# PRE - IMPLANTATION GENETIC SCREENING

As newer technologies emerge in the field of assisted reproduction, it may soon boil down to a statement made by a single cell called the blastomere. The technique of embryo biopsy is not new and has been a tool for more than a decade to diagnose inheritable disorders (PGD) or chromosomal screening (PGS) by FISH techniques. But, the need to use this tool as a screening procedure for embryo viability in terms of chromosomal numbers and integrity is being promoted intensely. Recently made popular in the Indian ART scenario, by the IVI group of clinics (Spain) and spear headed by IVIOMICS (Delhi), this emerging tool, using the new array CGH technology, seems to be gaining momentum as one of the most logical steps towards transfer of chromosomally viable embryos, thereby reducing the number of embryos for transfer, curtailing multiple pregnancies, and also enhancing pregnancy rates.

It’s almost given to understanding that if 100 grade-1 embryos were to be examined, almost 60 would be chromosomally incompetent which explains why success rates in ART stagnate or vary between a 50-65%, despite several private clinics claiming a startling 80-100% success rates or money back guaranteed schemes !

There are several factors controlling the journey of the gametes, right through extraction from its natural environment, through a laboratory process and thereafter into the womb. One of these is epigenetics, whereby there are changes in gene expressions which may not be related or do not share a cause-effect relationship with inherited genetic predispositions.

Embryo biopsy is done at different stages of embryo division, each giving its own piece of vital information on gametes. For example, polar body biopsy entails study of either first or second polar body or if effectively performed between 8-14 hours post fertilization, a study of both can be done (1). Now, studying only the first polar body may give information on the maternal oocyte but not the paternal contribution. Hence it is more suited for diagnosing monogenic disorders. In many others like recessive conditions a polar body biopsy may effectively rule out affection but may not rule out a carrier state thereby allowing for a bias in embryo selection. Also it has limited use in detecting post-meiotic aneuploidies by a conventional FISH or CGH array (2,3).

The cleavage stage biopsy is more informative but also more detrimental to embryo viability post biopsy. It is performed on day 3 embryos typically when there are 8-10 cells and not yet compacted. The concept behind timing a biopsy at a cleavage stage is that performing the procedure too early may lead to missing critical errors. Also the natural mechanism to self correct embryos occurs in the interim period and hence leads to an incorrect assigning of aneuploidy to actually viable embryos. Studies have shown that aneuploidies may not be limited to non dysjunction in meiosis 1 as speculated but in fact could occur in meiosis 2 and paternal meiosis.

To analyze the effect of epigenetics as an independent contributor to aberrations would be almost impossible and hence the use of PGS is almost justified in terms of screening. The current need is to enhance clinical pregnancy rates and thereby carry home baby rates by scrutinizing multiple factors that affect life in vitro namely the quality of gametes, culture medium and culture conditions.

Several techniques have been analyzed, updated or discarded to reach the stipulated current success rates for fresh cycles as opposed to a meager 10-15% when IVF was first established in practice . The variables that is amenable to research, modification, or upgradation have always been mainly the media and laboratory techniques.

The gametes are otherwise dependent on existing in vivo conditions which are intricately related to either genetic predisposing factors or acquired pathological conditions like anovulation with hormonal imbalance, endometriosis or severe pelvic inflammatory disease.

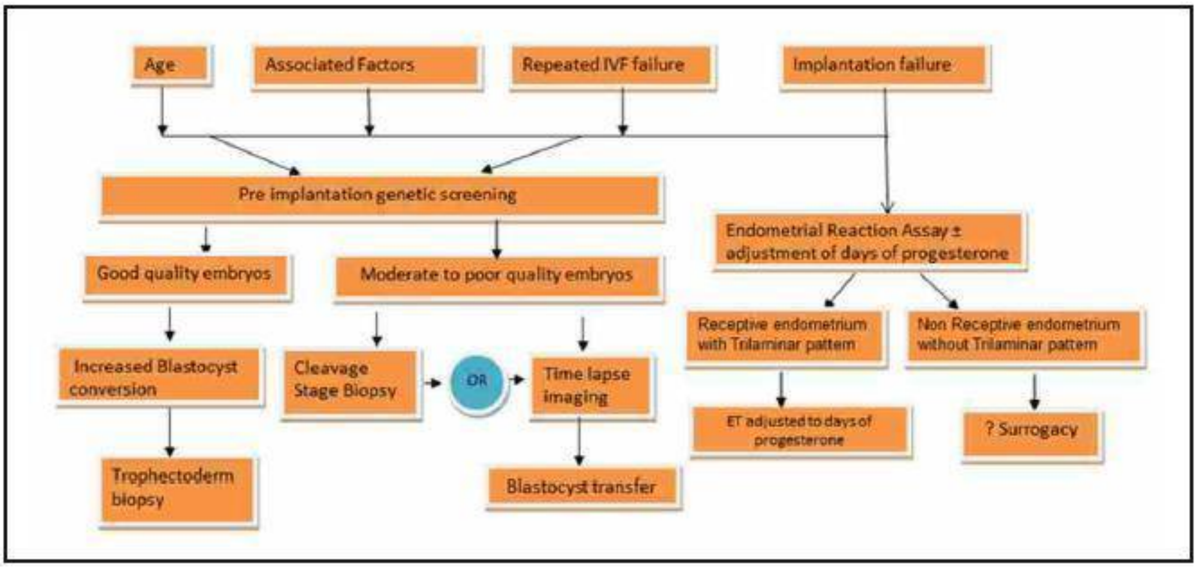
Age related aneuploidy is an independent risk and contributes to higher post transfer failure rates. Trending towards more number of women trying to conceive at an advanced age, these figures are likely to escalate and pose a dilemma for fertility experts. It then becomes essential to achieve a higher clinical pregnancy rate and include technical screening procedures in the form of embryo biopsies to rule out aneuploidies.

Hence it was inferred that biopsies performed at different stages of embryonic development could detect errors that occur at different timings. So this by itself is evidence that it's better to time a biopsy at a later stage like the blastocyst than limiting to polar body or cleavage stage (4,5,6).

However, other authors like Dr. Pere Mir, have shown a highly accurate and reliable analysis in cleavage stage biopsies using array CGH technology. These results are comparable with D5 embryo biopsies. In fact, they are applying D3 embryo biopsies for PGS, improving pregnancy rates and decreasing miscarriage rates too (7).

Ofcourse no method is completely foolproof in detecting aneuploidies since mosaicism is another entity that occurs in 29% of all embryos (2). So although chromosomally normal embryos are transferred, the ensuing pregnancy needs to be screened as per norms by noninvasive testing for aneuploidy such as NACE (non-invasive test for chromosomal examination) and by level 2 anatomical survey of fetus by ultrasound. NACE test promises a 99.9% specificity thereby almost ruling out the need for amniocentesis or chorionic villous biopsy.

It is interesting to note that by and large cleavage stage biopsy has been regarded as more harmful to embryos demonstrating a lowered implantation rate as opposed to trophectoderm biopsy.

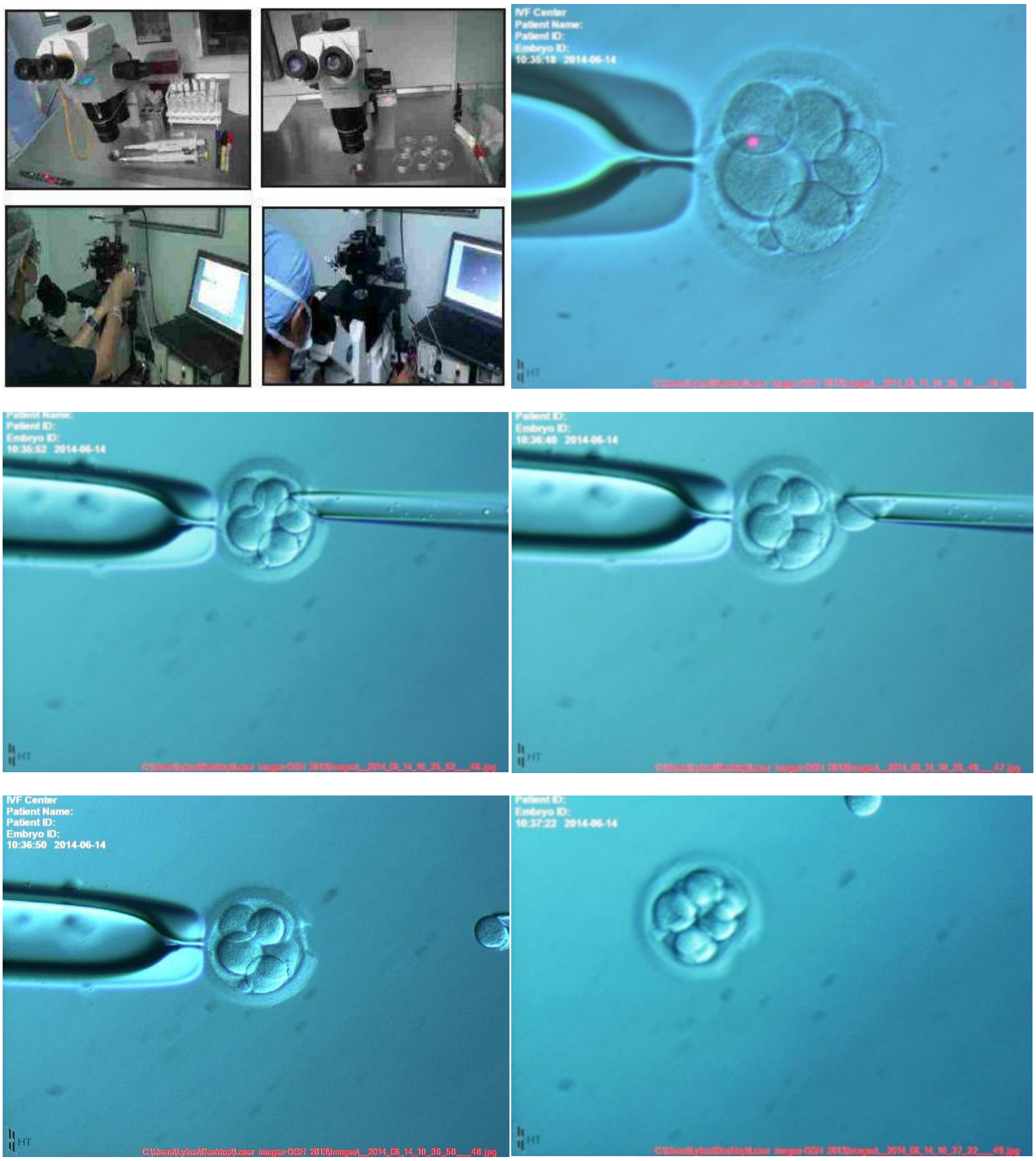



However it has been suggested that for PGD involving detection of balanced translocations it is more prudent to do a cleavage stage biopsy owing to better standards of fixation method for blastomeres for FISH in comparison to trophectoderm. The opposite is the case for detection of single gene defects which need a minimum of 5-7 cells that only a trophectoderm can provide. In conclusion, current studies have shed enough information to specifically assign advantages and disadvantages to timing of biopsy and what it may yield.

It is understood from all the above quoted studies that doing a trophectoderm biopsy and vitrifying the resultant biopsied blasts and transfer in a thawed cycle yields best results (4,8).

More studies are needed to elaborate on the application of the correct diagnostic technique for specific genetic conditions and also on the corresponding timing of biopsy.

Nevertheless, above all; one needs to have an understanding of the following before embarking on a biopsy trail:



- Choice of cases that may best benefit by this procedure
- An in depth knowledge of genetics and embryo dynamics in a laboratory environment
- A comprehensive knowledge of the diagnostic tools such as FISH, PCR and CGH
- The norms that need to be followed to have an in-house genetic lab or a referral lab

Dr.Marcos Meseguer, Senior Embryologist, IVI, Valencia, Spain shared his comments for our queries regarding PGS :

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