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Research Article

The Blastulation Rate as a Metric For an *In Vitro* Fertilization Laboratory, Patient And Physician Factors May Impact that Rate

Bruce I Rose[1,2,3*], **Kevin Nguyen**[2]

[1]*Department of Obstetrics and Gynecology, University of Vermont Medical Center, Burlington, USA;* [2]*Brown Fertility, 8149 Point Meadows Way, Jacksonville, FL 32256, USA;* [3]*Department of Obstetrics and Gynecology, School of Medicine, University of Central Florida, Orlando, USA*

ABSTRACT

Objective: To determine the blastulation rate for an *In Vitro* Fertilization (IVF) laboratory and determine how it was effected by patient and physician variables.

Design: Retrospective Cohort.

Setting: Community-based IVF center staffed by one embryologist and five physicians from 2017-2019.

Interventions: Controlled ovarian hyperstimulation, oocyte retrieval, and embryo culture for up to six days (1005 cycles and 11,022 oocytes).

Main Outcome Measure: The proportion of pre-zygotes that developed into blastocysts.

Results: The overall blastulation rate was 70%. The blastulation rate was not significantly affected by maternal age. The blastulation rate was decreased when surgically obtained sperm were used (59.2%; $p<0.0001$), but not significantly decreased with various degrees of oligozoospermia. The specific physician performing the retrieval significantly impacted the blastulation rate (up to a blastulation rate difference of 7.6%; $p<0.0002$). Individual physicians retrieved averages of different numbers of oocytes, which resulted in different average numbers of blastocysts for different physicians.

Conclusions: The blastulation rate is an easy-to-calculate statistic for an IVF laboratory. It is not significantly affected by the inclusion of patients with advanced maternal age undergoing extended culture or couples with poor sperm counts. Physician factors may affect both the blastulation rate and the average number of blastocysts produced.

Keywords: Advanced maternal age; Blastocyst; Blastulation rate; IVF laboratory; Physician skills; Oligozoospermia

INTRODUCTION

Most IVF programs incubate embryos for five days before transfer in order to be able to reduce the number of embryos transferred while maintaining a high pregnancy rate. One of the first studies advocating the value of this approach was by Gardner et al. who observed a linear relationship between the number of pronuclear embryos and the number of blastocysts formed. Gardner et al. also observed that there was no impact of age on this relationship [1]. Furthermore, Gardner et al suggested the need for additional study to confirm or determine if age and possibly other prognostic patient factors altered the blastulation rate. In contrast to Gardner, several researchers have reported that advanced maternal age decreased the blastulation rate [2,3].

It has been twenty years since the publication of Gardner et al. paper, and during that time, there has been a major effort in optimizing blastocyst culture. The effect of age and other patient factors on the blastulation rate merits re-visiting and with the widespread use of single embryo transfer at the blastocyst stage, it should be possible to examine this problem with a much larger database than was done twenty years ago.

The Gardner et al observation implied that laboratory factors, rather than intrinsic patient factors (such as age), played a larger role in determining an IVF laboratory's overall blastulation rate. If this were the case, the blastulation rate could serve as a marker for laboratory consistency. The embryology literature has often used small differences in the blastulation rate for groups of patients undergoing IVF to evaluate the potential superiority of

Correspondence to: Bruce I Rose, University of Vermont Medical Center, Department of Obstetrics and Gynecology, Burlington, VT 05401, USA; Phone: 1-610-653-7979; E-mail: brucerose50@gmail.com

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one technique or intervention compared to another [4-9].

At the extremes, blastocyst production may be impaired for subgroups of patients [3, 10-12]. This study's objective was to determine if couples with impaired blastocyst production, as a part of a program's unselected patient population, significantly altered the overall blastulation rate of a program. If these small subgroups of patients did not alter the overall blastulation rate, then the blastulation rate could serve as a measure of laboratory quality control.

In this study, subsets of cycles with differing sperm quality, oocyte retrieval number, and maternal age were examined to determine if they were statistically different from the remaining cycles. If they were not different, those subgroups were viewed as not materially affecting the overall blastulation rate. Since five different physicians were involved in this study, the blastulation rates for individual physicians were also examined.

MATERIALS AND METHODS

This was a retrospective review of all oocyte retrievals in which oocytes were obtained in a community-based IVF program from 2017 through 2019. The program was staffed by one embryologist and five physicians. The patient population was racially and ethnically diverse and drew from branch offices located in both urban and suburban sites in the states of Florida and Georgia.

The program cares for patients without limitations on age, ovarian reserve, or sperm quantity. Men with azoospermia were offered surgical sperm retrieval before the cycle in which their wives were to undergo controlled ovarian hyperstimulation. The program's policy, in all cases, was to utilize ICSI for fertilization. The culture media used was Continuous Single Culture NX IVF media (Irvine Scientific, Santa Ana, CA). Blastocyst freezing was limited to 5 or 6 days after fertilization. Embryos were not further evaluated after 6 days. The controlled ovarian hyperstimulation employed a Gonadotropin-Releasing Hormone (GnRH) antagonist suppression starting before the largest follicle was 14 mm. Choices in the use of starting medications were made by two physicians depending on the primary office for that patient. Decisions to freeze all blastocysts or to transfer embryos after three days of culture were determined by individual physicians. Rare patients with a prior poor response to the above protocol were sometimes offered controlled ovarian hyperstimulation with the long protocol. Some patients utilized *In Vitro* Maturation (IVM) rather than controlled ovarian hyperstimulation.

All aspects of laboratory procedures were performed by a single experienced embryologist at a single laboratory site. All retrievals were done in the same operative setting using assigned medical assistants and nurse anaesthetists. The schedule of dates for each physician to perform oocyte retrievals was created months before a patient's registration for a cycle. A patient's retrieval dates were determined by ultrasound findings. Physicians could not select particular patients or diagnoses for oocyte retrieval. Similarly, the particular physicians making dose change decisions and deciding when a patient was ready for her retrieval tracked a schedule for physicians created months in advance. Three of the physicians performed retrievals during the entire 36 months of this study, one participated for 23 months and another for only four months. All physicians had at least 15 years of experience in performing oocyte retrievals.

Chi-squared 2 × 2 contingency table calculations and two-tailed t-tests were used to determine statistical significance using statistical calculators available online. GraphPad for the two-tailed t-test was available at https://www.graphpad.com/quickcalcs/ttest1.cfm and the "social science statistics website" for chi-squared calculations, was available at https://www.socscistatistics.com/tests/chisquare/default2.aspx . To compensate for multiple pair-wise comparisons, $p<0.005$ was the level required for statistical significance. The only missing data in this study was the sperm concentration relating to seven cycles in which patient sperm was frozen and defrosted for use in that cycle. These cycles were excluded from the calculations involving sperm concentration.

The PROBE checklist for retrospective cohort studies was used for this publication. This study was approved by the institutional review board at Baptist Medical Center in Jacksonville, Florida (Baptist Research Institute).

RESULTS

During the period studied, there were 1105 retrievals in which 11,022 oocytes were obtained. These encompassed 983 different patients undergoing an average of 1.1 cycles. Of these cycles, 87.2% (962 cycles) utilized extended culture. The remaining cycles were either viewed as having too poor a prognosis to benefit from the extended culture and embryo transfer was performed 3 days after fertilization (10.3%) (114 cycles) or embryos failed to progress for day 3 transfer (2.4%) (26 cycles). There were 19 patients treated with IVM; these cycles were also excluded from further evaluation.

Women, having fewer than four pre-zygotes due to diminished ovarian reserve or other poor prognostic reasons, were largely transferred on day 3 after fertilization. All patients using the long protocol fell into this category. There were 130 cycles of women with a low number of pre-zygotes, who after cleavage of fertilized oocytes on day 2, were thought to have a good prognosis. All of these patients produced blastocysts after extended culture. After exclusion of the women transferred on day 3, women with failed fertilization or embryo development, and women using IVM, the remaining extended culture group contained 945 cycles. The total of oocytes was 10,204 with 93.8% found to be in metaphase II at the time of ICSI.

The blastulation rate for the entire group was 70%. To test the expectation that the blastulation rates would be influenced by patient age, the cycles with the extended culture of oocytes were divided into the following age distributions: Age <35 years, age 35 to <40 years, and ages 40 to 45 years. Table 1 describes the impact of age on studied parameters. Although the blastulation rate decreased with age, there were no significant differences in the blastulation rates of the various age groups. The proportion of blastocysts formed on day 6 compared to day 5 for these various age groups was also calculated and not found to be different.

Although 74.6% of cycles utilized sperm with a concentration of greater than 15 million/ml (including 44 cycles using donor sperm), 7.4% had a concentration between 5 and 15 million/ml, 5.4% had a concentration between 100,000 and 5 million, 6.7% had a concentration under 100,000, and 5.2% had required prior surgical sperm retrieval. Since a sperm concentration of greater than 15 million is considered in the normal range, it was used as a reference to compare to these smaller groups. The blastulation rate in women using surgically retrieved sperm was significantly lower than cycles in which at least 15 million sperm were used

Table 1: Progression-free survival in months.

Age range	Number	Blastulation rate	p-value compared to other ages	% blastocyst occurring on day 6	p-value compared to other ages
Less than 35 years	572[a]	70.7%	0.031	3.6%	0.025
35 to less than 40 years	275	70.2%	0.753	4.4%	0.345
40 to 45 years	96	72.6%	0.019	5.9%	0.017

[a]includes 151 cases using donor oocytes

Table 2: Blastulation rate for cycles using different sperm concentrations.

Sperm Category	Cycles	Blastulation Rate	p-value compared to sperm concentration greater than 15 M
Surgically obtained	50	59.2%	<0.0001
Less than 100,000 sperm	64	65.7%	0.006
100,000 to less than 5 M sperm	46	66.3%	0.065
5 M to less than 15 M sperm	68	72.6%	0.357
15 M or more sperm	708[a]	70.9%	reference group

[a]includes 44 cases using donor sperm

Table 3: Blastulation rate for cycles in which different numbers of oocytes were retrieved.

Oocyte retrieved	Number of cycles	Blastulation rate check	p-value compared to the remainder of the cohort
Less than 6	129	76.3%	0.001
6 or 7	178	74.3%	0.001
8 or 9	150	70.3%	0.947
10 or 11	142	71.6%	0.282
12, 13, 14	149	69%	0.805
15, 16, 17, 18	100	68.5%	0.065
More than 18	95	66.4%	0.002

(59.2%; p<0.0001). All other groups were not statistically different; although all subgroups trended lower with the subgroup with fewer than 100,000 sperm having had a blastulation rate of 65.7% (p=0.006) (Table 2).

The blastulation rate was also calculated for subgroups where a different number of oocytes were retrieved (Table 3). For these subgroups, the blastulation rate ranged from 67% to 76.3%, where the rate tended to decrease as the number of oocytes retrieved increased. Subgroups of cycles in which 1 to 5, 6 to 7, and greater than 18 oocytes were retrieved had blastulation rates significantly different from the blastulation rate of the entire group. Equivalently, when the number of oocytes retrieved was more than 7 and less than 19, the blastulation rate of subgroups was not statistically different from the overall mean.

One physician performed only 18 retrievals and was excluded from physician evaluations. For the remaining four physicians, blastulation rates per physician varied from 67.3% to 74.9% (Table 4). Physician D's blastulation rate was significantly lower than the remaining physicians (p=0.001). Physician C's blastulation rate was significantly higher (p=0.0002). The differences in the blastulation rate of other physicians were not statistically different from the overall rate.

Table 4: Physician on blastulation rate, oocytes retrieved, and blastocysts produced.

	Physician A	Physician B	Physician C	Physician D
Number of cycles	292	280	149	204
Blastulation rate	69%	71.1%	74.9%[a]	67.3%[b]
Oocytes per retrieval (SD)[c]	9.9 (4.8)	12.4 (5.9)	8.2 (3.8)	11.8 (6.1)
Blastocyst per retrieval (SD)[c]	6.1 (3.3)	8.0 (4.4)	5.7 (4.8)	7.1 (4.8)

[a]p<0.0002 compared to remaining physicians
[b]p<0.001 compared to remaining physicians
[c]see table 5 below

Table 5: Statistical data for pairwise comparisons of physicians.

Physicians compared	Oocyte number	Blastocyst number
A *vs* B	p<0.0001	p<0.0001
A *vs* C	p=0.0002	p=0.2065
A *vs* D	p=0.0001	p=0.0062
B *vs* C	p<0.0001	p<0.0001
B *vs* D	p=0.2767	p=0.0330
C *vs* D	p<0.0001	p=0.0018

The number of oocytes retrieved varied from a mean of 8.2 to a mean of 11.4 per physician. Similarly, the number of blastocysts produced varied from 5.7 to 8.0. As Table 4 demonstrates, there were significant differences between physicians in both the number of oocytes retrieved and the number of blastocysts produced. The worst physician in terms of the blastulation rate produced significantly more blastocysts than the physician with the best blastulation rate (7.1 blastocysts versus 5.7 blastocysts; p=0.0018), because of a higher oocyte retrieval rate (Table 5).

DISCUSSION

There are two common ways to calculate the blastulation rate for an IVF program. The first, which has been used exclusively to this point, was to simply count the total number of blastocysts produced and divide it by the total number of fertilized oocytes. The second was to calculate, for each cycle, the ratio of the number of blastocysts produced divided by the number of fertilized oocytes. The blastulation rate was then the average of these ratios. These calculations are not the same since $(x_1+...+x_n)/ (y_1+...+y_n)$ is not equal to $(x_1/ y_1+...+ x_n /y_n)/n$. However, in our clinical setting, the results were close to each other. In particular, for the first method of calculation, the blastulation rate was 70%; whereas, the second method yielded a blastulation rate of 71%. The first method was easier and potentially more applicable for use in IVF program quality improvement. An advantage of the second method was that

it allows us to calculate a Standard Error Of The Mean (SEM). The SEM was 0.7% which implies that 95% of the time using a similar database, the blastulation rate should fall in the interval of 69.6% to 72.4%.

The choice of 0.005 as statistically significant was made to compensate for multiple pairwise comparisons. For example, a pairwise comparison of four subgroups to determine if any pair is statistically different at the 0.05 level, has an 18.5% chance of making a type I error (1-(0.95)(0.95) (0.95)(0.95)); whereas, with a significance level of 0.005, the risk of a type I error drops to 2%.

Cycles in which fewer than eight oocytes were obtained had a higher blastulation rate and cycles in which less than 18 oocytes were obtained had a lower blastulation rate compared to the remaining cycles, respectively. The blastulation rate trended downward as the number of oocytes retrieved increased. These findings of differences in the blastulation rates for low or high oocyte retrieval numbers are likely an artefact of the statistical approach taken in this paper. When fewer oocytes were retrieved, each blastocyst had a greater impact on increasing the blastulation rate of a program than when a higher number of oocytes were retrieved. This same statistical bias has been used in all the references cited in this paper and has been commonly used in the IVF literature, for example, in evaluating a program's fertilization rate or oocyte immaturity rate. For this study's data set, the blastulation rate of the combined subgroups of cycles with less than 8 oocytes or more than 18 oocytes retrieved was similar to the blastulation rate of the remaining cycles (70.7% *versus* 70%).

An unexpected finding of this study was the impact of individual physicians on the blastulation rate. The difference in blastulation rates found, caused us to search for any differences between physicians which might affect this observed difference. Although all physicians could easily flush follicles, the physician with the lowest blastulation rate was the strongest proponent of follicle flushing. An evaluation of how this flushing was done suggested that the procedure room could do a better job of temperature maintenance of flushing fluids and aspirates [13,14]. It is too soon to determine if these procedure room changes will improve that physician's blastulation rate. However, note that this "worst" physician helped create more blastocysts, per patient on average, than the physician with the best blastulation rate, because of differences in the number of oocytes retrieved.

As suggested in Table 4, there are also significant differences in the retrieval success of individual experienced physicians. The most successful physician retrieved an average of more than three additional oocytes compared to the least successful physician. This suggests the need to further study retrieval techniques to determine why some physicians are more successful than others.

CONCLUSION

The blastulation rate is a single data point that summarizes the work of an IVF laboratory in producing blastocysts. It is easy to calculate and available for use even before cycles have been fully completed. Unlike the clinical pregnancy rate or the live birth rate, it provides information about the impact of the entire cohort of gametes presented to the laboratory in generating potentially usable products. Large changes in the blastulation rate may reflect a laboratory issue that requires explanation or a need for improvement.

Using statistical hypothesis testing, patient factors. such as maternal age or paternal sperm count, had little impact on the overall blastulation rate of 70% in the setting of contemporary blastocyst culturing techniques. The physician performing the retrieval may have a significant impact on the blastulation rate. The physician involved in a retrieval also had an impact on the average number of blastocysts available for use by that couple.

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