# EARLY PREGNANCY FETAL ANATOMIC SEX ASSIGNMENT BY ULTRASOUND - FASA[sm]

FASA is a new technique of ultrasound visualization of the fetal external genitalia. It was developed in conjunction with genetic amniocentesis in our prenatal diagnosis program.

It was developed by prospectively predicting fetal sex, subsequently verifying the prediction by comparison with the chromosome analysis. It also provides a unique quality assurance aspect to reporting results. The accuracy of FASA (and in concordance with genetic amniocentesis) was reported in the NEW ENGLAND JOURNAL OF MEDICINE, October 20, 1983. This series of 100 cases has now been extended to over 400 cases with the same accuracy -- in gestational age range of 16 to 18 weeks from the last menstrual period.

In an additional 100 cases, the patients were seen in gestational age range of 12 to 14 weeks. They subsequently had genetic amniocentesis and the same accuracy was achieved - with concordance between the first and second assessments.

The accuracy and relevance of FASA in prenatal diagnosis is further illustrated by the first prenatal diagnosis of complete sex reversal in a case of testosterone-receptor resistance in a case of testicular feminization syndrome (TF).

An additional application for the technique of FASA when performed in association with genetic amniocentesis is the ability to "resolve" the anticipated sex of an infant at birth in those cases where the sex chromosomes from genetic amniocentesis imply "ambiguity". This will be illustrated later in this presentation.

A potentially new application of FASA may be in association with chromosome analysis obtained from the chorionic villus biopsy (CVB). This may be particularly important when the CVB is performed for an X-linked indication.

If the chromosome result is female the remote possibility exists that only maternal tissue was sampled. The possibility that the CVB prenatal diagnosis

may have failed to detect a male fetus in an on-going pregnancy can be detected by FASA technique when performed in week 12 of gestation.

An additional advantage to a prenatal diagnosis program doing amniocentesis and CVB would be to develop the technique as a noninvasive genetic counseling technique for X-linked disorders. It can be offered as an alternative to invasive procedures such as amniocentesis and CVB for those X-linked conditions where there is currently no accurate male prenatal diagnosis exists - i.e., Duschenne muscular dystrophy - or where the condition is treatable (hemophilia A)when the couple wishes to avoid invasive procedures but would benefit from the improved maternal/paternal bond with the fetus if they knew the fetus was developing as a female.

Visualization of a male may permit the couple to pursue further in depth genetic counseling to take place to assist couples in exercising all the aspects of the reproductive options open to them. These may include termination of pregnancy - or to go on to pursue prenatal diagnosis to detect the normal or abnormal male where there is a specific prenatal diagnostic differentiating test.

Real-time, linear array ultrasound continues to improve images obtained, particularly because of the newer digital imaging process in these machines. The higher resolution capability - particular improved axial resolution - now permits smaller components of the fetus to be seen at even earlier stages of development. These structures can be imaged with greater accuracy and thereby improve the sensitivity and specificity of the test performance. The fetus at 10 weeks from the patient's LMP is essentially fully developed for almost all its external parts, as illustrated in slides and figures 1 and 2. The fetus is now at the stage where it is only undergoing enlargement. The last significant

external developmental changes are the fetal external genitalia. As illustrated in figure 3 in weeks 10 to 12 both males and females appear to have identical external genital anatomy. This is illustrated for a male at 10 weeks in figure 4.

The fetus which is destined to be chromosomally male undergoes a transformation process. The key to the technique of FASA is the labial/scrotal fold fusion which is illustrated in figure 5. This process is completed in week 12 from the patient's LMP. The genital tubercle destined to become the penis becomes more prominent as it incorporated the urethra and this is illustrated in figures 6 and 7. It also changes its direction from being a structure in a horizontal plane parallel to the longitudinal axis of the fetus to a direction perpendicular to the longitudinal axis of the fetus and the transverse plane of orientation and has the appearance of "sitting" on top of the early formation scrotum.

Once labial/scrotal fold fusion has been completed, usually in weeks 11 to 12 of gestation, the male pattern can readily be readily imaged by real-time ultrasound. The only change after week 12 in the male is growth and enlargement as the male genitalia develops to achieve its "mature" form in the gestational age range 15 to 20 weeks. This is illustrated in figure 8. Ultrasound imaging must be performed in at least 2 scan planes of orientation, as illustrated in figure 9.

To further address the issue of sensitivity and specificity of FASA, males can be identified in 11 to 12 weeks, but only a provisional FASA can be made in week 12 if the fetus is female. Also, only provisional FASA can be made when viewing the genitalia in the first selected plane of orientation. Visualization must be then continued and the mental picture made and noted so as to actively image in an alternative plane of orientation to establish exclusion of the opposite sex. Figure 10 illustrates that which is also seen in the polaroid photograph of the male genitalia pattern in week 12, as obtained in the longitudinal scan plane of orientation. Figure 11 illustrates the male genitalia pattern as obtained

in the transverse scan plane of orientation.

The female fetus external genitalia demonstrated in the "indifferent" stage of development in week 10 in figure 12 does not change substantially as it develops, as does the male. The key to the process of embryological development is the labial/scrotal fold. These do not fuse in the chromosomally female fetus, as illustrated figuratively in figure 13. It is the relative absence of the change of direction of the genital tubercle as seen in figure 14 as it develops into the clitorus that is a key factor in accurate sex determination. The clitorus does not become as prominent as the penis because it is not incorporating the urethra. Figures 14 and 15 demonstrate that the clitorus remains a horizontal structure relative to the longitudinal axis of the fetus - as is illustrated in figure 15 when the fetus is in week 14. Identification of the female fetus requires 2 scan assessments, one which can be done in week 12 and if it is not a male it can only be labeled presumptive female. This must be verified at the end of the range of the biological period of variation of development of external genitalia.

As further maturation occurs in weeks 14 through 20, the female external genitalia develops towards the mature pattern as illustrated in figures 16 and 17 - the labial/scrotal folds remain unfused and develop by enlargement of the labia majora. The clitorus which can be thought of as being "bound down" by the clitoral hood maintains its horizontal direction, as well as becoming relatively less apparent.

Ultrasound imaging in real-time is best seen by visualizing a videotape recording.

The female external genitalia figuratively illustrated in figure 18 shows the genital tubercle - now the clitorus - as a rectangular structure pointing in the caudal direction and horizontal as well as parallel to the longitudinal axis of the fetus - when viewed in the longitudinal scan plane of orientation.

In the transverse scan plane of orientation, as illustrated in figure 19, the unfused labial-scrotal folds appear as linear echos lateral to the clitorus and parallel to, as well as on either side of the rectangular echo which represents the clitorus as seen in a fetus at 14 weeks from the patient's LMP period.

The total time it takes to perform FASA varies from the optimal patient at 30 to 60 seconds up to 10 minutes in the more difficult patient. It is always possible to visualize external genitalia and perform accurate FASA with digital linear-array real-time ultrasound imaging, using the technique of FASA.

To achieve the same accuracy in weeks 12 to 14, it is important to demonstrate fetal maturity by a combination of deriving gestational age by the patient's LMP dates and confirming this by BPD measurements. It is necessary to be aware of the potential biological variation in the expression of the process of fetal external genitalia development, and particularly of the range-of-prediction error of fetal gestational age when reliance on the assessment of fetal maturity is being based on measurements when the LMP dates are either not known, not reliable, or discordant with measurements.

The male genitalia pattern when completed by labial/scrotal fold fusion is imaged as a characteristic ultrasound pattern which can be identified by a single ulrasound study in weeks 11 to 12.

However, a chromosomal male potential may not have yet undergone its characteristic change in weeks 11 to 12 and may visualize as an apparent female on the initial study, and then as a male visualize closer to week 14 of gestation. This is more likely to happen when gestational age is derived by measurements rather than by LMP-derived fetal maturity. At the initial scan of 11 to 12 weeks when one provisionally diagnoses female, the experience is that less than 1:2% of "females" change to "males" by week 14. It is necessary to stress once again that accurate diagnosis of female and avoidance of missing a genetic potential male, one must

only provisionally assign female in week 12 and confirm it as still visualizing as female by a second study in week 14.

By way of illustration of the validity of the claim of being able to accurately visualize fetal external genitalia and to perform FASA with the same accuracy as a chromosome analysis - obtained so far only in conjunction with genetic amniocentesis - but will shortly performed in association with CVB - to illustrate the additional power of this elegantly simple but also simply elegant technique, 2 cases were described in abstract #585 in the Proceedings of the AMERICAN SOCIETY OF HUMAN GENETICS, Toronto, October 1984.

In addition to the detection of sex reversal, by the diagnosis and subsequent infant verification of TF, it is necessary to consider the current standard of genetic counseling performed immediately after amniotic chromosome analysis revealed 45,XO/46,XY, but where the ultrasound prior to the genetic amniocentesis indicated a normal male external genitalia pattern. The couple in this case chose termination of the pregnancy because they focused on the uncertainty of potential ambiguity of genitalia development and the potential ambiguity of secondary sex characteristic development at puberty. Detailed examination of the fetus including histology of the undescended testes revealed normal male.

There have now been several cases of amniocentesis as attained 46,XO/46,XY (Hsu ref). The overall majority of these cases were found either at delivery or termination were found males. It is proposed that the ultrasound technique of FASA may add potential improvement to the genetic counseling for those couples wanting to consider continuation of the pregnancy by the demonstration of apparent normal male external genitalia development. The current prenatal diagnosis counseling should be revised.

The other example is that of where the ultrasound showed female, but the chromosomes revealed 46,XX15p+. This was shown to be a de novo Y q and c banding by not demonstrating any apparent "ambiguous genitalia" nor male genitalia. The genetic counseling was able to focus away from any consideration of the fetus

as having either Kleinfelter's syndrome or external genital ambiguity. This may also be implied by the determinant part of the Y chromosome is not on the short arm of the Y chromosome, but the ultrasound certainly added an additional beneficial perspective to the genetic counseling situation. The infant was born and is an apparently normal female and in all cases described, postdelivery confirmation has been obtained.

The only area of the sensitivity and specificity of the ultrasound technique which has yet to be addressed is the ascertainment of external genitalia ambiguity by ultrasound as the indication for them performing genetic amniocentesis or the visualization of external genitalia ambiguity in association with ambiguous sex chromosomes - neither of these situations has been experienced in my program. The potential value of learning FASA strictly in association with genetic amniocentesis in a prenatal diagnosis program will potentially permit these latter 2 situations to be diagnosed in the prenatal diagnosis age range - as further validation of the importance of the technique of FASA in genetic counseling in prenatal diagnosis.