Course Name :An Overview on Maternal Health Antenatal, Intranatal and Postnatal Care

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Fetal Imaging and Amniotic Fluid Study

Good morning students. Hope you are all doing good. I welcome you all to the session for the NPTEL online certified course on the topic and overview on maternal health, the antenatal, intranatal and postnatal care. I am Dr. Barnali Ghosh, an obstetrician and gynecologist working at B.C.Roy Medical College and Medical Research Center, IIT, Kharagpur. Today we are going to discuss regarding the pre-implantation genetic testing, right.

So, the concepts covered in today's class are the pre-implantation genetic screening, preimplantation genetic diagnosis, discussion regarding embryo biopsy and its various types, the methods of the genetic tests which are done in the embryo biopsy and lastly the indications for pre-implantation genetic tests, right. So, the concepts or the keywords for today's class are as follows. So, coming to the discussion proper prenatal genetic screening. So, prenatal genetic screening actually you can segregate it into prenatal meaning this is pre-implantation actually, right.

So, we are discussing we have already discussed the prenatal genetic screening methods and the diagnostic tests. In the previous classes what is you know to note is that the mother is already pregnant, she has conceived and it is a case of ongoing pregnancy when the mother has to the antenatal clinic to get screened for any anomaly within the fetus. But today we will be discussing regarding the pre-implantation genetic screening. So, before implantation, before implantation we go for genetic screening of the embryo and what is the advantage of this test? The advantage is that in this case if it is anomaly if any anomaly is present then the embryo is not implanted in the mother's uterus. So, the embryo is not implanted.

So, there is no risk of pregnancy termination where in the previous classes where the mother has already become pregnant and then you go for the diagnostic or the screening test and if the fetus comes out to have certain anomaly then you need to go for termination of pregnancy. So, in this case from beforehand we know that yes this embryo is anomalous and we are not going to put this embryo inside the mother's womb in the process of embryo transfer. So, this is possible only in case of ART pregnancies right. So, only in ART pregnancies or IVF in vitro

fertilization pregnancies we can do the pre-implantation genetic screening and to note is pre-implantation genetic test it has two parts pre-implantation genetic screening and pre-implantation genetic diagnosis right.

So, now coming to Embryo biopsy what is the most you know the concept behind? Embryo biopsy meaning that you take a biopsy of the embryo take some cells from the embryo and you see the detailed genetic structure of the embryo.

So, for the genetic makeup the genetic makeup will will mimic the whole fetus the whole fetal genetic makeup. So, the Embryo biopsy that embryo will actually grow grow grow and form the whole fetus. Once I go it will first divide by mitosis it will go for the cleavage stage and will form morula, it will form blastocyst, it will form you know hatching blastocyst, it will get implanted in the uterine wall and then it will form the three trilaminar structure that is the ectoderm endoderm and mesoderm and that will form the whole of the fetus. So, Embryo when it is taken and few embryonic cells are taken for fetal genetic study it actually depicts the total genetic makeup of the whole fetus right. So, that is one point and number two it should be done in such a way so as to least cause least damage to the fetus.

So, even if a Embryo some part of the cells are taken off, but that should be done in such a way that there is least detrimental effect on the fetal growth and subsequent development of the fetus from that Embryo. So, these two points need to be taken into account. Now, what is the principle there are various principles number one is you know that Embryo is actually Embryo is actually from the zygote, zygote dividing going to cleavage stage and dividing into morula right that is on day 4, 16 cell stage and this morula will be forming the blastocyst on day 5 and this will ultimately form the whole fetus. Whole fetal tissue. So, when you take cells from the morula or you take cells from the blastocyst they will actually you know depict the full fetal DNA right and number two sometimes we go for polar body biopsy.

Now, what is this polar body biopsy? Polar body biopsy will only be for the maternal genome right this will only be for the maternal genome study. If mother have any genetic defect, if mother have any mutation, if mother have any translocation, any type of you know structural rearrangement of the chromosome then we can detect from the polar body biopsy. So, that was regarding Embryo biopsy. Now, types of Embryo biopsy number one is the polar body biopsy which I have already discussed and it is sequential right both the first and the second polar body are biopsied are studied right and this is only going to say regarding the maternal genetic makeup and any abnormality present within the mother. Next is a blastomere biopsy.

Blastomere is the day 3 cleavage stage of the Embryo and this will ultimately form the whole fetus. It will form the inner cell mass which will form the fetus and the outer cell mass will form the placenta. So, day 3 cleavage Embryo biopsy or blastomere biopsy will depict the whole fetal genetic makeup of the offspring right. And then lastly is the blastocyst. Blastocyst formation is

on day 3 where the morula stage the compact cell stage will undergo fluid accumulation and this is called as the blastocoele.

This is the blastocoele, these are the outer cell mass or the trophoectodermal layer. So, this we have read in the implantation class right. So, Embryology this is related to Embryology and these are the inner cell mass which will actually form the fetus. Now, blastocyst biopsy what we do we take the trophoectodermal outer trophectodermal cell for biopsy. So, we can go for the trophoectodermal cell biopsy and sometimes we also take the blastocoel fluid for study because this blastocoele fluid will also contain fetal DNA you know floating in the fluid.

So, with that fetal DNA we also can go for genetic study. So, this is the picture of polar body biopsy this is the you know stabilizing pipette right it stabilizes the zygote. So, this you can see this is the first polar body this is the first polar body and this is the human oocyte right. So, you can go for the first polar body biopsy and the second picture this is the second polar body why because see here this is the male and the female pronuclei. So, this is the zygote right.

So, first polar body or second polar body can be taken for biopsy we fix the cell with the pipette and then with the know this is the micro pipette, micro pipette with the micro pipette with formation of negative pressure we suck the polar body and we take out the polar body and then we examine it under microscope and also go for the different genetic tests right. So, this was the first and the second polar body enclosed in zona pellucida. So, this is the zona pellucida this is the zygote with the male and female pronuclei and here the micro pipette will pierce the zona pellucida and it will suck the first polar body and the second polar body under negative pressure right. So, that was polar body biopsy now coming to cleavage stage day 3 embryo biopsy or blastomere biopsy where you see that this is the stage of morula or day 3 stage where you have the zona pellucida with the cell in mitotic division you know in the cleavage stage. Here also with the pipette this stabilizes the cell and with the micropipette we go inside we break the zona pellucida.

Zona pellucida is breached how it can be breached by laser, it can be breached by mechanical pressure or it can be breached by chemical methods and then we suck under negative pressure some of the cells. Number of blastomeres taken for biopsy is how many only 1 to 2 right. So, 1 is sufficient. So, this is the blastomere only 1 blastomere is sufficient to go for the total genetic assay of the fetus right because each cell is totipotent pluripotent each cell is capable of forming a whole of the fetus in this stage right. So, 1 is sufficient for assay and a maximum we can take 2 and then we go for assay and if it is normal then we implant the embryo inside the mother's womb that is we go for embryo transfer.

And lastly in case of blastocyst biopsy here you see this is the zona pellucida and this is the hatching blastocyst right. So, this is the hatching blastocyst this is the inner cell mass, this is the

inner cell mass, this is the outer trophoectodermal cell mass or outer cell mass and in the hatching this is the outer cell mass. And in the micro pipette here also we suck under negative pressure the outer trophoectodermal layer and this trophoectodermal layer with laser with laser we try to you know break the continuity with the blastocyst. And here it has broken see this laser this point here it has broken and this part is taken for genetic test right it will now go for various genetic tests. Before that we have to amplify the fetal genetic material by polymerase chain reaction or PCR.

So, coming to the pre-implantation genetic testing I have told there are 2 that is the screening test and the diagnostic test. Whatever test you do you first have to amplify the fetal DNA by PCR or polymerase chain reaction and on that amplified fetal DNA you will carry out the various tests number 1 the karyotyping to note the number of chromosomes right any case of aneuploidy, any case of trisomy, any case of monosomy can be detected any triploidy right can be detected. Number 2 is fluorescent in situ fluorescent in situ hybridization these are done to note the various alleles right the various alleles and you know particularly if we are no certain disease is present in the parent right particularly for that allele we use a antibody which is fluorescent act and if that allele is present in the fetal gene or fetal DNA then we say that yes the fetus is also affected. And number 3 are the array based method which are of different types number 1 is the comparative genomic hybridization, number 2 it can be single nucleotide polymorphism microarray and it can be next generation gene sequencing. So, these are the different array based genetic tests that can be performed and these also help to detect point mutation or single gene disorders right like cystic fibrosis, your Tay sachs disease right your your Huntington's disease these are you know single gene disorder or point mutation which can be detected by array based methods.

Now, coming to the types of prenatal genetic testing first is PGT-A, A meaning test for aneuploidy. So that means, any defect or change in chromosome number, chromosome number it will include all the aneuploidies, it will include all the trisomies, triploidies, monosomies. Then is PGT-M, M is monogenic disorder which I was telling like the point mutation or the single gene disorder as in case of cystic fibrosis as in case of BRCA1 and 2 mutation as in case of Tay sachs disease. So, all these are monogenic disease and it comes under the heading of PGT M and number 3 is for structural rearrangement. That means, if there is any say inversion or translocation right or say deletion, deletion will not come here inversion any you know structural rearrangement within the chromosome.

So, that is under the heading of PGT-SR. So, this is actually prenatal genetic test and these two will come under prenatal genetic diagnosis right. So, that was all regarding the theory part on pre implantation genetic testing. We go for embryo biopsy which can be polar body biopsy, but polar body biopsy will not go will not give an idea of the whole fetus, it gives an idea only of the mother or the human oocyte genetic makeup and nowadays it is not done because it is less

informative. Number 2 was the blastomere biopsy which is on day 3, but the number of cells are less 1 or 2 blastomeres can be obtained in blastomere biopsy.

So, it needs to be amplified further and you know sometimes we may cause some untoward damage to the embryo. Even if the embryo was normal during blastomere biopsy we may damage the other cells which are present within the cleavage stage within the zona pellucida and it can lead to the damage of the embryo. So, that was a drawback of blastomere biopsy and on day 5 we can go for the blastocyst biopsy where we get the trophoectodermal cells and how many cells it is as much as 5 to 10 cells, 5 to 10 tropho ectodermal cells can be you know can be extracted by the pipette micro pipette under negative suction and can be under and can be put for the genetic test. So, for tropho ectoderm biopsy what are the advantage we get a large amount of cell, we get more genetic material and also as the trophoectoderm is the outer cell mass which forms the placenta. So, the invasiveness is less the inner cell mass is not affected and that will have less deleterious effect on the embryo and it has been seen that these tropho ectoderm biopsy it is associated with no difference in implantation in the biopsies embryo and the non biopsy embryo.

So, it is less deleterious for the embryo itself. So, that was regarding embryo biopsy and then we did have a discussion regarding the different tests which are done PG, no your PGT, PGT meaning prenatal genetic testing. So, for structural rearrangement it is PGTSR, PGTM or PGTA right. Now, coming to some of the questions relating to this say number 1 question it is telling that as a couple who is under the risk of transmitting a genetic disease to their offspring. So, the couple have a genetic disease the mother or the father any one of the parent have a genetic disease and there is a risk of transmitting the genetic disease to their offspring.

So, now what can be offered to the couple so, as to ascertain that they transfer a disease free embryo. So, for these for these type of couple where there is a chance of transmitting a genetic disease to the offspring we go for prenatal genetic diagnostic testing right. Next is prenatal genetic screening is offered to couples who experience who experience multiple miscarriage recurrent implantation failure advanced age and all of the above. So, if there is history of multiple miscarriage you know there are previous 3 or 4 miscarriages in the first trimester more than equal to 3 in first trimester miscarriage. So, that will hint towards certain some type of chromosomal abnormality in the fetus which can be due to certain you know undiagnosed translocation or point mutation in the parent.

So, they need to go for prenatal genetic screening. Number 2 is recurrent implantation failure. So, in IVF pregnancy every time you go for the embryo transfer you put the embryo you see the endometrium is very receptive it is of you know the optimum thickness, but still the embryo is not getting implanted there is recurrent implantation failure that will also hint towards chromosomal abnormality of the fetus. Number 3 is advanced age. As the age of the mother

increases the more there is more chance of anomalous babies.

So, number 4 is all of the above. So, for prenatal genetic screening you should go for all of the above right. Next is cryopreservation of biopsied embryo play a significant role in aiding sufficient time to perform genetic test. So, what is it saying that yes embryo has been biopsied and the biopsied embryonic tissue we collect the fetal DNA and then we go for the different genetic test which will take some time. So, if we go for cryopreservation of this biopsied embryo then that will give us time right sufficient time to perform the genetic test right. So, that is a true statement and it is required you need to cryopreserve the embryo you need to go for the genetic test and if the genetic test are normal then the embryo is normal and we will go for transfer of this normal embryo inside the mother's womb.

Next question HLA typing of the embryo can be performed along with PGT along with pre implantation genetic diagnostic tests we can perform HLA typing whether it is true or false. Yes it is a true statement. So, HLA typing in certain cases like say bone marrow cancer one fetus or one offspring is having a bone marrow cancer or say certain disorder right and the next you know when the parents go for the next pregnancy they want the next pregnancy fetus to be normal right she should not she or he should not be carrying the same disorder as the first pregnancy. So, you need to go for prenatal pre implantation genetic tests right. Now, along with that pre implantation genetic test you can also perform HLA typing of the fetal tissue and depending upon that HLA typing you know you can the parents can ask that yes doctor we need our second offspring which is HLA matched with the first offspring.

So, that the second offspring now can be the donor for the first offspring in case of you know stem cell transplantation or bone marrow transplantation in case of blood cancer different blood cancer. So, HLA typing of the fetal tissue can be done and HLA matched embryo can be transferred to the maternal womb. So, as to make the second offspring having the same HLA type as the first offspring. So, that the second offspring now can act as the bone marrow donor for the first offspring after its birth.

So, it is a true statement. So, that was all regarding the pre implantation genetic tests screening as well as diagnosis and the different methods which we practice in embryobiopsy and the different tests that are done in pre implantation embryobiopsy to note here that yes this can be done in case of IVF pregnancy and it helps to segregate the normal embryos which are not anomalous which are not having any type of genetic disease and these only normal embryos right only one normal embryo can also be transferred into the maternal womb right. So, that was the importance of prenatal or pre implantation genetic testing. References taken are from D.C.Dutta book of obstetrics, the Williams obstetrics and the James book on high risk pregnancy right. So, thank you for today and keep reading keep taking notes and just go through the books for further understanding of these important concepts. Thank you.