fewer amniocenteses would have been done (assuming the screen-negative women with age risks exceeding 1:300 would not have chosen amniocentesis). In this group, given the limitations of sample size and follow-up, no cases of DS would have been missed.

Although we are not recommending the strategy based upon test risk versus age risk, it is interesting that patients themselves use this with regard to choice for amniocentesis. A woman of AMA with a test risk exceeding age risk is much more likely to have amniocentesis (and allow for fetal DS detection) as compared with a woman with a test risk lower than age risk.

The overall amniocentesis rate of 43 per cent for AMA women is comparable with the rate in younger women utilizing our screening programme. This alone markedly reduces our fetal DS detection rate regardless of definition of screen-positivity. We have recently investigated factors influencing choice of amniocentesis in our screening population to better understand their motivation (Priest *et al.*, submitted). For screen-positive women (all ages), those who chose amniocentesis were younger, had higher test-derived risks and were more concerned about their risk for fetal DS. Women who did not have amniocentesis indicated that attitudes about abortion and religion influenced their choice. Clearly, patient attitudes, as documented in that study, and the observed amniocentesis patterns, as described here, need to be accounted for in evaluating our screening programme. Women of AMA have generally been offered amniocentesis in preference to triple-marker screening, and have apparently declined this in favour of the screen. This test selection indicates that we are screening a biased subgroup. We wonder whether other programmes notice similar utilization patterns for amniocentesis in screen-positive AMA women.

John P. Johnson[1]*, Karen Streets[1], Joan Fitzgerald[1], Jean Priest[1], Marie Vanisko[2] and Mary Haag[1]
[1]*Shodair Hospital, PO Box 5539, Helena, MT 59604, U.S.A.*
[2]*Carroll College, 1601 N Benton Ave., Helena, MT 59601, U.S.A.*

*Correspondence to: J. P. Johnson, Shodair Hospital, PO Box 5539, Helena, MT 59604, U.S.A.

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Low ACTH and high melatonin concentrations in amniotic fluid as hormonal markers of high risk of fetal abnormalities. Preliminary studies

Studies conducted by Estin and Vernadakis (1986) indicate that cerebral fibroblasts strongly affect the metabolism and regular development of astroglial cells, and thus disturbances in the metabolism of embryonal mesenchymal cells could play a significant role in the process of differentiation of the fetal central nervous system (CNS). Increased secretion of melatonin, for example, has been found to have a direct inhibitory effect on connective tissue metabolism (Drobnik, 1993), so the fact that melatonin easily crosses the placental barrier (Muñoz-Hoyos *et al.*, 1992) indicates that maternal melatonin may also affect the mesenchymal cells of the fetus, being responsible for occurrence of fetal CNS defects.

It is also possible that high maternal melatonin concentration in the fetal circulation has a regulating effect on the pituitary–adrenal axis in the fetus, since it has been found to exert an inhibitory effect on the mitogenic activity of 1–39 ACTH in the adrenal cortex cells in rats (Sewerynek *et al.*, 1989). It should be stressed that in early pregnancy, the

Table I—ACTH and melatonin concentrations in amniotic fluid in normal pregnancies and those complicated with fetal pathology

No.	Weeks of gestation (w.g.)	Indications for antenatal diagnosis	Fetal pathologies and fetal karyotypes	AFP* (μg/ml), AChE band	ACTH (pg/ml)	Melatonin (pg/ml)
1.	14	Age of mother	Normal fetus 46, XY	10·8 AChE (−)	330·0	
2.	16	Previous acranius	Normal fetus 46, XY	21·48 AChE (−)	268·5	
3.	16	Spontaneous miscarriages	Normal fetus 46, XY	14·37 AChE (−)	135·5	6·83
4.	15	Psychological indications	Normal fetus 46, XX	20·67 AChE (−)	128·7	4·93
5.	15	Age of mother	Normal fetus 46, XX	15·71 AChE (−)	109·9	
6.	13	Previous acranius	Normal fetus 46, XX	16·3 AChE (−)	109·0	
7.	15	Down syndrome in child	Normal fetus 46, XY	10·0 AChE (−)	127·0	
8.	15	Age of mother	Normal fetus 46, XX	9·04 AChE (−)	123·1	
9.	15	Age of mother	Normal fetus 46, XX	6·15 AChE (−)	121·6	
10.	15	Age of mother; three infant deaths, between 1–2 years of life	Normal fetus 46, XX	9·47 AChE (−)	85·6	
11.	15	Previous acranius	Normal fetus 46, XX	7·65 AChE (−)	75·2	5·33
12.	14	CNS defects in child	Normal fetus —	11·0 AChE (−)	68·3	
13.	13	Age of mother	Normal fetus 46, XX	19·41 AChE (−)	65·0	6·51
14.	14	Age of mother	Symmetric hypotrophy of fetus (−2·5 w.g.), congenital heart defect (tetralogy of Fallot) 46, XX	20·0 AChE (−)	60·4	
15.	14	Hydrocephaly spina bifida	Anencephaly —	300·0 AChE (+)	45·1	
16.	14	Hydrocephaly spina bifida	Anencaphaly 46, XX	430·0 AChE (+)	53·9	
17.	14	Spontaneous miscarriages	Anencephaly 46, XX	366·7 AChE (+)	69·4	17·90
18.	14	Hydrocephaly spina bifida	Symmetric hypotrophy of fetus (−3 w.g.) 46, XY	21·43 AChE (−)	33·3	
19.	14	Age of mother	Symmetric hypotrophy of fetus —	6·54 AChE (−)	47·4	
20.	14	Age of mother; fetal multiple abnormalities	Symmetric hypotrophy of fetus (−2·5 w.g.) 46, XY/46, X	19·33 AChE (−)	24·4	

*Normal range of AFP: 13 w.g.: 7·9–27·6 μg/ml; 14 w.g.: 8·8–23·2 μg/ml; 15 w.g.: 11·25–23·69 μg/ml; 16 w.g.: 7·46–15·94 μg/ml.

pituitary gland of the fetus contains small quantities of 1–39 ACTH but relatively high amounts of peptides 1–13 ACTH (αMSH) and 18–39 ACTH (CLIP) (Silman *et al.*, 1976), which most probably originate from ACTH molecule cleavage. It has been found that the ratio of 1–39 ACTH to CLIP and αMSH changes in the course of pregnancy (Silman *et al.*, 1976). High concentration of CLIP has been found to influence the secretion of glial protein S-100 by glioblasts (Suzuki *et al.*, 1987).

The above data gave grounds for the assumption that disturbances in melatonin and ACTH concentrations in the fetal circulation (amniotic fluid) are concurrent with incidences of developmental abnormalities. Amniotic fluid (AF) samples from 20 women undergoing routine AF, AFP and AChE tests, as well as karyotype analysis of the fetus were collected by amniocentesis, between 13–16 weeks' gestation at the same time of day (1300–1400 hours). Three pregnancies with fetal CNS lesions were selected by ultrasound examination, but the other pregnancies were chosen at random. AF 1–39 ACTH (Nter ACTH, RIA kits, BYK Sangtec; Germany), as well as AF melatonin (RIA kit DDR-Mel 180, DRG, U.S.A.), were determined by radio-immunoassay. Routine ultrasonographic (USG) examinations were done at 20, 28 and 32 weeks of pregnancy in order to check for any other possible developmental pathologies in the second and third trimesters.

The results (see Table I) in the 13 normal pregnancies out of 20 showed a mean ($\bar{x}$) AF ACTH concentration of 146·7 pg/ml (85·3 pg/ml$<x\pm 2$SD$<$183·5 pg/ml, $P<0{\cdot}05$). Low concentrations of AF ACTH ($x<85{\cdot}3$ pg/ml) were found in 10 out of 20 women ($\bar{x}=54{\cdot}2$ pg/ml). Additionally, in 5 out of the 20 women, the amniotic fluid samples were assayed for melatonin concentration. In the pregnancy with anencephaly (patient 17), low AF ACTH concentration was accompanied by high melatonin concentration, which exceeded about three times the mean melatonin concentration as found in the four other amniotic fluid samples, in which cytogenetic, biochemical and USG examinations did not reveal any abnormalities of the fetus.

The preliminary results presented here confirm the supposition that low fetal AF ACTH concentrations in amniotic fluid are an element of a broader phenomenon of fetal pathology, not limited solely to CNS defects of the fetus. They are most probably an expression of more general hormonal disturbances, that are subject to an as yet unknown regulatory mechanism. Low ACTH concentrations in amniotic fluid of pregnancies with anencephalic fetuses, described by other investigators and accounted for by the missing pituitary gland, should be considered as an example of a particular condition.

It can be also supposed that very low AF ACTH concentrations in developmental abnormalities may be a result of more intensive cleavage of ACTH molecules into CLIP and αMSH. This idea is presented in hypothesis (Cieśla, 1998) suggesting that high maternal melatonin concentration (monomer forms) in the fetal circulation could cause the low ACTH and high beta-endorphin concentration in the fetus by selective stimulation of prohormone convertase 2 (PC2) synthesis (Berthagna, 1994). In consequence, it could lead to very low vimentin interfilament concentrations (Prieto *et al.*, 1989) in embryonic cells, the latter being directly responsible for inducing fetal pathologies. It is known that vimentin filaments play an important role in the processes of cell motility and spatial integration (Prieto *et al.*, 1989. On the other hand, dimer forms of melatonin would be responsible for the stimulation of prohormone convertase 1 (PC1) synthesis, and they would break up while crossing a placenta.

These results suggest that the risk of bearing a developmentally handicapped child would be highest in women with a high circadian secretion of melatonin, which may be additionally aggravated by the exposure of the mother to adverse environmental factors, or by immuno-hormonal disturbances from which she may suffer.

In conclusion, the estimation of ACTH and melatonin concentrations in amniotic fluid during routine antenatal diagnostic tests would render possible early selection of pregnancies with an increased risk of fetal developmental pathologies, like congenital heart defects, neural tube defects (e.g., hydrocephaly) or symmetric hypotrophy. The observations made are an introduction to further analyses.

WŁODZIMIERZ CIEŚLA*
Sterling's Hospital, Department of Endocrinology, Medical University of Łódź, 91-425, Sterling Str. 3, Poland

*Correspondence to: W. Cieśla, Department of Endocrinology, Sterling's Hospital, PSKi Ny3 Medical University of Łódź, 91-427 Łódź, Sterling Str. 3, Poland.

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Fetal cells in maternal blood as a second non-invasive step for fetal Down syndrome screening

Multivariate biochemical screening for Down syndrome has been widely described as a valid method in the second trimester of pregnancy between 15 and 22 completed gestational weeks (Wald *et al.*, 1995). If gestational age is estimated using ultrasound examination, and the marker levels are adjusted for maternal weight, then serum screening has a sensitivity of 72 per cent and a false-positive rate of 5 per cent (Wald *et al.*, 1995).

Nuchal translucency measurement has been introduced as a method for screening at 10–14 weeks of pregnancy, with a sensitivity of 86 per cent and a false-positive rate of 11 per cent (Nicolaides *et al.*, 1996). However, controversies exist in the literature regarding the quantification of such a method because of possible bias related to spontaneous fetal loss before the 16th gestational week and a possible positive correlation between increased nuchal translucency and increased fetal losses. These two conditions could, in theory, reduce the accuracy of the screening.

For these reasons, even if both methods use the same statistical algorithms but at different trimesters, the estimated posterior-risks are not easily overlappable, and the percentage of positive patients coming from serum screening and nuchal translucency will be dishomogeneous.

Serum screening by means of AFP, E_3 and hCG was described to have a positive predictive value of 1/40 (or 2·5 per cent), intended as the number of amniocenteses performed for every case of Down syndrome detected (Haddow *et al.*, 1992). Nuchal translucency, instead, was reported (in a case-control study) to have a positive predictive value of 36 per cent for Down, Patau and Edwards syndromes (with a cut-off $\geqslant 3$ mm) (Nicolaides *et al.*, 1994). If all the cases reported in that paper were due to Down syndrome, and taking into account an overall incidence of 1/700 of the disease, a theoretical positive predictive value of this marker alone, without age-specific risk adjustment, could be estimated as 1·77 per cent.

The detection of fetal cells in maternal blood could be useful as a second step in screening because the number of fetal cells detectable in the maternal circulation is higher in some aneuploid fetuses. In a recent paper, Bianchi *et al.* (1997) demonstrated that a higher mean number of fetal cells was detected by quantitative PCR in women carrying fetuses with Down syndrome when compared with women carrying normal fetuses.