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Approaches for prediction of the implantation potential of human embryos

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ABSTRACT

Optimization of assisted reproductive technologies (ART) has become the main goal of contemporary reproductive medicine. The main aspiration of scientists working in the field is to use less intervention to achieve more, and, if possible, in a more cost-effective way. A number of directions have been under development, namely – various stimulation protocols, ART with no stimulation whatever, all aiming at a single goal – the chase for Moby Dick, or the perfect embryo. Comprehensive embryo selection resulting in reducing the number of transferred embryos is one of the main directions for optimization of the ART procedures. Both clinical and laboratory procedures are being constantly improved, and today there is a significant number of clinics that report success rates of 30% and even higher. Based on results achieved, and analyzing data from millions of ART procedures, researchers from different centers are seeking to develop prognostic models in order to further improve success rates. One of the greatest challenges remains the reduction of the incidence of multifetal pregnancy, and that can be achieved only through reducing the number of embryos per transfer and a rise in single embryo transfer (SET) numbers. This, however, depends on reliable methods for preliminary embryo selection, employing a growing number of morphological, biochemical, genetic and other characteristics of the embryo. A primary concern in developing prognostic models for *in vitro* fertilization (IVF) outcome is selecting the prognostic parameters to be included. A number of publications define the main criteria that have an impact on fertilization outcome on the side of the embryo, and for the ultimate outcome of the ART procedure – on the side of the maternal organism as a whole. In this review, some of the most important parameters are discussed, with particular focus on their application for development of IVF prognostic models.

Key words: IVF, implantation, human embryo, assisted reproduction, prognostic models

1. EMBRYO QUALITY

1.1. Morphological criteria

The morphological parameters most often used for embryo quality assessment are blastomere size, cleavage rate, degree of fragmentation, cytoplasm appearance, blastomere nucleation (multinucleation has been shown to correlate to lower quality), but also zona pellucida peculiarities, time of the first mitotic division, etc**.** (Cumminis et al., 1986; Steer et al., 1992; Bertrand et al., 1995; Lundin et al., 2001). Morphological criteria for embryo quality assessment have changed over time. It has been shown that excellent quality zygotes sometimes result in poor quality embryos after *in vitro* culture, while some poor quality zygotes can result in excellent quality embryos. Because of this, it is generally

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assumed that oocyte quality is not a good prognostic parameter for ART outcome in comparison to embryo quality before transfer. Nevertheless, it has often been recommended that clinics use both zygote and late-stage embryo grading systems in combination (Figure 1).

1.2. Developmental stage

Most often embryos are transferred on day 2 or day 3 after fertilization. It is assumed that this provides sufficient time to perform tests for selecting the best quality embryo. Sometimes various culture media are used to sustain embryo development until day 5 through to the blastocyst stage.

The use of good quality blastocysts has been shown to improve success rates, as not all embryos survive to this stage. Embryo implantation in the endometrium takes place on day 5. Therefore, the blastocyst transfer at this moment improves synchronicity between embryo development and the endometrium, and has often been reported to improve successful pregnancy rates. Yet, in order to be possible to wait until day 5, it is necessary to have a sufficient number of embryos, which will then show their developmental competence. Thus, the blastocyst transfer is not a positive selection technique, but it is rather elimination.

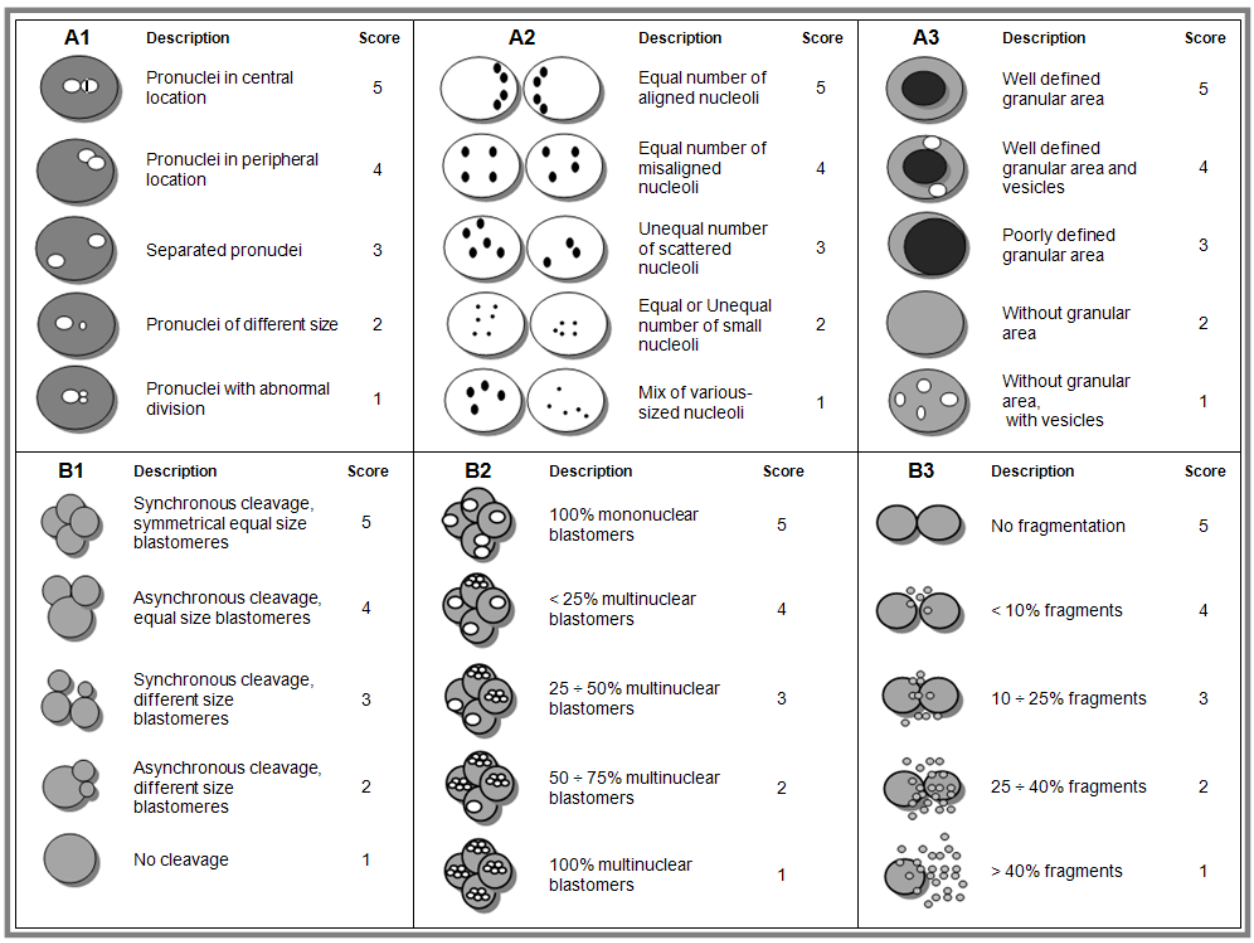

Figure 1. *Sample model of a quality grading system for oocytes and embryos: (**A**) Oocyte grading: score is equally distributed across three factors: (**A1**) – pronuclear location before first cell division; (**A2**) – location and appearance of nucleoli; (**A3**) – cytoplasm morphology; (**B**) Embryo morphology: once again score (15 points total) is equally distributed across three factors: (**B1**) – blastomere size and cleavage synchronicity; (**B2**) – level of multinuclear formation; (**B3**) – fragmentation level. (after De Placido et al., 2002).*

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1.3. Genetic factors

In the initial stages of embryo development the cell cycle control is carried out through a number of factors that are synthesized in the oocyte before fertilization. Genomic expression begins to gradually increase at the 8-cell stage. Preimplantation genetic aneuploidy screening (PGS) has failed. The big hopes that use to be put into this method unfortunately turned out to be in vain, because embryos tested for chromosome abnormalities by the FISH method at the 8-cell stage failed to show improved implantation as compared to controls (Blockeel et al., 2008). Two main reasons could explain this – on the one hand, the human embryo tends to eliminate mosaic blastomeres at later stages of its development, and on the other, not everything in the process of implantation depends on the right number of chromosomes. Because of this, analysis of the expression of specific genes is performed at later stages.

1.4. Metabolism

It has been suggested that embryo metabolism predetermines its survival at the different developmental stages. This has led to the development of new methods for analysis of embryonic metabolic processes, based on the interaction of the embryo with culture media. The application of spectroscopic methods has shown differences in spectral characteristics between successfully implanted embryos and embryos that failed to implant (Brison et al., 2007).

1.5. Processes of freezing / thawing

One of the key factors for selection of frozen-thawed embryos is the restarting of cleaving within 24 hours post-thaw. In post-thaw embryo culture overnight, cleaved embryos have been shown to have 10 times higher implantation potential in comparison to non-cleaved ones (Guerif et al., 2002). With the further development of methods and media for cryopreservation of embryos and oocytes in recent years, the significance of this factor has decreased.

1.6. Number of embryos transferred

The chances for successful embryo implantation and achievement of pregnancy rise with increasing the number of embryos to be transferred (Schieve et al., 1999; Salumets et al., 2006). The probability for successful implantation is increased by 22% with each additional embryo replaced. This rule can be explained with the molecular interactions between embryos and the endometrium that precede the actual implantation, as well as with the higher mass of the placenta during initial stages following implantation of multiple embryos, resulting in higher levels of hCG and progesterone (Matorras et al., 2005). The synergy between multiple embryos has beneficial effect on their long-term survival and decreases the risk for spontaneous abortion for any one of them (Lambers et al., 2007).

With the further development of ART methods, the understanding about the optimal number of embryos to transfer are also continually evolving; even opinions from 3-4 years ago are now changing. How many embryos to replace back in the uterus is the dilemma of each and every ART professional. Single embryo transfer cannot always be motivated as the right choice for each couple – sometimes it could actually decrease the chances for a successful procedure, which can eventually turn out to be expensive and demotivating for the patients. On the other hand remain the risks from pre-term birth and other complications of multifetal pregnancy, which are in no case easier to handle.

2. FEMALE FACTORS AFFECTING THE ART OUTCOME

The ultimate IVF result is much more dependent on maternal factors. This can be explained by the dominating role of oocyte components during the early stages of embryo development, as well as the direct involvement of the female organism in the processes of implantation and further development of the conceptus.

2.1. Oocyte quality

One of the main factors affecting fertilization outcome is oocyte quality. Various models for oocyte quality grading have been applied, most of them based on morphological criteria (Xia, 1997; Loutradis et al., 1999; Ebner et al., 2000).

Another significant parameter is the number of aspirated oocytes. An international study (Sunkara et al., 2011) reviewing 400 135 cycles, reports an average of 9 oocytes picked up per woman. The live birth rate reported from fresh IVF cycles rises with increasing the number of aspirated oocytes to 15, which is assumed to be the optimal number, and then begins to drop with further increase of the number of aspirated oocytes above 20. On the other hand there is a

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serious risk of ovarian hyperstimulation syndrome when the number of aspirated oocytes goes above 18 (Verwoerd et al., 2008; Lee et al., 2010).

2.2. Low ovarian response

In the cases when the result of ovarian stimulation is suboptimal we talk about poor or low ovarian response, which correlates with lower chances for implantation and successful pregnancy after IVF. Various methods for diagnosis, prognosis and management of this condition have been developed (Tarlatzis et al., 2003; Broekmans et al., 2007).

Low ovarian response is manifested in a low peak of estradiol level during stimulation (<300 pg/ml), estradiol level below 200 pg/ml on day 5 of stimulation, low number of aspirated oocytes (<3), and poor embryo quality (Fisch et al., 2008).

Among the prognostic parameters that are directly related to poor ovarian response are endocrine markers such as the base concentration of FSH, estradiol, AMH (which has an influence on follicle growth and development as well as on the number of preantral follicles) (Toner et al., 1991; Licciardi et al., 1995; La Marca & Volpe, 2006).

Stimulation of poor responders is a challenge for every IVF program, and there is no universal solution. It has to be noted that in the case of poor responders the number of embryos to be transferred is of lower significance, because the big question with those patients is not how many embryos to replace, but if there will be any embryos at all for transfer.

2.3. Endometrial receptivity

The receptivity of the uterus is of crucial importance for the success of the ART procedure, and it has been estimated to contribute between 30 to 64% to successful implantation (Rogers et al., 1986). Naturally the blastocyst is able to implant in the endometrium for a relatively limited period of only 48 hours per menstrual cycle. Using various types of stimulation this period can be significantly prolonged (Develioglu et al., 1999).

Embryo implantation is regulated by numerous endometrial factors. Glycodeline is a key component of endometrial secretions and its expression is regulated by progesterone. Glycodeline secretion is a known determinant of endometrium maturity, which is also an important factor in successful embryo implantation (Halttunen et al., 2000).

Most researchers share the understanding that the embryo is the most important factor in the initial stages of the process of implantation, while continuing pregnancy up to 6th g.w. depends to a greater extent on the combined effects of factors of both endometrial and embryo origin (Lambers et al., 2007). A coordinated molecular dialogue has been demonstrated to exist between the embryo and the endometrium, involving interactions of various growth factors and regulation factors such as leptin, αvβ3 integrin etc. (Irving & Lala, 1995).

At present, there exists a number of convenient methods for sampling and analysis of cytokines, chemokines, growth factors, and regulation factors, secreted by both the embryo and the endometrium (Boomsma et al., 2009a). There is a research study, launched to investigate and characterize key components of endometrial secretion that may have prognostic value as determinants for successful embryo implantation and successful pregnancy (Boomsma et al., 2009b).

Endometrium characteristics are among the key predictive parameters for the success of IVF procedures. Endometrial lining thickness of less than 6 mm is considered a poor prognosis, due to the difficulty it presents for good embryo adhesion. On the other hand, abnormal thickness of the endometrium is also bad news – endometrium lining of more than 12 mm during transfer seriously reduces the chances for successful implantation (Weissman et al., 1999; Kumbak et al., 2009).

2.4. Female age

Female age was the first indicator for the chances for successful pregnancy to be ever discussed as a prognostic factor for success in IVF procedures (Table 1) (Padilla & Garcia, 1989; Templeton et al., 1996). The main trend described is a decrease in success rates with advanced female age. This is especially well observed in patients beyond 38 years, with a dramatic drop in success rates. The key reason for this is the fact that with advanced age the oocytes produced demonstrate a marked decline in both numbers and quality. This is attributed to the prolonged exposure of chromosome telomeres to the detrimental effects of free radicals and reactive oxygen species in combination with the reduced synthesis of telomerase in the oocytes. This ultimately results in telomere shortening and risk of unwanted events like misalignment and wrong recombination of chromosomes during meiosis.

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Table 1. *Models including different types of prognostic factors for IVF procedures – age, embryo morphology, number of transferred embryos, type of treatment, diagnosis, serum biochemical markers of the endometrium, etc.*

Reference	Prognostic factors	Number repeats	Model
Chuang *et al*. 2003	**Age** (decline in success with aging)	1405 cycles	Logistic regression – pregnancy
Commengues-Ducos *et al*. 1998	**Age** (decline in success after >38 years)	923 transfers	Logistic regression – pregnancy and implantation
Haggarty *et al*. 2006	**1. Age** (decline in success with aging) 2. **Genetic factors** 3. **Dietary variables**	602 women (1 cycle)	Logistic regression – births
Lee *et al*. 2006	1. **Age** (decline in success with aging) 2. **Embryo quality** – cumulative score (top three)	584 transfers	Logistic regression – pregnancy
Ottosen *et al*. 2007	1. **Age** 2. **Embryo quality** – best and second best grade 3. **FSH** (follicular stimulating hormone)	2193 cycles	Logistic regression – pregnant vs non-pregnant, twins vs singletons
Roberts *et al*. 2009	1. **Age** (cubic) 2. **Embryo quality**– score 3. **Patient diagnosis** (idiopatic – higher success) 4. **Number of cycles** (>2 decline in success) 5. **FSH** 6. **Smoking** (decline in success) 7. **Alcohol consumption**	1198	EU – model – (multinominal response) live births
Rhodes *et al*. 2005	1. **Age** (decline in success with aging) 2. **Number of oocytes, % fertilized** 3. **ICSI** (increase in success) 4. **Cook catheter** (decline in success). 5. **Embryologis.**	205 (1 cycle)	Logistic regression – pregnancy
Sabatin *et al*. 2008	**Age** (relation to **FSH**)	1589	Significance tests, Logistic regression – live births
Terriou *et al*. 2001	1. **Age** (decline in success with aging) 2. **Embryo quality** – cumulative score 3. **Number of recovered oocytes, number of embryos transferred**	10000 transfers (5000 for model development & 5000 for model validation)	Logistic regression – pregnancy
Alsalili *et al*. 1995	1. **Age** (decline in success with aging) 2. **Male factor** (decline in success) 3. **Serum estradiol levels** (increase in success)	5209 cycles (2391 couples)	Cox regression – pregnancy
Hunault *et al*. 2002	1. **Age** 2. **Embryo development stage; morphological points** 3. **Number of oocytes** 4. **Day of transfer**	642 women (1 cycle)	Logistic regression / EU model – pregnancy and number of twins
Wilding *et al*. 2007	**Quality of oocytes** – score	822	Significance tests / Linear regression – fertilization result
Wheeler *et al*. 1998	1. **Age** (decline in success with aging) 2. **Embryo morphology** – total score	795 cycles	Logistic / Conditional logistic regression – implantation
Elizur *et al*. 2005	1. **Number of embryos** (two embryos double the chance for live birth) 2. **ICSI** (increase in success)	5310 cycles (with transfer)	Survival analysis (discrete) – live births
Tsafrir *et al*. 2007	1. **Age** 2. **Number of embryos transferred** 3. **Drug dose**	381 (women over 40)	Logistic regression – pregnancy
Wald *et al*. 2005	**1. Male infertility** **2. History of female infertility**	113 cycles	Nueral networks, discriminate analysis, logistic regression – pregnancy

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Templeton *et al*. 1996	1. **Diagnosis** (unexplained better) 2. **Donor oocytes** (improves success) 3. **Previous pregnancy** (improves success) 4. **Infertility period length** (decline in success)	36961 cycles (2893 cycles)	Logistic regression –births
de Klerk *et al*. 2008	1. **Treatment course** 2. **Depression** (slightly worse)	289	Logistic regression –births
Tan *et al*. 1994	**Treatment course**	2893 women	Survival table / Logistic regression
Engmann *et al*. 2001	1**. Previous births** (IVF) (better) 2. **Number of previous failures** (worse)	7700 cycles (4417 women)	Logistic regression – birth
Ferlitsch *et al*. 2004	1. **FSH** (lower level – higher success) 2. **BMI** (**Body Mass Index)** – (lower value/ increased success)	171 (1 cycle)	Logistic regression – pregnancy
Fujimoto *et al*. 2007	1. **FSH** (lower level –higher success) 2. **Normal menstrual cycle** (better)	112 (women over 40)	Significance tests – love births
Sneed *et al*. 2008	**BMI** (**Body Mass Indes) –** relation to age (higher values in younger patients – decline in success)	1273	Logistic and linear regression – various outcomes
Duran *et al*. 1998	**Sperm morphology and DNA status.**	66 couples	Logistic regression – fertilization
Shapiro *et al*. 2008	1. **Blastocyst diameter** (higher value – increased success) 2. **Early blastomere formation** 3 .Low preovulatory **serum levels of progesterone**	361 cycles (320 women)	Logistic regression – pregnancy
Esterhuizen et al. 2001	**ZIAR – zona pellucid – induces acrosome reaction**	35 couples	ROC analysis - fertilization
Boomsma et al. 2009b	**Cytokines** (analysis of endometrial secretions) **MCP-1, IP10** – implantation **IL-1β, TNF-*α*** – clinical pregnancy	210 women	Logistic regression – implantation. clinical pregnancy
Fasouliotis et al. 2004	**Cytokines (female serum levels) - INF-g, IL-2**	159 women	Significance tests – early pregnancy
Eldar-Geva et al. 2005	**Inhibin B, antimullerian hormone and estradiol**	56 women	Logiistic regression and ROC analysis – number of oocytes /pregnancy
Kim et al. 2012	**Serum biomarkers – chloroion gonadotropin, progesterone, and inhibin**	68 women	ROC analysis - pregnancy

Facts speak for themselves – every woman loses 20% in reproductive potential after the age of 40. In women over 43, nearly 99% of all retrieved oocytes show aneuploidy. Other possible causes include metabolic defects, abnormalities in the meiotic spindle and abnormalities in chromosome disjunction during meiosis, as well as other age-related processes (Maheshwari et al., 2008; Kuliev et al., 2011).

2.5. Body Mass Index (BMI)

Body mass index (BMI), which is the ratio between one's weight and the squared height, can be used to determine the energy levels of the organism and the mass of adipose tissue. Adipose tissue modifies steroid hormones and produces adipokines (Gonzales et al., 2000).

Leptin is probably the most extensively studied member of the adipokine family. Its serum levels are direct proportional to adipose tissue mass. Leptin is expressed in reproductive tissues as well, including the secretory endometrium where it probably contributes to angiogenesis regulation. Leptin expression has been detected in blastocysts as well, where it is thought to participate in the process of embryo implantation (Cervero et al., 2004).

Ghrelin is another adipokine with concentrations in reverse proportion to BMI (Budak et al., 2006). It has been demonstrated that ghrelin exerts inhibitory effect on in vitro embryo development and implantation (Kawamura et al., 2003).

Obesity, characterized by higher BMI values, presents with hormonal dysbalance and alterations in normal adipokine concentrations, and hence in the related metabolic pathways. As a result, women with high BMI are characterized by higher risk of reproductive failure and lower chances for successful embryo implantation after IVF. Obese women (BMI>25) have 20% to 50% lower chances for

successful pregnancy, with success rates decreasing with increase in BMI. These cases require modified treatment with higher hormone doses during ovarian stimulation (Fedorcsák et al., 2000).

Underweight women (BMI<18.5) also suffer from reproductive problems, particularly abnormalities in oocyte maturation and the menstrual cycle, as well as a higher risk of spontaneous abortion (Helgstrand & Andersen, 2005). Various studies indicate a negative effect of low BMI values on the chances for successful pregnancy after IVF (Wang et al., 2001). Until now, however, the precise molecular mechanisms involved in this remain to be unveiled.

3. A STRATEGIC APPROACH TO DEVELOPING A PROGNOSTIC MODEL

The larger part of existing models are founded on inclusion of different groups of prognostic parameters (factors) and the performance of linear and logistic regression analyses (Table 1). Most logistic regression analysis (LRA) models use as outcome measure variables such as biochemical pregnancy positive test results, number of live births per cycle, and total live births (Roberts et al., 2010).

A specific difficulty for the analysis of data from transfers with more than one embryo is the fact that it is impossible to determine which embryo(s) exactly have been involved in the successful pregnancy outcome. This requires that as prognostic determinants are used either average values of all embryos used or only the values of the embryo with the highest score (Roberts, 2007).

Another problem related to the requirement for observing the rule of independent data units for statistical analysis (for oocytes, embryos, patients etc.) is the probability of performing more than one IVF cycle on the same patients – the so-called "couple effect". In these cases using data from multiple cycles of the same patients requires special handling by hierarchical ordering and adding of standard random effects, which accounts for the non-independence of certain part of analyses (Ecochard & Clayton, 1998).

In certain cases the prognostic models include predictor parameters of different nature, for which separate regression equations are calculated, and then combined in a new mathematical dependency. An example for this is the so-called EU-model, where the success probability for embryo transfer is subdivided into two components (submodels) – the implantation potential of the embryo (E) and uterine receptivity (U). For a successful transfer at least one embryo has to implant and the uterus has to be receptive (Roberts et al., 2009).

The processes of folliculogenesis and oogenesis, the early stages of embryo development, endometrial receptivity, are all influenced by numerous factors, all acting in concertation and synchronicity. Pleiotropy is an additional factor that makes the choice of a "best" prognostic factor even more challenging. Employing a combination of factors in the context of a mathematical model would yield a significantly better prognostic value, based on the cumulative effect of prognostic value of separate markers.

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