Identification of embryo culture parameters that correlate significantly with high clinical pregnancy rates

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Abstract

Which IVF laboratory parameter(s) is/are directly linked to clinical pregnancy rates (CPR)? Retrospective analysis of routine laboratory data during periods when CPRs were good (48.2% & 46.9%) or poor (16% & 17.2%) was investigated. Parameters investigated were: fertilization rate (FR), zygote arrest rate (ZAR), mean blastomere number (MBN), mean embryo grade (MEG) [Embryos graded: 4 = excellent, 3=good,, 2=average, 1=poor], proportion of day 2 embryos at or above normal blastomere number (%≥NBN), proportion of embryos at or above a score of "3 or good" quality (%≥MEG). The data was statistically analyzed using Statistix™ and Medcalc™ statistical programs. Values obtained for parameters that appear to have an impact were: FR, 80.4, 75.5, 53.3, 56.4%; ZAR, 3.4, 7.7, 10.3, 17.7%. The higher the fertilization rate, higher the CPR (FRs of 80.4, 75.5, 53.3, 56.4% showed CPRs of 48.2, 46.9, 17.2, 16.0% respectively) whereas the ZAR has an inverse effect. The lower the ZAR rate the higher the CPR (ZARs of 3.4, 7.7, 10.3%,17.7% gave CPRs of 48.2, 46.9, 17.2,16.0% respectively). FR and ZAR were significantly correlated (FR vs CPR, r = + 0.8721, p=0.0010; ZAR vs CPR, r = - 0.8308, p=0.0029) with groups that gave higher CPRs (48.2%, 46.9%). These findings indicate that by keeping FR very high ≥80% and ZAR very low (≤3.4%) it may be possible to aim for CPRs of ≥45%. Efforts must be made to maximize FR and minimize ZAR. Other parameters tested did have an impact on the CPR but were not as consistent as FR and ZAR. While the findings of this small study can only be considered preliminary, it is however reasonable to speculate that a FR of ≥80% and a ZAR of ≤3.5% need be the target objectives of all IVF treatment cycles in order to attain high CPRs of ≥45%.

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Introduction

Are there ways by which we can increase the clinical pregnancy rate by carefully monitoring some specific laboratory parameter(s) in the IVF Laboratory?

Since the mid-2000's we have strived to identify specific indicators of quality that correlated highly significantly with the clinical pregnancy rate (CPR). To this end we utilized some parameters performed during routine IVF procedures as indicators of quality. We report in this communication statistical evidence on the impact of some laboratory parameters which could be utilized to aim for high CPRs. The indicators of quality that we investigated were: (a) the fertilization rate, (b) zygote arrest rate; (c) mean blastomere number, (d) mean embryo grade (embryos are graded 4=excellent,

1=poor), (e) proportion of embryos ≥4 blastomeres on day 2; and (f) proportion of embryos ≥grade 3 at the cleavage stages to determine their impact on the CPR.

We noted that the ESHRE Special Interest Group of Embryology and Alpha Scientists in Reproductive Medicine in their Vienna Consensus of 2017 have utilized some of these parameters (that we regularly employed since 2000's) for calculating their competency rates and performance indices (CR, PI; ESHRE and Alpha, 2017).

The objective of this study is to identify indicators of laboratory quality that correlate significantly with and could be used to aim for ≥45% clinical pregnancy rate (CPR) per oocyte retrieval (OR).

Materials and methods

Retrospective analysis of laboratory data in the authors' laboratory was performed during two periods, one, when the clinical pregnancy rate (CPR) was good (48.2% & 46.9%) with periods when the CPR (16-17.2%) was poor.

The following parameters were investigated, (i) fertilization rate (FR), (ii) zygote arrest rate (ZAR), (iii) mean day 2 blastomere number (MBN; microscopic examinations were performed at 7.30-8.30am), (iv) mean day 2 embryo grade (MEG), (v) proportion of day 2 embryos at or above normal blastomere number (%≥NBN), (vi) proportion of embryos graded as or above the score of "good" quality (%≥GEQ).

The embryos were graded in the range of 4 to 1, where 4=excellent, 3=good,, 2=average, 1=poor. The data were statistically analyzed using the statistical softwares, Statistix™ and Medcalc™.

Results

The results of the investigation is given in Tables 1 to 5. The impact of both the fertilization rate (FR) and zygote arrest rate (ZAR) on the clinical pregnancy rate (CPR) is readily discernible (Tables 1-5). Higher the FR, the higher the CPR. Whereas with the zygote arrest rate, the lower it is, the higher the CPR. Indeed this relationship is clearly demonstrated in the statistical results shown in Table 5 which shows a statistically highly positive correlation (r=+0.8721, p=0.0010) between the FR and CPR. In contrast, there is a highly negative correlation between the ZAR and CPR (r = -0.8308, p=0.0029). Low ZAR appears to impact the treatment outcome with high success rates while high ZAR is associated with poor treatment outcomes.

The values obtained for other parameters respectively also had an impact on the CPR. While all parameters appear to have an effect on the CPR however FR and ZARs appear to impact CPR more consistently. The rate of embryo development and embryo quality also has an impact on the pregnancy rate but their impact is not consistent and or as pronounced as that of FR and ZAR.

Discussion

IVF laboratories have customarily employed a variety of indicators to keep track of the quality of laboratory culture conditions and performance to sensitively discern and accurately maintain the total quality management (TQM) of the facility. TQM appear have a direct impact on treatment outcome. The commonly used indicators of laboratory quality include normal fertilization rates, polyspermic rates, embryo cleavage rates, intracytoplasmic sperm injection (ICSI) degeneration rates, implantation rates, pregnancy rates, and thaw survival rates (Wiemer et al., 2001).

Establishing the appropriate competency and benchmark values for all indicators of quality is a challenging but a vital task for the Total Quality Improvement (TQI) program to be effective. The Vienna Concensus of 2017 (ESHRE and Alpha Scientists, 2017a,b) that proposed benchmark and competency values for various parameters appear efficacious and need be adopted by all practitioners.

Table 1: Quality of day 2 human embryos generated during different ambient conditions

Medium/ Period	% Oocyte fertilized	Arrested at zygote stage	Mean Blastomere No. (MBN) (1SD)	Mean Embryo Grade (MEG)(1SD)	%≥4 blasto-meres	%≥3 Grade	%CPR (CP/OPU)
Good period Cook medium	75.5 (247/327)	7.7	3.9 ±1.7	3 ±1	62.3	63.8	46.9 (15/32)
Cook medium Poor period; dust storm	56.4 (243/431)	17.7	3.6 ±1.1	3 ±1	68.5	65	16 (10/62)
Significance	p=0.0000	p=0.0000	p=0.0334	p=1.0000	p=0.214	p=0.742	p=0.0032

Table 2: Quality of day 2 human embryos generated with drugs affected by poor storage

Medium/ Period	% Oocyte fertilized	Arrested at zygote stage	Mean Blastomere no. (MBN). (1SD)	Mean Embryo Grade (MEG)(1SD)	%≥4 blasto-meres	%≥3 Grade	%CPR (CP/OPU)
Good period Cook medium	75.5 (247/327)	7.7	3.9 ±1.7	3 ±1	62.3	63.8	46.9 (15/32)
Poor 2 (drug defect) Cook medium	53.3 (243/431)	10.3	3.8 ±1.1	3.3 ±1	70	76.9	17.2 (10/62)
Significance	p=0.0001	p=0.2351	p=0.3729	p=0.0001	p=0.0442	p=0.002	p=0.0045

Table 3: Quality of day 2 human embryos generated in different media and ambient conditions

Medium/ Period	% Oocyte fertilized	Arrested at zygote stage	Mean Blastomere no. (MBN). (1SD)	Mean Embryo Grade (MEG) (1SD)	%≥4 blasto-meres	%≥3 Grade	%CPR (CP/OPU)
Synbios medium	80.4 (320/398)	3.4	3.7 ±1.1	3 ±0.7	65	68	48.2 (55/114)
Cook medium (Dust storm)	56.4 (243/431)	17.7	3.6 ±1.1	3 ±1	68.5	65	16 (10/62)
Significance	p=0.0000	p=0.0001	p=0.3163	p=1.000	p=0.4707	p=0.544	p=0.0001

Table 4: Quality of day 2 human embryos generated in different media and poor drug quality

Medium/ Period	% Oocyte fertilized	Arrested zygotes	Mean Blastomere no. (MBN). (1SD)	Mean Embryo Grade (MEG)(1SD)	%≥4 blasto-meres	%≥3 Grade	%CPR (CP/OPU)
Synbios medium	80.4 (320/398)	3.4	3.7 ±1.1	3 ±0.7	65	68	48.2 (55/114)
Cook medium (Drug defect?)	53.3 (332/623)	10.3	3.8 ±1.3	3.3 ±0.7	70	76.9	17.2 (11/64)
Significance	p=0.0000	p=0.0001	p=0.3052	p=0.0022	p=0.2188	p=0.0018	p=0.0001

Grading system for Tables 1 to 4 above: 4 = Excellent; 3 = good; 2 = average; 1 = poor

Table 5. Impact of fertilization and zygote arrest rates on clinical pregnancies

Periods and factors Impacting Embryo Culture Conditions	% Fertili-zation Rate (FR)	% Zygote Arrest Rate (ZAR)	% CPR
Optimal culture conditions	80.4	3.4	48.2
Optimal culture conditions	75.5	7.7	46.9
Ovarian Stimulations Drugs less optimal	53.3	10.3	17.2
Dust-storm	56.4	17.7	16.0
Optimal Medium	82.8	5.3	50.0
Less-optimal Medium (batch variation)	75.5	9.1	27.8
Superior- Culture Conditions	80.6	3.4	62.1
Quality culture conditions	68.6	4.8	35.7
Moderate Quality Culture Conditions	73.4	6.8	29.2
Poor Quality Culture Conditions	60.3	19.8	12.7

Statistical analysis: Pearson's correlation

(i) FR vs CPR, r = + 0.8721, p=0.0010; (ii) ZAR vs CPR, r = - 0.8308, p=0.0029

In addition to existing methods of TQI and quality control there is a need to identify in vitro indicators that could be used to monitor quality in the laboratory. With identification of reliable indicators of in vitro quality it may be possible to aim for improved treatment outcome. Some less visited indicators could also sensitively and efficiently recognize sub-optimal laboratory conditions or sub-standard performance in the laboratory quite effectively.

The objective of this study is to identify indicators of quality that is directly correlated to CPR which can be used to aim for high CPRs (≥45%). All the identified laboratory indicators of quality collectively can serve as tools for TLI however only the FR and ZAR appear to be consistently correlated to CPR.

The present findings appear to suggest and is reasonable to speculate that if the FR and ZAR can steadfastly be maintained at very high ≥80% and low ≤3.4% rates respectively, the CPR obtained will likely be high (≥45%). These indicators of quality could be applied in routine

IVF treatment procedures to increase clinical pregnancy rates and treatment outcome.

To achieve this end, efforts must be made to maximize FR and minimize ZAR. The FR and ZAR, appear to be critical parameters that reflect on the TQM of the facility which can only be achieved if each IVF facility conducted itself in a manner that is compliant with the highest standards of the industry.

These include: careful monitoring of treatment cycles, the skills of all workers must be maintained at very high levels of competence (ESHRE and Alpha Scientists, 2017), the numbers of ovum pick-ups per day must not exceed the recommended number of treatment cycles beyond the capacity of the available workforce, workers must not be rushed to complete their work due to excessive workload, the quality of oocytes generated are good, ovarian hyper stimulation is maintained at very low rates, the quality of the culture conditions employed must be optimal, the quality of embryos generated are good, there is a high level of cryopreservation post embryo transfer and the skills of embryologists in handling oocytes and embryos must be optimal. In general, the entire team must work synergistically with singleness of purpose with the objective of overcoming the childlessness of every single client.

In conclusion, while the findings of this small study can only be considered preliminary, it is however reasonable to speculate that a FR of ≥80% and a ZAR of ≤3.4% need be the target objectives of all treatment cycles. This can be achieved through meticulous methodology and stringent adherence to TQM that borders or strive towards perfection. It is proposed that if these can be achieved, a CPR of ≥45% per OR can be anticipated.

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