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Gamete quality and ovarian reserve as markers for early pregnancy loss

Special Interest Group Early Pregnancy

14

1 July 2012
Istanbul, Turkey

Gamete quality and ovarian reserve as markers for early pregnancy loss

Istanbul, Turkey
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Organised by
the Special Interest Group Early Pregnancy

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Course coordinators

Ole B. Christiansen (Denmark)

Course description

There is uncertainly whether low quality of spermatozoa and low sperm count as well as low oocyte number and quality reflected in markers for ovarian reserve is predictive for an increased risk of biochemical pregnancies and miscarriages in subsequent pregnancies conceived with and without the use of ART.
The course will review the current knowledge about whether such associations exist and whether some of the markers for sperm and oocyte quality can be helpful in clinical practice.

Target audience

Reproductive physicians and biologists

Scientific programme

Chair: Mariette Goddijn (The Netherlands)

09.00 – 09.10	Introduction – **Ole B. Christiansen (Denmark)**
09:10 – 09:40	Subfertility, mode of conception and their effect on miscarriage rate – **Monique Brandes (The Netherlands)**
09.40 – 09.50	Discussion
09.50 – 10.20	Transmission electron microscopy and FISH studies of sperm in couples with recurrent miscarriage – **Gaia Terzuoli (Italy)**
10.20 – 10.30	Discussion
10.30 – 11.00	Coffee break

Chair: Siobhan Quenby (United Kingdom)

11.00 – 11.30	Markers of sperm quality and miscarriage rate – **Nicolas Garrido (Spain)**
11.30 – 11.40	Discussion
11.40 – 12.10	Sperm DNA damage and its effect on miscarriage after IVF/ICSI – **Armand Zini (Canada)**
12.10 – 12.30	Panel discussion with Nicolas Garrido, Gaia Terzuoli, Armand Zini, and delegates on the role of sperm factors in post-conception reproductive failure and its clinical implications
12.30 – 13.30	Lunch break

Chairs: Roy Farquharson (United Kingdom)

13.30 – 14.00	Are ovarian reserve tests predictive of miscarriage in women undergoing ART? – **Jayaprakasan Kannamannadiar (United Kingdom)**
14.00 – 14.10	Discussion
14.10 – 14.40	Ovarian reserve and early pregnancy – **Maaike Haadsma (The Netherlands)**
14.40 – 14.50	Discussion
15.00 – 15.30	Coffee break

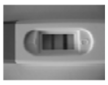
Chair: Ole B. Christiansen (Denmark)

15.30 – 16.00	Anti-Müllerian hormone levels and miscarriage rates after IUI – **Kelton Tremellen (Australia)**
16.00 – 16.30	Anti-Mullerian hormone levels in women with recurrent miscarriage and their value in predicting another miscarriage – **Elisabeth Clare Larsen (Denmark)**
16.30 – 17.00	Panel discussion with Banchhita Sahu, Maaike Haadsma, Kelton Tremellen, Elisabeth Clare Larsen and delegates on the role of ovarian reserve tests in miscarriage and their clinical importance

Subfertility, mode of conception and their effect on miscarriage rate

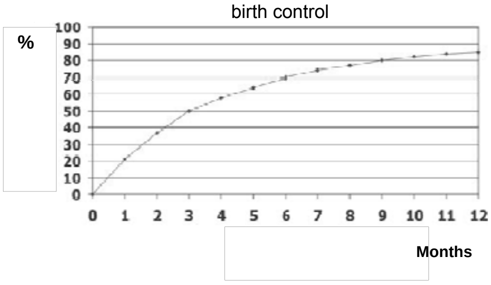
M. Brandes, MD PhD

Infertility



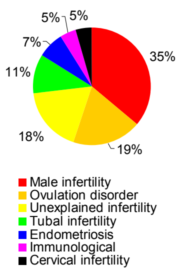
Normal fertility

Pregnancy chance in months after discontinuation of birth control



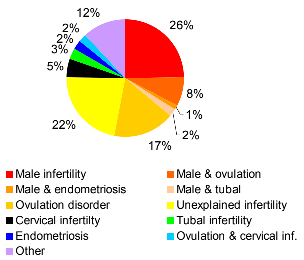
Diagnoses in the infertility clinic

Hull et al. 1985



N=708

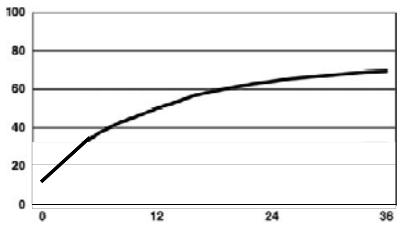
Brandes et al. 2010



N=2476

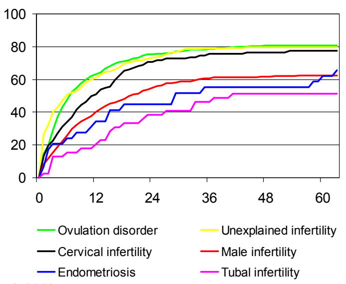
Overall outcome

Percentage ongoing pregnancies infertile patients



Brandes et al. 2010

Pregnancy chance per diagnosis



Brandes et al. 2010

Miscarriage rate

General population 10-15%
Infertile population 18-30%

Infertility and miscarriage

1572 women, 3269 pregnancies (1980-1990)

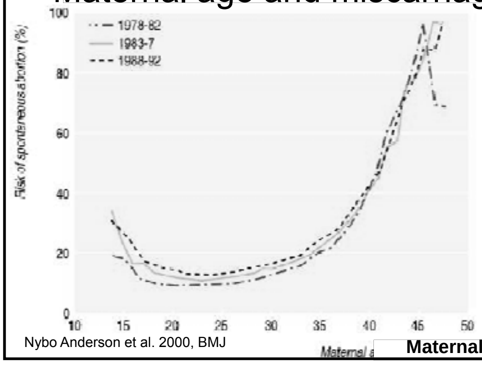
	Infertile couples	Fertile couples	Adj OR (95% CI)
Miscarriage	23%	14%	1.71 (1.26-2.94)

Infertile women experience more frequently a miscarriage compared to normal fertile women

Gray and Wu 2000, Am J Publ Health

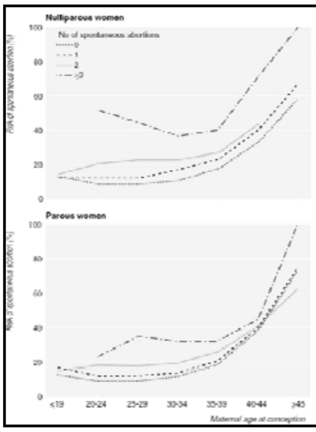
Risk factors for miscarriage

Maternal age and miscarriage



Nybo Anderson et al. 2000, BMJ

Maternal age



Nybo Anderson et al. 2000, BMJ

Maternal age and miscarriage

TABLE 1-Study Outcomes, by Characteristics of the Women at Time of Conception: New York and Vermont, 1980-1990

Characteristic	No of Pregn	**Spont abortion Rate %**	Odd Ratio (95% CI)		**Waiting Time >1y %**	Odd ratio of Infertility (95% CI)	
Age at conception, y							
<24	1001	10.4	1.0		5.8	1.0	
25-29	1277	13.6	1.36	(1.04-1.78)	9.6	1.73	(1.24-2.43)
30-34	573	22.3	2.48	(1.85-3.32)	14.3	2.72	(1.88-3.93)
>35	116	22.4	2.49	(1.50-4.13)	13.8	2.60	(1.38-4.86)

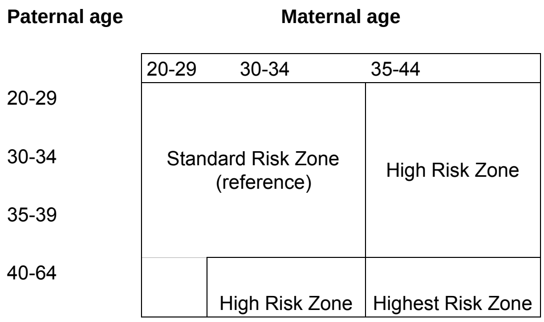
Gray and Wu 2000, Am J Publ Health

Maternal and paternal age and miscarriage

Paternal age	Maternal age 20-29	30-34	35-44
20-29	1.00 ref	1.72 0.62-4.74	9.18 1.8-46.66
30-34	1.06 0.61-1.86	1.62 0.93-2.82	3.87 1.24-12.02
35-39	1.31 0.56-3.07	1.06 0.52-2.17	3.38 1.76-6.47
40-64	1.80 0.52-6.24	2.90 1.26-6.67	6.73 3.50-12.95

de la Rochebrochard and Thonneau 2002, Hum Reprod

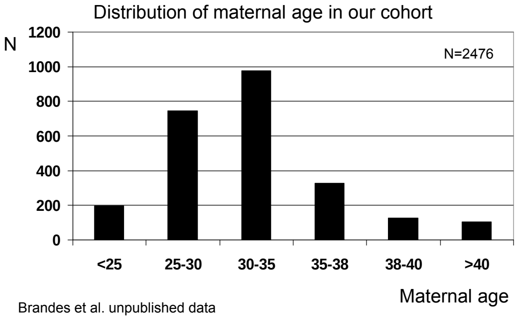
Maternal and paternal age and miscarriage



de la Rochebrochard and Thonneau 2002, Hum Reprod

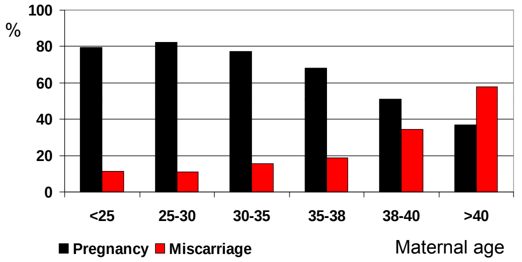
Maternal age

Distribution of maternal age in our cohort



Brandes et al. unpublished data

Maternal age and miscarriage



Brandes et al. unpublished data

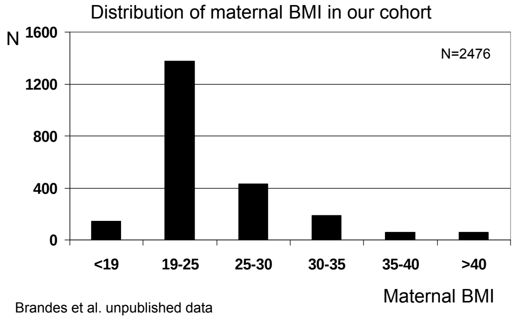
Maternal age and BMI and miscarriage

		n	Abn karyo type	p value			n	Abn karyo type	p value
AGE	<35	93	49.5	0.009	ART	IUI/nat	90	62.2	0.433
	≥35	111	67.6			IVF	111	56.8	
BMI	<25	153	63.4	0.040	ICSI	no	53	50.9	0.237
	≥25	51	47.1			yes	58	62.1	
PCOS	no	156	61.5	0.244	RPL	no	173	59.0	0.915
	yes	48	52.1			yes	30	60.0	

Landres et al. 2010, Hum Reprod

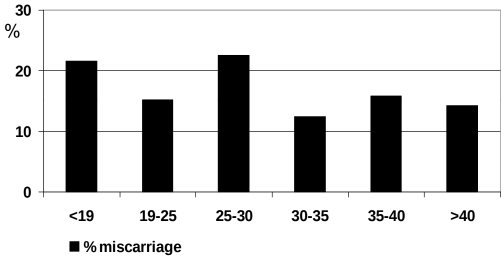
Maternal BMI

Distribution of maternal BMI in our cohort



Brandes et al. unpublished data

Maternal BMI and miscarriage



Brandes et al. unpublished data

Tobacco and Cocaine and miscarriage

400 women with miscarriage, aged 14-40 years

	Spontaneous abortion	No spontaneous abortion
Tobacco use	34.6	21.8
Cocaine use	28.9	20.5

Tobacco and cocaine use were associated with spontaneous miscarriage.

Ness et al. 1999, N Eng J Med

Cigarette, alcohol, caffeine and miscarriage

330 women with spontaneous abortion
1168 women with ongoing pregnancy

	OR (95% CI)
≥5 U alcohol/week	4.84 (2.87-8.16)
375mg caffeine/day	2.21 (1.53-3.18)
10-19 cigarettes	-
≥ 20 cigarettes	-

Consumption of ≥5 units alcohol p/wk and ≥ 375 mg caffeine p/day during pregnancy may increase the risk for spontaneous abortion.

Rasch et al. 2003, Acta Obstet Gynecol Scan

Fertility diagnosis and miscarriage

PCOS and miscarriage

Single center, retrospective study
PCOS: 631 patients
Controls (tubal path): 1423 patients

Pregnancies after IVF

409 miscarriage (23% vs 17%, $p<0.05$)

Yan et al. 2011, Zhonghua Fu Chan ke Za Zhi

PCOS and miscarriage

Single study, 1018 patients

Overall incidence of miscarriage 21%
Univariate:
- PCOS vs non-PCOS: 25% vs 18% $p<0.01$

Multivariate logistic regression:
- PCOS vs non-PCOS: not significant !

Wang et al. 2001, Hum Reprod

Endometriosis and miscarriage

140 patients with endometriosis

182 IVF cycles

Table II. Pregnancy outcome and implantation rate after in-vitro fertilization using gonadotrophi

Groups	No. of cycles	Implantation rate	Pregnancy rate/ transfer	Miscarriage rate
1. Male factor	45	23.4%	39% (9/23)	0
2. Unexplained	196	27.6%	48% (76/159)	3.9% (3)
3. Tubal factor	1136	23.4%	45% (465/1039)	3.4% (16)
4. Endometriosis	129	21.8%	40% (44/110)	0

Absolute numbers are given in parentheses. The differences between the groups for implantation

Geber et al. 1995, Hum Reprod

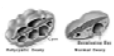
Relation of mode of conception to miscarriage

Infertility treatment and miscarriage

Single center, historical cohort, 418 patients

Spontaneous vs OI, vs IVF, vs non-OI depending treatment

No increased risk for miscarriage after either treatment



Pezeshki et al. 2000, Fertil Steril

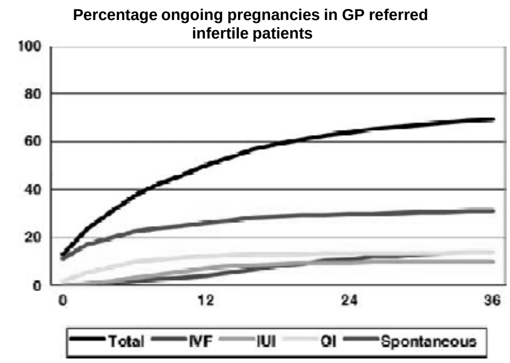
Infertility treatment and miscarriage

Multicenter, 6759 patients

Spontaneous vs ART pregnancies (2503 patients)

Corrected by female age (RR 1.20, 95%CI 1.03-1.46)

Wang et al. 2004, Hum Reprod



Brandes et al. 2010, Hum Reprod

Study design

2476 patients

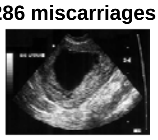
⇩

1809 clinical pregnancies

⇙ ⇘

1523 ongoing pregnancies

286 miscarriages



Brandes et al. 2011 RBM Online

Results

	Ongoing pregnancy	Early pregnancy loss	p value
	Mean ± SD	Mean ± SD	
Female age, years*	30.1 ± 4.1	32.1 ± 4.9	<0.001
Male age, years*	33.1 ± 5.1	34.1 ± 5.6	0.006
Female BMI kg/m2*	24.2 ± 4.8	23.8 ± 4.5	NS
Duration of infertility, months**	16.8 ± 12.3	17.9 ± 13.2	NS
Time to pregnancy, months**	25.8 ± 16.3	27.3 ± 16.7	NS

Brandes et al. 2011 RBM Online

Results

Multivariate analysis

Female age	(p<0.001)
Male age	(p=0.006)
Obstetrical history	(p=0.10)
Male alcohol use	(p=0.02)
Type of menstrual cycle	(p=0.14)
Diagnosis	(p=0.05)
Secondary or tertiary hospital	(p=0.03)
Female smoking behaviour	(p=0.12)

Brandes et al. 2011 RBM Online

Infertility treatment and miscarriage

Mode of conception	n	Miscarriage n	(%)	OR	95% CI
Spontaneous	864	125	(14.5)	1	-
OI	266	42	(15.8)	1.3	0.79-2.18
IUI	20	5	(25.0)	2.4	0.82-7.42
IUI/COH	203	37	(18.2)	1.3	0.85-2.10
IVF	190	31	(16.3)	1.1	0.67-1.75
ICSI	202	30	(14.9)	1.0	0.60-1.62
FET	**61**	**16**	**(26.2)**	**2.2**	**1.14-4.19**

Corrected for: female and male age, obst.history, diagnosis, male alcohol use, female smoking

Brandes et al. 2011, RBM Online

Infertility treatment and miscarriage

Distribution of the embryo quality for pregnancies after the different treatments.

	Grade 1 embryo Misc/pr	%	Grade 2 embryo Misc/pr	%	Grade 3 embryo Misc/pr	%	Grade 4 embryo Misc/pr	%
IVF	3/31	9.7	19/104	18.3	7/37	18.9	1/4	25.0
ICSI	4/21	19.0	22/128	17.2	3/27	11.1	0/1	-
FET	1/10	10.0	10/32	31.3	2/7	28.6	0/1	-

Brandes et al. 2011, RBM Online

Conclusions

The incidence of miscarriage in an infertile population is 18-30%.

But, most studies not corrected for risk factors, like female age

Age and lifestyle most important factors in an infertile population

Diagnoses give contradictory results

Conclusions

Modes of conception did not show an increased miscarriage rate, except for the replacement of frozen-thawed embryos

The quality of the embryo influences the chance of pregnancy but it does not influence the pregnancy outcome.

This information can be useful in counseling infertile couples.

Thank you for your attention!

University of Siena
Department of Biomedical Sciences, Applied Biology Section
Interdepartmental Centre for Research and Therapy of Male Infertility

Transmission electron microscopy and FISH studies of sperm in couples with recurrent miscarriage

Gaia Terzuoli

Disclosure of commercial and financial relationships and conflict of interest: none

RECURRENT MISCARRIAGE (RM)

- Three or more consecutive miscarriages before 20 weeks post-menstruation with the same biological father
- Around 1% of fertile couples
- More pregnancies are lost spontaneously than pregnancies carried to term
- The most common complication of pregnancy
- The experience can be painful for the couple

CAUSES OF RM

- Around 50% are associates with parental chromosomal anomalies, maternal thrombophilic disorders, structural uterine anomalies, maternal immune dysfunction, endocrine abnormalities
- Around 50 % are idiopathic

Any possible treatment for known causes?

- Treatment of genetic problems: in vitro fertilization
- Treatment of immunological factors (Antiphospholipid antibody syndrome and Systemic lupus erythematosus): Aspirin, Low-molecular-weight heparin, Prednisone
- Treatment of hormonal causes:
  - Luteal phase defect: Progesterone supplements, Clomiphene citrate
  - Polycystic ovary disease: Metformin

Male contribution to RM?

⇩

SEMEN ANALYSIS

ANALYSIS OF SEMEN PARAMETERS (WHO guidelines)

- Liquefaction
- Volume
- pH
- Motility
- Concentration
- Viability
- Morphology

Is this information enough?

Some studies, available in the literature, using different kind of sperm analysis to investigate male contribution

- Sperm aneuploidy and recurrent pregnancy loss, Bernardini et al. 2004
- Possible role of male factors in recurrent pregnancy loss, Saxena et al. 2008
- **TEM and FISH studies in sperm from men of couples with recurrent pregnancy loss, Collodel et al. 2009**
- Evaluation of sperm´s chromatin quality with acridine orange test, chromomycin A3 and aniline blue staining in couples with unexplained recurrent abortion, Kazerooni et al. 2009

Some studies, available in the literature, using different kind of sperm analysis to investigate male contribution

- Assessment of sperm factors possibly involved in early recurrent pregnancy loss, Gil-Villa et al. 2010
- Y chromosome microdeletions are not associated with spontaneous recurrent pregnancy loss in a Sinhalese population in Sri Lanka, Wettasinghe et al. 2010
- Y chromosome microdeletions, sperm DNA fragmentation and sperm oxidative stress as causes of recurrent spontaneous abortion of unknown etiology, Bellver et al. 2010
- Value of sperm chromatin dispersion test in couples with unexplained recurrent abortion, Absalan et al. 2012

First International Journal of Andrology
ANDROLOGIA

ORIGINAL ARTICLE

TEM and FISH studies in sperm from men of couples with recurrent pregnancy loss

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Keywords
Fluorescence in situ hybridisation—recurrent miscarriage—spermatozoa—transmission electron microscopy

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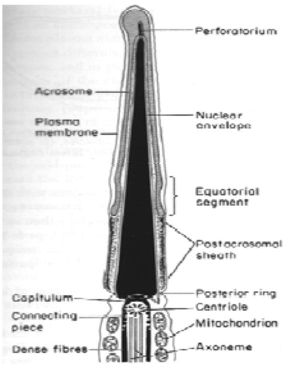
Summary

The role of the male partner in recurrent pregnancy loss (RPL) is not clear. In this study, semen characteristics of 22 men whose partners had experienced RPL were examined by light microscopy. Sperm morphology was analysed by transmission electron microscopy (TEM) and the data were mathematically elaborated to obtain numerical indices expressing the status of an ejaculate: the fertility index and the percentage of apoptosis, necrosis and immaturity. Sperm apoptosis and necrosis were also evaluated by annexin V/propidium iodide assay. To explore the status of meiotic segregation, fluorescence in situ hybridisation (FISH) with probes for chromosomes 18, X and Y, was applied directly on sperm nuclei. Sperm characteristics from a group of men of proven fertility were used as controls. Among the considered sperm characteristics, apoptosis ($P < 0.01$), 1818YY diploidy ($P < 0.05$) and 18YY disomy ($P < 0.01$) scores were significantly higher in men with RPL compared with controls. Our study showed that some patients with normal semen parameters can present a slight increase in aneuploidy compared with controls, indicating a possible involvement of sperm in some cases of RPL. Chromosomal FISH analysis and chromatin tests of sperm could be included in RPL work-ups when no other cause has been detected.

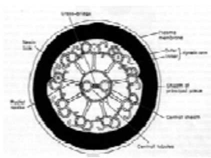
Why TEM analysis on spermatozoa?

- To improve the knowledge about their structure and physiology
- To verify pathologic conditions
- To verify the efficacy of treatments
- To investigate the presence and the causes of defects

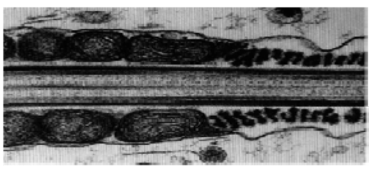
What it is possible to see through TEM Analysis



Axoneme and periaxonemal structures



Mitochondria and fibrous sheath



TEM analysis is NOT a quantitative analysis

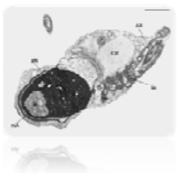
TEM data were elaborated using a mathematical formula, based on the Bayesian method (Baccetti et al. 1995), able to quantify electron microscopy results by calculating the number of spermatozoa probably free of structural defects in a semen sample (the fertility index) and the percentage of sperm pathologies as apoptosis, necrosis and immaturity (Collodel and Moretti 2008)

Bartoov et al. 1999 performed the quantitative ultramorphological methodology (QUM)

Fertility Index (FI) >$2x10^6$

IMMATURITY <55%

NECROSIS <21%



APOPTOSIS <5%

FLUORESCENCE IN SITU HYBRIDIZATION (FISH)

FISH is a simple technique able to highlight aneuploidy in sperm cells

PROS:

- quick, sensitive, specific
- it allows analysis of a large number of spermatozoa in a short time

CONS:

- you cannot analyze all the chromosomes at the same time
- it does not allow you to visualize the structural alteration of chromosomes

FLUORESCENCE IN SITU HYBRIDIZATION (FISH)

Centromeric probes (18, X, Y) were utilized on interphasic nuclei

1818YY 18XY 18YY

WHY a TEM and FISH study?

Presence of ultrastructural sperm defects and altered meiotic segregation
Their relevance in assisted reproduction techniques

- Ultrastructural studies of spermatozoa from infertile men with robertsonian translocation and 18, X, Y aneuploidies. Baccetti et al. 2005
- Necrosis in human spermatozoa. I. Ultrastructural features and FISH study in semen from patients with uro-genital infection. Collodel et al. 2005
- Necrosis in human spermatozoa. II. Ultrastructural features and FISH study in semen from patients with recovered infections. Moretti et al. 2005

WHY a TEM and FISH study?

Presence of ultrastructural sperm defects and altered meiotic segregation
Their relevance in assisted reproduction techniques

- Fluorescence in situ hybridization and molecular studies in infertile men with displasia of the fibrous sheath. Baccetti et al. 2005
- TEM, FISH and molecular studies in infertile men with pericentric inversion of chromosome 9. Collodel et al. 2006
- Cryptorchidism and semen quality: a TEM and molecular study. Moretti et al. 2007

PATIENTS:

- 22 Italian couples with at least three prior pregnancy losses after natural conception at less than 20 weeks of gestation
- All pregnancies were fathered by the same partner
- None of these couples have ever had a live birth
- Semen samples obtained from 25 men of proven fertility (aged 22–40 years) were used as controls for semen parameters (WHO guidelines), for TEM indices and for FISH values (Collodel and Moretti 2008)

WOMEN:

- 22 women aged 29-38 years
- Normal 46, XX karyotype (evaluated using conventional cytogenetic analysis)
- Normal Hysterosalpingogram, Thyroid function analysis and reproductive endocrine evaluation, Factor V Leiden status, lupus anticoagulant anticardiolipin antibody levels

MEN:

- 22 men aged 28–46 years
- Nonazoospermic patients
- Normal 46, XY karyotype (evaluated using conventional cytogenetic analysis)
- Normal hormonal profile
- No history of radiotherapy, chemotherapy, chronic illness or medication
- Absence of sperm defects of possible genetic origin

SEMINAL PARAMETERS

SUBJECTS	SP/ml x 10^6	MOTILITY (a+b)%	PAP %
1	106	61	30
2	78	50	32
3	625	67	36
4	150	53	30
5	73.5	54	32
6	20	50	32
7	58	50	34
8	93	50	35
9	138	54	36
10	332	50	30
11	161	50	33
12	120	50	36
13	114	50	35
14	20	50	36
15	58	50	34
16	28.5	68	40
17	84	50	32
18	74	57	38
19	10.5	57	20
20	34	22	20
21	135	41	20
22	105	21	18

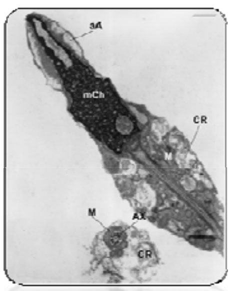
TEM analysis mathematically elaborated

SUBJECTS	APOPTOSIS %	NECROSIS %	IMMATURITY %	FERILITY INDEX
1	3.58	27.68	58.21	5457880
2	4.67	39.16	16.95	4781224
3	7	23	43	7335905
4	7	31	91	3907985
5	4.5	51.56	59.74	5282062
6	8.12	36.09	69.8	1433279
7	8.43	41.64	57.62	1374222
8	7.04	28.93	43.83	5810156
9	6.32	42.64	23.87	8647616
10	15	39	79	1352363
11	4.06	42.85	65.65	14487075
12	4.32	41.83	58.9	3229089
13	3.98	38.81	55.09	7162388
14	4.5	36.6	53.31	1058885
15	2.96	46.38	25.13	2021965
16	7.51	16.76	31.34	12578376
17	8.35	30.82	44.84	8251927
18	8.96	35.58	44.37	11219247
19	6.89	30.71	66.32	1213739
20	1.04	17.87	56.83	4661987
21	0.96	54.72	42.31	4499393
22	5.81	31.07	76.62	1008756

TEM analysis mathematically elaborated

	[b]APOPTOSIS %	NECROSIS %	IMMATURITY %	FERTILITY INDEX
Mean ± SD	5.95 ± 3.03	35.69 ± 9.68	52.89 ± 18.61	5307978 ± 3888738
[a]Mean ± SD	4.06 ± 2.05	32.13 ± 10.58	48.83 ± 13.93	7386080 ± 10464288
Median	6.06	36.34	55.96	4721606
[a]Median	4.06	34.63	47.29	3807391
Range	4.06-7.5	30.71-41.82	43-65.65	1433279-7335905
[a]Range	3.59-4.67	24.74-40.09	38.59-58.21	2057544-8308132

[a]Controls, Collodel and Moretti 2008
[b]Patients versus Controls Apoptosis $P<0.01$
Range: 25°-75° percentiles

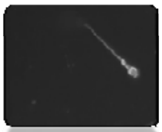


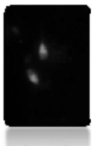
TEM micrograph of an apoptotic sperm characterised by a normal shape nucleus with marginated chromatin (mCh). The alterated acrosome (aA) shows sparse content. Cytoplasmic residue (CR) embeds mitochondria (M). A cross section of a mid-piece with well structured mitochondria (M) and axoneme (AX) is also shown. Bar: 1 µm.

Detection of membrane PS externalisation and membrane integrity using the Annexin V/Propidium Iodide Assay

Annexin V+/Propidium Iodide-

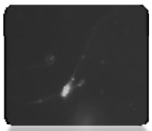
Apoptotic cell





Annexin V+/Propidium Iodide+

Apoptotic and Necrotic cell



Annexin V-/ Propidium Iodide+

Necrotic cell

Results from screening with Annexin V (AnV)-FITC and Propidium Iodide (PI) assay performed in sperm nuclei

SUBJECTS	AnV-/PI-%	AnV+/PI-%	AnV-/PI+%	AnV+/PI+%
3	80	7	8	5
4	80	7	10	3
6	79	8	10	3
7	78	8	11	3
8	80	7	9	4
9	78	6	14	2
10	71	11	14	4
14	80	5	10	5
16	81	10	8	1
17	80	8	10	2
19	76	9	13	2
20	80	7	12	1
21	79	4	15	2
22	78	6	10	6
Mean ±SD	78.6 ± 2.5	**7.4 ± 1.9	*11 ± 2.2	3.1 ± 1.5
[a]Mean ±SD	84.8 ± 4.8	2.8 ± 2.2	8.5 ± 2.2	3.9 ± 3.6

[a] Controls, Collodel and Moretti 2008
*<0.05
**<0.001

FISH RESULTS

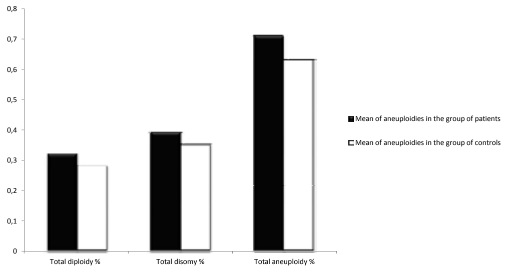
	% Diploidia			% Disomia			
	1818XX	* 1818YY	1818XY	1818	18XX	** 18YY	18XY
Media±DS	0,088±0,045	0,114± 0,06	0,076± 0,13	0,10±0,05	0,06±0,05	0,09±0,05	0,14±0,08
° Media±DS	0,087±0,039	0,075±0,043	0,115±0,048	0,103±0,052	0,06±0,057	0,048±0,032	0,137±0,071

° Controls, Collodel and Moretti 2008
* P<0,05; ** P<0,01

Diploidy 1818YY and disomy 18YY were significantly higher in the study group compared to the control group.

SUBJECTS	TOTAL DIPLOIDY %	TOTAL DISOMY %	TOTAL ANEUPLOIDY %
1	0.18	0.53	0.71
2	0.34	0.29	0.63
3	0.12	0.27	0.39
4	0.38	0.17	0.55
5	0.29	0.23	0.53
6	0.24	0.24	0.48
7	0.34	0.33	0.68
8	0.28	0.5	0.78
9	0.37	0.26	0.63
10	0.28	0.5	0.78
11	0.39	0.58	0.97
12	0.45	0.34	0.79
13	0.2	0.72	0.91
14	0.29	0.44	0.73
15	0.35	0.25	0.59
16	0.28	0.30	0.58
17	0.23	0.24	0.47
18	0.18	0.41	0.59
19	0.37	0.45	0.83
20	0.36	0.31	0.65
21	0.39	0.59	0.98
22	0.83	0.53	1.37

When in each patient disomies and dipolidies are evaluated, approximately 50% of patients presented a higher percentage diploidy and disomy compared to controls



FISH data, considered as total diploidy, total disomy, and total aneuploidy, were not significantly different from the control group

CONCLUSIONS

- Routinary semen parameter analysis is not enough in couples with unexplained RM
- Methods of investigations are reported in the literature to deeply investigate seminal samples of couple with RM
- FISH and TEM analyses of sperm are suggested in RM work-up when no other cause has been detected

THANK YOU FOR YOUR ATTENTION

Markers of sperm quality and miscarriage rate

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Instituto Universitario IVI Valencia
Plaza de la Policía Local, 3, 46015, Valencia (Spain)

Learning objectives:

To provide information about the relevance of early pregnancy loss in ART failures and describe the impact of male factor in assisted reproduction results

To define sperm quality markers involved in reproductive success

To describe the current knowledge about the link between early pregnancy loss and markers of sperm quality

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Course description:

"There is uncertainty whether <u>low quality of spermatozoa and low sperm count</u> ……… is predictive for an increased risk of biochemical pregnancies and miscarriages in subsequent pregnancies conceived with and without the use of ART."

What is quality, when referred to a sperm cell?

A quality indicator should be a feature linked to optimal results under a functional viewpoint

Optimal results → Healthy newborn achievement

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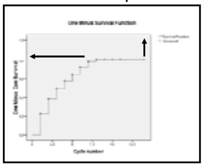
Markers of sperm quality and miscarriage rate

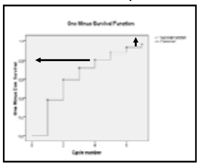
The upper limits of Assisted Reproduction Techniques: the male's perspective

Highest cumulative livebirth rates using donor sperm

IU - donor sperm



IVF - donor sperm



TAKE HOME MESSAGE:

EVEN USING THE BEST SPERM, THERE IS ROOM FOR IMPROVEMENT IN ART

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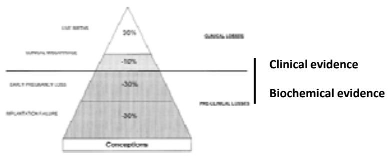
Markers of sperm quality and miscarriage rate



hCG produced by a conceptus is usually detected in maternal blood from implantation time onwards

clinical evidence of pregnancy is obtained from week 6th onwards

Biochemical pregnancies and early miscarriages can account up to 40% of all implantational events in natural pregnancies

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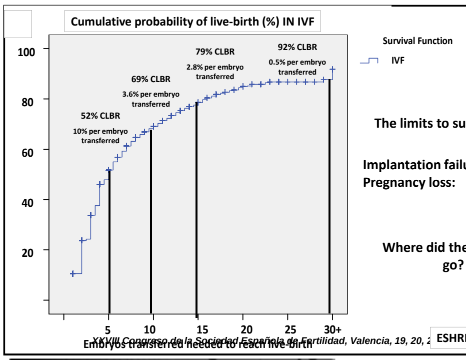
Markers of sperm quality and miscarriage rate



The limits to succeed:

Implantation failure
Pregnancy loss: pre-clinical
clinical

Where did the embryos go?

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Markers of sperm quality and miscarriage rate

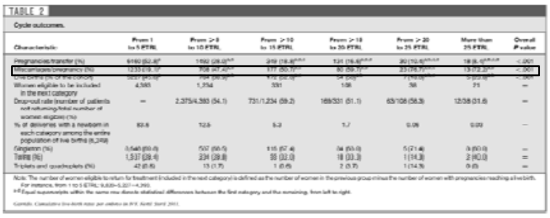

TABLE 2

Cycle outcomes.

Characteristic	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Pregnancies/transfer (%)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	<.001
Miscarriages/pregnancy (%)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	<.001
Live births (% of the cohort)	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	<.001
Women eligible to be included in the next category	[illegible]	1,234	331	108	[illegible]	21	—
Drop-out rate (number of patients not returning/total number of women eligible) (%)	—	2,375/4,393 (54.1)	731/1,234 (59.2)	169/331 (51.1)	63/108 (58.3)	12/38 (31.6)	—
% of deliveries with a newborn in each category among the entire population of live births (6,249)	[illegible]	12.5	[illegible]	1.7	0.09	0.03	—
Singleton (%)	[illegible]	[illegible]	[illegible]	[illegible]	5 (71.4)	[illegible]	—
Twins (%)	1,537 (29.4)	234 (29.8)	[illegible]	18 (33.3)	1 (14.3)	2 (40.0)	—
Triplets and quadruplets (%)	42 (0.8)	13 (1.7)	1 (0.6)	2 (3.7)	1 (14.3)	0 (0)	—

Note: The number of women eligible to return for treatment (included in the next category) is defined as the number of women in the previous group minus the number of women with pregnancies reaching a live birth. [illegible]

About 26% in IVF conceived pregnancies

Those patients needing a high number of embryos to achieve newborn are able to get pregnant, but experience several early pregnancy losses

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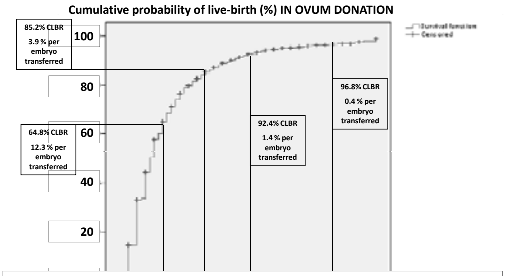
Markers of sperm quality and miscarriage rate

Cumulative probability of live-birth (%) IN OVUM DONATION



TAKE HOME MESSAGE:

OOCYTE QUALITY IS IMPORTANT, BUT NOT DEFINITIVE. SPERM CELLS SEEM TO PLAY A RELEVANT ROLE

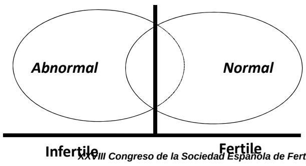
10

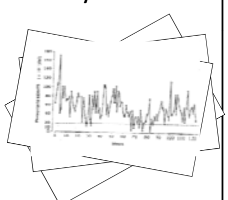
Markers of sperm quality and miscarriage rate

Diagnosis of male infertility:

Semen analysis, as stated by the World Health Organization is up to now the only worldwide accepted tool to diagnose male fertility, however its predictive value to forecast a male or a sperm sample possibilities <u>to reach livebirth</u> is relatively low.

Sperm analysis result





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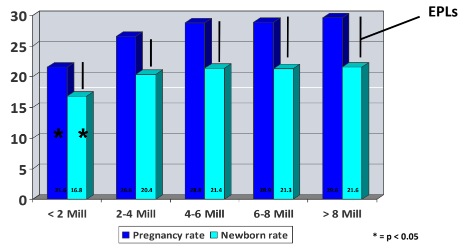
Regarding the number as a quality marker…

IUI with donor sperm depending on the total motile sperm inseminated



More does not mean better

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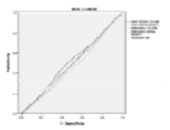
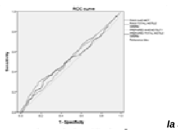
Regarding the number and motility as a quality marker…

Retrospective analysis of IVF with ovum donation pregnancies (n=4423, 27% miscarriages) from 2006



Predictive power of sperm parameters to forecast livebirth

	Area	95%CI inferior	95%CI superior
RAW SPERM VOLUME	.498	.478	.518
RAW SPERM DENSITY	.480	.460	.500
PREPARED VOLUME	.462	.442	.482
PREPARED SPERM DENSITY	.507	.487	.527



	Area	95%CI inferior	95%CI superior
RAW A+B MOTILITY	.547	.507	.587
RAW TOTAL MOTILE SPERM	.521	.482	.560
PREPARED A+B MOTILITY	.478	.439	.517
PREPARED TOTAL MOTILE SPERM	.520	.480	.560

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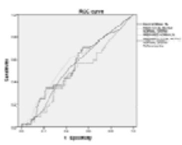
Regarding the morphology as a quality marker…



Predictive power of sperm parameters to forecast livebirth

	Area	95%CI inferior	95%CI superior
RAW NORMAL %	.533	.434	.631
RAW TOTAL MOTILE NORMAL SPERM	.483	.383	.582
PREPARED NORMAL%	.577	.477	.676
PREPARED TOTAL MOTILE NORMAL SPERM	.549	.450	.647

Basic sperm analysis is unrelated to EPLs

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Regarding the molecular profiles described as sperm quality markers…seems difficult to be a successful sperm!

Molecular markers

- **Nucleic acid-linked**
  - **Aneuploidies, DNA fragmentation, gene expression (Microarrays), epigenetic**
- **Non nucleic acid-linked**
  - **Apoptosis, Oxidative stress, Hialuronic acid, Ubiquitin, Platelet Activating Factor**

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The most challenging research in Andrology is on the causes making sperm cells unable to reach a term pregnancy with competent oocytes at any level, conception, implantation, or pregnancy maintenance:

- **DIAGNOSTIC TOOLS TO ASSESS SPERM AND FORECAST LIVEBIRTH**
- **SELECTION TOOLS TO UTILIZE THE MOST COMPETENT SPERM CELLS**

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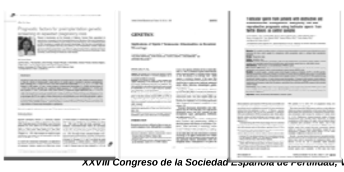
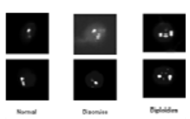
Sperm aneuploidies

FISH studies: up to 50% of the males with severe oligospermia or azoospermia and normal karyotype present FISH abnormalities (Rubio et al., 2001)

Linked to recurrent pregnancy loss

Effects mitigated by PGs





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Sperm DNA fragmentation

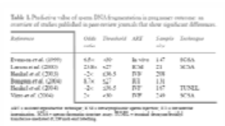
The theory:

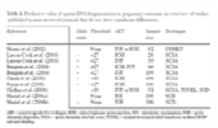
Sperm DNA is the vehicle to transmit males' genetic information to the offspring, several external and internal factors have been demonstrated to affect sperm DNA integrity.

To date, there are a lot of studies concerning DNA analysis of human semen suggesting that the determination of DNA fragmentation can be a diagnostic parameter of semen quality, directly implicated in male fertility (Agarwal and Said 2003).

Sperm DNA fragmentation

The facts (some of them, at least):





- **Highly controversial results**
- **Different techniques to be employed**
- **Different cut-off values and odds ratio of achieving a term pregnancy**
- **Outcomes not always related to miscarriage or livebirth**

Sperm DNA fragmentation

The conclusions (to date):

IN VITRO FERTILIZATION

Do sperm DNA integrity tests predict pregnancy with in vitro fertilization?

The clinical utility of sperm DNA integrity testing

There is a mild correlation between the sperm DNA integrity and embryo quality, that could potentially affect success.

The prognostic value of this test is very low, and it seems unnecessary to complement each semen analysis.

This information could help after repeated treatments with bad embryo quality and normal ovarian response and sperm count/motility.

Further information is needed.

All in one about recurrent pregnancy loss:

Y chromosome microdeletions

DNA fragmentation

8Oh guanosine (oxidative stress attack symptom on DNA: DNA oxidation)

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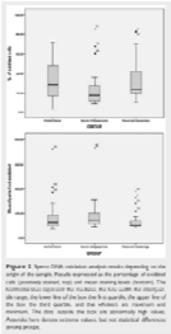

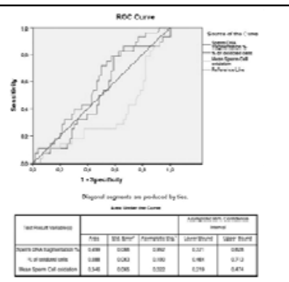

No Y-chromosome microdeletions found

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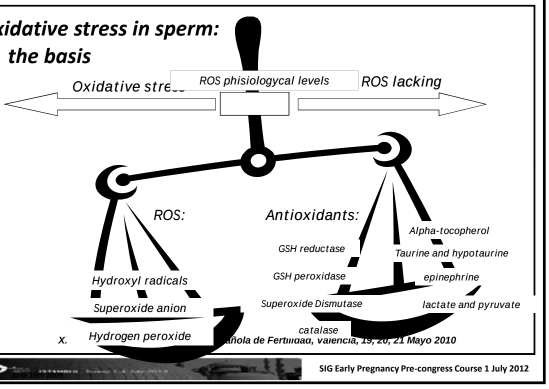
Oxidative stress in sperm: the basis



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IVI

Markers of sperm quality and miscarriage rate

Oxidative stress damage to cells:

Plasma membrane

Sperm DNA

Cytoskeleton

Motility

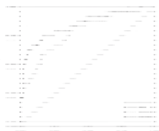
AJA

Review

Pro-oxidative and anti-oxidative imbalance in human semen and its relation with male fertility

ORIGINAL RESEARCH

IDENTIFICATION OF MALE FACTOR INFERTILITY USING A NOVEL SEMEN QUALITY SCORE AND REACTIVE OXYGEN SPECIES LEVELS



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IVI

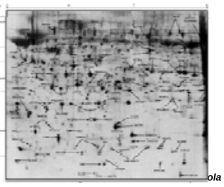
Markers of sperm quality and miscarriage rate

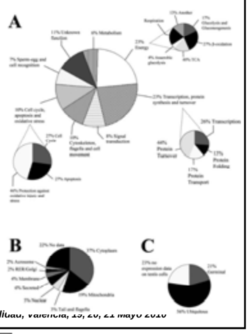
Proteomics in sperm:

Research Article

Proteomic identification of human sperm proteins

Juan Martínez-Heredia, Josep Maria Estanyol, José Luis Ballescà and Rafael Oliva





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IVI

New diagnostic tools: the -omics

Markers of sperm quality and miscarriage rate

Proteomics in sperm:

Very initial phase

Very expensive equipment

No clinical consideration

Human Reproduction Vol.19, No.6 pp.1409–1417, 2004

Advance Access publication April 22, 2004

Sperm proteome mapping of a patient who experienced failed fertilization at IVF reveals altered expression of at least 20 proteins compared with fertile donors: Case report

Katherine L.Pixton[1], Emma D.Deeks[1], Frits M.Flesch[1], Fleur L.C.Moseley[1,2], Lars Björndahl[1,3], Peter R.Ashton[2], Christopher L.R.Barratt[1,3,4] and Ian A.Brewis[1,3]

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Intracytoplasmic Morphologically Selected Sperm injection: the basis

Human Reproduction Vol.20, No.1 pp. 185–190, 2005
Advance Access publication October 7, 2004

The morphological normalcy of the sperm nucleus and pregnancy rate of intracytoplasmic injection with morphologically selected sperm

Arie Berkovitz[1], Fina Eltes[2], Shlomit Yaari[3], Nathan Katz[4], Ilya Barr[4], Ami Fishman[1] and Benjamin Bartoov[2,5]

- ✓fine morphological integrity of human sperm nuclei is positively associated with fertilization and pregnancy rates following IVF-ICSI.
- ✓a new method of unstained, real-time, high magnification motile sperm organellar morphology examination (MSOME)

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Markers of sperm quality and miscarriage rate

IMSI: the methods

- ✓Improved enhancement of the microscope resolution allowing 6300x.
- ✓The strict descriptive criteria for normally shaped nuclei were based on those defined by scanning electron microscopy, (Bartoov et al., 1981, 2002, 2003).
- ✓the average length and width of this configuration were estimated to be 4.75 ± 0.28 and 3.28 ± 0.20mm, respectively
- ✓the nuclear chromatin content was considered normal if it contained no more than one vacuole, which occupies 4% of the nuclear area (Bartoov et al., 1981)

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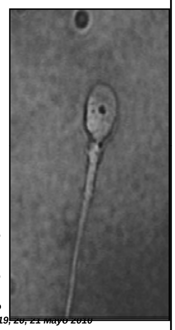
Markers of sperm quality and miscarriage rate

IMSI: the clinical aspects

Pregnancy rates are higher with intracytoplasmic morphologically selected sperm injection than with conventional intracytoplasmic injection

	Control group			Experimental group		
	previous (ICSI)	Actual (ICSI)		Previous (ICSI)	Actual (IMSI)	
Retrieved oocytes	13.3 ± 5.2	13.2 ± 5.9		13.4 ± 5.5	13.6 ± 5.8	
Injected oocytes	10.3 ± 4.7	10.2 ± 5.5		10.1 ± 4.6	10.6 ± 4.4	
Fertilization rates (%)	63.7 ± 18.9	65.5 ± 21.5		63.1 ± 25.3	64.5 _ 17.5	
Opotimal embryos (%)	31.1 + 16.4	31.0 ± 19.5		31.7 ± 21.6	45.2 ± 28.2*	
Transferred embryos (%)	3.6 ± 1.1	3.5 ± 1.2		3.6 ± 1.2	3.8 ± 1.1	
Implantation rates (%)	-	9.5 ± 15.3		-	27.9 ± 26.4*	
		nr	(%)		nr	(%)
Pregnancies		15	30		33	66*
miscarriages		5	[illegible]		3	9*



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IMSI: the clinical aspects

High-magnification ICSI overcomes paternal effect resistant to conventional ICSI

	ICSI	IMSI	P
transferences (*n*)	125	125	-
Transferred embryos (*n*)	265	261	>0.05
pregnancies (*n*)	8	51	<0.001
Clinical pregnancies (*n*)	3	47	<0.001
deliveries (*n*)	0	42	<0.001
newborns (*n*)	0	46	<0.001
Pregn. rates (%)	6.4	40.8	<0.001
Clin pregn rates (%)	2.4	37.6	<0.001
Clin impl. rates (%)	0.8	20.3	<0.001

Intracytoplasmic morphologically selected sperm injection: a prospective randomized trial

n=446		ICSI	IMSI
0 ICSI failed	**pregnancies %(*n*)**	28.0 (14/50)	42.5 (31/73)
	miscarriage %(*n*)	21.4 (3/14)	12.9 (4/31)
1 ICSI failed	**pregnancies %(*n*)**	33.6 (36/107)	45.5 (35/77)
	miscarriage %(*n*)	22.2 (8/36)	20.0 (7/35)
≥2 ICSI failed	**pregnancies %(*n*)**	12.9* (8/62)	29.9*(23/77)
	misacrriage %(*n*)	37.5 (3/8)	17.4 (4/23)

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Markers of sperm quality and miscarriage rate

The morphological normalcy of the sperm nucleus and pregnancy rate of intracytoplasmic injection with morphologically selected sperm

Arie Berkovitz[1], Fina Eltes[2], Shlomit Yaari[3], Nathan Katz[4], Ilya Barr[4], Ami Fishman[1] and Benjamin Bartoov[2,5]

Table III. Comparisons between the positive and negative study groups in IVF- IMSI outcome parameters

	Study groups	
	Positive (n = 38) per cycle[a] (total)	Negative (n = 38) per cycle[a] (total)
Continuous variables		
No. of retrieved ova	13.3 ± 5.7 (506)	13.1 ± 6.8 (488)
No. of injected ova	[illegible]	[illegible]
Fertilization rate (%)	71.3 ± 20.8 (264)	50.3 ± 21.1[b] (174)
Percentage of top embryos (%)	34.9 ± 31.3 (81)	19.4 ± 27.1[b] (32)
No. of embryos transferred	3.3 ± 1.2 (125)	3.5 ± 1.4 (113)
Implantation rate (%)	25.0 ± 25.9 (31)	5.9 ± 12.9[b] (8)
Discrete variables		
Pregnancy rate per transfer (%)	52.6 (20)	[illegible]
Abortion rate (%)	10.0 (2)	57.1[b] (4)

[a]Values are means ± SD per cycle.
[b]Significantly different from the positive group ($P \le 0.05$).

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Markers of sperm quality and miscarriage rate

IMSI: the clinical aspects

- ✓ **Good scientific evidences, clinically related to miscarriage**
- ✓ **Selected patient population**
- ✓ **Costs of the equipment**
- ✓ **Embryologists training**
- ✓ **Time-consuming (new microinjecting schedules?)**

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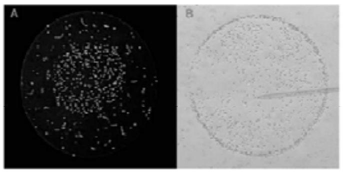
Hyaluronan binding assay (PICSI): the basis

- ✓**Diminished sperm fertility is associated with the retention of the surplus cytoplasm that is extruded from elongating spermatids in the course of normal sperm development**
- ✓**Only mature sperm without cytoplasmic retention were able to bind to the zona pellucida of oocytes. This finding led to hypothesize that the sperm plasma membrane undergoes a maturation-related remodeling.**
- ✓**This remodeling step facilitates the formation of the sperm binding sites for the zona pellucida of oocytes Indeed, immature sperm that fail to undergo membrane remodeling are unable to bind to immobilized HA, as is the case with immature sperm that fail to bind to the zona pellucida**

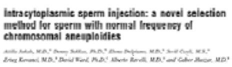
PICSI: the methods

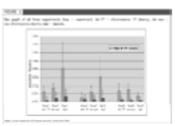


HA-bound plates are able to immobilize mature sperm

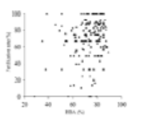
These can be selected for ICSI

PICSI: the clinical aspects


Intracytoplasmic sperm injection: a novel selection method for sperm with normal frequency of chromosomal aneuploidies




Relationship between human sperm-hyaluronan binding assay and fertilization rate in conventional in vitro fertilization



PICSI: the clinical aspects

"Physiologic ICSI": Hyaluronic acid (HA) favors selection of spermatozoa without DNA fragmentation

TABLE 3

Study 3: PVP-ICSI versus HA-ICSI.

	PVP-ICSI	HA-ICSI
No. treatments	107	125
Mean female age ± SE at oocyte retrieval	37.1 ± 0.4	37.5 ± 0.4
Fertilized oocytes (%)	236/275 (85.8)	304/332 (91.6)
Grade 1 embryos (%)	55/228 (24.1)[a]	101/282 (35.8)[b]
Mean embryo development rating ± SE	84.0 ± 1.1[c]	95.0 ± 0.8[d]
No. of embryo transfers	105	125
Clinical pregnancy rate per transfer (%)	22/105 (20.9)	31/125 (24.8)
Implantations (%)	23/226 (10.2)	35/282 (12.4)
Abortions (%)	4/22 (18.2)	6/31 (19.3)
No. of live births	19	29

Note: ICSI = intracytoplasmic sperm injection; other abbreviations as in Table 1.
[a,b] $P = .046$, PVP-ICSI versus HA-ICSI.
[c,d] $P \leq .001$, PVP-ICSI versus HA-ICSI.

Parmegiani. Sperm selection with hyaluronic acid. Fertil Steril 2009.

The -omics in sperm: the basis

Paternal contribution is not only one half of the embryo's genome: exclusive mRNAs, proteins, and structures are delivered into the oocytes.

IMPORTANT UNTIL THE EMBRYOS GENOMIC ACTIVATION

Sperm has no transcriptional activity, but possesses a pool of active mRNAS synthesized during spermatogenesis.

Moreover, the fertilized oocyte has mRNAs not found in the unfertilized oocytes. Failure of partenogenetic development.

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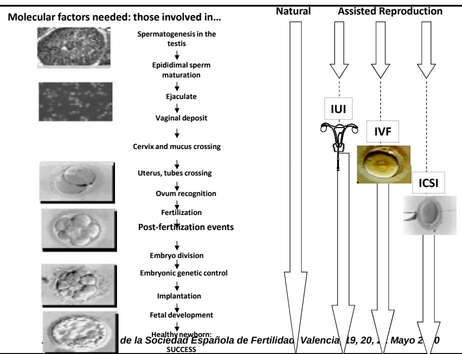

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Sperm Fertility Array (SFA) project:

Hypothesis:

Sperm cells with or without reproductive success pre transcriptomes

The molecular requirements of sperm cells to achieve pregnancies are different in vivo, or in vitro, and even different among different AR

TAKE HOME MESSAGE:

THERE ARE MANY MOLECULAR FEATURES POTENTIALLY ABLE TO BE EMPLOYED IN SPERM TO BETTER DIAGNOSE AND IMPROVE RESULTS

Obj

To te

FERTILITY AND INFERTILITY.

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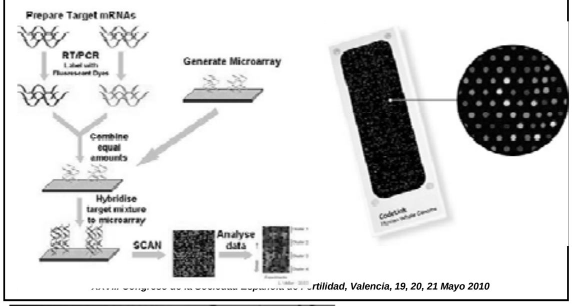
The genomics of sperm: the methods



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The genomics of sperm: how can we read and interprete the results?

- **Lists of genes**
  - **Those Differentially Expressed**
  - **Those present/absent**

  Sequences implicated in reproductive success or newly associated

- **Functional analysis:**

TAKE HOME MESSAGE:

MICROARRAY RESULTS CAN PROVIDE YOU WITH RELEVANT INFORMATION ABOUT THE KEY MOLECULAR FACTORS AND PROCESSES INVOLVED IN FERTILITY

molecular function

metabolic pathways

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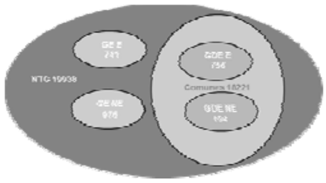
Microarray analysis of Intrauterine Insemination samples:



NTG: total number of genes expressed
GE: exclusive genes for this group
GDE: genes overexpressed in this group
E: pregnancy achieved
NE: pregnancy not achieved

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Markers of sperm quality and miscarriage rate

Name	GenBank #	Fold change	p-value
FCGR3A	NM_000569	20,71	0,0000
OSM	NM_020530	19,60	0,0000
FPR1	NM_002029	18,49	0,0000
CXCL1	NM_001511	17,83	0,0000
AQP9	NM_020980	17,75	0,0000
TMEM154	NM_152680	16,21	0,0005
PI3	NM_002638	15,96	0,0007
LILRB2	NM_005874	15,35	0,0000
VNN2	NM_004665	15,22	0,0000
APOBEC3A	NM_145699	14,10	0,0040

Overexpressed on sperm from pregnant couples

Name	GenBank #	Fold change	p-value
AK000872	AK000872	5,42	0,0254
ENST00000303697	ENST00000303697	5,13	0,0266
ZNF224	BC002889	4,68	0,0000
FLJ20581	ENST00000331849	4,55	0,0133
CAMK2N2	NM_033259	4,45	0,0097
CGB1	NM_033377	4,26	0,0166
THC2541992	THC2541992	3,98	0,0135
THC2609820	THC2609820	3,87	0,0150
LCE2D	NM_178430	3,75	0,0082
VPS18	NM_020857	3,70	0,0101

Overexpressed on sperm from NON-pregnant couples

Top10 genes differentially expressed

Intrauterine insemination

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Markers of sperm quality and miscarriage rate

Name	GenBank #	Fluorescence intensity
IL8RA	NM_000634	9,916
CYSLTR2	NM_020377	9,732
AK055428	AK055428	9,647
P2RY14	NM_014879	9,517
C8orf39	AF116672	9,500
PRG1	NM_002727	9,372
IFIT2	NM_001547	9,344
BTNL8	NM_001040462	9,218
STK4	NM_006282	9,175
SIKE	NM_025073	9,054

Exclusively found on sperm from pregnant couples

Fertility markers?

Name	GenBank #	Fluorescence intensity
THC2638360	THC2638360	10,428
CDC37L1	NM_017913	10,382
THC2547195	THC2547195	9,875
NOX1	NM_007052	9,461
CLCNKB	NM_000085	9,147
THC2555234	THC2555234	9,069
THC2648250	THC2648250	9,022
NETO1	NM_138966	8,873
THRSP	NM_003251	8,815
AADAC	NM_001086	8,264

Exclusively found on sperm from NON-pregnant couples

Infertility markers?

Top10 genes with exclusive expression

Intrauterine insemination

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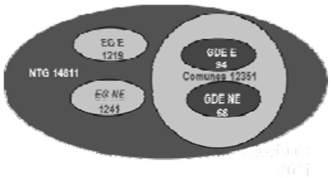
IVI

Markers of sperm quality and miscarriage rate

Microarray analysis of IVF samples:



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Markers of sperm quality and miscarriage rate

Name	GenBank #	Fold change	p-value
C10orf119	NM_024834	34,27	0,0060
SPP1	NM_001040058	32,72	0,0100
TGFBI	NM_000358	17,44	0,0050
CD163	NM_004244	15,24	0,0033
ADM	NM_001124	14,34	0,0025
RGS2	NM_002923	13,98	0,0020
MMP9	NM_004994	13,66	0,0050
CTSL	NM_001912	13,56	0,0043
MT1M	NM_176870	12,91	0,0038
IFI30	NM_006332	12,82	0,0030

Overexpressed on sperm from pregnant couples

Name	GenBank #	Fold change	p-value
PLA2G2A	NM_000300	42,31	0,0194
SH3RF2	NM_152550	8,05	0,0000
LELP1	NM_001010857	7,67	0,0050
HSPA1L	NM_005527	5,76	0,0125
DUSP21	NM_022076	5,50	0,0100
TSPAN16	NM_012466	5,42	0,0100
C10orf62	NM_001009997	5,40	0,0078
SMAD9	NM_005905	5,33	0,0363
SPATA20	NM_022827	5,15	0,0088
C16orf78	NM_144602	5,14	0,0117

Overexpressed on sperm from NON-pregnant couples

Top10 genes differentially expressed

IN VITRO FERTILIZATION

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Markers of sperm quality and miscarriage rate

Name	GenBank #	Fluorescence intensity
MMP12	NM_002426	9,276
C10orf119	NM_024834	9,252
CLEC4E	NM_014358	8,602
ADAMDEC1	NM_014479	8,526
PTPN12	NM_002835	8,248
PLEK	NM_002664	8,018
CXCR7	NM_001047841	7,870
MAP3K8	NM_005204	7,805
LYZ	NM_000239	7,788
INDO	NM_002164	7,734

Exclusively found on sperm from pregnant couples

Fertility markers?

Name	GenBank #	Fluorescence intensity
C10orf64	BC034937	8,369
MSH4	NM_002440	7,969
DCC	NM_005215	7,106
MYH7	NM_000257	7,031
INPP5F	NM_014937	7,008
C4orf6	NM_005750	6,952
ENST00000359466	ENST00000359466	6,926
TGM4	NM_003241	6,920
WDR87	ENST00000303868	6,909
HSFX1	NM_016153	6,772

Exclusively found on sperm from NON-pregnant couples

Infertility markers?

Top10 genes with exclusive expression

IN VITRO FERTILIZATION

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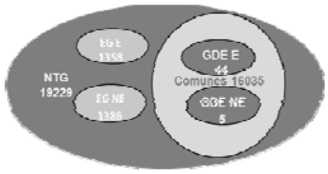
Microarray analysis of ICSI samples:



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Name	GenBank #	Fold change	p-value
APOE	NM_000041	28,27	0,0000
APOC1	NM_001645	27,90	0,0000
CFD	NM_001928	7,74	0,0067
CTSZ	NM_001336	6,85	0,0150
HMOX1	NM_002133	6,58	0,0120
FTL	NM_000146	6,35	0,0183
TGFBI	NM_000358	6,06	0,0157
CTSL	NM_001912	5,93	0,0163
LGALS3	NM_002306	5,60	0,0170
CD63	NM_001780	5,48	0,0183

Overexpressed on sperm from pregnant couples

Name	GenBank #	Fold change	p-value
ENST00000317633	ENST00000317633	4,09	0,0456
COX7B2	NM_130902	3,82	0,0490
C19orf36	NM_001039846	3,8	0,0464
ANKRD7	NM_001077708	3,79	0,0467
CDKN2D	NM_001800	3,70	0,0492

Overexpressed on sperm from NON-pregnant couples

Top10 genes differentially expressed

ICSI

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Name	GenBank #	Fluorescence intensity
FSTL4	NM_015082	10,636
TNMD	NM_022144	10,430
LOC646808	XR_017339	9,793
C22orf26	NM_018280	9,756
CXorf34	NM_024917	9,446
MBOAT4	AF359269	9,378
UBQLN4	NM_020131	9,361
ALDOC	NM_005165	9,136
ANGPTL4	NM_139314	8,869
SHFM3P1	AF174606	8,741

Exclusively found on sperm from pregnant couples

Fertility markers?

Name	GenBank #	Fluorescence intensity
DSG1	NM_001942	9,996
RPGR	NM_001023582	9,053
KLRC3	NM_007333	8,902
CYP3A7	NM_000765	8,794
ERN2	NM_033266	8,744
RP11-327P2.4	AK124707	8,670
GFM1	NM_024996	8,658
TMEM144	NM_018342	8,528
INSM2	NM_032594	8,528
C1orf74	NM_152485	8,474

Exclusively found on sperm from NON-pregnant couples

Infertility markers?

Top10 genes with exclusive expression

ICSI

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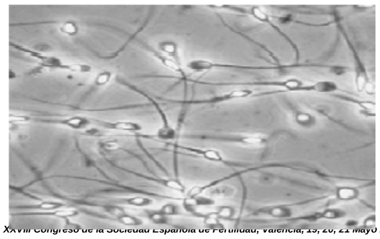
Markers of sperm quality and miscarriage rate

Is there any possibility to avoid early pregnancy losses?

Once defined the "ideal" molecular features in sperm, we could select those appropriate for ART



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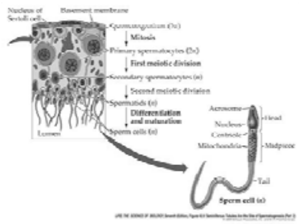
Markers of sperm quality and miscarriage rate

Why are sperm cells interesting?

a) excess: it is not a limiting factor (it is the oocyte!)



b) genetic uniqueness in each sperm provides a wide variety of sperm phenotypes (natural selection?)

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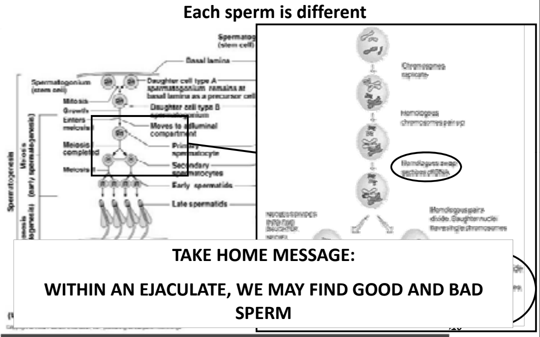
Markers of sperm quality and miscarriage rate

Each sperm is different



TAKE HOME MESSAGE:

WITHIN AN EJACULATE, WE MAY FIND GOOD AND BAD SPERM

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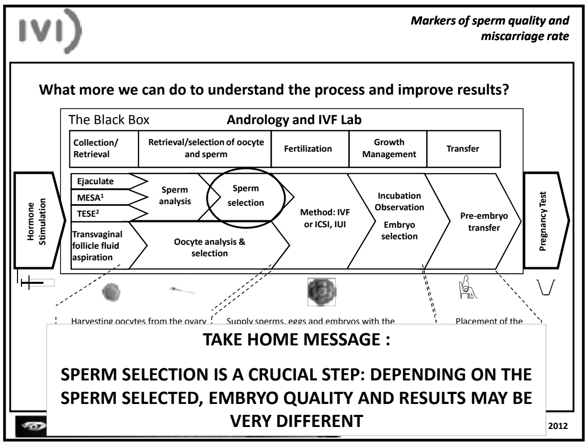
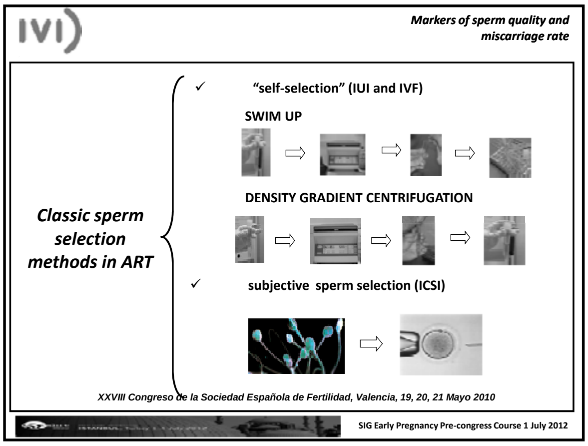

Markers of sperm quality and miscarriage rate
What more we can do to understand the process and improve results?
The Black Box
Andrology and IVF Lab
Collection/ Retrieval
Retrieval/selection of oocyte and sperm
Fertilization
Growth Management
Transfer
Hormone Stimulation
Ejaculate
MESA1
TESE2
Transvaginal follicle fluid aspiration
Sperm analysis
Sperm selection
Oocyte analysis & selection
Method: IVF or ICSI, IUI
Incubation Observation
Embryo selection
Pre-embryo transfer
Pregnancy Test
Harvesting oocytes from the ovary
Supply sperms, eggs and embryos with the
Placement of the
TAKE HOME MESSAGE :
SPERM SELECTION IS A CRUCIAL STEP: DEPENDING ON THE SPERM SELECTED, EMBRYO QUALITY AND RESULTS MAY BE VERY DIFFERENT
2012


Markers of sperm quality and miscarriage rate
Classic sperm selection methods in ART
✓ "self-selection" (IUI and IVF)
SWIM UP
DENSITY GRADIENT CENTRIFUGATION
✓ subjective sperm selection (ICSI)
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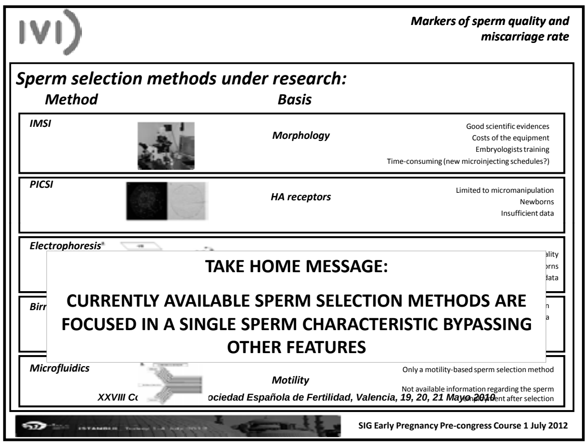
Markers of sperm quality and miscarriage rate
Sperm selection methods under research:
Method
Basis
IMSI
Morphology
Good scientific evidences
Costs of the equipment
Embryologists training
Time-consuming (new microinjecting schedules?)
PICSI
HA receptors
Limited to micromanipulation
Newborns
Insufficient data
Electrophoresis
Microfluidics
Motility
Only a motility-based sperm selection method
Not available information regarding the sperm
TAKE HOME MESSAGE:
CURRENTLY AVAILABLE SPERM SELECTION METHODS ARE FOCUSED IN A SINGLE SPERM CHARACTERISTIC BYPASSING OTHER FEATURES
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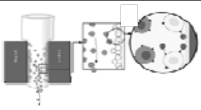
Sperm selection methods by molecular features:

Self/Subjective Sperm Selection → SMART SELECTION



Magnetic Activated Cell Sorting: MACS

- MICROBEADS: colloidal superparamagnetic particles (50 nanometers)
- Coupled to highly specific monoclonal antibodies or molecules recognizing targets of interest
- Specialized magnetic affinity-type columns to isolate sperm
- 2 fractions: bound and unbound, depending on molecular features
  - high purity
  - maintaining structure, viability and function

XXVIII Congreso de la Sociedad Española de Fertilidad, Valencia, 19, 20, 21 Mayo 2010

MACS, what's next?

Evaluation of new molecular candidates

Based on the previously available literature

Obtained information from the SFA project

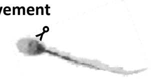
Gene lists of mRNA involved in reproductive success

XXVIII Congreso de la Sociedad Española de Fertilidad, Valencia, 19, 20, 21 Mayo 2010

MACS, what's next?

Molecular Candidate's required characteristics:

Having been related to successful livebirth achievement



To be present in sperm cells

To be located at the plasma membrane

Ab availability

XXVIII Congreso de la Sociedad Española de Fertilidad, Valencia, 19, 20, 21 Mayo 2010

MACS, what's next?

IVI Bilbao: **Dr. Fernando Quintana, Dr. Iratxe Peñalba**

Ubiquitin:

Defective mammalian spermatozoa become ubiquitinated during epididymal passage, a mechanism that may mark the abnormal spermatozoa for proteolytic destruction.

Those ubiquitinated sperm present in an ejaculate, or in a TESE sample (have not passed through the epididimis, could be used involuntarily in ART, impairing the results.

Then, removing those ubiquitinated sperm could help.

MACS, what's next?

Phospholipase A2

Has been demonstrated to be >40x downregulated in samples achieving pregnancy in IVF.

Then, the depletion of PLA2G2A presenting sperm cells may increase reproductive results (negative selection)

DOWNREGULATED			
Ranked#	GeneName	FC	Name
1	PLA2G2A	-42,31	phospholipase A2, group IIA (platelets, synovial fluid) (PLA2G2A), mRNA [NM_000300]
2	SH3RF2	-8,05	SH3 domain containing ring finger 2 (SH3RF2), mRNA [NM_152550]
3	LELP1	-7,67	late cornified envelope-like proline-rich 1 (LELP1), mRNA [NM_001010857]

Stabilin-2

Multifunction receptor, with seven domains FAS1, four repeats EGF-like, and a domain able to identifying and digesting old cells in apoptosis in macrophages.

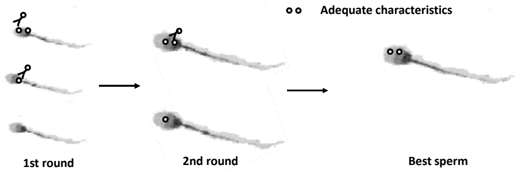
Also a phosphatidilserine recognizing receptor (Linked to apoptosis process)

Information from microarrays experiments: it has been linked to infertility.

MACS, what's next?

Complex sperm selection:

Successive positive selection rounds of appropriate sperm cells



Massive depletion of inadequate sperm cells (Ab cocktail)

MACS, what's next?

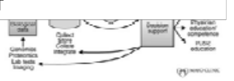
Complex sperm selection:

Study/characterization of molecular sperm defects in a patient

TAKE HOME MESSAGE:

MACS OPEN A UNIVERSE OF POSSIBILITIES IN SPERM SELECTION, BEING A PROMISING TOOL TO BE IMPLEMENTED IN ART

Individualized sperm selection strategy?



SUMMARIZING:

Sperm cells present very interesting and **unique properties** to enable the development of sperm selection strategies as a part of the ART

The development of objective sperm selection methods to be employed, and the design of complex strategies of sperm selection **could improve ART results**

MACS versatility enables sperm selection depending of very different molecular features, and can also be employed **in addition** to other techniques

In the future, the design of **customized sperm selection methods**, even individualized per patient, could solve the male infertility problems caused by altered molecular factors in the sperm samples

Conclusions:

Early pregnancy losses represent a significant % of all ART failures

There are clinical evidences about the sperm relevance on ART results, and several molecular sperm quality markers, seem relevant.

There are very few data directly linking sperm quality with pregnancy loss (biochemical or clinical), although the definition of the ideal features for a sperm may help to detect characteristics leading to pregnancy wastage.

The deep knowledge and identification of those molecular factors in sperm factors involved in ART may open the possibility of designing adequate sperm selection tools aiming to decrease EPL incidence.

Markers of sperm quality and miscarriage rate

Dr. Marcos Meseguer

Andrology Laboratory IVI Valencia

IVI International Sperm Bank

Markers of sperm quality and miscarriage rate

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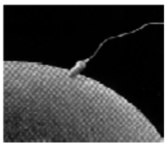
Sperm DNA Damage and Pregnancy Loss after IVF and ICSI

Armand Zini, MD
Associate Professor, McGill University



Disclosure: Shareholder in YAD Tech – Neutraceuticals Co.

Sperm DNA Damage & Pregnancy Loss

Learning Objectives

- **Recognize the etiologies of sperm DNA and chromatin damage**
- **Evaluate the relationship between sperm DNA damage and pregnancy outcomes**
- **Recognize the controversies regarding the studies on sperm DNA and reproduction**
- **Apply the results of sperm DNA damage tests into clinical practice**

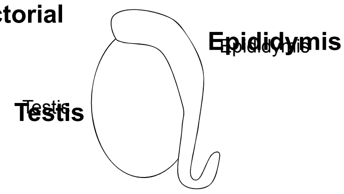
Sperm DNA Damage & Pregnancy Loss

Overview

- **Etiology of sperm DNA damage**
- **Rationale for examining sperm DNA**
- **Tests of sperm DNA damage**
- **Relationship between sperm DNA damage and reproductive outcomes**
  - Pregnancy and Pregnancy loss (in IVF & ICSI)
- **Clinical utility of sperm DNA tests**

Sperm DNA Damage: Etiology

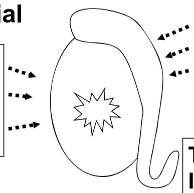
Multi-factorial



Sperm DNA Damage: Etiology

Multi-factorial

Hormonal
Temperature
Toxins
Oxidants

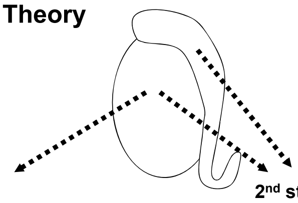


Temperature
Toxins
Oxidants

Testis dysfunction
Idiopathic
Genetic
Developmental

Sperm DNA Damage: Etiology

2 Step Theory



1st step
Poorly protaminated spermatozoa
(Defective spermatogenesis)

2nd step
Oxidative injury
(extrinsic or intrinsic)

Gatewood et al, *J Biol Chem* 1990
Carrell & Liu, *J Androl* 2001
Aoki et al, J Androl 2005

Aitken et al, *Mol Hum Reprod* 2010
Suganuma et al *Hum Reprod* 2005

Sperm DNA Integrity

Potential applications of sperm DNA tests?

1. To more accurately diagnose male infertility

Current markers of male fertility potential (i.e. conventional semen parameters) are inadequate

- **Exhibit a high degree of variability**
- **Modest predictors of male fertility potential**

Guzick et al, *NEJM* 2001
Menkveld et al, *Hum Reprod* 2001
Cooper et al, *Hum Reprod Update* 2009

Sperm DNA test results have a lower degree of variability

Oleszczuk et al, *Hum Reprod* 2011
Smit et al, *Int J Androl* 2011
Evenson et al, *Reprod Toxicol* 2011
Erenpreiss et al, *J Androl* 2011

Sperm DNA Integrity

Potential applications of sperm DNA tests?

2. To help predict pregnancy outcomes after ARTs (fertilization, pregnancy)

- **Conventional semen parameters are not predictive (only need viable & morphologically normal sperm)**

Nagy et al, *Hum Reprod* 1995
Creus et al, *Hum Reprod* 2000
De Vos et al, *Fertil Steril* 2003
Bartoov et al, *Fertil Steril* 2003

In animal studies, sperm DNA is a predictor of ART outcomes

Sperm DNA Damage (Animal studies): Influence on IVF outcomes

- Sperm DNA damage was induced by gamma radiation
- Spermatozoa (mouse model) were then used in IVF cycles

Ahmadi & Ng, *J Exp Zool* 1999

.

	Gamma radiation dosage (GY)				
Parameter	0	5	10	50	100
Fertilization(%)	**53**	**64**	**60**	**59**	**61**
Blastocyst (%)	50	20	8	3	2
Live Fetus	34	21	0	-	-

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Fatehi et al, *J Androl* 2006 – Bovine Model

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Perez-Crespo et al, *J Androl* 2008 – Mouse Model – frozen-thawed sperm

Experimental sperm DNA damage ≠ Clinical sperm DNA damage

Sperm DNA Integrity

Potential applications of sperm DNA tests?

3.To evaluate the relationship between sperm DNA damage and post-natal health (of the IVF - ICSI child) because:

- **Natural barriers to fertilization are removed at ICSI**
- **Infertile men exhibit high levels of sperm DNA damage**
- **Pregnancy is possible despite high levels of DNA damage**
- **Experimental (animal) studies suggest that sperm DNA damage might adversely impact the health of the child**

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Sperm DNA Damage and Fertility

Infertile men have higher levels of sperm DNA - Chromatin damage than fertile men

Chromatin Structure:	Evenson et al, *Hum Reprod* 1999
….	Spano et al, *Fertil Steril* 2000
….	Zini et al, *Fertil Steril* 2001
DNA Fragmentation:	Hughes et al, *Hum Reprod* 1996
….	Irvine et al, *J Androl* 2000
DNA Oxidation	Sen & Ong, *Free Rad Biol Med* 2000
Protamine Deficiency:	Gatewood et al, *J Biol Chem* 1990
….	Carrell & Liu, *J Androl* 2001
….	Zhang et al, *J Androl* 2006

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Sperm DNA Integrity: Influence on Health of the Offspring

- Mouse ICSI studies (fresh [**N**] and frozen-thawed spz [**DFS**])
- CD1 and B6D2F1 mouse strains

Fernandez-Gonzalez et al, *Biol Reprod* 2008

ICSI with DFS (compared to N sperm) →

- **Reduced embryo development**
- **Reduced number of live pups**
- **Development of atypical tumors in 33% of females (CD1)**
- **Reduced longevity (85% vs. 100% surviving at 25 weeks)**
- **Altered behavioral responses ("anxiety-like reactions")**

Sperm DNA Integrity/Damage

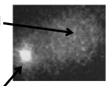
What do sperm DNA damage tests measure?

- Damage to the double stranded DNA
  - **Fragmentation, oxidation or denaturation**
  - **Presence of DNA adducts**
- Defects in the sperm chromatin
  - **Improper or incomplete compaction of the chromatin (DNA and nuclear proteins [protamines and histones])**

Estimation of Sperm DNA Damage

Direct Tests

- **Sperm DNA fragmentation**
  TUNEL assay – labeling of fragmented DNA
  COMET assay - Single cell gel electrophoresis



- **Sperm DNA oxidation**
  8-hydroxy deoxy guanosine (8-OHdG)

Indirect Tests (Assess susceptibility to DNA damage)

- **Sperm chromatin integrity/maturity**
  SCSA (susceptibility to DNA damage & chromatin compaction**)**
  Aniline / toluidine blue (detects histones**)**
  Chromomycin A3 (detects under-protamination**)**
  SCD – sperm chromatin dispersion (chromatin compaction**)**

Evenson et al, *Science* 1980
Sakkas et al, *Hum Reprod* 1996
Hughes et al, *Mol Hum Reprod* 1996
Erenpreiss et al, *J Androl* 2001

Estimation of Sperm DNA Damage

Advantages of sperm DNA damage tests

- Provide information on the quality of spermatogenesis
  - **Complementary to the conventional sperm parameters**
- Exhibit a low degree of biological variability
  - **Lower variability than conventional sperm parameters (SCSA)**
- Testing cryopreserved semen does not alter test results
  - **Cannot be done with conventional sperm parameters**

Zini, *SBRM* 2011

Estimation of Sperm DNA Damage

Limitations of Current Tests of DNA damage

- No test (direct or indirect) can measure:
  - **the full extent or degree of damage (quantitatively)**
  - **clinically relevant damage (e.g. gene-specific damage)**
- Results are dependent on chromatin compaction
  - **assay conditions can influence accessibility of the dye or enzyme to the target sites**
- No test can allow for use of sperm (e.g. for ICSI) after DNA testing

Barratt et al, Hum Reprod 2010

Sperm DNA Damage & Pregnancy Loss: What is the Evidence?

Are measures of sperm DNA damage associated with pregnancy loss after IVF-ICSI?

- Experimental-Animal Studies (Indirect)
- Indirect Clinical Evidence:
  - **Relationship between paternal age & sperm DNA damage**
  - **Relationship between paternal age & pregnancy loss (P-Loss)**
  - **Prospective Case-control studies on sperm DNA damage & P-Loss (natural pregnancy)**
- Direct Clinical Evidence:
  - **Systematic review of studies relating sperm DNA damage to pregnancy loss after IVF or ICSI**

Sperm (DNA) Damage (Animal studies): Influence on Pregnancy Loss

- **Administration of a germ cell toxin (drug) to the male leads to an increased risk of Post-implantation (PI) loss**

Study	Drug	Species	Results
Drug-induced damage			
Balasinor '02	Tamoxifen	rat	Increased PI loss
Salian '09	Bisphenol A	rat	Increased PI loss
Eustache '09	Endocrine Dis	rat	Increased PI loss
Anjum '11	Lead Acetate	rat	Increased PI loss

Sperm (DNA) Damage (Animal studies): Influence on Pregnancy Loss

- **Administration of a germ cell toxin (chemo) to the male leads to Post-implantation (PI) loss and sperm DNA damage**

Study	Drug	Species/Test	Results
Chemotherapy-induced damage			
Doerkson '96	5-azacytosine	rat/DNA methyl	Increased PI loss & hypomethyl
Vaisheva '07	CHOP	rat/SCSA+Tunel	Increased DD & PI loss
Delbes '10	CHOP	rat/COMET	Increased DD & PI loss

Sperm DNA Damage & Pregnancy Loss

Indirect Evidence

Paternal age & sperm DNA damage **Paternal age & pregnancy loss**

Relationship between sperm DNA damage & pregnancy loss

Sperm DNA Damage & Pregnancy Loss

Indirect Evidence

Relationship between paternal age & sperm DNA damage (DD)
(large retrospective studies)

Study	n	Assay	Population	Results
Trisini '03	257	COMET	Infertile men	Increased sperm DD with age
Singh '03	66	COMET	Infertile men	Increased sperm DD with age
Vagnini '07	508	TUNEL	Infertile men	Increased sperm DD with age
Belloc '09	1769	TUNEL	Infertile men	Increased sperm DD with age
Moskovtsev '09	2586	TUNEL	Infertile men	Increased sperm DD with age
Winkle '09	320	Flow-PI	Infertile men	No significant relationship
Brahem '11	140	TUNEL	Infertile men	No significant relationship
Hammiche '11	227	SCSA	Infertile men	Increased sperm DD with age
Nijs '11	278	SCSA	Infertile men	No significant relationship
Varshini '11	504	TUNEL	Infertile men	Increased sperm DD with age
Wyrobek '06	97	SCSA	Healthy men	Increased sperm DD with age

Most studies report higher sperm DNA damage with increasing paternal age
But, these are are largely from highly selected populations (infertile men)

Sperm DNA Damage & Pregnancy Loss

Indirect Evidence

Relationship between paternal age & pregnancy loss (natural-IUI)
(large retrospective or case/control studies)

Study	Design	Population	Results
Natural Pregnancy			
Selvin&Garfinkel'76	Retrospective	1.5 million certif.	Increased late P-Loss with age
Rochebrochard '02	Retrospective	3,174 pregnancies	Increased P-Loss with age
Slama '05	Prospective	5,129 (early preg)	Increased P-Loss with age
Kleinhaus '06	Case/Control	1,506 / 12,539	Increased P-Loss with age
Maconochie '06	Case/control	603 / 6,116	Increased P-Loss with age
IUI Pregnancy			
Bellver '08	Retrospective	2,204 cycles	Increased P-Loss with age
Belloc '09	Retrospective	17,000 cycles	Increased P-Loss with age

Natural-IUI studies report higher rates of P-Loss with increasing paternal age

Sperm DNA Damage & Pregnancy Loss

Indirect Evidence

Relationship between paternal age & pregnancy loss (IVF-ICSI)

Study	Design	Population	Results
IVF-ICSI Pregnancy			
Dain '11	Meta-analysis	7 studies (6,804)	No significant relationship

How do we reconcile the opposite findings of the Natural & IUI studies on the relationship between P-Loss and paternal age and those of the IVF/ICSI studies on the same relationship?

Selection process?
Natural & IUI pregnancies are from men with relatively homogeneous (and "normal") sperm parameters whereas with IVF/ICSI the population of men is so heterogeneous that an age effect may be diluted.

Sperm DNA Damage & Pregnancy Loss

Indirect Evidence

Sperm DNA damage & P-Loss (Natural pregnancies)
Prospective Case-control studies
(Cases: Couples with recurrent P-Loss, Ctls: Fertile Couples)

Study	Cases/Ctls		Assay(s)	Results
Bhattacharya '08	74	60	AO	Higher DNA damage in Cases
Saxena '08	35	20	NCD	Poorer NCD in Cases
Kazerooni '09	30	30	CMA3, AB, AO	Higher DNA damage in Cases
Bellver '10	30	30	SCD	Higher DNA damage in Cases
Talebi '11	40	40	5 tests of DD	Higher DNA damage in Cases
Absalan '12	30	30	SCD	Higher DNA damage in Cases

All studies report higher levels of sperm DNA-chromatin damage in cases

Sperm DNA Damage & Pregnancy Loss

Direct Evidence
Systematic Review & Meta-analysis:

Examined all studies on sperm DNA and …
- **Pregnancy loss (after IVF and ICSI)**

But also sperm DNA and …
- **IVF pregnancy**
- **ICSI pregnancy**

Systematic Review & Meta-analysis

Diagnostic test

- **Sperm chromatin / DNA damage (by SCSA, TUNEL, SCD, COMET)**

Reproductive outcomes (after IVF and ICSI)

- **Pregnancy rate (clinical pregnancy)**
- **Pregnancy loss**

Systematic Review & Meta-analysis

	Pregnancy **Disease +** (no preg)	 **Disease -** (+ preg)
Test + (>cutoff)	**a**	**b**
DNA damage **Test -** (<cutoff)	**c**	**d**

Systematic Review & Meta-analysis

	Disease + (no preg)	**Disease -** (preg)
Test + (>cutoff) **DNA damage**	**a** (true + test)	**b** (false + test)
Test - (<cutoff)	**c** (false - test)	**d** (true - test)

Sensitivity = a/(a+c) (true + test rate)
Specificity = d/(b+d) (true - test rate)
Odds Ratio = ad / bc (measure of assoc. b/n test and disease)
PPV (pos. predictive value)= a/(a+b)(disease prob if + test)
NPV (neg. predictive value)= d/(c+d)(no disease prob if - test)

Systematic Review & Meta-analysis

	Disease + (no preg)	Disease - (preg)
Test + (>cutoff)	**a** (true + test)	**b** (false + test)
DNA damage		
Test - (<cutoff)	**c** (false - test)	**d** (true - test)

Sensitivity = a/(a+c) (true + test rate)
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Systematic Review & Meta-analysis

	Disease + (no preg)	Disease - (preg)
Test + (>cutoff)	**a** (true + test)	**b** (false + test)
DNA damage		
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Sperm DNA Damage and IVF Outcomes

Systematic Review – March 2012

Sperm DNA Damage and IVF Outcomes

Study	n	Design	Fem - Selection	Fertilization
Filatov '99	176	not specified	none	0
Host, '00…	175	prospective, consecutive	none	↓
Tomlinson,'01	140	not specified	none	0
Henkel, '03	208	prospective	none	0
Huang, '05	217	retrospective	none	↓
Boe-Hansen,'06	139	prospective	fsh<10	NA
Borini, '06	82	not specified	none	↓
Benchaib, '07	84	prospective	none	0
Bungum, '07	388	prospective, consecutive	<40 yo, fsh<12	0
Lin, '07	137	prospective	<40, fsh<10	0
Frydman,'07	117	prospective	<38, fsh<15	0
Gu, '09	136	prospective	Tub obstrn	↓
Tarozzi, '09	82	not specified	none	↓
Speyer, '10	192	prospective	<45 yo	0
Simon, '10	224	prospective	none	↓
Nijs, '10	74	prospective	none	↓
Jiang, '11	116	not specified, consecutive	<38, fsh<12	0
Total	**17 studies**			

Sperm DNA Damage and IVF Outcomes

Study	n	Design	Fem - Selection	Fertilization
Filatov '99	176	not specified	none	0
Host, '00…	175	**prospective,consecutive**	none	↓
Tomlinson,'01	140	not specified	none	0
Henkel, '03	208	**prospective**	none	0
Huang, '05	217	retrospective	none	↓
Boe-Hansen,'06	139	**prospective**	fsh<10	NA
Borini, '06	82	not specified	none	↓
Benchaib, '07	84	**prospective**	none	0
Bungum, '07	388	**prospective,consecutive**	<40 yo, fsh<12	0
Lin, '07	137	**prospective**	<40, fsh<10	0
Frydman,'07	117	**prospective**	<38, fsh<15	0
Gu, '09	136	**prospective**	Tub obstrn	↓
Tarozzi, '09	82	not specified	none	↓
Speyer, '10	192	**prospective**	<45 yo	0
Simon, '10	224	**prospective**	none	↓
Nijs, '10	74	**prospective**	none	↓
Jiang, '11	116	not specified, consecutive	<38, fsh<12	0
Total	**17 studies**			

Sperm DNA Damage and IVF Fertilization

Study	n	Design	Fem - Selection	Fertilization
Filatov '99	176	not specified	none	0
Host, '00…	175	prospective, consecutive	none	↓
Tomlinson,'01	140	not specified	none	0
Henkel, '03	208	prospective	none	0
Huang, '05	217	retrospective	none	↓
Boe-Hansen,'06	139	prospective	**fsh<10**	NA
Borini, '06	82	not specified	none	↓
Benchaib, '07	84	prospective	none	0
Bungum, '07	388	prospective, consecutive	**<40 yo, fsh<12**	0
Lin, '07	137	prospective	**<40, fsh<10**	0
Frydman,'07	117	prospective	**<38, fsh<15**	0
Gu, '09	136	prospective	Tub obstrn	↓
Tarozzi, '09	82	not specified	none	↓
Speyer, '10	192	prospective	**<45 yo**	0
Simon, '10	224	prospective	none	↓
Nijs, '10	74	prospective	none	↓
Jiang, '11	116	not specified, consecutive	**<38, fsh<12**	0
Total	**17 studies**			

Sperm DNA Damage and IVF Outcomes

Study	n	Design	Female age	Fertilization
Filatov '99	176	not specified	not controlled	0
Host, '00…	175	prospective, consecutive	**controlled**	↓
Tomlinson,'01	140	not specified	**controlled**	0
Henkel, '03	208	prospective	not controlled	0
Huang, '05	217	retrospective	not controlled	↓
Boe-Hansen,'06	139	prospective	not cont, fsh<10	NA
Borini, '06	82	not specified	**controlled**	↓
Benchaib, '07	84	prospective	**controlled**	0
Bungum, '07	388	prospective, consecutive	**controlled**	0
Lin, '07	137	prospective	**controlled**	0
Frydman,'07	117	prospective	**controlled**	0
Gu, '09	136	prospective	**controlled**	↓
Tarozzi, '09	82	not specified	**controlled**	↓
Speyer, '10	192	prospective	not controlled	0
Simon, '10	224	prospective	not controlled	↓
Nijs, '10	74	prospective	not controlled	↓
Jiang, '11	116	not specified, consecutive	**controlled**	0
Total	**17 studies**			

Sperm DNA Damage and IVF Pregnancy

Study	n	PREG	Assay	Cutoff	Cutoff Justification
Filatov '99	176	↓	CC*	50%	Based on fertile population
Host, '00…	175	↓	TUNEL	4%	Based on fertile population
Tomlinson,'01	140	↓	ISNT	----	no cutoff
Henkel, '03	208	0	TUNEL	37%	Best CO from ROC analysis
Huang, '05	217	0	TUNEL	10%	Not justified
Boe-Hansen,'06	139	↓	SCSA	27%	Based on Evenson 2000, 2002
Borini, '06	82	↓	TUNEL	10%	Based on Benchaib 2003
Benchaib, '07	84	↓	TUNEL	15%	Based on IVF-ICSI results
Bungum, '07	388	↓	SCSA	30%	Based on Evenson 2000, 2002
Lin, '07	137	↓	SCSA	27%	Based on Evenson 2000, 2002
Frydman,'07	117	↓	TUNEL	35%	Median value
Gu, '09	136	↓	CMA3	----	no cutoff
Tarozzi, '09	82	↓	CMA3	29%	Best CO from ROC analysis
Speyer, '10	192	↓	SCSA	30%	Based on Evenson 2000, 2002
Simon, '10	224	↓	COMET	56%	Best CO from ROC analysis
Nijs, '10	74	0	SCSA	30%	Cannot construct 2 x 2 table
Jiang, '11	116	↓	SCSA	30%	Based on Evenson 2000, 2002

*Chromatin compaction (by flow cytometry)

Sperm DNA Damage and IVF Pregnancy

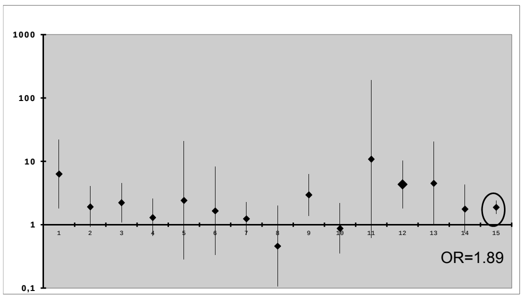
Study	n	PREG	Assay	Cutoff	Cutoff Justification
Filatov '99	**176**	↓	**CC***	**50%**	**Based on fertile population**
Host, '00…	**175**	↓	**TUNEL**	**4%**	**Based on fertile population**
~~Tomlinson,'01~~	~~140~~	~~↓~~	~~ISNT~~	~~----~~	~~no cutoff~~
Henkel, '03	**208**	**0**	**TUNEL**	**37%**	**Best CO from ROC analysis**
Huang, '05	**217**	**0**	**TUNEL**	**10%**	**Not justified**
Boe-Hansen,'06	**139**	↓	**SCSA**	**27%**	**Based on Evenson 2000, 2002**
Borini, '06	**82**	↓	**TUNEL**	**10%**	**Based on Benchaib 2003**
Benchaib, '07	**84**	↓	**TUNEL**	**15%**	**Based on IVF-ICSI results**
Bungum, '07	**388**	↓	**SCSA**	**30%**	**Based on Evenson 2000, 2002**
Lin, '07	**137**	↓	**SCSA**	**27%**	**Based on Evenson 2000, 2002**
Frydman,'07	**117**	↓	**TUNEL**	**35%**	**Median value**
~~Gu, '09~~	~~136~~	~~↓~~	~~CMA3~~	~~----~~	~~no cutoff~~
Tarozzi, '09	**82**	↓	**CMA3**	**29%**	**Best CO from ROC analysis**
Speyer, '10	**192**	↓	**SCSA**	**30%**	**Based on Evenson 2000, 2002**
Simon, '10	**224**	↓	**COMET**	**56%**	**Best CO from ROC analysis**
~~Nijs, '10~~	~~74~~	~~0~~	~~SCSA~~	~~30%~~	~~Cannot construct 2 x 2 table~~
Jiang, '11	**116**	↓	**SCSA**	**30%**	**Based on Evenson 2000, 2002**
14 studies					

Sperm DNA Damage and IVF Pregnancy

Study	n	%hDD	Sens	Spec	OR	(95% CI)
Filatov, '99	176	41	0.46	0.88	6.33	(1.82, 22.08)
Host, '00…	175	30	0.34	0.79	1.92	(0.92, 4.04)
Henkel, '03	208	69	0.35	0.81	2.24	(1.09, 4.58)
Huang, '05	217	19	0.22	0.83	1.30	(0.66, 2.56)
Boe-Hansen,'06	139	5	0.06	0.97	2.43	(0.28, 20.83)
Borini, '06	82	16	0.17	0.89	1.66	(0.33, 8.28)
Lin, '07	137	16	0.15	0.83	0.88	(0.35, 2.19)
Benchaib, '07	84	10	0.07	0.86	0.46	(0.11, 2.00)
Bungum, '07	388	16	0.17	0.86	1.24	(0.69, 2.26)
Frydman,'07	117	44	0.58	0.68	2.97	(1.39, 6.32)
Tarozzi, '09	82	17	0.22	0.97	10.9	(0.65, 191.5)
Speyer, '10	192	8	0.12	0.97	4.51	(1.00, 20.48)
Simon, '10	224	43	0.49	0.82	4.33	(1.82, 10.31)
Jiang, '11	116	25	0.30	0.84	1.77	(0.74, 4.25)
Total	**1805**	**23%**	**0.22**	**0.84**		

Fixed Effects Model: (Test for Homogeneity: (p > 0.1))
Combined **Odds ratio = 1.89** (1.48, 2.41), p < 0.01

Sperm DNA Damage and IVF Pregnancy



Sperm DNA Damage and IVF Pregnancy

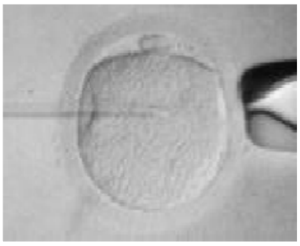
Fixed Effects Model:
Combined **Odds ratio = 1.89** (1.48, 2.41), p < 0.01

Clinical Application?

Positive predictive value (**PPV** median): 79% no PR (**21% PR**)
Negative predictive value (**NPV** median): **34% PR**

Clinical significance of an 13% difference in PR?

Sperm DNA Damage and ICSI Outcomes



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Sperm DNA Damage and ICSI Outcomes

Study	n	Design	Fem - Selection	Fertilization	
Hammadeh, '96	61	prospective	none	0	
Host, '00…	61	prospective, consecutive	none	0	
Virant-Klun, '02	183	prospective	none	↓	
Henkel, '03	54	prospective	none	0	
Huang, '05	86	retrospective	none	↓	
Check, '05	104	not specified, IVF failure	none	N/A	
Zini, '05	60	prospective, consecutive	<40	0	
Borini, '06	50	not specified	none	0	
Muriel, '06	85	prospective	none	↓	
Benchaib, '07	218	prospective	none	0	
Bungum, '07	223	prospective, consecutive	<40 yo, fsh<12	0	
Lin, '07	86	prospective	<40, fsh<10	0	
Bakos, '07	68	not specified	none	0	
Micinski, '09	60	prospective	<38	↓	
Tarozzi, '09	50	not specified	none	0	
Speyer, '10	155	prospective	<45 yo	0	
Simon, '10	127	prospective	none	0	
Nijs, '10	94	prospective	none	0	
Jiang, '11	63	not specified, consecutive	<38, fsh<12	0	n=19

Sperm DNA Damage and ICSI Outcomes

Study	n	Design	Fem - Selection	Fertilization	
Hammadeh, '96	61	**prospective**	none	0	
Host, '00…	61	**prospective, consecutive**	none	0	
Virant-Klun, '02	183	**prospective**	none	↓	
Henkel, '03	54	**prospective**	none	0	
Huang, '05	86	retrospective	none	↓	
Check, '05	104	not specified, IVF failure	none	N/A	
Zini, '05	60	**prospective, consecutive**	<40	0	
Borini, '06	50	not specified	none	0	
Muriel, '06	85	**prospective**	none	↓	
Benchaib, '07	218	**prospective**	none	0	
Bungum, '07	223	**prospective, consecutive**	<40 yo, fsh<12	0	
Lin, '07	86	**prospective**	<40, fsh<10	0	
Bakos, '07	68	not specified	none	0	
Micinski, '09	60	**prospective**	<38	↓	
Tarozzi, '09	50	not specified	none	0	
Speyer, '10	155	**prospective**	<45 yo	0	
Simon, '10	127	**prospective**	none	0	
Nijs, '10	94	**prospective**	none	0	
Jiang, '11	63	not specified, consecutive	<38, fsh<12	0	n=19

Sperm DNA Damage and ICSI Outcomes

Study	n	Design	Fem - Selection	Fertilization
Hammadeh, '96	61	prospective	none	0
Host, '00…	61	prospective, consecutive	none	0
Virant-Klun, '02	183	prospective	none	↓
Henkel, '03	54	prospective	none	0
Huang, '05	86	retrospective	none	↓
Check, '05	104	not specified, IVF failure	none	N/A
Zini, '05	60	prospective, consecutive	**<40**	0
Borini, '06	50	not specified	none	0
Muriel, '06	85	prospective	none	↓
Benchaib, '07	218	prospective	none	0
Bungum, '07	223	prospective, consecutive	**<40 yo, fsh<12**	0
Lin, '07	86	prospective	**<40, fsh<10**	0
Bakos, '07	68	not specified	none	0
Micinski, '09	60	prospective	**<38**	↓
Tarozzi, '09	50	not specified	none	0
Speyer, '10	155	prospective	**<45 yo**	0
Simon, '10	127	prospective	none	0
Nijs, '10	94	prospective	none	0
Jiang, '11	63	not specified, consecutive	**<38, fsh<12**	0

n=19

Sperm DNA Damage and ICSI Outcomes

Study	n	Design	Female age	Fertilization
Hammadeh, '96	61	prospective	not controlled	0
Host, '00…	61	prospective, consecutive	**controlled**	0
Virant-Klun, '02	183	prospective	**controlled**	↓
Henkel, '03	54	prospective	not controlled	0
Huang, '05	86	retrospective	not controlled	↓
Check, '05	104	not specified, IVF failure	not controlled	N/A
Zini, '05	60	prospective, consecutive	**controlled**	0
Borini, '06	50	not specified	**controlled**	0
Muriel, '06	85	prospective	not controlled	↓
Benchaib, '07	218	prospective	**controlled**	0
Bungum, '07	223	prospective, consecutive	**controlled**	0
Lin, '07	86	prospective	**controlled**	0
Bakos, '07	68	not specified	not controlled	0
Micinski, '09	60	prospective	not controlled	↓
Tarozzi, '09	50	not specified	**controlled**	0
Speyer, '10	155	prospective	not controlled	0
Simon, '10	127	prospective	not controlled	0
Nijs, '10	94	prospective	not controlled	0
Jiang, '11	63	not specified, consecutive	**controlled**	0

n=19

Sperm DNA Damage and ICSI Pregnancy

Study	n	PREG	Assay	Cutoff	Cutoff Justification
Hammadeh, '96	61	↓	A-Blue	29%	ROC analysis
Host, '00…	61	0	TUNEL	4%	Based on fertile population
Virant-Klun, '02	183	0	AO	56%	Based on Liu & Baker 1992
Henkel, '03	54	↓	TUNEL	24%	ROC analysis
Huang, '05	86	0	TUNEL	4%	Not justified
Check, '05	104	0	SCSA	30%	Based on Evenson 2000, 2002
Zini, '05	60	0	SCSA	30%	Based on Evenson 2000, 2002
Borini, '06	50	↓	TUNEL	10%	Based on Benchaib 2003
Muriel, '06	85	0	SCD	----	No cutoff
Benchaib, '07	218	↓	TUNEL	15%	Based on ART results (10%,'03)
Bungum, '07	223	0	SCSA	30%	Based on Evenson 2000, 2002
Lin, '07	86	0	SCSA	27%	Based on Evenson 2000, 2002
Bakos, '07	68	↓	TUNEL	35%	Cannot construct 2 x 2 table
Micinski, '09	60	↓	SCSA?	15%	Based on Filatov et al, 1998
Tarozzi, '09	50	0	CMA3	29%	ROC analysis
Speyer, '10	155	↓	SCSA	30%	Based on Evenson 2000, 2002
Simon, '10	127	↓	COMET	56%	Best CO from ROC analysis
Nijs, '10	94	0	SCSA	30%	Cannot construct 2 x 2 table
Jiang, '11	63	↓	SCSA	30%	Based on Evenson 2000, 2002

Sperm DNA Damage and ICSI Pregnancy

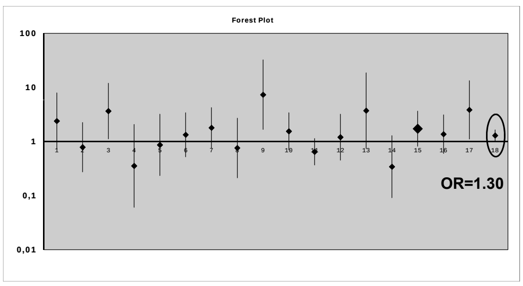
Study	n	PREG	Assay	Cutoff	Cutoff Justification
Hammadeh, '96	61	↓	A-Blue	29%	ROC analysis
Host, '00…	61	0	TUNEL	4%	Based on fertile population
~~Virant Klun, '02~~	~~183~~	~~0~~	~~AO~~	~~56%~~	~~Based on Liu & Baker 1992~~
Henkel, '03	54	↓	TUNEL	24%	ROC analysis
Huang, '05	86	0	TUNEL	4%	Not justified
Check, '05	104	0	SCSA	30%	Based on Evenson 2000, 2002
Zini, '05	60	0	SCSA	30%	Based on Evenson 2000, 2002
Borini, '06	50	↓	TUNEL	10%	Based on Benchaib 2003
~~Muriel, '06~~	~~85~~	~~0~~	~~SCD~~	~~—~~	~~No cutoff~~
Benchaib, '07	218	↓	TUNEL	15%	Based on ART data (10%,'03)
Bungum, '07	223	0	SCSA	30%	Based on Evenson 2000, 2002
Lin, '07	86	0	SCSA	27%	Based on Evenson 2000, 2002
~~Bakos, '07~~	~~68~~	~~↓~~	~~TUNEL~~	~~35%~~	~~Cannot construct 2 x 2 table~~
Micinski, '09	60	↓	SCSA?	15%	Based on Filatov et al, 1998
Tarozzi, '09	50	0	CMA3	29%	ROC analysis
Speyer, '10	155	↓	SCSA	30%	Based on Evenson 2000, 2002
Simon, '10	127	↓	COMET	56%	Best CO from ROC analysis
~~Nijs, '10~~	~~94~~	~~0~~	~~SCSA~~	~~30%~~	~~Cannot construct 2 x 2 table~~
Jiang, '11	63	↓	SCSA	30%	Based on Evenson 2000, 2002

Sperm DNA Damage and ICSI Pregnancy

Study	n	%DD	Sens	Spec	OR	(95% CI)
Hammadeh, '96	61	44	0.50	0.70	2.40	(0.72, 7.96)
Host, '00…	61	59	0.57	0.38	0.79	(0.28, 2.25)
Henkel, '03	54	48	0.68	0.63	3.67	(1.12, 12.0)
Huang, '05	86	57	0.64	0.50	1.80	(0.76, 4.27)
Zini, '05	60	18	0.17	0.81	0.87	(0.23, 3.22)
Check, '05	104	28	0.29	0.76	1.34	(0.52, 3.43)
Borini, '06	50	60	0.71	0.75	7.36	(1.67, 32.4)
Benchaib, '07	218	17	0.19	0.87	1.55	(0.70, 3.41)
Bungum, '07	223	33	0.29	0.61	0.65	(0.37, 1.14)
Lin, '07	86	24	0.26	0.77	1.21	(0.45, 3.23)
Micinski, '09	60	35	0.40	0.85	3.73	(0.74, 18.77)
Tarozzi, '09	50	56	0.49	0.23	0.34	(0.09, 1.29)
Speyer, '10	155	22	0.24	0.81	1.37	(0.60, 3.13)
Simon, '10	127	63	0.67	0.45	1.73	(0.82, 3.66)
Jiang, '11	63	33	0.44	0.83	3.86	(1.11, 13.43)
Total		**40%**	**0.40**	**0.71**		

Fixed Effects Model: (Test for Homogeneity: P > 0.1)
Combined **Odds ratio = 1.30** (1.02, 1.65), P <0.05

Sperm DNA Damage and ICSI Pregnancy



Sperm DNA Damage and ICSI Pregnancy

Fixed Effects Model:
Combined **Odds ratio = 1.30** (1.02, 1.65), P <0.05

Clinical Application?

Positive predictive value (**PPV** median): 70% no PR (**30% PR**)
Negative predictive value (**NPV** median): **37% PR**

Clinical significance of an 7% difference in PR?

Sperm DNA Damage and Pregnancy Loss after IVF and/or ICSI

Study	ART	Cycles	Preg	P-Loss	RISK*	Comment
Virro, '04	**Mixed**	---	---	---	↑	No 2 x 2 table
Check, '05	**ICSI**	104	34	47%	↑	Failed >2 IVF Rx
Zini, '05	**ICSI**	60	31	16%	↑	PL after CP
Borini, '06	**IVF**	82	18	6%	↑	PL after CP & BP
Borini, '06	**ICSI**	50	12	25%	↑	PL after CP & BP
Benchaib, '07	**IVF**	84	26	15%	↑	PL after CP
Benchaib, '07	**ICSI**	218	68	12%	↑	PL after CP
Lin, '07	**IVF**	137	81	10%	↑	PL after CP
Lin, '07	**ICSI**	86	44	18%	↑	PL after CP
Frydman,'07	**IVF**	117	59	19%	↑	PL after CP
Bungum, '07	**IVF**	388	148	24%	0	PL after BP
Bungum, '07	**ICSI**	223	106	19%	↑	PL after BP
Simon, '10	**IVF**	224	39	10%	↑	PL after CP
Simon, '10	**ICSI**	127	44	18%	↑	PL after CP
Jiang, '11	**Mixed**	179	76	17%	↑	PL after CP
Kennedy, '11	**Mixed**	233	141	8%	↑	No 2 x 2 table
Total 16 studies		**2079**	**776**			

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Sperm DNA Damage and Pregnancy Loss after IVF and/or ICSI

Study	ART	Cycles	Preg	P-Loss	RISK*	Comment
~~Virro, '04~~	~~**Mixed**~~	~~---~~	~~---~~	~~---~~	~~↑~~	~~No 2 x 2 table~~
Check, '05	**ICSI**	104	34	47%	↑	Failed >2 IVF Rx
Zini, '05	**ICSI**	60	31	16%	↑	PL after CP
Borini, '06	**IVF**	82	18	6%	↑	PL after CP & BP
Borini, '06	**ICSI**	50	12	25%	↑	PL after CP & BP
Benchaib, '07	**IVF**	84	26	15%	↑	PL after CP
Benchaib, '07	**ICSI**	218	68	12%	↑	PL after CP
Lin, '07	**IVF**	137	81	10%	↑	PL after CP
Lin, '07	**ICSI**	86	44	18%	↑	PL after CP
Frydman,'07	**IVF**	117	59	19%	↑	PL after CP
Bungum, '07	**IVF**	388	148	24%	0	PL after BP
Bungum, '07	**ICSI**	223	106	19%	↑	PL after BP
Simon, '10	**IVF**	224	39	10%	↑	PL after CP
Simon, '10	**ICSI**	127	44	18%	↑	PL after CP
Jiang, '11	**Mixed**	179	76	17%	↑	PL after CP
~~Kennedy, '11~~	~~**Mixed**~~	~~233~~	~~141~~	~~8%~~	~~↑~~	~~No 2 x 2 table~~
Total 14 studies		**2079**	**776**			

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Sperm DNA Damage and Pregnancy Loss after IVF and/or ICSI

Study	n	ART	Design	Female age
Check, '05	104	ICSI	not specified, IVF failure	not controlled
Zini, '05	60	ICSI	**prospective,consecutive**	controlled
Borini, '06	82	IVF	not specified	controlled
Borini, '06	50	ICSI	not specified	controlled
Benchaib, '07	84	IVF	**prospective**	controlled
Benchaib, '07	218	ICSI	**prospective**	controlled
Lin, '07	137	IVF	**prospective**	controlled
Lin, '07	86	ICSI	**prospective**	controlled
Frydman, '07	117	IVF	**prospective**	controlled
Bungum, '07	388	IVF	**prospective,consecutive**	controlled
Bungum, '07	223	ICSI	**prospective,consecutive**	controlled
Simon, '10	224	IVF	**prospective**	not controlled
Simon, '10	127	ICSI	**prospective**	not controlled
Jiang, '11	179	Mixed	not specified,consecutive	controlled

Sperm DNA Damage and Pregnancy Loss after IVF and/or ICSI

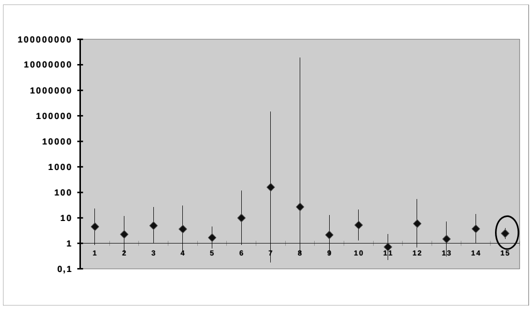
Study	n	ART	Design	Female age
Check, '05	104	ICSI	not specified, IVF failure	not controlled
Zini, '05	60	ICSI	prospective, consecutive	**controlled**
Borini, '06	82	IVF	not specified	**controlled**
Borini, '06	50	ICSI	not specified	**controlled**
Benchaib, '07	84	IVF	prospective	**controlled**
Benchaib, '07	218	ICSI	prospective	**controlled**
Lin, '07	137	IVF	prospective	**controlled**
Lin, '07	86	ICSI	prospective	**controlled**
Frydman, '07	117	IVF	prospective	**controlled**
Bungum, '07	388	IVF	prospective, consecutive	**controlled**
Bungum, '07	223	ICSI	prospective, consecutive	**controlled**
Simon, '10	224	IVF	prospective	not controlled
Simon, '10	127	ICSI	prospective	not controlled
Jiang, '11	179	Mixed	not specified, consecutive	**controlled**

Sperm DNA Damage and Pregnancy Loss (All) after IVF and/or ICSI

Study	ART	P-Loss	Ab Test	Sens	Spec	OR	(95% CI)
Check, '05	ICSI	47%	24%	0.63	0.83	2.27	(0.45, 11.59)
Zini, '05	ICSI	16%	19%	0.33	0.85	3.67	(0.46, 29.42)
Borini, '06	IVF	6%	11%	0.91	0.94	32.0	(0.62, 1663)
Borini, '06	ICSI	25%	25%	0.97	0.99	108.0	(1.73, 6729)
Benchaib, '07	IVF	15%	15%	0.50	0.91	10.0	(0.87, 114.8)
Benchaib, '07	ICSI	12%	15%	0.30	0.88	3.51	(0.89, 23.28)
Lin, '07	IVF	9%	15%	0.17	0.86	2.56	(0.44, 15.03)
Lin, '07	ICSI	18%	23%	0.40	0.83	5.00	(0.97, 25.77)
Frydman,'07	IVF	19%	32%	0.37	0.75	5.25	(1.31, 21.11)
Bungum, '07	IVF	24%	14%	0.19	0.85	0.73	(0.23, 2.33)
Bungum, '07	ICSI	29%	40%	0.24	0.63	1.69	(0.63, 4.49)
Simon, '10	IVF	18%	18%	0.50	0.86	6.00	(0.68, 52.90)
Simon, '10	ICSI	17%	55%	0.63	0.47	1.49	(0.31, 7.20)
Jiang, '11	Mixed	8%	18%	0.38	0.86	3.75	(1.00, 14.06)
				0.50	**0.85**		

Test for Homogeneity: Q test non-significant
Fixed Effects Model:
Combined **Odds ratio = 2.58** (1.67, 3.97), p < 0.0001 **2012**

Sperm DNA Damage and Pregnancy Loss (All) after IVF and/or ICSI



Sperm DNA Damage and Pregnancy Loss after IVF and/or ICSI

Pregnancy Loss
Combined **Odds ratio = 2.58** (1.67, 3.97), p < 0.0001

Clinical Application?

Positive predictive value (**PPV** median): **35% PL**
Negative predictive value (**NPV** median): 90% no PL (**11% PL**)

Sperm DNA Damage and Pregnancy Loss after IVF and/or ICSI

Pregnancy Loss (All)
Combined **Odds ratio = 2.58** (1.67, 3.97), p < 0.0001

Pregnancy Loss (IVF, 6 studies)
Combined **Odds ratio = 2.41** (1.19, 4.92), p < 0.05

Pregnancy Loss (ICSI, 6 studies)
Combined **Odds ratio = 2.50** (1.38, 4.54), p < 0.05

Sperm DNA Damage: Practical Application

What is clinical utility of sperm DNA tests?

In infertile couples with pregnancy loss post-IVF

Sperm DNA Damage: Practical Application

Infertile couples with pregnancy loss post-IVF

Test Characteristics:

Median prevalence of a + test is 25-30%
Median sensitivity 40% → many other causes for PLoss
Median specificity 85 % → + test points to male factor in PL

If +test → Increased risk of PL with IVF or ICSI

Evaluate the male & correct any male factor

Summary

The relationship between sperm DNA damage & pregnancy loss after IVF&ICSI is supported by…

Indirect Evidence:

- **Experimental studies**
- **Paternal age & sperm DNA --- paternal age & pregnancy loss studies**
- **Case-control studies on sperm DNA damage & P-Loss (natural)**

Direct Evidence:

- **Systematic analysis of studies relating sperm DNA damage to pregnancy loss after IVF or ICSI (OR=2.58)**

Summary

The relationship between sperm DNA damage & pregnancy loss after IVF&ICSI is supported by… But…

Indirect Evidence:

- **Experimental studies**
  (Experimental sperm DNA damage ≠ Clinical sperm DNA damage)
- **Paternal age & sperm DNA --- paternal age & pregnancy loss studies**
  (Large studies but relationship is speculative)
- **Case-control studies on sperm DNA damage & P-Loss (natural)**
  (Small studies, that may not necessarily reflect ART P-Loss)

Direct Evidence:

- **Systematic analysis of studies relating sperm DNA damage to pregnancy loss after IVF or ICSI (OR=2.58)**
  (Heterogeneous design, populations, sperm DNA tests)

Conclusions

Sperm DNA damage is related to pregnancy loss after IVF&ICSI

Future directions
Large, well-designed prospective studies on IVF and ICSI pregnancy and pregnancy loss (Multivariate analysis, well-defined parameters)

The University of Nottingham

Nurture Fertility

Are Ovarian Reserve Tests predictive of miscarriage in women undergoing ART?

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MRCOG, PhD
Associate Professor & Subspecialist in Reproductive Medicine
Queen's Medical Centre, Nottingham, UK

Declaration

I have no conflicts of interest or any commercial relationship

Learning objectives (Plan)

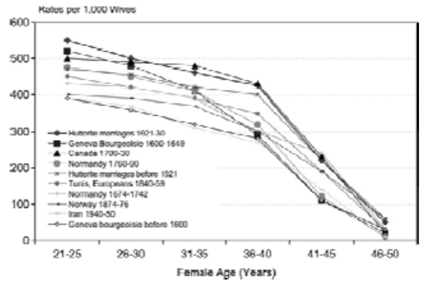
Reproductive ageing and ovarian reserve

Ovarian Reserve Tests (ORT)

Ability of ORTs to predict miscarriage: evidence

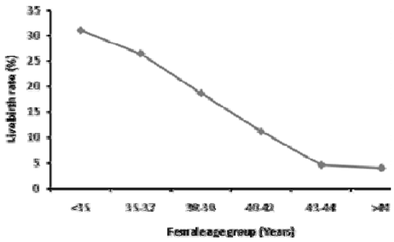
Conclusions

Reproductive ageing
Natural fertility rates by age



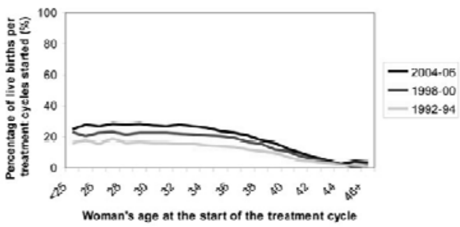
Menken et al., 1986; Baird et al., 2005

Reproductive ageing
Live birth rates by age per IVF cycle (2006)



(HFEA Database of 44275 IVF cycles)

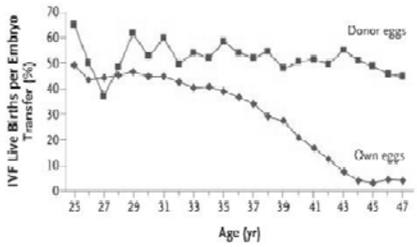
Reproductive ageing
Live birth rates by age per IVF cycle (1992-2006)



HFEA Database (http://www.hfea.gov.uk/2588.html)

Reproductive ageing

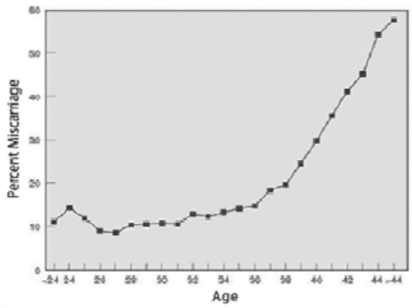
Live birth rates relate to egg quality



Van Voorhis, 2007 NEJM (IVF data)

Reproductive ageing

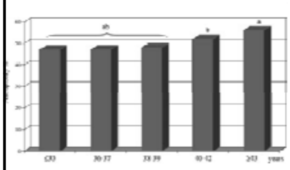
Rates of Miscarriage

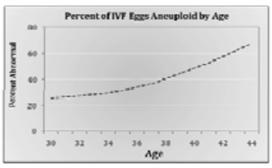


CDC report 2008

Reproductive ageing

Rates of Aneuploidy

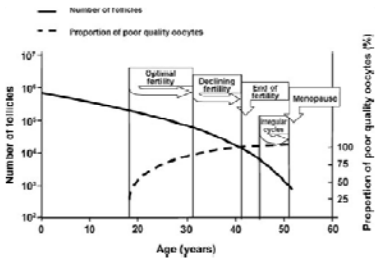




Gianaroli et. Al., Hum. Repro. 2010; Battaglia et.al. Hum. Repro. 1996

Reproductive ageing

Progressive loss of reproductive function



Broekmans et al., 2009 Endo. reviews

Reproductive ageing

OVARIAN AGEING

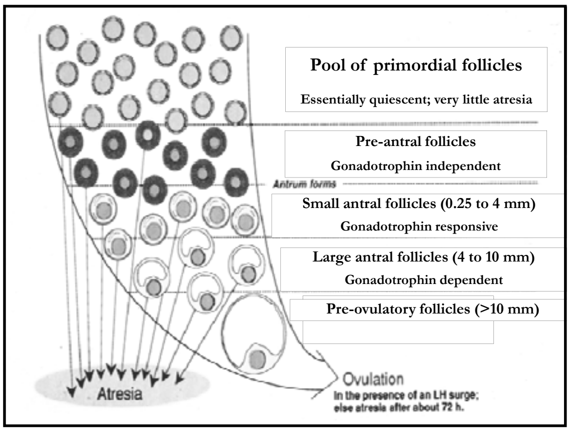
Decline in the quantity and quality of primordial follicles remaining with in the ovaries (Decline in "Ovarian Reserve")

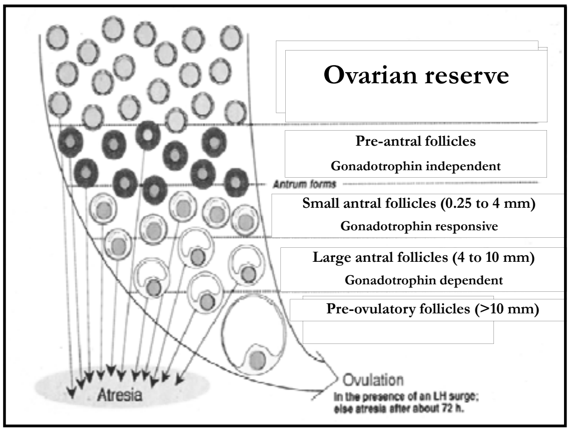
Broekmans et al., 2006 Hum. Repro. Update

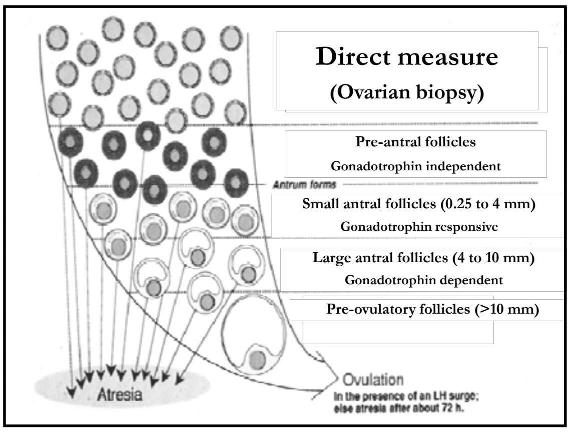
Ovarian reserve - Importance

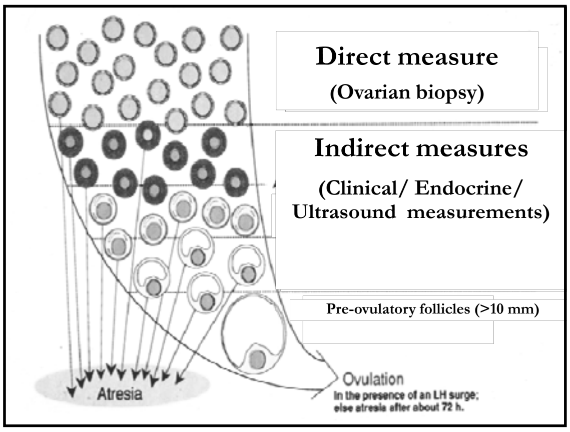
- ✓ **'Fertility potential' of women**
- ✓ **Prediction of ovarian response/ IVF success**
- ✓ **Make an individualized treatment plan**
- ✓ **Miscarriage/ Aneuploidy/ Pre-eclampsia**
- ✓ **Reproductive life span**

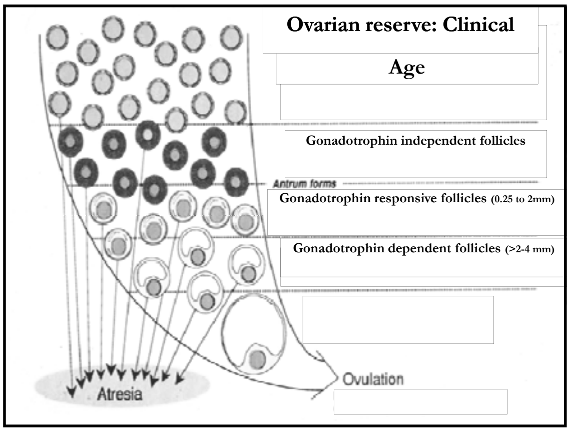
Trout & Seifer, 2000, Freeman et al., 2000, Broekmans et al., 2006, Woldringh et al., 2006, Nikolaou et al., 2002

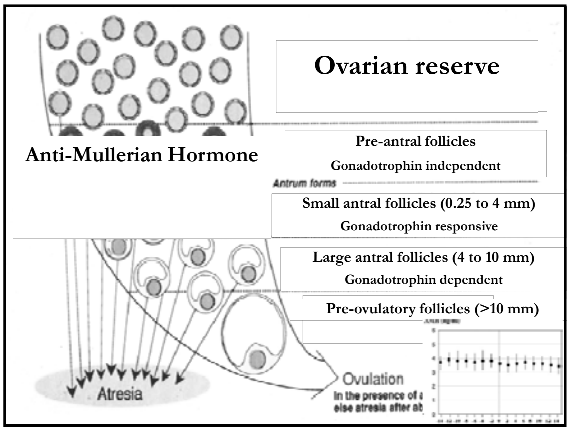

Pool of primordial follicles
Essentially quiescent; very little atresia
Pre-antral follicles
Gonadotrophin independent
Antrum forms
Small antral follicles (0.25 to 4 mm)
Gonadotrophin responsive
Large antral follicles (4 to 10 mm)
Gonadotrophin dependent
Pre-ovulatory follicles (>10 mm)
Ovulation
In the presence of an LH surge;
else atresia after about 72 h.
Atresia

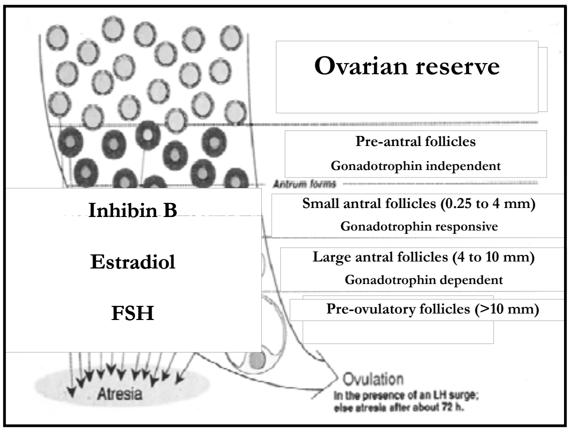

Ovarian reserve
Pre-antral follicles
Gonadotrophin independent
Antrum forms
Small antral follicles (0.25 to 4 mm)
Gonadotrophin responsive
Large antral follicles (4 to 10 mm)
Gonadotrophin dependent
Pre-ovulatory follicles (>10 mm)
Ovulation
In the presence of an LH surge;
else atresia after about 72 h.
Atresia

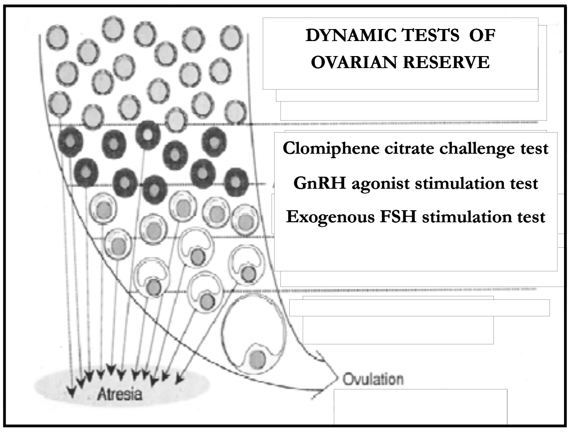

Direct measure
(Ovarian biopsy)
Pre-antral follicles
Gonadotrophin independent
Antrum forms
Small antral follicles (0.25 to 4 mm)
Gonadotrophin responsive
Large antral follicles (4 to 10 mm)
Gonadotrophin dependent
Pre-ovulatory follicles (>10 mm)
Ovulation
In the presence of an LH surge;
else atresia after about 72 h.
Atresia

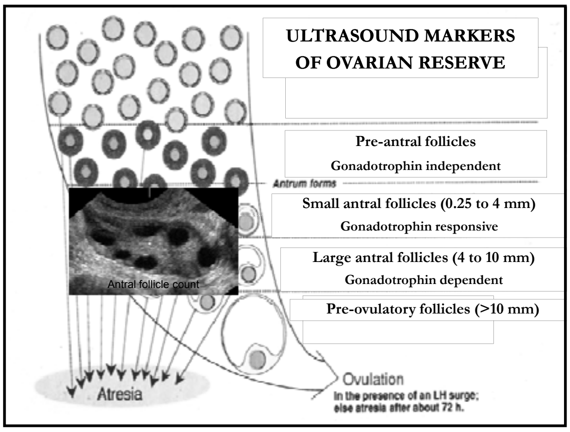

Direct measure
(Ovarian biopsy)
Indirect measures
(Clinical/ Endocrine/ Ultrasound measurements)
Pre-ovulatory follicles (>10 mm)
Ovulation
In the presence of an LH surge; else atresia after about 72 h.
Atresia

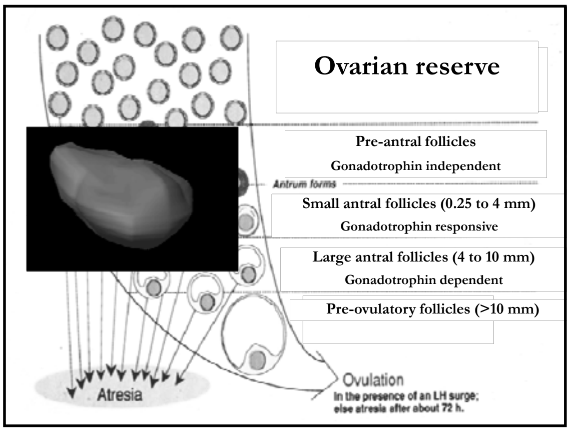

Ovarian reserve: Clinical
Age
Gonadotrophin independent follicles
Antrum forms
Gonadotrophin responsive follicles (0.25 to 2mm)
Gonadotrophin dependent follicles (>2-4 mm)
Ovulation
Atresia

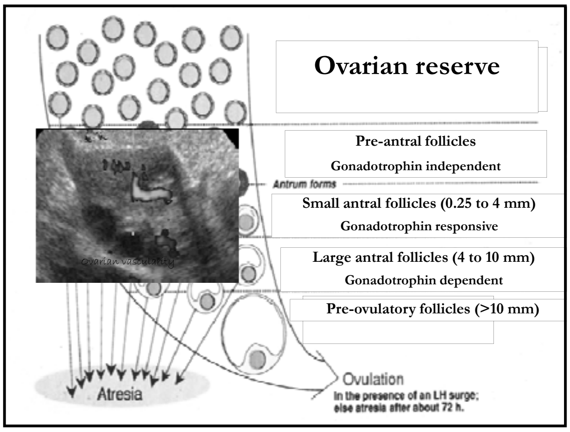

Ovarian reserve
Anti-Mullerian Hormone
Pre-antral follicles
Gonadotrophin independent
Antrum forms
Small antral follicles (0.25 to 4 mm)
Gonadotrophin responsive
Large antral follicles (4 to 10 mm)
Gonadotrophin dependent
Pre-ovulatory follicles (>10 mm)
Ovulation
Atresia

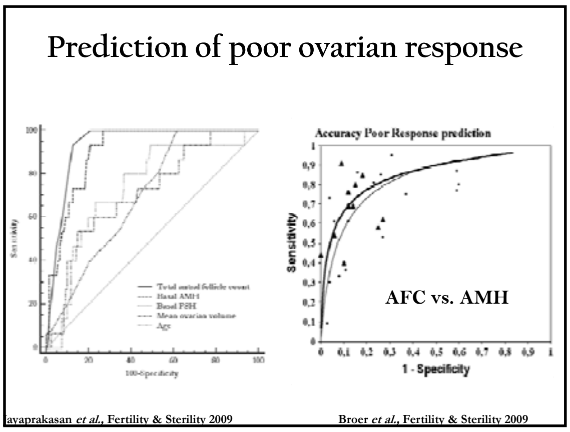

Ovarian reserve
Pre-antral follicles
Gonadotrophin independent
Antrum forms
Small antral follicles (0.25 to 4 mm)
Gonadotrophin responsive
Large antral follicles (4 to 10 mm)
Gonadotrophin dependent
Pre-ovulatory follicles (>10 mm)
Inhibin B
Estradiol
FSH
Atresia
Ovulation
In the presence of an LH surge;
else atresia after about 72 h.


DYNAMIC TESTS OF
OVARIAN RESERVE
Clomiphene citrate challenge test
GnRH agonist stimulation test
Exogenous FSH stimulation test
Atresia
Ovulation

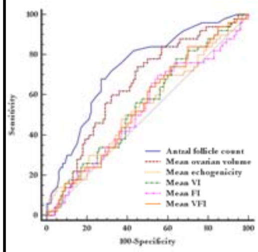

ULTRASOUND MARKERS
OF OVARIAN RESERVE
Pre-antral follicles
Gonadotrophin independent
Antrum forms
Small antral follicles (0.25 to 4 mm)
Gonadotrophin responsive
Large antral follicles (4 to 10 mm)
Gonadotrophin dependent
Pre-ovulatory follicles (>10 mm)
Antral follicle count
Atresia
Ovulation
In the presence of an LH surge;
else atresia after about 72 h.

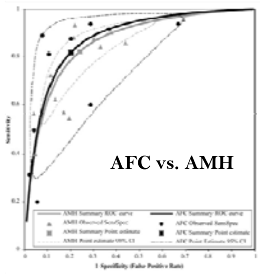

Ovarian reserve
Pre-antral follicles
Gonadotrophin independent
Antrum forms
Small antral follicles (0.25 to 4 mm)
Gonadotrophin responsive
Large antral follicles (4 to 10 mm)
Gonadotrophin dependent
Pre-ovulatory follicles (>10 mm)
Atresia
Ovulation
In the presence of an LH surge;
else atresia after about 72 h.


Ovarian reserve
Pre-antral follicles
Gonadotrophin independent
Antrum forms
Small antral follicles (0.25 to 4 mm)
Gonadotrophin responsive
Large antral follicles (4 to 10 mm)
Gonadotrophin dependent
Pre-ovulatory follicles (>10 mm)
Atresia
Ovulation
In the presence of an LH surge;
else atresia after about 72 h.


Prediction of poor ovarian response
Accuracy Poor Response prediction
Sensitivity
1 - Specificity
AFC vs. AMH
Jayaprakasan et al., Fertility & Sterility 2009
Broer et al., Fertility & Sterility 2009

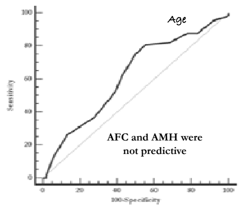
Prediction: excessive response

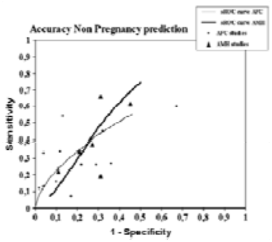




Jayaprakasan *et al.*, Fertility & Sterility 2008 — Broer *et al.*, Hum Repro update 2011

Prediction of conception





AFC & AMH are equally good at predicting ovarian response, but both less predictive of quality

Jayaprakasan *et al.*, Fertility & Sterility 2009 — Broer *et al.*, Fertility & Sterility 2009

Author	Study population	Protocol	ORT	RESULTS
Weghofer HR 2005 (Retrospective) N= 535 IVF	Age 25-40; FSH ≤10 IU/L	long protocol	Age specific FSH (lowest & highest quartile)	Not predictive, no difference in miscarriage rate (miscarriage not defined)
Chuang FnS 2003 (Retrospective) N= 1045 IVF	No inclusion or exclusion criteria described	long & short protocol ET with ?embryos	Age FSH	Not predictive of miscarriage
Levi FnS 2001 (Retrospective) N=9802 fertility	All fertility patients 1256 high FSH 9618 low FSH	CC IUI IVF	FSH (>14.2 & <14.2)	High 1st TM pregnancy loss (71.4% vs. 20%) in high FSH
Abdalla HR 2004 N=2057 (613 pregnant)	All IVF/ICSI pts	Long ag/ Antagonist	FSH 4 gps: <10,10-15,15-20, >20	No difference in miscarriage (<24 wks) rates across 4 gps
vanMontfrans HR2004 (prospective) N=129	Age>30, natural conception No infertility/ut anoma	F/U of 12 mo. UPT on day 1 of cycle	FSH	No difference in FSH levels between nonpreg, EPL, misc, ongoing prg gps

Author	Study population	Protocol	ORT	RESULTS
Hofmann FnS 2000 (Retrospective) N=692 (44 RPL vs.648 non-RPL)	Age >35; any age with unexplained infertility	long protocol D3 (ET with up to 4 emb)	FSH CCCT	Similar reduced ovarian reserve (18%) in both RPL and non-RPL group
Gurbuz AGO 2004 N=80 RPL (58unexpained vs.22cause found)	Any age	Conception by any means	FSH (≥10IU/L) E2 (≥50pg/ml) FSH/LH= ≥3.6	High FSH/LH ratio and high E2 levels in unexplained RPL (1st TM)
Trout FnS 2000 N-57 RPL (36 unexplained vs. 21 cause found)			FSH (≥10IU/L) E2 (≥50pg/ml)	High FSH & E2 level in unexplained RPL 31%vs.5% with high FSH 39%vs.14% with high E2 58%vs.19% with high FSH&/or high E2

Author	Study population	Protocol	ORT	RESULTS
Elter GE 2005 (Retrospective) N=62 pregnant (28misc vs.34 ongoing)	Age <45; FSH <15 IU/L; Excluded:PCOS/ ut. Anomaly/ habitual abortion	long protocol D3 (ET with up to 4 emb)	Age FSH E2 AFC (DR) of 2-10	AFC differs betn pregnant and miscarriage (12 wks) 6.8+2.4 vs. 8.8+3.6 (P=.02) At AFC cut-off 7.5: AUC 0.65; OR 4.2 Similar age/FSH/E2/BMI
Haadsma HR 2009 (Prospective) N=305 72 miscarriage 233 ongoing pr	Subfertility popn Ovulatory Excluded if bilateral tubal pathology	Expectant IUI with or with out stim IVF	FSH InhibinB CCCT AFC (2-6mm)	Similar ORTresults between the miscarriage and ongoing preg group (1st pregnancy followed up)

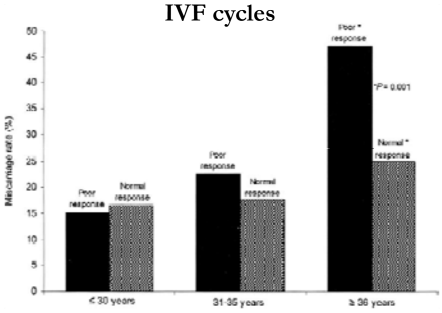
Table I Patient characteristics and results of ovarian reserve tests according to pregnancy outcome

	Ongoing pregnancy (n = 233)		Miscarriage (n = 72)		P-value
	Median (*No.)	10th–90th percentiles (*%)	Median (*No.)	10th–90th percentiles (*%)	
Results of ovarian reserve tests					
Antral follicle count (n)	11	5–23	11	5–22	0.06
Basal FSH (IU/l) (bFSH)	6.3	4.5–9.6	6.3	4.6–10.1	0.71
Stimulated FSH (IU/l) (sFSH)	6.2	3.9–10.7	6.5	4.2–9.7	0.56
CCCT (bFSH + sFSH)(IU/l)	12.9	9.0–19.6	12.8	9.1–19.6	0.71
Basal inhibin B (ng/l)	89.0	40.1–144.9	79.0	30.0–132.0	0.14
Stimulated inhibin B (ng/l)	230.0	98.0–144.9	238.5	113.2–416.8	0.98

Author	Study population	Protocol	ORT	RESULTS
Tremellen ANJOG 2010 (Retrospective) N=244 IUI	Age 18-43	Stimulated (Gn) IUI	AMH (4 quartiles)	Similar miscarriage (No FH on 1st TM US) rates across 4 AMH quartiles
Lekamge RBM 2007 (Retrospective) N=126 IVF	Age ? FSH ≤10IU/L PCOS excluded	Long DR protocol	FSH AMH AFC (2-5mm)	Miscarriage (?definition) high (33.3% vs 4.5%) in the low AMH (<14pmol/L) group. AMH may predict oocyte quality

Author	Study population	Protocol	ORT	RESULTS
Nasseri FnS1999 N=78 (34normal/44 aneuploid)	Karyotype of miscarriage			Greater proportion of abnormal karyotype had elevated FSH in women
Massie FnS 2008 (Retrosp) N=177 miscarriages	Karyotypes of 1st TM miscarriages done	53%IVF 23%IUI 24%natural	FSH	High FSH not predictive of aneuplody (70 euploid, 107 aneuploid)
vanMontfrans HR2001 118 cases with DS/ 102 controls	Age <41		FSH (11.5 IU/L cut-off level)	High mean FSH levels in DS mothers (6.9iu/l vs. 6.3) Higher proportion (14% vs. 5% with high FSH levels
Van der Stroom FnS 2011	Same population as in vanMontfrans HR2001	1998 study group. FU in 2009	AMH (0.5 mcg/l cut-off) Menopause	Similar AMH levels (2.3vs 2.6 mcg/l) High proportion of DS (12% vs.4%) with low AMH Similar menopausal status (15%vs13%) and age of menopause (47vs45yr)

Miscarriage and poor response
IVF cycles



Haadsma *et al.,* RBM online 2010 (N=1825; ongoing pregnancy=1468 & miscarriage = 357
(pregnancy loss betwee 4 and 16 wks)

Miscarriage and poor response
IVF cycles

Table 3 Odds ratios for miscarriage associated with poor response according to age category.

	n (%)	Odds ratio	95% CI	P-value
≤30 years	640 (35.5)	0.9	0.3–2.4	NS
31–35 years	826 (45.8)	1.4	0.7–2.7	NS
≥36 years	339 (18.8)	2.7	1.5–4.9	0.002
All ages	1805 (100)	1.9	1.3–2.8	0.001

CI, confidence interval; NS, not statistically significant.

Table 4 Odds ratios for miscarriage associated with number of retrieved oocytes.

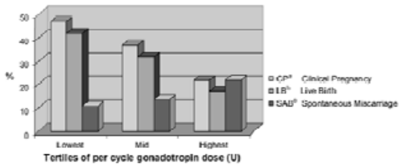
	n (%)	Odds ratio	95% CI	P-value
1–2 oocytes	52 (2.9)	2.6	1.4–4.6	0.001
3 oocytes	85 (4.7)	1.6	0.9–2.6	NS
4 oocytes	121 (6.7)	1.2	0.7–1.8	NS
≥5 oocytes (reference)	1547 (85.7)	1	–	–

Chi-squared test showed a statistically significant trend of increasing miscarriage risk with a lower category of oocyte number ($P = 0.004$).

Haadsma *et al.,* RBM online 2010 (N=1825; ongoing pregnancy=1468 & miscarriage = 357

Miscarriage and Gonadotrophin dose

Worsening of IVF outcomes is notable from the lowest to the highest gonadotropin dose tertile.



Gonadotropin dose (Units) per cycle (Mean ± SD)		Kruskall Wallis Rank Sum Test
Lowest tertile:	1931.76 ± 607.62	[a]p<0.001
Mid tertile:	3276.58 ± 392.71	[b]p<0.001
Highest tertile:	5550.01 ± 1308.34	[c]p=0.209

Pal *et al.*, FnS 2008 (N=806 IVF cycles

Miscarriage and NGF

Table 1. Miscarriage and parity in women with NGF above or below the 50th centile for their age

All women	n	Women < 50°	Women > 50°	p
n	75	25	50	Ns
Total number of pregnancies	222	64	158	Ns
Pregnancies ended in miscarriage, n (%)	64 (28.8)	17 (26.56)	47 (29.7)	Ns
Women with at least one miscarriage, n(%)	34 (45.3)	15 (60)	19 (38)	Ns
Miscarriage per pregnant woman, n	0.85	0.68	0.94	Ns
Number of deliveries,n	158	47	111	Ns
Mean number of deliveries per woman, n	2.1	1.88	2.22	Ns
Women > 41 years				
n	40	14	26	Ns
Total number of pregnancies	124	40	84	Ns
Pregnancies ended in miscarriage, n(%)	32 (25.8)	9 (22.5)	23 (27.3)	Ns
Women with at least one miscarriage, n(%)	19 (47.5)	8 (57.1)	11 (42.3)	Ns
Miscarriages per pregnant woman, n	0.8	0.64	0.88	Ns
Number of deliveries, n	95	34	61	Ns
Mean number of deliveries per woman, n	2.37	2.42	2.34	Ns

Ns, not significant.

LaMarca *et al.*, Gynae Endocrine 2011

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Do ovarian reserve tests predict miscarriage in women undergoing assisted reproduction treatment?

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Objective

To evaluate the role of Ovarian Reserve Tests for the prediction of miscarriage among ART pregnancies

Methodology

Design: prospective observational

Participants (n=978 subjects-320 pregnant):

- ✓ first cycle of IVF/ ICSI
- ✓ age <43 yrs; FSH ≤12 IU/L
- ✓ regular menstrual cycles (21 – 35 days)
- × PCOS/ Ovarian pathology on scan
- × Congenital/ acquired uterine pathology
- × Ectopic pregnancy

Early follicular phase assessment:

- ✓ TVS scan (AFC)
- ✓ Venepuncture (FSH/ E2/ FSH stimulation test)

Methodology

Treatment protocol:

- ✓ Long down-regulation using GnRH agonists
- ✓ HMG for ovarian stimulation (150-300 IU)

Main outcome measures:

- ✓ Miscarriage (pregnancy loss at ≤12 wks)

Statistical analysis:

- ✓ Mann-Whitney U test/ Chi-square test
- ✓ Regression analysis/ ROC curve analysis

n = 314

Results

Variables	Miscarriage (n=67)	Ongoing pregnancy (n=247)	P value
Age (years)	35.2 ± 4.5	34.3 ± 4	0.07
BMI (Kg/m^2)	24.6 ± 3.3	23.4 ± 2.9	<0.01
Basal FSH level (IU/L)	7.2 ± 1.7	6.9 ± 1.6	0.46
Basal oestradiol (pmol/L)	158.2 ± 49.7	162.7 ± 62.5	0.99
Delta oestradiol (FSH stimulation test)	311.5 ± 181.1	331.9 ± 201.8	0.51
Total AFC	10.9 ± 3.5	11.8 ± 3.3	< 0.05
Total gonadotrophins used (IU)	3404 ± 1255	3425 ± 1277	0.90
The number of oocytes collected	11.4 ± 3.6	12.1 ± 4.8	0.46
Fertilization rates	63.6 ± 21.5	65.5 ± 19.7	0.75
Subjects who had two embryos transferred	64 (95.5%)	234 (94.7%))	0.8
Twin pregnancy rates	20 (29.9%)	42 (17%)	<0.05

n = 314

Results

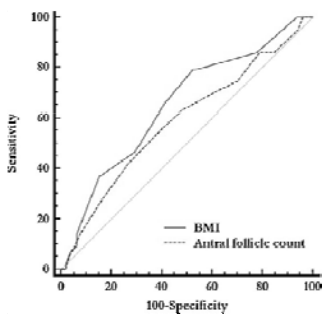
Logistic regression and ROC curve: Prediction of miscarriage

Parameters	Odds Ratio	95% CI	P-value	AUC
Age	1.059	0.988–1.135	0.11	0.571
Body mass index	1.132	1.040–1.232	<0.01	0.617
Basal FSH level	1.106	0.942–1.298	0.22	0.532
Basal oestradiol (pmol/L)	0.999	0.994–1.003	0.58	0.501
Delta oestradiol (FSH stimulation test)	0.999	0.998–1.001	0.45	0.526
Total AFC	0.917	0.843–0.9984	<0.05	0.588

n = 314

Results

ROC curve: Prediction of miscarriage



n = 314

Results

ROC curve: Prediction of miscarriage

Variables/ Cut-off levels	Sensitivity	Specificity	+LR	Post-test probability
AFC ≤12	0.68	0.40	1.2	24.5%
BMI ≥26 Kg/M²	0.82	0.37	2.1	36.2%
Combined test (AFC ≤12 & BMI ≥26 Kg/M²)	0.92	0.22	2.6	41.3%

+LR: positive likelihood ratio
The shift from pre-test probability (21.3%) to post-test probability of poor response is shown

Prediction of miscarriage: Summary

- ✓ Miscarriage is more common in women having increased BMI and reduced OR (as measured by AFC)
- ✓ ORTs (AFC) may be significantly predictive of miscarriage
- ✓ However, the clinical application of ORTs to predict miscarriage is limited as indicated by its low discriminative ability

The University of Nottingham

Nurture Fertility

Are Ovarian Reserve Tests predictive of miscarriage in women undergoing ART?

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Ovarian reserve and early pregnancy

The clinical relation between
oocyte quantity and oocyte quality

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No conflicts of interest to disclose

Learning objective

What is the current knowledge
on the relation between ovarian reserve and
miscarriage or trisomic pregnancy?

Content of the presentation

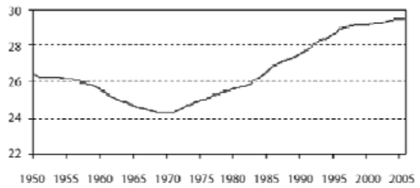
1. Introduction on female reproductive ageing

2. Relation between ovarian reserve and miscarriage

   Relation between ovarian reserve and trisomic pregnancy

3. General conclusions and future research

Mean age of the mother at the birth of her first child in the Netherlands



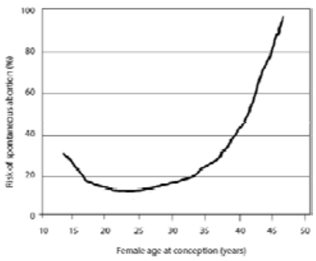
Mean maternal age at first child birth (Y-axis) during the last decades (X-axis)

Source: CBS, Dutch National Institute for statistics

Reproductive ageing

Reproductive ageing:
- ↓ chance to conceive
- ↑ risk of miscarriage

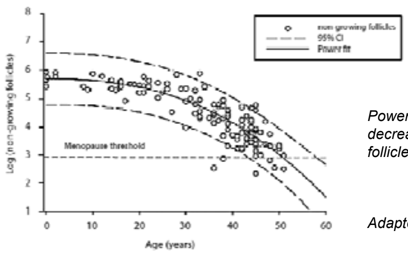
Attributed to:
- ↓ oocyte *quantity*
- ↓ oocyte *quality*



↑ Risk of spontaneous abortion with female age
Adapted from Nybo Andersen et al.

Oocyte quantity

- The number of oocytes decreases with age
- This process eventually leads to menopause

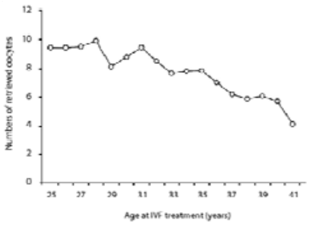


Power model for the decrease in non-growing follicles with age

Adapted from Hansen et al.

Clinical parameters for oocyte quantity

1. Ovarian reserve tests
2. Response to ovarian hyperstimulation
3. Ovarian surgery
4. Age at menopause



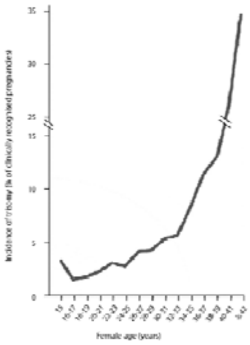
Decrease in the mean number of retrieved oocytes with age at IVF-treatment

Adapted from De Boer et al.

Oocyte quality

↑ Aneuploid oocytes with age
- ↑ risk of miscarriage
- ↑ risk of trisomic pregnancy

No non-invasive clinical tests available

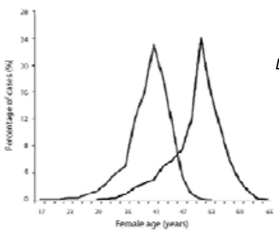


Increased incidence of trisomy in clinically recognized pregnancies with female age

Adapted from Hassold and Hunt

Are oocyte quantity and quality related?

- Parallel decline in oocyte quantity and oocyte quantity
- Resembling distribution curves for maternal age at last child birth and age at menopause



Distribution curves for the observed age at last child birth (left-hand curve) and age at menopause (right-hand curve)

Adapted from Lambalk et al.

Are oocyte quantity and quality related?

If so, the number of remaining oocytes
has predictive value for their quality

Hypothesis:
The ovarian reserve of a woman
is associated with her risk of
miscarriage and trisomic pregnancy

Overview

1. Ovarian reserve tests
2. Response to hyperstimulation
3. Ovarian surgery
4. Age at menopause

Miscarriage

Trisomic pregnancy

Ovarian reserve tests and miscarriage: findings from our research group

- Prospective cohort study
- 1999-2003
- Two hospitals in Groningen, the Netherlands
- Subfertile couples
- Follow-up of pregnancies and therapy

- Antral follicle count (AFC)
- Basal FSH en inhibin B
- Clomiphene citrate challenge test:
  'Stimulated' FSH en inhibine B values

Inclusion criteria

- Subfertility of ≥ 12 months
- Regular ovulatory cycle
- VCM ≥ 1.000.000
- At least one open Fallopian tube

- 474 couples → 320 achieve a pregnancy (67,5 %)

- Outcome of the first pregnancy during follow-up
  233 (75,1%) Ongoing pregnancy (>16 weeks)
  72 (23,2%) Miscarriage (4-16 weeks)

Table 1 Patient characteristics and results of ovarian reserve tests according to pregnancy outcome

	Ongoing pregnancy (n = 233)		Miscarriage (n = 72)		P-value
	Median (*No.)	10th–90th percentiles (*%)	Median (*No.)	10th–90th percentiles (*%)	
Patient characteristics					
Age (years)	32.4	27.0–38.1	34.2	28.0–39.6	<0.01
Body mass index (kg/m^2)	22.8	19.4–30.1	22.9	19.5–33.2	0.82
Smoking habit*	61	28.4%	16	27.1%	0.85
Duration of subfertility (years)	3.3	1.9–5.8	3.4	1.9–5.4	0.51
Primary subfertility*	162	69.5%	52	72.2%	0.79
Previous miscarriage*	35	15.1%	14	19.4%	0.35
Mean cycle length (days)	28	26–33	28	25–32	0.52
Semen analysis (TMC, ×10^6)	38.6	4.1–179.8	33.6	4.1–205.0	0.85
Diagnostic category of subfertility*					0.76
Unexplained	125	53.6%	35	48.6%	
Male factor	99	42.2%	34	47.2%	
Cervical factor	9	3.9%	3	4.2%	
Time to pregnancy (months)	8.8	1.2–28.4	10.6	0.8–34.4	0.22
Conception after ART*	52	22.3%	27	37.5%	0.01
Results of ovarian reserve tests					
Antral follicle count (n)	11	5–23	11	5–22	0.86
Basal FSH (IU/l) (bFSH)	6.3	4.5–9.6	6.3	4.6–10.1	0.71
Stimulated FSH (IU/l) (sFSH)	6.2	3.9–10.7	6.5	4.2–9.7	0.56
CCCT (bFSH + sFSH)(IU/l)	12.9	9.0–19.6	12.8	9.1–19.6	0.71
Basal inhibin B (ng/l)	89.0	45.1–144.9	79.0	30.0–132.0	0.14
Stimulated inhibin B (ng/l)	232.0	98.0–144.9	238.5	113.2–416.8	0.98

Ovarian reserve tests and miscarriage: findings in literature

- Multiple studies available, some presented in this session
  Abdalla et al, Elter et al, Lekamge et al, Levi et al, Luna et al, Van Montfrans et al (2004), Sahu et al, etc.

- Differences in study populations, sample size, ovarian reserve tests, cut-off values, outcome measures…

- Conflicting results → at least no clear-cut predictive value of ovarian reserve tests for miscarriage

Ovarian reserve tests and trisomic pregnancy: findings in literature

- ↑ levels of FSH in women with a trisomic pregnancy
  Kline et al (2011), Van Montfrans et al (1999).
- AMH and AFC are not related with trisomic pregnancy
  Kline et al (2011), Li et al, Plante et al,Seifer et al
- However, possibly very low AMH levels are associated with trisomic pregnancy – *Van der Stroom et al*

Overview

1. Ovarian reserve tests
2. **Response to hyperstimulation**
3. Ovarian surgery
4. Age at menopause

→ Miscarriage

→ Trisomic pregnancy

Ovarian response and miscarriage: findings from our research group

OMEGA project:

- National Dutch IVF-cohort from1983-1995
- Total cohort size: N=19,840
- Questionnaire in 1997-1998: response rate 73%
- Medical files: data abstracted for 75%
- Relation ovarian reserve and miscarriage
- Relation ovarian reserve and trisomic pregnancy

Ovarian response and miscarriage: findings from our research group

- Outcome of the first completed IVF-treatment
- Women with a miscarriage (4-16 weeks) vs. women with an ongoing pregnancy (>16 weken)
- Parameter of ovarian reserve: Poor response to ovarian hyperstimulation (<4 oocytes)

Basal characteristics

	Ongoing pregnancy N=1468 % or median	Miscarriage N=357 % or median	*P*-value
Poor response	6.6%	11.7%	0.001
Age	32.4 years	33.4 years	<0.001
Body mass index	21.7 kg/m²	21.6 kg/m²	0.76
Smoking	36.4%	43.8%	0.01
Primary subfertility	66.3%	59.9%	0.03
Duration subfertility	4.5 years	4.8 years	0.04
Ovarian surgery	9.2%	12.3%	0.08

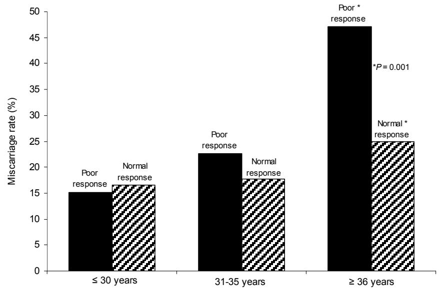
Interaction with female age

↑ Relation between poor response and miscarriage with ↑ age

	N (%)	Odds ratio	95% CI	*P*-value
≤ 30 years	640 (35.5%)	0.9	0.3 – 2.4	N.S.
31-35 years	826 (45.8%)	1.4	0.7 – 2.7	N.S.
≥ 36 years	339 (18.8%)	2.7	1.5 – 4.9	0.002
All ages	1805 (100%)	1.9	1.3 – 2.8	0.001

Interaction with female age

↑ Relation between poor response and miscarriage with ↑ age



Discussion

- Why does the relation between poor response and miscarriage ↑ with ↑ age?
  - Smaller chance of a 'coincidental' poor response?
  - ↑ of biological damage over time?
- These results support the hypothesis that oocyte quantity and quality **are** related, but
  - Poor responders: no possibility for embryo selection
  - Poor responders: fewer multiple pregnancies
- Two other large retrospective studies found no relation between a poor response **(defined as <5 oocytes)** and miscarriage (*Kumbak et al, De Sutter et al)*

Ovarian response en trisomic pregnancy: findings from our research group

- 28 cases — N=24 with trisomy 21; N=3 with trisomy 18; N=1 with trisomy 13
- Selection of 5 controls per case
- Controls are women with a live born child without a trisomy
- Matched for:
  - Age at IVF treatment
  - Mode of conception
  - Center of IVF treatment
  - Year of IVF treatment

Results

	Cases N = 28	Controls N = 140	OR for trisomic pregnancy	*P*-value
No. of oocytes				
1-4	9 (32.2%)	17 (12.1%)	3.7 (1.2 – 11.7)	0.03
5-8	8 (28.6%)	57 (40.7%)	0.9 (0.3 – 2.3)	0.76
≥ 9	11 (39.3%)	66 (47.1%)	1.0 (ref)	-
Poor response				
Yes (≤ 3 oocytes)	4 (14.3%)	9 (6.4%)	2.7 (0.7 – 10.7)	0.15
No (≥ 4 oocytes)	24 (85.7%)	131 (93.6%)	1.0 (ref)	-

No other studies available on ovarian response and trisomy

Overview

1. Ovarian reserve tests
2. Response to hyperstimulation
3. **Ovarian surgery**
4. Age at menopause

~~Miscarriage~~ — Trisomic pregnancy

No studies available

Results: ovarian surgery and ovarian response

	Cases N = 28	Controls N = 140	OR for trisomic pregnancy	*P*-value
Ovarian surgery				
Yes	5 (17.9%)	7 (5.7%)	3.3 (1.0 – 10.5)	0.04
No	23 (82.1%)	133 (94.3%)	1.0 (ref)	-

These findings are in line with *Freeman et al*:
Mothers of a child with Down syndrome more often had a history of ovarian surgery (7/189 cases vs 1/329 controls)

These findings also correspond to classic mouse studies:
↑ Aneuploid embryos in hemi-ovariectomised mice (*Brook et al*)

Overview

1. Ovarian reserve tests
2. Response to hyperstimulation
3. Ovarian surgery
4. **Age at menopause**

→ ~~Miscarriage~~ → Trisomic pregnancy

No studies available

Trisomic pregnancy and signs of menopause: findings from our research group

'Follow-up' within the previously described case-control study

Data complete: N=72 (43%)

- N=63 Premenopausal
- N=4 Hormonal replacement therapy
- N=5 Irregular cycle

Median age at questionnaire: 42.1 years
Median interval between IVF and questionnaire: 4.1 years

Results: signs of menopause

	Odds ratio	95% CI	P-value
Associated with trisomic pregnancy	5.5	1.2 – 24.9	0.03
Adjusted for - Age at the time of questionnaire - Smoking at the time of questionnaire	5.7	1.1 – 29.9	0.04

Two other studies available (*Kline et al (2000), Bartmann et al*): Women with a history of trisomic pregnancy enter menopause respectively 1.0 and 0.7 years earlier; not statistically significant

Conclusion

These latter results generally support a relation between diminished ovarian reserve and miscarriage and trisomic pregnancy

But…

Limitations

- Small numbers
- Studies not readily comparable
- Mostly IVF-populations
- Limitations of the parameters used

Clinical implications? Biological mechanism?
→ first confirmation of the results!

Current studies

- Collaboration with the fertility clinic of Rigs Hospitalet Copenhagen (professor A. Nyboe Andersen)
- Data from the various Danish registries are available on:
  - Pregnancy outcome (including terminated pregnancies)
  - Karyotype
  - Matching of mothers and their children is possible
  - Indication for hospital admission (ovarian surgery!)
  - Course of IVF treatment
  - …

Research questions

Within the general population

- Relation ovarian surgery and trisomic pregnancy?

Within the IVF-treated population

- Relation ovarian response and miscarriage?
- Relation ovarian response and trisomic pregnancy?

…

Learning objective

What is the current knowledge
on the relation between ovarian reserve and
miscarriage or trisomic pregnancy?

Take home message:
There may well be a relation between ovarian reserve and early pregnancy, but… more studies are needed.

?

Co workers

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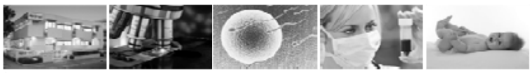
AMH Hormone levels and miscarriage rates after IUI

A/Prof. Kelton Tremellen

Repromed

University of South Australia



University of South Australia

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Commercial Disclosure

Consultancies for the following:

- MSD
- Merck Serono
- Bayer
- Hansen
- Beckman-Coulter (manufacturer of Immunotech and DSL AMH ELISA)

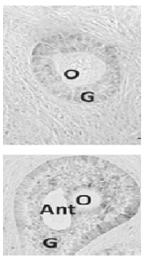
BECKMAN COULTER

AMH Gen II ELISA

repromed 2

AMH physiology- Background

- AMH is a glycoprotein from the TGFβ family. It was originally identified to be produced by the sertoli cells of the testis and cause the regression of the Mullerian ducts in males (*Jost 1946*). Hence AMH's alterative name- Mullerian Inhibiting Substance.
- AMH is produced by the granulosa cells of pre-antral and antral follicles (< 8 mm) in the ovary.



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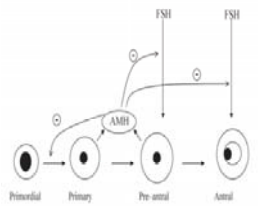
AMH physiology- Background

- The primary function of AMH is to inhibit the transition of primordial follicles into growing follicles.
- An absence of AMH function (gene “knock-out” mice) leads to accelerated primordial follicle recruitment and early onset of menopause- confirming the “oocyte development brake” role of AMH. *(reviewed in La Marca 2010)*



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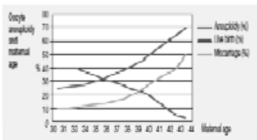
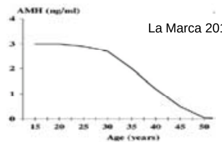
Why might serum AMH be linked to oocyte quality?

1. The decline in live-birth rates with advancing maternal age (primarily due to an increase in oocyte aneuploidy) mirrors the drop in serum AMH seen with advancing age.
2. As AMH is produced by the ovary and is known to play a role in oocyte physiology- it is possible to conclude that serum AMH levels <u>may</u> give a useful non-invasive insight into oocyte quality.





La Marca 2010

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Why might serum AMH be linked to oocyte quality?

3. The “bottom of the barrel” hypothesis of oocyte quality suggests the best quality oocytes ovulate first, leaving only the poor quality oocytes left at the end.

   As AMH is an excellent measure of quantitative ovarian reserve, it may therefore correlate with oocyte quality.

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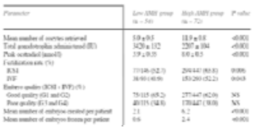
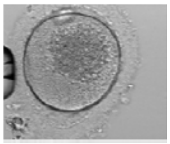
AMH and oocyte quality- lessons from IVF?

- A retrospective study suggested that oocyte fertilization rates (routine insemination and ICSI) are compromised in oocytes coming from women with low serum AMH. *(Lekamge and Tremellen 2007).*
- Low serum AMH has been linked with poor morphology oocytes (dark granular cytoplasm, aggregation SERs) in IVF cycles *(Ebner 2006).*



Parameter	Low AMH group (n = 54)	High AMH group (n = 72)	P value
Mean number of oocytes retrieved	5.0 ± 0.5	11.9 ± 0.8	<0.001
Total gonadotrophin administered (IU)	3420 ± 132	2207 ± 104	<0.001
Peak oestradiol (nmol/l)	3.9 ± 0.35	8.0 ± 0.5	<0.001
Fertilization rate (%)			
ICSI	77/146 (52.7)	294/447 (65.8)	0.006
IVF	38/90 (40.9)	153/293 (52.2)	0.043
Embryo quality (ICSI + IVF) (%)			
Good quality (G1 and G2)	75/115 (65.2)	277/447 (62.0)	NS
Poor quality (G3 and G4)	40/115 (34.8)	170/447 (38.0)	NS
Mean number of embryos created per patient	2.1	6.2	<0.001
Mean number of embryos frozen per patient	0.6	2.4	<0.001



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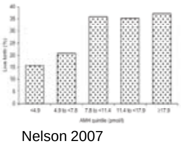
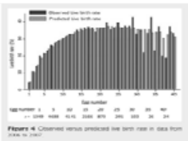
AMH and the prediction of live-birth in IVF- The Oocyte number confounder

- In a prospective study *Nelson (2007)* observed a significant correlation between serum AMH and cumulative LB rates (fresh and frozen embryo transfer of all embryos from 1 stimulated cycle). *However, AMH was not an independent predictor of LB when accounting for oocyte yields in regression analysis.*
- A retrospective study *(Lekamge and Tremellen 2007)* found AMH to only predict LB rates if all fresh and frozen transfers of embryos from 1 stimulated cycle were included in the analysis, not just fresh transfers.
- Therefore, it appears that in IVF the number of oocytes retrieved seems to be the primary confounder in the ability of serum AMH to predict LB rates.



Nelson 2007



Sunkara 2011

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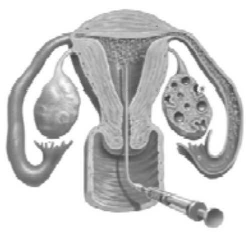
Low dose stimulation IUI as a model to test the ability of serum AMH to predict oocyte quality in the setting of *in vivo* conception (*Tremellen and Kolo 2010)*

IUI removes many of the non-oocyte quality related issues related to *in vivo* conception:

- confirmed tubal patency
- Ovular and no intercourse "timing" issues.
- No major semen defect issues
- No *in vitro* manipulation of the embryo, nor ability to select ideal embryos for transfer
- IUI is generally mono-ovulation in our clinic (similar to natural ovulation)



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IUI study protocol

- Retrospective study of 244 women undergoing 477 cycles of IUI (average 1.96 cycles per woman, mean age 33 years, mean number of motile sperm inseminated per IUI = 135 million).
- Indications for IUI treatment were male factor (35.5%), idiopathic (28.7%), anovulatory (17.2%), and combined infertility(14.8%).
- Low dose stimulation protocol (50 IU rFSH) with late start (Day 5) aiming for only 1-2 mature follicles.
- hCG "trigger" (5000 IU) and luteal support (1500 IU x 2).

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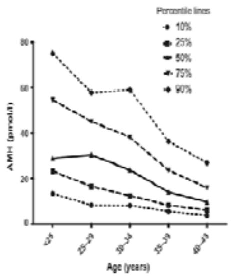
Development of AMH Quartile ranges for the purpose of ovarian reserve classification

- The study screened 1032 women aged 18-43 years undergoing infertility assessment with serum AMH measurements.
- We developed percentile charts using this data and then divided the IUI study participants into 4 groups corresponding to their respective age related AMH quartile.
- *Therefore, ovarian reserve status was classified comparing an individuals status to their age related peers, not just "raw" serum AMH measurements.*



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Serum AMH in successful v unsuccessful IUI cycles

Table 1 Characteristics of women undergoing IUI treatment in relation to their live birth outcome

	Live birth	No live birth	*P*-value
Maternal age (years)	32.1 ± 4.7	33.5 ± 5.1	0.076
BMI	26.9 ± 6.5	26.6 ± 6.2	0.79
Serum AMH (pmol/l)	35.4 ± 31.8	31.1 ± 28.0	0.30
Antral follicle count (2–5 mm)	14.6 ± 7.6	14.1 ± 8.8	0.53
Number mature follicles at insemination	1.31 ± 0.50	1.28 ± 0.46	0.84
Total motile sperm count in neat insemination sample ($\times 10^6$)	139.6 ± 181.3	127.5 ± 118.1	0.70

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Ovarian Reserve and IUI response in the AMH Quartile groups

Table 2 IUI treatment outcomes according to serum AMH assessed ovarian reserve status

AMH quartile (ovarian reserve status)	Serum AMH (pmol/L)	Antral follicle count (2–5 mm)	No. cycles IUI completed	Total no. mature follicles produced	Mean no. mature follicles per cycle IUI
Q1 (low ovarian reserve)	6.6 ± 4.3	5.4 ± 4.7	98	115	1.17 ± 0.4
Q2 (normal ovarian reserve)	16.8 ± 6.2	11.6 ± 5.1	117	151	1.29 ± 0.5
Q3 (normal ovarian reserve)	25.3 ± 9.6	14.6 ± 7.3	106	139	1.31 ± 0.5
Q4 (high ovarian reserve/PCOS)	62.6 ± 28.9	21.3 ± 7.6	156	209	1.34 ± 0.5
P-value	<0.0001	<0.0001	–	–	0.38

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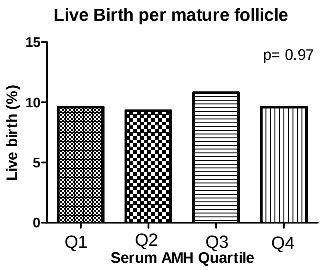
Live Birth rate in IUI treatment per mature follicle produced



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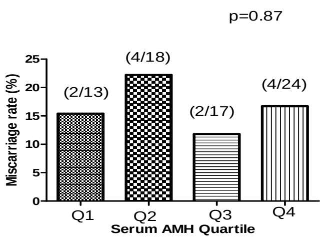
Miscarriage Rate per Clinical Pregnancy



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Are our results consistent with others?

There have been two similar studies conducted in France and Hong Kong analysing the ability of serum AMH to predict oocyte quality/ clinical pregnancy in IUI setting

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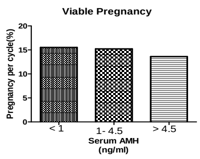
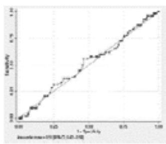
French IUI study

(Lamazou et al. J Gynecol Obstet Biol Reprod (Paris) in press.)

- 316 patients less than 39 years of age undergoing their first cycle of IUI.
- Patients were divided into three AMH groups (< 1 ng/ml, 1-4.5 ng/ml, > 4.5 ng/ml).
- No statistical difference was observed in the number of mature follicles, clinical pregnancy rates or spontaneous abortion rate.
- ROC analysis revealed AMH to have no ability to predict on going viable pregnancy.





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HK IUI study *(Li et al, Fert Steril 2010)*

- Retrospective study of 243 women (median age 35 years) undergoing IUI mainly for male factor (45.3%) and idiopathic (23.5%) infertility of duration between 1-3 years.
- High dose stimulation using a starting dose of 150 IU rFSH in most patients (75-100 IU in PCOS group).
- When analysing the cumulative chance of live birth (LB) in the first cycle of IUI or up to 3 cycles of IUI, serum AMH was significantly higher in LB group.
- A potential confounder is that outcomes were analysed per IUI cycle, not mature follicle. The LB group had more mature follicles (2 v 1) and higher E2 (3422 v 2541 pmol/l) at trigger than no LB group (not statistically significant though).

TABLE 1

Comparison of demographic and clinical characteristics of subjects in relation to live birth outcome in the first treatment cycle.

Demographic/clinical parameter	Live birth: Yes	Live birth: No	P value
Age (y)	35 (32–37)	35 (33–37)	[illegible]
Body mass index (kg/m^2)	21.2 (19.6–22.7)	20.5 (19.4–22.2)	.597
Previous live birth within the same relationship, n (%)			.625
Yes	2 (7.1)	8 (4.4)	
No	26 (92.9)	175 (95.6)	
Cause of subfertility, n (%)			.116
Male factor	49 (44.5)	34 (43.6)	
Unexplained	31 (28.2)	14 (17.7)	
Anovulation	7 (6.4)	12 (15.2)	
Other/mixed	23 (20.9)	19 (24.1)	
Serum AMH (pmol/L, ng/mL)	27.1 (15.8–48.4), 3.80 (2.21–6.78)	15.5 (7.8–27.1), 2.17 (1.09–3.80)	.002
Serum FSH (IU/L)	7.3 (6.1–8.3)	7.9 (6.8–9.6)	.036
Antral follicle count	13 (7–16)	9 (6–14)	.009
Serum E_2 on day of hCG trigger (pmol/L)	3,442 (1,502–5,583)	2,541 (1,745–3,593)	.330
Total dose of gonadotropin administered (IU)	1,069 (763–1,275)	975 (750–1,200)	.352
Duration of stimulation (d)	9 (8–13)	8 (7–10)	.017
No. of follicles ≥ 16 mm	2 (1–2)	1 (1–2)	.523
Total normal motile sperm count post-wash (millions)	10.5 (4.3–16.8)	11.3 (3.8–32.8)	.496

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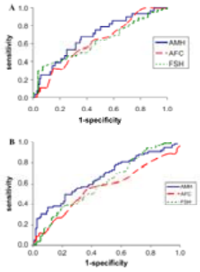
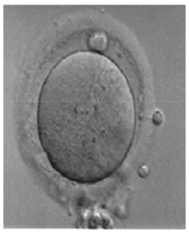
HK IUI Study outcomes

- ROC analysis suggested that serum AMH has a moderate but significant ability to predict LB in either the first cycle of IUI (Graph A, AUC 0.682) or over 3 cycles of IUI (Graph B, AUC 0.668).



AMH and oocyte quality

- The Australian and French IUI studies suggest that serum AMH does not predict oocyte quality (miscarriage rates, live birth rates) when analysed either as "raw" serum AMH values or AMH percentiles.
- The HK IUI study does suggest serum AMH may have a moderate ability to predict live birth, but it is possibly biased by the confounder of higher mature follicle responses in high AMH patients.



Serum AMH a marker of *in vivo* oocyte quality- summary

- Overall, the majority of IUI data suggests that serum AMH is not a useful marker of oocyte quality if comparing outcomes on a per mature oocyte generated during stimulation.
- While serum AMH is an excellent marker of quantitative ovarian reserve, the data from IUI do not support its use as a marker of oocyte quality.
- 6 prospective studies are presently being proposed to analyse serum AMHs ability to predict successful natural conception- these should answer the quality v quantity debate with certainty.

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Anti-Müllerian hormone levels in women with recurrent miscarriage and their value in predicting another miscarriage

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Conflict of interest

- I hereby confirm that I do not have any commercial and financial relationships related to this presentation and its contents

Learning Objectives

- A introduction to the condition Recurrent Miscarriage
- Maternal age and Recurrent Miscarriage
- Ovarian reserve and Recurrent Miscarriage
  - FSH
  - Estradiol
  - AMH
- AMH as a predictor of another miscarriage

Recurrent Micarriage

- Definition (ESHRE):
  - Three or more consecutive miscarriages before 20 weeks of gestation
  - 1% of fertile couples experience recurrent early pregnancy losses

Jauniaux E et al, Hum Reprod 2006

4 ESHRE 2012 Precongress course 14

Subgroups of Recurrent miscarriage

- Primary recurrent miscarriage group
  - Three or more abortions and no preceeding deliveries (livebirths nor stillbirths)
- Secondary recurrent miscarriage group
  - Three or more abortions after a delivery regardless of the outcome (liveborn or stillborn)
- *(Primary late recurrent miscarriage group)*
  - *Two or more second-trimester losses*

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Important predictors of another miscarriage

- Advanced maternal age
- Number of previous successive miscarriages

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Causes of Recurrent miscarriage

- Often the cause remains unexplained
  - Idiopathic recurrent miscarriage
- Often several risk factors in the same patient
  - Multifactorial disorder…..

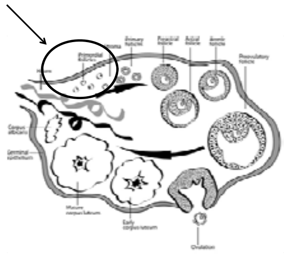
Well known risk factors of RM

- Structural uterine anomalies
- Parentel chromosomal abnormalities
- Maternal autoimmune disorders
- Maternal defects in coagulation factors
- Endocrine dysfunction
- Obesity

But what about ovarian reserve and recurrent miscarriage?

Ovarian reserve - definition

- *Quantity* and *Quality* of the existing pool of primordial follicles in the ovaries.

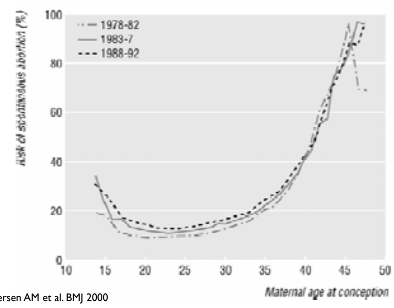


Quantity and Quality

Ovarian reserve declines with increasing age

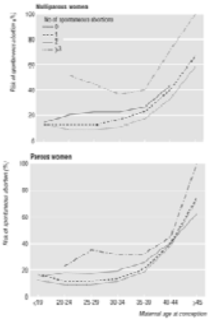
1. Decline in number of primordial follicles
2. Increase in number of poor quality oocytes

Risk of *sporadic* spontaneous abortion in relation to maternal age



Nybo Andersen AM et al. BMJ 2000

Risk of *recurrent* spontaneous abortion in relation to maternal age



13 Nybo Andersen AM et al. BMJ 2000 ESHRE 2012 Precongress course 14

Impact of age on *Recurrent* miscarriage rate:

- Register-based study:
  - 30-34 yr: 38-40%
  - 35-39 yr: 38-40%
  - 40-44 yr: 70%
- Multivariate analyses women < 40 yr:
  - Maternal age alone not a significant predictor of another miscarriage after adjustment for relevant independant variables

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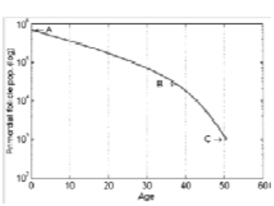
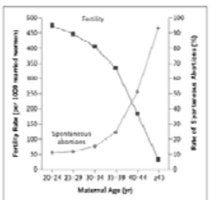
Connection between age, ovarian reserve and risk of miscarriage





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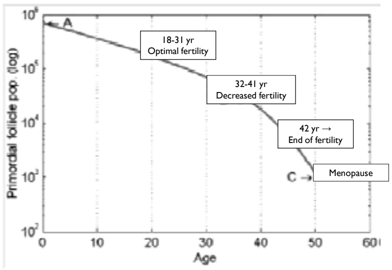
The rate of decline in ovarian reserve is unique in each woman



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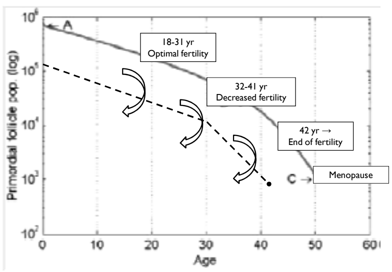
The rate of decline in ovarian reserve is unique in each woman



Challenge: When ovarian age is higher than chronological age….

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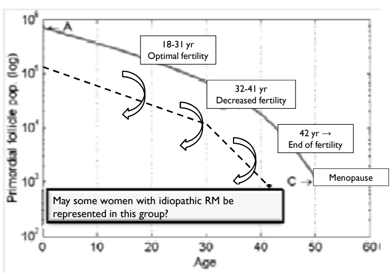
The rate of decline in ovarian reserve is unique in each woman



Challenge: When ovarian age is higher than chronological age….

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Evaluation of ovarian reserve

- (Chronological age – poor predictor)
- FSH
- Estradiol — Changes occur late in reproductive life
- Inhibin B
- Cycle length – good predictor
  - But a bit difficult to deal with
- Antral Follicle count – early and good predictor
  - But requires ultrasound equipment
- ***Anti-Mullerian hormone (AMH) – early and good predictor***
  - ***Only a blood sample – cycleday independant***

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Recurrent Miscarriage, basal FSH and Estradiol 3 studies.....

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1.


RECURRENT PREGNANCY LOSS

Recurrent pregnancy loss and diminished ovarian reserve

- Retrospective study
  - Routine fertility evaluation
- 44 women with RM and 648 without RM but infertile
- Intervention:
  - FSH measured on cycle day 3
  - Estradiol measured on cycle day 3
  - Cycle length
  - Clomiphene citrate challenge test

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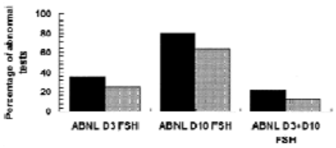
Results

TABLE 1

Comparison of demographic and CCCT hormonal data between +RPL and −RPL women.

Parameter	Group +RPL (n = 44)	−RPL (n = 640)	P value	Confidence interval
Age (y)	33 ± 4.6	35 ± 4.3	0.04	28.61–31.8
Gravity	4 ± 1	1 ± 1	0.0001	2.6–3.1
Cycle length (d)	29 ± 6	28 ± 5.6	0.13	−2.4–1.45
Day 3 FSH (mIU/mL)[a]	8.9 ± 7	11 ± 8	0.01	−4–.77
Day 3 E_2 (pg/mL)[b]	33 ± 19	36 ± 25	0.22	−5.7–7.5
Day 10 FSH (mIU/mL)[a]	11 ± 8	12 ± 11	0.16	−2.3–6.1

Results:
Gray bar (+RM) and Black bar (-RM)



- No differences in the incidence of abnormal day 3 FSH or day 10 FSH
- CCCT-results: 18% abnormal in both groups
  - +RM and abnormal CCCT
    - Delivery rate in the following year 0%
  - -RM and abnormal CCCT
    - Delivery rate in the following year 4.2%

2. **Do women with unexplained recurrent pregnancy loss have higher day 3 serum FSH and estradiol values?**

Susan W. Trout, M.D., and David B. Seifer, M.D.

Division of Reproductive Endocrinology and Infertility, UMDNJ-Robert Wood Johnson Medical School, New Brunswick, New Jersey

- Retrospective study
- 36 women with unexplained RM and 21 women with a known cause of RM
  - No difference in age and number of miscarriages
- Intervention
  - FSH cycle day 3
  - Estradiol cycle day 3

Results

TABLE 1

Elevated day 3 serum levels of FSH and E_2 in women with unexplained recurrent pregnancy loss and those with explained recurrent pregnancy loss.

Variable	Women with unexplained RPL (n = 36)	Women with explained RPL (controls) (n = 21)	P value
No. (%) with day 3 FSH level >10 mIU/mL	11 (21%)	1 (5%)	<.02
No. (%) with day 3 E_2 level >50 pg/mL	14 (39%)	3 (14%)	.05
No. (%) with day 3 E_2 level >50 pg/mL and/or day 3 FSH level >10 mIU/mL	21 (58%)	4 (19%)	<.001

Note: RPL = recurrent pregnancy loss.

- Unexplained RM:
  - 36 yr (+/- 5)
  - 58% had elevated levels of both day 3 estradiol and FSH
- Explained RM
  - 35 yr (+/- 5)
  - 19% had elevated levels of both day 3 estradiol and FSH

3.

Arch Gynecol Obstet (2004) 270:37–39
DOI 10.1007/s00404-003-0480-0

ORIGINAL ARTICLE

Birgül Gürbüz · Serap Yalti · Selcuk Ozden · Cem Ficicioglu

High basal estradiol level and FSH/LH ratio in unexplained recurrent pregnancy loss

- Retrospective study
- 58 women with unexplained RM, 22 women with explained RM *(uterine, chromosomal, anti-phospholipid syndrome)*, and 27 controls with no miscarriages
  - No differences in age and number of miscarriages (explained vs. unexplained)
- Intervention:
  - FSH cycle day 3
  - Estradiol cycle day 3
  - FSH/LH cycle day 3

Results

Table 2 Serum hormone levels and distribution of the cases according to different FSH, LH and E2 levels between explained and unexplained recurrent pregnancy loss groups

	Controls n=27	Unexplained n=58	p value[c]	Explained n=22	p value[d]
E_2[a] (pg/ml)	43.10±19.20	63.24±45.56	0.007	42.99±18.13	ns
FSH[a] (mIU/ml)	6.32±3.22	8.29±2.99	0.008	6.59±2.71	ns
LH[a] (mIU/ml)	3.57±1.50	4.60±2.04	0.001	3.58±1.64	ns
FSH>10 mIU/ml[b]	4 (14.8%)	20 (34.5%)	ns	3 (13.6%)	ns
E_2>50 mIU/ml[b]	5 (18.5%)	25 (43.1%)	0.008	4 (18.2%)	ns
FSH>10 mIU/ml and/or E_2>50 mIU/ml	5 (18.5%)	31 (53.4%)	0.004	4 (18.2%)	ns
FSH/LH≥3.6 mIU/ml[b]	7 (25.9%)	28(48.5%)	0.013	5 (25.8%)	ns

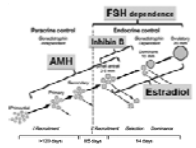
[a] Mean ± SD. [b] n, %. [c] Unexplained vs. controls. [d] Explained vs. controls

- Unexplained RM vs. Controls:
  - Nearly all ovarian reserve parameters significantly and negatively affected
- Explained RM vs. Controls:
  - No significant differences in any of the ovarian reserve parameters

To conclude: basal FSH and estradiol

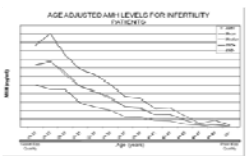
- Retrospective studies 2000-2004
- Small numbers
- *However:*
- Consistent results all concluding:
  - Young women with unexplained RM may have a diminished ovarian reserve as assessed with cycleday 3 FSH and estradiol.

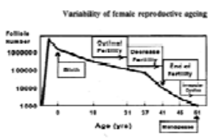
A bit about AMH



- Only produced in the granulosa cells in the ovary
- Measures the quantity of oocytes but not the quality
- Undetectable in serum 3-5 days after oophorectomi
- Undetectable after menopause

AMH and age





Anti-Müllerian Hormone decreases with age

Longitudinal observation study:
Measurement of AMH twice with a 3 year interval in women with regular menstrual cycles

 De Vet er al (2002) FertilSteril

AMH – a sensitive marker of ovarian reserve

	Visit 1	Visit 2	P-value
FSH	6.0 (1.4-13.5)	5.8 (2.1-13.4)	NS
Inhibin B	112 (12-213)	110 (4-206)	NS
AFC	14 (6-28)	14 (2-24)	NS
AMH	2.1 (0.1-7.4)	1.3 (0.0-5.0)	< 0.001

Probably the earliest marker of ovarian ageing

 De Vet er al (2002) FertilSteril

Recurrent Miscarriage and AMH
1 study….

RECURRENT PREGNANCY LOSS

Absence of follicular phase defect in women with recurrent miscarriage

Alka Prakash, M.D., Tin C. Li, Ph.D., Susan Laird, Ph.D., Geeta Nargund, F.R.C.O.G., and William L. Ledger, D.Phil.

- Prospective case-control
- 34 women with RM and 10 controls
  - Same age, regular cycles, equal length of follicular phase
- Intervention
  - AMH
  - FSH & LH
  - Inhibin B
  - Progesterone

Results

TABLE 2

Mean serum concentrations of various endocrinological markers for cases (women with history of recurrent miscarriage) and controls, taken once between days 2 and 3 of menstrual cycle.

Early follicular phase	Case Mean (± SEM)	Control Mean (± SEM)	P value
LH (IU/L)	4.4 (± 0.3)	5.1 (± 1.0)	.7
FSH (IU/L)	4.9 (± 0.5)	5.5 (± 0.6)	.4
E_2 (pmol/L)	170.1 (± 12.8)	258.7 (± 78)	.5
P (nmol/L)	1.8 (± 0.1)	3.1 (± 0.4)	.02
Inhibin B (pg/mL)	79.8 (± 7.3)	98.4 (± 15.1)	.3
AMH (ng/mL)	2.1 (± 0.2)	2.1 (± 0.2)	.5

Note: AMH = anti-Müllerian hormone.

[illegible]

- Apart from basal progesterone level NO differences between controls and women suffering from RM

AMH as a predictor of another miscarriage

Three conditions associated with recurrent miscarriage:

1. Advanced maternal age (> 40 years)
   1. Often low AMH
2. Polycystic ovarian syndrome (PCO´s)
   1. High AMH
3. Systemic Lupus Erythematosus (SLE)
   1. Often low AMH

Advanced maternal age

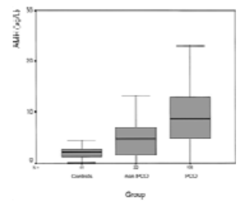
- The risk for miscarriage increases with age, and women in the advanced reproductive age who have low ovarian reserve are prone to a higher risk of recurrent miscarriage

PCO´s

- Common endocrine disorder in young women
- ~ 40% of pregnancies in PCO´s women result in spontaneous loss
- PCO´s and clinical features
  - Obesity → Independant risk factor for recurrent miscarriage
- PCO´s and paraclinical features
  - Insulin resistance & hyperandrogenism → Associated with recurrent miscarriage

Smith ML et al. Semin Reprod Med.2011

PCO´s and AMH



AMH in normo-gonadotrophic anovulatory women compared to controls *(Laven et al. JCEM 2004)*

- Women with PCO´s have elevated levels of AMH compared to non-PCO´s women with anovulation and to ovulatory controls

Systemic Lupus Erythematosus (SLE)

- Autoimmune disease
- 80-90% of affected individuals = women
- 20% miscarriage rate (< 20 weeks)
- Three-fold risk of miscarraige > 20 weeks
- High risk of recurrent miscarriage
- Risk of premature ovarian failure (treatment induced and/or ovarian antibodies)

Lawrenz B et al. Lupus 2011

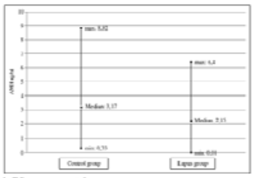
SLE and AMH

CONCISE REPORT

Impact of systemic lupus erythematosus on ovarian reserve in premenopausal women: Evaluation by using anti-Muellerian hormone

- 33 women with SLE (age 29.8 yr (21-39))
  - No previous gonadotoxic treatment
- 33 healthy age-matched controls (age 29.8 yr (21-40))
- Intervention
  - AMH
  - Number of previous abortions

Results



- AMH significantly lower in the SLE-group ($p < 0.05$)
- 5 miscarriages in the SLE-group vs. 2 in the control group (ns)

To conclude..

- Indeed, it is still unclear whether diminished ovarian reserve is an independent predictor of RM.
- Some conditions predisposing to RM have an impact on ovarian reserve
  - *F.x. SLE*
- The combination of *advanced maternal age* and *low level of AMH* is a risk factor for another miscarriage
  - *Low egg quantity and quality*
- *PCO´s* and corresponding *high levels of AMH* is a risk factor for another miscarriage
  - *Obesity, Insulin resistance, and hyperandrogenism*

Future perspectives

- Large prospective studies are needed to further evaluate ovarian reserve with AMH in women with recurrent miscarriage
- In particular in women with unexplained recurrent miscarriage

Thank You

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Mark your calendar for the upcoming ESHRE Campus events

- Basic Semen Analysis Course in Greek Language
  4-7 September 2012 - Athens, Greece
- Basic Genetics for ART practitioners
  7 September 2012 - Rome, Italy
- Regulation of quality and safety in ART – the EU Tissues and Cells Directive perspective
  14-15 September 2012 - Dublin, Ireland
- Basic Semen Analysis Course in Spanish language
  18-21 September 2012 - Galdakano,Vizcaya
- GnRH-antagonists in ovarian stimulation
  28 September 2012 - Hamburg, Germany
- The best sperm for the best oocyte
  6-7 October 2012 - Athens, Greece
- Basic Semen Analysis Course in Italian language
  8-11 October 2012 - Rome, Italy
- Accreditation of a preimplantation genetic diagnosis laboratory
  11-12 October 2012 - Istanbul, Turkey
- Endoscopy in reproductive medicine
  21-23 November 2012 - Leuven, Belgium
- Evidence based early pregnancy care
  29-30 November 2012 - Amsterdam, The Netherlands

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