INTRODUCTION

Infertility is failure to achieve a successful pregnancy after 12 months of regular unprotected intercourse. Earlier evaluation and treatment may be justified based on medical history and physical findings and is warranted after 6 months for women over 35 years of age. (1)

Studies revealed that at least one-quarter of all couples experience unexpected delays in achieving their desired family size, although only one half of these may seek treatment.(2) In recent years, there has been advances in reproductive medicine technologies that has helped in reducing both the stigma of infertility and the reluctance of couples to seek advice.(3,4)

Infertility as a complaint brought to medical attention is also on the increase. (5) Among factors responsible for this rise is advanced maternal age. The mean age of mothers at first birth is now approximately 29.5 years, as ooposed to 25 years two decades ago. (6) At age of 35, a woman has approximately 25,000 oocytes left in her ovarian reserve just over 1% of the number she was born with. There is data that suggests that the process of atresia is accelerated after the age of 35. (7)

Another important change that seems to be occurring in several countries is decline in male fertility. Enviromental pollution arising from oestrogenic industrial waste is thought to be the most likely cause. (8) Although the decline is most noticeable after 55 years of age, even men older than 35 have been shown to have one-half chance of achieving a pregnancy compared with men younger than 25. (9,10)

Indications of ART / IVF:

Assisted conception is indicated if the prognosis for tubal surgery is considered too poor or if conception has failed to occur within 6-12 months of tubal surgery. Consideration should be given to discussion of pre-treatment tubal sterilization, to minimize the risk of ectopic pregnancy after treatment, although in practice this discussion is seldom performed. (11)

In vitro fertilization (IVF) is indicated for moderate to severe endometriosis if conception has failed to occur within 12 months of ablative laparoscopic surgery, depending , of course, on age and other fertility factors. Consideration also should be given to pre-treatment management of endometriotic cysts. (12)

When there is severe sperm dysfunction, azoospermia, severe oligoasthenoteratozoospermia (OAT), sperm preparation providing an inadequate specimen for superovulation with intrauterine insemination (IUI) or if conception has failed to occur after three or four cycles of superovulation/IUI, IVF should be offered. Micromanipulation techniques such as intracytoplasmic sperm injection (ICSI) may be required to achieve fertilization if there is severe male factor infertility. [13,14]

Chronic anovulation is a common cause of infertility. Most anovulatory women have irregular menstrual cycles and normal serum FSH concentration. Depending on the criteria used, polycystic ovary syndrome (PCOS) is diagnosed in approximately 60-70% of these women. [15] IVF should be avoided as first line therapy in these patients, except for subgroups with a poor prognosis. Those women who may benefit from IVF as first line therapy can be identified by older age, longer duration of infertility, and higher insulin:glucose ratio. [16]

Women with malignancy or other illnesses who require treatments that may pose a negative effect to future fertility (i.e., chemotherapy, radiation therapy) may be candidates for urgent IVF and cryopreservation of embryos before the initiation of the treatment, if time and health allow. [17]

IVF can be used to generate embryos from which single cells can be obtained for genetic studies or simple sexing in cases where there are life-threatening sex-linked Genetic disease. Each cell in the pre-embryo is pluripotent, so a single cell can be removed up to blastocyst stage without damaging the development of the fetus. Using this technique, it is possible to transfer only healthy embryos therefor avoiding the risks of antenatal testing (e.g. chorion villus biopsy, amniocentesis). [18]

Complications of IVF:

The continued progress in the field of Reproductive Medicine has resulted in higher implantation rates, which also has provided a further motive to reduce the number of transferred embryos without affecting pregnancy rates. [19]

Infertile women are at a greater risk of having a baby born with a congenital malformation whether they conceive spontaneously or following treatment. [20] Some authors counsel couples that the incidence of birth defects in naturally conceived pregnancies is 2% to 3% and may be increased to 3% to 4% in babies following IVF treatment. [21]

Approximately 20% to 30 % of IVF patients develop mild OHSS and 1% to 2% develop symptoms compatible with severe OHSS. [22]

There is concern that the use of fertility medications could heighten the risk of ovarian cancer. While it is a fact that infertile women are at a greater risk of developing ovarian cancer, this risk is not heightened with the use of fertility medications. [23]

Assisted Reproductive Technologies:

Assisted reproductive technology includes all fertility treatments in which oocytes and/or sperms are manipulated in vitro. Assisted reproductive technologies procedures typically involve stimulating the growth of multiple ovarian follicles, aspirating follicles from a woman's ovaries, and then combining them with sperm in the laboratory. [24] In cases of gamete intra fallopian transfer (GIFT), a laparoscopy is performed, and sperm and unfertilized eggs are placed into fallopian tube immediately after egg retrieval. [25] Zygote intra fallopian transfer (ZIFT) is the laparoscopic transfer of fertilized eggs (zygotes) to the fallopian tube, usually 18-24 h after insemination. [26] Tubal embryo transfer (TET) is performed after 48 h when the pre-embryo has cleaved. [27] IVF is better than ZIFT (or related techniques) because of the avoidance of laparoscopy, which may carry significant morbidity and mortality. [28]

The intra cytoplasmic sperm injection (ICSI) Procedures entails the injection of a single spermatozoon directly into the cytoplasm of the oocyte, thus bypassing the zona-pellucida (ZP) and the oolemma. [29] The ability of ICSI to achieve higher fertilization and pregnancy rates regardless of sperm characteristics makes it the most powerful micromanipulation procedure to treat male factor infertility. [30]

The therapeutic possibilities of ICSI range from cases in which, after sperm selection, the spermatozoa show poor progressive motility, to its application to azoospermic men where spermatozoa are microsurgically retrieved from the epididymis or the testis. [31] Retrieval of a low number of oocytes represents a further indication for this procedure, because only after cumulus cell removal is it possible to identify the oocytes that have extruded the first polar body and then inseminate them accordingly. [32]

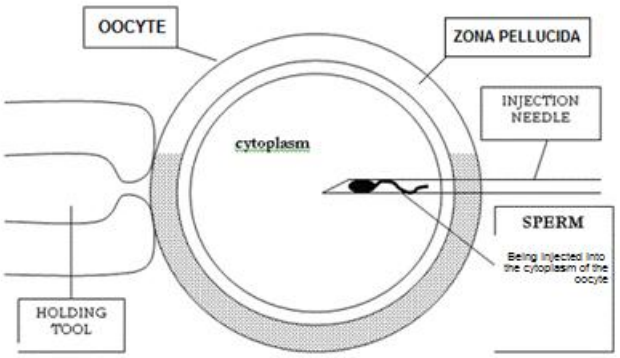

Figure (1): Intracytoplasmic sperm injection. [33]

Embryo Scoring :-

Non-invasive methods of embryo evaluation help assess embryos without damage. Until recently they were only of research value. However, since rapid advances in the field of assisted reproductive techniques (ART) took place they have gained more value in practice. All ART specialists, particularly embryologists who handle human germ cells and embryos, are now obliged to be familiar with precise, non-traumatic techniques of embryo evaluation. Moreover, due to ethical and legislative reasons the protocols of human embryo treatment are very restricted. Precise examination of embryos on particular days following in vitro fertilization (IVF) facilitates selection of the most potent embryos associated with highest pregnancy rates. [34,35]

Such management improves success rates of IVF programs. Also, selection of the best embryos for transfer reduces the number of transferred embryos and subsequently the incidence of multifetal pregnancies. The methods of embryo examination have been changing dramatically for the last 20 years. The routine embryo assessment has been supplemented with evaluation of numerous morphological features that enable prediction of developmental potential of one particular embryo and subsequently a highest chance of pregnancy among infertile couples. Nowadays, transfer of a single embryo at day 3 following IVF or intracytoplasmic sperm injection (ICSI) is related with 10-30%, and a day 5 blastocysts transfer with 40-60% implantation rates[36,37,38,39] Obviously, such an improvement in success rates was related not only to the improved embryo examination and selection, but also to enhancement of ovarian stimulation, the techniques of oocyte insemination, micromanipulation and embryo transfer, pre-implantation genetic diagnosis, and, finally the composition of embryo culture.

Non-invasive embryo examination is based on simple methods of observation focused on morphology and dynamics of embryo development. The analysis is performed under contrast-phase microscope with Hoffmann modulation contrast (HMC) or difference-interference contrast (DIC), enabling more precise assessment without fixing and staining. Initially, the embryologists assessed only several parameters corresponding to embryo quality. Increasing number of retro- as well as prospective studies determined a group of morphological features useful in predicting embryo quality. At present several classifications concerning many different criteria (embryo scores) are utilized in ART [40,41,42]

Since 1986 many different embryo scoring methods have been described. [43] The main features considered in embryo scoring systems include blastomere number, blastomere size, shape, equality, appearance of cytoplasm and degree of fragmentation. [44,45] These factors have been combined in complex numerous ways, to produce embryo scoring systems aiming at identifying embryos that would potentially result in a pregnancy.[46-49]

Embryos on day 3 should have around eight blastomeres that are equal in size and show no multinucleation, the cytoplasm of each blastomere should be pale and clear with some granulation, and fragmentation should be < 20%. It has been reported by several authors that too slow or too rapid embryo growth has negative impact on implantation rate. [50-54] In all these studies, the transfer of 8-cell stage embryos on day 3 resulted in a significantly higher implantation rate as compared with other cell stage embryos. [55,56] A correlation between embryo timely cell division and chromosomal constitution has also been reported. Slow cleavage and cell division arrest, as well as fast cleavage, has been related to embryo chromosomal abnormalities (such as mosaicism, polyspermia, and aneuploidies). [57,58]

The presence of anuclear small cellular fragments (fragmentation) is the norm in in vitro cultured human embryos. The degree of fragmentation varies from 5% or 10% to 100%, the fragments may be either localized or scattered and initially appear from the first mitotic division on. However, when the degree of fragmentation exceeds 10% of embryonic volume it can have a negative effect on development. Large fragments that are found in the cells of a 2- or 4-cell embryo are due to the electro-density of the mitochondria in each fragment. Mitochondria are more electro-dense in later stages of development than in earlier stages. When large fragments are released in the early stages of

development the embryo loses important organelles such as the mitochondria. When the fragmentation occurs the section of the cell that is left with the nucleus, arrest of division can take place due to the loss of important organelles. [59] Fragmentation has also been attributed to spermatozoa. [60] Spermatozoa were implicated as a cause of fragmentation through DNA damage occurring before fertilization, [61,62] whereas others have reported that metabolic disturbances in the oocyte may play a role. [63] Fragmentation percentage has also been known to be associated with chromosomal abnormalities [64,65,66] Both apoptotic and necrotic processes have been suggested as causes of blastomere fragmentation in human embryos. [67]

According to Van Royen et al., a top quality embryo will have no multinucleated blastomeres; eight blastomeres on day 3; and < 20% fragments. [68] On the other hand small and scattered or localized fragments did not significantly impact implantation potential [69,70] Fragments can be removed by microsurgical techniques, which improve the developmental potential because the spatial relationship between the blastomeres is restored. Frozen embryos also show fragmentation and are detrimental to the embryo. Elliott et al. showed that by removing lysed cells in mouse embryos the developmental potential was restored to that of the control. [71]

Uneven cellular cleavage is an indicator of poor quality and slow development; this can lead to an uneven distribution of genetic material. Embryos with unevenly sized blastomeres displayed a much higher multinucleation and aneuploidy rate resulting in a lower implantation rate.[72]

Multinucleation is the presence of two or more nuclei inside a single blastomere. Multinucleated blastomeres can be observed more easily on day 2. However, multinucleation is only visible for those blastomeres that are at the interphase stage. [73] Multinucleation results from a failure in cytokinesis during early cleavage and also has a detrimental effect on developing embryos. [74] Observing multinucleation in day 2 embryos is a simple, non-invasive detection and therefore should be added to the embryo grading system. Day 2 embryos have a larger dimension and less overlap than day 3 embryos, therefore day 2 is the best day to assess multinucleation. [75] Van Royen et al. also found that multinucleation is correlated with early cleavage. As mentioned earlier, early cleavage is associated with embryo quality. Day 2 embryos that had an optimal cleavage pattern exhibited a lower number of multinucleated blastomeres. Embryos not only with a lower number but also with a

higher number of blastomeres on day 2 showed significant increase in multinucleation rate. Normal day 3 embryos, those with eight blastomeres, showed the lowest frequency of multinucleation. Van Royen et al. also found that a shorter than average stimulation, higher than average number of oocytes retrieved, and a higher than average FSH dose for stimulation was related to a higher incidence of mutinucleation. [75]

When the cytoplasm of a developing embryo is pale and clear or finely granular in appearance it is considered normal. Unusual colour or granular texture is considered abnormal, as well as vacuoles or dense bodies. [76] There is no correlation established between the cytoplasm of blastomeres and embryo morphology in terms of regularity and fragmentation rate and blastomere number. [77]

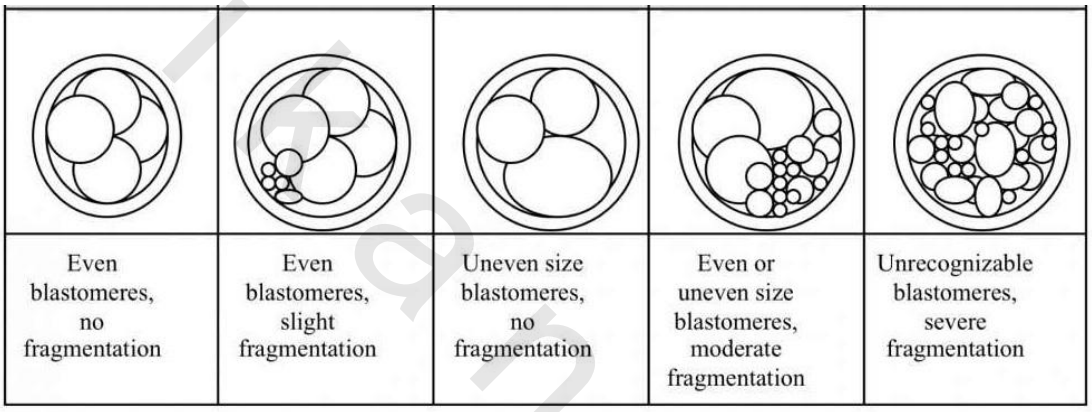

Figure (2): Developing embryos of different quality.

The first reports of embryo scoring [78] have concentrated on embryo growth rates with attention to its morphology. In 1987 Puissant et al., [79] suggested that consideration of an embryo scoring system including cell number, blastomere size and shape, degree of fragmentation is essential to identify high quality embryos that would lead to pregnancy. This idea was followed by Steer et al. [80] who proposed a mathematical scoring known as cumulative embryo score (CES), created by the summation of the individual scores of all embryos transferred. It is based on five criteria; the number of blastomeres or cells observed in relation to number of hours post-ICSI, the uniformity of cells in terms of size and shape, the clarity of the cytoplasm in terms of presence or absence of granulation, as well as the degree of anuclear fragmentation. The best embryos obtained a score of five, while the minimum cut-off score for embryos deemed suitable for transfer is three. (81,82)

Cumulative Embryo Score (CES) is a clinically useful tool

reflecting embryo developmental potential, which will enable the selection of the optimal number of embryos to transfer in order to achieve the maximum pregnancy rate with the lowest possible incidence of high order multiple pregnancies. Such scoring system would have the definite practical advantages of being easily performed and interpreted with little room for inter-observer variation. [83,84]

However further research and effort are needed in order to reach the optimal scoring system having the highest correlation with both pregnancy and implantation rates, so as to reach the final goal of a single fruitful embryo transfer.