Is accreditation necessary for ART laboratory?

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Accreditation

- A procedure by which an authoritative body gives a formal recognition that a body or person is competent to carry out specific tasks

(ISO definition)

International Quality Control levels in Assisted Reproduction

- **COMMISSION DIRECTIVE 2006/17/EC of 8 February 2006**
- **implementing Directive 2004/23/EC of the European Parliament and of the Council**

- • ESHRE guidelines for good practice in IVF laboratories
- • EN ISO 9001 certification
- • EN ISO 170 25 accreditation
- • EN ISO 151 89 accreditation

Why quality control systems are needed?

- To assure the reproducibility of all methods and competence in all duties performed by the personell

ISO standards

ISO 17025 standard (1999)

- General requirements for the competence of testing and calibration laboratories

ISO 15189 standard (2003)

- Particular requirements for quality and competence of medical laboratories

ISO 15189 - Management requirements

- Organization and management
- Quality management system
- Documental control
- Review of contracts
- Examination by referral laboratories
- External services and supplies
- Advisory services
- Resolution of complains
- Identification and control of nonconformities
- Corrective action
- Preventive action
- Continual improvement
- Quality and technical records
- Internal audits
- Management review

ISO 15189 - Technical requirements

- Personnel
- Accommodation and environmental conditions
- Laboratory equipment
- Pre - examination procedures
- Examination procedures
- Assuring quality of examination procedures
- Post-examination procedures
- Reporting of results

Documental control

- The laboratory shall define, document and maintain
- procedures to control all documents and information that
- form its quality documentation
- (ISO 15189 section 4.3.1)

Effecting parameters

1.Patient age and infertility factors
2. Gamets
3.Hiperstimulation protocol
4.Culture media
5.Laboratory techniques
6. Performance of incubators
7.Laboratory conditions
8.Manuplation of phsician or embryologist

General Procedures

- Opening of the laboratory
- Closing the laboratory
- Cryostorage of biological material
- Cleaning of incubators
- Patient sample collection and management
- Safety
- Storage of supplies
- Preparations for the following day
- Cleaning of the laboratory

Selected first priority subjects to be addressed

- Appropriately educated and trained personnel
- Documental control and detailed written standard procedures
- Proper air quality
- Correct operation and calibration of all laboratory instruments
- Control of disposables and culture media
- Definite Identification of patients and their biological material
- Identification and correction of deviations from laboratory Procedures selected

Staff requirements

- The number of staff has to be adjusted according to the number and nature of the procedures performed in the laboratory

Cycles per year	Positions *
< 250	1.5
250 – 500	2.5
500 - 750	3.0
750 - 1000	4.0
1000 - 1500	5.0
1500 - 2000	6.0
2000 – 2500	7.0
2500 – 3000	8.0

- * Including laboratory director

Laid by the German Society for human reproductive Biology

Education program for the IVF laboratory team

- There shall be a continuing education program for new as well as senior embryologists
- The competency of each person to perform assigned tasks shall be assessed following training and periodically thereafter

Embryologist should know

- ROS production
- Apoptosis
- Twins after blastosist culture
- Metilation and gen expresions

- Angelman sendromu
- Prader –Willi sendromu
- Backwith wiedeman senromu

Evaluation of performance of all team members

- Reliable database indicating the identity of the embryologists
- performing the different tasks

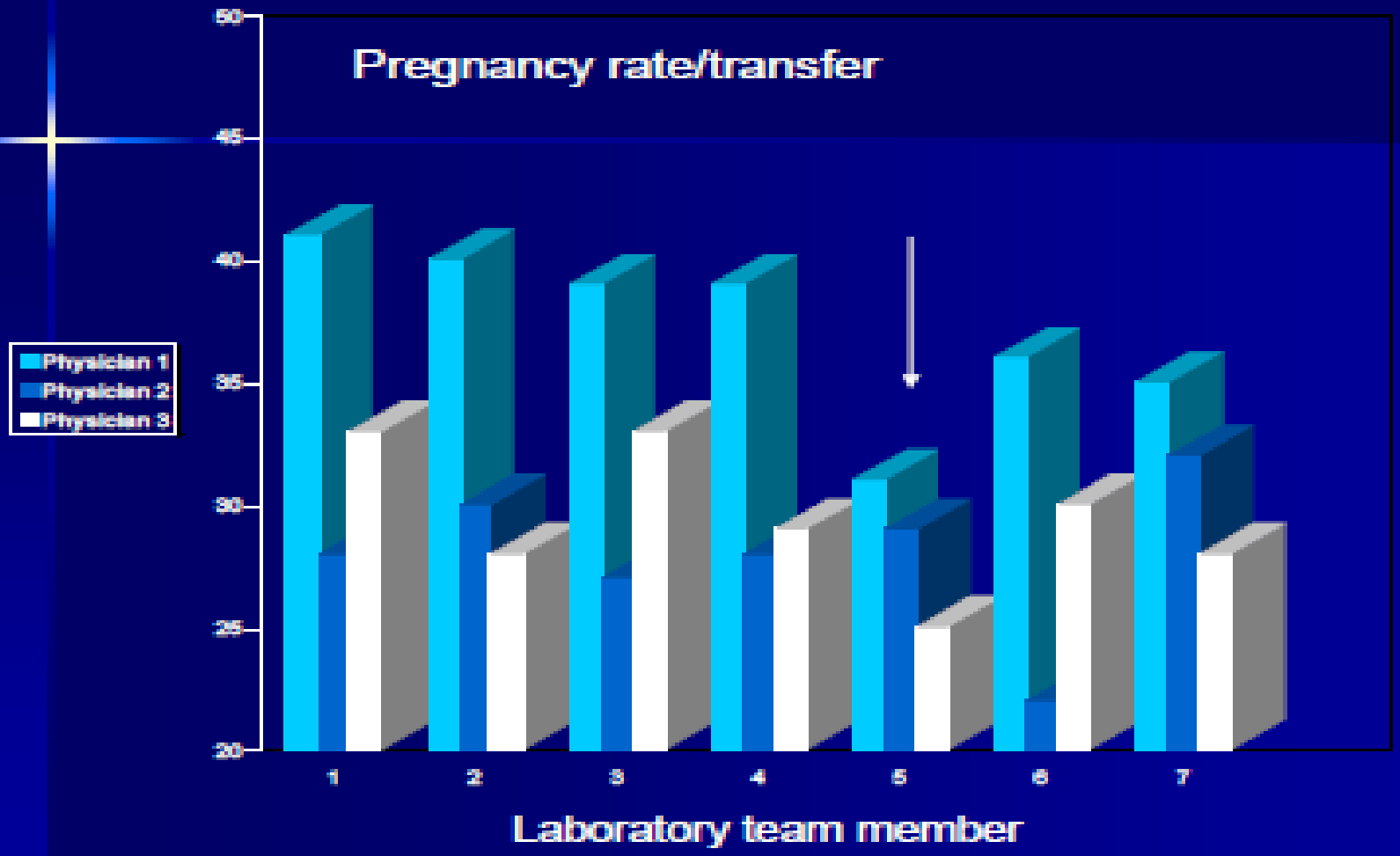
Short term intervals

- Grading of oocyte maturity
- Grading embryo quality
- Evaluation of sperm parameters

Long term intervals

- Pregnancy rate / transfer
- Fertilization/degeneration rates following ICSI

Effect of physician and laboratory team on pregnancy rate



Detailed Standard Operating Procedures (SOPs) - Why?

- Ensures uniform execution of all laboratory procedures by all team members
- Enables introduction of the protocols to new team members

Standardisation of semen analysis

- Sperm counting method
- Counting with neubauer or makler
- Heating tables
- Collecting method
- Timing
- Correct morphological evaluation

Standard Operating Procedures

Procedure

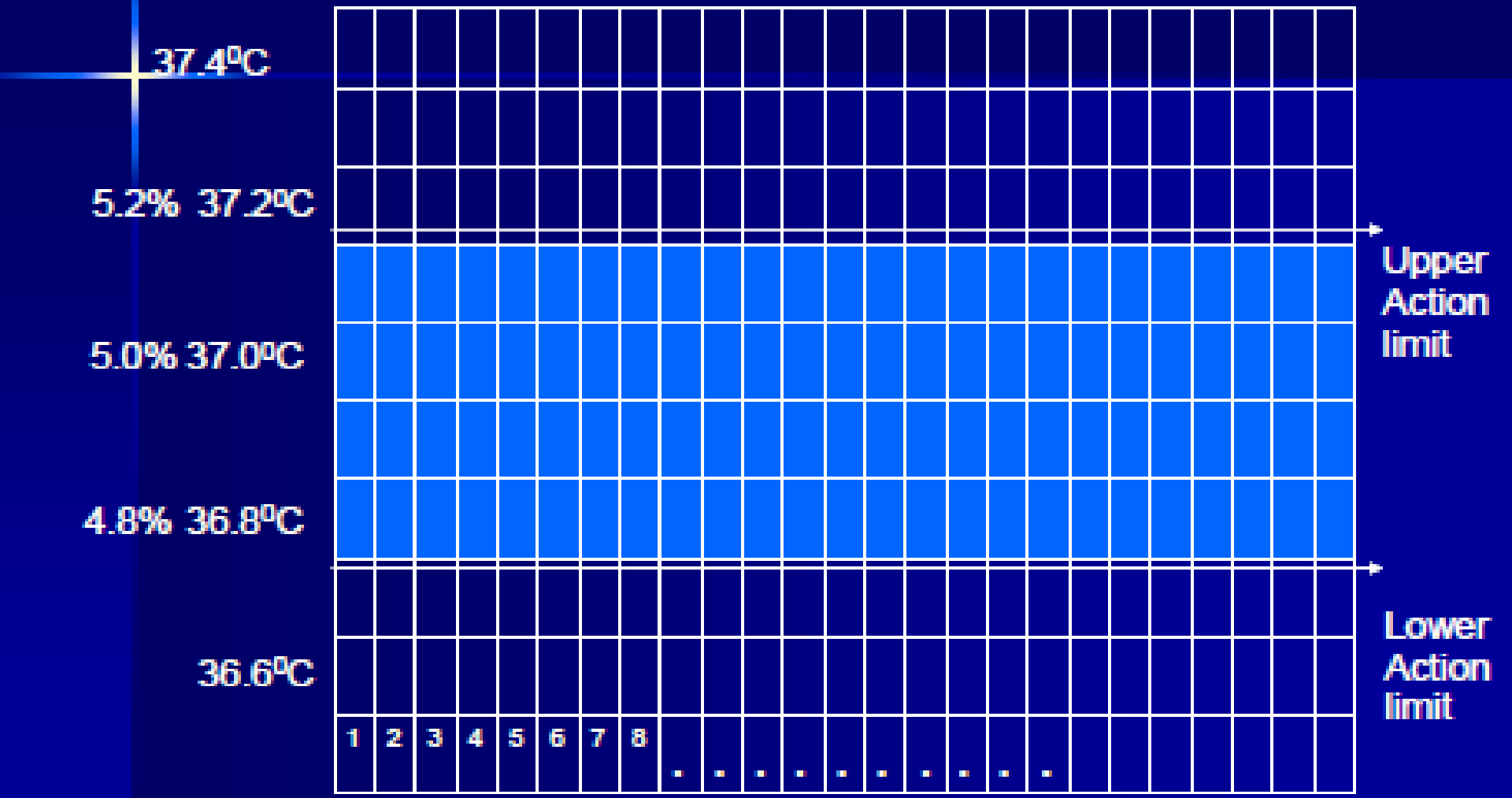
- Oocyte collection and preparation for insemination
- Sperm preparation for IVF or ICSI
- Conventional insemination
- IntraCytoplasmic Sperm Injection (ICSI)
- The analysis of fertilization and embryo development
- Embryo transfer
- Embryo cryopreservation
- Embryo thawing
- The preparation epididymal and testicular sperm

Daily monitoring of other instruments

Refrigerators and freezers

- Monitor using calibrated thermometer inside the cabinet
- In freezer (with no alarm), conduct frozen sample test
- Heated working surfaces and water baths
- Check tube warmers and water baths with a calibrated thermometer in a tube of medium
- Check warm surfaces with surface thermometers
- Liquid Nitrogen tanks
- Monitor level using dipstick/ruler

Monitoring CO_2 and temperature levels in the incubator



Safety concerns - Definite patient and sample identification

- All material obtained from the patients, should bear unique identification
- Incubators should be organized in order to facilitate identification of embryos, oocytes and spermatozoa
- Verification of patients' identity should be performed at critical steps: before ovum retrieval, at semen recovery and embryo transfer procedures

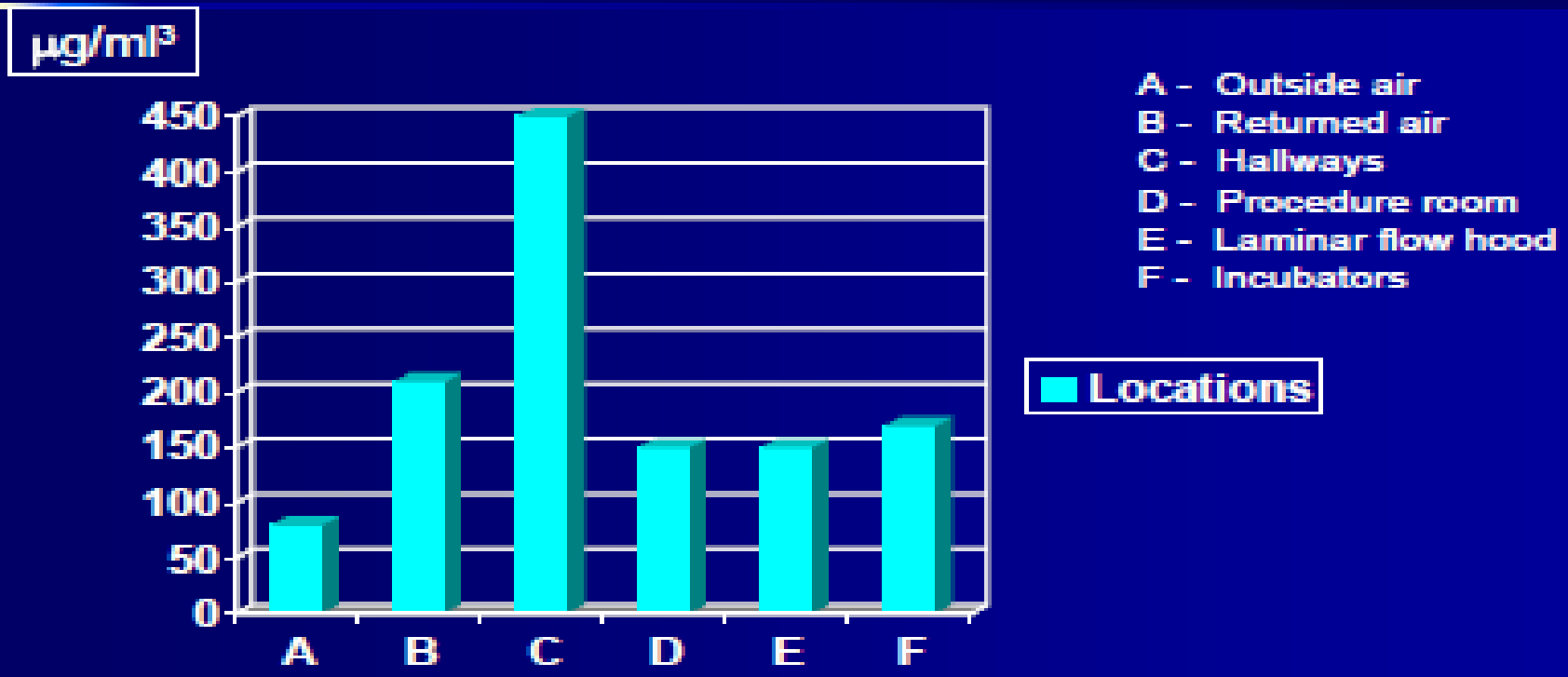
Double checks need to be considered at least at: insemination of oocytes, replacement of embryos, embryo freezing and thawing

- Selected first priority subjects to be addressed
- Appropriately educated and trained personnel
- Documental control and detailed written standard procedures
- Proper air quality
- Correct operation and calibration of all laboratory instruments
- Control of disposables and culture media
- Definite Identification of patients and their biological material
- Identification and correction of deviations from laboratory procedures

Proper air quality

- Chemical air contaminants (COC) are believed to exert a range of effects, from fertilization failure and delayed embryonic development to a reduction in viability and pregnancy rates
- These effects may or may not be evident morphologically

The distribution of Enflurane at different locations in and around the IVF laboratory



Effect of CODA incubator system on outcomes in an IVF program Mayer et al 1999

	CODA	No CODA	Significance
Cycles	66	63	NS
Age	34.6±0.82	33.0 ± 0.83	NS
Embryos			
Transfer	3.7 ± 0.15	3.7± 0.21	NS
% Preg	52 %	30%	p< 0.02

Coda Tower and Coda incubators (Racowsky et al 1999)

	-	Tower	Tower+ink	p
Emb transfer	170	149	147	
Emb hücre sayısı	5.74± 0.11	6.02 ± 0.11	6.00 ± 0.10	0.10
Ort.fragm.skoru	1.28 ± 0.05	1.81 ± 0.06	1.89 ± 0.06	<0.001
+ HCG/ET %	50.6	59.1	50.0	0.21
Klinik gebelik %	37.1	45	40.5	0.36
% abortus	14	5.7	1.4	<0.007

Developmental Arrests

Three main mechanisms can explain the high level of developmental arrest reported previously;

1. Chromosomal abnormalities
2. Intrinsic defects in the oocyte and preimplantation embryos
3. Suboptimal culture conditions

Edwards 1984,1999,Sakkas 1998,2001

Control of disposables and culture media

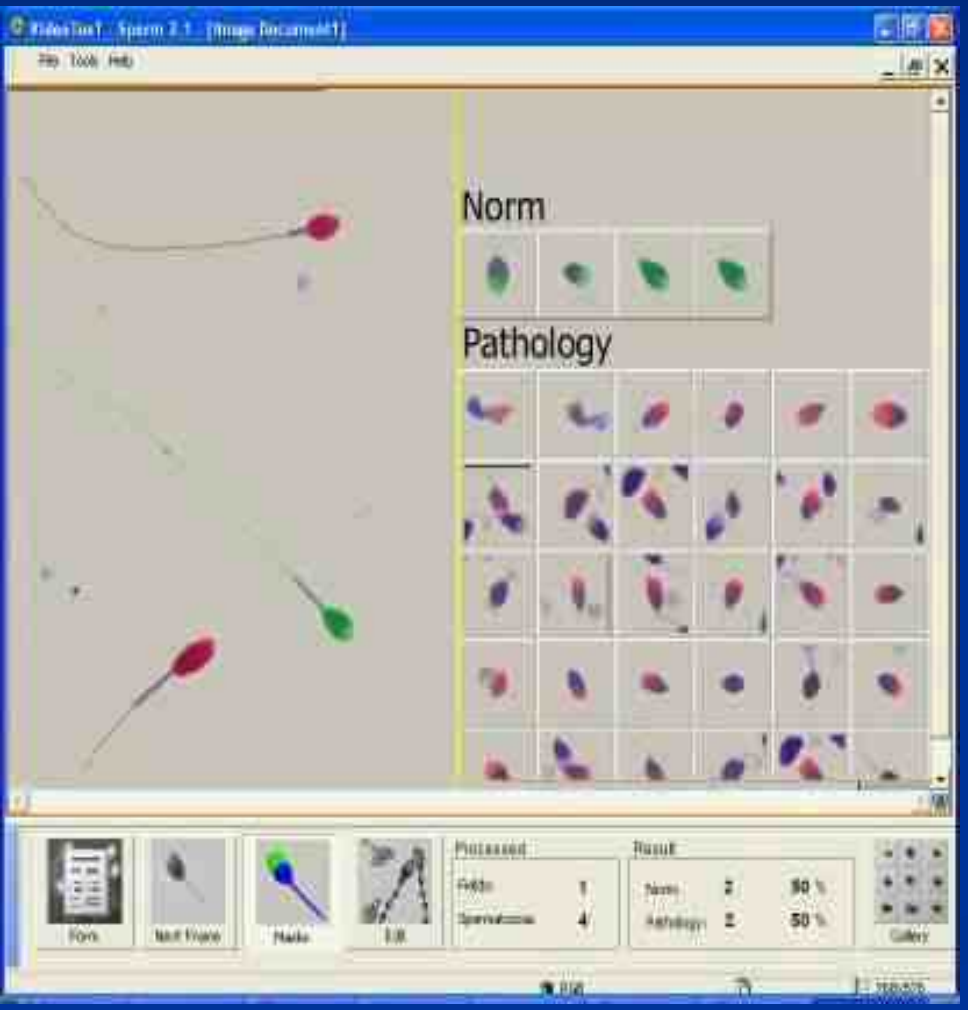
- Purchased supplies should meet the laboratory's quality requirements
- All tissue culture media prepared in the laboratory should undergo quality control using an appropriate bioassay system
- When commercially produced media are used, integrity of the packages and appropriate delivery conditions should be controlled.
- Documentation of quality control testing specifies in the certificate of analysis (COA) should be supplied by **the** manufacturer for any commercially produced media distributed
- Reagents and media should always be used prior to the manufacturer's expiry date

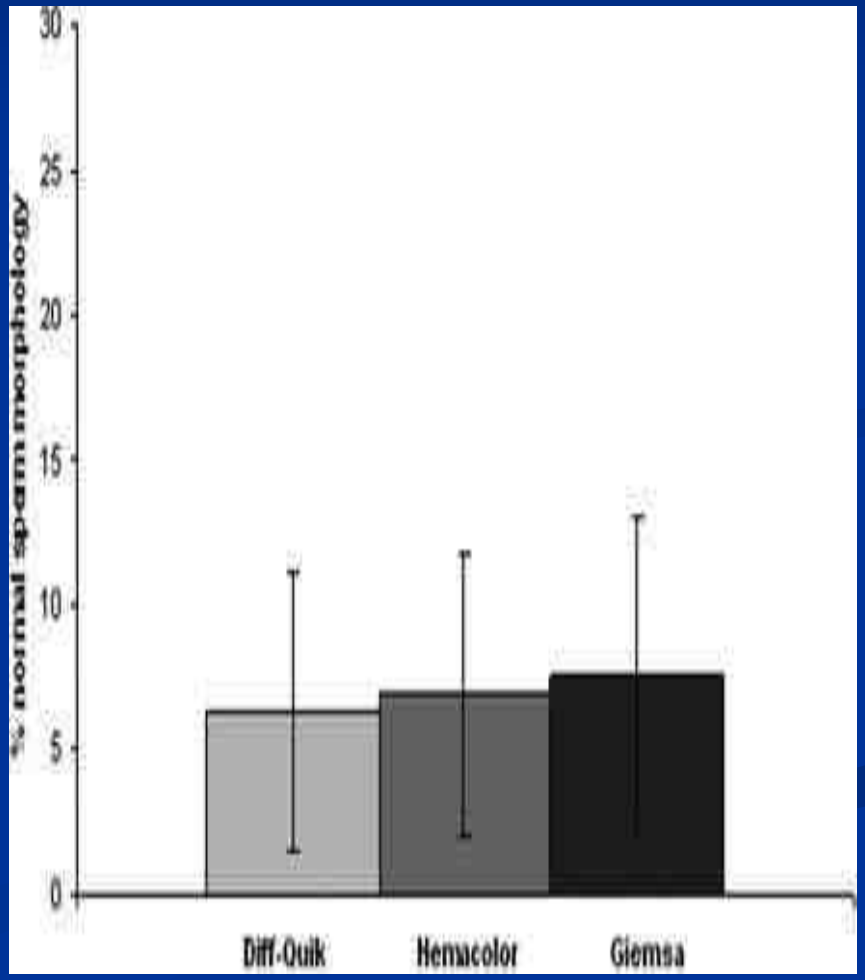
Quality in Andrology laboratory

		1980	1987	1990	1999	2002
Concentration	Hemositometri	+	++	++	++	++
	Makler	-	+	+	-	+
	Coulter	+	-	-	-	-
	CASA	-	-	-	+	+
Motility	Heated	-	-	-	+	+

WHO,1999,ESHRE 2002

Sperm staining procedures





LIMITES IN EMBRYOLOGY LAB
D.K.Gardner et al.2004

Procedures	
Normal fertilization rate	% 60
Percentage of polyspermy	% 10
ICSI degeneration rate	% 15
Embryo clavage	%80
Cryopreservation viability	% 50
PR	% 30
IR	% 20

Preimplantation Embryos

- Several key events control preimplantation development
- Cleavage of the fertilized egg through about five mitotic divisions
- Switch of control from maternal to embryonic genome
- Differential expression of imprinted genes

- Initial steps in differentiation(Blastomere orientation and fate)
- Expression of key molecules mediating communication among blastomeres
- Stage specific respiration,metabolic requirements

Strategies For Improved Embryo Selection

CONVENTIONAL MORPHOLOGIC CHARACTERISTICS

A.Number of cells

B.Degree of asymmetry

C.%fragmentation

NEW PARAMETERS

A.Pronuclear scoring

B.Early cleavage

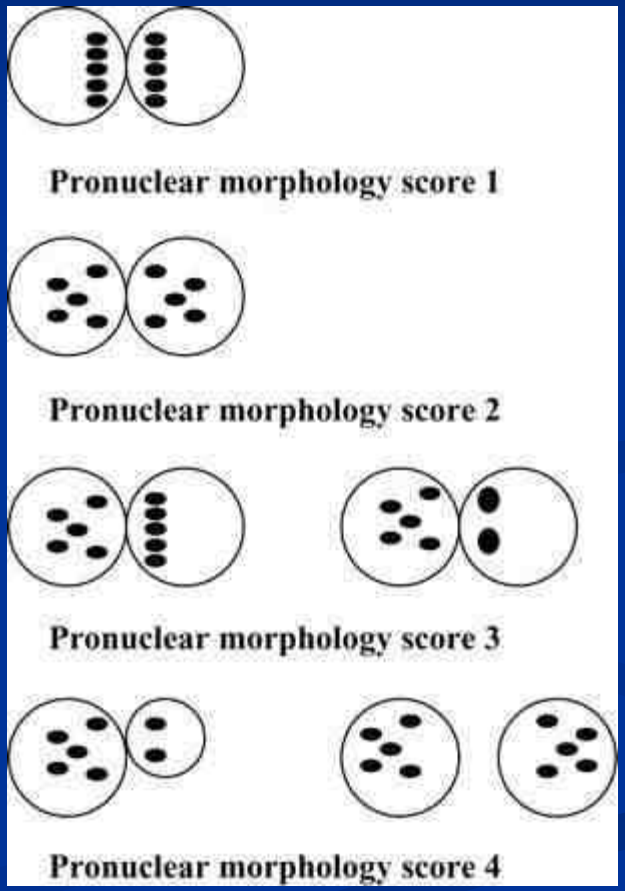
C. Early compaction

D.day3 or 5 transferes

Z skores and chromosomal abnormalities

- Normal embryo
- Z1 % 40
- Z2 % 29.7
- Z3 % 22.7
- Z4 % 13.6

- W.R.Edirisinghe et al. 2004



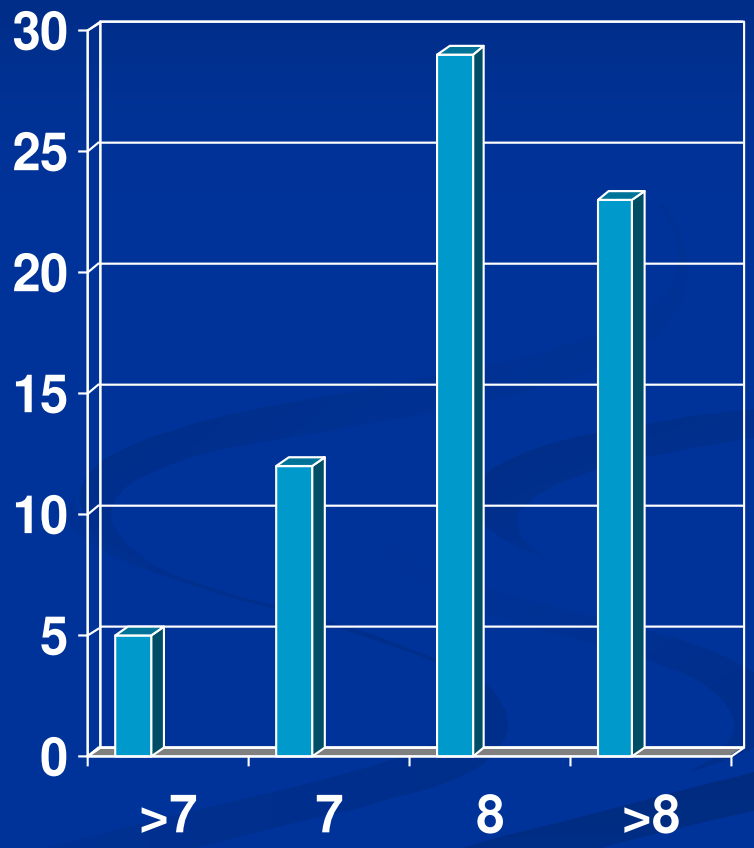
Multinucleation and Implantation

	Multinucleation (+)	(-)
Pelinck et al (1998) %	13,2	23,2
Jackson et al (1998) %	3,4	14,7

3. day selection

- Blastomer number=8
- Fragmentationon<%10
- Symmetry of blastomer

- Racowsky et al.2003



Blastomere Asimetry and pregnancy

Racowsky et al 2003

IVF/ICSI Results in CTF 1998 - 2005

	old system	improved system
Female age	< 37	< 37
Cycles	600	940
No of oocytes	5930	9710
fertilized	3200	6950
(% 2PN zygote)	(53.9 %)	(71.5 %)
Cleavage(%)	3010 (94 %)	6925 (98.6 %)
Clin Preg rate	15.9 %	37.9 %

Conclusion

- Personel education and correct manuplation
- Timing
- Quality control on each steps
- Perfect laboratory conditions
- Equipments
- Each person believe the quality
- Gives high success rate

TEŞEKKÜRLER