New Developments in the IVF-Lab and their Impact on the Patient

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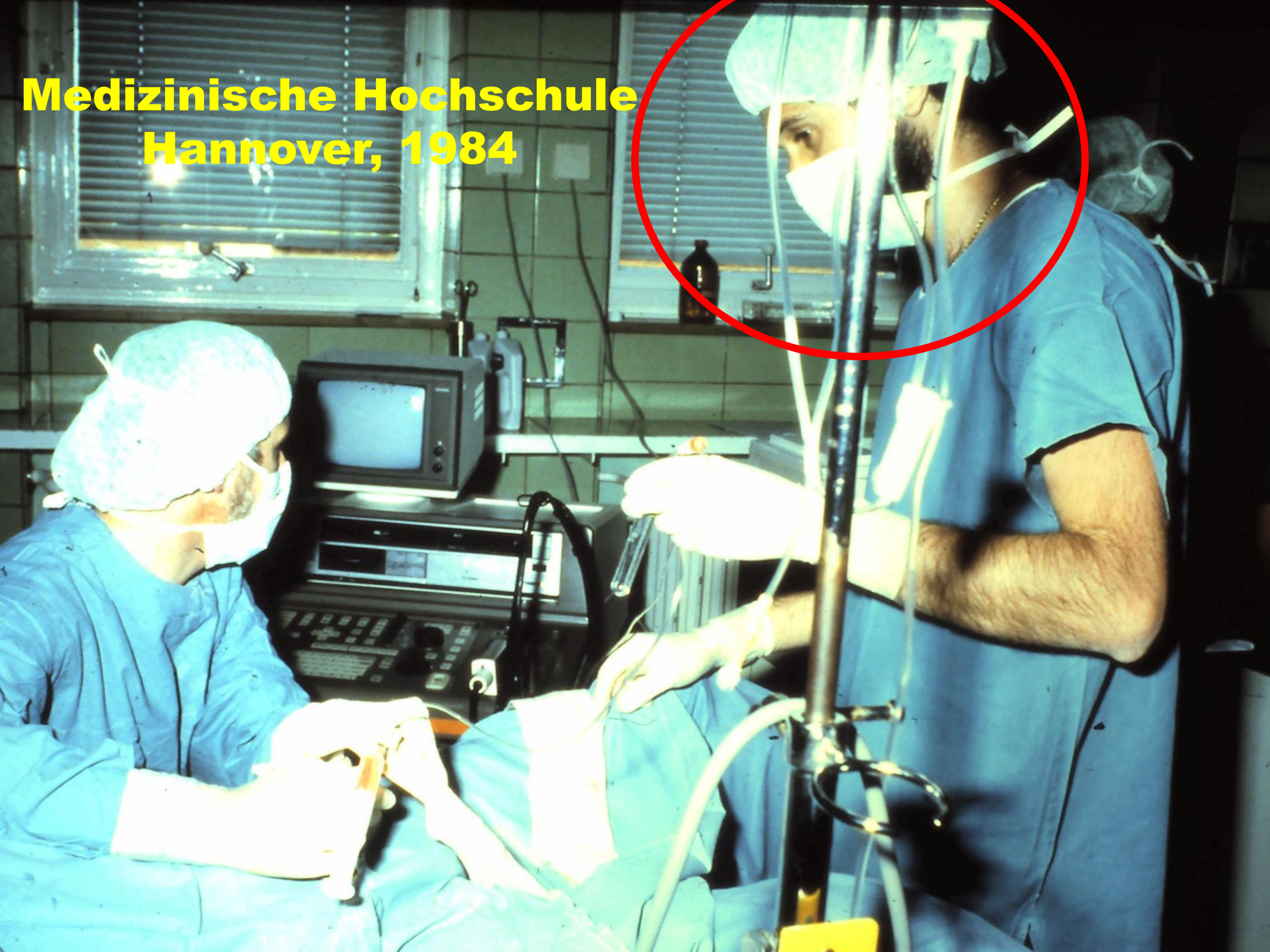
Kinderwunsch-Zentrum
Stuttgart

FRAUENKLINIK DER MEDIZINISCHEN HOCHSCHULE HANNOVER

Erfolgsrate nach Extrakorporaler Befruchtung

Jahr	1982	1983	1984	1985
Follikelpunktionen	25	56	53	130
Embryotransfers	5	20	12	69
Schwangerschaften	0	3	4	14
			30 %	**20 %**

Medizinische Hochschule Hannover, 1984



DIR

German IVF Registry

	IVF Foll. punct.	IVF Pregn. rate	ICSI Foll. punct.	ICSI Pregn. rate
2006	9927	27,39 %	25051	26,46 %
2009	9882	26,87 %	31289	27,36 %
2012	9237	26,89 %	31340	26,31 %

New developments in the IVF-Lab

Is it all a matter of selection?

Sperm selection
- IMSI
- PICSI

Embryo selection
- Blastocyst culture
- Quality Mixed Embryo Transfer

Endometrium selection
- Scratching
- Seminalplasma-Flushing

IMSI

Intracytoplasmatic
Morphologically-selected
Sperm-
Injection

Fertil Steril 2013(100)379

Sperm vacuoles negatively affect outcomes in intracytoplasmic morphologically selected sperm injection in terms of pregnancy, implantation, and live-birth rates

IMSI

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[a] Centre for Reproductive Medicine, European Hospital, Rome, Italy; and [b] Clinica Margen, Molecular and Genetics, Granada, Spain

Objective: To retrospectively evaluate whether sperm vacuoles influence clinical results, with a pa
101 intracytoplasmic morphologically selected sperm injection (IMSI) cycles.
Design: Retrospective, observational study.
Setting: Medical center.
Patient(s): A total of 101 couples with at least two failed intracytoplasmic sperm injection (I
morphology.
Intervention(s): Patients divided into two groups according to sperm morphology and vacuoliz
patients with good quality spermatozoa (type I and/or type II spermatozoa) (n = 63 patients); gro
quality spermatozoa (type III and/or IV spermatozoa) (n = 38 patients).
Main Outcome Measure(s): Fertilization rate, embryo quality, pregnancy, implantation, and live
Result(s): No statistically significant differences were observed between group A and B with rega
outcomes (fertilization rate and embryo quality). However, the "late" outcomes (pregnancy, impla
statistically significantly higher in group A.
Conclusion(s): These results confirm a correlation between sperm vacuoles and a negative IMSI outcome, suggesting that sperm vacuoles are related to the late paternal effect. (Fertil Steril® 2013;100:379–85. ©2013 by American Society for Reproductive Medicine.)
Key Words: Clinical outcomes, intracytoplasmic, IMSI, live-birth rate, morphologically selected sperm injection, vacuoles

Discuss: You can discuss this article with its authors and with other ASRM members at http://fertstertforum.com/grecoe-sperm-vacuoles-imsi-live-birth-rate/

Human sperm head vacuoles are physiological structures formed during the sperm development and maturation process

Atsushi Tanaka, M.D.,[a] Motoi Nagayoshi, M.D.,[a] Izumi Tanaka, Phar.B.,[a] and Hiroshi Kusunoki, Ph.D.[b]

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Objective: To clarify whether human sperm vacuoles affected intracytoplasmic sperm injection [ICSI] success rates.
Design: Retrospective study.
Setting: A private infertility clinic.
Patient(s): Spermatozoa and spermatids were obtained from 11 normozoospermic, 10 oligozoospermic or asthenozoospermic, 4 obstructive azoospermic, and 3 nonobstructive azoospermic men.
Intervention(s): Differential interference contrast observation and intracytoplasmic injection of morphologically selected sperm.
Main Outcome Measure(s): Incidence, size, and position of vacuoles of sperm cells were recorded. Ability of fertilization and blastocyst development were compared between cells with and without vacuoles.
Result(s): More than 97.4% of ejaculated, 87.5% of epididymal, 87.5% of testicular spermatozoa, and more than 90.0% of Sc-Sd2 spermatids had vacuoles of various sizes. The incidence of vacuoles on ejaculated cells was significantly higher than that on the other types of cells, but there was no difference between sperm from normozoospermic men and those from the other donors. Removal of plasma membrane and/or acrosome did not affect the incidence of vacuoles. Although more than 60% of spermatozoa had small vacuoles in the acrosomal regions, 52.6% of Sb1-2 spermatids had large vacuoles. After injection of a motile spermatozoon with large and small vacuoles, 60.9% and 85.7% of metaphase II oocytes could be normally fertilized, respectively, and almost half of the zygotes developed to the blastocyst stage. When using sperm without vacuoles, the fertilization rate was 80.0%, but only 25% of them developed to the blastocyst stage.
Conclusion(s): Human sperm head vacuoles did not affect ICSI outcomes. (Fertil Steril® 2012;98:315–20. ©2012 by American Society for Reproductive Medicine.)
Key Words: Vacuole, spermatozoa, spermatid, spermiogenesis, epididymal maturation

Discuss: You can discuss this article with its authors and with other ASRM members at http://fertstertforum.com/tanakaa-sperm-vacuoles-epididymal-maturation/

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Fertil Steril 2012(98)315

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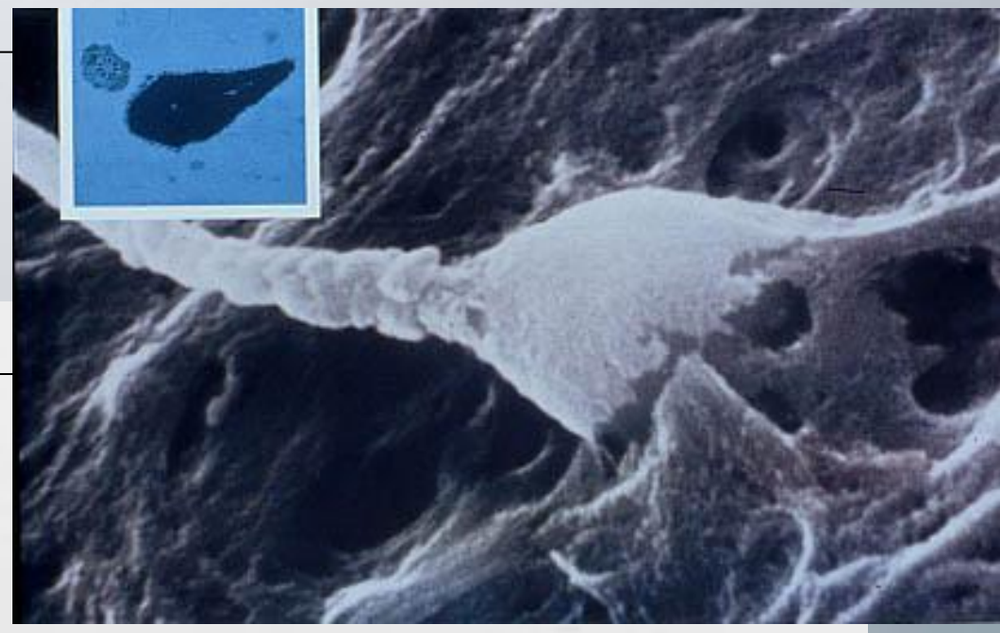
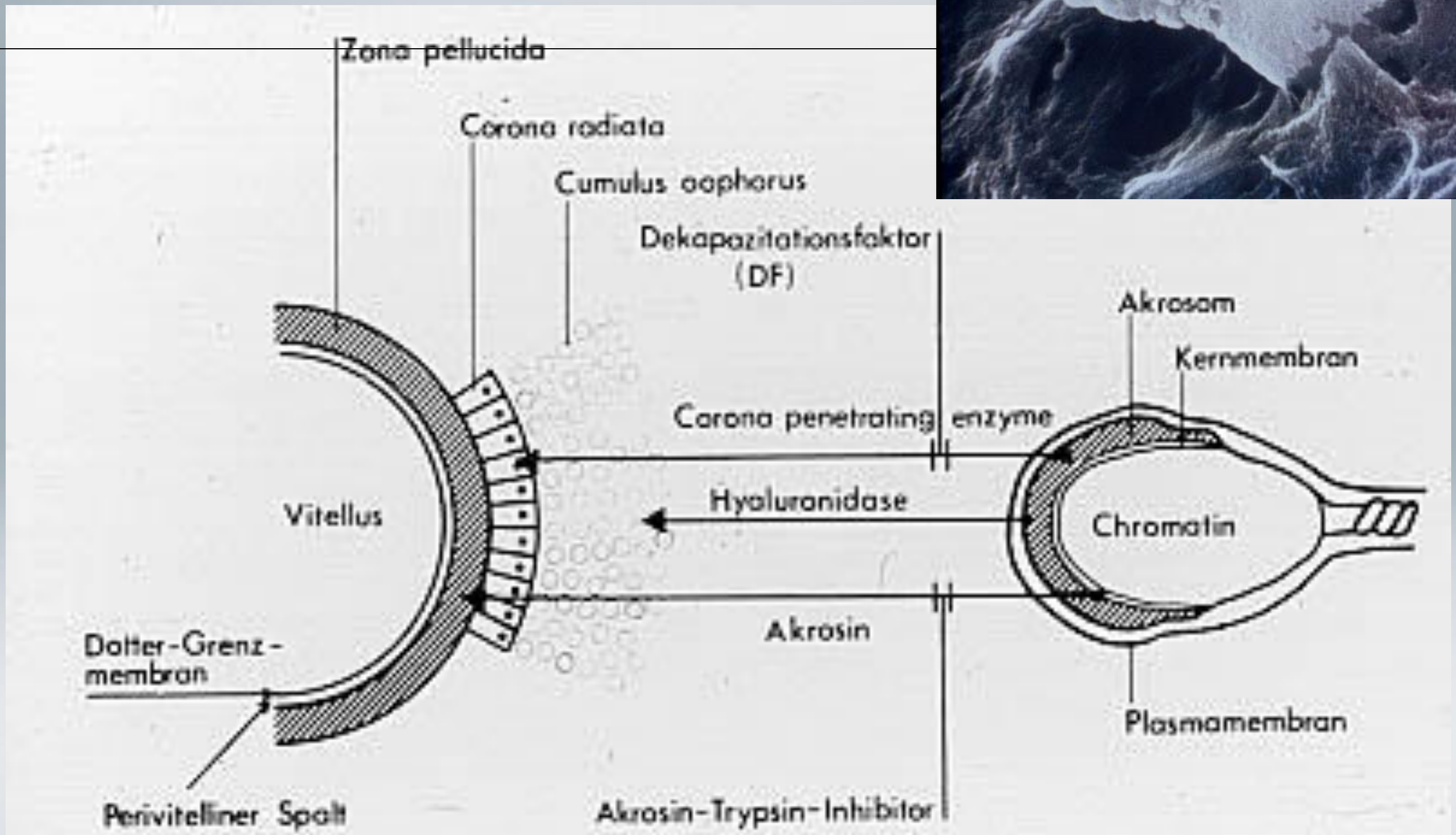
Endometrium selection

Scratching

Seminalplasma-Flushing

Acrosome-Reaction





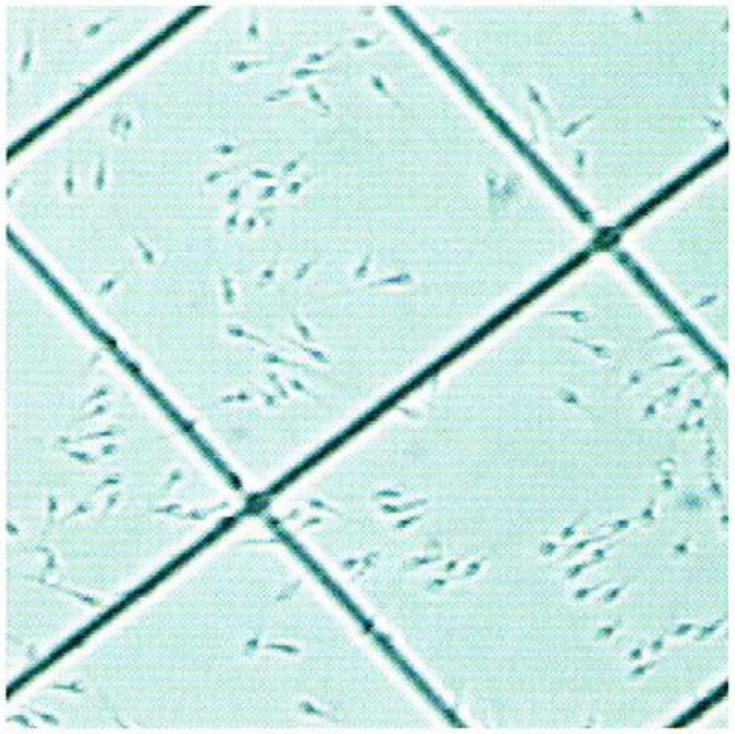
Schill Lab.med. 9(1985)63

Experiences with sperm selection by hyaluronic acid coated pertri dishes (PICSI) in an ART program

N. Saymé, K. Sollmann, D.H.A. Maas, T. Krebs

ASRM 2013, Boston

	Fertilization rate			Embyo Quality			Pregnancy rate		
	ICSI	PICSI	p	ICSI	PICSI	p	ICSI	PICSI	p
All samples	73,50%	73,86%	0,912	2,48	2,48	0,991	27,1%	23,4%	0,691
Normospermic	71,16%	64,20%	0,464	2,56	2,02	0,204	not evaluable		
Oligospermic	68,47%	63,88%	0,546	2,56	2,76	0,421	26,7%	16,7%	0,674
Asthenospermic	74,96%	75,88%	0,833	2,46	2,56	0,524	27,8%	26,7%	0,554
Teratospermic	72,43%	74,50%	0,630	2,54	2,60	0,697	32,3%	23,7%	0,266



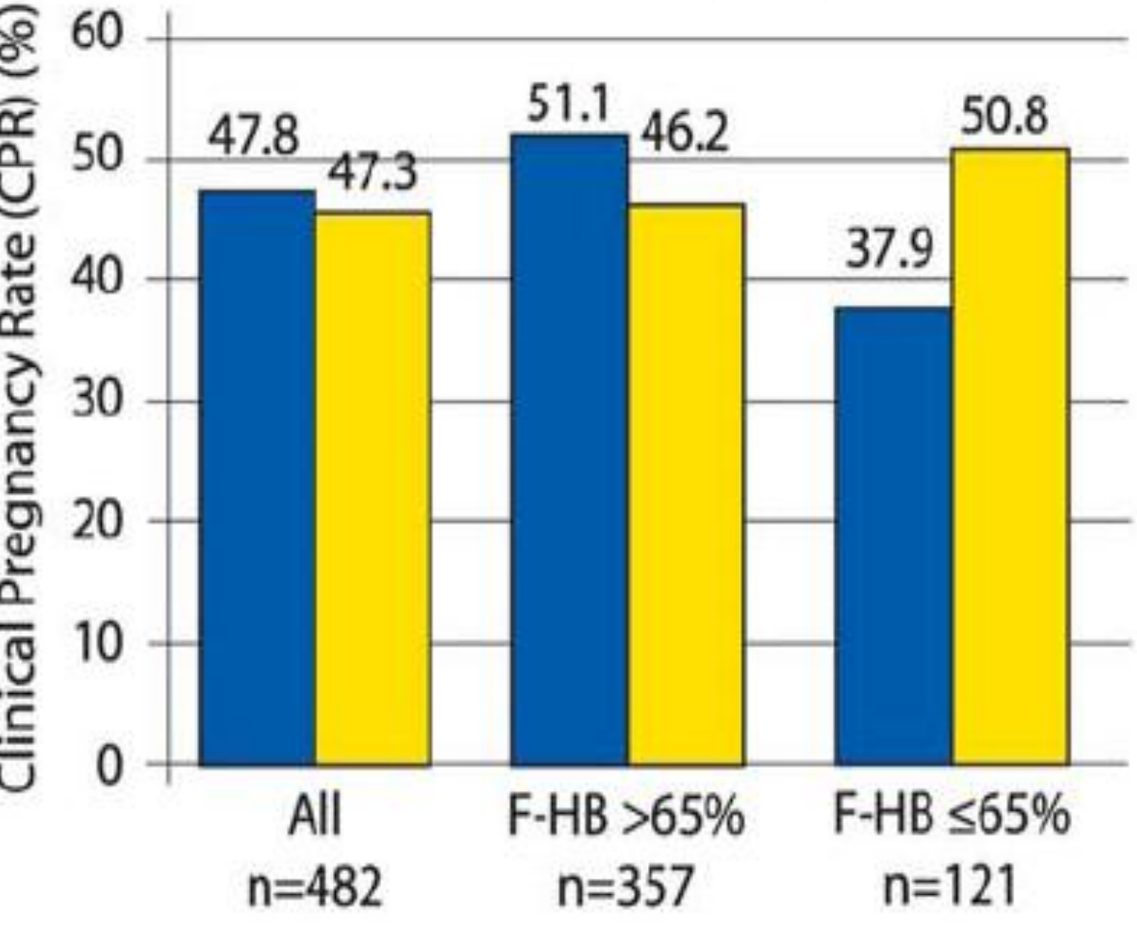
HBA

The sperm-Hyaluronan Binding Assay

HBA is designed to provide a qualitative assessment of sperm quality, maturity, and fertilizing potential

- HBA provides a rapid assessment of sperm quality, maturity and structural integrity
- Correlations with fertility outcomes and morphology assessments
- Simple, objective, and rapid assessment (<60 seconds)

Worrilow et al., HR 2012 (289) 306



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STANDARD-CULTURE

Embryotransfer on
day 2 in a (2-)4-(8-)cell-stage

LONGTERM-/ BLASTOCYST-CULTURE

Embryotransfer on
day 5 in the blastocyst-stage

Blastocyst culture

Pregnancy rates after Embryotransfer on Day 2/3 versus Day 4/5

age	Day 2/3	Day 4/5
<35	40,5 %	55,9 %
35-40	26,9 %	44,7 %
>41	12,1 %	21,2 %
Total	**28,4 %**	**46,8 %**

2013(Jan-Dec) 582 pat.
2014(Jan-Mar) 138 pat.

Kinderwunschzentrum Ulm/Germany

I.El-Danasouri pers.comm 2014

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Quality mixed embryo transfer

Embryo scoring:

Morphology

- Grade A: regular cleavage, no fragmentation
- Grade D: irregular cleavage, highly fragmented

In-phase cleavage stages

- Day 2: 4 cells
- Day 3: 8 cells
- Day 4: morula
- Day 5: blastocyst

I. El-Danasouri et al., pers.comm. 2014

Quality mixed embryo transfer

Embryo scoring

Good: Grade A + B
Cleavage stages in phase
or faster

Poor: Grade C + D
Cleavage stages slowlier
and out of phase

I. El-Danasouri et al., pers.comm. 2014

Quality mixed embryo transfer

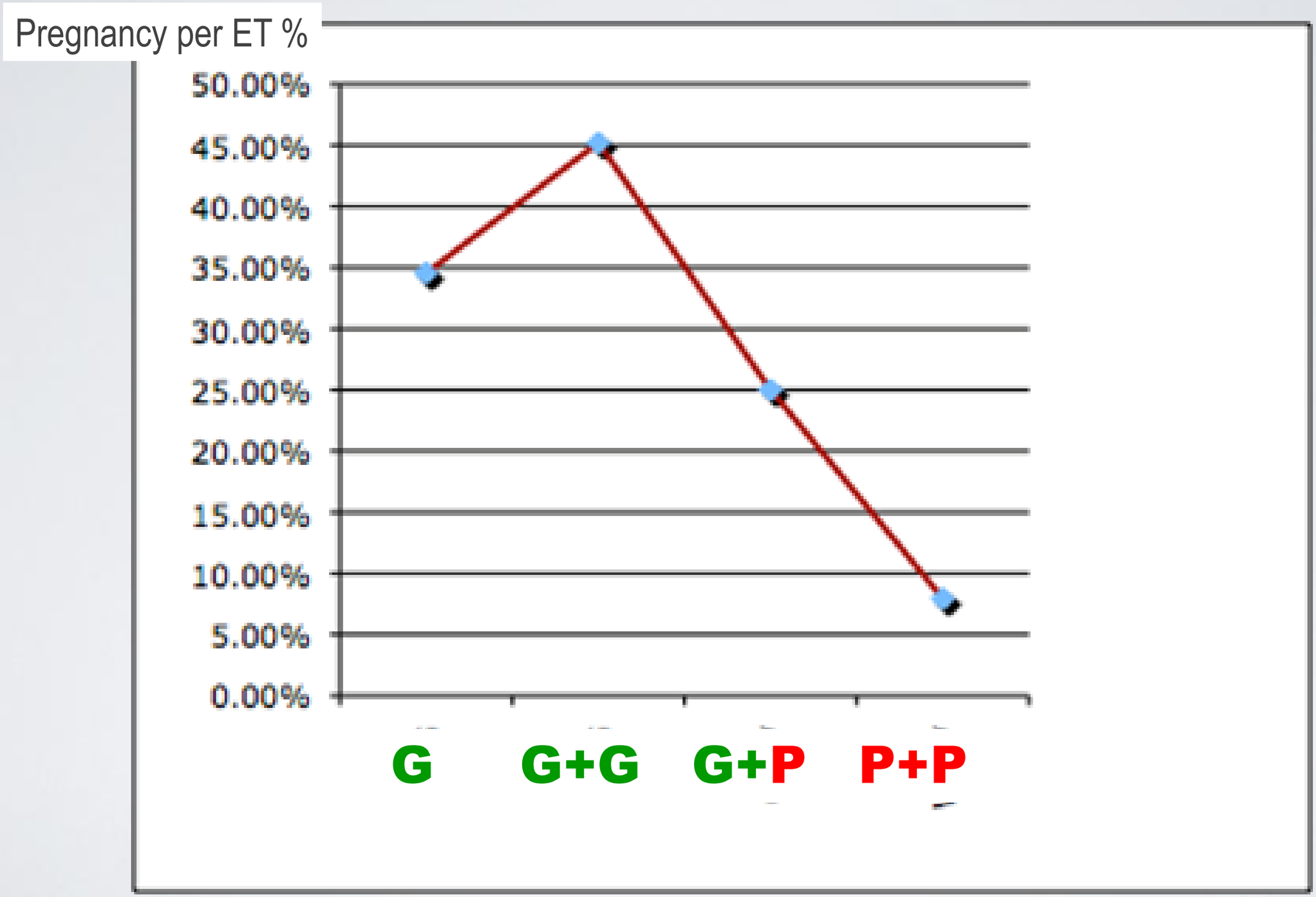
785 patients at the age of ≤ 35 yrs. with transfer of one or two embryos

G	1 embryo	55 pat	31,8 +/- 2,7 yrs
G+G	2 embryos	323 pat	32,0 +/- 2,0 yrs
G+P	2 embryos	236 pat	32,6 +/- 2,6 yrs
P+P	2 embryos	126 pat	33,1 +/- 2,9 yrs
P	1 embryo	45 pat	32,6 +/- 1,6 yrs

Kinderwunschzentrum Stuttgart, Germany, and
RISEL-One Day Medical Center, Rome, Italy

I. El-Danasouri et al., pers.comm. 2014

Quality mixed embryo transfer



I. El-Danasouri et al., pers.comm. 2014

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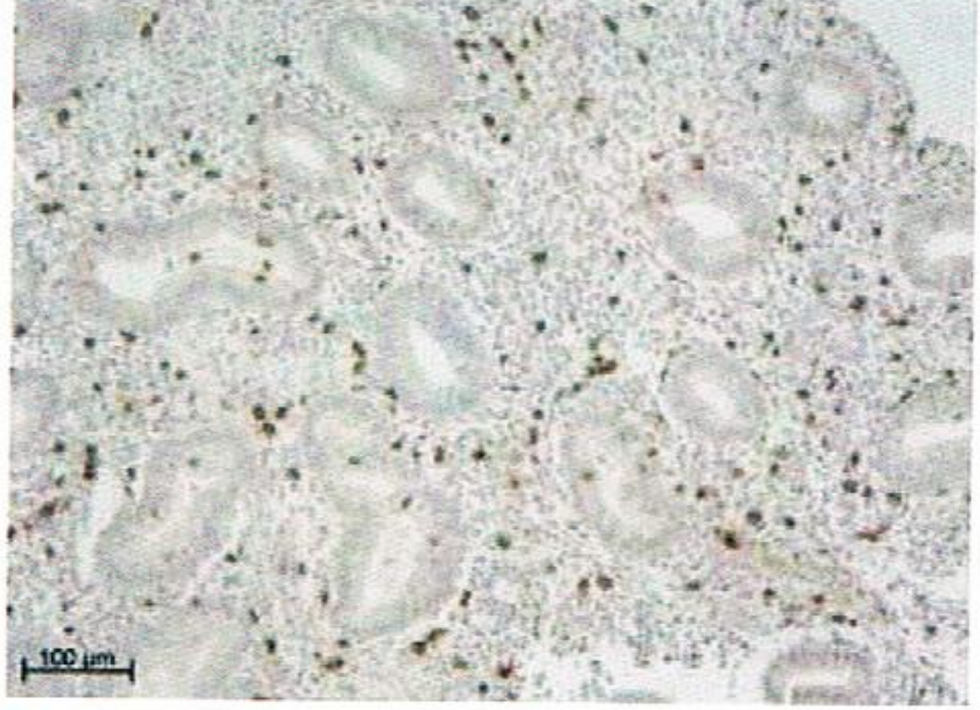

Bild 1

Before ovulation

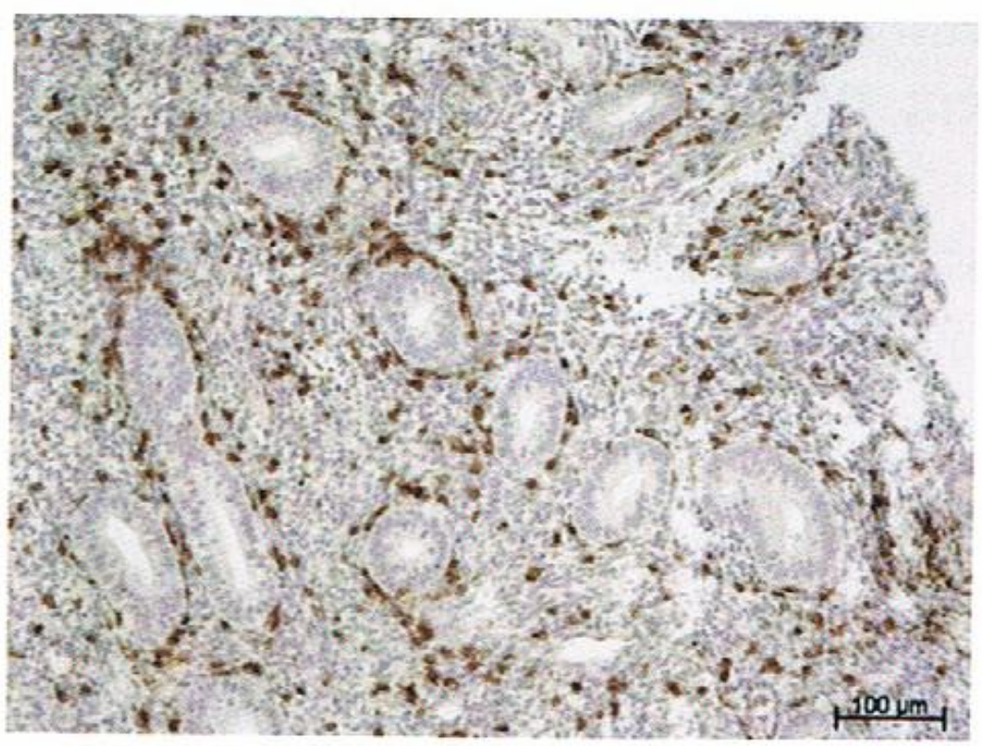

Bild 2

After ovulation

U. Markert, Jena, 2014

Endometrial natural killer cells

NK-cells invade into the endometrium after ovulation until week 20 of pregnancy. They are regarded responsible (besides destroying virus-infected and tumouros cells) for ***inducing vascular growth and immunological tolerance.***

Present therapeutic studies:

Inducing the NK-cell number by

- **Endometrial scratching**
- **G-CSF-injections**
- **seminal plasma flushing**

Reducing the NK-cell-number by

- intra venous **infusions of lipid-preparations** (omega-3/-6-fatty acids)

Seminal plasma flushing

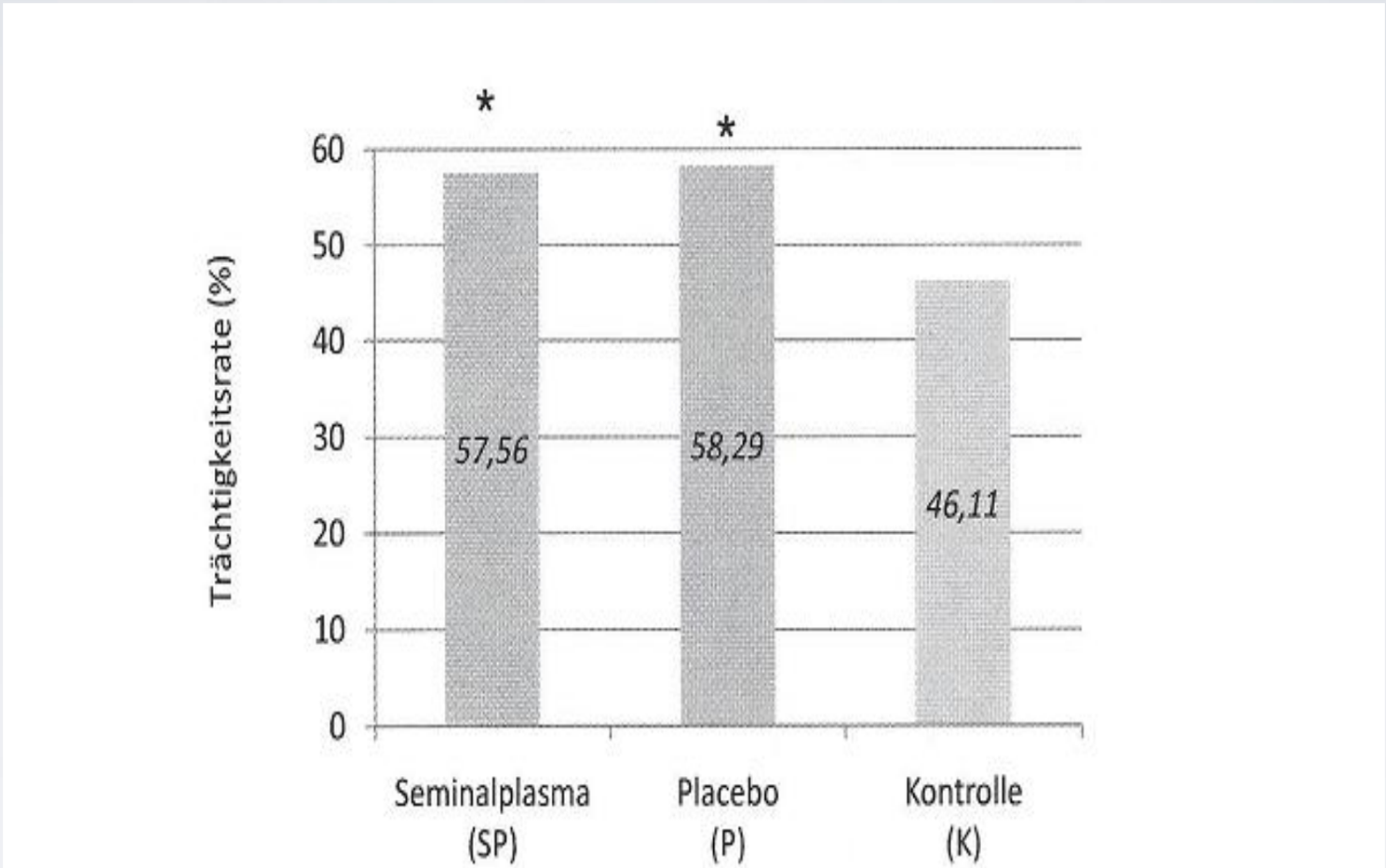

Abb. 12: Trächtigkeitsergebnisse in den Versuchsgruppen in Prozent ($n^{SP} = 172$; $n^{P} = 175$; $n^{Kontrolle} = 167$; * = signifikant im Vergleich zur Kontrollgruppe: $P_{SP} = 0,035$; $P_{P} = 0,024$)

Pregnancy rate after insemination in the bovine with and without flushing the vagina/cervix with seminal plasma or culture medium as placebo.

M. Scherhoff, School of Veterinary Medicine, Hannover, 2012

Thank you for your attention !

New developments in the IVF-Lab

Is it all a matter of selection?

Sperm-selection IMSI
PICSI

Embryo-selection Blastocyst culture
Micro Vibration
Mixed Embryo Transfer

Endometrium Stimulation
Scratching
Seminalplasma-Flushing

„Rüttler“
(„shaker“)

Three-dimensional vibration at 56 Hz delivered over 5 sec. every hour.

Micro vibration

	Micro vibration (1-4 cell group-culture) 244 pat.	Static culture (single cell-culture) 291 pat.	p
Fertilization rate	82,1%	78,2 %	0.02
Implantation rate	41,9 %	35,5 %	0,043
Clinical pregnancy rate	46,7 %	43,3 %	0,427

Kinderwunschzentrum Ulm
and Kinderwunschzentrum Stuttgart

I. El-Danasouri et al., pers. comm. 2014