NOVE TEHNOLOGIJE U INFERTILITETU - STVARNA POTREBA, HIR ILI MARKETING?

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Klinički centar Vojvodine

VANTELESNA OPLODNJA DANAS

- OBIM pojave
- USPEH i njegovo tumačenje
- NADA koja nosi izazove

OPTEREĆENJE USPEHOM

- Gde je ključ uspeha
- Potraga za svetim gralom

NOBELOVA NAGRADA - MEDICINA

- Nobelov komitet je 04.10.2010. objavio dobitnika Nobelove nagrade iz fiziologije ili medicine za 2010. godinu za otkriće postupaka vantelesne oplodnje
  - Robert G. Edwards

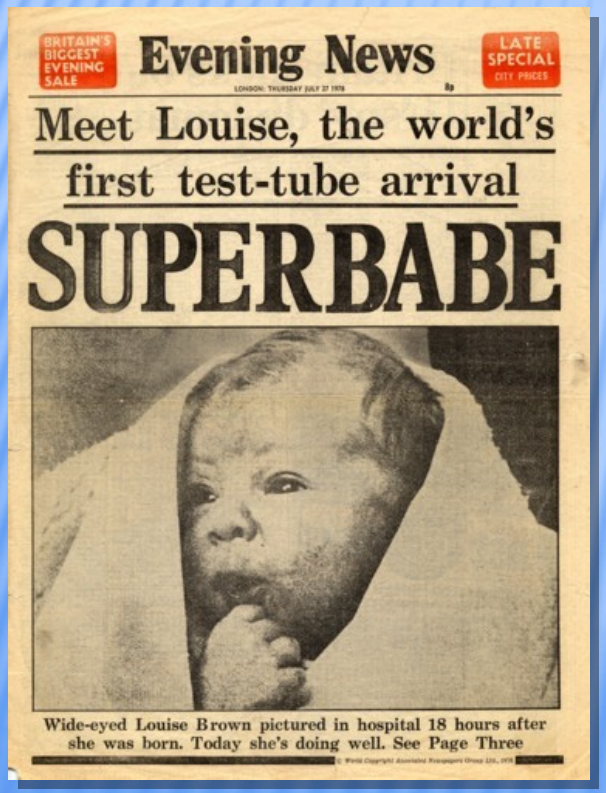
The Nobel Prize in Physiology or Medicine has been awarded 101 times to 196 Nobel Laureates between 1901 and 2010

Rođen 25.09.1925.
Batley, UK
University of Cambridge, Cambridge, United Kingdom

PIONIRI VANTELESNE OPLODNJE

Nakon više od 100 neuspelih pokušaja, 25.07.1978.
Patrik Stepto i Robert Edvards objavljuju rezultat zajedničkog rada – rođenje prve bebe začete vantelesnom oplodnjom.

Ime joj je Lujza Džoj Braun (Oldham, Engleska).


BRITAIN'S BIGGEST EVENING SALE

Evening News

LATE SPECIAL CITY PRICES

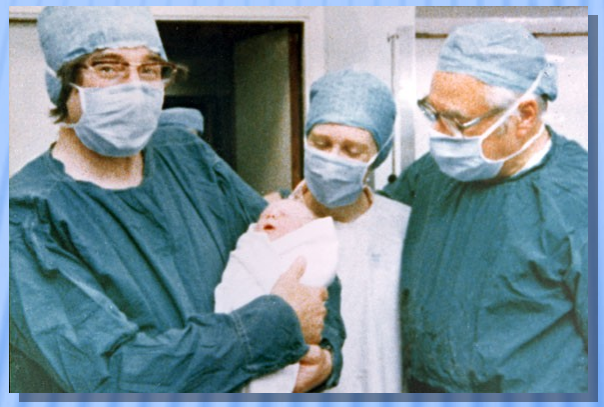
LONDON: THURSDAY JULY 27 1978 8p

Meet Louise, the world's first test-tube arrival

SUPERBABE

Wide-eyed Louise Brown pictured in hospital 18 hours after she was born. Today she's doing well. See Page Three

Rođenje Lujze Braun,
25. juli 1978.




Robert G. Edwards


Patrick C. Steptoe


Bourn Hall klinika, Kembrdž, Engleska,
prvi IVF kongres 1982.


Lujza Braun (35) danas sa sinom Kameronom

ORIGINALNO PISMO UREDNIKU LANCETA

Letters to the Editor

BIRTH AFTER THE REIMPLANTATION OF A HUMAN EMBRYO

SIR, — We wish to report that one of our patients, a 30-year-old nulliparous married women, was safely delivered by cæsarean section on July 25, 1978, of a normal infant girl weighing 2700 g. The patient had been referred to one of us (P.C.S) in 1976 with a history of 9 years' infertility, tubal occlusions, and unsuccessful salpingostomies done in 1970 with excision of the ampullæ of both oviducs followed by persistent tubal blockages. Laparoscopy in February, 1977, revealed grossly distorted tubal remnants with occlusion and peritubal and ovarian adhesions. Laparotomy in August, 1977, was done with excision of the remains of both tubes, adhesolysis, and suspension of the ovaries in good position for oocyte recovery.

Pregnancy was established after laparoscopic recovery of an oocyte on Nov. 10, 1977, in-vitro fertilization and normal cleavage in culture media, and the reimplantation of the 8-cell embryo into the uterus $2^{1}/_{2}$ days later. Amniocentesis at 16 weeks' pregnancy revealed normal α-fetoprotein levels, with no chromosome abnormalities in a 46 XX fetus. On the day of delivery the mother was 38 weeks and 5 days by dates from her last menstrual period, and she had pre-eclamptic toxæmia. Blood-pressure was fluctuating around 140/95, œdema involved both legs up to knee level together with the abdomen, back, hands, and face; the blood-uric-acid was 390 µmol/l, and albumin 0·5 g/l of urine. Ultrasonic scanning and radiographic appearances showed that the fetus had grown slowly for several weeks from week 30. Blood-œstriols and human placental lactogen levels also dropped below the normal levels during this period. However, the fetus grew considerable during the last 10 days before delivery while placental function improved greatly. On the day of delivery the biparietal diameter had reached 9·6 cm, and 5 ml of amniotic fluid was removed safely under sonic control. The lecithin: sphingomyelin ratio was 3·9:1, indicative of maturity and low risk of the respiratory-distress syndrome.

We hope to publish further medical and scientific details in your columns at a later date.

Department of
Obstetrics and Gynæcology,
General Hospital,
Oldham OL1 2JH

P. C. STEPTOE

University Physiology Laboratory,
Cambridge CB2 3EG

R. G. EDWARDS

MAR (MEDICALLY ASSISTED REPRODUCTION) – BIOMEDICINSKI POTPOMOGNUTA OPLODNJA (BMPO) OBIM POJAVE

Preko 5 miliona dece

- 2009: Evropa 537 463 ciklusa (> 50% svetskog broja ciklusa)
- 1067 ciklusa na milion žena reproduktivnog doba (166-2726)
- MAR deca (od 0,6% do 4,5%)
- 1179 IVF centar
- Preko 15 različitih vrsta MAR procedura (od IUI, IVF, ICSI … do PGD, TESE, Surogacy)

Human Reproduction, Vol.28, No.9 pp. 2318–2331, 2013
Advanced Access publication on July 9, 2013 doi:10.1093/humrep/det278

human reproduction ORIGINAL ARTICLE *ESHRE pages*

Assisted reproductive technology in Europe, 2009: results generated from European registers by ESHRE†

A.P. Ferraretti*, V. Goossens, M. Kupka, S. Bhattacharya, J. de Mouzon, J.A. Castilla, K. Erb, V. Korsak, and A. Nyboe Andersen, The European IVF-monitoring (EIM)‡, Consortium, for The European Society of Human Reproduction and Embryology (ESHRE)

ESHRE Central Office, Meerstraat 60, Grimbergen B-1852, Belgium

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Submitted on May 30, 2013; resubmitted on May 30, 2013; accepted on June 7, 2013

STUDY QUESTION: The 13th European *in vitro* fertilization (IVF)-monitoring (EIM) report presents the results of treatments involving assisted reproductive technology (ART) initiated in Europe during 2009: are there any changes in the trends compared with previous years?

SUMMARY ANSWER: Despite some fluctuations in the number of countries reporting data, the overall number of ART cycles has continued to increase year by year and, while pregnancy rates in 2009 remained similar to those reported in 2008, the number of transfers with multiple embryos (3+) and the multiple delivery rates declined.

WHAT IS KNOWN ALREADY: Since 1997, ART data in Europe have been collected and reported in 12 manuscripts, published in *Human Reproduction*.

STUDY DESIGN, SIZE, DURATION: Retrospective data collection of European ART data by the EIM Consortium for the European Society of Human Reproduction and Embryology (ESHRE); cycles started between 1st January and 31st December are collected on a yearly basis; the data are collected by the National Registers, when existing, or on a voluntary basis.

PARTICIPANTS/MATERIALS SETTING, METHODS: From 34 countries (−2 compared with 2008), 1005 clinics reported 537 463 treatment cycles including: IVF (135 621), intracytoplasmic sperm injection (ICSI, 266 084), frozen embryo replacement (FER, 104 153), egg donation (ED, 21 604), *in vitro* maturation (IVM, 1334), preimplantation genetic diagnosis/screening (PGD/PGS, 4389) and frozen oocyte replacements (FOR, 4278). European data on intrauterine insemination using husband/partner's semen (IUI-H) and donor (IUI-D) semen were reported from 21 and 18 countries, respectively. A total of 162 843 IUI-H (+12.7%) and 29 235 IUI-D (+17.3%) cycles were included. Data available from each country are presented in the tables; total values (as numbers and percentages) refer to those countries where all data have been reported.

MAIN RESULTS AND THE ROLE OF CHANCE: In 21 countries where all clinics reported to the ART register, a total of 399 020 ART cycles were performed in a population of 373.8 million, corresponding to 1067 cycles per million inhabitants. For IVF, the clinical pregnancy rates per aspiration and per transfer were 28.9 and 32.9%, respectively and for ICSI, the corresponding rates were 28.7 and 32.0%. In FER cycles, the pregnancy rate per thawing was 20.9%; in ED cycles, the pregnancy rate per transfer was 42.3%. The delivery rate after IUI-H was 8.3 and 13.4% after IUI-D. In IVF and ICSI cycles, 1, 2, 3 and 4+ embryos were transferred in 24.2, 57.7, 16.9 and 1.2%, respectively. The proportions of singleton, twin and triplet deliveries after IVF and ICSI (combined) were 79.8, 19.4 and 0.8%, respectively, resulting in a total multiple delivery rate of 20.2%, compared with 21.7% in 2008, 22.3% in 2007, 20.8% in 2006 and 21.8% in 2005. In FER cycles, the multiple delivery rate was 13.0% (12.7% twins and 0.3% triplets). Twin and triplet delivery rates associated with IUI cycles were 10.4/0.7% and 10.3/0.5%, following treatment with husband and donor semen, respectively.

LIMITATIONS, REASONS FOR CAUTION: The method of reporting varies among countries, and registers from a number of countries have been unable to provide some of the relevant data such as initiated cycles and deliveries. As long as data are incomplete and generated through different methods of collection, results should be interpreted with caution.

†ESHRE pages content is not externally peer reviewed. The manuscript has been approved by the Executive Committee of ESHRE.

‡EIM Committee 2011–2013: chairman: A.P.F.; chairman elect: M.K.; past chairman: J.d.M. members: A.N.A. (special advisor), S.B., J.A.C., V.K. and K.E. V.G. is a science manager at ESHRE Central Office, Brussels. See also Appendix for contributing centres and contact persons representing the data collection programmes in the participating European countries.

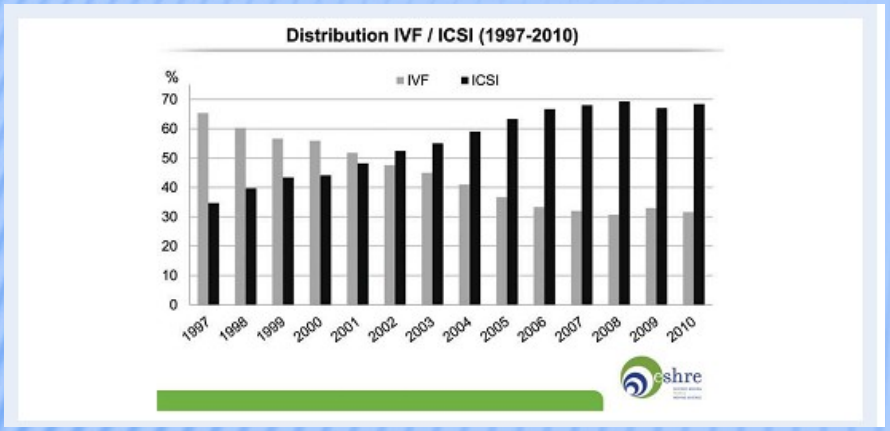
MAR (MEDICALLY ASSISTED REPRODUCTION) – BIOMEDICINSKI POTPOMOGNUTA OPLODNJA (BMPO) NEOPHODNOST MONITORINGA I KONTROLE

Ekspanzivan rast obima i vrsta procedura, kao i naučnog i tehnološkog razvoja zahteva i prateće legislativne mere, koje uglavnom kasne za naukom i strukom.

TRENDOVI U VTO

STABILNI REZULTATI, POZITIVNI ALI LAGANI TRENDOVI, I DALJE DALEKO OD SAVRŠENIH

SVE VEĆI BROJ CIKLUSA, VIŠE ICSI CIKLUSA, MANJE MULTIPLIH TRUDNOĆA



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human reproduction ESHRE PAGES

Assisted reproductive technology in Europe, 2010: results generated from European registers by ESHRE†

M.S. Kupka*, A.P. Ferraretti, J. de Mouzon, K. Erb, T. D'Hooghe, J.A. Castilla, C. Calhaz-Jorge, C. De Geyter, V. Goossens, and The European IVF-monitoring (EIM)‡ Consortium, for the European Society of Human Reproduction and Embryology (ESHRE)

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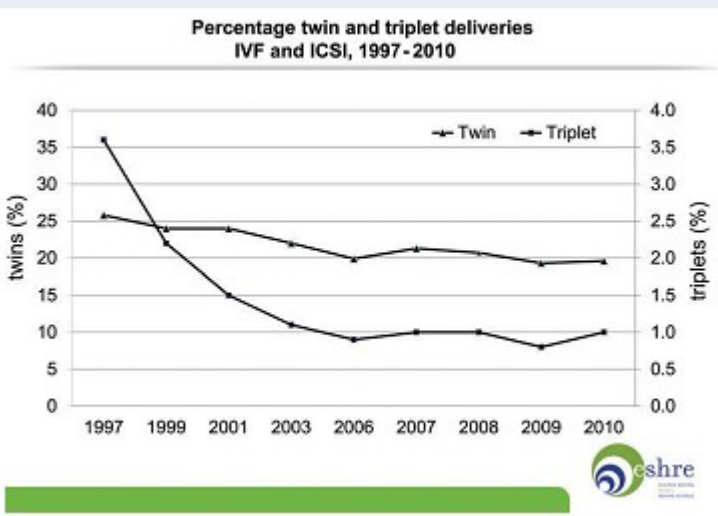
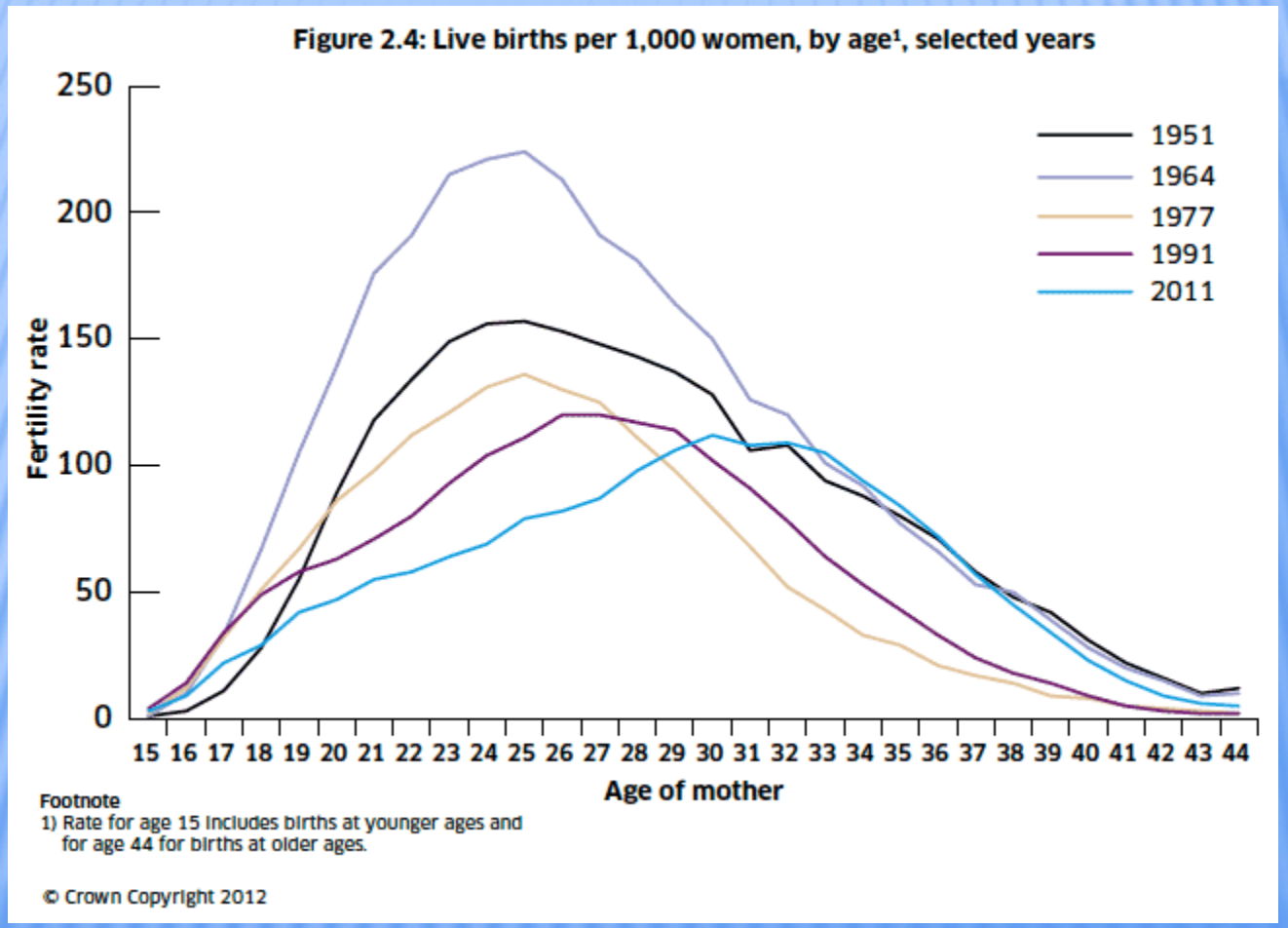
EIM, 1997-2010

year	countries	clinics	cycles	Increase (%)	ART infants
1997	18	482	203,225		
1998	18	521	232,225	+ 14.3	
1999	21	537	249,624	+ 7.5	
2000	22	569	275,187	+ 10.2	
2001	23	579	289,690	+ 5.3	
2002	25	631	324,238	+ 11.9	
2003	28	725	365,103	+ 12.6	68,931
2004	29	785	367,056	+ 0.5	67,973
2005	30	923	419,037	+ 14.2	72,184
2006	32	998	458,759	+ 9.5	87,705
2007	33	1029	493,420	+ 7.6	96,690
2008	36	1051	532,260	+ 7.9	107,383
2009	34	1033	537,287	+ 1.0	109,239
2010	31	1202	548,734	+ 2.1	120,676
total			5,295,845		593,877



USPEH VTO

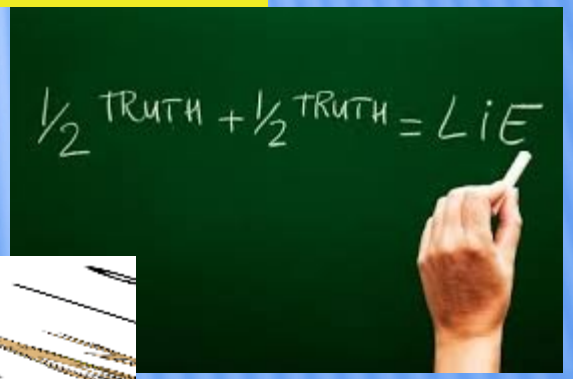
LAGANO RASTE IZ GODINE U GODINU, I DALJE NA PROSEKU OKO 30%, NAJVIŠE ZAVISI OD GODINA ŽENE



KLJUČ USPEHA .... IPAK

NEKRITIČNOST U POTRAZI ZA USPEHOM

- Stopa trudnoće se koristi u marketinške svrhe
- Mulitpli embriotransferi
- Neprimenjivanje stope implantacije kao merila
- Primena brojnih intervencija bez naučne osnove
- „Kuvanje“ u IVF-u



POSLEDICE NEKRITIČNE POTERE ZA USPEHOM VISOKA STOPA MULTIPLIH TRUDNOĆA

- Procenjeno je da jedna troplodna trudnoća košta zdravstveni sistem i do 2000 pokušaja VTO
- Implementacija eSET-a u najvećoj meri zavisi od dobre reimbursement politike.

One child at a time

Reducing multiple births after IVF

Report of the Expert Group on Multiple Births after IVF

Professor Peter Braude

- Doing 30% eSET cycles leads to a twin birth rate of around 20%;
- Doing 50% eSET cycles leads to a twin birth rate of around 10%;
- Doing 70% eSET cycles leads to a twin birth rate of around 5%.

after the babies themselves was even more striking: neonatal twin costs were 16 times higher than singleton costs; triplet costs 109 times higher.[50] Neonatal and paediatric services in the

work differently from the UK: generally patients have better access to publicly funded IVF treatments, which directly influences patients' attitudes to eSET, and their patient profile

KLJUČ USPEHA LEŽI DAKLE U ....

NOVE TEHNOLOGIJE I POSTUPCI U INFERTILITETU DA LI NAS ONE VODE DO CILJA?

- PGS/PGD
- TIME LAPSE
- Assisted hatching
- ERA
- Faktori rasta za endometrijum (G-CSF)
- DHEA za „poor respondere“

NOVE TEHNIKE U EMBRIOSELEKCIJI
POTRAGA ZA PRAVIM EMBRIONOM

- TIME LAPSE
- PGS

TIME LAPSE ANALIZA
ŠTA NAS UČI MORFOKINETIKA

PRIMO-VISION

EMBRYOSCOPE

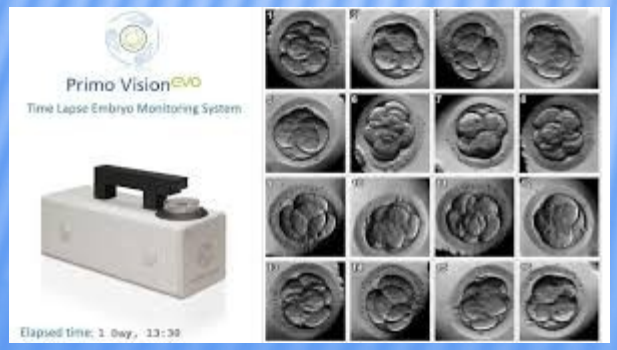
EEVA

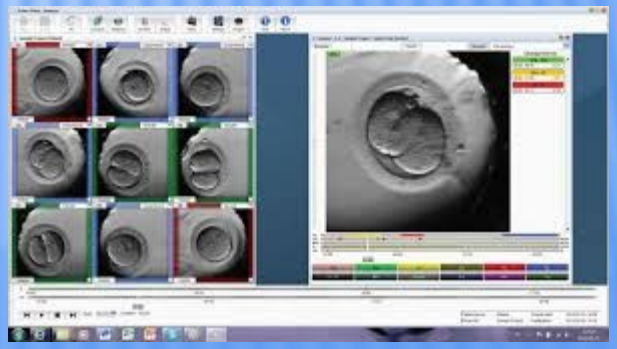
Bolja embrioselekcija?

Otpimalan softver

Standardizovano za datu populaciju.

Da li imamo više trudnoća?





COCHRANE 2015, ZAKLJUČUJE BEZ ZNAČAJA

Time-lapse systems for embryo incubation and assessment in assisted reproduction (Review)

Armstrong S, Arroll N, Cree LM, Jordan V, Farquhar C

THE COCHRANE COLLABORATION®

Authors' conclusions

There is insufficient evidence of differences in live birth, miscarriage, stillbirth or clinical pregnancy to choose between TLS and conventional incubation. Further data explicitly comparing the incubation environment, the algorithm for embryo selection, or both, are required before recommendations for a change of routine practice can be justified.

OSNOVNE DEFINICIJE

- PGD – Preimplantaciona genetska dijagnostika

metoda za identifikaciju zdravih i nezdravih embriona u fertilnoj populaciji pacijenata u cilju sprečavana prenošenja naslednih bolesti.

- PGS – Preimplantacioni genetski skrining

Metoda za identifikaciju euploidnih embriona u infertilnoj populaciji pacijenata

ISTORIJAT PGD/PGS

- 1985 PCR
- 1990 PRVI PGD NA LJUDIMA (Winston)
- 1992 PGD Cistična fibroza (Handyside)
- 1993 PGS (Delhanty 1993, Munne 1993)

ZAŠTO PGS?

- Šta je PGS?
- Kome ga ponuditi?
- Šta očekujemo od procedure – zadovoljstvo?
- Sa kakvim izazovima i razočaranjima se suočavamo –zabrinutost?

NOVE TEHNOLOGIJE I POSTUPCI U INFERTILITETU KOJI NAS VODE DO CILJA?

- PGS/PGD

Izbor najboljeg embriona i posledice po efikasnost i bezbednost postupka

PGS

- Pre svega i jedino najsuverenija metoda embrioselekcije.
- Ne može stvoriti embrion boljim nego što jeste.
- Može nam pomoći da izaberemo najbolji iz prve i skratimo vreme do trudnoće, smanjimo breme neuspeha i implementiramo SET sa sigurnošću.

OBIM PGS –A – DA LI JE TO NOVINA ILI RUTINA?

- ESHRE PGD consortium
- 1997-2010.
- 60 centara
- 39431 ciklusa
- 6231 dece rođeno

- De Rycke HR 2015
- Jan.– Dec. 2010
- 62 centra

- 5780 ciklusa

1071 hromozomske abnormalnosti

108 X vezane bolesti

1574 monogenske bolesti

2979 PGS

- 1503 trudnoće
- 1152 živorođenih

PGS

PGS/PGD

- Hromozomske aneuploidije su najčešći uzrok prekida trudnoće nakon prirodne koncepcije i In Vitro fertilizacije.
- Pojava aneuploidnih embriona ima eksponencijalni rast u deceniji pred menopauzu.

- Najveći skorašnji pomak u preimplantacionoj genetskoj dijagnostici je uvođenje metode komparativne genomske hibridizacije (mArrays) za detekciju hromozomski abnormalnih oocita i embriona, uz implementaciju NGS tehnologije.

KLJUČNA PITANJA

- Zašto PGS za selekciju
- Koje tehnike za genetsku analizu koristiti
- Kada izvoditi biopsiju (polarno telo, D3, D5)
- Klinički benefit na osnovu dokaza
- Tehnički i organizacioni izazovi

Zašto PGS

- Visoka stopa aneuplidnih oocita i embriona u ljudskoj populaciji koja raste sa godinama (<5% oocita sa 20 godina i preko 50% nakon 37. god aneuploidno).

MEDICINSKO OPRAVDANJE ZA PGS

GODINE I ANEUPLOIDIJE, FRANASIAK ET AL 2014. FERTIL STERIL

The nature of aneuploidy with increasing age of the female partner: a review of 15,169 consecutive trophectoderm biopsies evaluated with comprehensive chromosomal screening

Jason M. Franasiak, M.D.,[a] Eric J. Forman, M.D.,[a,b] Kathleen H. Hong, M.D.,[a,b] Marie D. Werner, M.D.,[a,b] Kathleen M. Upham, B.S.,[b] Nathan R. Treff, Ph.D.,[a,c] and Richard T. Scott Jr., M.D.[a,b]

[a] Division of Reproductive Endocrinology, Department of Obstetrics, Gynecology and Reproductive Science, Robert Wood Johnson Medical School, Rutgers University, New Brunswick, and [b] Reproductive Medicine Associates of New Jersey, Morristown, New Jersey

Objective: To determine the relationship between the age of the female partner and the prevalence and nature of human embryonic aneuploidy.
Design: Retrospective.
Setting: Academic.
Patient(s): Trophectoderm biopsies.
Intervention(s): Comprehensive chromosomal screening performed on patients with blastocysts available for biopsy.
Main Outcome Measure(s): Evaluation of the impact of maternal age on the prevalence of aneuploidy, the probability of having no euploid embryos within a cohort, the complexity of aneuploidy as gauged by the number of aneuploid chromosomes, and the trisomy/monosomy ratio.
Result(s): Aneuploidy increased predictably after 26 years of age. A slightly increased prevalence was noted at younger ages, with >40% aneuploidy in women 23 years and under. The no euploid embryo rate was lowest (2% to 6%) in women aged 26 to 37, was 33% at age 42, and was 53% at age 44. Among the biopsies with aneuploidy, 64% involved a single chromosome, 20% two chromosomes, and 16% three chromosomes, with the proportion of more complex aneuploidy increasing with age. Finally, the trisomy/monosomy ratio approximated 1 and increased minimally with age.
Conclusion(s): The lowest risk for embryonic aneuploidy was between ages 26 and 30. Both younger and older age groups had higher rates of aneuploidy and an increased risk for more complex aneuploidies. The overall risk did not measurably change after age 43. Trisomies and monosomies are equally prevalent. (Fertil Steril® 2014;101:656–63. ©2014 by American Society for Reproductive Medicine.)
Key Words: Comprehensive chromosomal screening, embryonic aneuploidy, IVF, preimplantation genetic screening, trophectoderm biopsy

Discuss: You can discuss this article with its authors and with other ASRM members at http://fertstertforum.com/franasiakj-aneuploidy-age-trophectoderm-biopsy/

Use your smartphone to scan this QR code and connect to the discussion forum for this article now.*

* Download a free QR code scanner by searching for "QR Scanner" in your smartphone's app store or app marketplace.

TABLE 1

Distribution of samples evaluated relative to the age of the female partner and the ensuing comprehensive chromosomal screening results.

				Euploid		Aneuploid	
Age (y)	Oocytes retrieved ($\mu \pm$ SEM)	Cohorts of embryos evaluated (n)	No. of biopsies evaluated (n)	n	Percentage of total	n	Percentage of total
22	23.5 ± 3.5	9	72	40	55.6	32	44.4
23	19.3 ± 1.7	12	76	45	59.2	31	40.8
24	21.5 ± 2.1	13	79	57	72.2	22	27.8
25	12.9 ± 1.1	17	90	50	55.6	40	44.4
26	15.1 ± 1.1	29	175	132	75.4	43	24.6
27	16.2 + 0.6	36	240	175	72.9	65	27.1
28	13.0 ± 0.7	57	335	259	77.3	76	22.7
29	13.9 ± 0.4	106	585	464	79.3	121	20.7
30	12.9 ± 0.4	126	802	616	76.8	186	23.2
31	13.9 ± 0.3	164	862	595	69.0	267	31.0
32	11.1 ± 0.2	193	1,023	705	68.9	318	31.1
33	13.9 ± 0.4	231	1,324	913	69.0	411	31.0
34	13.7 ± 0.4	221	1,156	794	68.7	362	31.3
35	11.6 ± 0.3	226	1,222	800	65.5	422	34.5
36	12.8 ± 0.3	267	1,284	828	64.5	456	35.5
37	10.1 ± 0.3	257	1,153	662	57.4	491	42.6
38	8.7 ± 0.2	280	1,123	585	52.1	538	47.9
39	10.5 ± 0.2	272	1,008	475	47.1	533	52.9
40	11.2 ± 0.3	249	953	398	41.8	555	58.2
41	9.2 ± 0.3	234	750	233	31.1	517	68.9
42	8.7 ± 0.2	150	453	113	24.9	340	75.1
43	6.0 ± 0.3	79	217	36	16.6	181	83.4
44	5.7 ± 0.2	41	85	10	11.8	75	88.2
45	8.0 ± 0.4	22	39	4	15.7	35	84.3
46	11.8 ± 0.5	4	43	12	27.9	31	72.1
47	6.3 ± 0.3	4	17	0	0.0	17	100.0
48	2	1	1	0	0.0	1	100.0
49	4	1	2	0	0.0	2	100.0
Total		3,301	15,169	9,001		6,168	

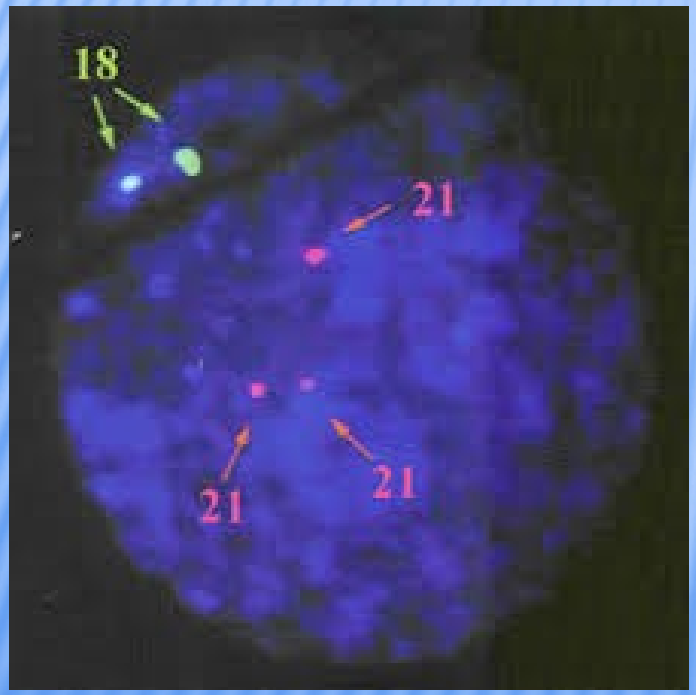
Franasiak. Aneuploidy versus age. Fertil Steril 2014.

PORED OOCITA I VISOKA STOPA ANEUPLIDIJA U EMBRIONIMA

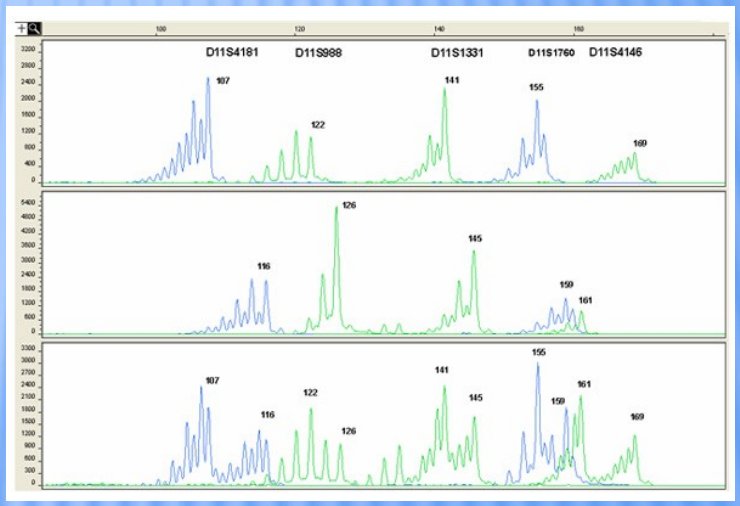
- Preko 2/3 humanih embriona aneupolidno
- 58% do 30 te god
- 77% preko 30 god
- Mantzouratou et al 2007.

TEHNIKE PGS-A ISTORIJAT I IZAZOVI

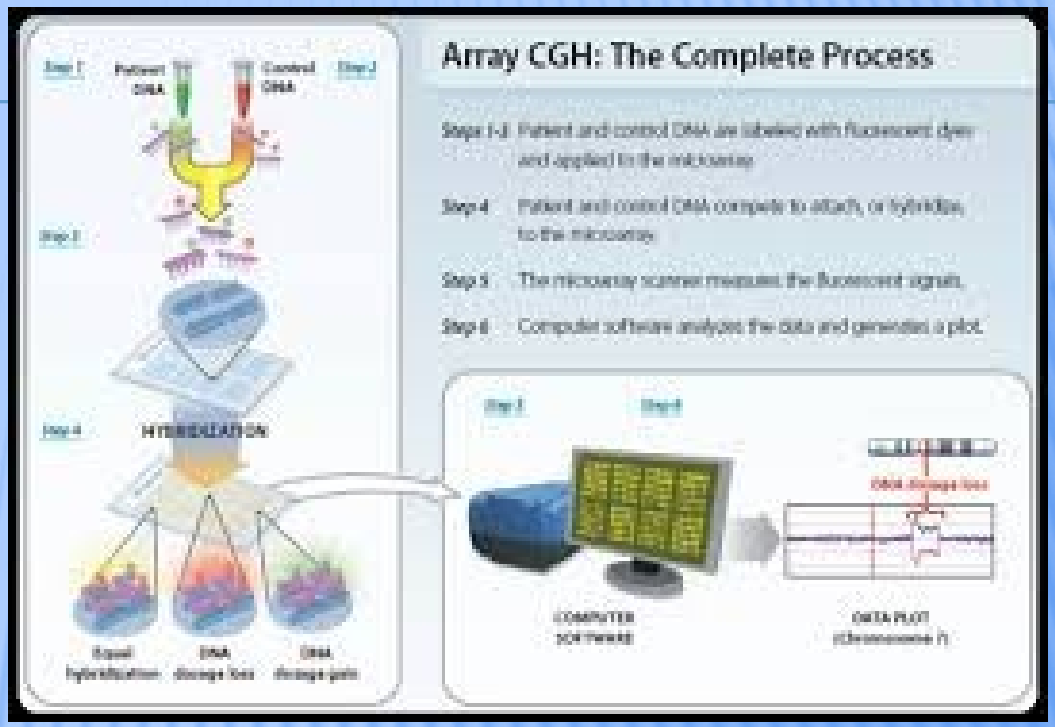
PGS – FISH (mali broj hromozoma, brojne tehničke poteškoće)

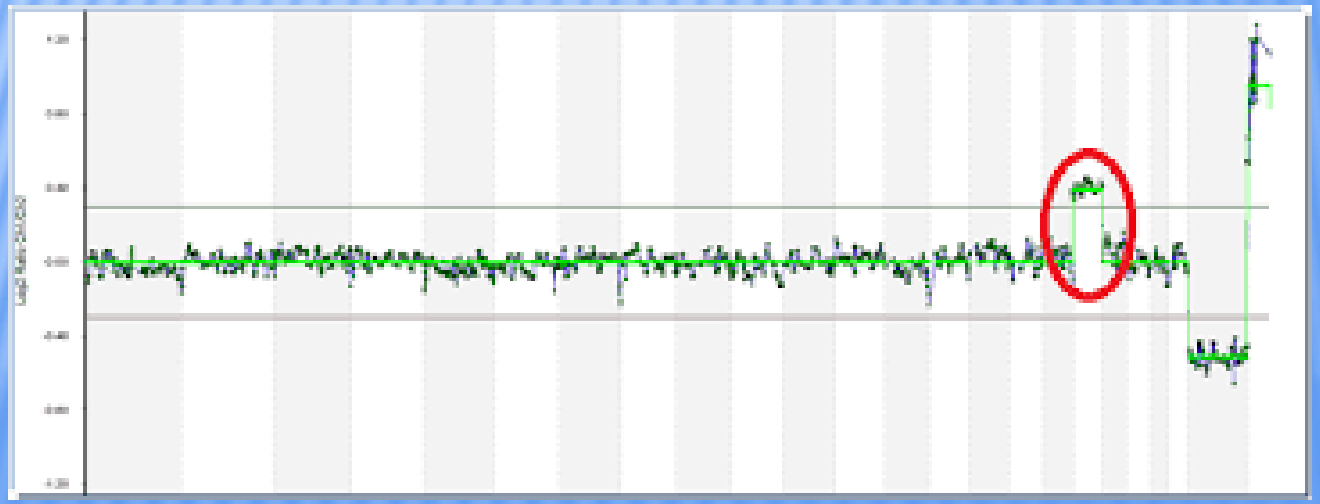


PGS - PCR



MICROARRAY CGH



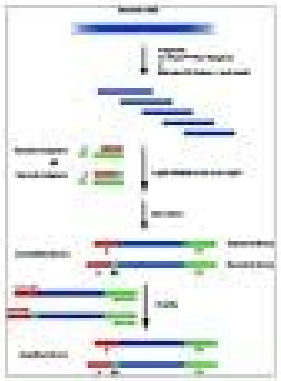


PGS - NGS

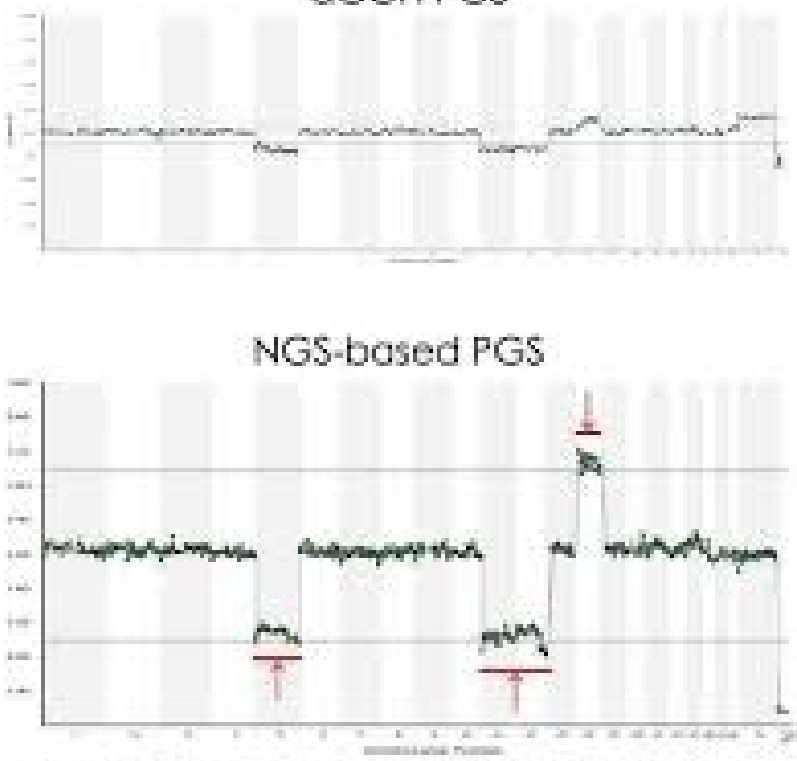
PGS with NGS: Method

Reprogenetics

- Whole Genome Amplification of Sample
- Library preparation:
  - Fragment DNA
  - Ligate adapters and barcodes (≥16)
- Sequence



D Wells, K Kaur, A Rico, I Grifo, S Andersen, J Sherlock, JC Taylor, S Munne (2013) ESHRE



NGS-based PGS is an easier and faster method for physicians to find out the healthy embryo for the patients.

RAZLIČITI ASPEKTI PGS – KADA GA RADITI?

D5 – BLASTOCISTA, MANJE MOZAICIZMA, MANJA TRAUMA ZA EMBRION, UZ BRZE REZULTATE OPCIJE SVEŽ I KRIO ET

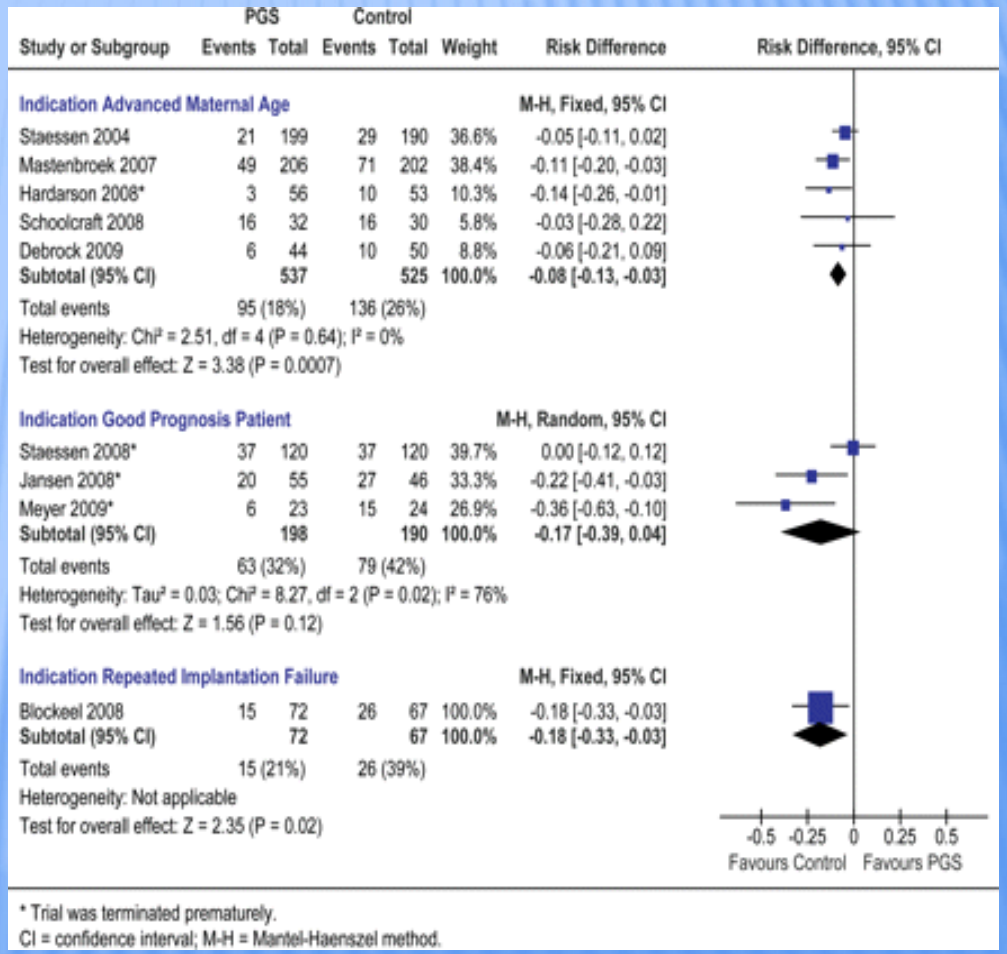
Table 3. Advantages and drawbacks of different embryo-stage biopsies

Biopsy stage	Polar body (oocyte)	Day 3 blastomere	Day 5–6 trophectoderm
Advantages	• no effect on development • ample time for genetic testing • excellent for maternal origin • avoids legal and ethical concerns	• low number of cells required • all indications • time for genetic test	• low number to test • more cells available • all indications • less mosaicism
Drawbacks	• high number tested • sequential biopsy • no information on mutations of paternal origin	• mosaicism • ADO • possible lower implantation rates	• blastocyst culture • needs vitrification • expertise required

ISTORIJAT PGS

Mastenbroek et al, HRU, 2011
Manja stopa trudnoća nakon PGS-a D3
18% NASPRAM 26%

- Prvi rezultati obeshrabrujući (FISH, D3, problemi sa kultivacijom, suboptimalna krioprezervacija).
- Visoka stopa mozaicizma 3. dana



Klinički rezultati sa prvim PGS cikluslima razočaravajući ...

Gotovo napušten PGS

Onda je usledio napredak
CGH
VITRIFIKACIJA
BLASTOCIST KULTIVACIJA

A ONDA ...

- Kultura do blastociste
- Prelazak na biopsiju trofoektoderma.
- Unapređenje CGH, microarray
- Unapređenje vitrifikacije
- Manji mozaicizam na nivou blastociste
- Bolji rezultati sa D5 PGS i povratak procedure na velika vrata

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doi:10.1093/humrep/deu163

human reproduction OPINION

Preimplantation genetic screening: back to the future

Sebastiaan Mastenbroek* and Sjoerd Repping

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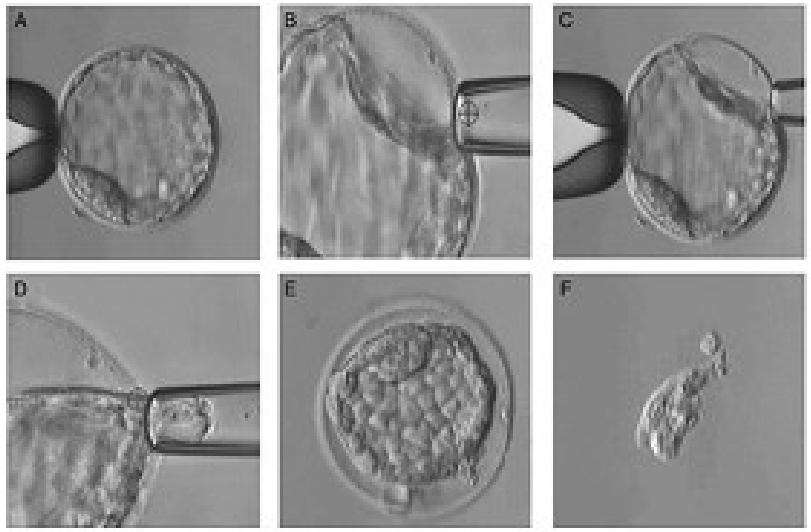
Submitted on April 1, 2014; resubmitted on May 9, 2014; accepted on June 3, 2014

Blastocyst biopsy: LAH on D5



Capalbo et al., HR, 2014

PRAVI IZBOR

BIOPSIJA TROFOEKTODERMA UZ CGH TRENUTNI STANDARD

- OBEĆAVAJUĆI USPEH
- NEDOSTAJU DODATNE STUDIJE
- JASNO DEFINISATI GRUPU PACIJENATA KOJI MOGU OČEKIVATI BENEFIT
- SMANJUJE VREME DO TRUDNOĆE I NEPOTREBNE NEUSPEHE I POBAČAJE

Clinical application:
Blastocyst biopsy for PGD SGD

	D5 biopsy/ D6 transfer	D5 biopsy/ Vitrification	D5 biopsy vitrified/ D6 transfer
Cycles treated	177	40	13
diagnosed	93%	90%	92%
Cycles to transfer	113	34	13
Implantation rate	48.8%	50%	46%
Pregnancies/ transfer	51.3%	70.5%	63%
	McArthur *et al*. (2008) Prenat Diagn	Chang *et al*. (2013) Hum Reprod	Lathi *et al*. (2012) RBMOnline

Clinical application:
Blastocyst biopsy, CGH and vitrification

	Cycles	Mat. Age	Prev. Failed Cycles	Embryos Replaced	Implantation (+sac)
CGH:	45	37.7	2.4	2.0	72%
Control:	113	37.1	1.2	2.7	46%
					p=0.03

Schoolcraft et al., FS, 2010

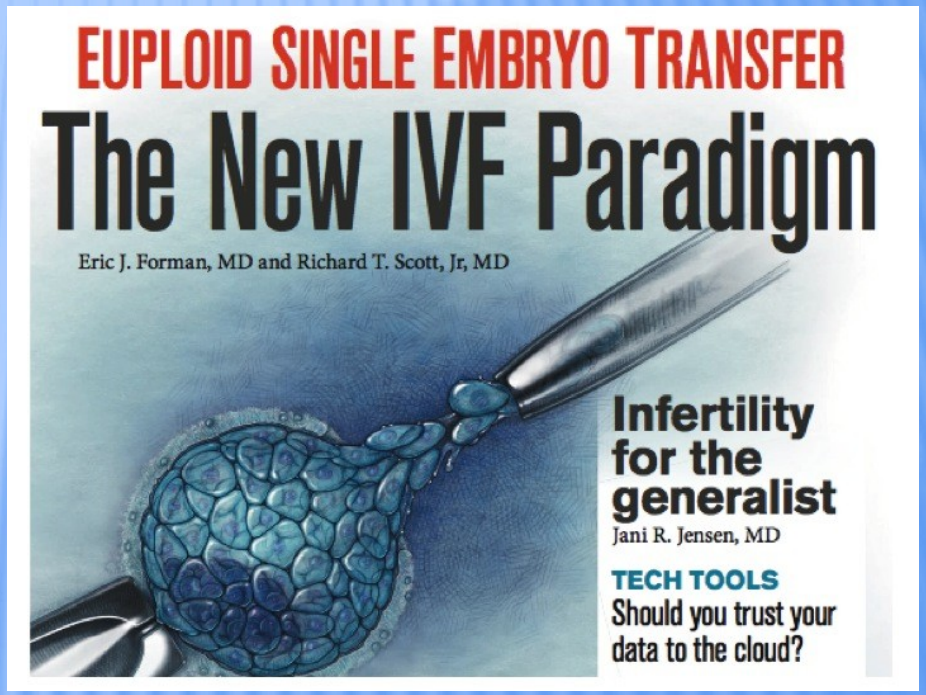
Randomised Trial:
<35, blastocyst biopsy, CGH, fresh transfer

	Control	CGH	
Patients	48	55	
Maternal age	<35	<35	
Biopsy on D5	No	Yes	
Transfer on	Day 6	Day 6	
Embryos euploid (N)	n/a	53.2% (425)	
Embryos replaced (aver)	48 (1)	55 (1)	
Pregnancy rate (sac)	45.8%	70.9%	p=0.017
Ongoing pregnancy rate	41.7%	69.1%	p=0.009
Multiple pregnancies	0	0	

Yang et al., Molec Reprod, 2012

KOME RADITI PGS?

- Pacijenti koji su u IVF postupku u starijoj starosnoj dobi (38 ili starije),
- Pacijenti sa učestalim IVF neuspehom, bilo koje starosne dobi (3 ili vise neuspelih pokušaja),
- U svrhu screening-a na porodično prenosive genetske bolesti
- Pacijenti koji su nosioci hromozomskih translokacija
- Pacijenti koji imaju učestale pobacaje



PREDUSLOVI ZA IMPLEMENTACIJU PGS-A

- ODLIČNI LABORATORIJSKI USLOVI
- RUTINSKA KULTIVACIJA DO BLASTOCISTE
- VISOKO USPEŠAN KRIO-VITRIFIKACIONI PROGRAM
- OBUČEN KADAR – GINEKOLOG, EMBRIOLOG, GENETIČAR
- DOBRA KOORDINACIJA I MULTIDISCIPLINARAN RAD
- ADEKVATNA OPREMLJENOST – GENETSKA LABORATORIJA – SEKVNCIONIRANJE, CGH, NGS ...
- LEGISLATIVNI PREDUSLOVI

PGS U SRBIJI – NAŠI REZULTATI

- **2015** započet program (inkluzioni kriterijumi 2 ili više neuspešnih pokušaja, habitualne pobačaje ili starije od 38. godina).

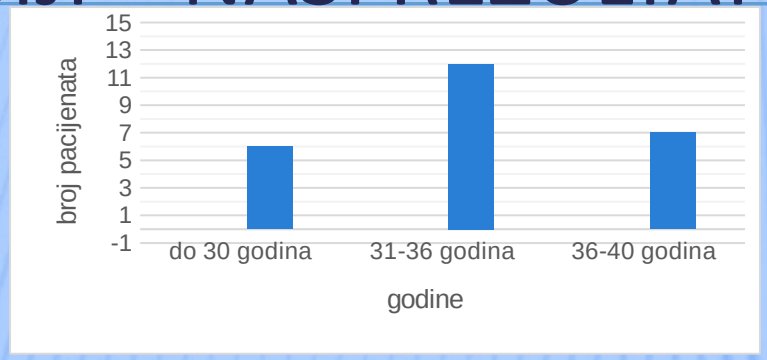
- **GAK Kliničkog centra Vojvodine**, uzorkovane su blastomere ili blastociste
- Preimplantacione genetske analize po protokolu 24sure za aneuploidije su urađene na **Institutu za zdravstvenu zaštitu dece i omladine Vojvodine.**

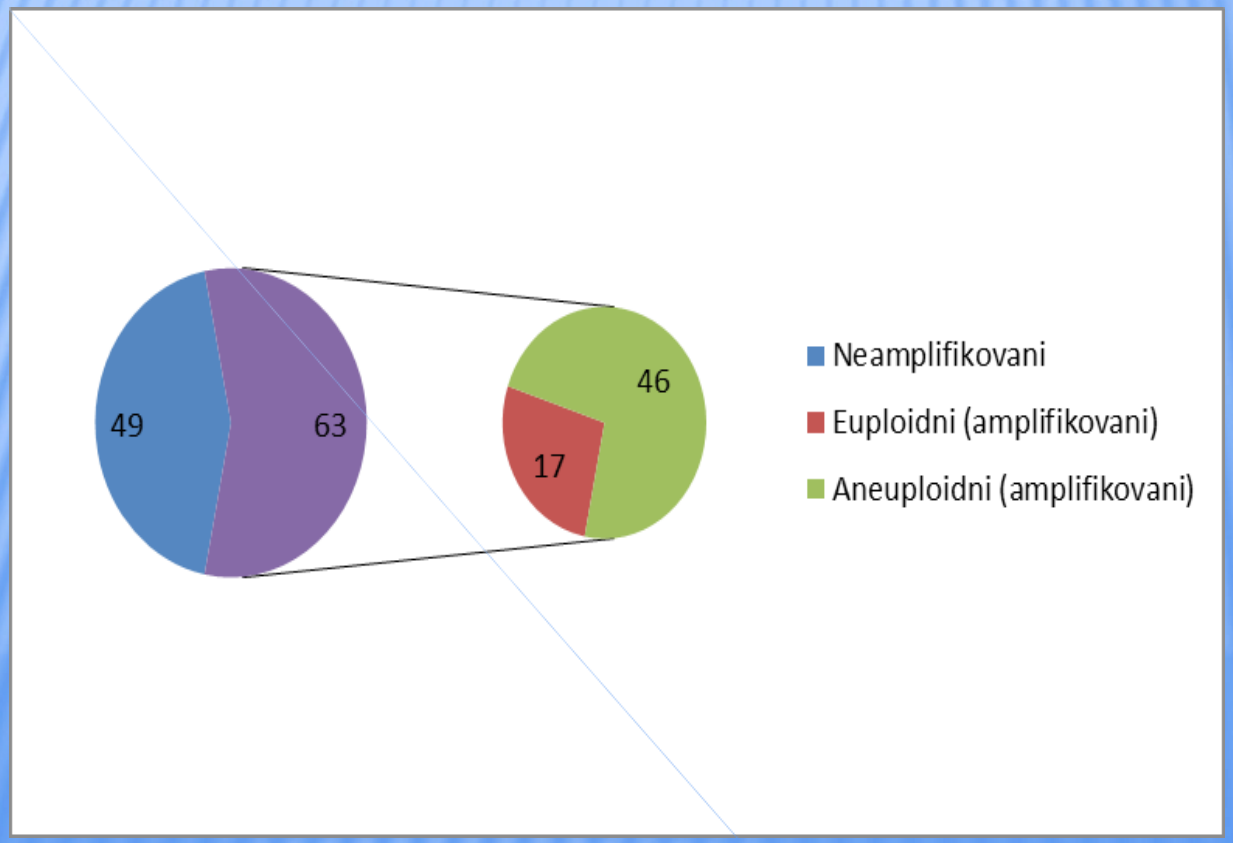
- **24 polarna tela**
- **80 blastomera**
- **5 biopsija trofoektoderma**

- Amplifikacija je bila uspešna kod 63 od 109 uzoraka.
- 46 uzoraka nije uspešno amplifikovano u početnim ciklusima.
- **Aneuploidni nalazi** su dobijeni kod **46 uzoraka (73,02%).**
- **Sve pacijentkinje su imale neki ili sve aneuploidne embrione**.
- Euploidni nalazi su dobijeni kod 17 uzoraka (26,98 %), što ih je kvalifikovalo za eventualni transfer.

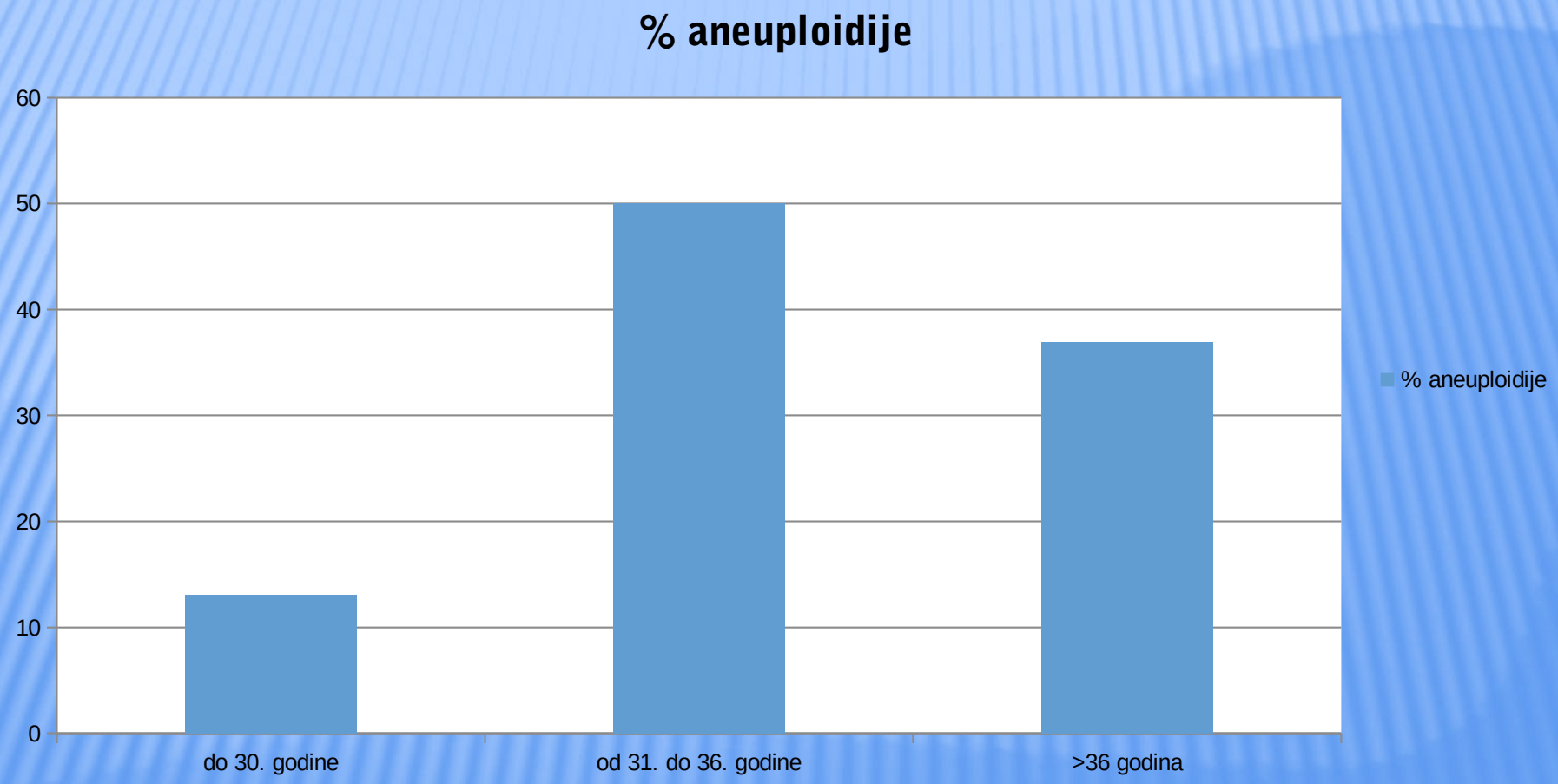
- Procenat euploidnih nalaza nakon preimplantacionog genetskog skrininga se uklapa u podatke iz literature.

PGS U SRBIJI – NAŠI REZULTATI, 2015.





GODINE I ANEUPLIDIJE – OČEKIVANI REZULTATI



ŠTA ISKRENO MOŽEMO OČEKIVATI OD PGS-A

Kraći put do cilja

Ušteda vremena, smanjenje anksioznosti i bremena neuspeha!

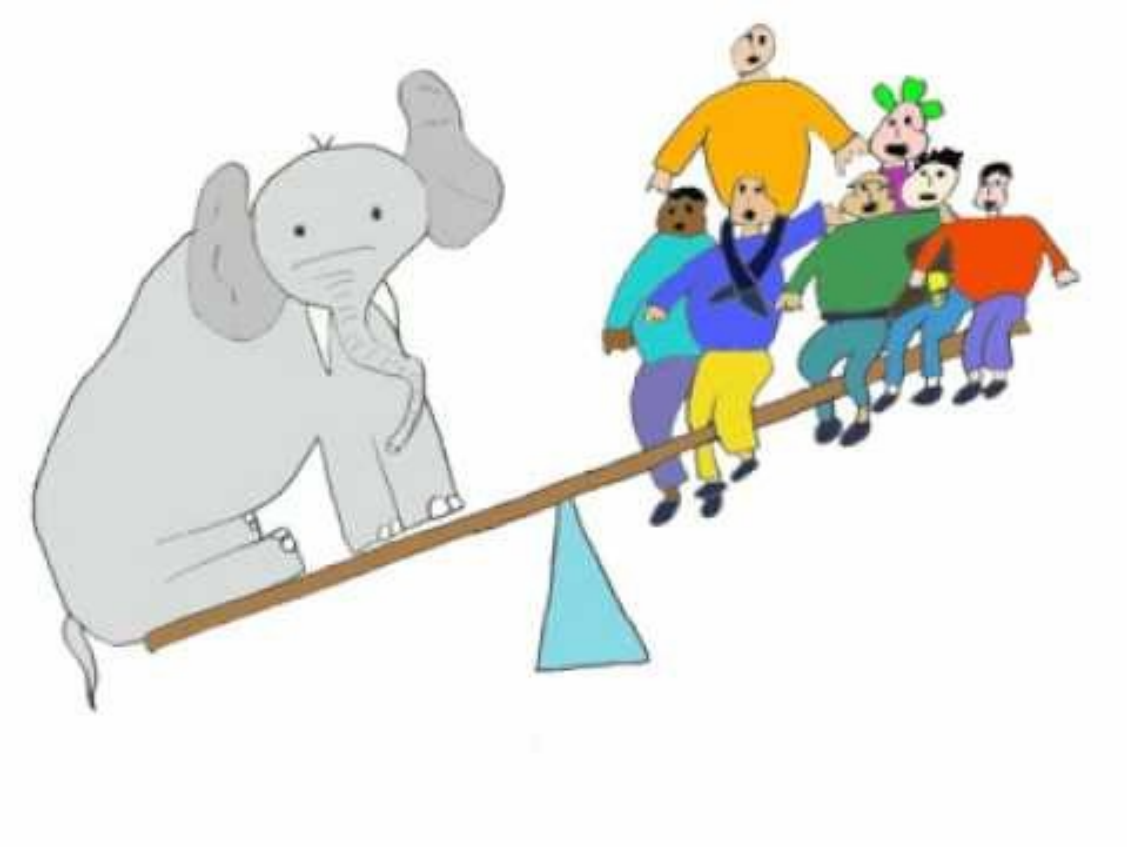
Da li to znači da je on rešenje za sve?

Ko će sve to da plati?

KLJUČ NEUSPEHA

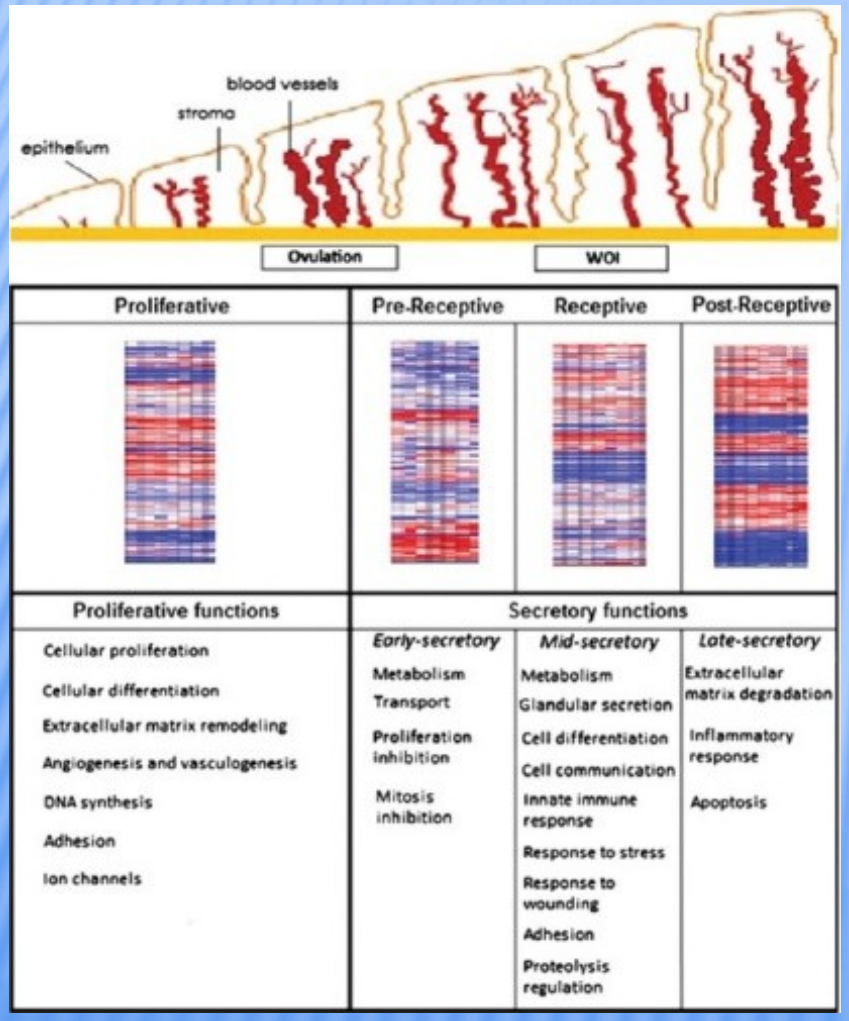
Embrion (80%?) - PGS

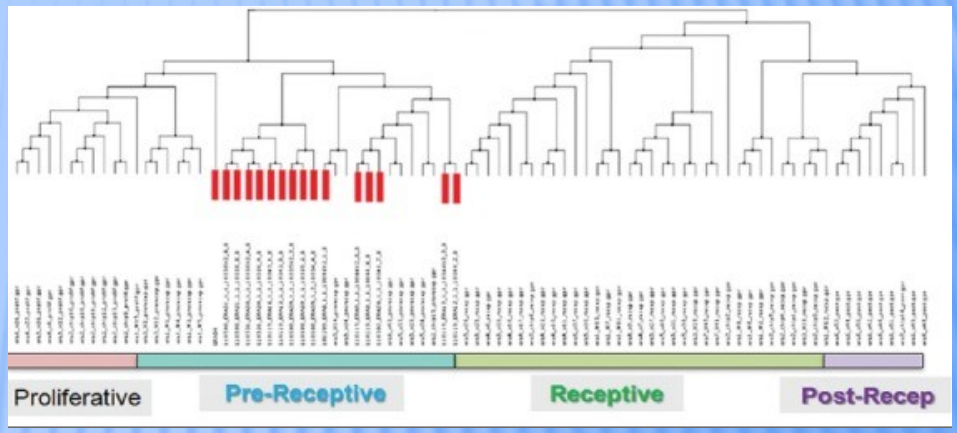
Endometrijum (20%?) - ERA



ERA, DA LI SMO NAŠLI PROZOR IMPLANTACIJE?

ANALIZOM OKO 250 GENA I NJIHOVE EKSPRESIJE GLEDAMO DA LI JE ENDOMETRIJUM RECPTIVAN





Otvorena ERA genetskih biomarkera optimalizacije uspeha VTO.

Ipak ... Da li imamo podatke koji nam nedvosmisleno ukazuju na benefit testa, i za koju grupu pacijenata

Poslednja tvrdnja odnosi se na sve intervencije u sterilitetu

ERA SE PRE SVEGA PRIMENJUJE ZA REKURENTNI POREMEĆAJ IMPLANTACIJE DA LI SE SLAŽEMO ŠTA JE TO?

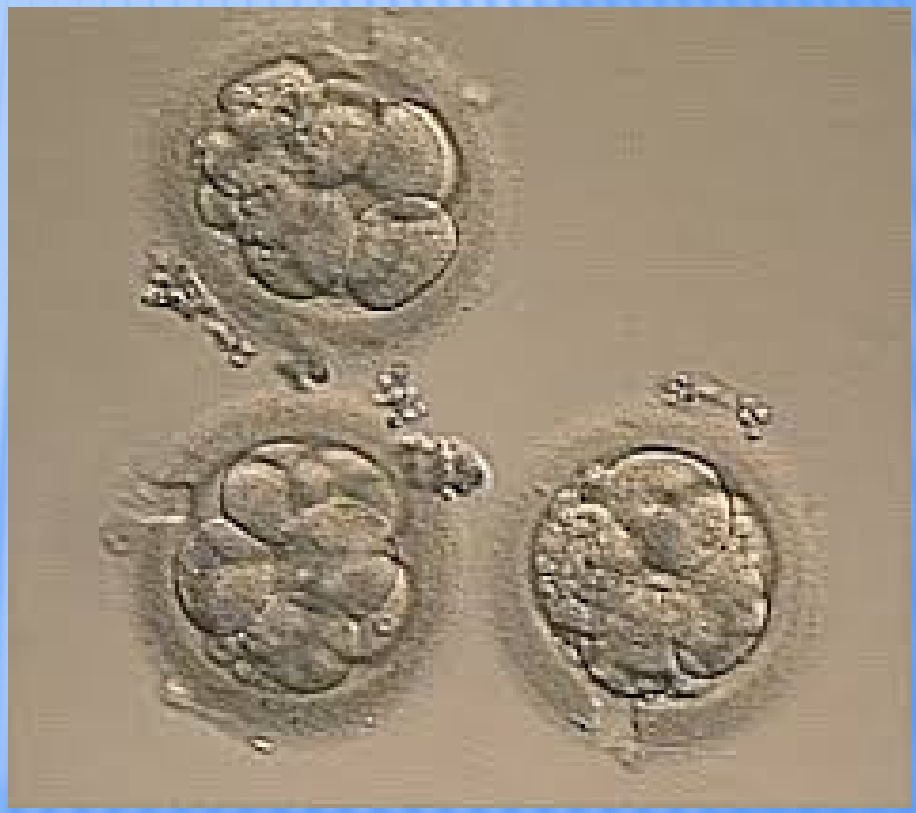
- Recurrent implantation failure (RIF) klinički entitet koji se odnosi na situaciju u kojoj se ni nakon više pokušaja implantacije ne dolazi do stadijuma trudnoće detektabilnog ultrazvukom.
- I dalje ne postoji univerzalno prihvaćena definicija RIF-a, zavisi od broja embriona, ciklusa, ... Što otežava metodologiju naučnoistraživačkog rada
- Das and Holzer, 2012
- Laufer and Simon, 2012
- Penzias, 2012
- Simon and Laufer, 2012a
- Urman et al, 2005

ERA REZULTATI
RUIZ-ALONSO, SIMON. 2013, FERTIL STERIL

- 85 RIF i 25 kontrolnih pacijenata
- Receptivni endometrijum **74.1% RIF** pacijenti i **88% kontrolni** subjekti
- Kod personalizovanog ET u RIF pacijenata 51.7% PR.
- Kod pacijenata sa „pomerenim“ prozorom implantacije, potvrđenim sa 2 ERA testa, kao i sa naknadnim pomeranjem vremena transfera 50.0% PR.
- Preliminarni rezultati.

ASITIRANI „HATCHING“ – AHA

- Asistirani hečing se kao novi metod za povećanje implantacionog potencijala embriona pojavio devedesetih godina (Cohen, 1990).
- Kontradiktorni rezultati
- Nije rutinski preporučljiv svim pacijentima (ASRM, 2014).
- Mali broj studija dokazao korist u stopi živorođenih, čak i gde su stope kliničkih trudnoća bile veće.
- Moguće povećanje stope multiplih trudnoća
- RIF mogući benefit, ali ne veliki



Assisted hatching on assisted conception (in vitro fertilisation (IVF) and intracytoplasmic sperm injection (ICSI)) (Review)

Carney SK, Das S, Blake D, Farquhar C, Seif MM, Nelson L

THE COCHRANE COLLABORATION®

This is a reprint of a Cochrane review, prepared and maintained by The Cochrane Collaboration and published in *The Cochrane Library* 2012, Issue 12

http://www.thecochranelibrary.com

Authors' conclusions

This update has demonstrated that whilst assisted hatching (AH) does appear to offer a significantly increased chance of achieving a clinical pregnancy, the extent to which it may do so only just reaches statistical significance. The 'take home' baby rate was still not proven to be increased by AH. The included trials provided insufficient data to investigate the impact of AH on several important outcomes. Most trials still failed to report on live birth rates.

FAKTORI ZA RAST ENDOMETRIJUMA

- Sildenafil
- Vazodilatatori
- G-CSF (Neupogen)

- Oprečni rezultati, uglavnom bez benefita.
- Nedostaju adekvatne studije
- Samo eksperimentalno

ZADACI ZA BUDUĆNOST U UNAPREĐENJU ART EDUKACIJA EMBRIOLOGA

Master akademske studije

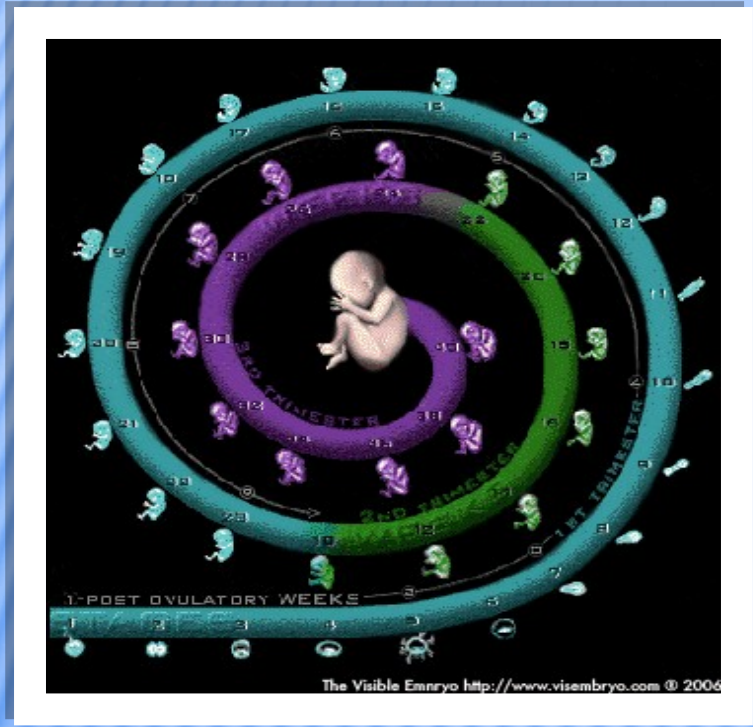
Reproduktivni biolog – embriolog

PMF Departman za biologiju

Medicinski fakultet

Univrezitet u Novom Sadu

BUDUĆNOST VTO



- Banka ovarijalnog tkiva uzimanog od žena u reproduktivnom dobu i IVM širih razmera
- Razvoj veštačke materice?
- Da se etička razmatranja promene i prilagode tehničkom napretku u ovoj oblasti!

KLJUČNE PORUKE

- Kontinuirano unapređenje laboratorijskih uslova
- Ne postoji jedan „čarobni štapić“
- Multidisciplinaran rad
- Iskrenost u implementaciji novina (medicina zasnovana na dokazima, dobro dizajnirane studije)
- Regulacija, kontrola, standardizacija rada, manje marketinga
- Novine doživeti kao komplementarne dosadašnjem tretmanu
- Prikupiti objektivne dokaze o koristi od navedenih procedura
- Definisati grupu pacijenata i kliničke okolnosti u kojima možemo očekivati dobit.

HVALA NA PAŽNJI