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Mathematical methodology to obtain and compare different embryo scores

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ABSTRACT

In Vitro Fertilization (IVF) units need to decrease multiple pregnancies without affecting their overall success rate. In this study we propose a mathematical model to evaluate an embryo's potential ability to implant in the uterus. Embryos are graded by the embryologist based on the number of blastomeres, evenness of growth and degree of fragmentation. Therefore, the following variables were considered: number of blastomeres produced by division of the egg after fertilisation (blastomeres), symmetry and fragmentation of the embryo (grade). This model evaluates the embryos assigning them a score which represents their quality. The main result derived from this model is the estimation of the significant improvement in the implantation rate due to the increase in blastomere values and the decrease in grade factor values. But the increase from two–three to four produces more improvement in the implantation rate than two–three to five–six blastomeres.

First, statistical models were used to study embryo traceability from transfer to implantation and to evaluate the effect of the quality of the embryos (embryo score) and women's age on implantation potential. This score was obtained by making predictions from the fitted model which was used to rank embryos in terms of implantation potential. Then we totalled the scores of embryos that had been transferred to each woman for obtaining the Embryo Quality Index (EQI). In addition, we studied the effects of EQI and women's age on pregnancy. Finally, statistical techniques such as Receiver Operating Characteristics (ROC) and bootstrap procedures were used to assess the accuracy of this model. This embryo score is a quick, efficient and accurate tool to optimise embryo selection for transfers on the second day after fertilisation. This tool is especially useful for transfers involving non-top embryos.

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1. Introduction

The increased obstetric and perinatal risks involved in multiple pregnancies urge the clinician to reduce the number of embryos to transfer following in vitro fertilization (IVF)-intracytoplasmic sperm injection (ICSI) cycles [1–3]. IVF and ICSI assisted reproduction techniques are highly complex. They are so called because it is necessary to have an embryology laboratory for the handling of gametes and embryos in vitro, i.e. outside the woman's body. Embryo transfer refers to a step in reproduction techniques in which embryos are placed in the uterus of a female with the intent of establishing a

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pregnancy (embryo implantation). High multiple pregnancy rates correlate both to the number and quality of transferred embryos, therefore [3–5] propose to select only one top quality embryo to transfer (SET). The implementation of a top quality single-embryo transfer (SET), should produce an important decrease in multiple pregnancies without a significant decrease in pregnancy rates [6–10]. However, the implementation of SET determines an unacceptably low pregnancy rate, particularly in older women and in those with poor embryo quality [10]. Thus, it is important to increase our knowledge of the implantation potential of each individual embryo in order to select top quality embryos for transfer. At the same time a ranking selection, which allows the reduction of multiple pregnancies without reducing pregnancy rates, should be established.

The two factors that most influence the implantation rate are a woman's age and embryo quality [11–18]. A woman's age is unchangeable. However, when there is a sufficient number of embryos available, we can select the embryos to be transferred with the greatest implantation potential according to morphological criteria such as blastomere number and blastomere symmetry, equality and multinuclearity [14,19–25,18]. Evaluation of the implantation potential of transferred embryos has generally been based on the construction of accumulated embryonic scores. Assumptions need to be made about the overall quality of transferred embryos and their subsequent implantation due to a lack of knowledge about the exact quality of the embryo finally implanted [26–28,13,29,11,16,30]. In more recent studies, logistic regression models have been used to predict the possibility of embryo implantation [18]. The inclusion of embryonic quality as continuous instead of categorical variables or factors in the logistic regression models, forces those authors to use transformed variables. It also does not provide knowledge about which values of the variable produce significant increases in the implantation rate.

In the present study a distinctive methodology for estimating the embryos' implantation potential has been developed. This methodology allows us to evaluate the effect of each variable's value when it is considered as a factor. In addition, we propose the validation of models by using Receiver Operating Characteristics (ROC) curves. The aim of this paper is to propose a mathematical methodology to obtain and compare different embryo scores adapted to the number and nature of our database variables.

2. Materials and methods

2.1. Data

The paper is a retrospective study of 5242 cycles of IVF-ICSI with transfers of one, two or three embryos on day 2 (second day after fertilisation) in the Human Reproduction Unit at the University Hospital La Fe in Valencia from January 2003 to January 2007.

2.2. IVF-ICSI procedure

The women were treated using a controlled ovarian hyper-stimulation protocol (COH), including down regulation with a gonadotropin-releasing hormone (GnRH) agonist in a long protocol. Stimulation was performed with recombinant follicle stimulating hormone (FSH: Gonal or Puregon). Oocytes were retrieved 36–38 h following human chorionic gonadotrophin (HCG) using transvaginal sonographically guided puncture.

Fertilisation was performed by conventional IVF or ICSI, following standard techniques [31]. A commercial culture media was used according to local routines (MediCult Denmark). The oocytes were inseminated (or injected with sperm after denudation for ICSI) after 2–6 h of incubation and cultured in an IVF medium (MediCult, Denmark) in a 5% CO_2 incubator at 37 °C. All ICSI were performed with motile spermatozoa. When both ICSI and IVF embryos were available, the best quality embryos were transferred. Fertilisation was checked 16–20 h after insemination. The embryos were evaluated and transferred on day 2 after oocyte retrieval. Selection was performed immediately before embryo transfer.

The embryos were selected depending on cleavage rate and blastomere symmetry size and fragmentation. The embryo classification was modified from the system described by [28] as follows:

- Grade 1 (G1) embryos consisted of symmetrical blastomeres of approximately equal size and without anucleate fragments.
- Grade 2 (G2) embryos had blastomeres of even or uneven size and had less than 15% of the volume of embryos filled with anucleate fragments.
- Grade 3 (G3) embryos had anucleate fragments occupying between 15% and 50% of the volume of the embryos.
- Grade 4 (G4) embryos had anucleate fragments occupying more than 50% of the volume of the embryos.

Then, all the transferred embryos were scored considering the following variables: the number of blastomeres (2, 3, 4, 5 and 6) and the grade of fragmentation and variation in the size of the blastomeres (Grades 1, 2, 3 and 4). To evaluate both the number of blastomeres and fragmentation grade of the embryos, the cumulative embryo score (CES) [11] for each embryo transfer was calculated. The CES was calculated multiplying the number of blastomeres from each embryo by their grade recoded numerically as G1 = 4, G2 = 3, G3 = 2 and G4 = 1. Thus the embryos with the highest score were those which had a higher number of blastomeres and less degree. It is a criterion used to select higher quality embryos for later transfer.