

Morfokinetik kriterlere dayalı embriyo seçimi: Bu sadece bir başlangıç mı?



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Time-lapse morfokinetik analiz

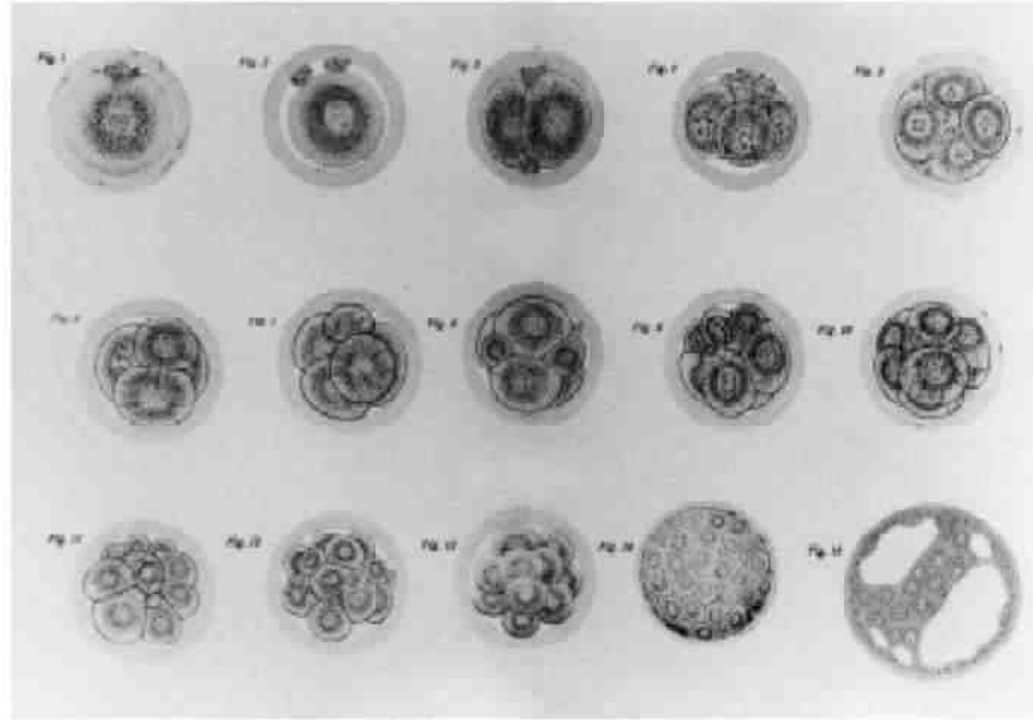


Fig. 1. Plate showing the successive preimplantation stages of the egg in two bat species, *Vesperugo dasycnema* (Fig. 1) and *Myotis* sp. (Fig. 2-15). The eggs were observed in March and April from 1876 (Fig. 1) through 1888 (Figs. 12-15). Reproduced from E. Van Beneden (1911).

Lewis ve Gregory, gelişen tavşan embriyolarını erken klivaj aşamasından blastosist aşamasına kadar büyütüp sinematografik olarak izlemeyi başardı (1929) .

Time-lapse morfokinetik analiz

Reference	Species	Observation(s)
Cassini (1961)	Mouse	Development from 2-cell to blastocyst
Cole (1967)	Mouse	Blastocyst hatching
Brackett (1972)	Rabbit	Fertilization and development to morula
Massip <i>et al.</i> (1982)	Cattle	Blastocyst expansion and hatching
Massip <i>et al.</i> (1983)	Cattle	Atypical blastocyst hatching resulting in twin half-blastocysts
Cohen <i>et al.</i> (1988)	Human	Morphological assessment of cleavage-stage embryos
Selwood and Smith (1990)	Marsupials	Cleavage and development to blastocyst
Gonzales <i>et al.</i> (1995)	Hamster	Cleavage, and timing of cleavage
Gonzales and Bavister (1995)	Hamster	Blastocyst hatching
Gonzales <i>et al.</i> (1996)	Cattle, horse, human	Blastocyst expansion, contraction and hatching, trophoctoderm projections and locomotion of hatched blastocysts
Payne <i>et al.</i> (1997)	Human	Polar body extrusion and pronuclear formation
Holm <i>et al.</i> (1998)	Cattle	Developmental kinetics of early cleavage with respect to viability
Van Blerkom <i>et al.</i> (2001)	Human	Fragmentation and subsequent development
Peippo <i>et al.</i> (2001)	Cattle	Effect of sex and glucose on developmental kinetics
Holm <i>et al.</i> (2002)	Cattle	Effect of serum on developmental kinetics
Lequarre <i>et al.</i> (2003)	Cattle	Effect of oxygen on cell-cycle duration
Mateusen <i>et al.</i> (2005)	Pig	Relationships among developmental kinetics, fragmentation and apoptosis
Zaninovic <i>et al.</i> (2005)	Human	Development from zygote to hatched blastocyst
Mio and Maeda (2008)	Human	Fertilization, development to blastocyst and hatching
Gendelman <i>et al.</i> (2010)	Cattle	Effect of season on developmental kinetics
Lopes <i>et al.</i> (2010)	Cattle	Relationship of cleavage to oxygen consumption
Wale and Gardner (2010)	Mouse	Effect of oxygen on cell-cycle duration

(Don Rieger, 2013)

YÜT Tedavilerinde başarı nelere bağlı?

- Maternal /paternal faktörler
- Optimal Stimulasyon protokolleri/gamet maturasyonu
- Optimal gamet ve embriyo manipulasyonu
- **Optimal kültür şartları**
- **En uygun embriyonun seçimi**
- Başarılı embriyo transferi
- Optimal uterin ortam



Time-lapse
Morfokinetik Değerlendirme

Morfoloji-temelli **STATİK** embriyo seçimi

Human Reproduction, Vol.26, No.6 pp. 1270– 1283, 2011

Advanced Online Publication on April 18, 2011 doi:10.1093/humrep/dar100

Table IV Timing of observations and embryos, and each time point.

Type of observation	Number of embryos
Fertilization check	
Syngamy check	
Early cleavage check	2
Day-2 embryo assessment	4
Day-3 embryo assessment	68
Day-4 embryo assessment	92
Day-5 embryo assessment	116

ICSI, intracytoplasmic sperm injection.

Table VIII Consensus scoring system for blastocysts.

	Grade	Rating	Description
Stage of development	1		Early Blastocyst
	2		Expanded
	3		Hatched/hatching
	4		
ICM	1	Good	Prominent, easily discernible, with many cells that are compacted and tightly adhered together
	2	Fair	Easily discernible, with many cells that are loosely grouped together
	3	Poor	Difficult to discern, with few cells
TE	1	Good	Many cells forming a cohesive epithelium
	2	Fair	Few cells forming a loose epithelium
	3	Poor	Very few cells

The scoring system for blastocysts is a combination of the stage of development, and of the grade of the ICM and of the TE (e.g. an expanded blastocyst with a good ICM and a fair TE would be scored as 312). It is a numerical interpretation of the Gardner scale (Gardner and Schoolcraft, 1999a,b).

Consensus scoring system for cleavage-stage embryos (based on cell number).

Description

- < 10% fragmentation
- Stage-specific cell size
- No multinucleation
- 10–25% fragmentation
- Stage-specific cell size for majority of cells
- No evidence of multinucleation
- Severe fragmentation (>25%)
- Cell size not stage specific
- Evidence of multinucleation

Scoring system for Day-4 embryos

to a fourth round of cleavage. If compaction that involves virtually all cells is observed, the embryo volume. to a fourth round of cleavage. involves the majority of the embryo. late compaction involving less than half of the embryo, with two or three cells forming discrete blastomeres

and, E-mail: ... (Beigem),

(ALPHA., 2011)

YÜT Tedavilerinde başarı nelere bağlı?

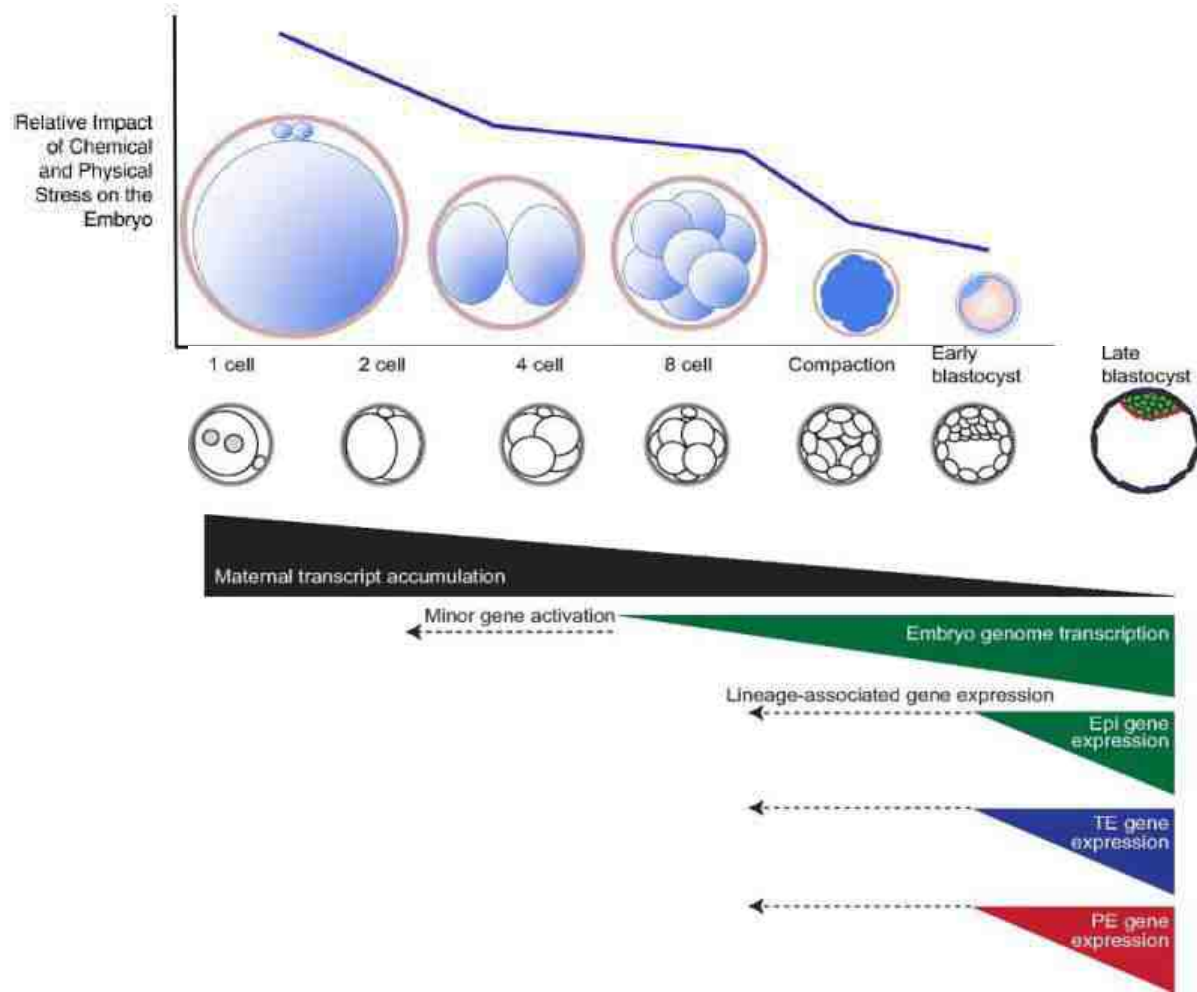
- **Optimal kültür şartları**

Minimum stres
Minimum in vitro kültür
Maksimum in vivo'ya yakın şartlar

- **En uygun embriyonun seçimi**

Statik+Dinamik Seçim
De-selection
Re-selection?

İmplantasyon öncesi embriyo kültürü



(Niakan ve ark., 2012; Wale ve Gardner, 2015)

Morfokinetik kriterlere dayalı embriyo seçimi: Nihai Amaç

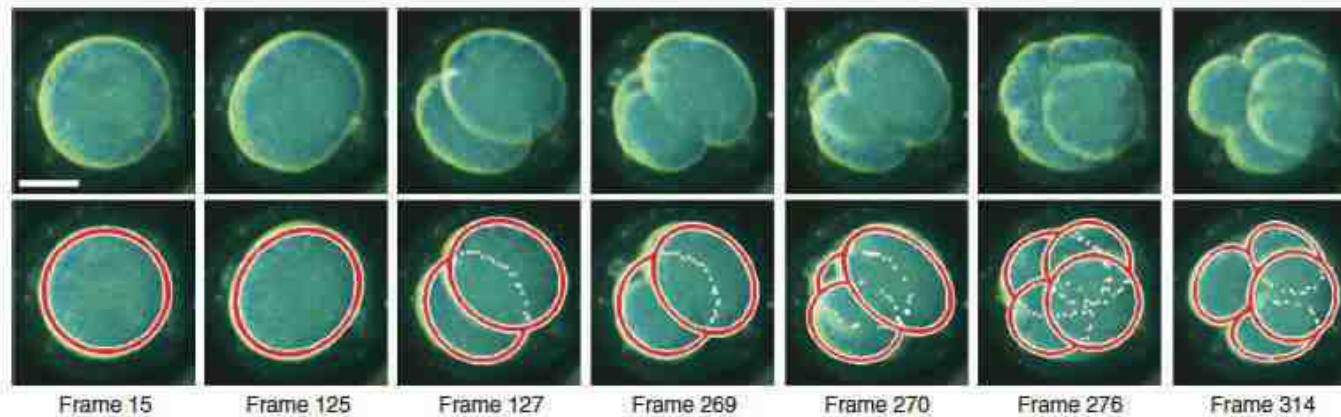
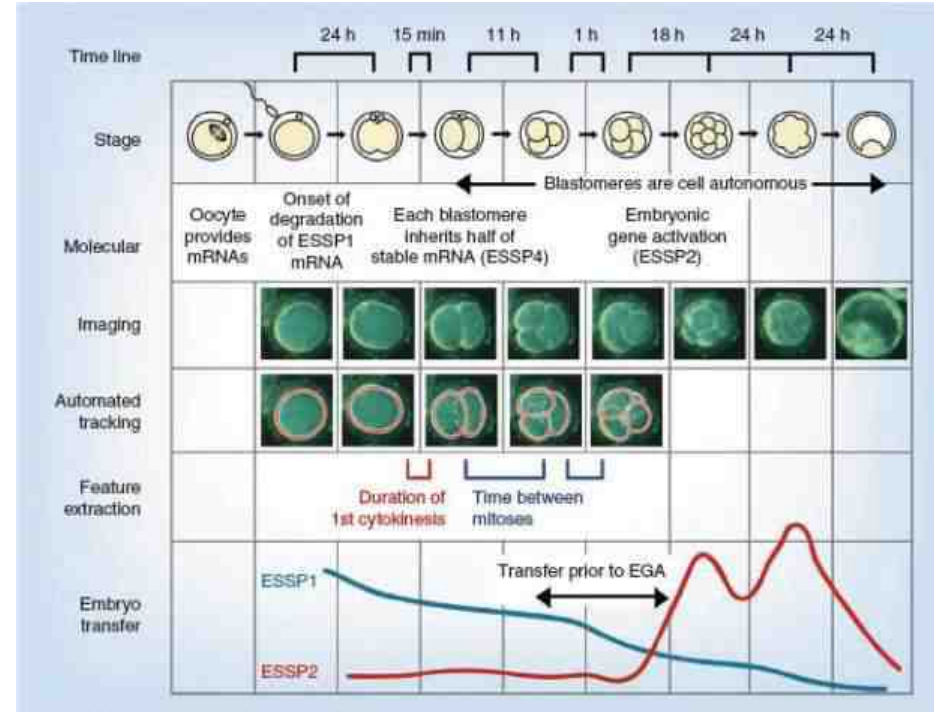
Erken dönem morfokinetik parametrelere göre:

- **Blastosist aşamasına ulaşacak embriyonun seçimi**
- **implante olacak embriyonun seçimi**
- **Canlı doğum ile sonuçlanacak embriyonun seçimi**

Non-invasive imaging of human embryos before embryonic genome activation predicts development to the blastocyst stage

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We report studies of preimplantation human embryo development that correlate time-lapse image analysis and gene expression profiling. By examining a large set of zygotes from *in vitro* fertilization (IVF), we find that success in progression to the blastocyst stage can be predicted with >93% sensitivity and specificity by measuring three dynamic, noninvasive imaging parameters by day 2 after fertilization, before embryonic genome activation (EGA). These parameters can be reliably monitored by automated image analysis, confirming that successful development follows a set of carefully orchestrated and predictable events. Moreover, we show that imaging phenotypes reflect molecular programs of the embryo and of individual blastomeres. Single-cell gene expression analysis reveals that blastomeres develop cell autonomously, with some cells advancing to EGA and others arresting. These studies indicate that success and failure in human embryo development is largely determined before EGA. Our methods and algorithms may provide an approach for early diagnosis of embryo potential in assisted reproduction.



The use of morphokinetics as a predictor of embryo implantation[†]

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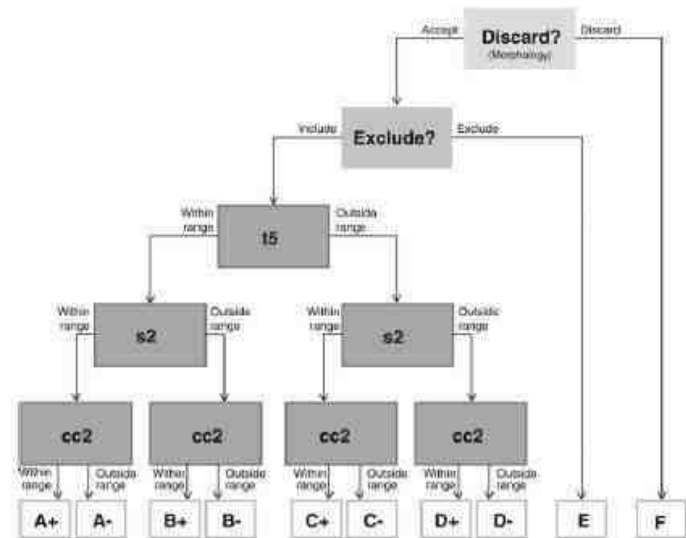
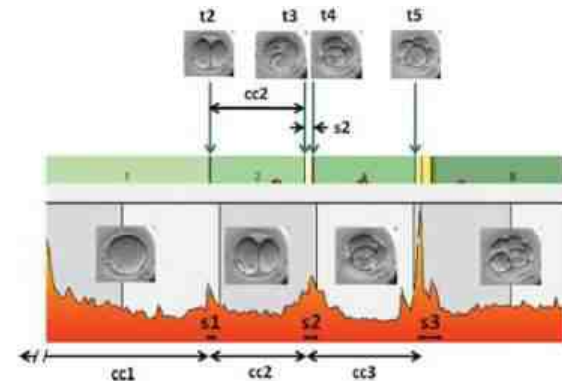
BACKGROUND: Time-lapse observation presents an opportunity for optimizing embryo selection based on morphological grading as well as providing novel kinetic parameters, which may further improve accurate selection of viable embryos. The objective of this retrospective study was to identify the morphokinetic parameters specific to embryos that were capable of implanting. In order to compare a large number of embryos, with minimal variation in culture conditions, we have used an automatic embryo monitoring system.

METHODS: Using a tri-gas IVF incubator with a built-in camera designed to automatically acquire images at defined time points, we have simultaneously monitored up to 72 individual embryos without removing the embryos from the controlled environment. Images were acquired every 15 min in five different focal planes for at least 64 h for each embryo. We have monitored the development of transferred embryos from 285 couples undergoing their first ICSI cycle. The total number of transferred embryos was 522, of which 247 either failed to implant or fully implanted, with full implantation meaning that all transferred embryos in a treatment implanted.

RESULTS: A detailed retrospective analysis of cleavage times, blastomere size and multinucleation was made for the 247 transferred embryos with either failed or full implantation. We found that several parameters were significantly correlated with subsequent implantation (e.g. time of first and subsequent cleavages as well as the time between cleavages). The most predictive parameters were: (i) time of division to 5 cells, t5 (48.8–56.6 h after ICSI); (ii) time between division to 3 cells and subsequent division to 4 cells, s2 (<0.76 h) and (iii) duration of cell cycle two, i.e. time between division to 2 cells and division to 3 cells, cc2 (<11.9 h). We also observed aberrant behavior such as multinucleation at the 4 cell stage, uneven blastomere size at the 2 cell stage and abrupt cell division to three or more cells, which appeared to largely preclude implantation.

CONCLUSIONS: The image acquisition and time-lapse analysis system makes it possible to determine exact timing of embryo cleavages in a clinical setting. We propose a multivariable model based on our findings to classify embryos according to their probability of implantation. The efficacy of this classification will be evaluated in a prospective randomized study that ultimately will determine if implantation rates can be improved by time-lapse analysis.

Key words: embryo / cell division / pregnancy / exact timing / time-lapse



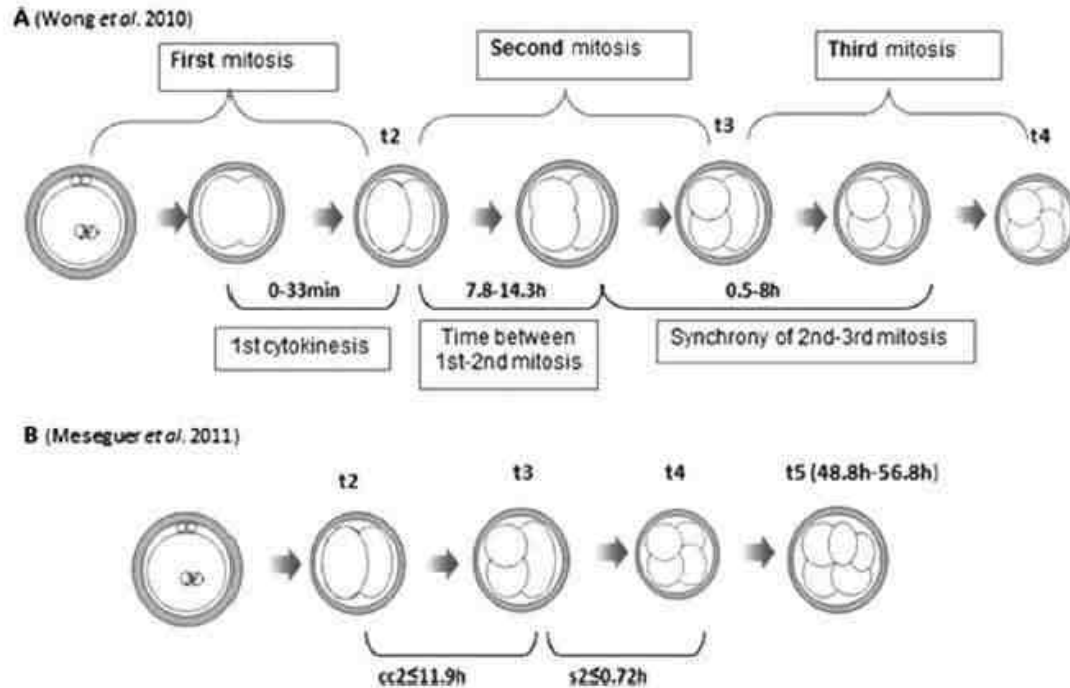
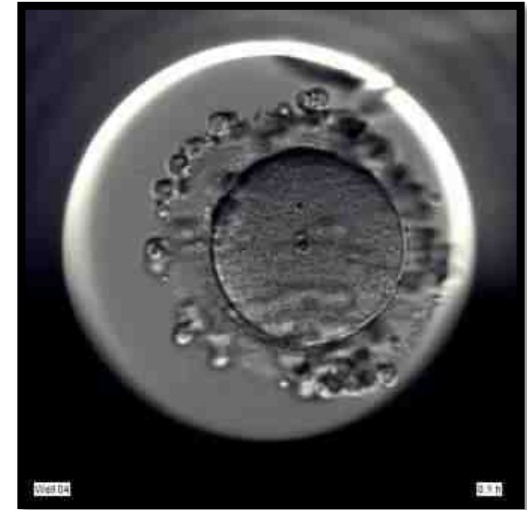
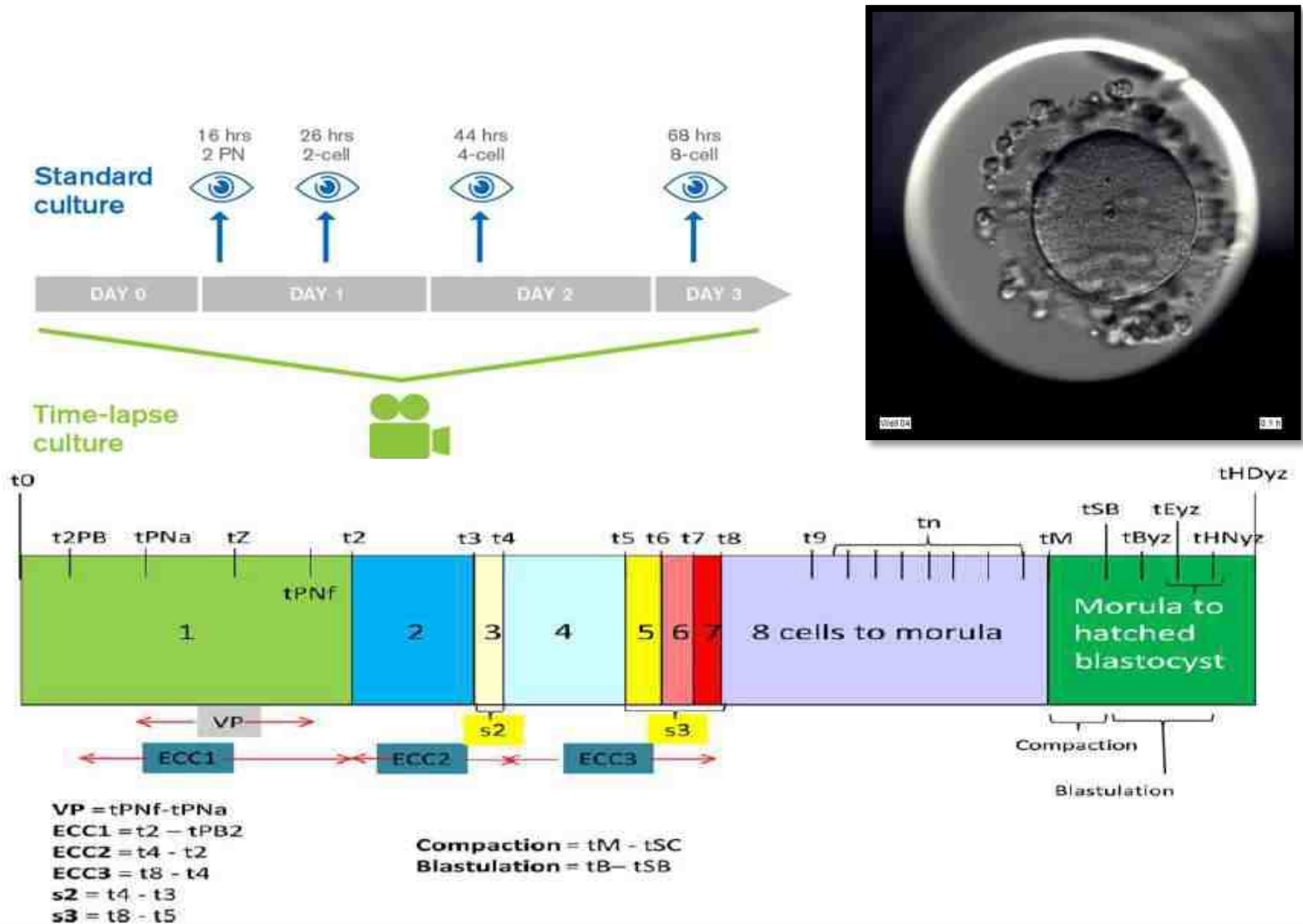


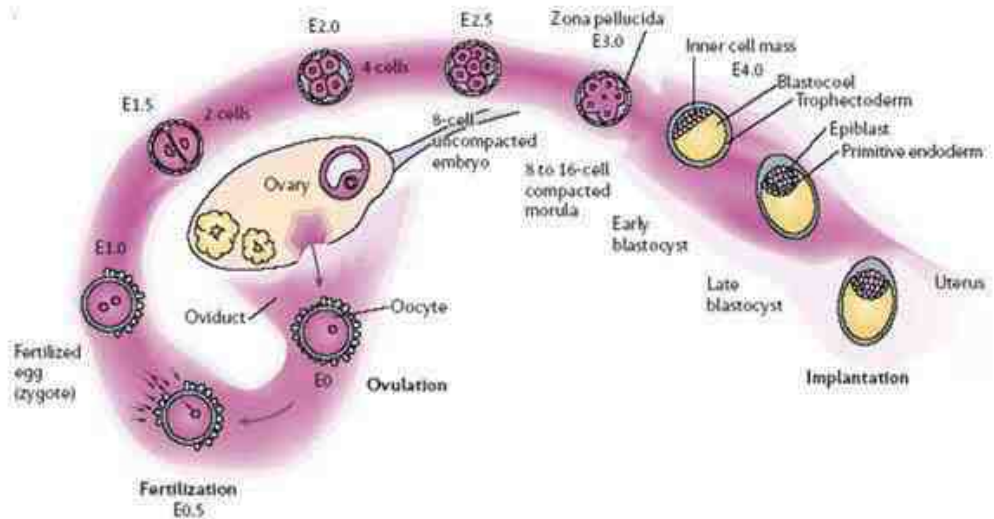
Figure 2 Comparison of two algorithms designed to select the best embryos with high implantation potential. (A) Algorithm designed by Wong et al. (2010). Embryos that reached the blastocyst stage could be predicted according to timings of three parameters: the timing from the appearance of a cleavage furrow to complete cell separation of first mitosis; time from the completion of the first mitosis to the appearance of cleavage furrow of the second mitosis; timing between the appearance of the cleavage furrows of the second and third mitosis. (B) Algorithm designed by Meseguer et al. (2011). Embryos are morphologically evaluated, excluded according to criteria, and then observed for the exact times from cleavage to cc2, s2 and t5. cc2 = duration of second cell cycle ($t_3 - t_2$); s2 = synchrony in cleavage from 3 to 4 cells ($t_4 - t_3$); t5 = time of cleavage to a 5-cell embryo.

Morfokinetik-temelli DİNAMİK embriyo seçimi



(Ciray ve ark., 2014)

Güncel Durum



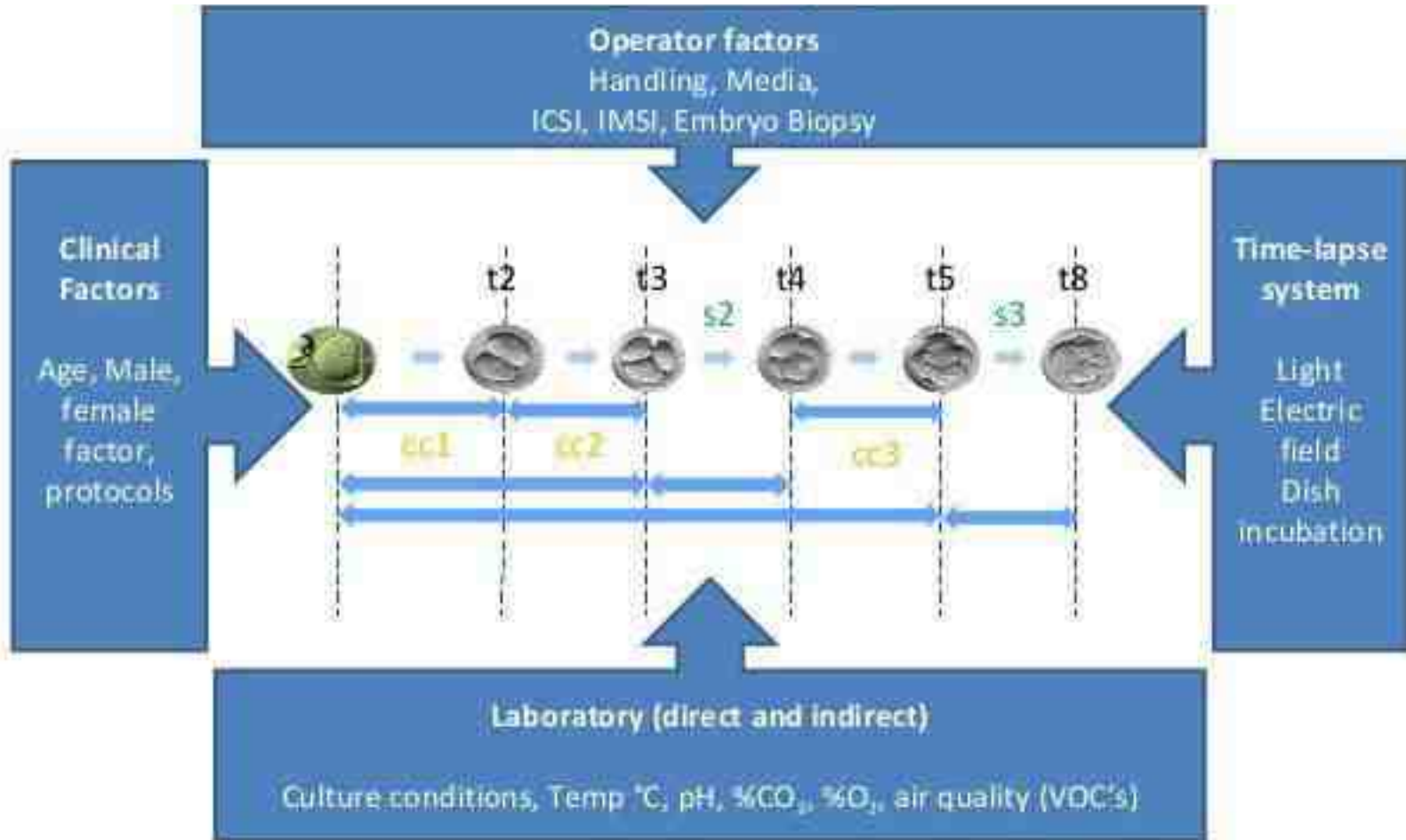
Güncel Durum



Time Lapse Monitoring Systems	Studies
TLM Analysis chambers that are built around an inverted microscope	
Olympus IX-70 inverted microscope equipped with an environmental chamber	(Payne et al. 1997)
Nikon Diapot 300 microscope with camera in a closed system	Lemmen et al. (2008)
Olympus IX-71 inverted microscope acrylic resin environmental chamber	Mio and Maeda (2008)
Olympus IX-70/71 microscopes with an aperture for dark-field illumination	Wong et al. (2010)
Inverted digital microscopes with LED illumination modified for dark field illumination.	Chavez et al. (2012)
Nikon TiU epifluorescence microscope with a 20 0.75-NA objective	Swann et al., 2012
Modified incubators that have flexible and customized microscopes for TLM Analysis	
Biostation CT (Nikon Corporation)	Hashimoto et al. (2012)
Sanyo MCOK-5 In vitro Live Cell Imaging Inc. System	Nakahara et al., 2010
Modular image/video capture systems that can be placed in conventional incubators	
PrimoVision (Cryo-Innovation)	Hlinka et al. 2008; 2012; Pribenszky et al., 2010; Knez et al., 2013
Eeva (Early Embryo Viability Assessment)	Conaghan et al., 2013
All-in-one systems	
EmbryoScope (Unisense Fertilitech, Denmark)	Meseguer et al. 2011; 2012; Rubio et al. (2012); Azzarello et al., 2012; Dal Canto et al., 2012; Kirkegaard et al. 2013a, 2013b; Chamayou et al. (2013); Freour et al., 2013; Cruz et al., 2012; Ciray et al., 2012; Basile et al., 2013; Cruz et al., 2013; Kirkegaard et al. (2013)(c); Bellver et al., 2013; Serdarogullari M et al., 2014; Bronet et al., 2014; Munoz et al., 2012

(Serdarogullari ve ark., 2015-in press)

Embriyo gelişimini etkileyen dış faktörler



Time-lapse in the IVF-lab: how should we assess potential benefit?

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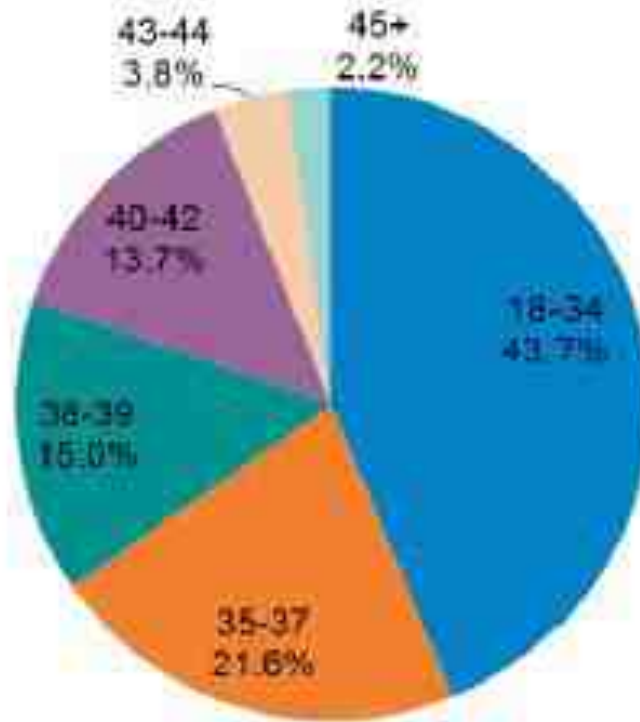
Submitted on July 27, 2014; resubmitted on July 27, 2014; accepted on August 21, 2014

ABSTRACT: Time-lapse imaging of embryos has been widely introduced to fertility laboratories worldwide with the aim of identifying the best quality embryos to transfer that will ultimately improve IVF success rates. In this opinion paper, we explore the lack of evidence of benefit of this novel intervention, analyse the methodological flaws of current studies, offer ideal study designs that assess the various features of time-lapse imaging, and discuss forthcoming studies. In particular, we emphasize the ethical aspects of hastily adopting a costly technology without current high level evidence of improved live birth rates, safety and cost effectiveness.

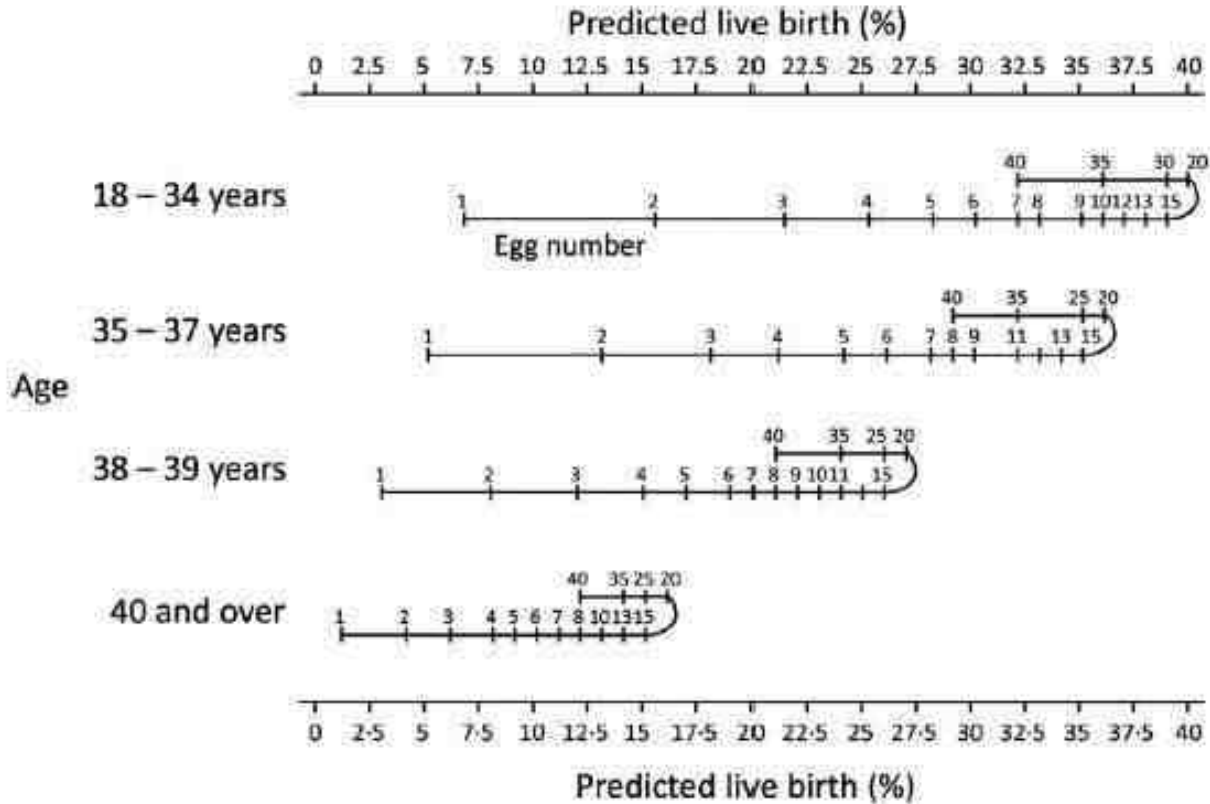
Key words: IVF / time-lapse / live birth / IVF outcome / embryo incubation

Yaş gruplarına göre siklus dağılımı-UK (2013)

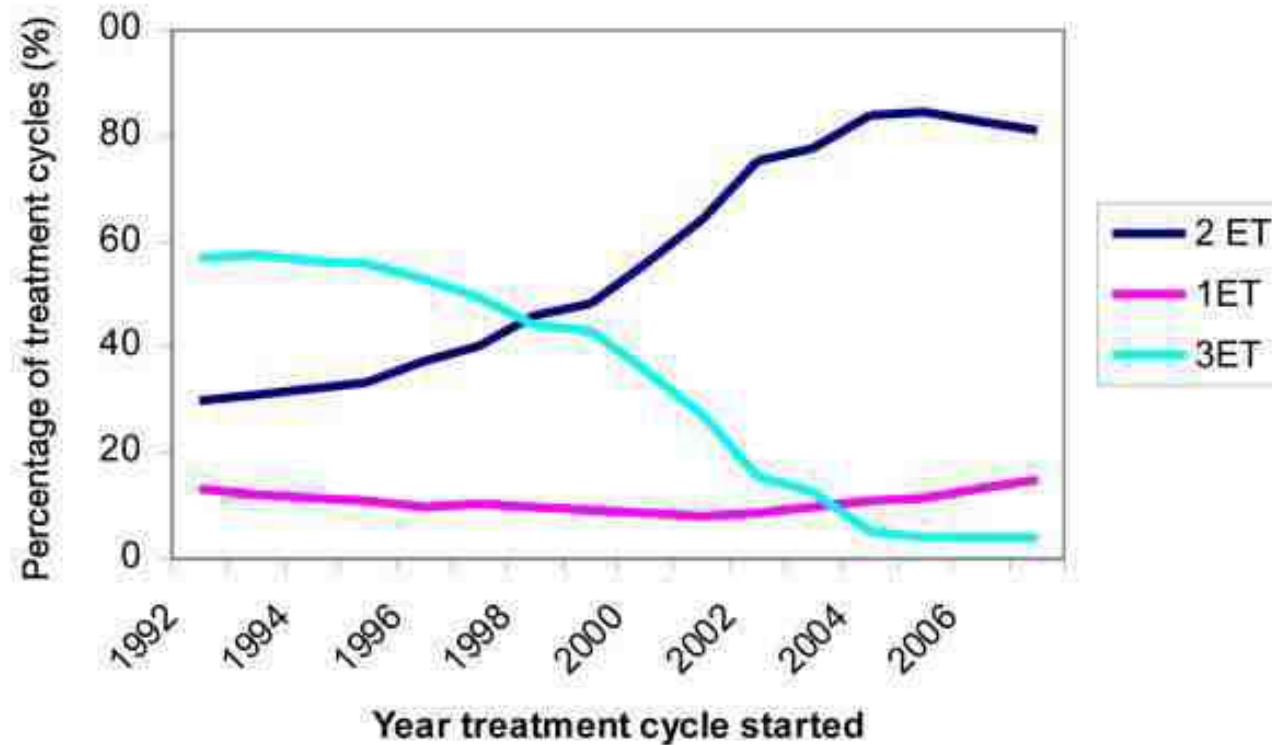
Percentage of all IVF cycles performed by age group (2013)



Morfolojiye göre embriyo seçimi: Ne kadar gerçekçi?



Transfer edilen embriyo sayıları (1992-2007) - UK



Morfokinetik Analiz – Kromozomal anomali ilişkisi

Reproductive BioMedicine Online (2013) 26, 528–530



www.sciencedirect.com
www.rbmonline.com



COMMENTARY

Morphokinetics and embryo aneuploidy: has time come or not yet?

Markus Montag

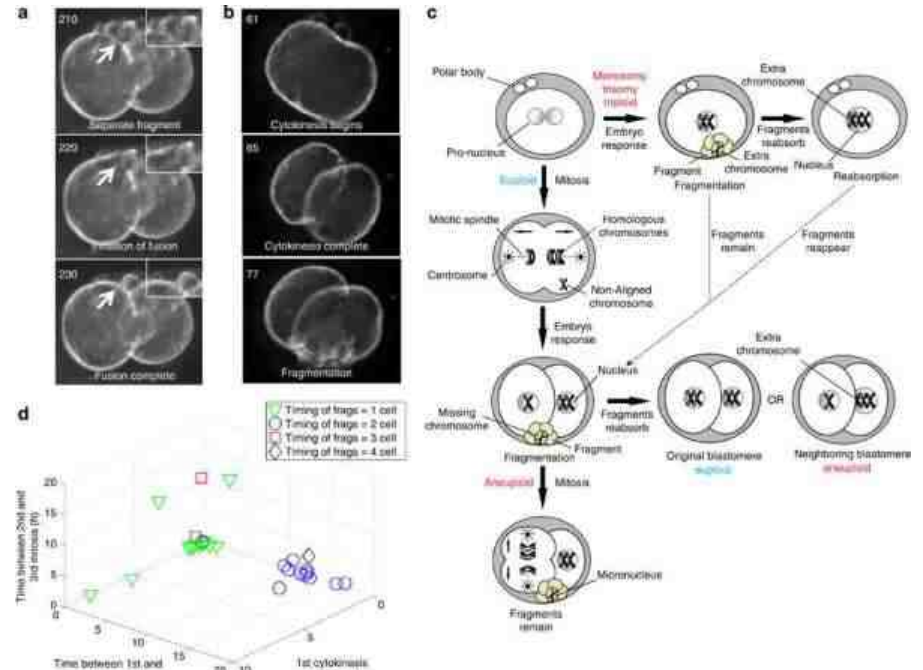
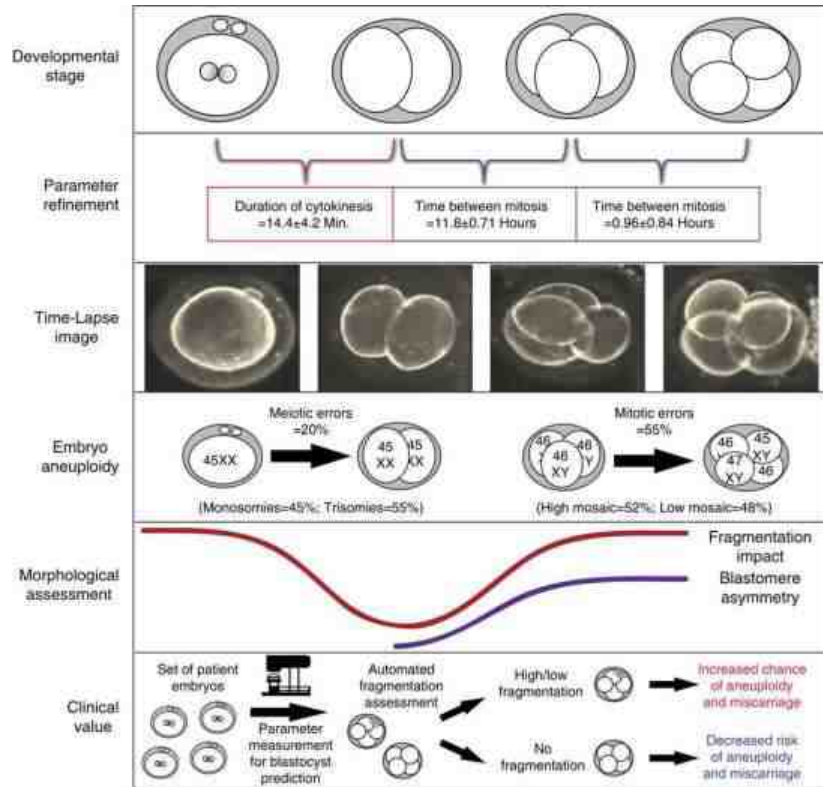
Korelasyon var

Campbell ve ark. 2013
Basile ve ark. 2014

Korelasyon yok

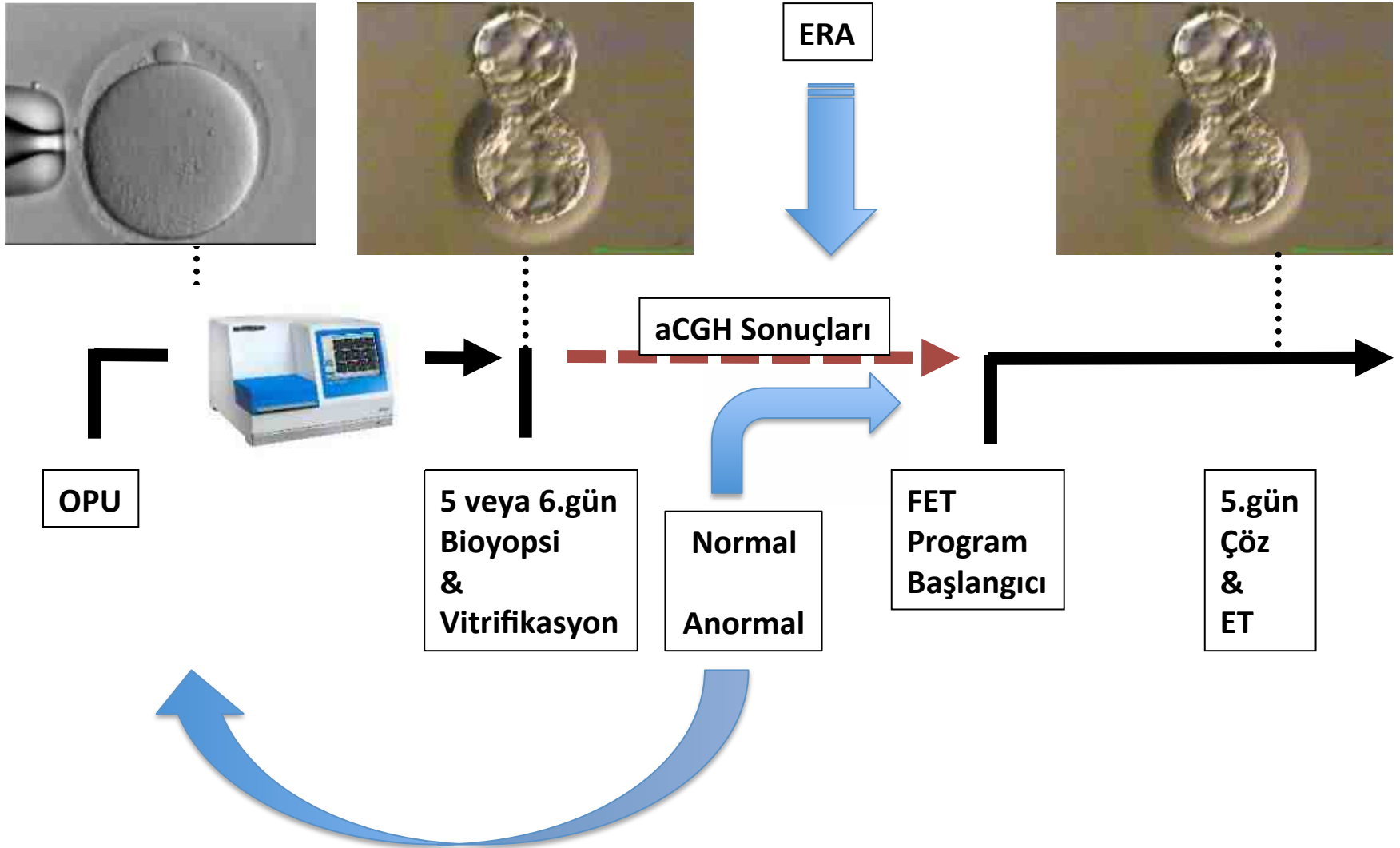
Rienzi ve ark. 2014
Ottolini ve ark. 2014
Kramer et al. 2014

Morfokinetik Analiz – Kromozomal anomali ilişkisi



(Chavez ve ark., 2012-Nature)

İdeal senaryo



De-selection

Desai et al. *Reproductive Biology and Endocrinology* 2014, **12**:54
<http://www.rbje.com/content/12/1/54>



RESEARCH

Open Access

Analysis of embryo morphokinetics, multinucleation and cleavage anomalies using continuous time-lapse monitoring in blastocyst transfer cycles

Nina Desai^{1*}, Stephanie Ploskonka, Linnea R Goodman, Cynthia Austin, Jeffrey Goldberg and Tommaso Falcone

Abstract

Background: Time-lapse imaging combined with embryo morphokinetics may offer a non-invasive means for improving embryo selection. Data from clinics worldwide are necessary to compare and ultimately develop embryo classifications models using kinetic data. The primary objective of this study was to determine if there were kinetic differences between embryos with limited potential and those more often associated with in vitro blastocyst formation and/or implantation. We also wanted to compare putative kinetic markers for embryo selection as proposed by other laboratories to what we were observing in our own laboratory setting.

Methods: Kinetic data and cycle outcomes were retrospectively analyzed in patients age 39 and younger with 7 or more zygotes cultured in the Embryoscope. Timing of specific events from the point of insemination were determined using time-lapse (TL) imaging. The following kinetic markers were assessed: time to syngamy (tPNf), 12; time to two cells (t2), 3c (t3), 4c (t4), 5c (t5), 8c (t8), morula (tMor), start of blastulation (tSB), tBL, blastocyst (tBL); expanded blastocyst (tEBL). Durations of the second (cc2) and third (cc3) cell cycles, the t5-t2 interval as well as time to complete synchronous divisions s1, s2 and s3 were calculated. Incidence and impact on development of nuclear and cleavage anomalies were also assessed.

Results: A total of 648 embryos transferred on day 5 were analyzed. The clinical pregnancy and implantation rate were 72% and 50%, respectively. Morphokinetic data showed that tPNf, t2,t4, t8, s1, s2,s3 and cc2 were significantly different in embryos forming blastocysts (ET or frozen) versus those with limited potential either failing to blastulate or else forming poor quality blastocysts ultimately discarded. Comparison of embryo kinetics in cycles with all embryos implanting (KID+) versus no implantation (KID-) suggested that markers of embryo competence to implant may be different from ability to form a blastocyst. The incidence of multinucleation and reverse cleavage amongst the embryos observed was 25% and 7%, respectively. Over 40% of embryos exhibiting these characteristics did however form blastocysts meeting our criteria for freezing.

Conclusions: These data provide us with a platform with which to potentially enhance embryo selection for transfer.

Keywords: Blastocyst, Time-lapse, Morphokinetic parameters, Implantation, Embryo development, Pregnancy, Embryoscope, Cleavage

Frequency of embryo multinucleation detected by time-lapse system and its impact on pregnancy outcome

Elif G. Ergin, M.D., Eray Caliskan, M.D., Ender Yalcinkaya, M.Sc., Zeynep Öztepe, B.Sc., Kevser Çokeles, B.Sc., Alev Özyay, M.D., and Hakan M. Özörnek, M.D.

Eurofertil IVF Center, Istanbul, Turkey

Objective: To compare the detection rate of multinucleation with the time-lapse system and conventional control timing proposed by European Society of Human Reproduction and Embryology (ESHRE) consensus and evaluate its impact on pregnancy rates.

Design: Retrospective study.
Setting: A private IVF center.

Patient(s): A total of 606 embryos from 511 intracytoplasmic sperm injection (ICSI) cycles.

Intervention(s): None.

Main Outcome Measure(s): A time-lapse system was used to acquire embryo images until ET; the stored data were reviewed for the presence and persistence of multinucleation. The detection rate of multinucleation was compared with ESHRE/ALPHA consensus-proposed embryo evaluating times (23 ± 1, 26 ± 1, 44 ± 1 hours). Morphokinetic characteristics of multinucleated embryos and the effect of multinucleation on pregnancy rate were researched.

Results: Multinucleation was detected in 159 embryos of 145 ICSI cycles. Using ESHRE/ALPHA consensus embryo evaluating times, only 44 (27.6%) out of 159 multinucleated embryos could be identified. In cycles with multinucleated ETs compared with cycles with no multinucleated embryos, clinical pregnancy rates (respectively, 23.4 vs. 44) and implantation rates (respectively, 23.1 vs. 43.6) were significantly lower. Time to 2-cell, 4-cell, and 6-cell stage was significantly longer in multinucleated embryos. Patient age (odds ratio [OR], 0.95; confidence interval [CI], 0.92-0.98) and presence of multinucleation (OR, 0.37; CI, 0.24-0.56) were the only significant predictors of clinical pregnancy rate.

Conclusion(s): The time-lapse monitoring system seems to be a valuable tool to identify all cases with multinucleation. We conclude that the detection of multinucleation by time-lapse monitoring is associated with lower implantation and clinical pregnancy rates. (Fertil Steril® 2014; 102:1029-33. ©2014 by American Society for Reproductive Medicine.)

Key Words: Embryo development, time-lapse, multinucleation, morphokinetic, pregnancy rate

Discuss: You can discuss this article with its authors and with other ASRM members at <http://fertilityforum.com/ergin-a-multinucleation-time-lapse-pregnancy>



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

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(Desai ve ark., 2014; Ergin ve ark., 2014)

De-selection

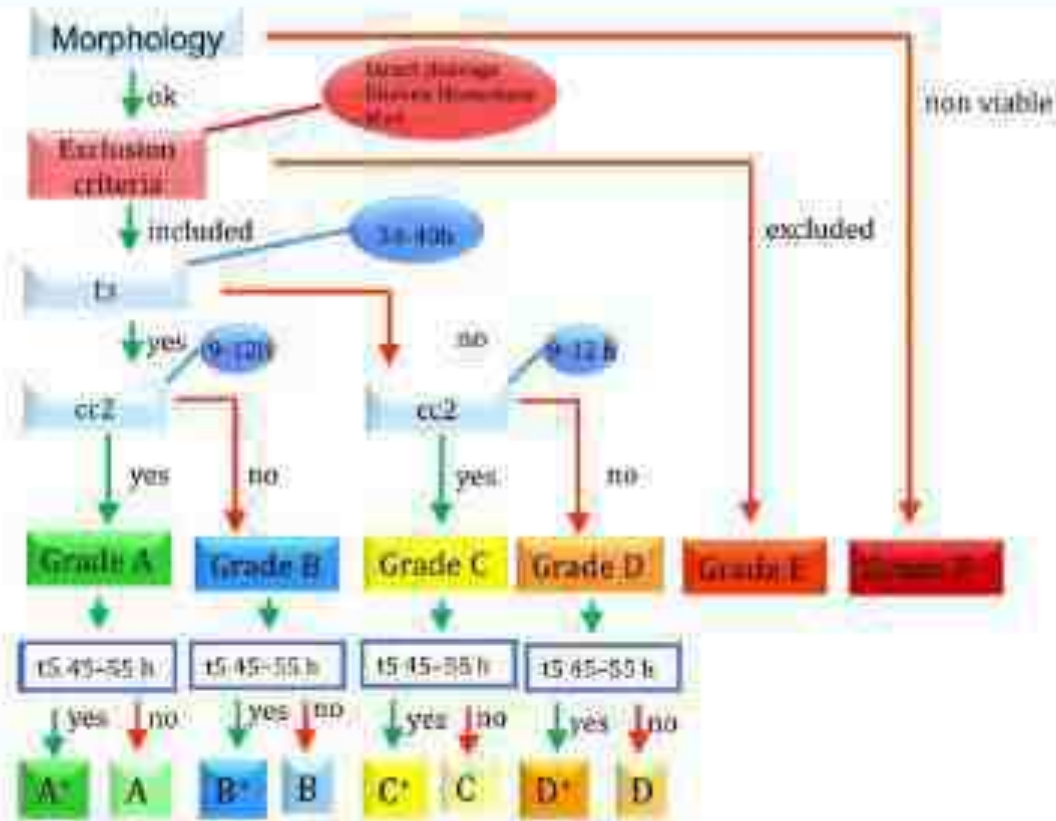


Figure 3 Hierarchical classification of embryos based on: (i) Morphological screening; (ii) the new morphological criteria; (iii) timing of cell division to three cells (t3); (iv) duration of second cell cycle, cc2, i.e. the time from division to a two blastomeres until division to a three blastomeres embryo; (v) timing of cell division to five cells. The classification generates 10 categories of embryos with increasing expected implantation potential (right to left) and almost equal number of embryos in each.

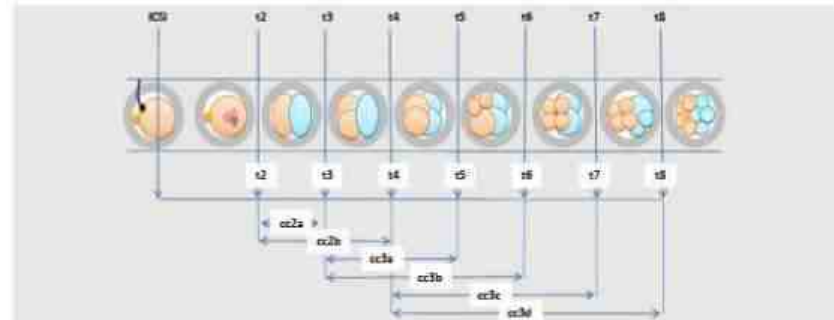
Re-selection



Morphokinetics as a predictor of self-correction to diploidy in trippronucleated intracytoplasmic sperm injection-derived human embryos

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Graph representation of embryo development events (in order A) to determine direct and indirect morphokinetic variables. The time reference (t0) is the intracytoplasmic sperm injection (ICSI) event. The direct morphokinetic variables were the time points of embryo cleavage to the 2-, 3-, 4-, 5-, 6-, 7-, and 8-cell stages (t2, t3, t4, t5, t6, t7, and t8). The indirect morphokinetic variables were: duration of the second cell cycle of the first blastomere to cleave from the 2- to the 3-cell stage (cc2a = t3 - t2); duration of the second cell cycle of the second blastomere to cleave from the 2- to the 4-cell stage (cc2b = t4 - t2); and duration of the third cell cycle of the first (cc3a = t5 - t3), second (cc3b = t6 - t4), third (cc3c = t7 - t5), and fourth (cc3d = t8 - t6) blastomeres to cleave to the 5-, 6-, 7-, and 8-cell stages, respectively.

Gray: Kinetics of trippronucleated (TPN) embryos. *PNB* (Black) 2015.

Objective: To describe, in morphokinetic terms, a trippronucleated embryo (TPN) population according to ploidy and to explore the value of such variables for predicting ploidy.

Design: Experimental.

Setting: In vitro fertilization laboratory.

Patient(s): Seventy-nine TPN embryos obtained after intracytoplasmic sperm injection (TPN-ICSI) were cultured in a time-lapse incubator for 6 days.

Intervention(s): Ploidy determinations were carried out for 35 TPN-ICSI at the cleavage and/or blastocyst stage. Their morphokinetics were then retrospectively compared.

Main Outcome Measure(s): Direct (cleavage time from 2- to 8-cell stages) and indirect (cell cycle duration and blastomere synchrony at cleavage) morphokinetic variables; ploidy determination by FISH; in vitro development to the blastocyst stage.

Result(s): TPN-ICSI cleaved later than bipronucleated control embryos (BPN). Diploid TPN displayed morphokinetic behavior closer to BPN than triploid TPN regarding almost all of the direct and indirect morphokinetic variables measured. Variable t5 was found to be a predictable variable of ploidy in TPN.

Conclusion(s): TPN-ICSI are not homogeneous in ploidy, cleavage, or morphokinetic terms.

Diploid, but nontriploid, TPN are morphokinetically similar to diploid BPN. The ploidy of TPN can be predicted by variable t5. (*Fertil Steril*® 2015;104:728-35. ©2015 by American Society for Reproductive Medicine.)

Key Words: Trippronucleated, diploidy, triploidy, time-lapse, morphokinetics, human.

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Direct and indirect variables of trippronucleated (TPN) and bipronucleated (BPN) fertilized embryos.

Timing (h)	TPN (n = 23)	BPN (n = 17)	Average
Cleavage to stage			
2-cell (t2)	29.9 ± 3.8 (28.2-31.8) ^a	26.2 ± 2.1 (25.2-27.2) ^a	N/A ^b
3-cell (t3)	41.3 ± 5.4 (38.6-44.0) ^a	38.0 ± 2.7 (36.6-39.4) ^a	N/A
4-cell (t4)	42.3 ± 5.6 (38.6-45.0) ^a	38.6 ± 2.9 (37.1-40.0) ^a	N/A
5-cell (t5)	56.7 ± 12.9 (50.9-63.1)	52.8 ± 3.8 (50.9-54.6)	54.8 ± 9.6 (51.5-58.1)
6-cell (t6)	61.8 ± 11.2 (56.0-67.2) ^a	53.8 ± 3.7 (51.8-55.7) ^a	N/A
7-cell (t7)	62.9 ± 12.1 (58.2-68.6) ^a	54.8 ± 3.9 (52.8-56.8) ^a	N/A
8-cell (t8)	63.9 ± 10.2 (58.0-69.7) ^a	57.0 ± 5.3 (54.3-59.7) ^a	N/A
Indirect variable			
cc2a	12.7 ± 1.4 (11.9-13.4) ^a	11.8 ± 0.9 (11.3-12.3) ^a	N/A
cc2b	13.1 ± 1.5 (12.8-12.9) ^a	12.3 ± 1.2 (11.7-12.9) ^a	N/A
cc3a	13.0 ± 1.4 (12.3-13.7) ^a	12.1 ± 1.0 (11.2-12.6) ^a	N/A
cc3b	18.8 ± 6.8 (18.3-22.3) ^a	14.7 ± 1.9 (13.2-15.6) ^a	N/A
cc3c	22.8 ± 7.1 (19.1-26.4) ^a	18.2 ± 2.0 (16.4-18.5) ^a	N/A
cc3d	21.9 ± 7.7 (17.6-26.3) ^a	18.2 ± 2.2 (15.1-17.0) ^a	N/A
cc3e	22.0 ± 5.5 (18.5-25.5) ^a	18.3 ± 3.8 (16.3-20.3) ^a	N/A
cc3f	18.6 ± 4.1 (16.8-22.0) ^a	16.0 ± 2.2 (14.9-17.2) ^a	N/A
SD2, at the 2-cell stage	0.86 ± 0.28 (0.73-1.00)	0.96 ± 0.04 (0.94-0.98)	0.91 ± 0.21 (0.84-0.98)
SD3, at the 4-cell stage	0.79 ± 0.14 (0.70-0.88)	0.87 ± 0.07 (0.83-0.91)	0.83 ± 0.11 (0.79-0.88)

Note: The direct variables were embryo (mean ± SD) cleavage (stage) to the 2-, (t2), 3-, (t3), 4-, (t4), 5-, (t5), 6-, (t6), 7-, (t7), and 8-, (t8) cell stages, referred to as hours after intracytoplasmic sperm injection. The indirect variables, referred to as hours, were: time span for the first and second blastomeres to cleave at the 3-cell stage (cc2a and cc2b). Duration of the third cell cycle of each blastomere (cc3a-cc3f), and average duration of the second (cc2c) and third (cc3c) cell cycles. Blastomere synchrony in cleavage in the second (SD2) and third (SD3) cell cycles are also shown. Values are presented as mean ± SD (95% confidence interval).

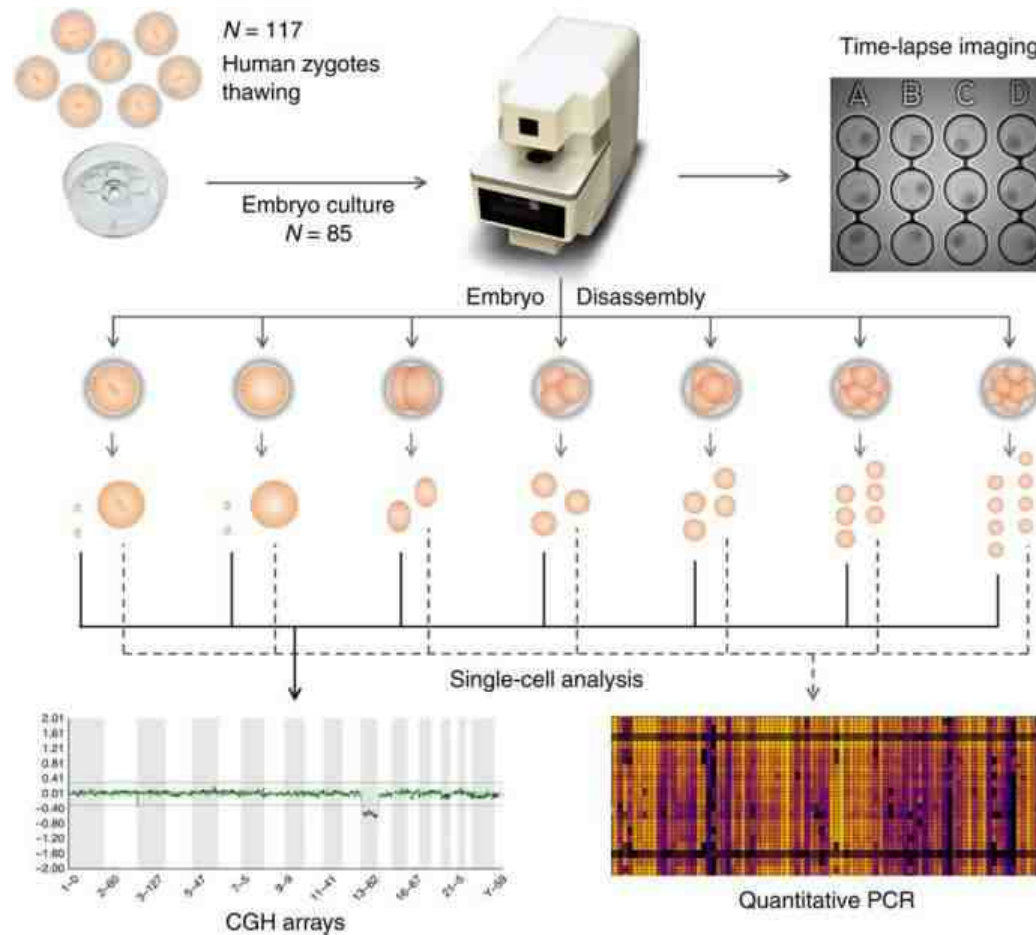
^a Different from control in the same row indicate statistically significant differences (P < .05).

^b N/A = not applicable. It was not possible to provide the average mean of the variable, because there were statistically significant differences between TPN and BPN embryos regarding the corresponding morphokinetic variable.

Gray: Kinetics of trippronucleated (TPN) embryos. *PNB* (Black) 2015.

Morfokinetik Analiz – Kromozomal anomali ilişkisi

TLM+CCS+Gen ekspresyonu analizi



(Rodriguez ve ark., 2014-Nature)

TLM+miRNA ekspresyonu analizi

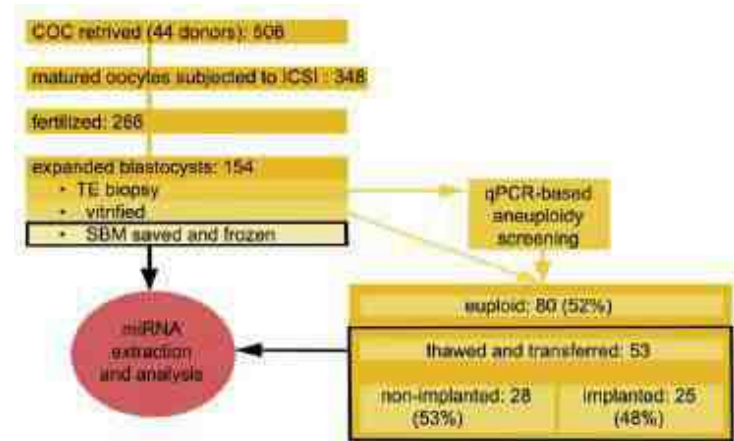
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ORIGINAL ARTICLE: REPRODUCTIVE SCIENCE

MicroRNAs in spent blastocyst culture medium are derived from trophectoderm cells and can be explored for human embryo reproductive competence assessment

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Objective: To assess whether extracellular microRNAs (miRNAs) can be accurately profiled from spent blastocyst culture media (SBM) and used as embryonic biomarkers.

Design: Prospective cohort study.

Setting: Private and academic in vitro fertilization centers.

Patient(s): Inner cell mass-free trophectoderm (TE) samples and their relative SBM from five good-quality human blastocysts.

Intervention(s): Protocol for mRNA purification and analysis based on quantitative polymerase chain reaction set and validated on human embryonic stem cells (hESCs) and on SBM with and without biological variability.

Main Outcome Measure(s): Analysis of miRNAs in culture media in relation with TE cells and comparison of miRNA profiles between implanted and unimplanted euploid blastocysts.

Result(s): Culture media from embryos in the cleavage, morula, and blastocyst stages were collected to investigate the presence of miRNAs. The SBM were prospectively collected from euploid implanted (n = 25) and unimplanted blastocysts (n = 28) for comparison. We hypothesized that human embryos secrete miRNAs in culture media that can be used as biomarkers. The comparative analysis of TE and SBM samples revealed that 96.6% (57 of 59; 95 CI, 88.3–99.6) of the miRNAs detected in the SBM were expressed from TE cells, suggesting a TE origin. The culture media collected from cleavage and morula stage embryos showed a pattern similar to blanks, suggesting that miRNAs profiling from spent culture media applies only for blastocysts. MicroRNAs analysis of SBM from euploid implanted and unimplanted blastocysts highlighted two miRNAs (miR-20a, miR-10c) that showed increased concentrations in the former and were predicted in silico to be involved in 23 implantation-related pathways.

Conclusion(s): MicroRNAs secreted from human blastocysts in culture media can be profiled with high reproducibility, and this approach can be further explored for noninvasive embryo selection. [Fertil Steril® 2015;■:■–■. ©2015 by American Society for Reproductive Medicine.]

Key Words: Biomarkers of implantation, blastocyst-endometrial dialogue, blastocyst evaluation, embryo quality, microRNA

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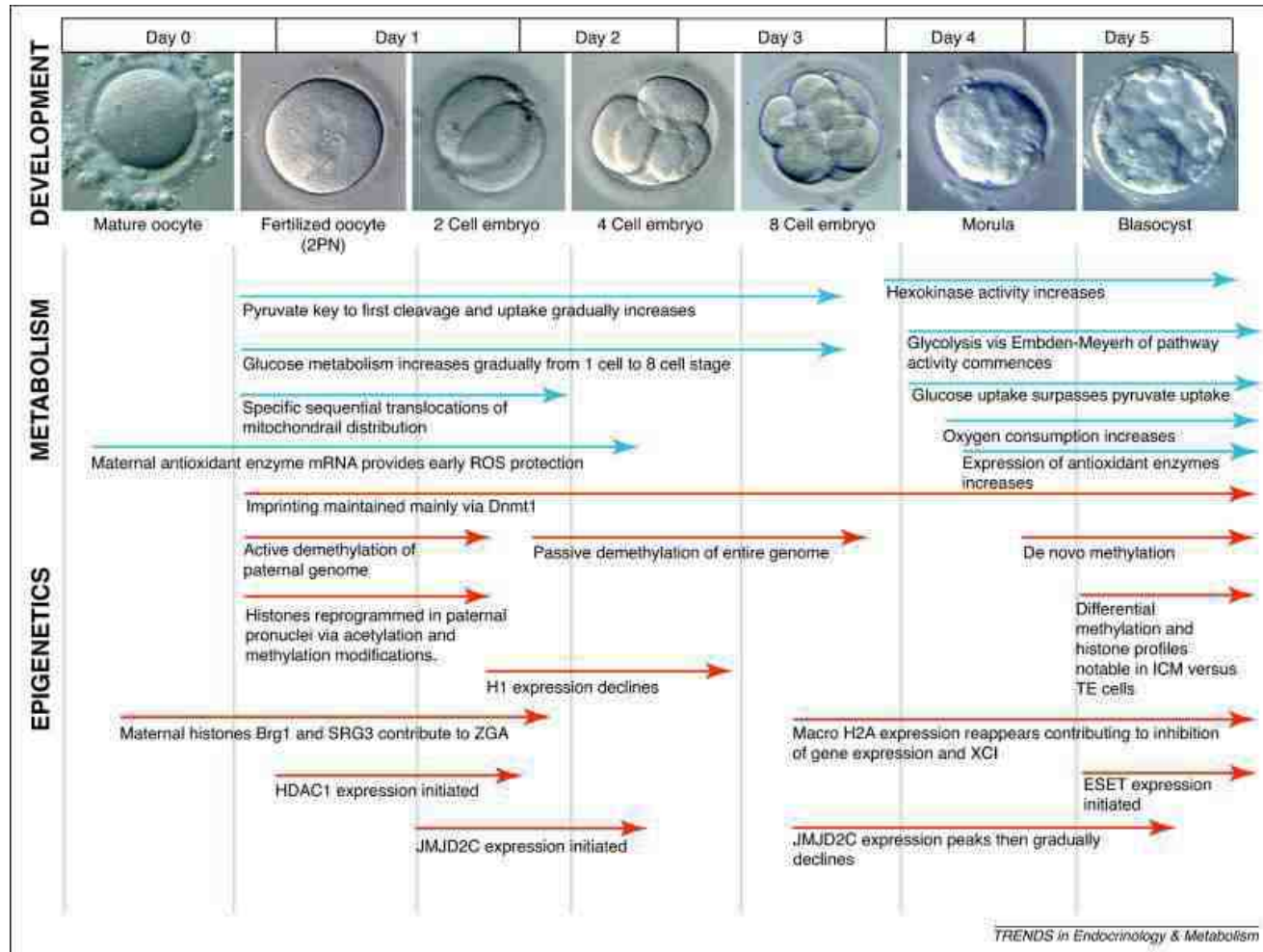


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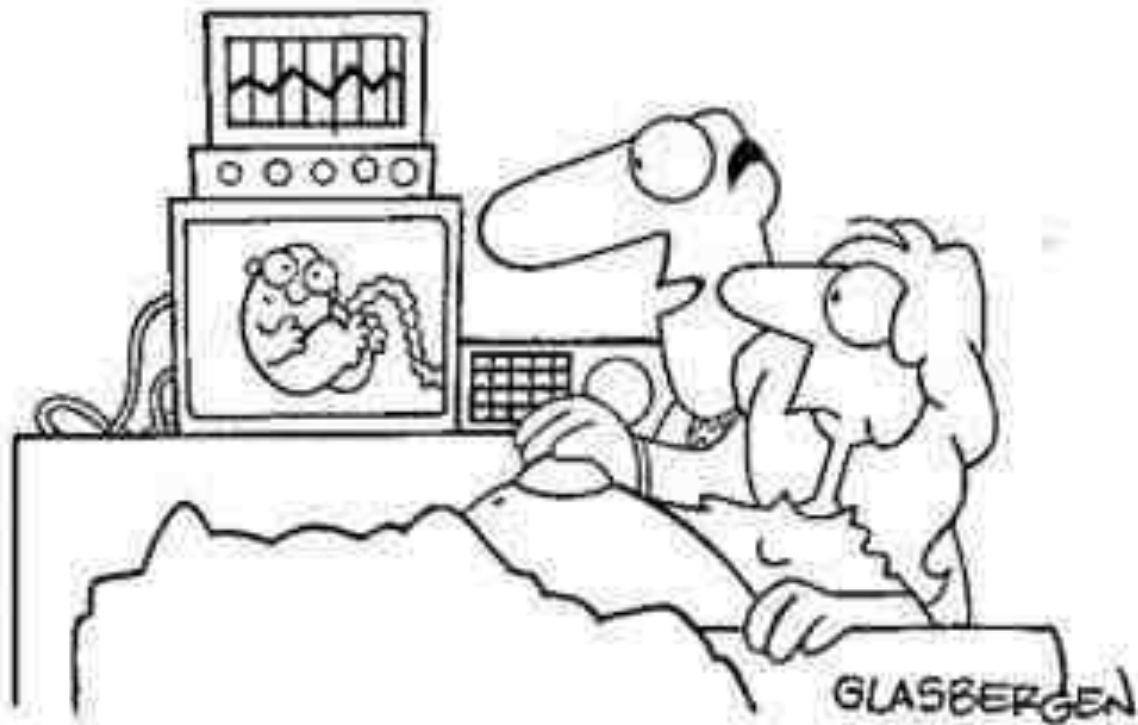
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(Rodriguez ve ark., 2014-Nature)

Yakın gelecek...



(Chason ve ark. 2011; Gardner ve ark, 2015)



**“Your baby is developing very nicely.
Would you like to send him an e-mail?”**

Thank you😊