The dos and don’ts of the insemination kitchen.

Nijs Martine
PhD, MSc, Senior Clinical embryologist
Genk Institute for Fertility Technology

Recepy for Belgian fries

1

2

3

4

5

6

7

The Walking Egg

Ziekenhuis Oost-Limburg

Recepy of Belgian fries

- Some fine potatoes (Bintje is the best, but in the US Yucon Gold or Russett will do fine), a sharp knife, a deep fryer, frying oil (any oil that can take the heat will do but will determine the taste of the fries), some kitchen paper and a bowl or an ovenplate.
-
- Start by peeling the potatoes. Cut them in slices 1 cm (3/8”) thick and finally cut them into fries of 1 cm square .. Dry the fries well in paper or a towel before putting them into the oil.

- Heat the oil in a hot frying pan or deep fryer to a temperature of 160° C (320° F). Put in a handfull of fries: not more at once because the oil will cool down too much. Fry for a few minutes (4-8 depending on the thickness and the kind of potatoes), stir regularly to prevent sticking. Put the fries into a large bowl with kitchenpaper (or even better on an ovenplate) and let them cool down and ’sweat’ for at least 1/2 hour.

- Finally heat to 190° C (375° F) and fry for 2 minutes until crispy and golden brown. This way the fries will be crispy on the outside and soft on the inside, the way they should be! Good fries make a nice sound!

The Walking Egg

Ziekenhuis Oost-Limburg

IUI outcome linked to

- Better Sperm morphology (>4%)
- Better Sperm morphology in processed sperm
- High motile count before preparation (>20 10^6/ml)
- High Inseminating Motile Count (IMC)
- Low Sperm DNA fragmentation (%DFI)

Duran et al., 2002; Ombelet et al., 2003; Bensdorp et al., 2007; Ombelet and Nijs, 2007; Bungum et al., 2007; Guven et al., 2008, ……

Ziekenhuis Oost-Limburg

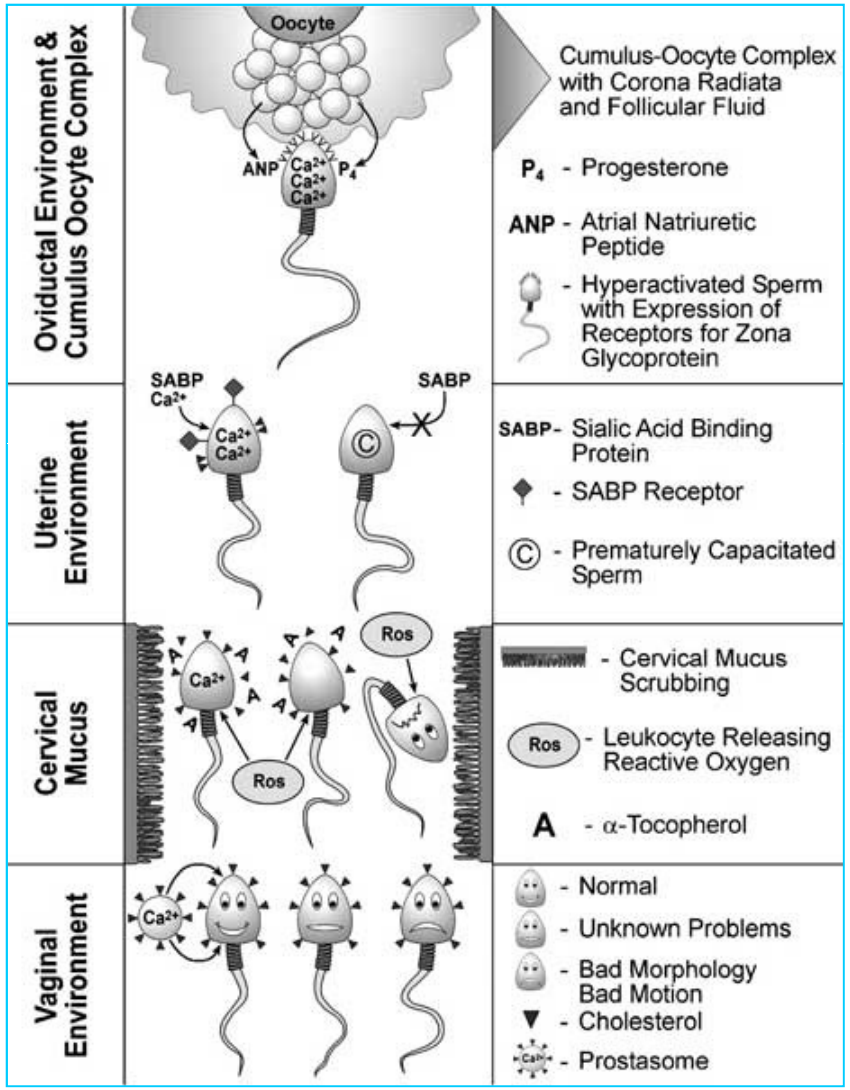
Why sperm 'preparation'?

- Removal of seminal plasma and prostaglandines
- Removal of dead sperm cells, epithelial cells from urinary tract, prostate cells, leucocytes, spermatogenic cells
- Removal of ROS originating from different germ cells, bacteria, leucocytes
- Concentration of most motile, morphologically normal mature sperm cells
- Preparation of 'process of capacitation' of sperm cells

The Walking Egg

Ziekenhuis Oost-Limburg

Why sperm 'preparation'? Human spermatozoa 'in vivo'



De Jonge C, Hum Reprod Update, 2005

The Walking Egg

Ziekenhuis Oost-Limburg

Standard procedure

- Sperm production
- Liquifaction of semen sample
- Quick semen analysis for quality: volume, motility, concentration, agglutination, viscoscity
- Sperm 'preparation'
- Analysis according to WHO
- 2 X wash in medium supplemented with albumin
- equilibaration
- Inseminating motile count determination
- Registration
- Equilibration in 6% CO_2 (>15 minutes) at 36.5° C

The Walking Egg

Ziekenhuis Oost-Limburg

Sperm production

- Time of abstinence:
  - <3 days (Jurema et al., 2005)
  - 42-54 hours (Amman and Chapman, 2009)
  - >1 day (%HDS-DFI, De Jonge et al., 2004)

  : 2-4 days

- Sperm production
  - Production at home vs lab (Elzanaty & Alm, 2008)
  - Time to ejaculation (Pound et al., 2002; Elzanaty, 2008)

- Delivery to laboratory
  - Body temperature, < 60 minutes

The Walking Egg

Ziekenhuis Oost-Limburg

Sperm preparation: techniques 1

- Simple washing technique
  - Cheap
  - Quick
  - Highest yield of spermatozoa
  - Quality of spermatozoa? (morphology, maturity, maturity,….)
  - Concentration of 'debris'
  - Risk for transmission of infectious agents persists
- Swim-up technique
  - Cheap
  - Easy
  - Only selection for motility
  - Less effective for ROS removal
  - Less effective for removal of seminal components
  - Risk for transmission of infectious agents persists

Ziekenhuis Oost-Limburg

Sperm preparation: techniques 2

- Glass wool separation
  - Effective for ROS removal
  - Risk for glass chips

- Gradient centrifugation

  Sakkas et al., 2000, Tomlinson et al., 2001
  - Expensive
  - Easy
  - Selection for morphology, intact DNA, good condensed chromatin, low degree of apoptosis
  - Effective for ROS removal
  - Effective for removal of seminal components
  - Removal of blood cells - removal of infectious agents

Ziekenhuis Oost-Limburg

Sperm preparation

- Electrophoretic sperm selection

  Ainsworth et al., 2002; 2007

  - Cheap
  - Easy
  - Very fast
  - Selection for normal and DNA intact spermatozoa
  - Effective for ROS removal

- Magnetic–activated cell sorting

  Said et al., 2008

  - Expensive
  - Easy
  - Selection for non-apoptotic sperm cells

Ziekenhuis Oost-Limburg

Little changes – big improvements ???

Ziekenhuis Oost-Limburg

Choice and QC of materials and products

J.Critchlow et al, Hum Reprod,1989; Lierman et al., Fertil Steril, 2007; Nijs et al., Fertil Steril, 2009

Patient-related procedures

143 SpST's
12 products
33 brands
7 types of products reprotoxic

Product	Brand/product type	Number of tests	SpST index 24 hours	96 hours
Needle guide	CIVCO 610-604	10	0.96	1.10
OPU needle	Gynetics, 4551 NS1	1	0.78[a,b]	0.77[a,b]
	Gynetics, 4551 FAS E2	2	1.01[c]	0.98[c]
	Cashmed, CA SC1SE	6	1.11	1.13
	Vitrolife 14122	4	1.05	0.97
Monoject needles	Kendall 8881202348	5	1.05	0.85
Condoms	Aloka RB-945BP-NS	5	0.95	0.88
	IM Services 2050-cover latex	1	0[a]	—
Hysterometer	Unomedical 02914182	1	0.14[a]	0.2[a]
	Osfinder, Coopere Surgical 1176	1	0[a,d]	—
	Osfinder, Coopere Surgical 1176	3	1.10[e]	0.90[e]
Latex tubing OPU	VWR 201050080	3	0.96	0.20[a]
IUI catheter	Gynetics, 4220	2	1.10	1.05
	Genitor, CCD 120400M	12	0.96	1.26
	Gynotec, PM IUI catheder	4	0.96	0.85
	Gynetics, Emtrac set 4219	1	0.96	0.88
Embryo transfer catheter	Frydman,CCD 1306045	15	0.91	0.87
	TDT, CCD 1308000	9	1.30	0.88
	Gynetics, Emtrac set 4219	1	0.95	0.88
	Gynetics, Emtrac plus 4219	1	1.1	1.07
	Gynetics, Emtrac A 4219	1	0.94	0.87
	Gynetics, Semtrac set 2000	1	0.77[a]	0.70[a]
Unpowdered gloves, nitrile	Ansell, TNT 92-760	1	0[a]	—
	Ansell, TNT 92-760	3	0.88	0.87
Unpowdered gloves, latex	Health Line, GS22 Long C	1	0.93	0.87
	Regent, Biogel latex 30460	1	0[a]	—
	Regent, Biogel Supersensitive S751	1	0[a]	—
	Health Line, Cision GS75	1	0[a]	—
	Health Line, GS22 long	1	0[a]	—
	Health Line, GS22N Long	1	0[a]	—
Unpowdered, polyisoprene gloves	Regent, Biogel Skinsense N 31465	7	0[a]	—
Unpowdered, nonlatex gloves, composition not defined	Ansell, Derma Prene type 2-RT	24	1.10	0.85
	Kimberly-Clarck, Safeskin NXT 62992	12	1.05	0.87
	Regent, Biogel Indicator gloves 31270	1	0[a]	—

Choice and QC of materials and products

Gamete and embryo related procedures

207 SpST's
24 products
39 brands

3 types of products reprotoxic

Product	Brand/product type	Number of tests	SpST index 24 hours	SpST index 96 hours
Holding pipettes	Gynetics, 001-100-20	1	1.06	0.96
	Fertipro, HOLD-20	7	1.09	0.92
ICSI pipettes	Swemed, H-33311	1	1.31	1.00
	Fertipro, ICSI-20	5	1.08	0.88
	Gynetics, ICSI pipette 20°	4	1.33	1.09
	Humagen, MIC-50-20	2	1.21	1.11
Denudation pipette	Vitrolife H-130-33	12	0.97	0.96
	Vitrolife H-156-90	21	1.17	0.93
Syringes 1 mL	BD 30015	10	0.96	0.89
1-mL tuberculin	BD 300013	5	0.90	0.90
10 mL	BD 302188	14	0.94	0.92
20 mL	BD 300613	12	1.00	0.92
Tubes 5 mL	BD 352003	6	1.00	0.95
12 mL	BD 352001	7	1.10	1.00
15 mL	BD 352097	2	1.00	0.94
15 mL	BD 352095	8	1.10	1.00
Pipette 1 mL	BD 357521	4	1.00	1.10
5 mL	BD 357543	7	1.00	1.10
10 mL	BD 357551	8	0.97	1.10
Flask 50 mL	Falcon, BD	5	0.98	1.45
Specimen container and lid	Kendall	2	1.00	1.10
	VWR, D11181	9	1.00	1.05
	VWR, GOSSTPS1-019	3	1.01	1.06
Container lid →	Labline	1	0.80	0.07[a]
Petri dish →	Nunc 150270	2	0.93	0.16[a]
	Nunc 150255	1	0.91	0.81
→	BD 353004, cover	2	1.14	0.56[a]
	BD 353004, dish	4	1.04	0.90
	BD 353653	3	1.14	0.94
	BD 353652	4	0.85	0.9
Multidishes	Nunc, 144444	8	0.95	0.93
22-μm filter	Millex, SLGV033RS	6	0.94	0.97
	Millex, SVGV01015	1	1.34	1.00
Freezing straws	CBS 010288	4	1.01	1.00
Filling tips	CBS008656	5	1.31	1.00
Pasteur pipette →	Pould, 1812/VA	1	0.88	0.56[a]
	Humagen, 16-PP-9	2	1.08	1.13
	IM series, Repromed 1685	3	1.08	0.97
Tips	Biopur, EPPE 00300310035	5	0.95	0.88

Evaluation 1 wash – 2 wash

period: 01.06.07 – 06.10.07

455 cycles week/week

1 x wash: PR 8.7%

2 x wash: PR 13.7%

Evaluation 7.5 ml wash – 10 ml wash

period: 01.01.08 – 15.04.08

190 cycles week/week

7.5ml wash: PR 16.6%

10 ml wash: PR 19.6%

The Walking Egg

Ziekenhuis Oost-Limburg

Evaluation Gsperm wash – GIVF wash

period: 10.10.07 –20.12.07
85 cycles week/week

Gsperm wash: PR 16.2%

GIVF wash: PR 21%

Evaluation GIVF wash – EBBS suppl wash

period: 01.01.08 – 15.04.08
145 cycles

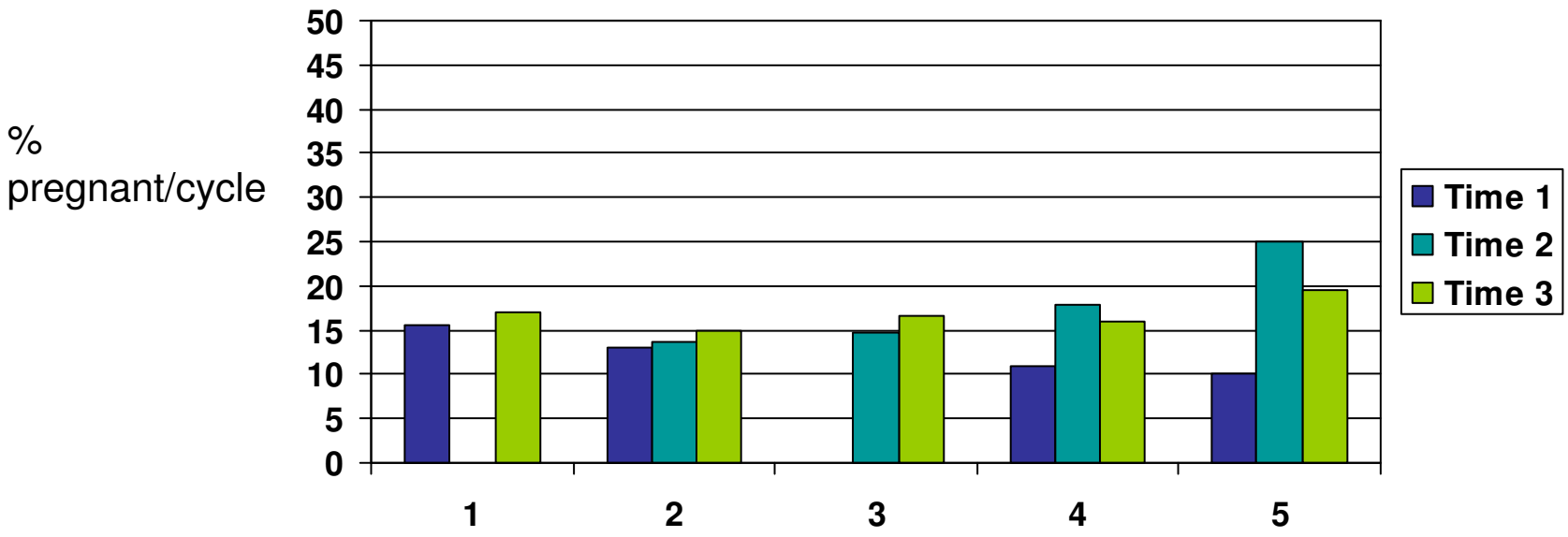
GIVF wash: PR 19.7%

EBBS suppl wash: PR 23.1%

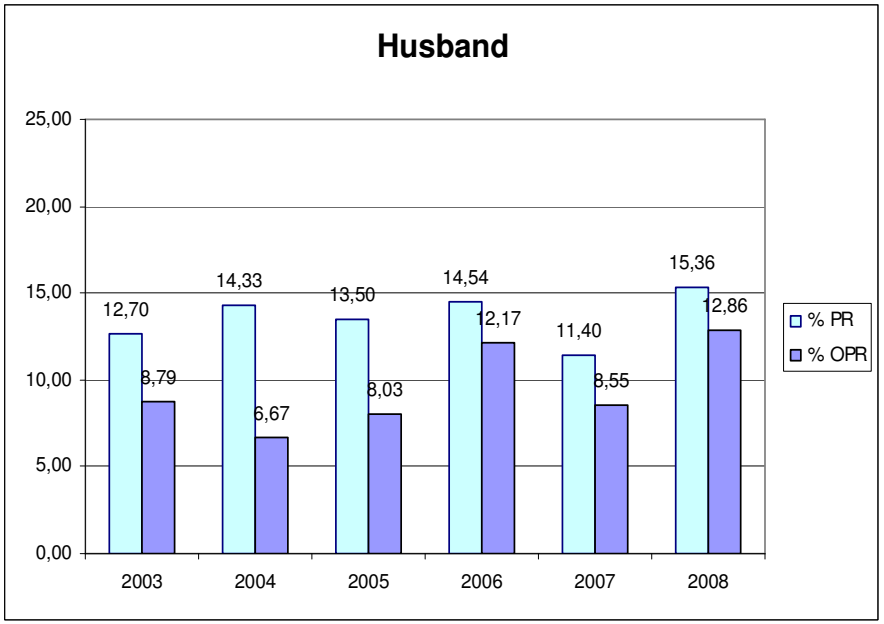
The Walking Egg

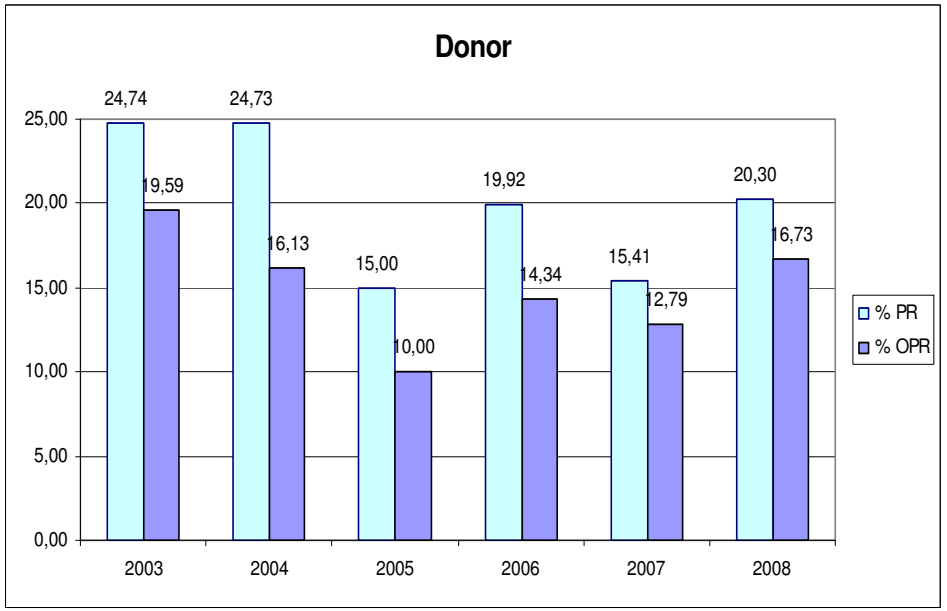
Ziekenhuis Oost-Limburg

Inter operator evaluation: every six months



Pregnancy rate and ongoing pregnancy rate
Partner IUI
GIFT Genk

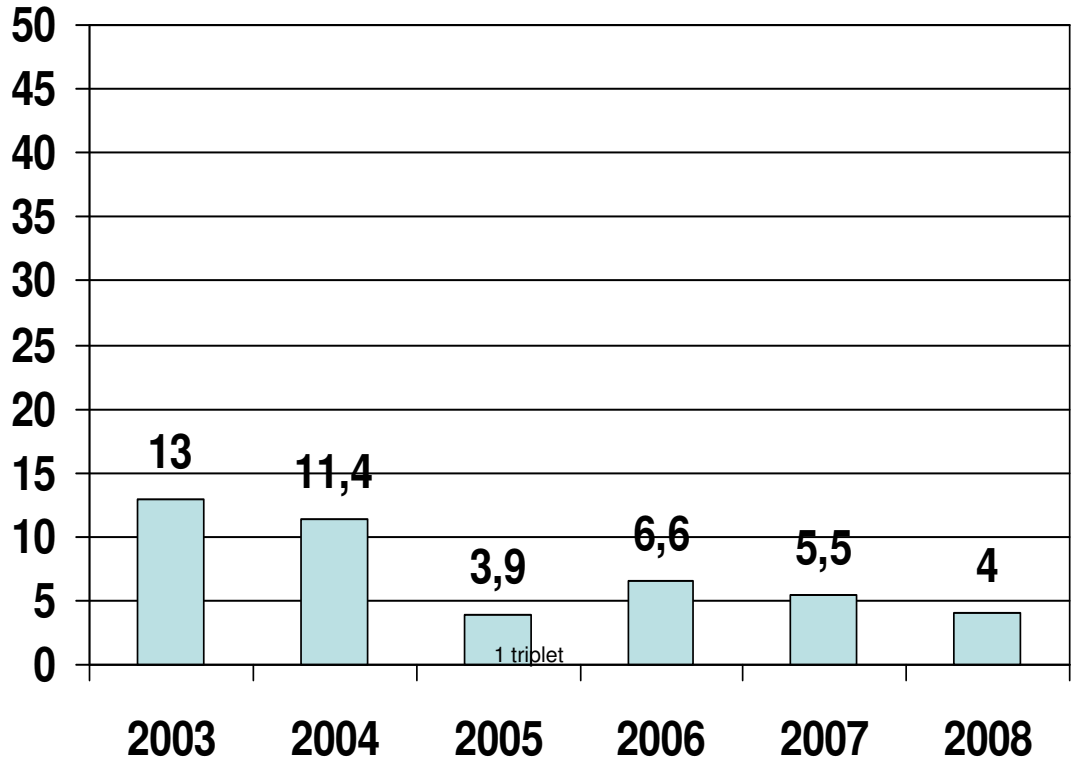




The Walking Egg

Ziekenhuis Oost-Limburg

Multiple pregnancy rate
GIFT Genk



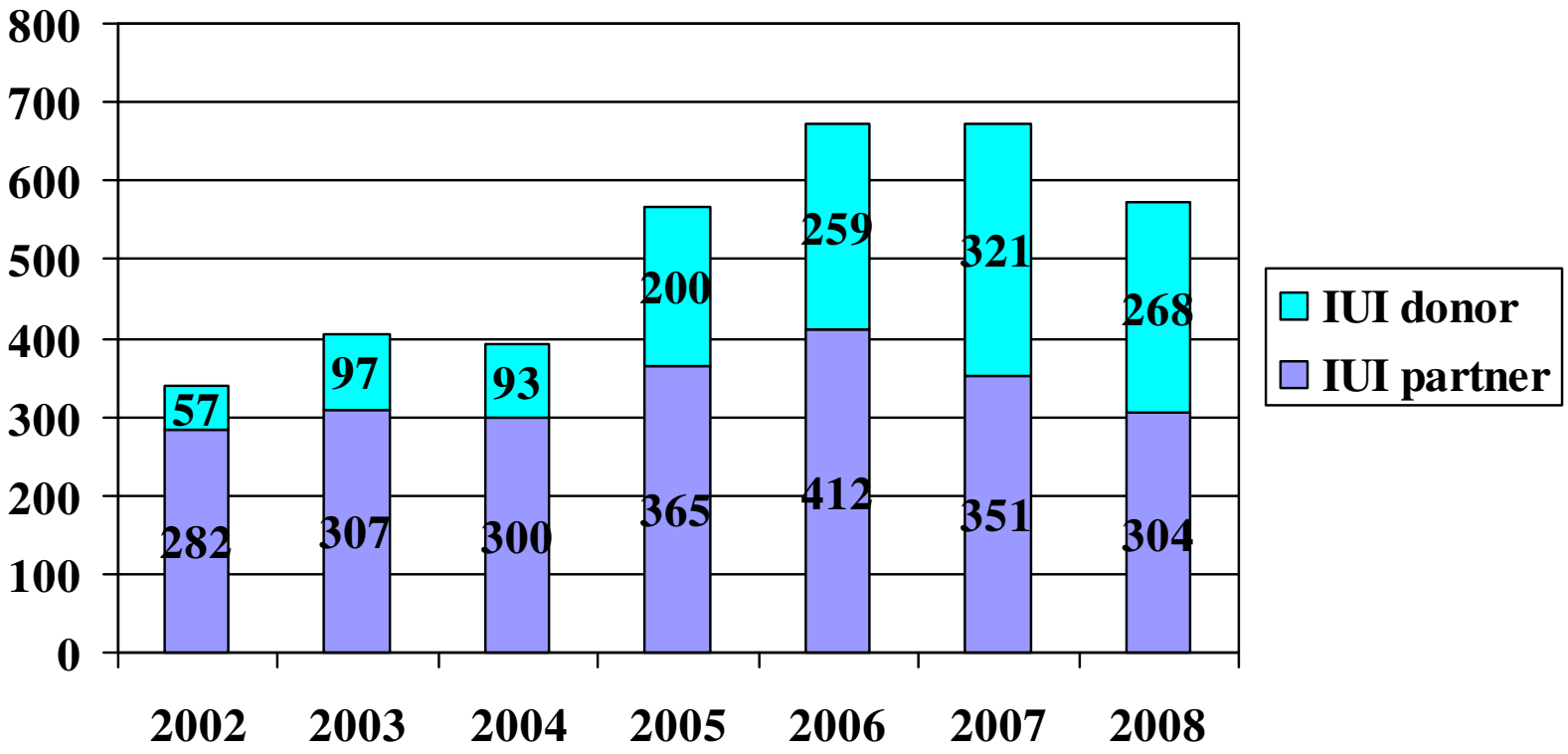
The Walking Egg

Ziekenhuis Oost-Limburg

The dos and don’ts of the insemination kitchen.

Nijs Martine
PhD, MS, Senior Clinical embryologist
Genk Institute for Fertility Technology
Belgium

IUI partner and IUI donor
Genk Institute For Fertility Technology



The Walking Egg

Ziekenhuis Oost-Limburg