The association between quality of supernumerary embryos in a cohort and implantation potential of the transferred blastocyst

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Abstract

Purpose Despite studies focused on the association between embryo morphology and implantation potential, it is unknown how the collective quality of the supernumerary embryos in a cohort is associated with the implantation rate (IR) of the transferred embryo. This study tested the hypothesis that a relationship exists between the quality of the supernumerary cohort and IR.

Methods A retrospective cohort study of first fresh autologous IVF cycles from 05/2012 to 09/2016, with ≥ 3 blastocysts, resulting in a single blastocyst transfer ($n = 819$) was performed. Cohorts were grouped in two ways: by mean priority score (PS; 1 being best) of supernumerary embryos and by percent supernumerary embryos with low implantation potential. The relationship between cohort quality and IR was assessed using logistic regression.

Results As mean cohort PS increased, IR of the transferred embryo decreased (test for linear trend, $p = 0.05$). When $\geq 75\%$ of the supernumerary cohort was predicted to have low implantation potential, IR of the transferred embryo was significantly lower compared to when $< 75\%$ of the cohort was predicted to have low implantation potential (OR 0.71; 95% CI (0.53–0.94)). All associations were attenuated when adjusting for PS of the transferred embryo.

Conclusions Our findings suggest that quality of supernumerary embryos is associated with IR of the transferred embryo, among patients with ≥ 3 blastocysts available on day 5. As cohort quality declines and the proportion of low implantation potential embryos increases, the IR of the transferred embryo declines. These associations are attenuated when controlling for quality of the transferred embryo, suggesting that the relationship between embryo cohort quality and implantation is not independent of the transferred embryo quality.

Keywords Embryo quality · Embryo score · Embryo morphology · Implantation · Supernumerary embryo

Introduction

Practitioners have sought to improve the success of in vitro fertilization (IVF) since inception of the technology. While the transfer of multiple embryos generally increases the likelihood of success, it also increases the rate of multifetal gestations, and subsequently obstetric complications [1]. The optimal number of embryos to transfer continues to be one of the major challenges in the field.

Morphological criteria for identifying embryos with high implantation potential have evolved from evaluating cleavage stage embryos for cell number [2], blastomere symmetry, and fragmentation [3] to assessment of blastocyst stage and quality of the inner cell mass and trophectoderm [4, 5]. Validated scoring systems for both cleavage [6–8] and blastocyst [9, 10] stage embryos have been developed. Evaluation of embryo grade and morphology help to determine not only which embryos have the highest potential for implantation [11, 12] and live birth rate [13, 14], but also might influence the number of embryos to transfer [15] or the optimal day for embryo transfer [16].

In addition to established relationships between the quality of a transferred embryo and its potential to implant, previous research has suggested that implantation rates may also be positively correlated with the presence of un-transferred or supernumerary embryos with high implantation potential [17–20]. However, these earlier studies focused only on supernumerary embryos assessed as high quality; it is unknown

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how lower quality supernumerary embryos may be associated with implantation potential of the transferred embryo.

The objective of the present study was to determine whether the quality of supernumerary embryos in a cohort is associated with the implantation potential of the transferred embryo and to determine whether the quality of all supernumerary embryos within an embryo cohort should be taken into consideration when advising physicians and patients on the number of embryos to transfer.

Methods

Institutional review board approval

This study was approved by the Partners Human Research Committee at the Brigham and Women's Hospital (Protocol #2016P002232). Given the retrospective nature of this study and that all data were retrieved from standard records collected during clinical care, informed consent was not required.

Study population and design

This was a retrospective cohort study of women undergoing their first fresh autologous IVF cycle, with or without ICSI, resulting in a day 5 blastocyst transfer between 06/01/2012 and 10/31/2016 at the Brigham and Women's Hospital. Data were collected from our prospectively maintained departmental database and the hospital electronic medical record system. Women having a single blastocyst transfer were included in the primary analysis while those who had a double blastocyst transfer were included for a sensitivity analysis. Exclusion criteria included patient age ≥ 43.0 years, use of preimplantation genetic testing, gestational carriers, donor oocytes, a mixed source of fresh and cryopreserved blastocysts transferred, or cohorts having < 3 blastocysts available on day 5.

Study definitions

A cohort was defined as all day 5 embryos from one cycle, regardless of developmental stage. Supernumerary embryos were defined as all the un-transferred embryos within a cohort.

Clinical protocols

Stimulation protocols utilized gonadotropin-releasing hormone (GnRH) antagonists, GnRH agonists for downregulation, estradiol priming, or low-dose GnRH agonist flare protocols [21–24]. Following retrieval, oocytes were either inseminated in groups (3–5 oocytes) or underwent intracytoplasmic sperm injection (ICSI). A fertilization check was then performed at 16–18 h and zygotes with 2 pronuclei (2pn) were cultured individually in 25 μ l droplets overlain with mineral oil. All

embryos were evaluated and scored on day 5 for stage (arrested, early or late morula or blastocyst with extent of expansion) and quality of the inner cell mass, and the trophectoderm.

Selection of patients to day 5 transfer and number of embryos transferred was determined according to internal protocols and patient history. Luteal support was generally provided with vaginal progesterone gel (one applicator daily), beginning 2 days after oocyte retrieval and continued up to 10 weeks of gestation. Patients who received a combination trigger with Lupron and hCG additionally received luteal support with 3 mg estradiol orally (twice daily), beginning 1 day after oocyte retrieval and discontinued once there was a positive pregnancy test. Approximately 2 weeks after embryo transfer, patients had serum human chorionic gonadotropin (hCG) levels checked at 2-day intervals. If the serial hCG rise was $> 66\%$ over 2 days, an obstetric ultrasound was obtained at 7–8 weeks of gestational age. If at any point the hCG rise over 2 days was below 66%, an early ultrasound was obtained at approximately 5 weeks of gestational age, with further monitoring and management, as dictated by clinical findings.

Clinical outcome

For each cycle, successful implantation was defined as the number of fetal hearts recorded on ultrasound between 7 and 8 weeks of gestation divided by the number of embryos transferred.

Assignment of priority scores

At our institution, each embryo is assigned a priority score (PS) to help determine which embryo(s) to preferentially select for transfer. The PS system is categorical, ranging from 1 to 8. The system was developed based on a dataset of historical implantation rates at our institution and was retrospectively validated on a second dataset of implantation rates at our institution. For each PS, the implantation rates from the validation dataset were within 5% of the implantation rates derived from the original dataset. A PS of 1 was given to embryos deemed to have the highest implantation potential and an 8 was given to embryos with the lowest implantation potential. The PS was derived from the stage (1–9) and grades of the inner cell mass (A–D) and trophectoderm (a–d). For example, an 8Ab embryo would be assigned a PS of 1, based on the historical implantation rate of 65.8% for embryos of this quality at our institution. Priority scores within a cohort are not mutually exclusive, and multiple embryos within the same cohort could be given the same PS. During the study period, a total of 9 embryologists graded embryos in our laboratory. All embryologists participated in twice yearly external quality control assessments and intermittent internal quality control evaluations.

The mean PS of the supernumerary embryos was calculated for each cohort. Cohorts were then categorized into four

groups based on their mean PS: Group A (1.0–4.9), group B (5.0–5.9), group C (6.0–6.9), and group D (7.0–8.0) (group A being the “best” and group D being the “worst” quality).

An individual embryo was considered to have low implantation potential if assigned a PS ≥ 5 . This cut-off was chosen because embryos with PS of 5 have a relatively low implantation potential of $< 40\%$ and are considered to yield poorer prognoses. Accordingly, cohorts were separated into two groups: those having $\geq 75\%$ of supernumerary embryos with low implantation potential and those with $< 75\%$ of supernumerary embryos with low implantation potential. A threshold of $\geq 75\%$ was chosen as the cut-point to capture cohorts with all or the great majority of supernumerary embryos scored as having low implantation potential. The implantation rates of the transferred embryos originating from these two groups were then compared.

Statistical analyses

Embryo cohorts were grouped by the mean day 5 PS of the supernumerary embryos, as defined above. Logistic regression models were used to estimate the odds ratio (OR) with a 95% confidence interval (CI) of implantation in single blastocyst transfer cycles. Patient age, body mass index (BMI, kg/m²), AMH, day 3 FSH, and number of embryos within a cohort were included *a priori* in all analyses to calculate the adjusted odds ratios (aOR). A secondary adjustment for the day 5 PS of the transferred blastocyst was performed (aOR²). A test for linear trend was performed for the mean day 5 PS analysis (Table 1). A sensitivity analysis was performed grouping embryo cohorts by the percentage of embryos with PS ≥ 5 (i.e., those with low implantation potential, as defined above). Additional sensitivity analyses were then performed among women who underwent double blastocyst transfers. The day 5 PS of the transferred embryo was tested for effect modification to determine whether the relationship between embryo cohort quality and implantation differed by different values of the PS of the transferred embryo. Statistical analyses were performed using Statistical Analysis Software version 9.3 (SAS Institute, Inc.).

Results

There were 819 fresh day 5 single embryo transfers and 179 fresh day 5 double embryo transfers analyzed in this study. Demographic characteristics for patients in the study group are shown in Table 1. Women had a mean age of 33.7 ± 3.4 years and a mean BMI of 25.9 ± 6.4 kg/m². The mean number of embryos frozen was 5.1 ± 3.3 .

Implantation rates for fresh day 5 single blastocyst transfers classified by the mean day 5 PS of the supernumerary embryos in the cohort are shown in Table 2. The implantation rate for group A was 54%. Group D was the only group with a statistically

Table 1 Demographic characteristics among single embryo transfers

Characteristic	Fresh day 5 SET (<i>n</i> = 819)
Patient age at retrieval (year)	33.7 $\pm$ 3.4
BMI at retrieval (kg/m ²)	25.9 $\pm$ 6.4
Race	
Caucasian	593 (72.4)
African American	45 (5.5)
Hispanic	41 (5.0)
Asian	114 (13.9)
Other/unknown	26 (3.2)
Primary infertility diagnosis	
Decreased ovarian reserve	47 (5.7)
Anovulatory	114 (13.9)
Uterine factor	14 (1.7)
Tubal factor	53 (6.5)
Endometriosis	52 (6.4)
Other/unknown	313 (38.2)
Male factor	226 (27.6)
Number of oocytes retrieved	17.4 $\pm$ 7.2
Number of mature oocytes	14.0 $\pm$ 6.0
Number of 2pn zygotes	11.4 $\pm$ 4.9
Number of embryos frozen	5.1 $\pm$ 3.3
Stimulation protocols	
Antagonist	547 (66.8)
Long protocol*	210 (25.6)
Poor responder†	38 (4.6)
Ovulation induction/other	24 (2.9)
Day 3 FSH (IU/L)	6.97 $\pm$ 2.0
AMH (ng/mL)	4.2 $\pm$ 3.7
Peak estradiol (pg/mL)	2182.1 $\pm$ 938.0
ICSI	386 (47.1)
Clinical pregnancy	359/819 (43.8)
Implantation	359/819 (43.8)
Endometrial thickness (mm)	10.9 $\pm$ 2.8

SET, single embryo transfer; BMI, body mass index; 2pn, 2 pronuclei; FSH, follicle stimulating hormone; AMH, anti-müllerian hormone; ICSI, intracytoplasmic sperm injection; IM, intramuscular

Data are mean $\pm$ standard deviation or *n* (%) unless otherwise noted

*Long protocol: LDL, VLDL, ULDL, FDL, or pseudoluteal

† Poor responder protocol: Microflare, patch

significantly decreased implantation rate when compared directly to group A (37.4%, aOR 0.56 (95% CI 0.33–0.95)). However, there was a statistically significant linear trend of decreasing implantation rate across groups as mean quality of the supernumerary embryos decreased (group B 44.8%, aOR 0.72 (95% CI 0.43–1.21); group C 44.4%, aOR 0.73 (95% CI 0.45–1.19); group D 37.4%, aOR 0.56 (95% CI 0.33–0.95); test for linear trend, *p* = 0.05). A secondary adjustment for the day 5 PS of the transferred embryo attenuated the effect of declining implantation rates with decreasing mean day 5 quality of the cohort (group B

Table 2 Association between the mean priority score of the supernumerary embryos within an embryo cohort and the implantation rate of the transferred blastocyst

Cohort group* (PS range)	N	Implantation rate <i>n</i> , (%)	Adjusted OR [†] (95% CI)	Secondary adjusted OR [‡] (95% CI)
A (1.0–4.9)	87	47 (54.0)	1.00 (Ref)	1.00 (Ref)
B (5.0–5.9)	201	90 (44.8)	0.72 (0.43–1.21)	0.82 (0.49–1.38)
C (6.0–6.9)	336	149 (44.4)	0.73 (0.45–1.19)	1.00 (0.60–1.66)
D (7.0–8.0)	195	73 (37.4)	0.56 (0.33–0.95)	1.09 (0.60–1.97)
Linear trend <i>p</i> value	–	–	0.05	0.43

OR, odds ratio; CI, confidence interval; Ref, referent

*Embryo cohorts were stratified into one of four cohort groups based on the mean priority score of the supernumerary embryos within the cohort: A (1.0–4.9), B (5.0–5.9), C (6.0–6.9), D (7.0–8.0)

[†] Adjusted a priori for number of embryos within the cohort, patient age at retrieval, BMI, AMH, and day 3 FSH

[‡] Secondary adjustment for the priority score of the transferred embryo

aOR² 0.82 (95% CI 0.49–1.38); group C, aOR² 1.00 (95% CI 0.60–1.66); group D, aOR² 1.09 (95% CI 0.60–1.97); test for linear trend, *p* = 0.43).

Table 3 shows the sensitivity analysis for the association between the proportion of supernumerary embryos having low implantation potential (PS ≥ 5) and implantation rate of a single day 5 transferred embryo. Among cohorts with < 75% of supernumerary embryos having a day 5 PS ≥ 5 (*n* = 322), the implantation rate was 49.7%. In cohorts having ≥ 75% of supernumerary embryos with a day 5 PS ≥ 5 (*n* = 497), the implantation rate was 40.0% (aOR 0.71 (95% CI 0.53–0.94)). Adjustment for the PS of the transferred embryo attenuated the observed decrease in implantation rate in those cohorts with ≥ 75% of supernumerary embryos with a day 5 PS ≥ 5 (aOR² 0.95 (95% CI 0.70–1.31)).

A sensitivity analysis of fresh day 5 double embryo transfers showed a similar pattern of association, but did not identify a statistically significant association between the mean day 5 PS of the supernumerary embryos and the implantation rate of the transferred embryos.

Discussion

The primary aim of this study was to identify how the collective quality of supernumerary embryos in a cohort may be associated

with the implantation potential of the transferred embryo. We found that the quality of the supernumerary embryos does, indeed, influence the rate of implantation of the transferred blastocyst. Additionally, as the average quality of the supernumerary embryos declines, the implantation rate of the transferred blastocyst also declines. However, these relationships between quality of the cohort and implantation are not independent of the quality of the transferred embryo as these associations were attenuated after controlling for this variable.

Despite the increasing use of time-lapse imaging [25, 26] and preimplantation genetic testing for aneuploidy [27, 28], conventional morphological assessment continues to be used either in conjunction with these approaches or as a first-line method for embryo selection. However, the sensitivity and specificity of morphological evaluations for predicting implantation, whether at the cleavage or the blastocyst stage, have limitations [29–31]. Therefore, while morphological grading is helpful when determining which embryo to transfer and the optimal number of embryos to transfer, not all embryos with even the highest possible score successfully implant [10]. The cause of unsuccessful embryo transfer is certainly multifactorial, and the identification of external influences on implantation is necessary in order to improve embryo selection and transfer techniques.

To the best of our knowledge, this is the first study to summarize the quality of every embryo within a cohort and

Table 3 Association between the proportion of supernumerary embryos with low implantation potential within an embryo cohort and the implantation rate of the transferred blastocyst

Percent of cohort with PS ≥ 5*	Implantation rate [†] <i>n</i> , (%)	Adjusted OR [‡] (95% CI)	Secondary adjusted OR [§] (95% CI)
< 75% (<i>n</i> = 322)	160 (49.7)	1.00 (Ref)	1.00 (Ref)
≥ 75% (<i>n</i> = 497)	199 (40.0)	0.71 (0.53–0.94)	0.95 (0.70–1.31)

PS, priority score; OR, odds ratio; CI, confidence interval; Ref, referent

*Embryos with a priority score of ≥ 5 were defined as having low implantation potential

[†] Implantation rate for each group was defined as the number of single embryo transfers with fetal cardiac activity recorded on ultrasound between 7 and 8 weeks of gestation divided by the total number of single embryo transfers

[‡] Adjusted a priori for number of embryos within the cohort, patient age at retrieval, BMI, AMH, and day 3 FSH

[§] Secondary adjustment for the priority score of the transferred embryo

the relationship between the collective quality and implantation potential of the transferred embryo. Previous literature evaluating supernumerary embryos has focused only on high-quality embryos [17–20]. It is known that the presence of high-quality supernumerary embryos increases the rate of implantation and that a direct correlation may exist as the number of high-quality embryos available increases [17–20]. As such, this information can be useful in determining the optimal number of embryos to transfer. However, whether the implantation potential of a transferred embryo is associated with the quality of the entire embryo cohort from which it came was previously unknown.

The observed linear decline of implantation rate associated with the decline of overall mean embryo quality in a cohort is consistent with prior studies which observed that implantation potential may be influenced by supernumerary embryo quality [17–20]. This effect of the total cohort, however, was mitigated by accounting for the PS of the highest quality embryo, the one that was transferred. Similarly, a higher proportion of embryos with low implantation potential within a cohort was observed to result in a lower implantation rate of the transferred embryo. This association was similarly mitigated by accounting for the PS of the highest quality embryo for transfer. This suggests that although the implantation rate of embryos may be impacted by the overall quality of their cohort, this effect can be overcome by selecting the highest quality embryo for transfer.

In our analyses of double embryo transfers, we failed to identify any association between supernumerary embryo quality and implantation rates. This may be due, in part, to the difficulty in accounting for the variability between the qualities of the two embryos transferred.

We acknowledge the limitations of our study. Our study only included patients with a single or double embryo transfer, and therefore, our results may not be generalizable to IVF populations undergoing transfer of three or more embryos. Additionally, our cohort only included patients with ≥ 3 blastocysts on day 5. Patients with < 3 blastocysts, who may have a worse prognosis, were excluded because they were less likely to have any supernumerary blastocysts after embryo transfer. The results of our study should not be applied to this population. Furthermore, we assumed that the average score (cohort groups A–D) and the proportion of low implantation potential embryos ($< 75\%$ and $\geq 75\%$) to be reasonable proxies of overall supernumerary embryo cohort quality, though it is possible that these calculations were unable to fully capture and report the complex interactions among embryos within a cohort.

In summary, this study demonstrates that the quality of supernumerary embryos influences the implantation rate of the transferred embryo among cohorts with ≥ 3 blastocysts available on day 5. This suggests that the overall quality of the supernumerary embryos may be used to help predict the implantation potential of a selected embryo for transfer. These

findings highlight the interaction of external influences on embryo implantation and are consistent with the fact that current morphologic evaluation methods of embryo quality do not reflect all characteristics of an embryo that promote successful implantation. Importantly, the association between supernumerary embryo quality and implantation is attenuated when controlling for the quality of the transferred blastocyst, suggesting that this relationship is not independent of the quality of the transferred embryo. Therefore, we recommend against allowing the quality of the entire cohort to influence the optimal number of embryos to select for transfer. However, further investigation is warranted to explore these intricate relationships.

References

1. Beral V, Doyle P, Tan SL, Mason BA, Campbell S. Outcome of pregnancies resulting from assisted conception. *Br Med Bull*. 1990;46:753–68.
2. Trounson AO, Leeton JF, Wood C, Webb J, Wood J. Pregnancies in humans by fertilization in vitro and embryo transfer in the controlled ovulatory cycle. *Science*. 1981;212:681–2.
3. Puissant F, Van Rysselberge M, Barlow P, Deweze J, Leroy F. Embryo scoring as a prognostic tool in IVF treatment. *Hum Reprod*. 1987;2:705–8.
4. Thompson SM, Onwubalili N, Brown K, Jindal SK, PG MG. Blastocyst expansion score and trophoctoderm morphology strongly predict successful clinical pregnancy and live birth following elective single embryo blastocyst transfer (eSET): a national study. *J Assist Reprod Genet*. 2013;30:1577–81. <https://doi.org/10.1007/s10815-013-0100-4>.
5. Hill MJ, Richter KS, Heitmann RJ, Graham JR, Tucker MJ, DeChemey AH, et al. Trophoctoderm grade predicts outcomes of single-blastocyst transfers. *Fertil Steril*. 2013;99:1283–1289.e1. <https://doi.org/10.1016/j.fertnstert.2012.12.003>.
6. Steer CV, Mills CL, Tan SL, Campbell S, Edwards RG. The cumulative embryo score: a predictive embryo scoring technique to select the optimal number of embryos to transfer in an in-vitro fertilization and embryo transfer programme. *Hum Reprod*. 1992;7:117–9.
7. De Placido G, Wilding M, Strina I, Alviggi E, Alviggi C, Mollo A, et al. High outcome predictability after IVF using a combined score for zygote and embryo morphology and growth rate. *Hum Reprod*. 2002;17:2402–9.
8. Visser DS, Fourie FR. The applicability of the cumulative embryo score system for embryo selection and quality control in an in-vitro fertilization/embryo transfer programme. *Hum Reprod*. 1993;8:1719–22.
9. Heitmann RJ, Hill MJ, Richter KS, DeChemey AH, Widra EA. The simplified SART embryo scoring system is highly correlated to implantation and live birth in single blastocyst transfers. *J Assist Reprod Genet*. 2013;30:563–7. <https://doi.org/10.1007/s10815-013-9932-1>.
10. Gardner DK, Lane M, Stevens J, Schlenker T, Schoolcraft WB. Blastocyst score affects implantation and pregnancy outcome: towards a single blastocyst transfer. *Fertil Steril*. 2000;73:1155–8.
11. Tesarik J, Junca AM, Hazout A, Aubriot FX, Nathan C, Cohen-Bacrie P, et al. Embryos with high implantation potential after intracytoplasmic sperm injection can be recognized by a simple,

- non-invasive examination of pronuclear morphology. *Hum Reprod*. 2000;15:1396–9.
12. Van Royen E, Mangelschots K, De Neubourg D, Laureys I, Ryckaert G, Gerris J. Calculating the implantation potential of day 3 embryos in women younger than 38 years of age: a new model. *Hum Reprod*. 2001;16:326–32.
 13. Racowsky C, Stern JE, Gibbons WE, Behr B, Pomeroy KO, Biggers JD. National collection of embryo morphology data into Society for Assisted Reproductive Technology Clinic Outcomes Reporting System: associations among day 3 cell number, fragmentation and blastomere asymmetry, and live birth rate. *Fertil Steril*. 2011;95:1985–9. <https://doi.org/10.1016/j.fertnstert.2011.02.009>.
 14. Vernon M, Stern JE, Ball GD, Wininger D, Mayer J, Racowsky C. Utility of the national embryo morphology data collection by the Society for Assisted Reproductive Technologies (SART): correlation between day-3 morphology grade and live-birth outcome. *Fertil Steril*. 2011;95:2761–3. <https://doi.org/10.1016/j.fertnstert.2011.02.008>.
 15. Hu Y, Maxson WS, Hoffinan DI, Ory SJ, Eager S, Dupre J, et al. Maximizing pregnancy rates and limiting higher-order multiple conceptions by determining the optimal number of embryos to transfer based on quality. *Fertil Steril*. 1998;69:650–7.
 16. Racowsky C, Jackson KV, Cekleniak NA, Fox JH, Hornstein MD, Ginsburg ES. The number of eight-cell embryos is a key determinant for selecting day 3 or day 5 transfer. *Fertil Steril*. 2000;73:558–64.
 17. Salha O, Dada T, Levett S, Allgar V, Sharma V. The influence of supernumerary embryos on the clinical outcome of IVF cycles. *J Assist Reprod Genet*. 2000;17:335–43.
 18. Wang JG, Douglas NC, Dicken C, Nakhuda GS, Guarnaccia MM, Sauer MV. Cryopreservation of supernumerary high quality embryos predicts favorable outcomes for patients undergoing repeated cycles of in vitro fertilization. *Fertil Steril*. 2008;89:368–74.
 19. Hill MJ, Richter KS, Heitmann RJ, Lewis TD, DeCherney AH, Graham JR, et al. Number of supernumerary vitrified blastocysts is positively correlated with implantation and live birth in single-blastocyst embryo transfers. *Fertil Steril*. 2013;99:1631–6. <https://doi.org/10.1016/j.fertnstert.2013.01.130>.
 20. Stern JE, Lieberman ES, Macaluso M, Racowsky C. Is cryopreservation of embryos a legitimate surrogate marker of embryo quality in studies of assisted reproductive technology conducted using national databases? *Fertil Steril*. 2012;97:890–3. <https://doi.org/10.1016/j.fertnstert.2011.12.050>.
 21. Cheung LP, Lam PM, Lok IH, Chiu TT, Yeung SY, Tjer CC, et al. GnRH antagonist versus long GnRH agonist protocol in poor responders undergoing IVF: a randomized controlled trial. *Hum Reprod*. 2005;20:616–21.
 22. Dragisic KG, Davis OK, Fasouliotis SJ, Rosenwaks Z. Use of a luteal estradiol patch and a gonadotropin-releasing hormone antagonist suppression protocol before gonadotropin stimulation for in vitro fertilization in poor responders. *Fertil Steril*. 2005;84:1023–6.
 23. Surrey ES, Bower J, Hill DM, Ramsey J, Surrey MW. Clinical and endocrine effects of a microdose GnRH agonist flare regimen administered to poor responders who are undergoing in vitro fertilization. *Fertil Steril*. 1998;69:419–24.
 24. Tummmon IS, Daniel SA, Kaplan BR, Nisker JA, Yuzpe AA. Randomized, prospective comparison of luteal leuprolide acetate and gonadotropins versus clomiphene citrate and gonadotropins in 408 first cycles of in vitro fertilization. *Fertil Steril*. 1992;58:563–8.
 25. Armstrong S, Arroll N, Cree LM, Jordan V, Farquhar C. Time-lapse systems for embryo incubation and assessment in assisted reproduction. *Cochrane Database Syst Rev*. 2015;(2):CD011320. <https://doi.org/10.1002/14651858.CD011320.pub2>.
 26. Racowsky C, Kovacs P, Martins WP. A critical appraisal of time-lapse imaging for embryo selection: where are we and where do we need to go? *J Assist Reprod Genet*. 2015;32:1025–30. <https://doi.org/10.1007/s10815-015-0510-6>.
 27. Ginsburg ES, Baker VL, Racowsky C, Wantman E, Goldfarb J, Stern JE. Use of preimplantation genetic diagnosis and preimplantation genetic screening in the United States: a Society for Assisted Reproductive Technology Writing Group paper. *Fertil Steril*. 2011;96:865–8. <https://doi.org/10.1016/j.fertnstert.2011.07.1139>.
 28. Chang J, Boulet SL, Jeng G, Flowers L, Kissin DM. Outcomes of in vitro fertilization with preimplantation genetic diagnosis: an analysis of the United States Assisted Reproductive Technology Surveillance Data, 2011–2012. *Fertil Steril*. 2016;105:394–400. <https://doi.org/10.1016/j.fertnstert.2015.10.018>.
 29. Paternot G, Wetzels AM, Thonon F, Vansteenbrugge A, Willemen D, Devroe J, et al. Intra- and interobserver analysis in the morphological assessment of early stage embryos during an IVF procedure: a multicentre study. *Reprod Biol Endocrinol*. 2011;9:127. <https://doi.org/10.1186/1477-7827-9-127>.
 30. Storr A, Venetis CA, Cooke S, Kilani S, Ledger W. Inter-observer and intra-observer agreement between embryologists during selection of a single day 5 embryo for transfer: a multicenter study. *Hum Reprod*. 2017;32:307–14. <https://doi.org/10.1093/humrep/dew330>.
 31. Racowsky C, Ohno-Machado L, Kim J, Biggers JD. Is there an advantage in scoring early embryos on more than one day? *Hum Reprod*. 2009;24:2104–13. <https://doi.org/10.1093/humrep/dep198>.