Genetic Engineering: Theory & Applications
Professor Vishal Trivedi
Department of Biosciences and Bioengineering,
Indian Institute of Technology Guwahati Assam
Module XI
Biotechnology in Social Welfare
Lecture 37
Applications of Biotechnology Part III

Hello everybody, this is Doctor. Vishal Trivedi from department of Biosciences and Bioengineering, IIT Guwahati. And what we were discussing in this module about the applications of biotechnology for the society. And in this context in previous 2 lectures we have discuss about the role of biotechnology in the agriculture. And then we have discussed about the role of biotechnology in the animal sciences. And within the animal sciences, in the previous lecture we have discussed about how to produce the vaccines, how the gene therapy is working and so on.

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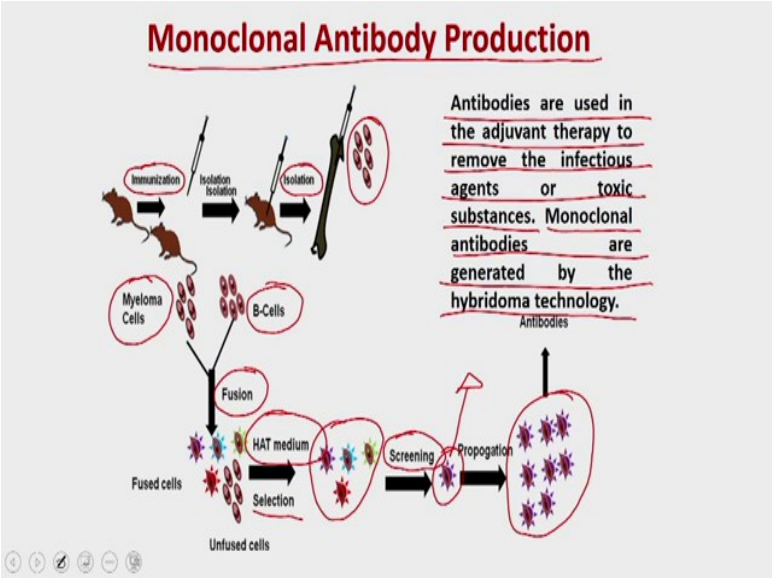

So now today, we are going to discuss about the production of monoclonal antibodies. So the monoclonal antibody is an antibody which is used in the adjuvant therapy to remove the infectious agent or the toxic substances. For example, if someone is getting a snake bite, the snake bite is actually contains different types of enzyme. And these different types of enzymes are having the neurotoxicity as well as the hemotoxicity. So these

enzymes can be neutralized simply by using a monoclonal antibody against those enzymes.

So what the antibody is going to do is, if you inject a patient these monoclonal antibodies, what they will do is, they are going to bind these enzymes. And then, the cellular system is going to recognize these antibodies. And that is how these enzymes or the proteinaceous substances or the infectious organism can be removed from the circulation. Monoclonal antibodies are generated by a technology known as the hybridoma technology. So as you can see in this hybridoma technology what you need is, you need the 2 different types of cells. You need myeloma cells, then you need the B-cells. These B-cells you have to have it from the mice.

So in the total technology, what you are supposed to do is, first you are going to immunize the animal. And once you are immunize the animal, you are going to isolate the B-cells from the bone marrows. And then, these B-cells can be used along with the myeloma cells for fusion reactions to generate the hybridoma cells. And these hybridoma cells are then going to be go through with their screening process in the presence of a specialized media, which is called as HAT media.

And what HAT media is going to do is, it is going to kill all the unfused cells. And then you are going to have the hybridoma cells, which are going to contain the different types of B-cells, fused with the myeloma cells. And then you are going to do a further screening with the help of the antigen to isolate the monoclonal hybridoma, which is going to express your antibody or the monoclonal antibodies.

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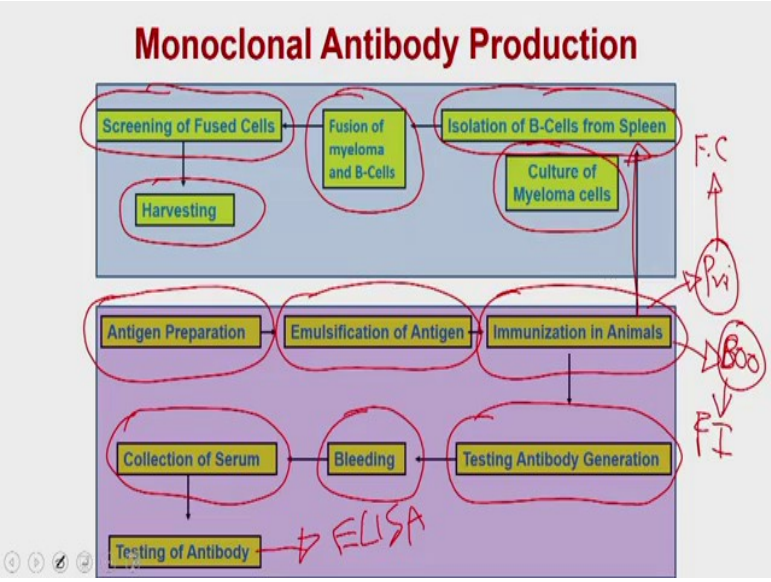

So the complete procedure what you see is actually divided into two part. One is that where you are going to first prepare the antigens. And then, once the antigen is being prepared, then you are going to prepare the emulsification of these antigens. So if you remember, we have already discuss about how to prepare the antigen either by using a recombinant DNA technology or you can purify these antigen using the classical chromatography techniques, either of these source.

Once you have the antigen, then you are going to emulsify these antigens with the help of the different types of the adjuvants. Either you will use the Freund's complete adjuvants or the incomplete adjuvants. The purpose of making the emulsification or the emulsification of the antigen is that it is going to make the antigen trapped within the lipid vesicles. And because of that the removal of the antigen side of your injection is going to be very slow. And because of that it is going to keep challenging the immune system for a very very long time. And that actually is going to give you a robust immune response or it was going to give you very high amount of antibodies producing cells.

So once the emulsification of antigen is done, then you can do the immunization of the animals. Now once the immunization of the animal is done, then you can test whether the antibodies are being produced. So immunization has to be done by 2 times. One, you are going to do the primary injections. And then you are going to do a, booster injections. So

primary injections you always use the Freund's complete adjuvants whereas the booster dose, you always use the Freund's incomplete adjuvants. The difference between the Freund's complete adjuvant as well as the incomplete adjuvant is that the Freund's complete adjuvant contains the mycobacterium cell wall whereas the Freund's incomplete adjuvant only contains the oil as well as the, some amount of detergents.

Then you have, so you have to test the antibody generation. So what you are going to do is you are going to collect the blood from the animals. And then, you will collect the serum. And you are going to check the presence of antibodies simply by the ELISA. And once your sure that the antibodies are being produced and the animal what you are using is actually going to give you very high amount of antibodies, then you can use this animal for generating the monoclonal antibodies using the hybridoma technologies.

So, to explain these steps- how to prepare the, how to emulsify the antigens, how to immunize the animals either from the primary injections or the boost injections. And then, how to test the presence of antibodies, how to bleed, how to collect the serum and how to test the antibodies in ELISA, we have prepare a small demo clips to explain all these steps. And with this clip you will be able to understand the each and every step in detail.

(Video Started)

I am Amogh Anant Sahasrabuddhe. I work in CSIR-CDRI, Lucknow and in today's demo we will be discussing different steps involved in generation of antibodies in the (()) (7:26).

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So for the first step, we require several things like Freund's complete adjuvant, here it is from Sigma. We need a micro emulsifying agent which has two openings, connected with a fine needle. We need antigen which is purified and filtered, so there are no contaminations, it is sterile solution of antigen. Then we take out some of Freund's complete adjuvant in a (()) (8:02) and then mix them together. Since one, since this adjuvant is oil based, it does not mix easily with a watery system like the antigen is in PBS. So, therefore we mix them rigorously, vigorously and forcefully.

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Step 1
Emulsification of antigen


Step 1
Emulsification of antigen

For that purpose, we take these two, we mix this emulsion and we mix this PBS and PBS containing antigen and the adjuvant (()) (8:40). After mixing them, we take out in a needle. Using a needle, we take out in the syringe like this. And then we fix the micro emulsifying needles into it. Attach another syringe into it like this. So once you, once you have filled your antigen and the adjuvant in this needle, you push it here. And then keep pushing from one side, keep pulling from another side. Keep pushing from one side and keep pulling from another side. So this process forcefully pushes your material in the oil and the antigen through this fine needle. And with that in that process the emulsion is formed. Emulsion can be called as water in oil or oil in water because both are in the same concentration, same volumes. So you can call them anyway. So it is, the emulsion, by this method the emulsion is formed.

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So for (()) (9:56), you have already prepared the emulsion. This emulsion looks like white. Initially it was 2 phase. And then slowly it has turned into single phase. Now we can push this emulsion from one unit to another side and from another; this syringe to another syringe. So this process creates very good emulsion. This does not separate out later on, when you are ready to inject.

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So how do you check them? For checking purposes, we drop 1 of this emulsion, a drop of emulsion on water surface like this. If emulsion is not formed perfectly, this will spread out. Otherwise, it will not spread. So this is a check that your emulsion is formed correctly. So once you find that this drop is not spreading, your emulsion is actually ready for usage. So this was the process by which you prepare the emulsion for usage. So this is the first step of preparing the emulsion. So now let us understand why we prepare the emulsion. We have checked that the emulsion is formed. Now, the purpose of making

the emulsion- because you have antigens and through antigen you can raise antibodies. But after emulsifying them, you actually make the antigen releases slowly.

So it is a substituent release kind of preparation. So, that the antigen is exposed to system in systematic manner. So, that the more and more memory cells is generated. And that is the sole purpose of having emulsion. Otherwise, if you inject antigen as such in PBS or other watery system, it will spread out in the body and it will be cleared out by the immune system rapidly and no memory cells will be generated. So these are, this is the main purpose of preparing the emulsion.

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So now we have prepared the emulsion. We have come to animal house. This is our rabbit which will be immunized. And before immunization, we have to take the pre-immune meal, so that we can compare whether the serum and the antiserum. So we will now start how we immunize it. So now we are preparing to immunize. The first and very important thing is in all this immunization processes is we have to avoid the pain to the animal. So for that purpose we have to strain the animal because we have to inject.

So we strain the animal in a way that it has less and less pain. And the movement is also very less. So we will inject this emulsion into the thigh. Catch hold up both the legs and we have to sterilize the area using alcohol. You have to look at the thigh muscles. Muscles with (()) (13:26) cleanly visible, skin should be cleanly visible. There are two kind of injection that we give. One is intra-dermal and other one is subcutaneous. So today we will be doing subcutaneous injection.

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This is our emulsion that we have prepared by micro emulsifying needle, we have seen earlier. This is the area where we would like to inject. You have to take out all the air from the syringe and the needle. We have taken out, clean the area again and then we apply some antiseptic powder. Here it is betadine powder. So the infection cannot develop later on. Sprinkle some of it at the area of injection. And then slowly release, give the animal relax and it is immunized.

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To stain an animal in we take a towel or this kind of cloth. So the advantage of having this cloth, to strain the animal have its claw inside, outside of this cloth and then it cannot move. So we have to restrict the movement very similar in stomach and in the (()) (15:52) not on this cloth so we use this. Now we will strain the animal in this cloth. We will put the animal relaxed on this cloth (()) (16:06). Make sure that their ears are outside and animal is strained properly so as to reduce its movement. And now it is ready to bleed.

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Step 3
Bleeding


Step 3
Bleeding

We will bleed the animal from this (()) (16:46) ear vein and we rub it so it gets heated up and the circulation is faster. Vein is also expand and more and more flow will be there. This is the method which we normally we apply. When the vein is properly visible, we will slightly sterilize using alcohol and this a (()) (17:32) needle which is wide enough to give sufficient bleed. We will pick the vein and collect the bleed. To stop it, we just, with less and less pain you can collect the bleed like this.

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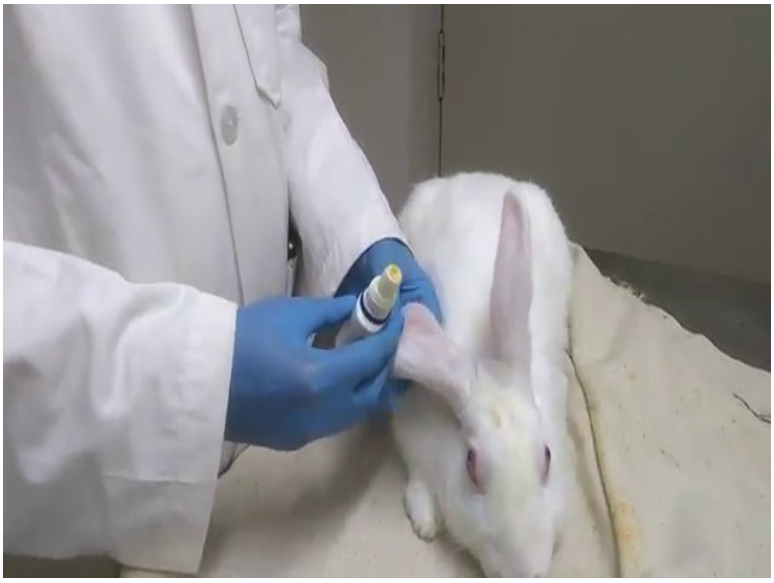

Now we have you make sure that no further bleeding occurs. And we wipe out whatever bleed is outside using sterile water. Then wipe out outer blood here and there (()) (19:04). Then wipe out all over with water so that the vein becomes cool and gets shut. We will just check. Still it is bleeding so we keep it pushed until the bleeding stops. So now, I think the blood has stopped coming out. And then, now we apply some antibiotic. Here in this case is betadine powder, so that there is no further infections or inflammation with rabbit. And this also ensures that there is (()) (20:20). If there is new inflammation, it will have some pain. So it will avoid that kind of pain also.

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So we have isolated approximately 10 to 12 ml of blood. This will give us approximately half of the value of the blood serum. Blood will be coagulated at 37 centigrade for 1 hour and then it will be kept at 4 degree centigrade for whole night. So that clot is shrunken properly and the serum is maximally taken out. And then we will add it to the serum, add some preservative like sodium azide and keep it at minus 20 or minus 80 as per requirement. And then we also test some (()) (21:10) the title of it. The best test using (()) (21:15) test. Now we can see this whole of the animal is thus this animal is completely relaxed. It has, I think it has very less pain or negligible pain.

And see this is very important for handling that in whatever procedure you go through with animal, animal should be ensured not to have pain. Maybe you can, if the procedure is painful to understand, it is painful procedure. You anesthetized it. Since this procedure is not painful we have not anesthetized it. So this is very important step to ensure that the animal (()) (21:55), it should be relaxed. So in the metabolic process, I think you have got to know all steps of antibody development in rabbit. And there we have prepared emulsion, we injected the emulsion, we isolated the blood after giving him (()) (22:16) booster doses and full of the process is (()) (22:22). I think you have understood most of these processes and you liked it. Thank you.

(Video Ended)

So with this clip, I am sure you could have been seen that what are the different types of precaution you have to take. One of the important precaution what you have to take when you are working with the animals is that you should not give them a very very severe level of the pain when you are doing the procedures. For example, you might have seen in this particular video that when we were even bleeding the animals or when we were injecting, when were immunizing them with the injections, the animal was not feeling lot of pain. And that is what the purpose is that when you work with the animals, they should be having the very little or no pain at all.

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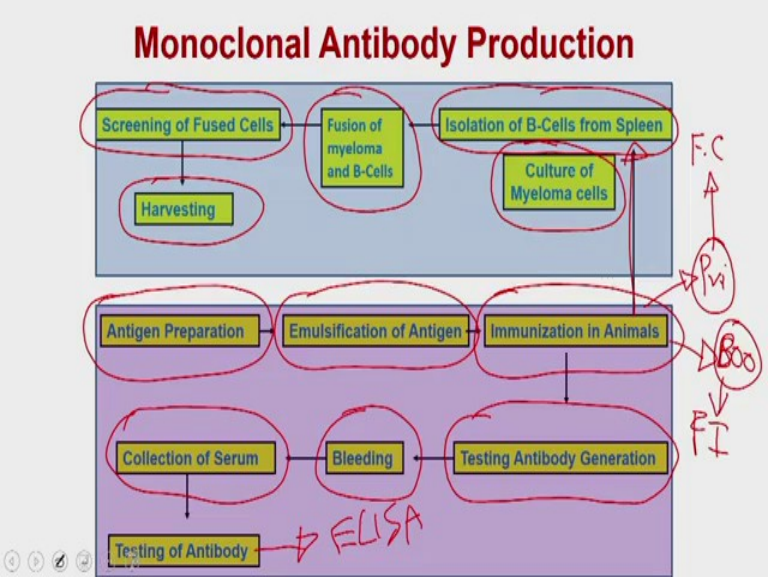

Now once you are sure that the antibodies are been produced using the ELISA, then what you can do is- you can take these animals and then you isolate the B-cells from the spleen. So you can collect the B-cells from the spleen. And you can also culture the myeloma cells. And now, what you have to do is, you have to put these 2 types, the B-cells as well as the myeloma cells for fusion reactions.

In the fusion reactions, you are going to do lot of different types of steps. And once the fusion is done, then you are going to do the screening of the fused cells which are called as hybridomas. And once you are going to get your desirable hybridomas, then you can do the harvesting of the antibodies from the hybridomas. So once you are sure that the animal is being immunized, the antibodies are produced. This means this animal is good

enough to be used for production of monoclonal antibodies. So now what you can do is, you can take this animal and isolate the B-cells. And take these B-cells for its fusion reactions with the myeloma cells. And once the myeloma cells are going to be fused, then it will give you the hybridoma.

So there are some cells which are not going to be fused. And then what you have to do is, you have to do a screening with the help of the media which is called as HAT media. And that HAT media is going to kill all the cells which are not going to be fused. And once that happens, then you are going to get the selected pool of hybridoma cells. These selected pool of hybridoma cells are going to be, can be used for further screening with the, to identify the specific clones which are actually expressing your monoclonal antibodies. And then you can use those clones for harvesting the antibodies.

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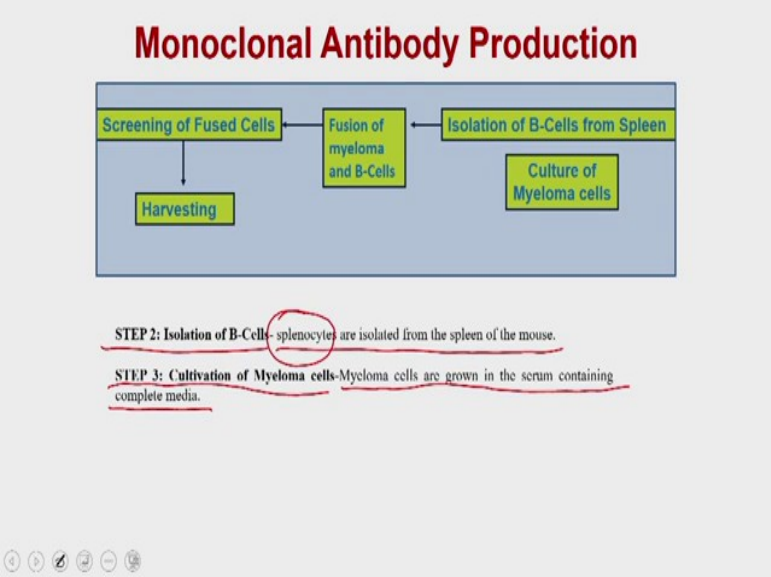

So now, the step 2 is the isolation of B-cells. So you can collect the splenocytes from the spleen of the mouse. So what you have to do is, once the rabbit or the mouse is immunized, then you can isolate the spleen. And then you fractionate that spleen into the individual cells. And if you remember, you can actually use the strategies what we have discussed, some in our lecture number 3 of 4, how you can actually, how you can be able to isolate different types of cells from the tissue using the different density gradient centrifugations or the differential centrifugations. And with the help of this, you can be

able to isolate the splenocytes. And these splenocytes can be used for the downstream hybridoma preparations. Then the step 3, you are going to do the cultivation of myeloma cells. So myeloma cells are grown in a serum containing complete media.

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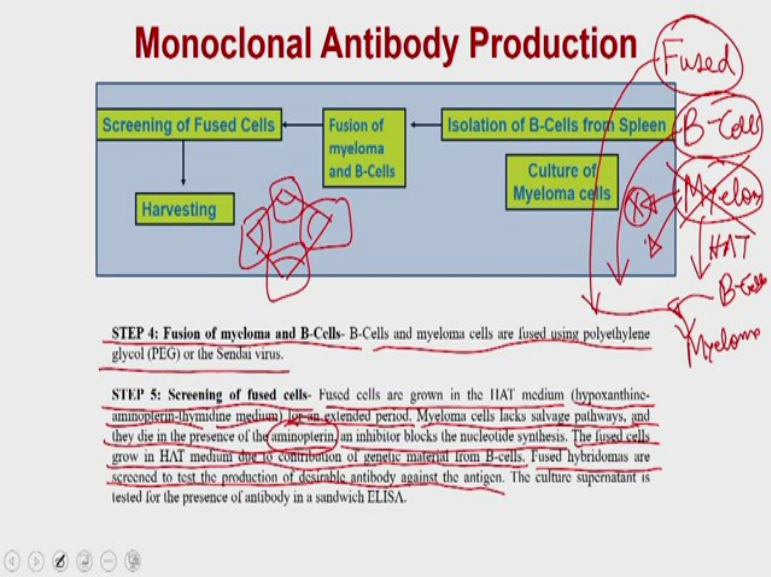

Now once these 2 steps are over then you can put it for the next step which is the fusion of the myeloma cells and the B-cells. So the B-cells and myeloma cells are fused using the polyethylene glycol for the Sendai virus. So PEG is an agent which can be used to fuse the cell. So what PEG is doing is actually bringing the plasma membranes of the two cells. And that is how it is actually fusing the cells. So when you incubate the cells with the PEG, the cells are going to come together. And in this process, the myeloma cells are going to be fused with the splenocytes. And that is how you are going to get a hybrid cells.

Then the step 5, you are going to do the screening. So the fused cells are grown in the HAT media. The HAT media contains hypoxanthine, aminopterin, and thymidine for an extended period of time. So myeloma cells lack salvage pathways. So that is the problem. So myeloma cells actually lack the salvage pathway. So they will die in the presence of aminopterin cause the aminopterin I going to cut the production of the execution of the salvage path, and inhibitor which blocks the nucleotide synthesis.

The fused cells grow in HAT media due to the contribution of genetic material from the B-cells. And the fused hybridomas are screened to test the production of desirable antibodies against the antigen. So once you got the hybridoma cells or once you got the fused cells, the fused; so what you are going to get is, you are going to get the fused cells, you are going to get the B-cells which are not been to get fused or you are going to get the myeloma cells.

Now once you put these 3 different types of cells and you put under them in the HAT media, what will happen is that the myeloma cells are actually not having the salvage pathway, which means they cannot be able to utilize the preformed or the precursors of the nucleotides because if they will have a salvage pathway, they can be able to use the nucleotide precursors and run the salvage pathway to synthesize the nucleotide. What the myeloma cells actually can do is, they can actually run the denovo nucleotide synthesis. And that actually are, you are going to block with the help of an inhibitor which is known as aminopterin.

So because of that myeloma cells are not going to survive, if you kill the nucleotide synthesis in the help of aminopterin. The B-cells, which are actually the primary cells, are anyway having a very very short half-life. So they are going to die in due course. The only population which is going to survive is the fused cells because the fused cells are going to give the production of the nucleotide by utilizing the machinery of the B-cells. Whereas the myeloma cells are actually going to make these cells, this particular fused cells as a transformed cell.

So the fused cells are going to propagate for a very very long time because they are having the transformations. Because they have the component of the myeloma cells, which are transformed cell. So they are having the tendency to grow for a very very long time. On the other hand, myeloma cells will not grow because their nucleotide synthesis is been blocked with the help of the aminopterin. So that pathway is going to be provided by the B-cells. So as a result, the only the fused cells are going to survive in the HAT media, which actually contains the hypoxanthine and thymine. But it also contains the inhibitor which is called aminopterin.

Now once you got the fused cells, these fused cells are also going to be screen with the help of the antigen because once you produce, once you isolate the B-cells at any moment of time the animal may have the B-cells for your antigen. Animal may have the B-cells for many different types of antigens. Or different types of antigen what have been exposed by to the animal. On the other hand, within the single antigen also, you could actually have the monoclonal antibodies of different epitopes.

For example, if you have an antigen like this, you could be able to have the 4 different types of epitopes. And all these 4 different epitopes probably can give you 4 different types of monoclonal antibodies. And that is why it is important that you use the antigen as a screening material and that is how you screen the hybridomas to select, further select the hybridoma which are going to produce the antibodies. So you can actually get the 4 different types of monoclonal antibodies from a single antigen, so that you are going to do. So the culture supernatant is going to be tested for the presence of antibodies in a sandwich ELISA.

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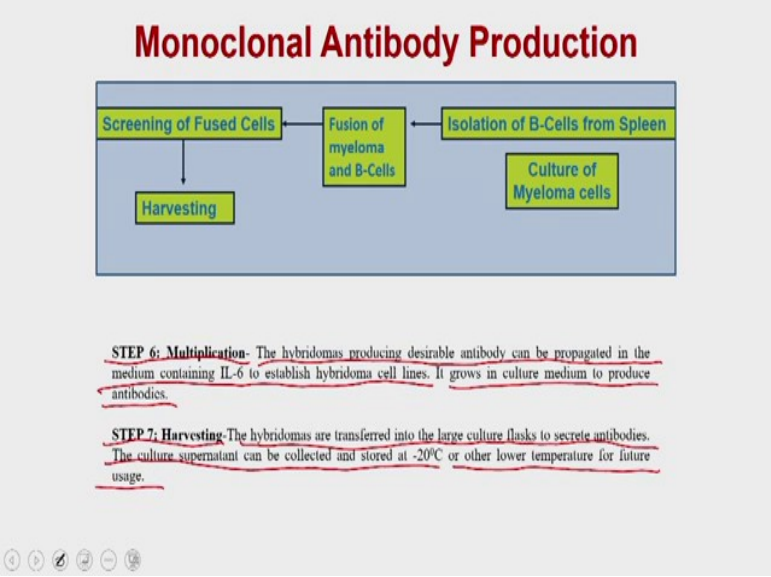

Now in the step 6, once you got hybridomas or once you got the fused cells, the hybridoma producing desirable antibodies can be propagated in a medium containing IL-6 to establish hybridoma cell lines. It grows in culture media to produce the antibodies. Step 7, which is actually the harvesting. The hybridomas are transferred into the large

culture flask to secrete the antibodies. The culture supernatant can be collected and stored at minus 20 degree Celsius or other lower temperature for future usage.

So the hybridomas actually secrete the antibodies because the antibody does not contain the transmembrane regions. So once it reduces antibodies, it goes into the secretory pathway. The antibodies are secreted into the culture supernatant. And then what you can do is, you can just spin down the cells. You can get the supernatant and that supernatant can be stored in minus 20 or other any other minus 80 or any other temperature for future use.

So this is all about the production of monoclonal antibodies. What we have discussed so far? We have discussed about how to use the antigen, how to immunize the animals and how you can isolate the B-cells from the animals. And then, how to produce the hybridomas, how to screen the hybridomas and ultimately how to harvest the antibodies from these hybridomas.

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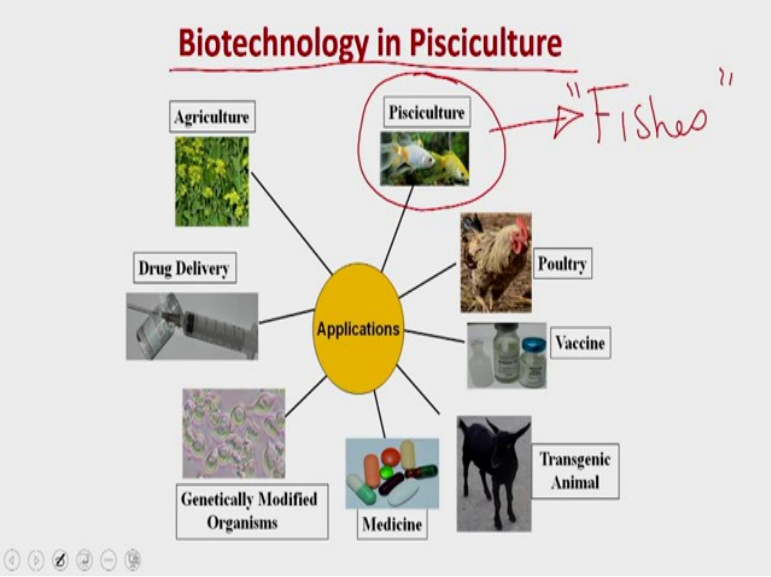

So the biotechnology has lot of applications. One of the classical application is that the biotechnology can be used for the pisciculture. Pisciculture means the production of fishes. And with the help of the genetic engineering as well as the advances in the

technology to know what are the requirement of the fishes, the biotechnology has significantly contributed into the pisciculture or the fish culture.

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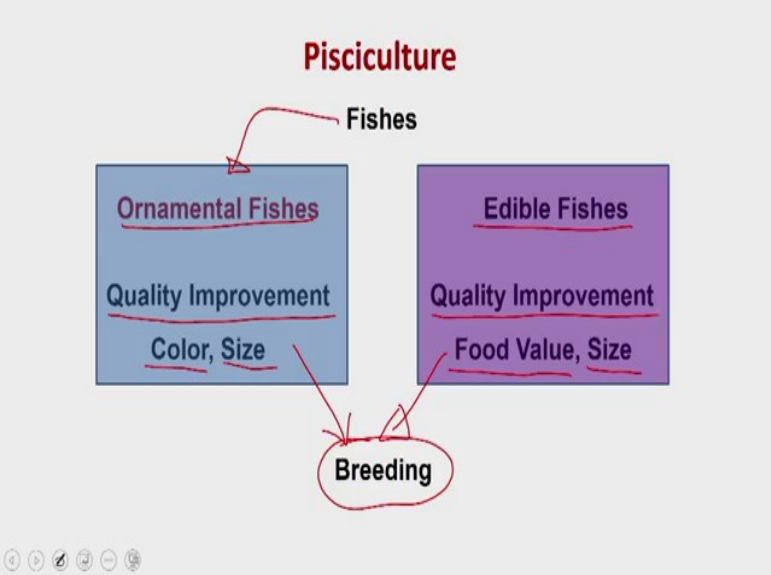

So the fish culture is being done for 2 purposes. You have the fish which are actually been for the ornamental fishes and you have the fishes which are for the edible fishes. For both, the ornamental fishes as well the edible fishes, the quality improvement is one of the area where the biotechnology can easily contribute. The quality improvement in terms of the color as well as the size.

Whereas in the case of edible fishes, the quality improvement means you can increase the food values, you can increase the size, you can increase the shelf life of that particular fish or you can actually have some kind of fishes which are actually going to have lower layer cholesterol or high level of this and that. So those kind modifications are possible only if you could be having up technology to breed these animals under the control conditions. So for both of these aspects, whether you want the fishes for the ornamental fishes or the edible fishes, the in-vitro breeding or the breeding in a controlled condition is the key to supply or make the desirable features to the both of these classes.

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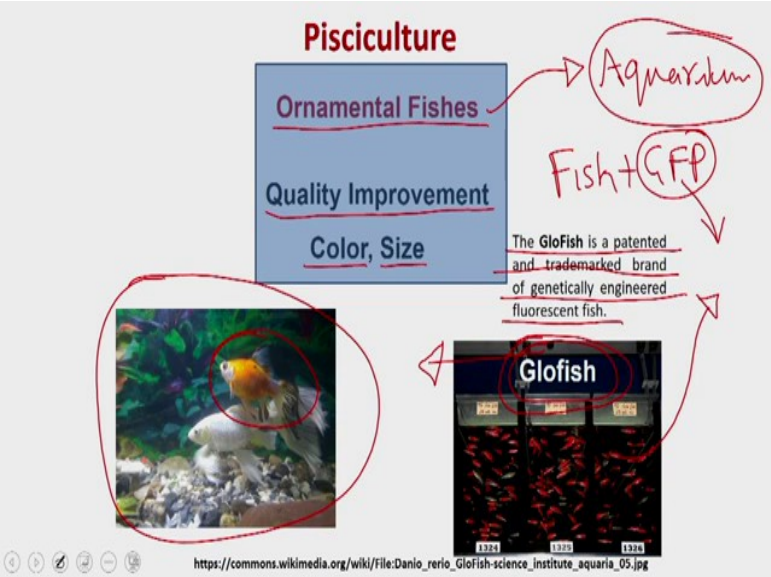

Now, in the case of ornamental fishes, you want to improve the quality which means actually the color as well as the size. So ornamental fishes are always been used in aquarium and they have a very big market in terms of that they are going to be used in the aquarium only for decoration purposes. And because of that what you want is that, these fishes are going to be as attractive, as good possible. So because of that you can actually induce or you can make the genetically modified fishes, so that they will have different types of color patterns.

You can have the different sizes and you can have the different; and on the other hand, you can also make the fishes which are actually going to be acclimatize in a different types of water environment. So that actually will help to bring these fishes into the aquarium. Because most of the time, the aquarium is actually been run by the fishes who are at least running in a freshwater fishes. You are not using the ocean water or any other kind of salt water for the aquarium purposes.

So this is just a classical example of the aquarium fishes, what is available. You can see that what you can improve is the pattern of the color what is available for these fishes and as well the size and other kind of parameter. To keeping this in mind, a series of the couple of the scientist have decided that okay we are going to generate the genetically

modified fishes. And what they have done is, they have started over expressing or started expressing different color proteins.

So because of that the fishes started developing the different types of color or the patterns And on the other hand, some scientist are also decided that okay will go with the different types of fish,, where the fish is going to express the fluorescent protein. So what they have done is, they have started expressing the green fluorescent proteins or red fluorescent proteins or yellow fluorescent proteins and so on. And they have also discovered some other fluorescent proteins. So because of that they could be able to generate a fluorescent fishes. And they named these fishes as the Glofishes.

The Glofish is a patented and trademark brand of genetically engineered florescent fishes. So what you can see, these glofishes that they are actually going to give you the fluorescence when they are being kept in the aquarium. And these glofishes are; what they are doing is, there are just adding, what they are doing is- for example, if they take the zebra fish and what they do is they add the green fluorescent protein. Because of that what will happen is, that the zebra fish is going to be fluorescent. It is going to give you the green fluorescence when it is going to be in the aquarium in the night or in the daytime.

So what they have done is they have ensure that the, fluorescence what you are going to get is a going to be from the sunlight instead of the UV light. In a typical fluorescent experiment, what you have to do is you have to exposed the, for example GFP with a UV light for and then only it is going to give you the fluorescence. But what they have done is they have over expressed the GFP, in such a way that it is actually going to give you the fluorescence in the sunlight.

And these glofishes have a very very big market. These glofishes are costing somewhere around the 50 dollar to 200 dollars and depending on what kind of variety of fish. So this glofishes are been developed for different variety of fishes, like tiger fish, gold fish and all those kind of fishes, people have developed the glofishes.

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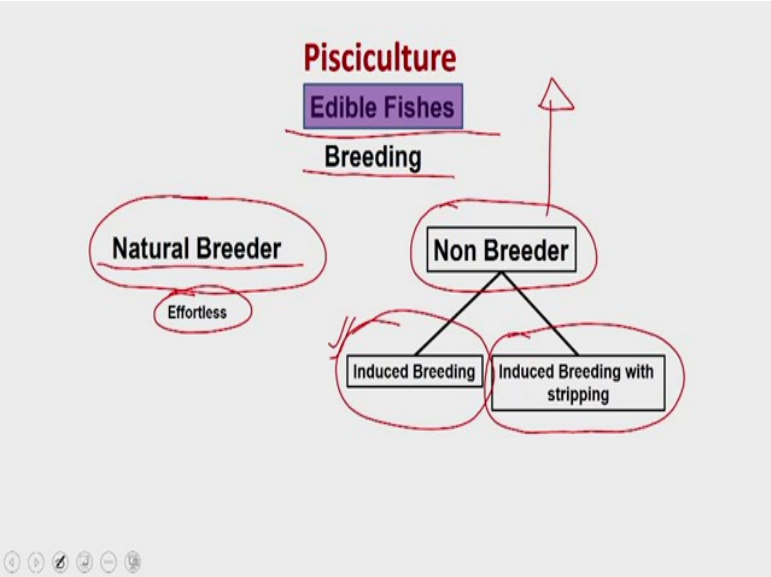

Now come back to the edible fishes. In the edible fishes, as far as the breeding is concerned you have the three different types of fishes. You have the natural breeders. So you have the fishes which are actually very easy to breed and those are called as the natural breeders. The breeding of these fishes are effortless because what you have to do if you just buy the egg or what you have to buy the seeds from the companies. And then you have to just make a pond and put these seeds into the pond. And these seeds are going to grow and give you the fishes. And out of these fishes, some are going to be male fishes; some are going to be female fishes. And then, these male and female fishes are going to produce the more seeds or more eggs.

And that is how you can actually be able to keep breeding the cells; keep breeding these fishes in a pond. So for these kind of fishes which are actually natural breeders, do not need a special procedure to adopt and these are the effortless. On the other hand, you have the class of fishes which are actually the non-breeders. What is mean by the non-breeder- is that these fishes are natural breeders, if you keep them in the river or the larger natural water sources. For example, if you keep them in the river, they are going to breed because they are acclimatized to kept in the river.

But once you keep these fishes under the pond or once you keep them in the aquarium or a smaller artificial water reservoir, then they did they do not breed actually because that

disturbs they are hormonal cycles or there are so many reason could be possible that these fishes have to stop breeding, which means you still have the male and female but they will not breathe because you have changed the water environment, you have changed many things.

So in this category, you can actually do the induced breeding with the help of the hormone or you can do induced breeding with the stripping. So what is meant by the induced breeding is that you are going to induce the breeding with the help of the chemical agents whereas in the stripping, the procedure is completely different.

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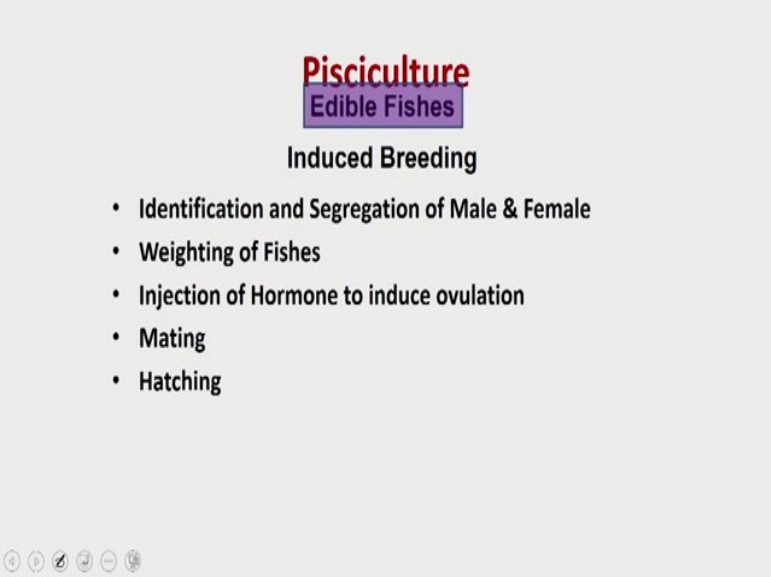

Now let us talk about the induced breeding. So in the induced breeding, you are actually going to have the induced breeding when the fishes are not been able to breed in the natural environment. So they are breeding in the natural environment which means if you keep the fishes in river, they are going to breed. But if you keep them in a captivity means in small pond or a small aquarium, they are not going to breed.

So in induced breeding what you have to do is first you have to identify the male and female and then you have to keep them in a separate container. Then you have to weigh the fishes, so that you know what are the dozing off the hormone you have to give. Then you have to inject the hormone to the female for ovulation. Then you have to keep this

female for mating and the ultimately you are going to get the embryos, which are going to be go for the hatching process.

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So what you have to do is, you have to identify the male and female fish. So for every fish kind you are going to have a discrete identification pattern, whether it is some kind of the spots or some kind of growth which actually will allow you to identify the male and female fishes. Once you identify using these characteristic patterns. And that would

be exclusive to that particular species or class of those fishes. Then you segregate the male and female separately.

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Then you are going to inducing. So what are the inducing agents for breeding? Mostly you are going to use the gonadotropin hormones or you are going to use the ovaprim or sometime people are also using the HCG, which is called as the human chorionic gonadotropins. So in general, you are actually going to use the gonadotropin hormones for inducing the ovulation. So now you have segregated the male and female. You can use the hormones.

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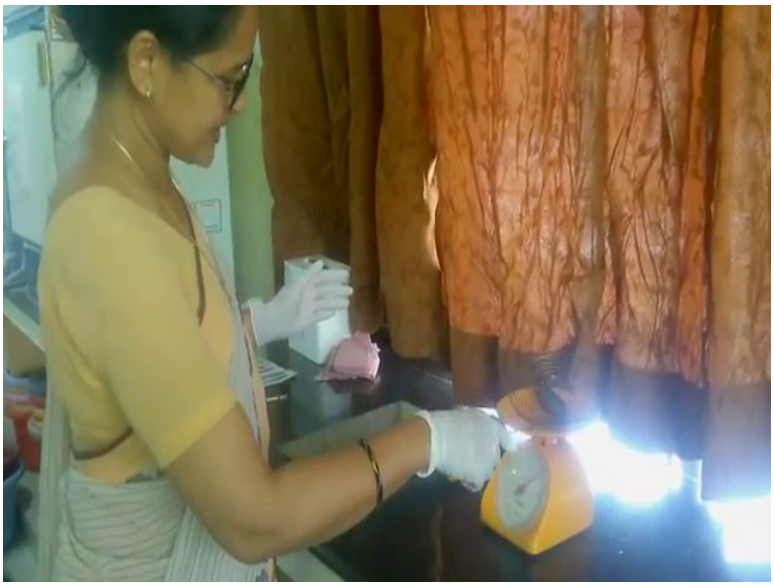

First you take out the fish. Weigh it in a weighing balance. So you, according to the weight you can actually give the dose for the hormones.

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Then you have the two options. So you have the two sides for inducing the brooders for the ovulations. You have the intra-peritoneal injection. So you can give the intra-peritoneal injection to the fish. Or you can give the intra-muscular sites, which means you can give the intramuscular injections of the hormones to the fishes.

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And then you are going to keep the male and female together for the mating. Once they mate, they are going to produce the embryos. They are going to produce the eggs which are going to be fertilized.

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And then ultimately, they are going for the hatching. So what you see is a classical picture of the hatching of Channa punctatus and where you can see that the small small hatchlings are been coming out from the eggs. And these hatchlings will further develop. When you provide them the food, they will get converted into the small fishes and now if you can take this hatchlings and put it into the pond. And slowly slowly you can increase the size of that particular pound. And that is how you are going to have the big fish farms. And that is how you can actually increase the fish breeding.

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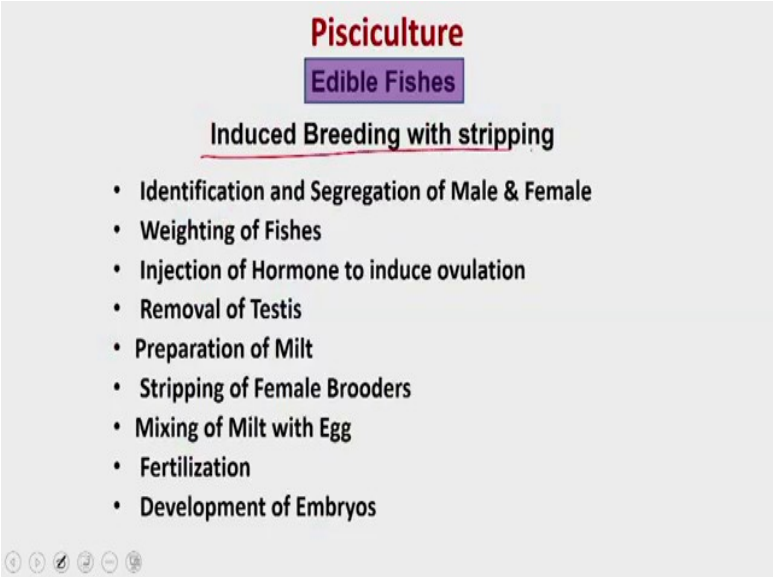

Now let us come to the induced breeding with the stripping. So what is mean by the induced breeding with the stripping is the initial few steps are identical, which means you have to identify and segregate the male and females. Then you have to weigh the fishes. Then you have to inject the hormones to induce the ovulation. You have to inject the hormone; in this case you also have to inject the hormones to the male as well. Then what you have to do is, you have to remove the testis from the male. And you have to prepare the milt. So milt is actually the preparation which you are going to prepare from the testis of the male fishes.

And then what you have to do is you have to strip the eggs from the female brooders. So you have to manage, in such a way so that the milt preparation as well as the stripping of female should be of same time or almost the same time. Then you are going to mix the milt with the eggs. So what will happen? It is going to go for the fertilization. And once the fertilization is over, so the next step is, it will go for the fertilization. Once the fertilization is over, then it is going to give you the fertilized eggs. And these fertilizers eggs are going to develop into the embryos. And these embryos are going to give you the hatchlings. And these hatchlings are eventually going to develop into the fishes.

So in the stripped breeding, what you are going to do is this is actually called as the artificial breeding, which means, you are; so in the induced breeding, you are not doing

anything in the pond. In the induced breeding with the stripping is, that you are doing every within the water itself, which means you are bringing the egg, you are taking out the egg from the fish, you are taking out the sperms from the male and then you are incubating them together to fertilize. And that is how you are actually doing the complete process what is happening in the natural environment, under the in-vitro reactions.

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So as I said, you have to identify the male and female. So in the catfish, you may have the different types of pattern which you have to use to identify the male and female fishes.

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Then you have to segregate the male as well as the female into the separate container, so that you can be able to give them the hormone treatments.

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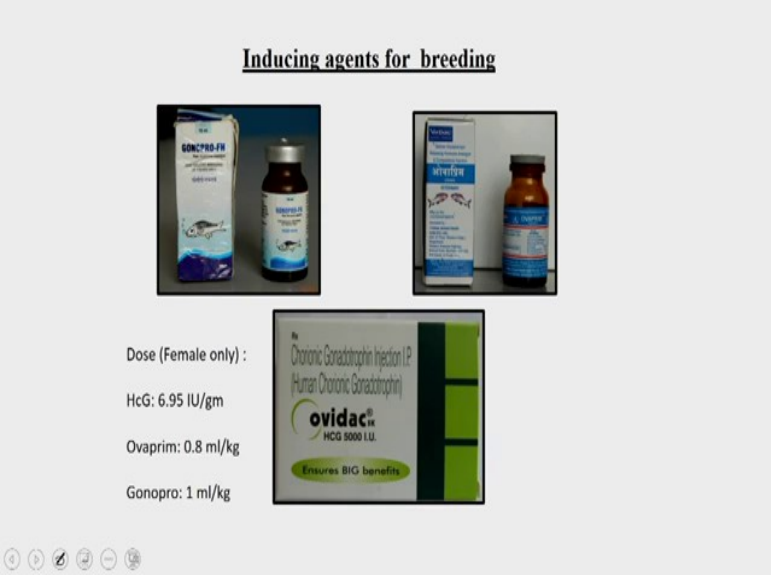

You can use this gonadotropin hormones. Dose for SCG is 6.95 International units per gram of weight of the fish. Ovaprim is 0.8 ml per kg and as well as the GonoPro is 1 ml per kg. So you can follow this particular dozing and these dozing is only for the reference

purposes. It could be vary from fishes to fishes sometime. And it required some kind optimization as well. For the male, the dozing is slightly on the lower side.

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So then what you have to do is, you have to weigh the fishes.

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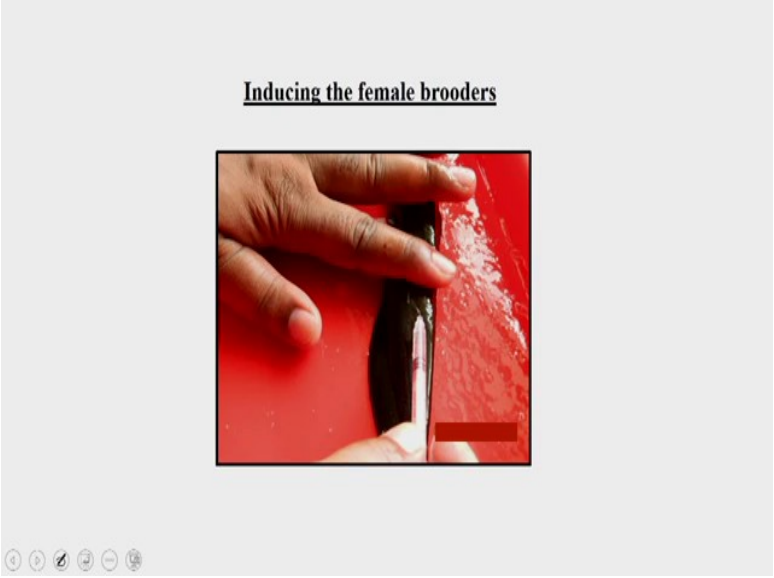

And you are going to give the injections. So first you are going to induce the female brooders with the intra-peritoneal or the intra-muscular injections of the hormones.

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And then, you are going to remove the testis from the male fish. So once you remove the testis, you are going to prepare; you can you can take these testis and prepare the milt.

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So once you prepare the milt, so actually the milt; you can put in into pestle mortar and then it is going to give you the solution of the milt. And that milt is nothing but the sperms which actually contains with in the testis. So what you are going to do is, you are only going to crush the testis in such a way, that all the sperms which are present in the

testis are going to come out into the environment or into the solutions. Now this solution is ready for added to the eggs. So that you can be able to fertilized or you can use these sperms to go and do the fertilization.

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But before that you have to treat the fishes and then you have to withdraw the eggs from the fishes into the water or into the reservoirs. So what you can do is, you can just strip female with the all the eggs. So what you can do is, you can see all the eggs are been collected into the tub. And now this tub is containing the eggs. You have already prepared the milt which is present in the pestle mortar.

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Then you can add these two together. And, so mixing of milt with the eggs. So you can mix the eggs with the milt. And in this process, what will happen is the milt which actually contains the sperms are going to fertilize these eggs. And it will give you the fertilized eggs.

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So these are the fertilized eggs which are actually slightly brownish in color. And now these fertilized eggs are going to grow, are going to take up the nutrition and they will going to differentiate and give you the embryos.

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And during this process, since you have to keep changing the aquaria. So you have to keep transferring the eggs in the aquaria, so that they will be having the more oxygen and other kind of conditions. So that, that will actually going to help these eggs to develop into the embryos and then it develops into the hatchlings. And that is how you actually can overcome the breeding problems in different types of fishes. Either you use the induced breeding or you can you use the induced breeding with the stripping. Both are these procedures are going to help the breeders to achieve the breeding in that particular fish. And they will get the fish in the ponds.

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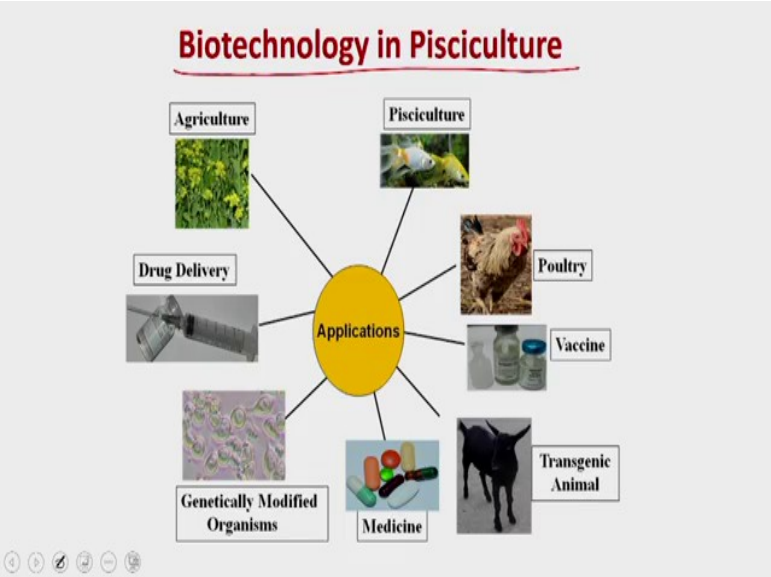

So this is all about the application of biotechnology in the pisciculture. As you can see, the biotechnology is effecting the human society or human standard of living in a many ways. What you can see is that the biotechnology is effecting the agriculture field, biotechnology is effecting the pisciculture, biotechnology has a role in medical biotechnology, biotechnology has a role in devising the formula so that you can be able to produce the drugs.

So the vast applications of biotechnology is cannot be covered in a single lecture or a couple of modules. That is why I strongly encourage you to go through with these applications by following the some of the reference books. And with this I would like to conclude our lecture here. And in the subsequent lecture, we are going to discuss about the overall, the summary as well as the conclusion of this particular course and with this I would like to conclude our lecture here. Thank you.