FETUS SEXING BY MEANS OF SIMPLE AND QUICK STAINING

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It has been discovered that sex chromation is found in the stationary nuclei in the somatic cells of females and may be detected under a microscope, but not in males. Recently, Serr et al. (1955), James (1956), Suzuki et al. (1956), Dewhurst (1956) and Shettles (1956) have reported that the sex of a fetus in the uterus can be determined by a application of this principle. For staining the smear preparations to be examined, James (1956) used Papanicolau’s and Giemsa’s stainings, Dewhurst (1956) and Moore and Barr’s (1955) cresyl echt violet staining method and Shettles (1956) the Feulgen’s staining. We have also reported on our study using Feulgen’s, Giemsa’s and haematoxylin stainings. More recently, I have devised a new simpler and quicker staining method and found the superiority of this method over the methods available in the past, as reported in the following.

SEXING METHOD

At first, the pregnant is subjected to vaginal examination or abdominal palpation for determining the size and the position of the uterus and the location of the amnion water. Next, in the early stage of pregnancy, puncture is effected through the vaginal wall, but in the later stage, it is made through the abdominal wall. A puncturing needle of 1 mm in diameter and 10 cm long, provided with a mandolin in it, is used for the purpose. The position to be punctured is in the early stage of pregnancy on the fornix vaginae posterior, in the middle stage, two finger-breadths below the navel on the median line of the abdominal wall, and in the later stage of the pregnancy, two finger-breadths below the navel but three finger-breadths aside from the median line to the side away from the back of the fetus. The cessation of resistance shows by touch that the tip of the needle has run into the amnion water. Then draw out the mandolin and the amnion water will flow out. No outflow of the water indicates that the tip of the needle is not yet in the amnion water. Draw the amnion water with an injecting syringe upon assuring the flow of the water, and no blood will be sucked out. In the case of twins, the amnion water is sampled per fetus by puncturing at two appropriate positions.

Thus sample 5-10 ml of the amnion water, centrifuge it, place a modicum of the sediment on a slide-glass, add a drop of the staining solution and stir with a slender glass rod. Cover with a cover-glass and fix its edges with molten wax. The staining solution should be mixed with the sediment in the proportion of about 2 to 1 volume of the sediment when the sediment is solid, but when the sediment is small in quantity so that only a liquid suspension can be tested, in the proportion of 1 to 1 in volume. Any one of the following 5 kinds of staining solutions can be used: a. 120 mg of cresyl

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echt violet dissolved in 20 ml of 30% acetic acid solution, b. 160 mg of cresyl echt violet dissolved in 20 ml of 40% glacial acetic acid solution, c. 80 mg of gentiana violet dissolved in 20 ml of 30% glacial acetic acid solution, d. 60 mg of fuchsin dissolved in 20 ml of 30% glacial acetic acid solution, and e. 80 mg of fuchsin dissolved in 20 ml of 40% glacial acetic acid solution.

After the preparation is completed, leave the samples for about 30 minutes to effect through staining and examine them under a good microscope at ×1000-1500 magnification under strong illumination. Focus upon the nuclear membrane and make observations manipulating the micromotion screw. Find out 50 to 100 fresh nuclei with their internal structure distinctly stained and count the percentage of the nuclei containing visible sex chromatin. If the fetus is female, 30-50% of the nuclei would be found containing sex chromatin but if it is male no typical sex chromatin would be found in any nucleus. By this method, only the nuclei are stained but the cytoplasm remains nearly unstained.

Most of the cell nuclei in the sediment of the amnion water have to be excluded from the test due to degeneration or inadequate staining. Of a properly stained fresh nucleus, only the nuclear membrane is well stained, so that the outline of the nucleus is clearly shown, but the interior is not much affected, so that the inside is very light in view. Some stained lines are sometimes seen in the nucleus — perhaps wrinkles produced by the difference in the osmotic pressure of the staining solution and the cytoplasm. The nucleoli are scarcely stained. In most cases, only the large-sized nuclei are of service in my tests, for the smaller nuclei are usually stained without leaving the interior and look dark throughout, so that their inner structure is hard to observe.

Under my staining method, the sex chromatin is seen as flat semicircle plates of uniform size closely adhering to the nuclear membrane, clearly demarked from the surrounding and uniformly very dark-staining. If once such positions, form, size and the darkness of sex chromatin are learned, there would be little chance of mistake in recognizing them.

Amnion water was taken again about one month after the previous examination and used for the sexing fetus in order to minimize the erroneous diagnosis.

RESULTS OF TESTS

I tried determining the sex with fetus of 4 to 10 months as subjects. The fetus proved to be male in 67 cases and female in 70 cases after births, and my predictions proved correct in 65 cases of male fetus and in 66 cases of female fetus as shown in Tab. 1. The rate of fulfilled predictions came up to 96%.

As side effects of our tests, I saw miscarriages following puncture in one case in the early and one case in the middle stage of the 4th month of pregnancy. Thus, it is advised that the puncture be made in the late 4th month or later. One gravida in the 6th month of pregnancy had cerebral anemia after the puncture, but soon recovered from it. In 21 cases among the total 137 bleeding in the amnion was observed, but the bleeding was always slight, no case ever showing severe bleeding. Such cases of bleeding are probably minimized by higher skill in puncturing. No case of injury to the uterus or the fetus as well as to any other organ or infection ever occurred. Interuterine puncture can be done with almost no risk, if undertaken with adequate caution.

Next, for following up the origin of the cells suspended in the amnion water, I made examinations for the sex chromatin in the mucous membranes of the tongue, the oral cavity, the esophagus, the stomach, the intestine, the bladder and the vagina and the skin of 4 dead fetus (2 male and 2 female), using Feulgen's staining and haematoxylin staining. The chromosomal sex was female in
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SEXING FETUS

Table 1. Relationship between the fetal sex after births and the predicted fetal sex

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<thead>
<tr>
<th>The month of pregnancy</th>
<th>Male fetus</th>
<th>Female fetus</th>
<th>Total</th>
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<td>Correct</td>
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<td>19</td>
<td>36</td>
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<tr>
<td><strong>Total</strong></td>
<td><strong>65</strong></td>
<td><strong>2</strong></td>
<td><strong>67</strong></td>
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all the tissue of female fetus and male in all the tissues of male fetus. Considering the incomparably large superficial area of the skin, I may take that the sex chromatin found in the nuclei in the sediment of the amniotic fluid have their origin in the skin of the fetus.

DISCUSSION

In comparison of the merits of the staining pigments cresyl echt violet, gentiana violet and fuchsin, as used in my method of sexing fetus in the uterus, I find that cresyl echt violet stains strictly the nucleus only, so that the field under the microscope is very light, while the other two pigments partially diffuse to the other parts outside and obscure the field. Cresyl echt violet and gentiana violet stain violet, as the names suggest, but fuchsin stains red, and in a slide, violet is seen more distinct. Cresyl echt violet is superior to the other two in better definition of the fine structure. Even when the concentration of the pigment in the solution of cresyl echt violet comes off the optimum, its staining power suffers less than in the case with the other pigments. Thus, cresyl echt violet is the best adapted for use in my method of sexing.

The staining method I have adopted for staining sediments of the amniotic fluid has the following merits and demerits in comparison with the other methods proposed heretofore: 1) This method is very simple in operation to anyone, requiring no special training and skill. 2) The time required for adequate staining is very short. 3) Since the operation consists of only one process, the nucleus tested remains uninjured and its fine structure is very distinct. 4) No specific equipment is required, so that it can be used at any minor health center. 5) As demerit, we may point out that the stained preparations are not good for permanent preservation.

CONCLUSION

I have conducted successful experiments of determining the sex of fetus in the maternal uterus, by staining cell nuclei in the amniotic fluid for detecting
sex chromatin, using a speedy and simple staining method of my own. This staining method has many specific points of merit over the methods in general use, and I am convinced of its high serviceability.

REFERENCES


