

First mitotic division: criterion for selection of potential IVF embryo – A Systematic Literature Review

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-----ABSTRACT-----

Background: *In-Vitro-Fertilization (IVF) is a technique in which an egg is fertilized by sperm outside the body i. e. in vitro ("in glass"). It is a procedure designed to enhance likelihood of conception in couples for whom other fertility therapies have been unsuccessful or are not possible. Importance of various stages of IVF embryos for successful IVF treatment has been reported. However, there are studies which assume that embryo that shows first mitotic division within 27 hr post insemination (Early Cleavage –EC) has higher potential to get implanted than embryo that cleaves later. Objective: To correlate all the available literature on studies related to selection of EC embryos for IVF treatment and the subsequent outcome of the procedure. Inference: Present study advocates selection of EC embryo as a dependable criterion for IVF success.*

KEY WORDS - Embryo potential, Embryo quality, First mitotic division, Invitro fertilization, Pregnancy.

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I. INTRODUCTION

Over a period of time there is a gradual development in drugs used for controlled ovarian hyper stimulation in IVF therapy. Improved oocyte retrieval technique and culture conditions have resulted in high fertilization rate and availability of more number of good quality embryos for transfer. It was observed that pregnancy rate increases with the number of embryos transferred. However this may result in multiple pregnancies, which is undesirable. Goal of an IVF unit is to avoid multiple pregnancies without affecting success rate. This can be achieved by identifying potential embryo from a cohort and limiting the number of embryos to be transferred.

Assessment of zygote morphology, cleavage pattern, embryo morphology and degree of fragmentation are the routinely used parameters for the selection of potential embryos. The intention of all these evaluation systems is to find out an easy, non invasive and non subjective assessment of potential embryo. Common selection method is to evaluate the embryo for cell number and symmetry prior to transfer [1]. Early onset of first mitotic division is defined as a cell division resulting in a two celled embryo at 25 to 27 hour after Intra Cytoplasmic Sperm Injection (ICSI). Edward et al, have shown for the first time that embryos which show early cleavage have more chance to implant than non early cleaved embryos, which is subsequently confirmed by many studies [2-18]. Assessment of embryos for early onset of first mitotic division is easy, noninvasive and non subjective.

This review is focused on studying the importance of onset of Early Cleavage (EC) as a useful tool for selection of potential embryo. Correlation of onset of EC with subsequent quality of embryos at all possible stages during invitro culture along with Implantation Rate (IR) and Pregnancy Rate (PR) is also studied.

II. MATERIALS AND METHODS

a. Literature search

An electronic literature search of published articles on Pub med, Medline, NCBI about prognostic value of early cleavage of human IVF embryos was performed. All the published articles during last 25 years were reviewed for content, along with the references of interest. The search was limited to English language literature. Only studies concerned with early onset of first mitotic division were analyzed. Case studies with retrospective or prospective data were included in the search.

b. Study selection

All studies were assessed and publications to be included were selected independently by all three authors. At the first screening, the titles were investigated and studies with lack of any relevance were excluded. The second screen was performed by reading the selected abstracts. For third screen, all available full papers were read with a focus on EC criteria for selection of IVF embryos. Selected studies were grouped according to their scope i.e. correlation of EC with Pronuclear (PN) morphology, quality, cleavage pattern, ability to develop up to blastocyst stage, IR and PR. Correlation of EC with IR, PR and blastocyst development was concluded with graphical representation.

III. RESULT

The search of databases resulted in 102 titles. Irrelevant publications were excluded after first screen. The second screen was performed on basis of abstracts of the remaining 53 relevant publications. 32 items did not meet selection criterion and were excluded for the further considerations. Thus 24 publications were subjected to third screen. The third screen was performed by reading the available full papers. After this investigation 3 papers having completely different emphasis were excluded from the present study. This process of selection is illustrated in Fig 1.

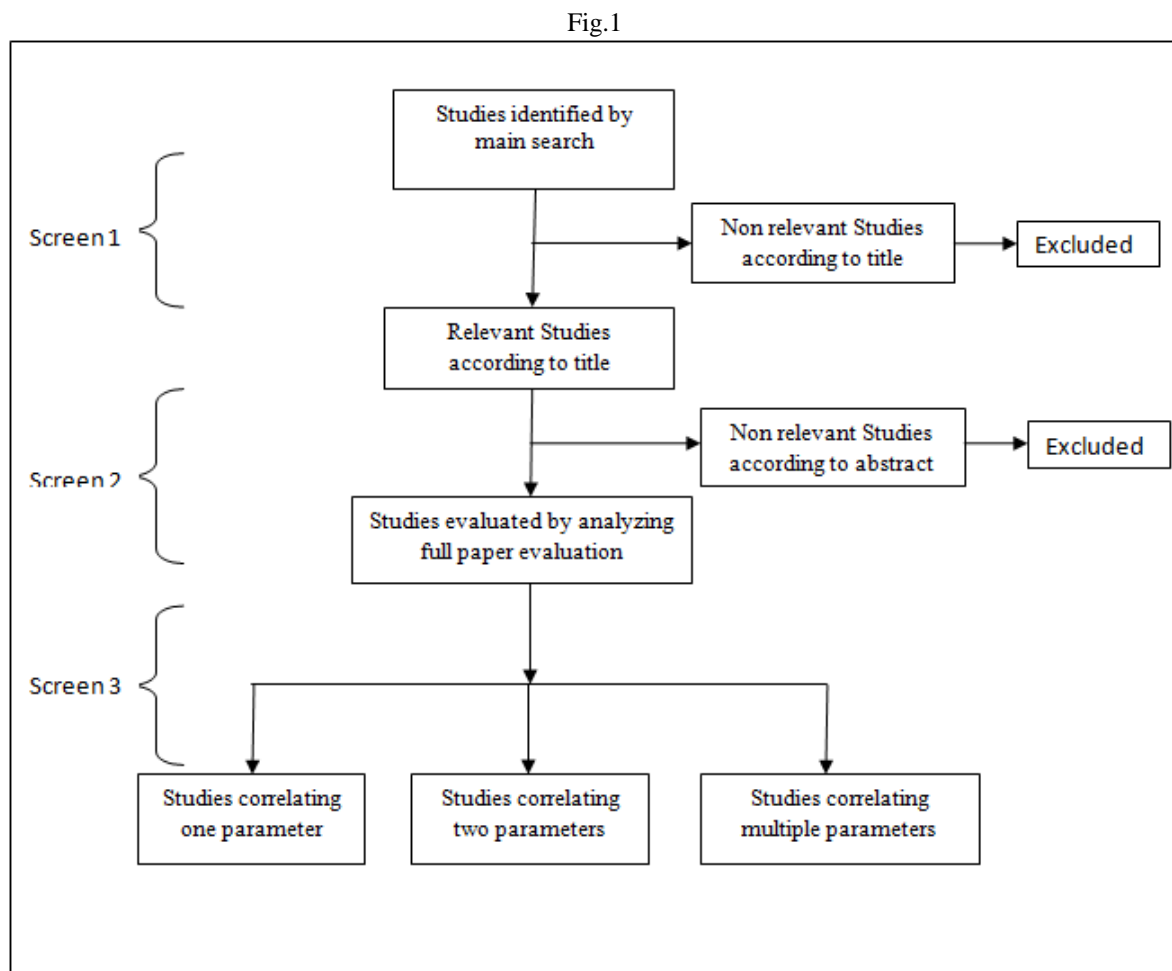


Figure1. Flow chart:-Systematic review of literature investigating use of early cleavage assessment

Out of 21 selected relevant publications there were seventeen studies correlating PR with EC, 12 correlating IR and PR, nine studies correlating multiple parameters of subsequent embryo development. Two studies compared the EC system with the system which scores PN morphology for embryo assessment. Literature search indicated the correlation between EC and PN morphology, cleavage pattern, blastocyst formation, multinucleation, rate of spontaneous abortion and birth rate.

Fertilization is a complex process involving many steps viz. activation of oocyte, decondensation of sperm head, extrusion of second polar body, formation of male and female PN and their rearrangement for the formation of zygote. This single celled embryo is now ready for first mitotic division [19]. IVF program involves controlled ovarian hyper stimulation, surgical oocyte retrieval, invitro fertilization and embryo transfer at certain stage by selecting viable embryo from a cohort.

3.1 Pronuclear morphology

PN morphology is one of the criteria used in assessment of embryo viability. Several studies designate importance of PN morphology for embryo selection [20-25]. Pronuclear morphology has a potential to predict EC and subsequent quality of embryo. According to these studies assessment of progression of embryos in addition to PN score is not necessary in the selection for transfer on D3 [27]. Embryo quality assessment at the time of embryo transfer in combination with PN morphology is recommended by some studies [28-30]. However some studies fail to show any correlation between zygote score and PR. [31-33]. This discrepancy may be because the PN morphology changes over time and the grading may vary with observer. As compared to EC assessment the PN assessment needs careful observation which may need more time for embryos to be kept outside the incubator. Positive correlation was observed with good PN morphology and early onset of first mitotic division [26, 27].

3.2 Early cleavage and Non Early Cleavage (NEC)

All embryos from a cohort do not have ability to cleave earlier. Positive correlation of EC embryos with IR and PR was observed in conventional IVF cases where oocyte maturity and time of fertilization is not defined [3, 34]. To understand the influence of these factors; studies were carried out on cases where fertilization was achieved using ICSI technique. During ICSI cumulus cells surrounding the oocyte are removed to assess oocyte maturity and sperm is mechanically inseminated into oocyte cytoplasm where difference in oocyte maturity and timing of fertilization were ruled out. Studies showed positive relation of EC with IR and PR when fertilization was achieved by ICSI technique [7, 35]. Improvement in the PR of an IVF unit was observed when early cleavage parameter was included in assessment protocol [7].

Results of IR and PR with transfer of EC and NEC embryos, analyzed by different studies are tabulated (Table 1 and Table 2 respectively) and graphically represented in Fig. 2 and Fig.3

During retrospective evaluation of merits of two grading systems i.e. PN morphology and EC assessment no difference was found in IR and PR with transfer of embryos having different zygote patterns, however significant difference in IR and PR has been reported between EC and NEC embryos [36]. Studies also document better prediction of implantation potential when both the criteria were considered together [27, 36].

3.3 Cleavage pattern

It has been established that duration of the cell cycle is usually around 10-12 h. It is a time required for a cell to undergo cytokinesis and replication of whole cell genome. Abnormal length of the cell cycle i. e. prolonged or shorter duration negatively influences embryo competence [17, 37- 39]. Division of ooplasm by meridian axis results in even distribution of cellular and genetic material, producing exactly equal sized blastomeres. Uneven distribution of cellular and genetic material results in unequal sized blastomeres which are detrimental to embryo viability and negatively correlate with IR and PR. The significantly higher percentage of early cleaved embryos show normal cleavage pattern and even sized blastomeres as compared to non early cleaved embryos [17, 37,39, 40].The results suggest a positive correlation of normal cleavage pattern with normal morphology, blastocyst formation rate and pregnancy rate [41]. Morphological anomalies in first few cleavages and poor quality of embryonic development are strongly associated with error in duration of cell cycle [38, 40-42]. EC embryo showing even blastomeres shows higher potential for implantation [43].

Table 1. -Summary of the studies analyzing IR with EC and NEC embryos.

	Year	Author	IR with EC	IR with NEC
1	1997	Youssef Shoukir1 et al	23.60%	7.50%
2	2001	Sakkas et al	25.50%	14.80%
3	2001	K.lundin et al	28.00%	19.50%
4	2001	A bos-Mikich etal	18.00%	11.60%
5	2002	J. Fenwick et al	21.40%	6.00%
6	2002	Y.C Tsai	18.80%	11.60%
7	2003	E.Neuber	21.90%	14.30%
8	2003	Brezinová J	38%	33%
9	2004	Denny Sakkas et al	14.00%	3.20%
10	2004	Brezinová J	27%	25.00%
11	2012	Meng et at	48.10%	24.00%
12	2013	Edessy M	58.60%	35.00%

Fig. 2

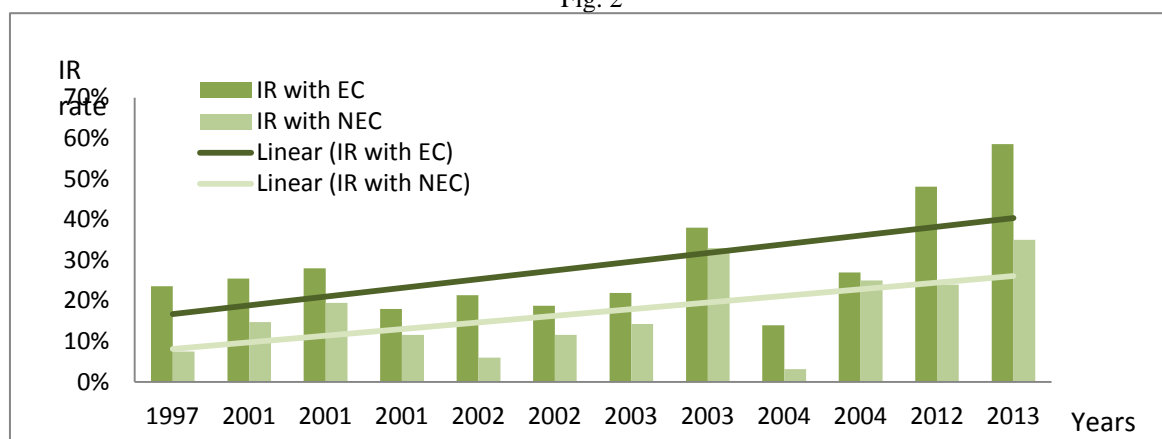


Fig. 2. Comparison of IR with EC and NEC embryos in different studies.

Table 2. -Summary of the studies analyzing PR with EC and NEC embryos.

Sr.No	Year	Author	PR with EC	PR with NEC
1	1997	Youssef Shoukir1 et al	33.30%	14.70%
2	1998	Denny Sakkas et al	25.90%	3.20%
3	2001	Sakkas etal	45.00%	23.80%
4	2001	K.lundin et al	40.50%	31.30%
5	2001	A bos-Mikich etal	55.00%	25.00%
6	2002	J. Fenwick et al	31.30%	10.50%
7	2002	Y.C Tsai	38.80%	25.60%
8	2003	Salumets et al	50.00%	26.40%
9	2003	E.Neuber	40.00%	26.00%
10	2003	Brezinová J	61%	56%
11	2004	Denny Sakkas et al	25.90%	3.20%
12	2004	M.L. Windt	41.30%	20.20%
13	2004	Aafke PA Van Montfoort	46.00%	18.00%
14	2004	Brezinová J	40%	33%
15	2007	Giorgetti C1	42.40%	25.90%
16	2012	Meng et at	68.00%	51.00%
17	2013	Edessy M	43.30%	21.80%

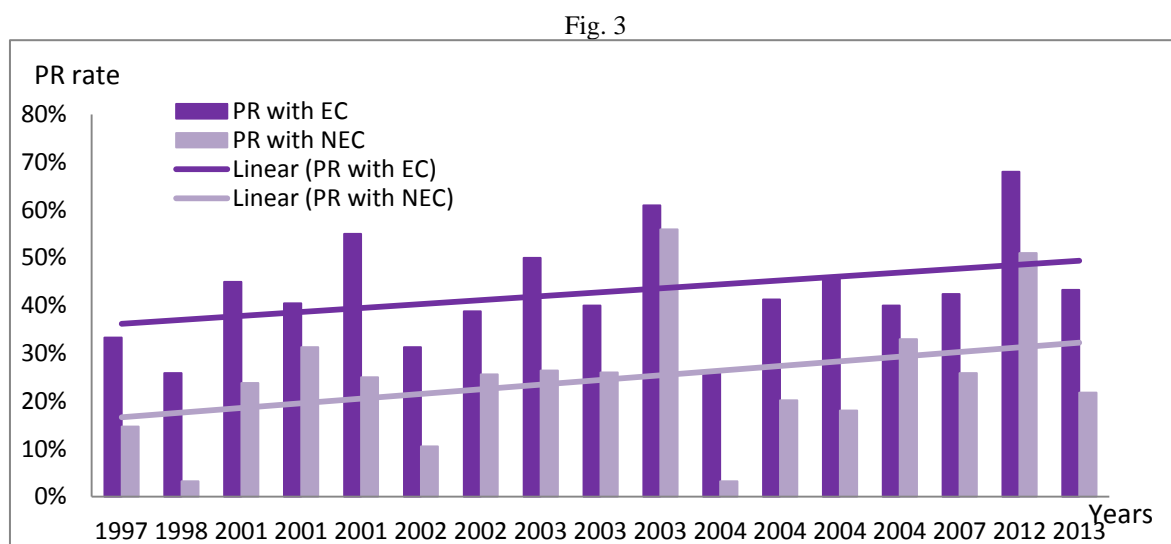


Fig. 3. Comparison of PR with EC and NEC embryos in different studies.

3.4 Mononucleation

One of morphological criteria for selection of potential embryo in IVF treatment is to observe the nuclear status of the blastomeres. IVF embryos frequently exhibit multinucleated blastomeres [44] which is usually associated with developmental incompetence, poor IR and impaired cleavage. [44-46]. Mononucleated blastomeres on the other hand are an index of chromosomal normality of blastomere nucleus [43-48]. Improvement in the rate of implantation has been documented in early cleaved mononucleated embryos [49]. Availability of higher percentage of EC embryos showing mononucleated blastomeres than NEC embryos has been reported [13,41, 49].

3.5 Blastocyst development

Extending the culture up to blastocyst stage allows selection of more advanced embryos with stronger developmental competence for IR and PR than cleavage stage embryo [50-53]. However some studies do not recommend the benefit in transferring blastocyst stage embryo over cleavage stage embryo in terms of IR and PR [54-56]. Whereas there are studies insisting upon the need to carry out properly designed work to compare the benefit of blastocyst stage embryo transfer over cleavage stage embryo transfer [54]. Standard IVF culture conditions support only about 25 to 60% of human embryos to progress up to the blastocyst stage after 5 days of invitro culture. This is attributed to the inherent weakness of the particular human embryos or weak culture environment which imparts stress on development of the embryo. Comparative studies on the development of EC and NEC embryos up to blastocyst stage have been reported (Table 3). Positive correlation has been observed between early onset of first mitotic division and ability to develop up to blastocyst stage invitro as compared to non early cleaved embryos [9, 11, 14]. Time lapse monitoring system supports close relation between exact timing of overall early development events and potential to develop up to blastocyst [39]. Effective selection of embryos at earlier stages which will help to identify the ability of embryos to develop up to blastocyst stage for successful IR and PR is necessary.

Table 3. Results of Blastocyst development of different studies with EC and NEC embryos.

Sr. No	Year	Author	Blastocyst With EC	Blastocyst with NEC
1	2002	J. Fenwick et al	32.20%	16.60%
2	2003	E. Neuber	39%	19%
3	2004	Aafke PA Van Montfoort	66%	40%

Fig .4

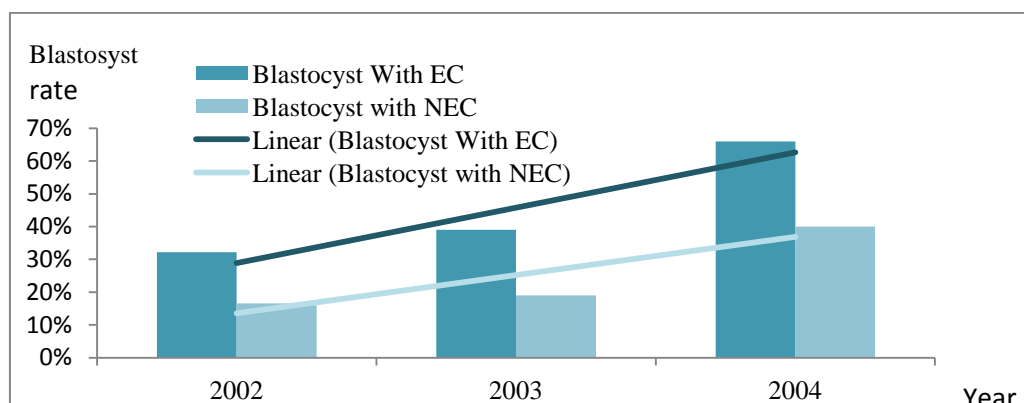


Fig .4 Comparison of blastocyst development of EC and NEC with different studies.

3.6 Birth rate

Improvement in birth rate or take home baby rate is the ultimate goal of IVF unit. It has been found through the literature search that implantation of EC embryos show reduction in rate of spontaneous abortion as compared to implantation of NEC embryos. This also results in improvement in IR and PR as well as birth rate. [6, 17, 37]. This is trend lines demonstrate the gradual improvement in success of IVF outcome in terms of pregnancy and implantation rate over a period of time with persistent difference with transfer of EC and NEC embryos.

Very few embryos reach the 2 cell stage at 25h post ICSI and few patients benefit from such an early assessment and selection to transfer. It is not known, however, at which time point of completion of the first cell cycle embryo viability starts to drop. 50% of embryos show cleavage at 29hr. post insemination and has equal potential. This extended period maximize the number of potential embryos available to be transferred [5].

IV. DISCUSSION

Present review makes a systematic literature search of the articles related to the importance of first mitotic division in selection of embryos for successful IVF treatment in last twenty five years. Development in biotechnology enables manipulation of human gametes for in-vitro-fertilization (IVF) treatments. Developments in IVF protocols have enable the availability of more number of good quality embryos for implantation. This has resulted in increasing the rate of implantation and rate of pregnancy in the subsequent years. Increase in the rate of take home baby – which is the aim of every IVF unit has also been improved due to the research and developments in this field. However, since the total success is too far there is a scope for probing into the selection criteria for potential IVF embryos.

In IVF programs most routinely used criteria for embryo selection has been based on cell number and morphology at the time of embryo transfer. Biochemical methods analyzing follicular fluid or metabolic activity of embryo are also available to assess gametes and embryo quality. These methods are very complex, time consuming, expensive and impractical in most of the busy IVF laboratories. Hence, in the present scenario the selection of potential IVF embryo on the basis of study of its morphology has been, and will remain the first choice for selection. Study of zygote morphology (PN score) and morphology at cleavage as well as blasocyst stage are also considered for selection. PN assessment requires expertise, is subjective and time consuming. Whereas, cultivation up to blastocyst stage may impart stress to the embryo resulting decrease in IR and PR. Analysis of publications suggests that early onset of first mitotic division is a strong biological marker of embryo viability, quality as well as developmental potential. Within a cohort of embryos, embryo that shows first mitotic division within 27 hr post insemination (Early Cleavage –EC) has always been assumed to have higher potential to get implanted than embryo that cleaves later. Comparative results showed significantly higher pregnancy and implantation rate with early cleaving embryos than non early cleaving embryos [2-18]. The quality of pre-implantation embryos changes over a time during in-vitro culture and it is disagreed to use this criterion as an independent tool for embryo selection. Effective selection of embryos at earlier stages which will help to identify the ability of embryos to develop up to blastocyst stage for successful IR and PR is necessary. The recent data suggests early cleavage ability is a good marker for embryo selection but should be used in accordance with other parameters like PN score and morphology at cleavage as well as blasocyst stage at the time of transfer. Better results are observed when different scoring systems are used jointly. Most of the studies correlate one or the other subsequent development stage with early onset of mitotic division. It is

necessary to correlate early cleavage status with quality of embryo at all possible stages during in-vitro culture as well as with pregnancy and implantation rate cumulatively. Early cleavage assessment is noninvasive, non-subjective and less time consuming thus giving a great advantage for selection process of potential embryo.

Trend lines revealed a gradual improvement in IR and PR over a period of time with persistent difference in IR and PR by transfer of EC and NEC embryos. Present review validates the selection of early cleaved embryo for successful IVF treatment. Coordinated and intensive work with large sample size will endorse importance of the selection of EC embryos for transfer.

REFERENCES

- [1] Catherine Racowsky, Lucila Ohno-Machado, Jihoon Kim, and John D. Biggers, Is there an advantage in scoring early embryos on more than one day?, *Hum Reprod*, vol. 24, no 9 pp. 2104-2113, 2009.
- [2] Edwards RG, Fishel SB, Cohen J, Fehilly CB, Purdy JM, Slater JM, Steptoe PC, Webster JM 1984 Factors influencing the success of in vitro fertilization for alleviating human infertility, *Journal of In Vitro Fertility and Embryo Transfer:IVF* 1 3-23.
- [3] Youssef Shoukir, Aldo Campana, Tim. Farley and Denny Sakkas, Early cleavage of in-vitro fertilized human embryos to the 2-cell stage: a novel indicator of embryo quality and viability, *Human Reproduction* vol.12 no.7 pp.1531-1536, 1997.
- [4] Sakkas D, Shoukir Y, Chardonnens D, Bianchi PG, Campana A, Early cleavage of human embryos to the two-cell stage after intracytoplasmic sperm injection as an indicator of embryo viability, *Hum Reprod* 1998;13:182-7.
- [5] Bos-Mikich A, Mattos AL, Ferrari AN, Early cleavage of human embryos: an effective method for predicting successful IVF/ICSI outcome, *Hum Reprod*. 2001; 16:2658-61.
- [6] Lundin K, Bergh C, Hardarson T, Early embryo cleavage is a strong indicator of embryo quality in human IVF, *Hum Reprod*. 2001; 16:2652-7.
- [7] Sakkas, D., Percival, G., D'Arcy, Y., Sharif, K. and Afnan, M. (2001). Assessment of early cleaving in vitro fertilized human embryos at the 2-cell stage before transfer improves embryo selection, *Fertil. Steril.*, 76, 1150-1156.
- [8] Tsai YC, Chung MT, Sung YH, Tsai TF, Tsai YT, Lin LY, Clinical value of early cleavage embryo, *Int J Gynaecol Obstet*. 2002 Mar;76(3):293-7.
- [9] Fenwick J, Platteau P, Murdoch AP, Herbert M, Time from insemination to first cleavage predicts developmental competence of human preimplantation embryos in vitro, *Hum Reprod*. 2002;17:407-12.
- [10] Brezinová J, Oborná I, Svobodová M, Krsková M, Fingerová H, Machac S, [Early cleavage embryos and their effect on the results of standard in vitro fertilization], *Ceska Gynekol*. 2003 Nov;68(6):449-53.
- [11] Neuber E, Rinaudo P, Trimarchi JR, Sakkas D, Sequential assessment of individually cultured human embryos as an indicator of subsequent good quality blastocyst development, *Hum Reprod*. 2003;18:1307-12.
- [12] Andres Salumets, Christel HydeÅn-Granskog, Sirpa MaEkinen, Anne-Maria Suikkari, Aila Tiitinen and Timo Tuuri, Early cleavage predicts the viability of human embryos in elective single embryo transfer procedures, *Human Reproduction* Vol.18, No.4 pp. 821-825, 2003
- [13] Brezinová J, Svobodová M, Krsková M, Fingerová H, Machac S, [Effect of early cleavage of embryos after intracytoplasmic sperm injection (ICSI) into oocytes on results of fertilization in vitro (IVF) and embryo transfer (ET)], *Ceska Gynekol*. 2004 Jan; 69(1):37-42.
- [14] Van Montfoort AP, Dumoulin JC, Kester AD, Evers JL, Early cleavage is a valuable addition to existing embryo selection parameters: a study using single embryo transfers, *Hum Reprod*. 2004; 19:2103-8.
- [15] M.-L.Windt, T.F.Kruger, K.Coetzee and C.J.Lombard, Comparative analysis of pregnancy rates after the transfer of early dividing embryos versus slower dividing embryos, *Human Reproduction* Vol.19, No.5 pp. 1155-1162, 2004.
- [16] Giorgetti C, Hans E, Terriou P, Salzman J, Barry B, Chabert-Orsini V, Chinchole JM, Franquebalme JP, Glowaczower E, SitriM-C, Thibault M-C, Roulier R, Early cleavage: an additional predictor of high implantation rate following elective single embryo transfer, *Reprod BioMed Online*. 2007; 14:85-91.
- [17] Meng-Ju Lee, Robertkuo-Kuuang lee, Ceavage speed and implantation potential of early-cleavage embryos in ivf or ICSI cycles, *Embryo biology* 2012.
- [18] Edessy M, Ali AEN, Fata A and Hamed W, Early cleavage of human embryos is a strong predictor for embryo implantation in ICSI, *New York Science Journal* 2013;6(12).
- [19] David K. Gardner, *Textbook of Assisted Reproductive Techniques* (Informa, Health care). Fourth edition.
- [20] Jan Tesarik, and Ermanno Greco, The probability of abnormal preimplantation development can be predicted by a single static observation on pronuclear stage morphology, *Human Reproduction* vol.14 no.5 pp.1318-1323, 1999.
- [21] Scott, L., Alvero, R., Leondires, M. and Miller, B. (2000). The morphology of human pronuclear embryos is positively related to blastocyst development and implantation, *Hum. Reprod.*, 15, 2394-2403
- [22] Tesarik J, Junca AM, Hazout A, Aubriot FX, Nathan C, Cohen-Bacrie Pand Dumont-Hassan M (2000) .Embryos with high implantation potential after intracytoplasmic sperm injection can be recognized by a simple, non-invasive examination of pronuclear morphology, *Hum Reprod* 15,1396-1399.
- [23] M. Montag, and H. van der Ven, Evaluation of pronuclear morphology as the only selection criterion for further embryo culture and transfer: results of a prospective multicenter study *Human Reproduction* vol.16 no.11 pp.2384-2389, 2001.
- [24] Wittemer, C., Bettahar-Lebugle, K., Ohl, J, Rongieres, C., Nisand, I. and Gerlinger, P. (2000), Zygote evaluation: an efficient tool for embryo selection, *Hum. Reprod.*, 15, 2591-2597.
- [25] Mohammad Ali Khalili, Ph.D. Vida Razavi, M.D., Farahnaz Mardanian, M.D., Navid Esfandiari D.V.M., Ph.D., H.C.L.D, The predictive value of pronuclear morphology screening on embryo development and pregnancy outcome in ART cycles, *Middle East Fertility Society Journal*, Vol. 13, No. 1, 2008, pp. 44-51
- [26] Balaban B, Urman B, Isiklar A, et al, The effect of pronuclear morphology on embryo quality parameters and blastocyst transfer outcome, *Hum Reprod* 16: 2357- 61, 2001.
- [27] Christopher Chen, F.R.C.O.G., and Suresh Kattera, Ph.D., Comparison of pronuclear zygote morphology and early cleavage status of zygotes as additional criteria in the selection of day 3 embryos: a randomized study, *Fertility and Sterility* Vol. 85, No. 2, February 2006 347 doi:10.1016/j.fertnstert.2005.07.1319.
- [28] Giuseppe Placido, High outcome predictability after Ivf using combined score for zygote and E M and growth rate, *Hum. Reprod*. 2002.

- [29] Kau-Chund Lan, Predictive value of using a combined Z score and d3 E M score in the assessment of E survival on d5.
- [30] Yu-li QIAN, Ying-hui YE, Chen-ming XU, Fan JIN, He-feng HUANG, Accuracy of a combined score of zygote and embryo morphology for selecting the best embryos for IVF, *Qian et al. / J Zhejiang Univ Sci B* 2008 9(8):649-655.
- [31] Salumets A, Hyden-Granskog C, Suikkari AM, The predictive value of pronuclear morphology of zygotes in the assessment of human embryo quality, *Hum Reprod* 16: 2177-81, 2001.
- [32] Nicoli A, Valli B, Di Girolamo R, Di Tommaso B, Gallinelli A, La Sala GB, Limited importance of pre-embryo pronuclear morphology (zygote score) in assisted reproduction outcome in the absence of embryocryopreservation, *Fertil Steril* 2007;88:1167-1173.
- [33] James AN, Hennessy S, Reggio B, Wiemer K, Larsen F, Cohen J, The limited importance of pronuclear scoring of human zygotes, *Hum Reprod* 2006;21:1599-1604.
- [34] Biezinová J, Svobodová M, Oborná I, Fingerová H, Dostá J, Krsková M., [Embryo quality evaluation according to the speed of the first cleavage after conventional IVF], *Ceska Gynekol.* 2006 May; 71(2):105-10.
- [35] Biezinová J, Svobodová M, Oborná I, Fingerová H, Dostá J, Krsková M. Embryo quality evaluation according to the speed of the first cleavage after intra Cytoplasmic Sperm Injection (ICSI), *Ceska Gynekol.* 2006 May; 71(3):204-8.
- [36] Jana Brezinova, Ivana Oborna, Magda Svobodova and Helena Fingerova, Evaluation of day one embryo quality and IVF outcome – a comparison of two scoring systems, *Reproductive Biology and Endocrinology* 2009, 7:9 doi:10.1186/1477-7827-7-9.
- [37] d. hlinka, b. kařatová, uhrinová, s. dolinská, j. rutarová, j. řezáčová, s. lazarovská1, m. dudáš, Time-Lapse Cleavage Rating Predicts Human Embryo Viability, *Physiol.Res.* 61:523-525, 2012.
- [38] Irene Rubio, Ph.D., Reidun Kuhlmann, Inge Agerholm, Ph.D., John Kirk, M.D., Javier Herrero, Ph.D., María-Jos Escrib, Ph.D. Jos_Bellver, Ph.D., and Marcos Meseguer, Ph.D., Limited implantation success of direct-cleaved human zygotes: a time-lapse study, *Fertility Sterility* 2012.
- [39] María Cruz Nicolás Garrido Javier Herrero Inmaculada Pérez-Cano Manuel Muñoz Marcos Meseguer, Timing of cell division in human cleavage-stage embryos is linked with blastocyst formation and quality blastocyst *Reprod Biomed Online.* 2012 Oct;25(4):371-81. doi: 10.1016/j.rbmo.2012.06.017. Epub 2012 Jul 7.
- [40] Mina Alikani, Gloria Caderon, Tomkin, John Garrisi, Magdalena Kokdand Jacques Cohen, Cleavage anomaly in early human embryo and survival after culture in vitro, *Hum Reprod.* vol. 15 no 12 pp. 2634-2643, 2000.
- [41] Thorir Harderson, Charles Hnasen, ANITA Sjoren and Kersti Lundin, Human embryo with unevenly sized blastomeres have lower pregnancy and implantation rates: indications for aneuploidy and multinucleation, *Human Reproduction* vol 16 no 2 pp 313-318, 2001.
- [42] Laëtitia Hesters, Pharm.D. Nadia Prisant, M.D., Renato Fanchin, Ph.D., Daniel H. M_endez Lozano, M.D. Estelle Feyereisen, M.D., Ren_eFrydman, Ph.D. Gerard Tachdjian, Ph.D., and Nelly Frydman, Pharm.D., Impact of early cleaved zygote morphology on embryo development and in vitro fertilization-embryo transfer outcome: a prospective study, *Fertility and Sterility* Vol. 89, No. 6, June 2008 1677 doi:10.1016/j.fertnstert.2007.04.047.
- [43] P Terriou1, C Giorgetti, E Hans, J Salzmman, O Charles, L Cignetti, C Avon, R Roulier, Relationship between even early cleavage and day 2 embryo score and assessment of their predictive value for pregnancy, *RBMOnline - Vol 14. No 3. 2007 294-299.*
- [44] Eric Van Royen, Katelijure Mangelschots, Miet Vereruyssen, Diane DC Neubourg, Marion Vakenburge, Greet Ryckaert and Jan Gerrig, Multinucleation in early cleavage embryo, *Hum Reprod.* vol. 18 no pp. 1062-1069, 2003.
- [45] Isaac Kligman, Claudio Benadiva, Mina Alikani1 and Santiago Munne'12, The presence of multinucleated blastomeres in human embryos is correlated with chromosomal abnormalities, *Human Reproduction* vol 11 no 7 pp 1492-1498, 1996.
- [46] M.J. Pelinck, M. De Vos, M. Dekens, J. Van der Elst, P. De Sutter and M. Dhont1, Embryos cultured in vitro with multinucleated blastomeres have poor implantation potential in human in-vitro fertilization and intracytoplasmic sperm injection, *Human Reproduction* vol. 13 no. 4 pp. 960-963, 1998.
- [47] M Takayuki Moriwaki, Nobuhiko Suganuma, Mina Hayakawa, Hiroko Hibi, Yoshinari Katsumata, Hidenori Oguchi and Madoka Furuhashi, Embryo evaluation by analysing blastomere nuclei, *Human Reproduction* Vol. 19, No. 1 pp. 152-156, 2004.
- [48] Katharine V. Jackson, B.S., Elizabeth S. Ginsburg, Multinucleation in normally fertilized embryos is associated with an accelerated ovulation induction response and lower implantation and pregnancy rates in in vitro fertilization-embryo transfer cycles, *Fertility and Sterility* vol. 70, no. 1, July 1998.
- [49] Haydar Nadir Ciray, M.D., Ph.D., Levent Karagenc, Ph.D., Ulun Ulug, M.D., Faruk Bener, M.D., and Mustafa Bahceci, M.D, Use of both early cleavage and day 2 mononucleation to predict embryos with high implantation potential in intracytoplasmic sperm injection cycles, *Fertility and Sterility* vol. 85, no. 2, Feb. 2006.
- [50] David K. Gardner1,3 and Michelle Lane1,2 Culture and selection of viable blastocysts: a feasible proposition for human IVF? *Human Reproduction*, Vol. 3, No. 4 pp. 367-382, 1997
- [51] David K Gardner, William B. Schoolcraft, Lyla Wagley, Treay Schlenker, John Stevens and John Hesla, A prospective randomized trial of blastocyst culture and transfer in In-Vitro fertilization, *Human Reproduction* Vol. 13, No. 12 pp. 3343-3440, 1998.
- [52] William B. Schoolcraft, M.D., and David K. Gardner, D. Phil, Blastocyst culture and transfer increase the efficiency of oocyte donation, *Fertility and Sterility* vol. 74, no. 3, September 2000.
- [53] Evangelos G. Papanikolaou, Elke D'haeseleer, Greta Verheyen, Hilde Van de Velde, Michael Camus, Andre Van Steirteghem1, Paul Devroey and Herman Tou, Live birth rate is significantly higher after blastocyst transfer than after cleavage-stage embryo transfer when at least four embryos are available on day 3 of embryo culture. A randomized prospective study, *Human Reproduction* Vol. 20, No. 11 pp. 3198-3203, 2005 doi:10.1093/humrep/dei2173198.
- [54] Efstratios M Kolibianakis1, Paul Devroey, Blastocyst culture: facts and fiction, *RBMOnline - Vol 5. No 3. 285-293.*
- [55] Takafumi Utsunomiya, Tac Naitu and Miyuki Nagali, A prospective trial of blastocyst culture and transfer, *Human Reproduction* Vol. 17, No. 7 pp. 1846-1851, 2002.
- [56] Serder Coskun, Johannes Hollander, Saad AL-Hasson, Hamad Al-Sufyan, Hend Al Moyman and Komal Jaraudi, Day 5 versus day 3 embryo transfer: a controlled randomized trial, *Human Reproduction* Vol. 15, No. 9 pp. 1947-1952, 2000.