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Cryopreservation: the problem of evaluation

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A simple evaluatory formula for reporting pregnancy rates involving cryopreserved material has so far remained elusive. It is highly desirable to have a method which would allow not only an evaluation of cryotechnology *per se*, but also an evaluation of the role of cryotechnology in enhancing the total reproductive potential of a single cycle. In this paper, nine separate formulae are described which can be used for the evaluation of cryopreservation. It is concluded that none of the existing formulae used alone expresses the total potential of cryopreservation, and that while each formula provides information regarding some aspect of the role of cryopreservation, the most comprehensive evaluation requires the answer to five different equations. The other four formulae, while often used, are regarded as providing information which is unsubstantiated or does not allow complete evaluation.

Key words: cryopreservation/evaluation procedures/formulae

Introduction

A simple evaluatory formula for reporting pregnancy rates involving cryopreserved material has remained elusive, although suggestions have been made (Testart, 1988; Alam *et al.*, 1993; Veeck *et al.*, 1993). It is highly desirable to have a method which would allow not only an evaluation of cryotechnology *per se*, but also an evaluation of the role of cryotechnology in enhancing the total reproductive potential of a single cycle. For evaluation, the cryobiologist/clinician needs to have information about four clusters of variables of the freeze–thaw process: (a) the percentage of patients with a fresh transfer who also have material available for cryopreservation, and the percentage of pre-zygotes/pre-embryos assigned to fresh transfer and to cryopreservation; (b) the efficacy of the cryoprocedure, i.e. the percentage of pre-zygotes/pre-embryos which survive the freezing process; (c) the pregnancy potential of the surviving pre-zygotes/pre-embryos. This can be determined on (i) a per zygote/pre-embryo basis, or (ii) on a per transfer basis. Furthermore, the type of pregnancy can be ascertained, i.e., all, clinical, ongoing, or term. This information cluster might be referred to as cryo-based; (d) the contribution of the cryopreserved results to the fresh pregnancy rate determines the total pregnancy potential of a particular, or all, stimulation cycles. This cluster might be referred to as fresh + cryo-based.

The situation becomes extremely complicated and obfuscated if one tries to express all desired information by a single formula. It seems necessary to have several separate formulae which, together, will provide a meaningful evaluation. For general use, all formulae must be limited to the same time interval or patient population. For investigational purposes, if cryopreservation is involved, the formulae can be used to examine specific points, for example, the type of transfer—uterine or tubal, natural or steroid controlled reception cycles—or other variables.

A goal is to eliminate the pitfall of attributing more than one pregnancy to a single retrieval cycle when there are pregnancies from both fresh and thawed material, or from more than one transfer of thawed material from the same cycle. It is our view that such multiple pregnancies from a single harvest cycle should be treated as a single pregnancy, but as a multiple birth, for example, twins, triplets, etc., although their birthdates may be different. It must be emphasized that throughout, the word 'cycle' in this paper means a single retrieval harvest of oocytes.

(A) Percentage of patients and eggs assigned to fresh transfer and to cryopreservation

This requires three formulae:

(i) Number of cycles with cryopreservation / Number of cycles with fresh transfers = Cycle cryopreservation rate

(ii) Number of pre-zygotes/pre-embryos transferred fresh / Total number of two-pronuclear pre-zygotes/pre-embryos = Pre-zygote/pre-embryo fresh transfer rate

(iii) Number of pre-zygotes/pre-embryos cryopreserved / Total number of two-pronuclear pre-zygotes/pre-embryos = Pre-zygote/pre-embryo cryopreservation rate

(B) Efficiency of cryopreservation (assuming only viable thawed pre-zygotes/pre-embryos are transferred)

This requires one simple formula:

(iv) Number of surviving* thawed pre-zygotes/pre-embryos transferred / Number of pre-zygotes/pre-embryos thawed = Pre-zygote/pre-embryo survival rate

*Survival judged by morphology after thaw or preferably by cleavage 24 h after thaw.

(C) Pregnancy potential of surviving pre-zygotes/pre-embryos

One method of reporting pregnancies after thawing is to report rates from the cryopreserved material more or less as pregnancy rates are reported from fresh material. This method provides a limited but useful, and even necessary, measure of the pregnancy potential of the surviving cryopreserved material.

There are several sub-options within this cryo-based option, e.g. it is possible to ascertain a procedure-based pregnancy rate. Thus, a patient must have had a procedure (e.g. at least one transfer) to be included, and the number of procedures becomes the denominator of the pregnancy rate fraction. Several numerators can be used, such as pregnancies, clinical pregnancies or deliveries. The formula:

(v) Number of pregnancies from thawed pre-zygotes/pre-embryos / Number of transfers of thawed pre-zygotes/pre-embryos = Thawed pre-zygote/pre-embryo transfer specific pregnancy rate

Ascertaining transfer-specific pregnancies and deliveries, the 1992 Registry data published in 1994 showed that the national transfer-specific pregnancy rate per procedure (transfer) was 24.1%, and the delivery rate per procedure was 19.2% (Society for Assisted Reproductive Technology, 1994).

Formula (v), while helpful, can include repeat pregnancies from the frozen material of the same harvest cycle. It can therefore be referred to as a transfer-specific evaluation, rather than a cycle-specific evaluation. In order to eliminate duplicate thawed pregnancies from the frozen material of a single cycle, it is necessary to modify formula (v) as follows:

(va) Number of cycles with one or more pregnancies from thawed material / Number of cycles with thawed transfer = Thawed pre-zygote/pre-embryo cycle-specific pregnancy rate

(D) Fresh plus cryo-based evaluation (including the total reproductive potential of stimulation cycles)

One fresh + cryo-based option often used is simply to add the gross fresh rate to the gross cryo rate according to the following formula:

(vi) Number of pregnancies from cycles with cryopreservation + number of pregnancies from thaw transfers / Number of cycles with transfers with cryopreservation = Augmented pregnancy rate

One can add to this the projected number of thawed pregnancies from the unthawed material, making the assumption that the pregnancy rate for the yet unthawed material will be the same as for the already thawed material, and making the additional assumption that all cryopreserved material will be thawed and transferred. The formula for this is:

(vii) Number of pregnancies from cycles with cryopreservation + number of pregnancies from thaw transfers + number of pregnancies projected from yet unthawed material / Number of cycles with transfers with cryopreservation = Projected augmented pregnancy rate

Neither of the methods of expressing the pregnancy rate in formulae (vi) or (vii) are without fault as they duplicate pregnancies, similar to formula (v). Both the augmented pregnancy rate and the projected augmented pregnancy rate can be falsely high. They can be falsely high if one or more patients has a pregnancy both with a fresh transfer and a thawed transfer, or from still unthawed material. This has the effect of crediting a patient with more than one pregnancy per cycle when in fact she is having a multiple pregnancy with oocytes inseminated at the same time, but born on different dates. In our view, such multiple births should be counted as one pregnancy, but as a multiple pregnancy with different birthdates, and noted separately in a footnote.

To compensate for the double counting, it is necessary to limit the cryopregnancies, and but one of them only to the patients who have not had a pregnancy from a fresh transfer according to the following formula:

(viii) Number of cycles with pregnancy from cycles with cryopreservation + number of cycles with one or more pregnancies from thaw transfers among patients without a pregnancy from the same cycle* / Number of cycles with transfers with cryopreservation = Cycle-specific augmented pregnancy rate

In order to overcome the problem of over-reporting pregnancies by repeat pregnancies in the yet unthawed cryopreserved material, the potential augmentation should be confined to material from patients who have not had a pregnancy from either a fresh or a frozen transfer. The formula:

(ix) Number of cycles with pregnancy from cycles with cryopreservation + the number of cycles with one or more pregnancies from thaw transfers among patients without a pregnancy from the same cycle + number of cycles with pregnancy projected from yet unthawed material among patients without a fresh or thawed pregnancy from the same cycle / Number of cycles with transfers with cryopreservation = Cycle specific projected augmented pregnancy rate

Discussion

The nine formulae taken together seem extremely formidable and are probably too complicated for routine use. Indeed, they are not presented or proposed for that purpose. However, they are presented to call attention to the fact that no single formula yet devised expresses the total potential of cryopreservation.

Indeed, there are some nuances that can bias the use of the formulae. An example might be the use of selection of pre-embryos as a policy, either for fresh transfer or for cryopreservation. However, extreme selection would become manifest by the use of formula (i), which gives the cycle cryopreservation rate. If this figure is very low, there is a presumption that substantial selection is being practised, or that cryotechnology is not being used by that particular programme for whatever reason.

There are other nuances which could influence the 'projected augmented pregnancy rate'. This is the rate that assumes that

*The number of patients who have a thawed pregnancy who have also had a fresh pregnancy can be noted separately.

unthawed cryopreserved material will have the same pregnancy potential as fresh or, in some instances, previously thawed material. Such nuances might include pre-embryo selection by grade, either for fresh transfer or for cryopreservation, or transfer of cryopreserved material in rank order of grade. A bias can surely be introduced into any cryoevaluation system, if in a particular laboratory the survival rate [formula (iv)] differs depending on developmental stage (i.e. two-pronuclear stage or later) at cryopreservation. If four-to-eight cell cryopreservation results in a lower survival rate, it might be compensated for by a higher implantation rate, and therefore pregnancy rate [formula (v)]. However, if the goal is to evaluate the total pregnancy potential from fresh plus cryopreserved material in a given harvest cycle, all of these nuances will be totally evaluated by formula (v) – the augmented pregnancy rate, if and when all cryopreserved material for a given harvest cycle is used.

While each formula provides information about some aspect of the role of cryopreservation in an assisted reproductive technology programme, the most comprehensive evaluation requires the answers to five equations, namely: (i); (ii); (iii); (iv); and (viii). While formulae (v), (vi), (vii), and (ix) are often used, they give information which is unsubstantiated or does not allow a comprehensive evaluation of the role of cryopreservation. It is exceedingly important for the cryobiologist/physician and the patient to understand and comprehend the methods of expressing the potential in the cryopreserved material in order to be able to evaluate the total pregnancy potential of a particular stimulation cycle of assisted reproductive technology.

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