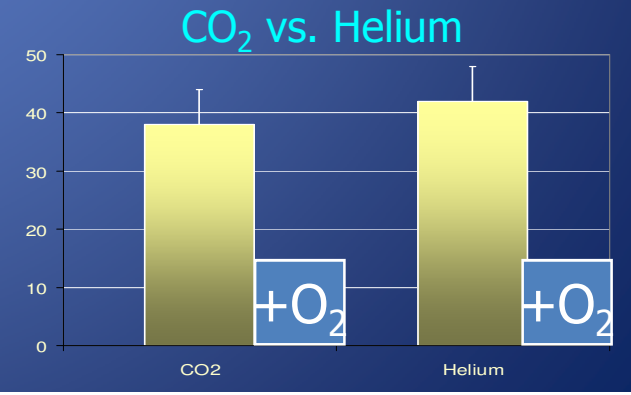
SOME OF OUR RESULTS

CO_2 pneumoperitoneum is a cofactor in adhesion formation

- Adhesions increase with duration of surgery
- Adhesions increase with insufflation pressure
- Adhesions are similar with CO_2 and Helium pneumoperitoneum
- Adhesions decrease after addition of oxygen



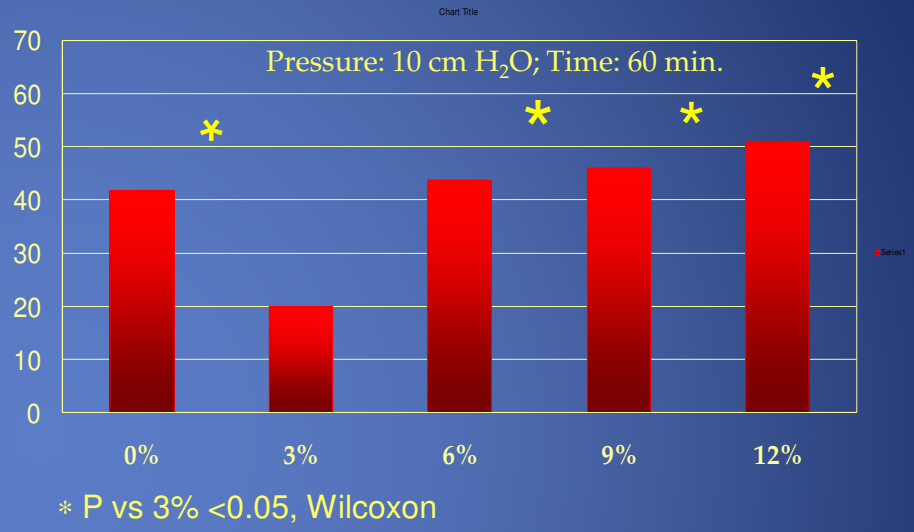




Molinas CR and Koninckx PR, Fertil Steril, 2001

CO_2 pneumoperitoneum is a cofactor in adhesion formation

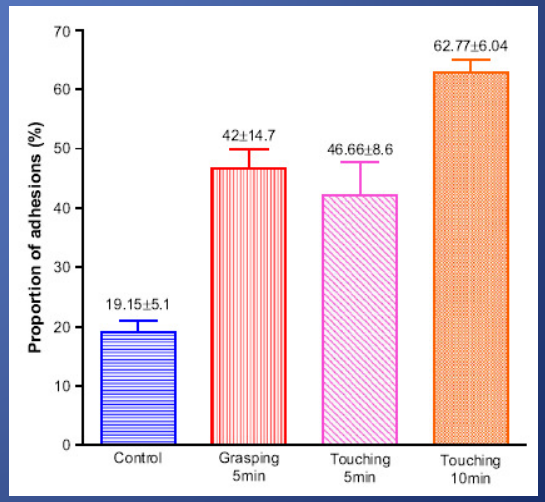
- Adhesions increase with the addition of more than 3% Oxygen to the pneumoperitoneum
  (Elkelani OA, Binda MM *et al* . Fert Steril 2004)



* P vs 3% <0.05, Wilcoxon

- Hypercarbia/Acidosis
  (Molinas *et al*, Fertil Steril 2004)

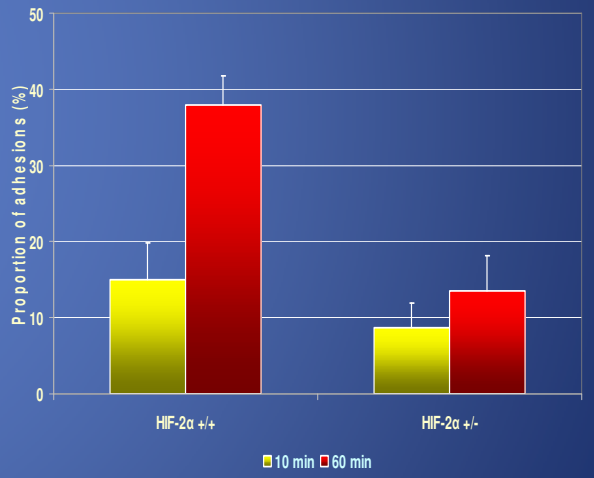
- Manipulation during pneumoperitoneum increases adhesions
  (Schonman R *et al*, J Minim Invasive Gynecol. 2009)



CO_2 pneumoperitoneum is a cofactor in adhesion formation

Hypothesis Mesothelial Hypoxia

✓ Consistent with the absence of pneumoperitoneum-enhanced adhesion in mice knockout for HIFs, VEGF-A, VEGF-B, PlGF and PAI-1.

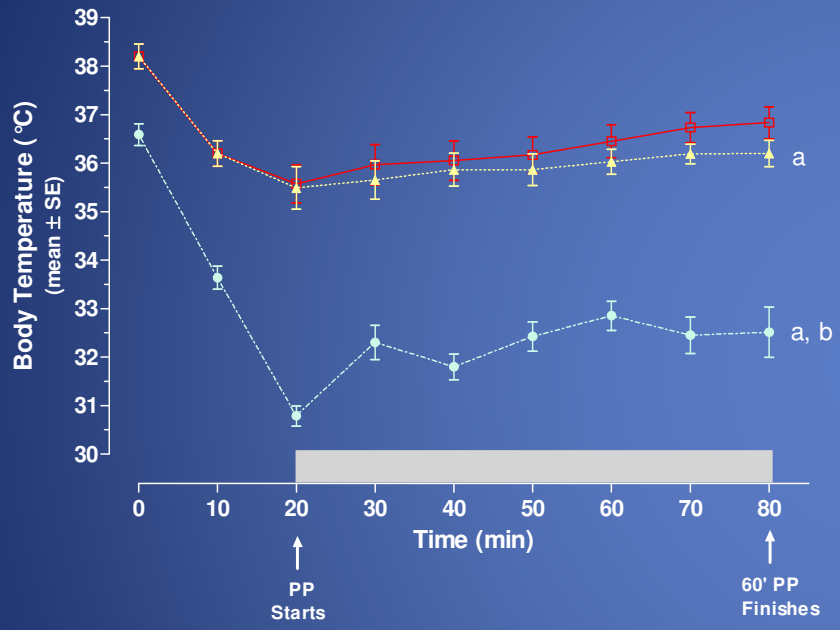


Molinas *et al*, Fertil Steril 2003
Molinas *et al*, Fertil Steril 2003
Molinas *et al*, Fertil Steril 2003

Genetic background has an influence in adhesion formation

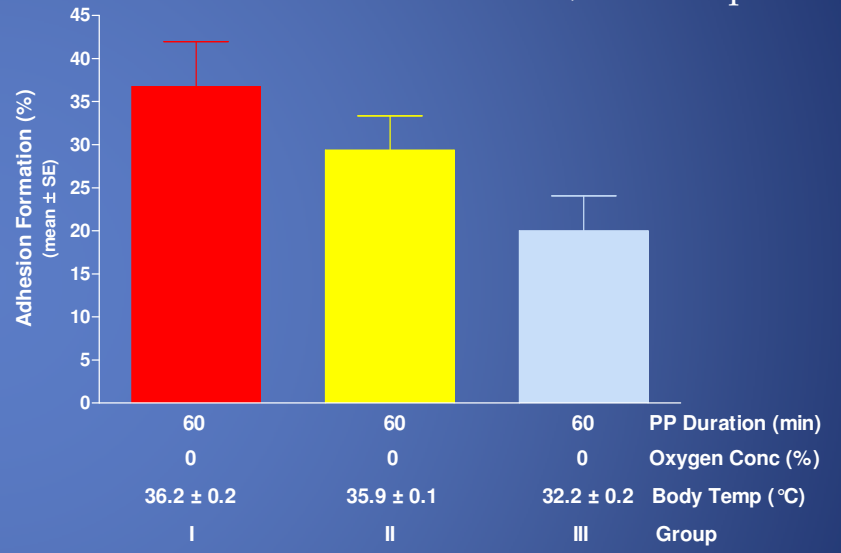
Molinas CR, Binda MM *et al*, Fertil Steril, 2005

Hypothermia reduces adhesion formation



Two way ANOVA
[a] p<0.01 vs group I
[b] p<0.0001 vs group II

Binda MM *et al*, Hum Reprod 2004.



P=0.02, Pearson

Experimental design: 60 min CO_2 PP, humidified gas for PP, little flow = No desiccation, Chamber at 37°C, modulate mouse body temperature: around 37°C: ventilation with humidified gas, around 36°C: ventilation with non-humidified gas, around 32°C: placing mouse in/outside the 37°C chamber

Hypothermia reduces adhesion formation

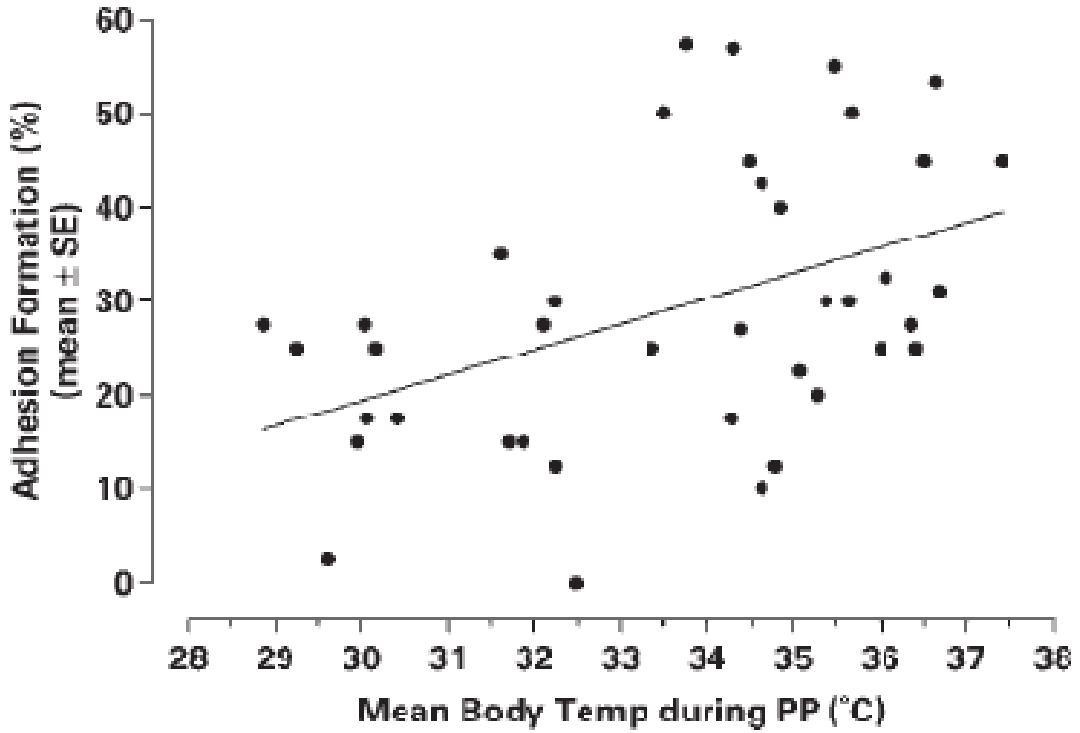
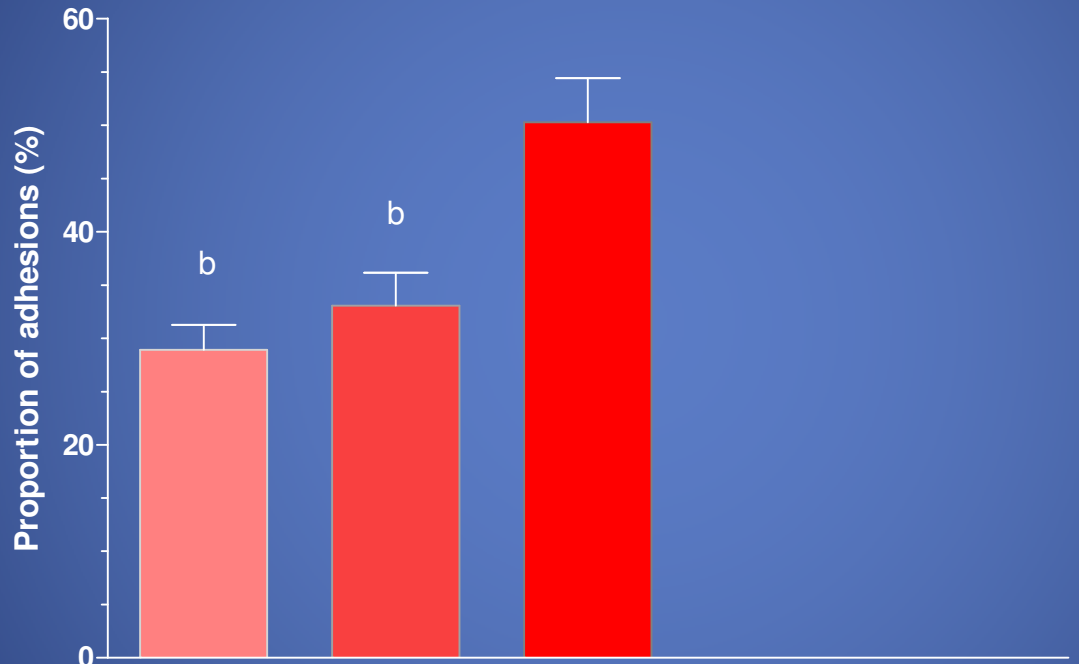


Figure 4. Relationship between body temperature and adhesion formation. Individual values of the mean of body temperature between T_{20} and T_{80} with their respective proportion of adhesions are depictured for pneumoperitoneum-enhanced adhesion for experiments I and III. $P = 0.004$ (Pearson correlation).

Binda MM *et al*, Hum Reprod 2004.

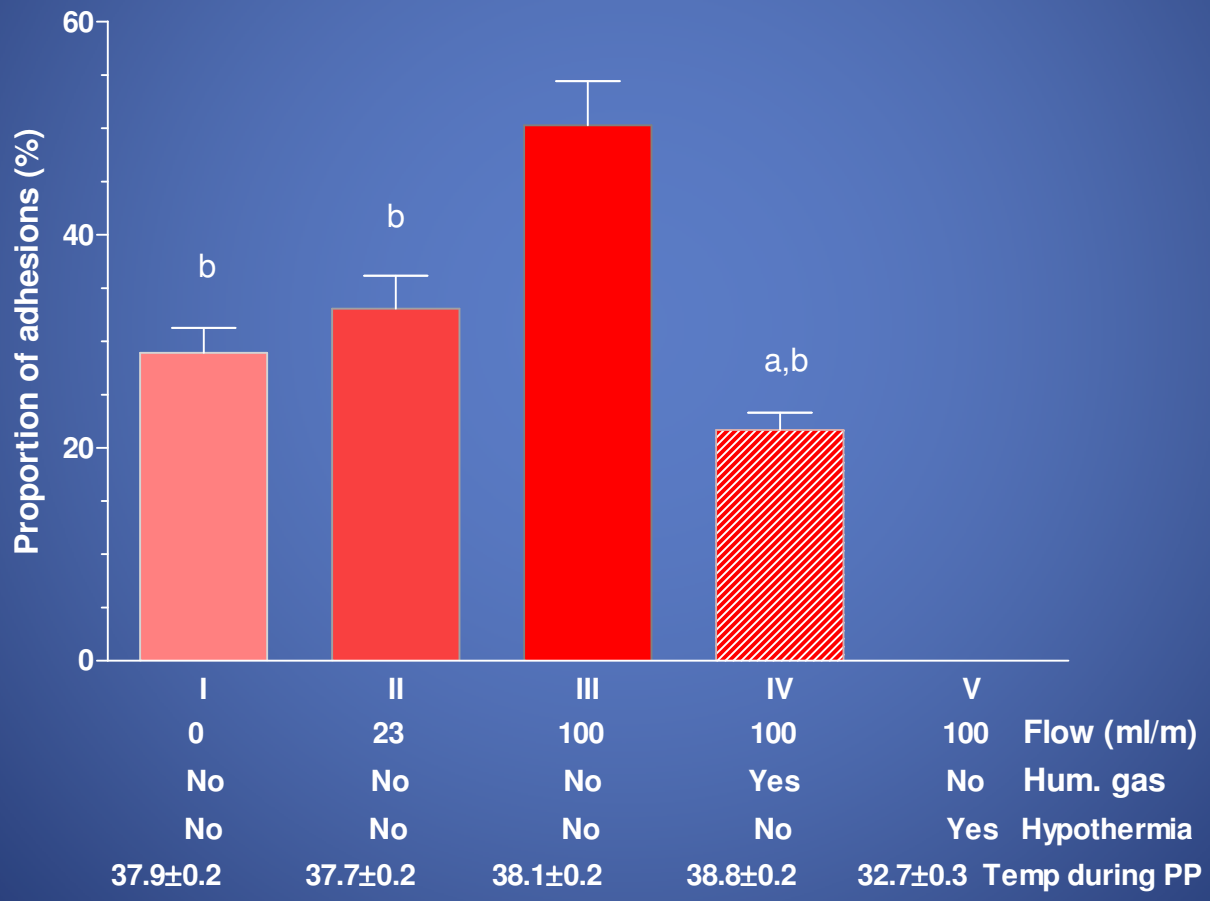
Desiccation increases adhesion formation



Wilcoxon
[a] p<0.05 vs group I
[b] p<0.05 vs group III

Binda MM *et al*, Fertil Steril 2006.

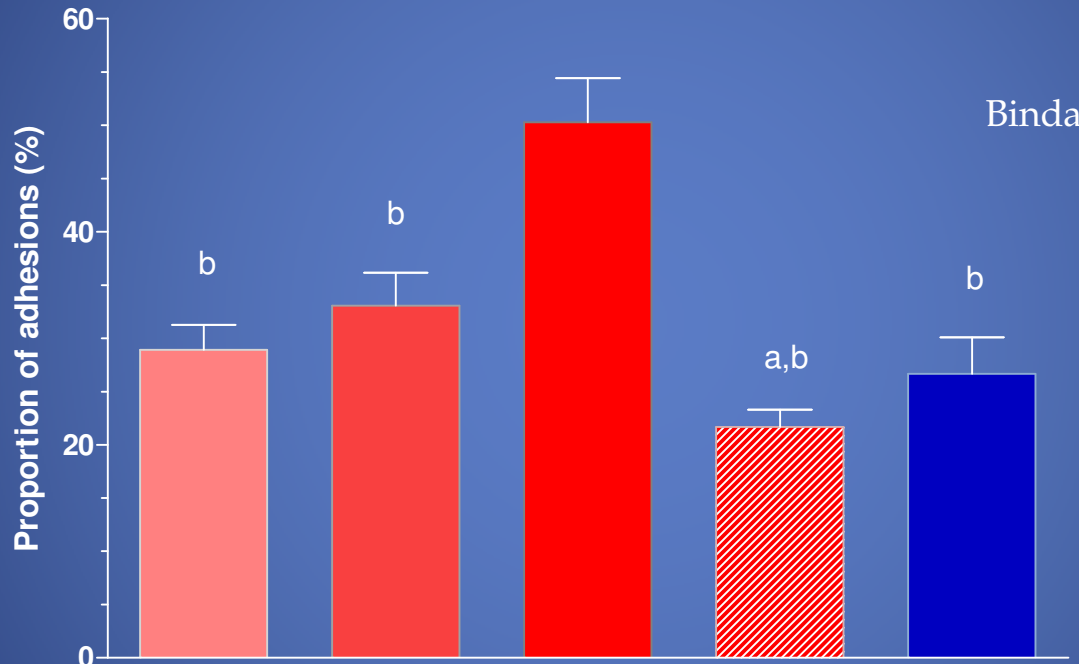
Desiccation increases adhesion formation



Wilcoxon
[a] p<0.05 vs group I
[b] p<0.05 vs group III

Binda MM *et al*, Fertil Steril 2006.

Hypothermia reduces adhesion formation

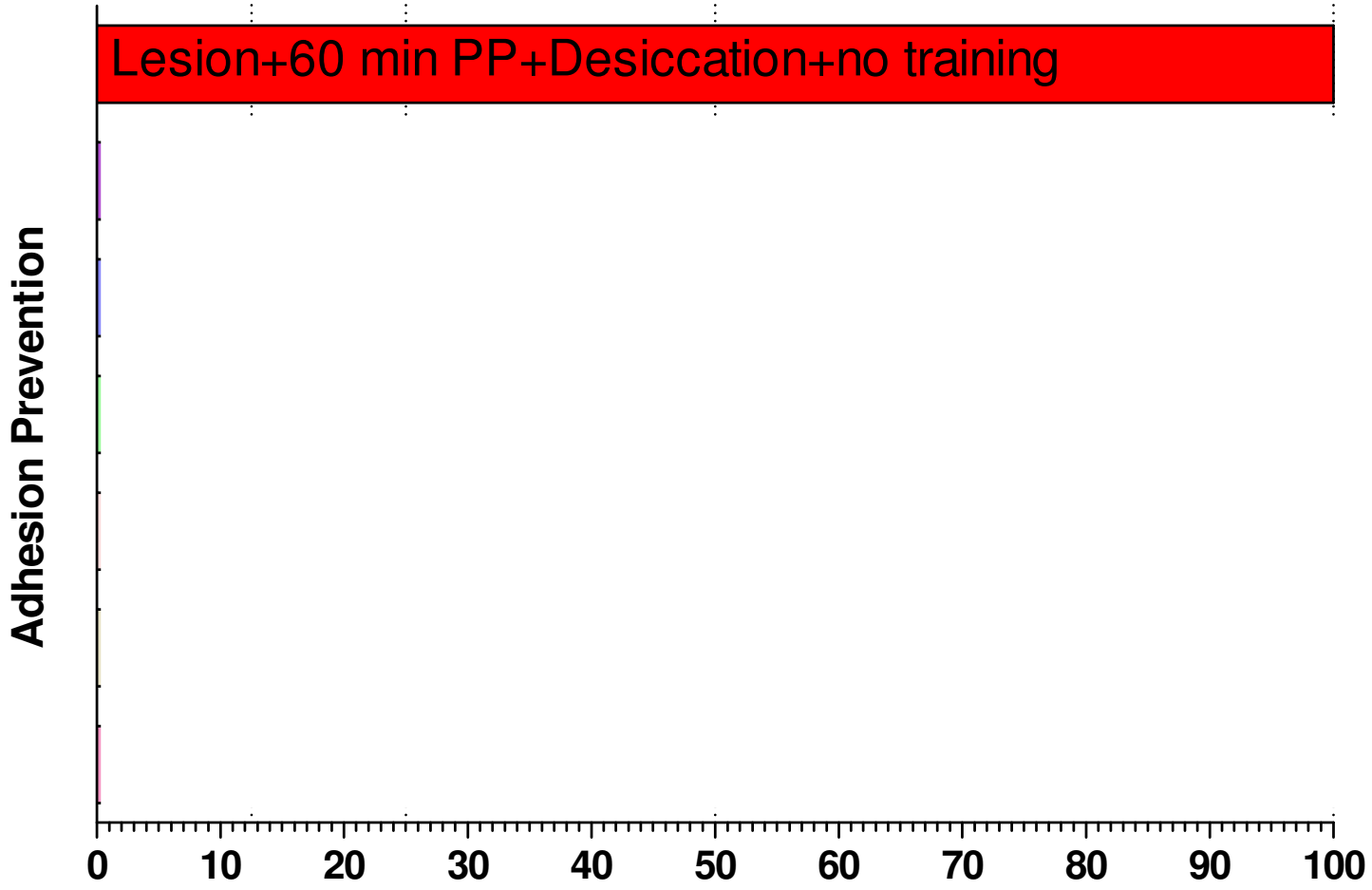


I	II	III	IV	V	
0	23	100	100	100	Flow (ml/m)
No	No	No	Yes	No	Hum. gas
No	No	No	No	Yes	Hypothermia
37.9±0.2	37.7±0.2	38.1±0.2	38.8±0.2	32.7±0.3	Temp during PP
0	0	0	0.6±0.1	0	PPT - BT
100	100	100	100	100	PPRH (%)

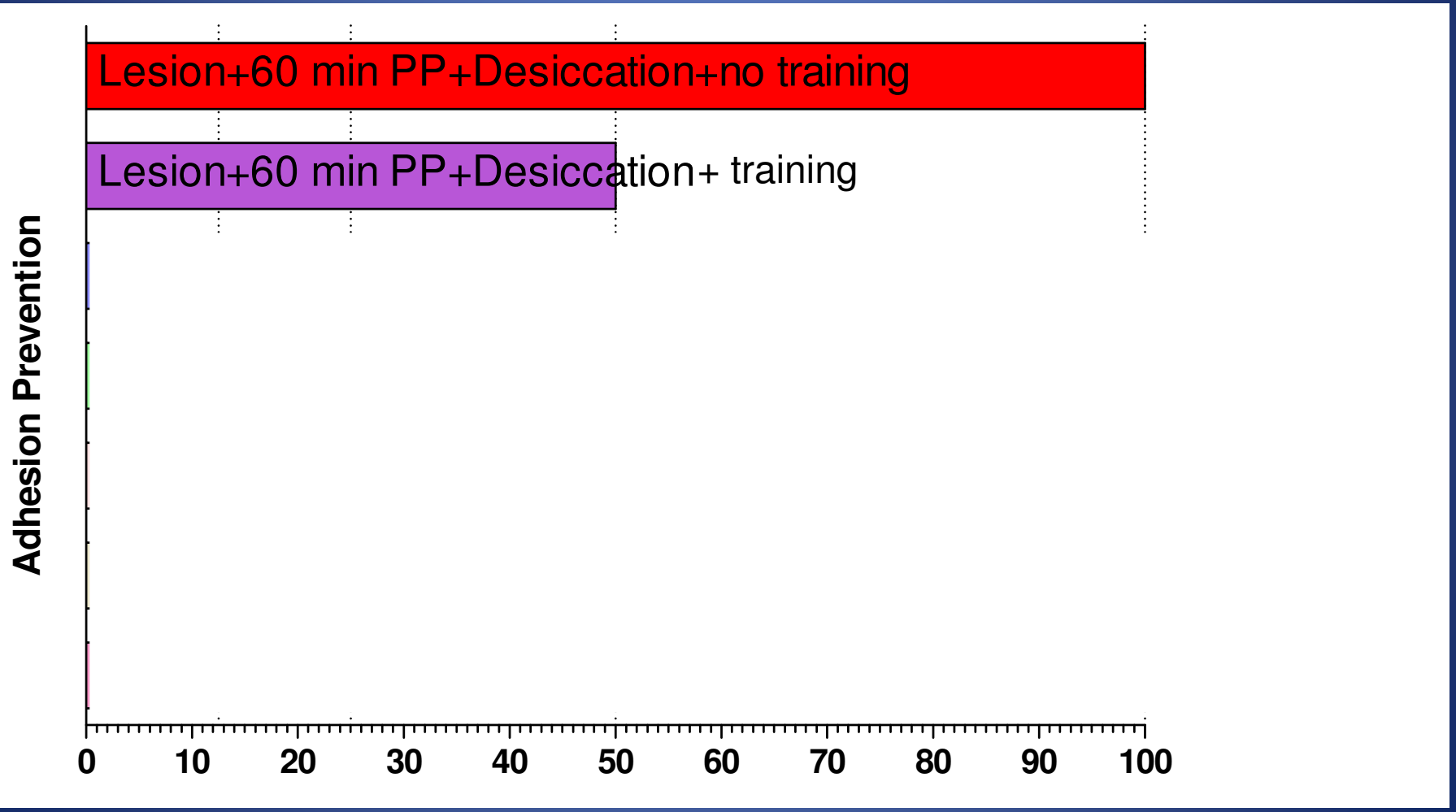
Binda MM *et al*, Fertil Steril 2006.

Wilcoxon
[a] $p<0.05$ vs group I
[b] $p<0.05$ vs group III

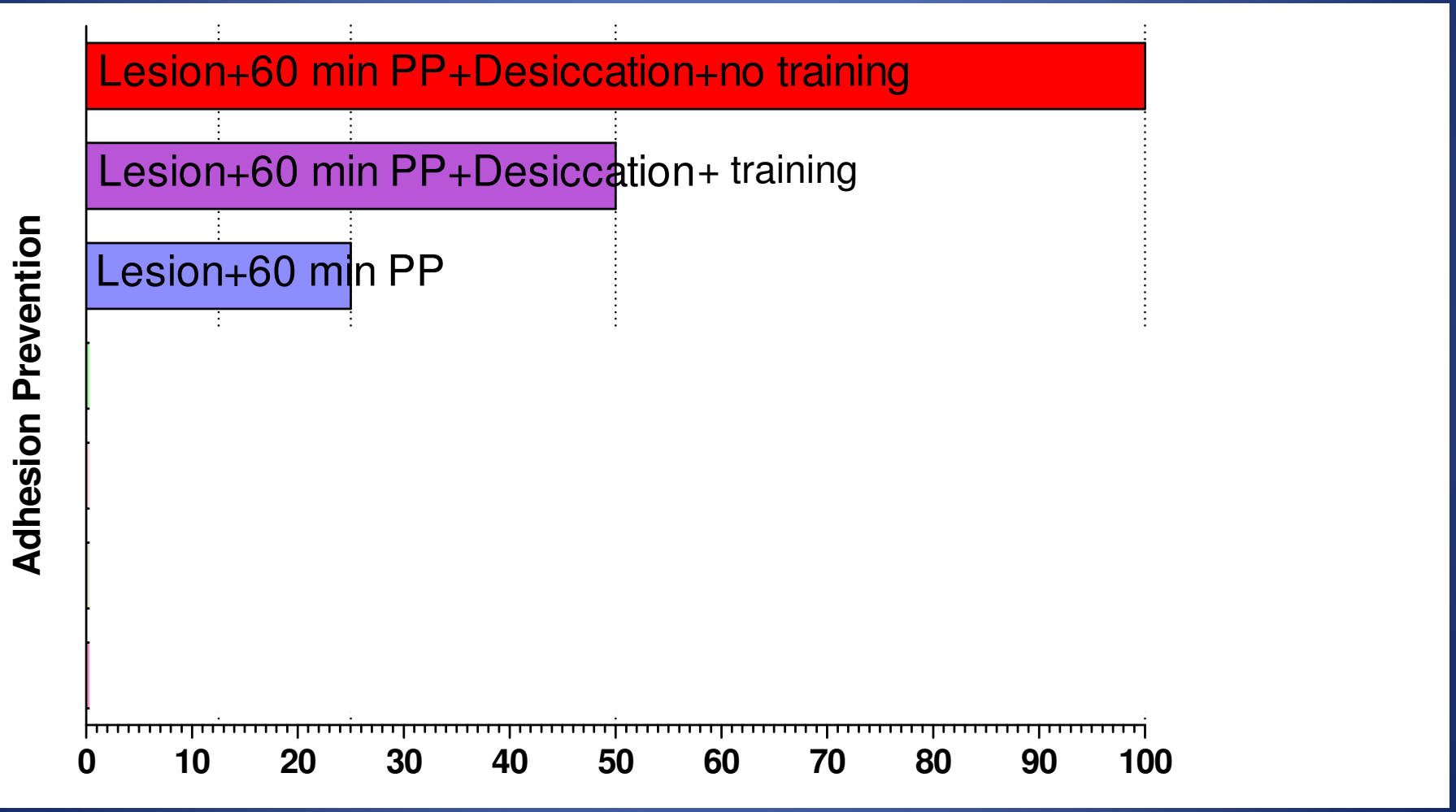
Adhesion Prevention in our laparoscopic mouse model



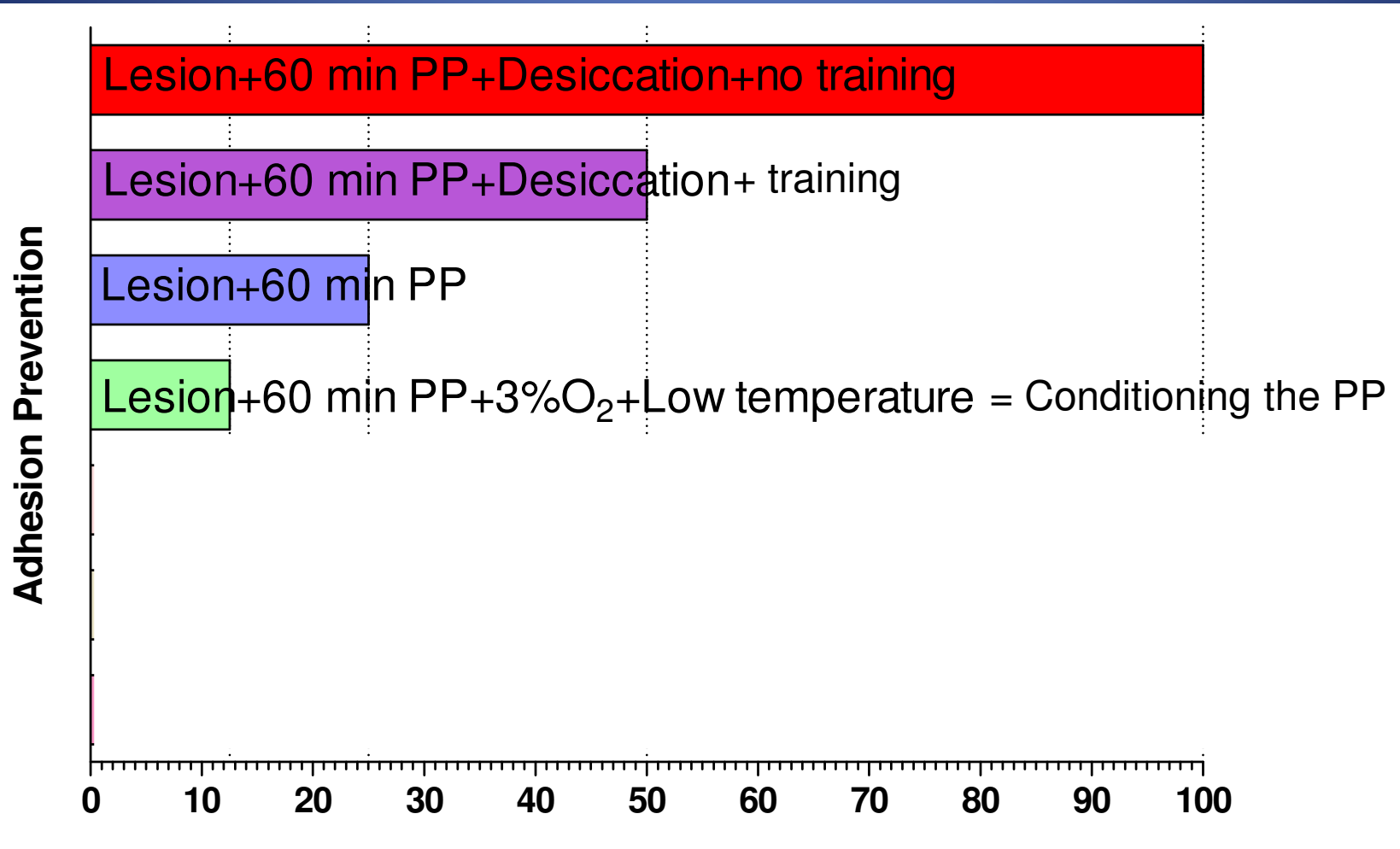
Adhesion Prevention in our laparoscopic mouse model



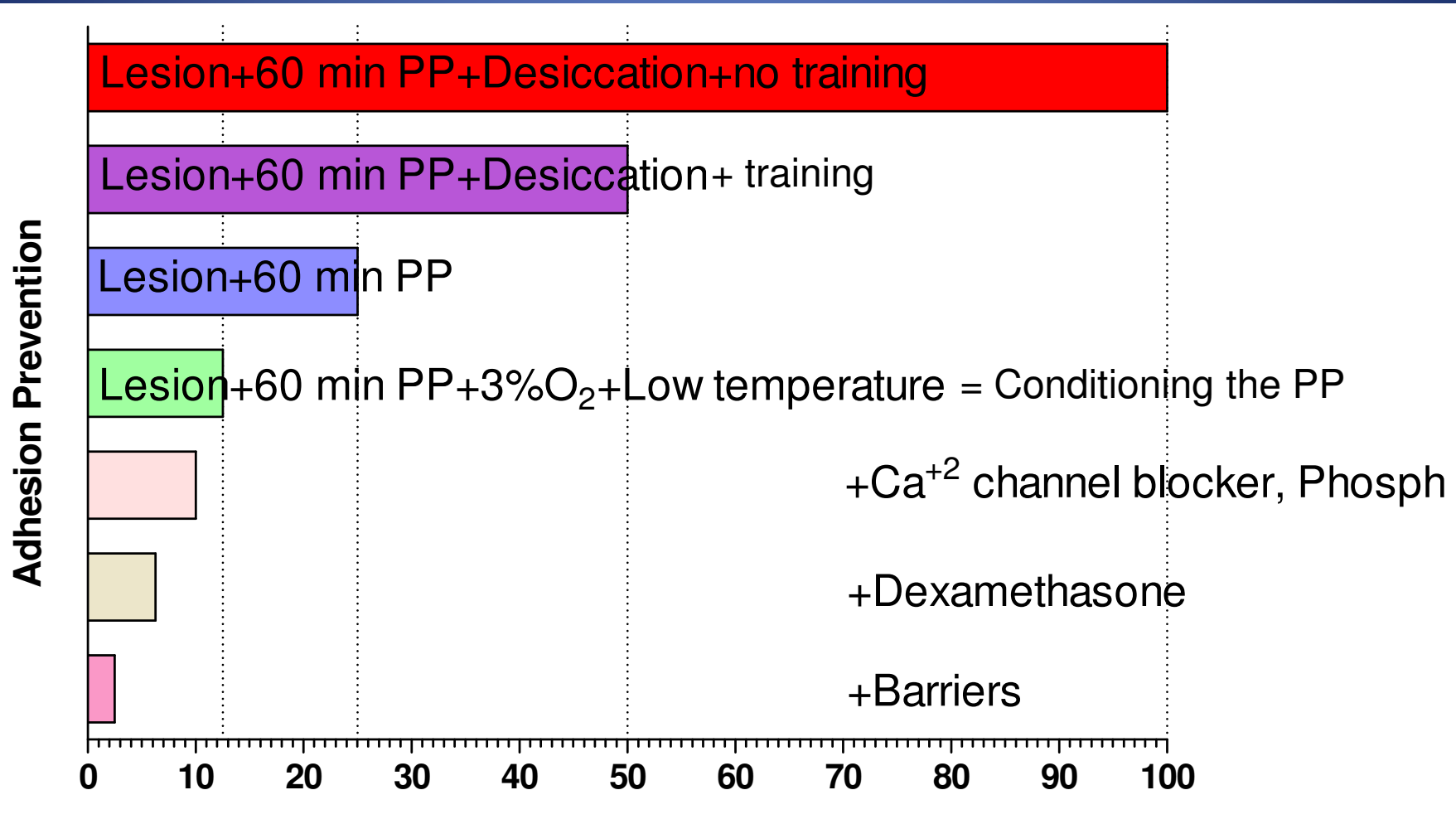
Adhesion Prevention in our laparoscopic mouse model



Adhesion Prevention in our laparoscopic mouse model



Adhesion Prevention in our laparoscopic mouse model



Binda and Koninckx, Human Reprod, 2009.

Summary of our results in the laparoscopic mouse model:

Adhesion formation is influenced by

- ✓Genetic background
- ✓Duration and pressure of PP
- ✓Type of gas and its humidification
- ✓Body temperature: hypothermia reduces AF
- ✓Manipulation= good surgeon training is very important

Summary of our results in the laparoscopic mouse model:

✓Best way to reduce adhesion: conditioning the PP (humidified CO_2 + 3% O_2 and low temperature) + combination of products + good surgeon training.

Binda and Koninckx, Human Reprod, 2009.

Conclusions: Animal models for adhesion formation

- Small animal models, i.e. mouse, rat and rabbit are the most used models for screening experiments (mouse and rat: inbred strains, no need of sterility during surgery)
  - ✓Good price, easy to handle, available quickly
- Before starting any study, the consistency, reliability and reproducibility of the animal model should be checked.

Many Thanks

City Hall, Leuven, Belgium