REVIEW ARTICLE

Embryo and Oocyte Wastage in ART: Is it Inevitable?

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Abstract

Natural human reproduction is very inefficient in achieving live births. Number of studies have shown that the maximum chances of conceiving a clinically recognized pregnancy in one natural menstrual cycle is about 30%, under optimal conditions for conception. Which implies in nature almost 70% of human embryos are lost at various stages from preimplantation embryo to full term pregnancies.

From the time of number of oocytes retrieved, to cleavage stage embryos formed, IVF technologies are also marred with high loss of oocytes and embryos, low implantation and high early pregnancy loss rates. In fact right from the time of fetal germ cell production to live births—human reproduction is an extremely wasteful exercise both in nature and also in assisted reproduction. A process of continuous reduction or selection against aneuploid embryos starts right from the time of fertilization. Current methods used in the laboratory for embryo selection, do help choose better embryos but are still inaccurate. PGS and metabolomic profiling are technique that may help select chromosomaly normal embryos, how ever are not practical and cost-effective yet.

This raises the obvious question of how far we can take the success of IVF? Have we reached the limits of improving pregnancy rates in IVF? Is it possible that majority of oocytes and embryos are intrinsically abnormal and not capable for implantation or developing further?

Keywords: Oocytes, preimplantation genetic diagnosis (PGD), preimplantation genetic screening (PGS), recurrent pregnancy loss, implantation failure.

NATURAL HUMAN REPRODUCTION

Although planet earth is facing a population explosion, it is a known fact that the fecundity of the human race is very low. Human reproduction is very inefficient in achieving live births right from the time of the formation of the primary germ cell line in the fetal ovaries. This was first highlighted in a publication by Roberts and lowe in 1975, where the number of births registered in England in 1970 was compared with the number of births that might have been expected, given the estimated number of fertile ovulatory cycles exposed to coitus in the same year and population.[1] The authors put forth the question "where have all the conceptions gone ?" Since only 22% of cycles at risk of pregnancy resulted in live birth.

Number of studies have since reported[2,3] that the maximal chance of conceiving a clinically recognized pregnancy in one cycle is about 30%, when circumstances for conception are optimal. Pregnancies may be lost at any time between fertilization and implantation, or up to term. Clinical pregnancy loss is only the tip of the iceberg. Vast majority of conceptions are lost even before the woman realizes she might be pregnant.

The reproductive loss that occurs even before a first missed period is substantial. Following fertilization about 30% embryos fail to implant, another 30% are lost after the embyo has started implanting but before the pregnancy is clinically recognized.[4]

Availability of sensitive assays for hCG and IVF technologies have made it possible to observe the events from ovulation to an on-going pregnancy. Intense research is on in the field of implantation, biomarkers of early embryos, cytogenetics, genetic regulation of implantation, etc.

This has literally opened up the previously elusive "black box" of early pregnancy.[5] Our understanding of the natural limits of human fecundity has thus improved. In nature, human pregnancy wastage occurs on a scale that only about 25 to 30% of conceptions will progress to live birth.

This raises the obvious question of how far we can take the success of IVF? Have we reached the limits of improving pregnancy rates in IVF?

SUCCESS OF ART: AS IT STANDS TODAY

Assisted reproductive technique (ART) has come a long way since 1978 birth of the first test tube baby. In clinical practice, availability of purified and recombinant products have made ovulation induction very comfortable for the patients and clinician. Many softer ovulation induction protocols are being tried without compromising on success rates. Highly researched and scientifically prepared culture media are now easily available, along with improved culture conditions in the laboratory, have ensured optimal embryos being produced in

the lab. Success of frozen embryo transfer has made IVF cycles more cost-effective in many patients. Assisted laser hatching and preimplantation genetic diagnosis are likely to further improve pregnancy rates in assisted reproduction.

Over the last decade clinicians are striving to reduce multiple pregnancies. Embryologists have concentrated on choosing top quality embryos using various morphological criteria.

Elective single embryo and double embryo transfer policy are being adopted by many centers. Emphasis is on choosing high quality embryos, and the process starts right from grading the oocytes.

2003 ART surveillance date from the USA[6] reported–overall 42% of ART transfer procedures resulted in a pregnancy, and 35% resulted in a live birth delivery (delivery of one or more live born infants). The highest live birth rates were observed among ART procedures utilizing freshly fertilized embryos from donor eggs (51%), multiple pregnancy rate ranged from 35 to 40%. Current data available from *in vitro* fertilization practices in the USA indicate that there is a 27% live birth rate from all initiated cycles, which does show some improvement over previous years.[7] Despite improvements in laboratory and clinical practice, ongoing pregnancy rates from IVF remain 20 to 25% per started cycle.[5]

The role of early pregnancy loss in determining clinical out comes of IVF is uncertain as there are very few studies reporting the true rate of early pregnancy loss following IVF. In one of the early studies liu et al, 1988[8] reported a 4% occult pregnancy loss rate which was much lower than following natural conception. Later studies have shown IVF premenstrual pregnancy loss to be more prevalent.

Data from oocyte donation studies suggest that impaired implantation may explain the early pregnancy loss observed in IVF. Oocyte donation is associated with higher implantation rates than routine IVF (SART/ASRM 2002). A possible explanation to this observation is the more physiological endometrial milieu into which embryos are transferred in oocyte donation cases. Unlike routine IVF, the endometrium of the recipient is not exposed to supra physiological levels of hormones in the follicular phase, or the high luteal progesterone levels which may alter the endometrial receptivity.[9-12]

Thus IVF technologies are marred with low implantation rates and high early pregnancy loss rates.

ENORMOUS BIOLOGICAL LOSS: BOTH IN NATURE AND IN ASSISTED REPRODUCTION

In nature almost 70% of human embryos are lost at various stages- from preimplantation embryo to full term pregnancies. Similarly there are high rates of embryo wastage with the use of assisted reproductive technology. This biological wastage of gametes and embryos becomes even more pronounced, if comparison is made between the number of oocytes retrieved with the live birth rate. This section will discuss two different issues of embryo and oocyte wastage.

EMBRYO AND OOCYTE WASTAGE

In a recent study reported in fertility sterility (kovalevsky et al 2005),[13] statistics for ART cycles using fresh, nondonor eggs and embryos were derived, and the percentage of embryos wasted each year was calculated. Trends over time were evaluated for percentage of embryos wasted, the average number of embryos transferred, and the delivery per transfer rate. The percentage of embryos transferred that did not produce a live birth was 90.8 in 1995 and decreased to 84.9 in 2001. It was also noted that this trend corelated with a reduction in the number of embryos transferred (3.9-3.1) and an improvement in delivery rate per transfer (25-33.4%). The authors concluded that possibly only a small fraction of embryos has the capacity to become a live birth. Clinicians should strive to reduce embryonic wastage without an adverse effect on delivery rates by perfecting methods of ovarian stimulation, embryo screening and reducing the number of embryos transferred.

Patrizio P et al 2007[14] in a very interesting study reported the overall biological wastage from oocytes inseminated to ongoing pregnancy. The study group consisted of patients undergoing preimplantation genetic screening (PGS) for advanced maternal age, recurrent pregnancy loss and multiple failed IVF cycles. Of 333 oocytes inseminated 183 (55%) provided embryos for biopsy, of which only 33 (18% per embryo and 9.9% per oocytes) were normal. 26 embryo were finally found suitable for transfer (14% per embryo and 7.8% per oocyte), of which five (1.5%) implanted and three (1%) resulted in live birth. Thus 333 oocytes resulted in 3 live births!

Cytogenetic studies of oocytes and preimplantation embryos support the concept that majority of embryos and oocytes obtained during IVF are intrinsically chromosomally abnormal and therefore lack the capacity to develop into good quality embryos and implant. Under current *in vitro* culture conditions high rates of oocyte and embryo wastage are observed. Underlying causes for embryo demise could be DNA damage, poor embryo metabolism, suboptimal culture conditions, or intrinsic chromosomal imbalance. Abnormalities in zygotes and preimplantation embryos are observed during IVF/ICSI right from fertilization.

Rates of abnormal fertilization vary from 2 to 9%.[5] The two main categories are haploid (one pronucleus) or triploid (three pronulei). One pronucleus may be due to nondecondensation of the sperm nuclear material or by parthenogenetic activation of the oocyte. Embryos originating from a single pronuclear zygote may be considered for transfer only if conventional insemination has been carried out and normally fertilized two

pronucleus zygotes are not available.[15] Tripronuclear zygotes may be as a result of dispermy or nonextrusion of 2nd polar body. They cleave fast, may develop into blastocysts. But should not be considered for transfer. Tetraploidy or more zygotes are almost 100% aneuploid.

CHROMOSOMAL ABNORMALITIES IN PREIMPLANTATION EMBRYOS

Analysis of cleavage stage embryos: Embryos cultured *in vitro* show various morphological features, which are very commonly used as "embryo selection criteria".

a. Morphologically normal embryos
b. Multinucleation in blastomeres
c. Cytoplasmic fragmentation
d. Dominant blastomere
e. Abnormal or delayed cell division
f. Embryo arrest.

1. *Karyotype analysis of morphologically normal embryos:* Reports have been published giving 20 to 40% rate of chromosomal abnormalities in normal appearing preimplantation embryos. Munne et al[16] report a 29% abnormality rate. Similar rates were reported for human blastocysts, although there have also been reports of almost 100% chromosomal mosaicism at the blastocyst stage.[17]

   The observations reported from these studies is complicated and there are disparity in results. From recent data it is becoming apparent that many factors could determine chromosomal aneuploidy rates. Also the timing and technique used for analysis could give variable results. There is a need to find improved techniques for chromosomal analysis of cleavage stage embryos.
2. *Chromosomal abnormalities in fragmented and multinucleated embryos*: Percentage of fragmentation has been associated with chromosomal abnormalities, mostly mosaicism.[18] Detected by FISH, embryos with 45 to 100% fragmentation showed 89% mosaics and 11% aneuploids, i.e. almost 100% abnormality rate. Where as 0 to 15% fragmentation showed 29% and 11% respectively.[19] Embryos with multinucleated blastomeres are normally associated with abnormal embryo development and/or dysmorphism. A recent study has reported that presence of multinucleated cells in viable embryos could indicate up to 74% chromosomal abnormality (extensive mosaicism and/or polyploidy).
3. *Chromosomal abnormalities and embryo development*: Majority (71%) of arrested embryo are chromosomaly abnormal. Dominant disorder being polyploidy followed by mosaicism and aneuploidy. 57% of slow developing embryos are abnormal showing aneuploidy (23%) in majority, followed by mosaisism (22%) and polyploidy (13%).[16]

Few external factors have been implicated in high rates of chromosome abnormalities in cleaving emblyos. Type of hormonal stimulation, left, temperature, water and air quality may influence chromosomal abnormalities (19%).

PROGRAMMED CELL DEATH AND EMBRYO WASTAGE

Programmed cell death is a finely coordinated set of events involving atleast 100 gene products that can either suppress or activate cellular self destruction.[20] The fate of a cell, i.e. Whether it lives, differentiates or dies is determined by the balance between cell death suppressor *vs* cell death inducers. Increasing evidence now indicates that cell fate is determined by out come of specific intracellular interactions between pro and antiapoptotic proteins, many of which are expressed during oocyte and preimplantation embryo development.

Recent data shows that the onset of apoptosis seems to be developmentally regulated in a stage specific manner, however underlying molecular mechanism are yet to be determined. Off course chromosomal abnormality are one cause of developmental arrest, fragmentation of oocytes and embryos. Activation of programmed cell death pathways play an important role in pre implantation embryo survival. Cell death triggers could be DNA damage, poor embryo metabolism, suboptimal culture conditions, etc.

Current concepts and advances in the understanding of the regulation of cell death gene expression during pre-emplantation embryo is beyond the scope of this chapter.

PROGRAMMED CELL DEATH AND MASSIVE OOCYTE LOSS IN THE HUMAN OVARIAN GERM CELL LINE

Primordial germ cells are the direct precursors of ova. They start proliferating by sixth gestational week reaching 6 to 7 million oogonia by 16 to 20 weeks. Loss of germ cells starts from this time onwards. A massive loss of oocytes (close to 4.5 million) occurs over the next 20 weeks. The new born female is born with 1 to 2 million oocytes per ovary, having lost 80% of her oocytes much before even attaining reproductive potential. This huge loss of oocytes in such a short time has been described as "mass cellular suicide".[21]

Functional life span of the female gonads is determined by the size and rate of depletion of the oocyte stock in the ovaries at birth. This is also described as the female biological clock – which is driven by a genetic program of cell death. This programmed cell death (apoptosis) claims up to 99.9% of mammalian germ cell line, which is ultimately responsible for

menopause, ovarian failure and infertility.[2] The study of germ cell death is still at its infancy. However, many interesting questions arise:

How and why does the female body create so many germ cells only to delete them?

Is it possible to prolong the lifespan of a female by manipulating oocyte depletion? Can this information be used therapeutically to treat infertility and aging process?

IMPLICATIONS FOR ART

Despite advances today IVF has a low implantation and high embryo wastage rates. Current studies put for the hypothesis that a large number of human oocytes and embryos are chromosomally abnormal (aneuploides) and possibly not capable of producing a healthy pregnancy. Also genes regulating programmed cell death claims a large number of gametes and zygotes which may be morphologically and chromosomally normal. Thus it is fair to presume that success rates greater than the maximal rates reported in spontaneous cycles will not be achieved unless the "aneuploid embryo" is eliminated from the selection process.

In both IVF and ICSI the most challenging and difficult step is to select the most competent embryo for transfer.

WHAT IS A "GOOD EMBRYO" (THE SEED)?

Selection of the most competent embryo is generally based on morphological criteria.

Morphological characteristics are however notoriously hard to describe, are often unambiguous, findings aften do not co-relate between early and late stages of development. More over, considerable inter-observer variability is not unusual.

Many scoring systems currently being used are crude using only a few characteristics like fragmentations, cell number, general appearance of blastomeres and that too only on the day of embryo transfer. Such traditional practices do not tap the full potential of morphological scoring. Careful observation and documentations should be a part of every IVF lab. The embryologist should consider information from all stages of development starting from the oocyte, zygote and embryo, by which, much better prediction can be achieved rather than only using day two morphology.

Oocyte morphology and embryonic development have been well corelated.[23,24] Many transcription factors (indicating upregulation of certain genes) have a polarized distribution in the oocyte. Oocytes with a dark, coarse or pitted cytoplasm are much more likely to be aneuploid. Abnormal cytoplasm in the oocyte is associated with poor development and should be considered when selecting embryos for transfer or freezing.[25]

Polar body morphology has been well corelated with subsequent blastocyst development and implantation. Polar body shape (round or ovoid), fragmentation, orientation and size of perivitallene space has been used by researchers to grade oocytes. Round or ovoid, unfragmented polar bodies with small perivitallene space was associated with better development potential.[25]

Scoring the *pronuclear (PN*) and nucleolus morphology for predicting the developmental potential of zygotes has been in use. Normally the two PNs appear within a short interval and rapidly migrate to the center of the cytoplasm. Nucleoli form and became polarized at adjacent poles of the apposition PNs. Asynchrony of nucleolar dynamics are associated with lower developmental potential. PN and nucleolar morphology seem to have strong corelation with blastocyst development and should be considered for embryo selection.[25]

Blastomere size, shape and number are a regular part of all embryo scoring systems. It is generally agreed that uneven irregular cleared and unequal blasto meres have poor potential. Multinucleated blastomeres should be assessed with multiple charts. They are highly likely to be chromosomely abnormal, especially if all blastomeres are multinucleated. If normal embryos are not available only then those with few and late multinucleation may be considered for embryotransfer.

Appearance of *zona pellucida* as an indicator of embryo quality has been described.[26,27] Embryos with uneven zona may make it easier to hatch and thus implant.

Embryo *cytoplasmic fragmentation* is commonly used for embryo scoring. Pattern and degree of fragmentation both are used according to the definition of Alikari et al.[28] Pattern comprises of:

a. Minimal volume in one blastomere
b. Localized fragments in PVS
c. Small fragments all over the embryo and
d. Large fragments resembling a whole blastomere. Degree of fragmentation is expressed in percentage and defined as the volume of the PVS and/or the cleavage cavity occupied by the enucleated cytoplasmic fragments.

"*Cleavage rate*" has now emerged as a major determinant of development.[25] Embryos which start cleaving early and are at four cell stage 42 hours postinsemination have a better developmental potential.

Thus it would appear that systematic observation, recording and analysis of morphological characteristics starting from the oocyte, increase significantly the ability to select good embryos.

Yet to improve current pregnancy rates we need newer techniques to identify the aneuploid embryos. Many of the morphologically normal embryos are aneuploid or chromosomaly abnormal.

ROLE OF PREIMPLANTATION GENETIC SCREENING (PGS) IN EMBRYO SELECTION

Preimplantation genetic diagnosis (PGD) was first introduced in 1990. Today PGD has became a clinically established procedure in ART. Initially PGD was being performed for monogenic disorders like X-linked disorder, cystic fibrosis, Tay Sachs disease, etc. with the development of FISH (fluorescent in situ hybridization) PGD is now being used for aneuploidy detection for a number of clinically significant chromosomes.

Genetic material for PGD is derived from three possible source:

a. Polar bodies
b. Blastomeres from early cleaving embryos (D3,6 to 10 cell stage)
c. Trophectoderm cells from blastocysts.

PGDS IS BEING USED GENERALLY FOR THE FOLLOWING INDICATIONS

- Advanced maternal age
- Repeated IVF failure
- Repeated miscarriage
- TESE-ICSI.

Currently PGD can be used for about 50 monogenic disorders chromosomal aneuploidy screening for chromosome number X, Y, 13, 14, 15, 16, 18, 21, 22.

Although preimplatation genetic screening for aneuploidy is being used more and more often for selecting embryos for transfer, its effectiveness is still unclear.

Cochrane database review[29] reported the latest analysis results in 2006. The authors concluded that there is insufficient data to determine whether PGS is an effective intervention in IVF/ICSI to improve live birth rates.

Available data on PGS for advanced maternal age showed no difference in live birth rate and ongoing pregnancy rates. More properly conducted randomized controlled trials are needed.

ROLE OF METABOLOMIC PROFILING OF THE EMBRYO

Today there are no biological criteria or objective analytical methods to assist in the process of embryo selection. Morphological criteria is the primary determinant of embryo viability unfortunately morphological analysis does not equate to biological functionality.

Embryologists have to depend on this grading in the absence of alternative methods. New technology that is capable of selecting only functionally competent oocytes and embryos can lead to markedly improved success rates in IVF program. PGS and genomic testing have limitations. It is a labor intensive procedure, lacks sensitivity and specificity, is considered controversial, since it requires single cell biopsy at the early embryo.

PGD is still regarded as an experimental procedure by FDA and ASRM.

Metabolomic profiling is new technology used in the laboratory. Semen, follicular fluid, culture media of the embryo during culture and prior to transfer are specimens that are usually discarded, can be analyzed for multiple biomarkers of oxidative stress. This is a noninvasive test, performed on normally discarded culture media. An embryo which is healthy and likely to cause pregnancy has a different metabolism than a non-healthy embryo and these difference can be picked up from the fluid in which the embryo is cultured. The embryo literally eats and breath into it.

Quantification may be possible using spectroscopic analysis and advanced bioinformatics. The technology of metabolomic profiling of the embryo is now commercially available in the USA from a company called molecular biometrics. If proven to be accurate and reliable this break through technology could provide IVF practitioners a new tool for accurate selection of embryo.

THE EMBRYO OR THE ENDOMETRIUM (SEED OR THE SOIL)

Role of the endometrium in establishing a pregnancy is now becoming very clear.

The implantation window is the self-limiting period of endometrial receptivity during which the endometrium opens up to receive the embryo. Various morphological and biochemical markers are being proposed to define the implantation window. Recently genes regulating implantation window are being recognized. It is obvious that extreme fine tuning or perfect co-ordination of ovulation induction, luteal support and timing of embryo transfer is of prime importance for successful nidation.

However, within the "implantation window" it will have to be the biological competence of "the embryo" which will determine pregnancy.

It has became clear that right from the time of fetal germ cell production to live births - human reproduction is an extremely wasteful exercise both in nature and also in assisted reproduction. A process of continuous reduction or selection against aneuploid embryos starts right from the time of fertilization.

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