Characterization of a top quality embryo, a step towards single-embryo transfer

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In most in-vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI) programmes approximately one ongoing pregnancy in three is multiple. The need to characterize embryos with optimal implantation potential is obvious. We retrospectively examined all of 23 double transfers resulting in ongoing twins, occurring between January 1, 1996 and May 19, 1997. Characteristics of these top quality embryos were absence of multinucleated blastomeres, four or five blastomeres on day 2, seven or more cells on day 3, and ≤20% anucleated fragments. In a subsequent series of 400 IVF/ICSI cycles (out of which 372 were selected for embryo transfer) from May 20, 1997 to July 31, 1998, only women <38 years of age had multiple pregnancies: after 221 transfers of two embryos, 45/116 (39%) were multiple, and after 77 transfers of ≥2 embryos, 11/31 (35%) were multiple. We applied our top quality criteria to the 221 double transfers: 106 transfers with two top embryos resulted in 65 (63%) ongoing pregnancies with 37 (57%) twins, 65 transfers with one top embryo in 38 (58%) ongoing pregnancies with eight (21%) twins. In the group without top embryos, 12/52 (23%) ongoing singletons occurred, with no twins. The corresponding ongoing implantation rates were 49, 35 and 12%. This analysis suggests that single embryo transfer with an acceptable pregnancy rate might be considered if a top quality embryo is available.

Key words: embryo characterization/ICSI/implantation potential/IVF/multiple pregnancies

Introduction

There is growing concern about the risks of multiple pregnancies. Some authors strive to limit multiple pregnancies to twins, but it should not be forgotten that the ultimate goal of assisted procreation is to achieve singleton pregnancies. The only guaranteed way to reach this goal is to restrict oneself to single embryo transfer (Coetsier and Dhont, 1998). Until now there has been a conflict of interest between this ambition of good medical practice on the one hand and the economic and emotional aspects of a low pregnancy rate on the other, because it is widely accepted that the number of embryos transferred is closely related to success rate. This dilemma could be overcome, if it were possible to select embryos with a very high implantation potential. Culturing for a prolonged period of time until the blastocyst stage is a way of tackling this problem. However, as culture conditions are still imperfect, the longer culture lasts, the fewer embryos suitable for transfer are left. It would be more convenient if an equally effective selection could be performed, but at an earlier stage. In an attempt to establish better selection criteria, we decided to examine retrospectively the characteristics of embryos that all had resulted in an ongoing implantation. We also examined the consequences of the application of these criteria.

Materials and methods

All patients were treated with the long protocol for ovarian stimulation. Desensitization was initiated in the midluteal phase with buserelin acetate (Suprefact®, Hoechst, Frankfurt, Germany) six times 100 µg per day intranasally. For follicular stimulation purified follicle stimulating hormone (FSH; Metrodin HP, Serono, Geneva, Switzerland) was used. When three or more follicles reached a size of 18 mm or more in diameter, human chorionic gonadotrophin (HCG) 10 000 IU i.m. (Profasi, Serono) was administered. A transvaginal ultrasound-guided ovum retrieval was performed 37 h later. Standard in-vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI) procedures were used. Culture medium on the day of oocyte retrieval was Mênézé B2 in 25 µl droplets under oil (Sigma no. M8410; Sigma-Aldrich, Bomen, Belgium). Oocytes were inseminated, each in a separate droplet with 20 000 spermatozoa having a linear motility >22 µm/s in case of IVF. In case of ICSI, up to 10 injected oocytes were incubated together in a 10 µl Mênézé B2 (Laboratoire C.C.D., Paris, France) droplet under oil. On day 1, oocytes were examined for the appearance of two pronuclei and up to 10 fertilized oocytes were cultured together in a 10 µl droplet Mênézé B2 under oil. On day 2, embryos were rinsed and transferred to individual 10 µl droplets of Medi-Cult M3 medium (Medi-Cult, Copenhagen, Denmark) under oil in order to follow their further individual development. All transfers were performed on day 3. A maximum of two embryos was transferred in the first two attempts in women <38 years of age (van Kooij et al., 1996).

All embryos were scored for three parameters on day 2 (41–44 h after insemination/injection) and again on day 3 (66–71 h post-insemination/injection): (i) fragmentation (A = no fragmentation, B = 20% or less by volume of anucleated fragments, C = 20–50% by volume of anucleated fragments); (ii) number of blastomeres; (iii) number of multinucleated blastomeres.

From January 1, 1996 to May 19, 1997 the Jansen–Anderson embryo transfer catheter (Cook, Queensland, Australia) was used.

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All data concerning the establishment of top quality embryo criteria originate from this period.

In May 1997 we changed our transfer procedure to the Edwards–Wallace embryo replacement catheter (Simms Portex Ltd., Hythe, Kent, UK) with the use of a stylet (Naaktgeboren et al., 1997). From May 20, 1997 to July 31, 1998 a total of 409 ovum retrievals were performed. Because of personal preferences, another procedure was used in nine transfers. To preserve the homogeneity of the group results from these nine cycles (ending in two ongoing singleton pregnancies) were not included in this study. Main causes of infertility were male related in 211 cycles (53%): 193 cycles with oligoterato-asthenozoospermia and 18 cycles with a male immunological factor. Main causes of infertility were female related in 107 cycles (27%): 39 tubal, 22 tuboperitoneal, 25 endometriosis, four immunological, 16 polycystic ovaries, and one cycle with oocyte donation. In 75 cases (19%) clinical diagnosis was idiopathic infertility. Seven cycles (2%) were originally planned as non-IVF stimulations, but were converted to IVF because of an unacceptably high number of maturing follicles. The mean age of patients was 31.5 years with a standard deviation of 4.82. This mean age is underestimated by 0.5 years because only the integer number of years was recorded. ICSI was performed in 162 cycles (40.5%) of which 21 were with non-asthenozoospermia. The mean age of patients was 39 years with a standard deviation of 4.82. This mean age is underestimated by 0.5 years because only the integer number of years was recorded. ICSI was performed in 162 cycles (40.5%) of which 21 were with non-asthenozoospermia.

A biochemical abortion was recorded when there were at least two HCG values >5 IU/ml and incremental. A clinical abortion was recorded when a fetal sac had been seen on ultrasound. An ongoing pregnancy was defined as a pregnancy that was ongoing past the first trimester. For the calculation of the ongoing implantation rate, only concepti reaching the second trimester were considered. Confidence interval analysis (Gardner and Altman, 1986) was used for statistical analysis.

Results

Characterization of a top quality embryo

In order to characterize embryos with optimal implantation capacity, we examined embryos which we were certain had implanted. For the period January 1, 1996 to May 19, 1997 we reviewed all transfers where two embryos had been transferred and that had resulted in an ongoing twin pregnancy. No monozygotic twins were recorded in this series. There were 23 such transfers. Table I shows the characteristics of these 46 embryos both on day 2 and on day 3. All fragmentation scores were A or B meaning fragmentation was 20% or less in all embryos. Because fragmentation will not decrease during development, it was sufficient to take into account the score on day 3 only. None of the embryos showed any multinucleated blastomeres, either on day 2 or on day 3.

Table II shows the frequency distribution for day 2 and day 3 according to the number of blastomeres.

On day 2 the vast majority (37) of embryos had four blastomeres, while a considerable number (seven) had five. Only one embryo had three and another six. Omitting these extreme values, 44/46 (96%) of all embryos lay within the narrow interval of four to five blastomeres. On day 3 the vast majority of embryos (29) had eight blastomeres, but the distribution seemed to be wider, especially towards the higher limit. Omitting the extremes gave a lower limit of seven blastomeres and a higher limit of 10. However, we preferred not to set an upper limit because the faster an embryo cleaves, the more likely it is to implant successfully. Nevertheless, 43/46 (93%) of these embryos fitted into the following description of a ‘top embryo’: four or five blastomeres on day 2, and seven or more on day 3; 20% fragmentation or less on day 3 and no multinucleated blastomeres ever.

Top quality embryo and implantation potential

Table III shows an overview of all 400 cycles recorded between May 20, 1997 and July 31, 1998, after we had established top quality characteristics, according to the patient’s age group (<38 or ≥38 years) on one hand and the number of transferred embryos on the other.

Only two categories showed multiple pregnancies, both in women <38, namely 221 transfers of two embryos resulting in 116 ongoing pregnancies, of which 45 were twins (39%) and 77 transfers of more than two embryos resulting in 31 ongoing pregnancies, of which six were twins (19%) and five were triplets (16%). All triplets were reduced to twins.

In this paper we only considered the former category, because it offered a much simpler analysis of the impact of embryo quality on implantation and twinning rate.

These 221 transfers of two embryos were divided into three groups according to the number of top embryos as defined above: 2, 1 or 0. Results are shown in Table IV. Mean age was not different in the three groups consisting of 104, 65 and 52 transfers. Ongoing pregnancy rates were 63, 58 and 23%. Pregnancy rates were not significantly different between the first two groups, i.e. the two groups containing either one or two top embryos.

Pregnancy rates were significantly different between the group with two top embryos and the group without any, as well as between the group with only one top embryo and the group without any.

Twinning rates are significantly different between groups: 37 twins in 65 pregnancies (57%) in the first group, and eight twins in 38 pregnancies (21%) in the second group. With the third group where no twins occurred in 12 pregnancies, no statistical analysis was possible due to the small sample size.

Ongoing implantation rates of 102/208 (49%) versus 46/130 (35%) were significantly different between both groups with top embryos and there was a highly significant difference between both groups containing two or one top embryo versus the group containing none which had an ongoing implantation of only 12/104 (12%).

There was no difference between IVF and ICSI results.

In order to check what percentage of transferred embryos in non-pregnant cycles were top quality embryos, we attempted to match transfers of two embryos with the same main cause of infertility and the same maternal age to the 23 transfers that led to the establishment of our criteria (Tables I and II). We managed to find 21 matching transfers. Of the 42 embryos involved in these, only 18 (43%) showed all top embryo characteristics. Failure to comply with these characteristics was due to presence of multinucleated blastomeres (three embryos), >20% fragmentation on day 3 (six embryos), but most frequently to a discrepancy in the number of blastomeres on day 2 (15 embryos) and on day 3 (20 embryos). Most often
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Table I. Scores for fragmentation, number of blastomeres, and number of multinucleated blastomeres for all 46 embryos that implanted in 23 ongoing twin pregnancies, recorded both on day 2 and on day 3. Embryos with identical scores were grouped

<table>
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<th>Blastomeres (n)</th>
<th>Multinucl. blast (n)</th>
<th>Fragmentation</th>
<th>Blastomeres (n)</th>
<th>Multinucl. blast (n)</th>
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Table II. Frequency distribution of the number of blastomeres on day 2 and day 3 for 46 embryos that all implanted in 23 ongoing twin pregnancies

<table>
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<th>Blastomeres (n)</th>
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<th>Day 3 Embryos (n)</th>
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</tbody>
</table>

it was due to a combination of these parameters. Table V shows the frequency distribution for these non-implanting embryos on day 2 and day 3 according to the number of blastomeres.

Discussion

There is general agreement that a positive relationship exists between embryo quality and pregnancy rate. Two parameters are mainly involved in this quality notion: cleavage speed and fragmentation (Cummins et al., 1986; Claman et al., 1987). Several attempts were made to quantify the implantation potential of an embryo by means of a scoring system combining both these parameters (Puissant et al., 1987; Visser and Fourie, 1993). This awareness of the higher pregnancy chance offered by transferring good quality embryos led to the reduction of the number of transferred embryos to two and consequently to the avoidance of triplet pregnancies (Staessen et al., 1992, 1995). Still the concept of quality was predominantly based on the results of transfers with a mixture of embryos of different types (Zhu et al., 1997; Hu et al., 1998). Only Ziebe (Ziebe et al., 1997), who considered exclusively transfers of identical cleavage stage and identical fragmentation, and Giorgetti (Giorgetti et al., 1995) who only examined single-embryo transfers were able to describe the implantation potential of a well-defined type of embryo.

Recently it was demonstrated that embryos displaying multinucleated blastomeres have a severely impaired implantation potential (Jackson et al., 1998; Pelinck et al., 1998). This means the appearance of multinucleated blastomeres is another important quality related parameter. In 1995, immediately after the appearance of the article by Pickering (Pickering et al., 1995), we tried to avoid transferring embryos with multinucleated blastomeres.

Instead of using the common concept of embryo quality and just extending it with this parameter, we decided to approach it from a different perspective. We decided to analyse the properties of embryos that had proved to be of top quality, i.e. embryos that beyond any doubt had implanted and evolved into an ongoing pregnancy. Then we tried to find the common features of such embryos in the same way that reference values are established. These features would characterize a top quality embryo.

To our knowledge there is no article where an attempt has been made to describe embryos with a maximal implantation potential based on the observation of implanted embryos cultured to day 3. By doing so we have defined a type of embryo with an ongoing implantation rate of 49%. This would suggest that, if there has not been an embryo-helping effect in these double transfers, we might expect similar implantation rates and ongoing pregnancy rates in single embryo transfers with this type of embryo.

It must be emphasized that the number of blastomeres (and maybe even the fragmentation) may vary with culture conditions and with timing of evaluation. This means our criteria are not absolute.

As only 52 out of 221 transfers did not involve any top embryos, we might expect to be able to treat 75% of the population now receiving two embryos with single embryo transfer. Later a new transfer strategy will have to be developed for the third transfer onwards, for the group <38 years now
receiving more than two embryos. This will not be simple because a good embryo quality alone is not enough to achieve pregnancy. The better the embryo quality, the clearer the impact of pregnancy-preventing factors not related to embryo quality, such as lack of endometrial receptivity and shortcomings in the transfer procedure. Although we obtained a significant difference in implantation rate (and twinning rate) between the group with one and the group with two top embryos (Table IV), there was no difference in pregnancy rate: although implantation rate increased by 14% (from 35 to 49%), pregnancy rate increased only by 5% (from 58 to 63%) but the twinning rate almost tripled (from 21 to 57%). At 63% pregnancy we may be close to the barrier formed by these non-embryo-quality-related pregnancy-preventing factors. By transferring two embryos with an implantation rate of 49%, one would expect 49% + 0.51×49% = 74% pregnancy rate (Gardner and Schoolcraft, 1999).

The implantation rate of these top embryos seems very similar to those of blastocysts reported by Gardner (Gardner et al., 1998). Our approach of transferring on day 3, however, implies a shorter culture time and less cost, and avoids the risk of having no blastocyst embryo available for transfer in about 40% of patients (Scholtes and Zeilmaker, 1998; Shoukir et al., 1998). A remarkable attempt to successfully evaluate and transfer pronuclear embryos has been reported (Scott and Smith, 1998). These workers managed to select a group with 28% implantation. In accordance with the suggestion of Edwards and Beard, who found it essential to combine this pronuclear evaluation together with blastocyst culture on the same embryos to see if both systems would select the same embryos (Edwards and Beard, 1999), we would suggest also to apply our selection system. As our approach fits right in the middle of these two extremes, maybe the use of all three types of evaluation on the same embryos might lead us to conclude what is the optimal time to transfer: when there will be no further gain in selection by prolonged culturing.

In the group \( \geq 38 \) years old no multiple pregnancies occurred, thus we see no reason to change our strategy of transferring three embryos in those <40 and four or more in patients of \( \geq 40 \) years of age.

A controlled, prospectively randomized, study has meanwhile been completed to test our speculations.

### References


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