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Original Research Article

Embryo quality before and after slow freezing: Viability, implantation and pregnancy rates in 627 single frozen-thawed embryo replacement cycles following failure of fresh transfer

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ABSTRACT

Frozen embryo transfer cycles are now common practice, however, various aspects regarding the potential of frozen embryos remain unclear. The main goal of the present study was to assess embryo quality before and after slow freezing procedure, and more specifically blastomere loss and embryo quality as indicator of viability. A single center retrospective analysis of single frozen-thawed embryo replacements (s-FER) was performed. The embryo quality before and after slow freezing and thawing, implantation, and pregnancy rates were recorded. One hundred and twenty seven s-FER were included in the final analysis. The probability of achieving an ongoing pregnancy was significantly associated with embryo quality and the percentage of blastomere loss after thawing. Considering thawed embryos, a non-significant difference in term of implantation rate was observed, regardless to their post-thawing quality and the percentage of blastomeres loss. In conclusion, current data suggest that thawed embryos are capable of implantation regardless of their morphological quality and the degree of cryoinjury sustained.

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1. Introduction

Since the first pregnancy and live birth resulting from frozen embryo replacement (FER) in the early '80s [1,2], embryo freezing has become an established procedure in assisted reproduction treatments all over the world [3,4]. Its applications are many and include the storage of surplus embryos, prevention of ovarian hyperstimulation syndrome (OHSS), embryo donation and, more recently, fertility preservation and preimplantation genetic screening (PGS) [5–7].

Despite implementation on a large scale, many aspects of the technique remain open to discussion, such as the impact of pre-freeze and post-thaw embryo quality on implantation potential [8,9]. Specifically, there is no consensus regarding the clinical significance of the number and/or percentage of post-thawing blastomere loss, and consequent impact on ongoing pregnancy rates after FER [10–12]. In the clinical practice, FER is recommended only if at least one-half of the initial blastomeres remain intact after thawing [7,10]. This recommendation however is based on very weak clinical evidences. Successful pregnancies have been described following FER with less than 50% of intact blastomeres [12]. Of note, this datum [12] has been published over 25 years ago, and innovation in technology and culture media are very likely to have a potential beneficial effect on the primary endpoints.

In our Unit, the number of and/or the percentage of blastomere loss did not influence decision to transfer. Thus, our biological and clinical data could be an interesting model to study the reproductive potential of embryos irrespective of blastomere loss. Based on these considerations, the aim of the current study was to clarify the clinical impact of embryo quality assessed before and after slow freezing/thawing procedures with particular regard to number and/or percentage of blastomere loss after embryo thawing.

2. Materials and methods

We present a retrospective cohort study on a population of infertile patients scheduled for *in vitro* fertilization (IVF)/intracytoplasmic sperm injection (ICSI) programs. All procedures were carried out at the Center of Reproductive Medicine and Surgery, Arcispedale Santa Maria Nuova of Reggio Emilia (Italy). The study period was from September 2009 to June 2015.

Analysis was confined to patients who underwent single non-donor embryo transfer in the context of single-FER (s-FER) cycles and in which the controlled ovarian stimulation protocol consisted of gonadotropin releasing hormone (GnRH) agonist pituitary desensitization attained by long protocol followed by daily recombinant follicle stimulating hormone (rFSH) administration. Exclusion criteria included the following: s-FER cycles in which embryos were obtained by homologous fertilization in the event of a severely azoospemic male partner, or through the use of heterologous gametes; all cases of incomplete data regarding embryo morphology, the presence of genetic disorders (*i.e.* cystic fibrosis or abnormal karyotype) in at least one partner, history and/or diagnosis of pelvic diseases (such as uterine malformations, endometriosis or pelvic inflammatory disease) and/or systemic diseases (such as diabetes mellitus, thyroid diseases, autoimmunity diseases, *etc.*).

The Institutional Review Board (IRB) approval was not required because the study protocol required exclusively the use of non-sensitive patient data in patients receiving standard non-experimental treatments. All eligible patients were adequately counseled regarding the potential use of non-sensitive data for scientific purposes and a signed informed consent was obtained at IVF/ICSI cycle scheduling.

2.1. *Embryo freezing and thawing protocols*

All embryos were morphologically scored prior to freezing. Classic parameters [13] were used until December 2014 after which the Istanbul consensus workshop parameters [14] were applied. The embryos evaluated in the first time period were revisited and subsequently re-scored according to the newly proposed international criteria [14].

Top quality embryos were transferred in the fresh cycle with the exception of patients at risk of OHSS in which case all embryos were frozen, regardless of quality. Of all surplus embryos frozen, we considered eligible for the analysis only those frozen on days 2 or 3 post-insemination (2–10 cells stage). Applying the above mentioned score system at the time of freezing, embryos were distinguished into good and poor quality embryos (group 1 and 2, respectively).

A slow freezing method based on increasing concentrations of cryoprotectant agents (CPAs) was adopted. The embryos were incubated for 5 min in Cryo-PBS (without 1,2-propanediol or sucrose), and for 10 min in embryo freezing solution 1 (EFS 1) (1.5 M 1,2-propanediol without sucrose). The embryos were transferred to EFS 2 (1.5 M 1,2-propanediol and 0.1 M sucrose) and loaded into straws by attaching the straw to a 1 mL syringe. The straws were sealed and the temperature was lowered through an automated Kryo 10 series III biological freezer (Planer Kryo 10/1,7 GB Planer, Plc, Sunbury-on-Thames, UK) from 20 °C to −6 °C at a rate of 0.2 °C/min. Manual seeding was performed at −6 °C and this temperature was maintained for 10 min in order to allow uniform ice propagation. The temperature was then decreased to −30 °C at a rate of 0.3 °C/min, decreased to −150 °C at a rate of 50 °C/min. The straws were then directly plunged into liquid nitrogen at −196 °C and stored for later use. Embryo thawing was performed by the rapid protocol based on a stepwise lowering of CPAs. The paillettes containing the frozen embryos were taken to room temperature for 30 s and in 30 °C bath for other 30 s. The embryos were expelled in a Petri capsule and incubated for 5 min in thawing solution 1 (1 M 1.2-PROH and 0.2 M sucrose), 5 min in thawing solution 2 (1.2-PROH 0.5 M and 0.2 M sucrose), 10 min in thawing solution 3 (0.2 M sucrose), 10 min in thawing solution 4 (buffered solution without CPAs). Thawed embryos were checked by an inverted microscope with Hoffman modulation contrast using a magnification of ×400 (TE 2000 U, Nikon Corp., Tokyo, Japan). If one or more blastomeres survived the thawing, the embryos were incubated in G1-Plus medium at least one hour before s-FER. Completely degenerated embryos were discarded (group 3tw). Viable embryos were re-scored and allocated to either group 1tw (good quality) or group 2tw (poor quality). The blastomeres present in each viable thawed embryo were counted and then compared, in