The improvement of oocyte selection for social freezing application

Federica Cariati[1,2,3], Francesca Bagnulo[4], Stefania Ramona Peluso[4], Marialaura De Simone[4], Alessandro Conforti[4], Rosario Pivonello[5], Antonella Vaccina[6], Carlo Alviggi[4]

[1]CEINGE-Biotecnologie Avanzate scarl, Napoli

[2]KronosDNA s.r.l., spin-off dell'Università degli Studi di Napoli Federico II, Napoli

[3]Dipartimento di Medicina Molecolare e Biotecnologie Mediche, [4]Dipartimento di Neuroscienze, Scienze Riproduttive e Odontostomatologiche e [5]Dipartimento di Medicina Clinica e Chirurgia, Università degli Studi di Napoli Federico II, Napoli

[6]IBM Italia S.p.A.

ABSTRACT

Lifestyle, educational opportunities, career choices and new unions lead to pregnancy in more advanced age, increasing the emerging preventive solution to freeze oocytes at a young age for later use. In this scenario, the oocyte selection has a great importance in order to choose the best ones capable of a good subsequent embryo development and implantation. The aim of this study was to develop a decision support system, able to classify oocytes according to a score based on morphological features and patients' clinical data. The approach would offer a more effective selection method because it is not dependent on the doctor's experience or on an "at-first-sight" impression. As a first step, a prototype system able to support embryologists in oocyte selection was presented and an experimental evaluation on a real set of data provided. The developed pipeline included the identification of main morphological features influencing oocyte quality and the assignment of a weight and of a better way of measuring them. After that, a standard data format collecting in an organized way all morphological features of oocytes, zigote and embryos and patients' clinical data was developed. More than 150 oocytes images, taken in standard and comparable conditions, from 35 women were collected. Morphological features were extracted manually and automatically. A preliminary version of the scoring algorithm was tested on these data.

INTRODUCTION

The number of women requesting oocytes cryopreservation is increasing, because it represents a precautionary measure to preserve fertility (1). In fact, through the application of the traditional techniques for the oocytes cryopreservation, the later access to assisted reproductive technology (ART) procedures is possible. Taking into account changes in lifestyle, educational opportunities, career choices and unions, the "social oocytes freezing" appears a good solution for a young woman who is not ready for a pregnancy and projects for later (2). Furthermore, the oocytes cryopreservation may be offered to those women who have to undergo chemotherapy, radiotherapy or surgery that irreversibly affect the reproductive capacity (3). Finally, it is also indicated in the case of a family history of premature menopause (4).

The oocytes cryopreservation is the final stage of an articulated procedure that includes the hormonal profile for the assessment of ovarian reserve and transvaginal ultrasound in menstrual phase with the measurement of ovarian volume and the count of the antral follicles, the induction and ovulation monitoring, which allows the simultaneous maturation of several follicles, and the oocytes pick-up. Many are the variables that interfere with the whole process of obtaining a pregnancy by ART, but the crucial points are the preliminarily evaluation of the ovarian reserve, the definition of an appropriate stimulation protocol to obtain an adequate number of oocytes and their culture conditions and, finally, the identification of the oocytes with high probability to develop in a good embryo.

Before the ART procedure, a hormone stimulation protocol on the female patient is carried out, which, in the majority of women, causes mild effects connected with the hormone therapy, even if the ovarian hyperstimulation syndrome can rarely occur (5). Therefore, it is mandatory

Correspondence to: Carlo Alviggi, Dipartimento di Neuroscienze, Scienze Riproduttive e Odontostomatologiche, Università degli Studi di Napoli Federico II, Via Sergio Pansini 5, 80131 Napoli. Tel. 0813737890, Fax 0813737808, E-mail alviggi@unina.it

Ricevuto: 07.04.2017 Revisionato: 07.06.2017 Accettato: 08.06.2017

Pubblicato on-line: 15.11.2017 DOI: 10.19186/BC_2017.046

to perform a preliminary assessment of the number and quality of oocytes, due to age and ovarian reserve of the patient, by the evaluation of anti-Müllerian hormone (AMH), follicle-stimulating hormone (FSH) and 17β-estradiol in menstrual phase, the count of antral follicles and the measurement of ovarian volume. Furthermore, clinicians can adopt different stimulation protocols according to their experience and, more importantly, according to the patients' health conditions (5).

After the *in vitro* fertilization (IVF), the oocyte is placed in cell culture and checked during the following day for signs of fertilization. The fertilized oocyte develops into embryo in laboratory in one to 5 days, then it is transferred in the woman's uterus. However, it is possible that the oocyte may not develop into an embryo even after it is injected with sperm or the embryo may stop the development, so that the transfer cannot be performed.

The oocyte and embryo quality can be determined by genetic alterations (6). For this scope, the preimplantation genetic screening (PGS) for the identification of chromosomes abnormalities is the most effective selection method (7). Recent data show that the analysis is also applicable on frozen-thawed embryo (8).

Many studies discuss about the topic of oocytes and embryos non-invasive evaluation showing a set of morphology parameters to be examined for both oocytes and embryos. Regarding the oocyte, these parameters include oocyte and cytoplasm dimension, perivitelline space (PS) and zona pellucida (ZP) thickness, first polar body (FPB) conformation, and more subtle abnormalities (dysmorphisms) of cytoplasm, such as central granularity, inclusions and vacuolation, features that can influence the assisted fertilization process (9). It has been suggested that regular patterns of granularity are related to oocyte maturity (10-13) and in a pronuclear stage oocyte score based on cytoplasmic substructures has been addressed by considering abnormalities such as excessive granularity (14).

It is clear that there are numerous factors that contribute to determining a good oocyte quality. Consequently, the choice of the most suitable oocyte is based on various parameters. The embryologists are recording a score about each oocyte to freeze to perform the selection of the best oocytes to thaw, but to date this evaluation is based on subjective experience. Hence, it could be useful to have at clinicians' disposal a system able to support them in both processes of defining a therapeutic plan for patients, with the aim of obtaining a great number of oocytes, and of selecting the most promising ones obtained after the stimulation process, with the goal of improving the success rate of fertilization and implantation. In particolar, a computer-assisted system, able to exploit past experiences and to provide suggestions about the oocyte quality, could be useful to support embryologists in making decisions for selecting the best quality oocytes.

The aim of this work was the application of a pipeline to support oocyte selection (SOS) for social freezing application.

MATERIAL AND METHOD

Samples

158 metaphase-II oocytes data were collected from 37 patients encountered at the IVF center of Federico II University of Naples from January 2009 to November 2010. From each patient, the following data were registered to be inserted in the system: patient's reference number, age, weight, height, body mass index (BMI), basal follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels, female and male physical state, stimulation protocol parameters [levels of gonadotropin-releasing hormone (GnRH), recombinant FSH (rFSH), recombinant LH (rLH), human menopausal gonadotropin (HMG), FSH]. All patients provided informed consent to anonymous use their clinical and laboratory data for scientific purposes.

The oocyte related data of interest for scoring and evaluation were: diameter, refractal body (number and dimensions), ZP thickness, vacuoles (number and dimensions), PS thickness, FPB (fragmented or not), cytoplasm polarity (if present), cytoplasm granularity classification, intracytosplasmic sperm injection (ICSI) information (if done), zigote and embryo information. Oocyte images were acquired by a Nikon Eclipse TE200 inverted microscope; each image has a size of 1280x960 pixels.

The SOS pipeline

As detailed below, it includes 3 different sections: 1) insert and search data, 2) features extraction and 3) scoring system.

Insert and search data

This module allows inserting all data related to a patient, searching for an existing one and eventually add new information in a relational data base that has been designed to contain the heterogeneous kind of data required by the system to perform analysis. Finally, it allows storing all the oocyte images to be analyzed. In this way, this module allows creating an exhaustive patient's record that will constitute the base for the analysis and the next steps for the system control flow.

Features extraction

It aims to automatically extract some morpho-structural features from oocyte images by means of image processing and machine learning techniques, as previously described (15). The module develops in cascade: morphological operators to extract the oocyte diameter from the image (16), the Hough transform to exactly delineate the cytoplasm region of the oocyte (17) and finally the Haar transform to represent the texture of a rectangular central region of the oocyte by means of statistical measures with the aim of analyzing the cytoplasm central granularity (18). Then, machine learning techniques and, specifically, a k-means clustering algorithm is performed to obtain a clustering model of these regions and to classify new unseen

extracted central regions according to the learned model to provide a label for the central granularity of the new incoming oocytes.

Scoring system

This system has been developed from data available in literature (19-22) and experience of embryologists. It evaluates 8 morphological features measured from oocytes microscope images. According to the experience of clinicians, these parameters have been divided into 8 different weight classes according to a priority index (Table 1); each of them should satisfy particular conditions or fit in a particular range of values.

Table 1
Oocytes morphological features and related weights

Parameter	Weight
Oocyte diameter	1
Refractal body	2
Zona pellucida thickness	3
Vacuoles	4
Perivitelline space	5
First polar body	6
Cytoplasm polarity	7
Cytoplasm granularity	8

Table 2 summarizes feature values related to both positive and negative contribution; we assumed a tolerance equal to ±10% to feature values. Each parameter contributes differently to the calculation of the score. For this reason, in accordance with the clinicians, we assigned a different weight in case of positive/negative value for each of them, as reported in Table 3. The score was evaluated as a weighted average on standardized parameters as follows:

$$s_k = \frac{\sum_i Cp_k(i)}{\sum_i Cp(i)}$$

where Cp_k is the weight of the feature (i) for the oocyte k normalized to the sum of all values of Cp.

RESULTS

We tested the SOS pipeline by analyzing how the automatic evaluation was in agreement with the clinician evaluation for the task of oocyte scoring and features extraction. The results of applying the proposed SOS pipeline are presented in two case studies.

Table 2
Morphostructural feature values related to both positive and negative contribution

Parameter	Positive feature	Negative feature
Oocyte diameter	[115-δ; 165+δ] μm	]115-δ; 165+δ[μm
Refractal body	Not present OR diameter <5 μm	Diameter ≥5 μm
Zona pellucida thickness	[15-δ; 20+δ] μm	]15-δ; 20+δ[μm
Vacuoles	Not present OR diameter <9 μm	Diameter ≥9 μm
Perivitelline space	[14-δ; 16+δ] μm	]14-δ; 16+δ[μm
First polar body	[14-δ; 16+δ] μm	]14-δ; 16+δ[μm
Cytoplasm polarity	Present	Absent
Cytoplasm granularity	Homogeneous, bright, medium granularity	Not homogeneous, dark, high granularity

Table 3
Weights of positive and negative contribution of the features in the oocyte scoring

Parameter	Positive contribution	Negative contribution
Oocyte diameter	2	1
Refractal body	2	1
Zona pellucida thickness	2	1
Vacuoles	3	0
Perivitelline space	3	1
First polar body	4	3
Cytoplasm polarity	4	2
Cytoplasm granularity	4	1

Score evaluation (case study 1)

To test the scoring algorithm, we compared the oocyte score assigned by the system with the selection manually made by the clinicians and with the result of ICSI procedure and embryo growth in case of oocyte real selection for fertilization. Specifically, a threshold value t was defined and considered of good quality, and hence potentially able to give rise to a right cell development, for the oocytes that obtained a score value s_k >t. In this evaluation t=0:5 and, according to this value, 77 out of 157 oocytes (49%) obtained a score >0:5 and therefore considered of good quality, according to the automatic system. The remaining 80 (51%) obtained a score ≤0:5 and hence they were considered poor quality oocytes.

Based on the manual evaluation provided by the clinicians, oocytes were divided in 3 classes: C1, oocytes that were selected for the fertilization and that had a correct development after fertilization; C2, the ones that were selected for the fertilization, but that had not a correct development after fertilization; C3, the oocytes that were not fertilized and probably rejected by the embryologists. A deep analysis, which was performed according to further information provided by clinicians about the oocyte fertilization and growing, revealed that among the oocytes that the system considered good for fertilization according to the assigned score, i.e. s_k >0:5, 37 belong to C1, 24 belong to C2 and 16 belong to C3. Among the oocytes that the system considered of poor quality and hence not appropriate for fertilization according to the assigned score, 43 belong to C1, 29 belong to C2 and 8 belong to C3.

Features extraction (case study 2)

The SOS system is able to automatically extract two oocyte morpho-structural features: diameter and cytoplasm texture. To test whether these measures were properly acquired by the system, we compared them with manual evaluations made by clinicians.

About oocytes diameter evaluation, we obtained that the automatic measure was in agreement with the manual one for 98 oocytes (62% of measure reliability). However, in the other cases, the SD of the difference between the automatic extracted measure and the real diameter was ~11 μm, representing the 0.06% of the real measure.

Regarding the central granularity evaluation, currently clinicians evaluate the morphology of the cytoplasm through visual inspection by considering the presence of a central area that is characterized by a low or medium degree of granularity as an important feature for good quality oocytes. In addition, the presence of a high not homogeneous central granularity is considered an indicator of not-good-quality oocytes. For these reasons, we aimed at obtaining two classes from the clustering step: homogeneous-clear-medium cytoplasmic granularity and non homogeneous-dark-high cytoplasmic granularity. Hence, the k-means algorithm was set to generate two clusters on the set of images representing the extract central region of the cytoplasm. A qualitative analysis of these clustered regions carried out by an expert confirmed that they correspond to a good classification of the oocytes. Indeed, the resulting clustered images (i.e., oocytes with low-medium and high central granularity) have been submitted to the opinion of clinicians, who found the overall performance of the proposed method very satisfactory.

A quantitative validation was performed by comparing the automatic result with the manual evaluation provided by clinicians. As a result of this comparison, the system was able to correctly cluster oocytes with an error estimated at 30%. After this, the learned clustering model was saved and employed to automatically evaluate the new incoming oocyte images provided to the system. The new set of data was made up of 43 oocytes images from which the central region of the cytoplasm was automatically extracted and represented along with its first order statistical measures to obtain the feature-vector representation of the texture. Then, the clustering learned model was applied resulting in a correct classification of the central region in ~85% of the new analyzed oocytes.

DISCUSSION

All the collected data will constitute the system knowledge base accessed by an “Oocyte scoring” and a “Features extraction” modules. The first one assigns a score value to each oocyte according to a scoring metrics that takes in consideration 8 morphological features, some automatically extracted and some values manually inserted by technicians. By analyzing the results obtained by the score evaluation, it is possible to affirm that the SOS pipeline was right in 62% of the analyzed cases, being in accordance or disagreement with the clinicians’ evaluation. Moreover, the SOS pipeline allowed selecting further 16 oocytes (21%) that were rejected by manual evaluation and rejecting 29 oocytes (36%) that were fertilized but did not generate embryos.

The second module automatically extracts some morphological features by means of image processing techniques. In the cases where the automatic measure did not work, we observed that the cause was in oocyte images having a not spherical shape, due to the holding pipette used to steady the cell during the observation and, mostly, to very noisy images (acquired according to different or not standard acquisition protocol by the microscope operator), in which the image background was confused with the oocyte region.

In a published paper, a simple and semiquantitative human oocyte scoring system was described, based on the assignment of points to the following morphological parameters: cumulus/corona expansion and appearance, amount of cumulus and oocyte appearance (14). The experimental results confirmed that the oocyte score was significantly correlated to maturity and fertilization rate. Hence, the oocyte scoring procedure can be useful for selecting oocytes for IVF and embryo transfer and also for training new laboratory personnel in recognizing the important characteristics of a mature oocyte. Following this direction, in a more recent work a system for the morphological scoring of human oocytes that consists of

central granularity, inclusions, vacuolations and the injection properties of the oocyte after the injection step, demonstrated that top quality oocytes had a significantly higher level of fertilization as compared to low scoring oocytes (23).

In the future, the SOS pipeline could also embed a knowledge learning step able to elaborate all collected data and define a model that will support the scoring metric in the oocyte evaluation and classification for fertilization.

CONCLUSION

The SOS pipeline, a prototypical computer-assisted system, is able to acquire images in consistent and standard conditions, to quickly collect data in a structured format and to finally provide a quantitative metric in order to support embryologists in oocyte objective scoring process and in oocyte selection at the thawing time. In addition, a database of all medical and biological information about each patient may help clinicians and researcher to have a clear picture of hormonal stimulation response to analyze or examine it in future studies. Moreover, it allows to compute and to assign a quality score to the acquired oocyte images exploiting morpho-structural features, some of which are automatically extracted by the system.

The SOS pipeline will be used to support embryologists in making decisions during their work and in evaluating and selecting the best quality oocytes. Future work would concern the extension of the clinical data by considering more parameters in the asset of ovarian reserve, in the stimulation protocol and in the description of health conditions and by providing a component for analyzing such data. Furthermore, the inclusion of the genetic data is planned with the aim of inferring new knowledge by putting in relation all the variables involved in the domain with genetic alterations, if any.

ACKNOWLEDGMENT

This work was supported by the Italian Project MIUR-FAR 08-09: Laboratorio di Bioinformatica per la Biodiversità Molecolare (MBLab-DM19410).

CONFLICTS OF INTEREST

None.

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