GENETIC CONTROL OF THE SURVIVAL OF MURINE TRISOMY 16 FETUSES

BY

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Abstract

A mouse model that allows for the experimental induction of an aneuploid state has been employed to investigate the factors that control the survival of trisomy 16 fetuses. The prevalence of trisomy 16 fetuses on day 15 of gestation was shown to vary significantly with the genetic background of the female parent. The ability to spontaneously abort a trisomy 16 conceptus was shown to be higher in the mouse strain with a low prevalence of trisomy 16, compared to those mouse strains with a high prevalence of trisomy 16. Furthermore, the maternal ability that selects against, or promotes the survival of a trisomic conceptus was shown to be specific for the trisomy in question.

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Résumé

Un modèle permettant d'induire expérimentalement un état aneuploide chez la souris fût employé pour examiner les facteurs contrôlant la survie des foetus trisomiques pour le chromosome 16. Il a été démontré que la predominance des foetus possédant un chromosome 16 surnuméraire au quinzieme jour de la gestation varie de façon significative selon le génotype de la mère. Les données présentées montrent que la capacité d'avorter spontanément un foetus trisomique murin est plus élevée dans les lignées à basse fréquence de trisomie 16, en comparaison des lignées ayant une haute fréquence de trisomie 16. En outre, il a été démontré que l'habileté maternelle de sélectionner contre ou de favoriser la survie d'un foetus trisomique est spécifique pour le chromosome impliqué.

"Build today, then strong and sure
with a firm and ample base;
and ascending and secure
shall tomorrow find its place"

Longfellow

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Section I. Literature Review
Antenatal Screening for Down Syndrome

1. Introduction

Penrose was the first to report an association between advanced maternal age and the birth of a child with autosomal trisomy (Penrose, 1933). In 1975, the National Institutes of Health endorsed the use of amniocentesis as a means of prenatal diagnosis. Since this time, women 35 years of age and older have been deemed eligible for amniocentesis due to their increased risk of bearing conceptuses with trisomy (Hook and Chambers, 1976).

The maternal age limitation ($\geq$ 35) for amniocentesis was implemented for socio-economic reasons including, limited resources and personnel, as well as the cost-effectiveness of the procedure itself (Hagard and Carter, 1976; Hook and Chambers, 1976). It was determined that offering an amniocentesis to mothers of a particular risk group would only be cost-effective if that risk group was set at a maternal age at birth of 35, which translates to a risk of approximately 1% (Hagard and Carter, 1976).

Despite the implementation of a prenatal diagnostic program to identify fetuses with autosomal aneuploidies (specifically trisomy 21-Down syndrome) in a high risk group, the incidence of trisomy 21 in the United States has remained quite stable at 1/1000 livebirths (Adams et al, 1981). A possible rationale for this finding has to do with the considerable changes in planned parenthood within the past two decades. In 1969, women aged 35 or greater gave rise to

approximately 9% of the total number of livebirths in the United States. This proportion was reduced to 4.7% when the same study was repeated in 1979 (Adams et al, 1981). Fewer older mothers are bearing children, therefore, they are contributing a smaller proportion of trisomic births than are younger mothers, even though the incidence of autosomal trisomy is higher in the older mother. These proportions are sure to change now that the "baby boom" generation has entered their fourth decade of life.

The incidence of Down syndrome can be reduced by at most 20% with the present practice of offering women an amniocentesis based on the maternal age limitation of 35 years and older (Adams et al, 1981). Even by increasing the rate of prenatal testing among the women aged 35 and older to as high as 50%, the crude incidence would drop by only an approximated 10% (Adams et al, 1981). Another means to reduce the incidence of Down syndrome would be to routinely test younger women who conceive the majority of Down syndrome fetuses. Unfortunately, this practice is unfeasible due to the limited funding and resources allotted to prenatal diagnostic centers. If young mothers are to benefit from prenatal diagnosis, efforts must be concentrated in identifying among this group, individuals who are at an increased risk of bearing a Down syndrome conceptus.

The ensuing pages focus on disclosing methods of screening pregnant mothers whose conceptuses are at risk of

being born with trisomy 21. The manuscript is divided into two sections. The first section examines potential practices of screening pregnant women whose conceptuses are at an increased risk for Down syndrome. Maternal serum levels of alpha fetoprotein, chorionic gonadotropin, and unconjugated oestriol will be discussed in relation to possible screening programs. Screening for Down syndrome fetuses by ultrasonic detection of morphologic markers will also be considered. Section two covers an experimental approach, employing a mouse model, designed to identify mothers at risk of carrying an aneuploid conceptus to term.

Antenatal Screening for Down Syndrome with Biochemical Markers

2.1. Characteristics of Alpha-Fetoprotein

Alpha fetoprotein (AFP) was first described in 1956 as a fetal specific protein (Bergstrand and Czar, 1956) detectable as early as the fourth week after conception in the human embryo (Gitlin and Boesman, 1966). AFP synthesis takes place initially in the yolk sac, gastrointestinal tract, and then primarily in the fetal liver. Fetal plasma levels (measured in mg/ml) peak between 10 and 13 weeks of gestation and then decline progressively until term (UK collaborative study, 1977). AFP enters the amniotic fluid (measured in ug/ml) via fetal urination and likewise peaks in the second trimester (Weiss et al, 1976). AFP appears in the maternal circulation (measured in ng/ml), partly by direct transfer across fetal membranes (Macri et al, 1979), and continues to rise, probably due to enhanced placental perfusion (Nicolini, 1988) until the twenty-eighth to thirty-second week of gestation. Thus, neither amniotic fluid AFP (AFAFP) nor maternal serum AFP (MSAFP) can be measured independently of gestational age. A normal measurement at one stage in gestation may be considered an overestimation for an earlier stage in gestation.

Although AFP has been well characterized, its function remains unknown. One of the more popular theories regarding the role of AFP focuses on an immunoregulatory function during pregnancy (Merkatz et al, 1984). It is hypothesized that AFP

is involved in the prevention of rejection of the fetus by the mother. Even though an obvious function has not yet been assigned to AFP, researchers are continuing to find ways to make use of the glycoprotein.

The identification of AFP as a marker molecule present in the amniotic fluid of fetuses with anencephaly and/or spina bifida (Brock and Sutcliffe, 1972) prompted a means of screening pregnant women at risk of carrying fetuses with neural tube defects (UK collaborative study, 1977). The basis for such a screening program lies in the finding that significant increases in AFAFP have been associated with neural tube defects (Brock and Sutcliffe, 1972). AFP is able to cross the placental barrier and can therefore be measured in maternal serum with a sensitive radioimmunoassay. Women with MSAFP levels 2.5 times the normal median at 16-18 weeks of gestation will have an approximately 1 in 10 chance of having a fetus with a neural tube defect (UK collaborative study, 1977).

2.2. Association Between MSAFP, AFAFP, and Trisomy

MSAFP screening programs for neural tube defects rapidly became a part of prenatal care in many countries. It was through one of these routine MSAFP screening programs that Merkatz et al (1984) made the initial association between significantly low MSAFP concentrations and trisomy. The proband case involved a 28 year old primigravid woman who gave birth to an infant with trisomy 18, while twice recording

MSAFP values below the sensitivity of the testing procedure. An association between significantly low MSAFP and second trimester fetal loss had previously been reported (Davenport and Macri, 1983), however, the contribution of chromosomal anomalies to the makeup of these fetal losses was not analyzed. For this reason, Merkatz et al (1984) proceeded to undertake a comprehensive retrospective study of pregnant women with known chromosomal anomalies to investigate the clinical significance of low maternal serum and amniotic fluid AFP values.

MSAFP values obtained from mothers bearing aneuploid fetuses showed a significant difference in their distribution about the normal median compared to normal (diploid) pregnancies. Of 41 cases of autosomal aneuploidy from two centres, 36 (88%) had MSAFP values below the median. This distribution was significantly different from the gestational age matched controls, in which 45 values were above and 37 were below the median ($X^2=20.64$, $p<0.001$).

The MSAFP values of women bearing specific trisomies revealed that 20 of 25 cases with trisomy 21, 12 of 13 cases with trisomy 18, 3 of 3 cases with trisomy 13, and 7 of 12 cases with a sex chromosome aneuploidy were below the normal median. When compared to the distribution of the MSAFP values of the age matched controls, only the sex chromosome aneuploidies failed to show significant differences. This

finding suggests that low MSAFP is specific for autosomal trisomies.

When AFAFP values from aneuploid fetuses were examined, a random distribution about the normal median was observed. This finding allowed Merkatz et al (1984) to suggest that altered trophoblastic production or diminished placental clearance is responsible for the decrease in AFP in the maternal serum of women bearing Down syndrome fetuses.

Merkatz's presentation of the above findings at the New York State Fourteenth Birth Defects Symposium in 1983, prompted a group from Britain to review their data. Cuckle et al (1984) compared MSAFP values taken between 14 and 20 weeks of gestation from mothers of 61 pregnancies with trisomy 21, to MSAFP values obtained from mothers of 36652 unaffected singleton pregnancies of the same range of gestational age. The median MSAFP value for the cases with trisomy 21 was 0.72 multiples of the median (MoM), 28% lower than that for the unaffected pregnancies ($p<0.0001$, $z=5.53$, Wilcoxon rank sum test). This finding agreed with the results of Merkatz et al (1984) and was further supported by the following studies: Tabor et al, 1984; Fuhrmann et al, 1984; Seller, 1984; Guibaud et al, 1984; Murday and Slack, 1985; and Doran et al, 1986, all of which found a significant relationship between low MSAFP concentrations and Down syndrome.

Not all of the studies performed were in accord with Cuckle et al's findings. Spencer and Carpenter (1985) observed that the extent of the decrease in the median MSAFP for cases with trisomy 21, in their study 0.82 MoM, was not as significant a difference from the normal median as was determined by Cuckle et al (1984). However, Cuckle et al (1985) rebuked that Spencer and Carpenter's finding could easily have arisen by chance, so the two results are consistent with each other. Similarly, Hershey et al (1985) observed that the median MSAFP value for cases with trisomy 21, in their study 0.83 MoM, approached but did not reach statistical significance ($p=0.086$ with the Wilcoxon rank sum, for a test of difference from 1.0). Cowchock and Ruch (1984) were also unable to reproduce the findings of Cuckle et al. However, as Hershey et al (1986) mention, their test protocols were different from other centres. Their storage of frozen MSAFP samples may have been responsible for the inconsistent results.

Significant differences in AFP measurements were not restricted to maternal serum. The median AFAFP value from 27 pregnancies with trisomy 21 was found to be 0.60 MoM ($p<0.001$) (Cuckle et al, 1984). Based on this and similar findings (Tabor et al, 1984; Trig et al, 1984; Baumgarten et al, 1985; Nelson et al, 1985; Hershey et al, 1985; and Jones et al, 1986) an anonymous editorial (Lancet, 1985) offered an explanation for why low AFP values in maternal serum and

amniotic fluid are associated with Down syndrome pregnancies. The editorial states that one of the main characteristics of Down syndrome is growth retardation. The effects of growth retardation on the liver of a Down syndrome fetus may result in a lower production of AFP, and hence, lower concentrations of both maternal serum and amniotic fluid AFP. This explanation was not supported unanimously for as already stated, Merkatz et al (1984) did not find an association between low AFAFP and Down syndrome. Further to these studies, a preliminary report found that fetal levels of AFP are not significantly lower in Down syndrome compared to normal pregnancies (Nicolini, 1988). This finding suggests that placental or membrane passage of AFP from fetal to maternal compartments rather than decreased fetal synthesis is the cause for reduced levels of MSAFP in Down syndrome pregnancies (Nicolini, 1988).

Cuckle et al restricted their efforts to fetal trisomy 21 data. Down syndrome has a far greater incidence at birth than any of the other autosomal aneuploidies, and is the only autosomal trisomy associated with any degree of viability at birth. Nonetheless, trisomy 21 is not the only autosomal trisomy associated with a low MSAFP value in the second trimester. Merkatz et al (1984), Doran et al (1986), and Lindenbaum et al, (1987), have all determined that like trisomy 21, trisomy 18 is also associated with low MSAFP values. Such findings were not reported for trisomy 13 (Doran

et al, 1987), although sample sizes were considerably lower in this group. AFAFP levels showed no association with any other trisomy except for trisomy 21 (Davis et al, 1985; Nelson and Peterson, 1985; Doran et al, 1986; and Lindenbaum et al, 1987).

2.3. Screening for Down Syndrome with MSAFP

Once Cuckle et al (1984) were convinced of an association between Down syndrome pregnancies and significantly low MSAFP values, they proposed that this relationship could be used to form the basis of a screening test for mothers at risk of carrying a fetus with trisomy 21. According to Macri (1986), a screening test must result in the identification of a subgroup in which the following criteria are met: (1) screened gravid women should be at a sufficient risk to warrant further offering of a diagnostic test (amniocentesis) and (2) fetuses with the specific defect (whether autosomal aneuploidy or neural tube defects) in the screened population should be found within the identified subgroup. Cuckle et al (1984) were determined to investigate whether MSAFP screening could satisfy these criteria. They determined the number and proportion of Down syndrome and unaffected pregnancies in the second trimester with MSAFP values less than or equal to specified cut-off levels. This enabled the calculation of the relative risk a mother has in carrying a Down syndrome fetus at any particular MSAFP cut-off level.

By screening pregnant women at 14-20 weeks gestation with MSAFP values at or below 0.5 MoM and whose fetuses' gestational age have been verified by ultrasound biparietal measurements, 5% of all unaffected pregnancies and 21% of pregnancies with Down syndrome would be selected for amniocentesis (Cuckle et al, 1984). This screening performance can be further improved when combined with maternal age risk estimates for Down syndrome. MSAFP values are independent of maternal age, therefore, the use of both will yield a better screening performance than either alone (Cuckle et al, 1984). For instance, Cuckle's group recommended the offering of amniocentesis to all women aged 38 and over and to younger women with serum AFP values equal to or less than specified cut-off levels. This policy would select 6.8% of unaffected pregnancies for amniocentesis while at the same time detecting 40% of the Down syndrome pregnancies. Such a policy would be favorable to simply lowering the maternal age eligibility for amniocentesis, for a far greater proportion of normal pregnancies (16% versus 7%) would have to be screened to disclose the same number of trisomic fetuses (Cuckle et al, 1984).

The fulfillment of Macri's two criteria for the initiation of a screening program appear to have been met on a qualitative basis. That is, 1) women do exist who are at an increased risk of carrying a fetus with trisomy 21, and 2) these women can be detected with knowledge of their MSAFP

measurement. However, the true performance of a screening test must be measured quantitatively as well. The factors used to evaluate the use of MSAFP in an antenatal screening test for Down syndrome are, 1) sensitivity (the ability to detect pregnancies with trisomy 21) and 2) the number of false positives (the number of unaffected pregnancies that fall into the affected range).

2.4. Sensitivity and False Positive Rate in AFP Screening

The sensitivity of MSAFP screening has been evaluated on the basis of six published reports totalling 204 cases of Down syndrome (Cuckle et al, 1985). When applying an MSAFP cut-off of 0.5 MoM, approximately 20% of the Down syndrome pregnancies would be selected for amniocentesis and therefore detected. With the maternal age specific cut-off values for MSAFP measurements suggested by Cuckle et al, 36% of the Down syndrome pregnancies would be selected for amniocentesis and therefore detected.

The false positive rate has been estimated on the basis of four published reports totalling 100000 pregnancies (Cuckle et al, 1985). The combined false positive rate is 5.6% using a cut-off of 0.5 MoM and 7.4% using the maternal age specific cut-off levels.

To summarize, applying a MSAFP cut-off value of 0.5 MoM would select 5.6% of all pregnant women for amniocentesis in which 20% of Down syndrome fetuses would be detected. Applying the maternal age specific MSAFP cut-off values would

select 7.4% of all pregnant women for amniocentesis in which 36% of Down syndrome fetuses would be detected.

The implementation of MSAFP screening programs that select between 5% and 7% of all pregnant women for amniocentesis was felt by some to be unacceptable (Houlsby, 1985; Spencer and Carpenter, 1985; Wyatt, 1985; Wu, 1986). The concern over the number of false positives was two-fold. Firstly, Houlsby (1985) states that by selecting as much as 6% of all pregnant women for amniocentesis using the method suggested by Cuckle et al (1984), more normal fetuses would be lost due to the risk of amniocentesis (utilising the 1% miscarriage rate from Tabor et al, 1986) than Down syndrome fetuses actually detected by the MSAFP screening policy. This finding was confirmed by Wyatt (1985), Wu (1986), and Ager and Oliver (1986). However, their results were reversed when a more liberal risk figure of 0.5% was used (Verp and Gerbie, 1981). That is, the number of Down syndrome fetuses detected in midtrimester by amniocentesis was now greater than the number of non Down syndrome fetuses lost as a result of the procedure (Ager and Oliver, 1986).

Spencer and Carpenter (1985) felt that their finding of a ratio of normal fetal loss/Down syndrome cases detected of 1.2 was too high and lent towards their conclusion that MSAFP screening for Down syndrome is unacceptable. However, Cuckle et al (1985) as well as Macri (1988), point out that the current obstetric practice of offering amniocentesis to women

above the age of 35 or 38, depending on the centre, has a ratio of fetal loss/Down syndrome cases detected of anywhere from 1 to 3.65. Despite the value laden context of this policy, many women are willing to accept the higher risks of spontaneous abortion caused by amniocentesis than the risk of having a child with Down syndrome (Thornton et al, 1986).

Cuckle et al (1985) suggest that if a normal fetal loss/Down syndrome cases detected ratio is unacceptable to a particular centre then the MSAFP cut-off level can be adjusted. They cite an example from Baumgarten et al (1985), whose centre offers amniocentesis to women under the age of 35 based on a low MSAFP value equivalent to a Down syndrome risk of at least 1:250. Their expected ratio of normal fetal loss/Down syndrome cases detected was 0.3.

The second concern over the high number of false positives deals with the fact that some prenatal diagnostic centres would not be able to cope with cytogenetic analyses on 6% of all pregnancies (Houlsby et al, 1985). The policy of offering amniocentesis to women over 34 years of age already selects approximately 6% of all pregnant women (Brock, 1984). The difference between this maternal age policy and the proposed MSAFP policy is that utilization of amniocentesis by the older mother is generally less than 50% (Brock, 1984), while MSAFP screening for neural tube defects has reached 80% of pregnant women in one survey (Brock et al, 1978). Brock (1984) suggests that a pregnant woman aged 35 with a low MSAFP

value would be more apt, upon receiving prenatal counselling of her risk of carrying a Down syndrome fetus, to request an amniocentesis, than would a 35 year old mother who did not have her MSAFP tested. Cytogenetic laboratories would find themselves overwhelmed by the increase in demand for the karyotypic analysis of fetal chromosomes. Furthermore, a cytogenetics laboratory with limited resources would surely add to the emotional stress of a pregnant woman who must wait up to four weeks for the assessment of her amniocentesis.

2.5. Screening for Down Syndrome with AFAFP

To relieve the potential burden of excess karyotypic analyses with the advent of a MSAFP screening program for fetuses at risk for Down syndrome, a revision of the cytogenetic policies in use was recommended (Anonymous, Lancet, 1985). At present, some centres only perform karyotype studies on amniotic fluid samples from women who have had an amniocentesis for reasons of an increased risk of a fetal chromosomal abnormality. There are also centres that will routinely determine a fetal karyotype when the amniocentesis has been performed as a result of a raised MSAFP level (i.e. risk for a neural tube defect). The risk of bearing a fetus with Down syndrome in women having an amniocentesis because of a raised MSAFP level is only 1:500, a prevalence much rarer than other indications for amniocentesis (Cuckle and Wald, 1986). Cuckle and Wald (1986) determined that the cost of detecting one case of Down syndrome is 50

times more expensive when women with a raised MSAFP level are offered an amniocentesis than when women with a high risk for a fetal chromosomal abnormality are offered an amniocentesis.

Cuckle et al (1985b) and Hullin et al (1985) both suggest a new cytogenetic policy that would effectively reduce the cost and volume of a cytogenetic centre. The policy would involve starting the amniotic fluid cell culture in the usual fashion but completing it only if a low AFAFP is detected. This policy could be applied on all cell cultures regardless of the potential for chromosomal abnormalities (Hullin et al, 1985), or only on those not at an increased risk for chromosomal abnormalities (Cuckle et al, 1985b). Hullin et al reported that if their cytogenetic policy would have been adopted, none of the 37 cases of Down syndrome in their retrospective study would have gone undetected. Furthermore, only 500 karyotypic analyses would have been necessary as opposed to the 750 actually performed.

A major drawback to the above policy is that it applies only to cases of trisomy 21. As was already mentioned, only trisomy 21 is associated with a low AFAFP. In Hullin et al's study, 5 of 27 cases of non trisomy 21 aneuploidy would have the potential to proceed to term because of the inability to detect them. Furthermore, AFAFP offers no predictive value for the course of events in later gestation such as, fetal or neonatal death, preterm delivery, or low birth weight (Brumfield et al, 1987). Without a strong directive from the

American College of Obstetricians and Gynecologists and the American Society of Human Genetics, Hullin et al's policy would leave the obstetric community exposed to costly litigation (Evans et al, 1987).

A policy that terminates amniotic fluid cultures based on AFAFP levels may reduce the volume and cost of a cytogenetics unit that screens for Down syndrome, but such a policy would not reduce the actual number of false positives being spontaneously aborted due to the risk of amniocentesis. There are means by which the reduction of false positives can occur without interfering with the sensitivity of the screening program.

2.6. Reducing the Number of False Positives in an MSAFP Screening Program

One method to reduce the number of false positives in an MSAFP screening program for Down syndrome would be to correct for gestational age errors with the use of ultrasound biparietal diameter measurements (Cuckle et al, 1984). The reasoning behind this finding has to do with the fact that MSAFP increases with gestational age in the second trimester. Women with low MSAFP values will include pregnancies with trisomic fetuses as well as a relatively large proportion of unaffected fetuses with overestimated gestational ages. MSAFP values will be increased to normal levels in the unaffected pregnancies when the gestational age is corrected for by

ultrasound biparietal diameter measurements, thus reducing the number of false positives.

Another means of reducing the number of false positives was determined by Haddow et al (1981) and Wald et al (1981). They were both able to show that between 15 and 20 weeks gestation, MSAFP concentrations are influenced by maternal blood volume, as estimated by maternal weight. Heavier women have a significantly lower concentration of MSAFP presumably because their larger blood volume acts to dilute the AFP that passes into the maternal circulation (Haddow et al, 1981).

Palomaki et al (1985), showed that women aged 33, whose maternal weight is corrected for when undergoing MSAFP screening, have their odds for a Down syndrome pregnancy reduced from 1:170 to 1:150. Thus, an increase in specificity is obtained without a subsequent loss in sensitivity. A similar reduction in the number of false positives was determined in a New England collaborative research project studying the efficiency of a MSAFP screening program for the detection of Down syndrome pregnancies (Palomaki, 1986). Only 2.1% of all pregnant women under 35 whose maternal weight was adjusted were selected for amniocentesis in order to detect 21% of the Down syndrome pregnancies. This false positive rate is less than half of that obtained by Cuckle et al (1984). The reduction of false positives was also partly due to the use of an AFP assay kit that is more sensitive at the low end.

Macri et al (1986) were not able to verify the aforementioned finding that an inverse relationship exists between maternal weight and MSAFP concentrations. Data on over 81000 pregnancies did not support the use of correction formulas for maternal weight for those women undergoing MSAFP screening for neural tube defects. Furthermore, Macri et al state that if correction formulas for maternal weight would have been implemented in their study a larger and different proportion of women would have been considered at risk for neural tube defects, with a reduction in the detection efficiency (sensitivity). Macri et al did not, however, investigate the effects of applying maternal weight correction factors for those women at risk of having a Down syndrome pregnancy, i.e. with low MSAFP concentrations.

MSAFP concentrations are also known to be associated with ethnicity. Black women are associated with a MSAFP concentration that is on average 10% higher at each week of gestation (Crandall et al, 1983). Baumgarten (1986) noted that of 39259 MSAFP samples from white women, 1710 (4.4%) were interpreted as raised, whereas, in 3057 black women, 290 (9.5%) were considered raised, a statistically significant difference ($p<0.001$).

Macri et al (1987) also noted this racial difference in MSAFP concentrations, however, they did not recommend the use of a 10% uniform correction factor (for gestational weeks 14-22). The finding of a significant difference in the

variance of MSAFP concentrations between white and black women invalidates the use of a correction factor (Macri et al, 1987). This finding reveals that the amount of difference between black gravid women and others is by no means consistent through successive weeks of gestation. These findings may subject blacks to an increased risk of falling into the false positive category in the screening for neural tube defects. Macri et al emphasize the necessity of evaluating MSAFP concentrations in black gravid women on the basis of a distribution of MSAFP levels established in a suitable population of black gravid women. More studies should be performed in order to determine if racial differences in MSAFP concentrations effect the number of false negatives in the screening for Down syndrome at the low range of MSAFP values.

2.7. Retrospective Versus Prospective Studies

The staunchest critics of a MSAFP screening program that identifies fetuses at risk for chromosomal abnormalities have not been so harsh as to condemn its outright use, but merely suggest that it continue to be designated under an investigational status until collaborative prospective studies are completed (Macri, 1986; Lippman and Evans, 1987; and Wu, 1988). The studies that associated low MSAFP values with the risk for fetal chromosomal abnormalities have virtually all been performed retrospectively with the exception of Tabor et al (1984) and Baumgarten et al (1985). The variation in the

way the studies were carried out (use of different assay kits, correcting or not for gestational age, maternal weight, race, etc...) have led to variations in MSAFP levels associated with Down syndrome pregnancies, as was previously discussed (Spencer and Carpenter, 1985; and Hershey et al, 1985). Furthermore, these variations in the testing procedure have created uncertainties in the sensitivity and specificity of the screening program.

Baumgarten et al (1985) and DiMaio et al (1987) (both from the Yale group) contributed to some of the first studies to prospectively examine the association between low MSAFP and fetal trisomies. Their MSAFP screening program is based on the calculation of a woman's individual risk of carrying a Down syndrome pregnancy, taking into account maternal age, gestational age, MSAFP concentration, maternal weight and race. Women under 35 years of age were offered an amniocentesis if their individual risk for a Down syndrome pregnancy, based on the above criteria, equaled or exceeded the threshold risk of 1 in 270. This threshold risk is the approximated risk of Down syndrome based solely on the maternal age risk of a 35 year old woman. The results revealed that of 35797 MSAFP samples (96% of which were from women younger than 35 years), 1814 (5.3%) were considered to have a Down syndrome risk of 1 in 270 or greater (DiMaio et al, 1987). Of the couples that considered amniocentesis (76%), eight cases of trisomy 21, three cases of trisomy 18,

and one case of trisomy 13 were identified. One other case of trisomy 21 was identified at birth from the sample of women who refused the amniocentesis. In total then, of 5.3% of pregnant women who were offered an amniocentesis based on their individual risk of having a fetus with Down syndrome, the MSAFP screening program hereby employed, identified 9 of 27 cases (33%) of trisomy 21 as well as several other fetal trisomies.

DiMaio et al (1987) state that the 33% detection ability of the MSAFP screening program is complicated by incomplete ascertainment. Approximately 29% of fetuses with Down syndrome die between midtrimester and term (Hook et al, 1983). Assuming a 29% mortality rate for Down syndrome fetuses, 34 instead of 27 fetuses with trisomy 21 would have resulted, with a conservative estimate of 26% (9/34) being detected. These figures are comparable to those obtained in their retrospective study (DiMaio et al 1987), as well as other studies previously cited.

A New England collaborative group (Palomaki, 1986) performed their studies prospectively as well. Their study also offered amniocentesis to women whose individual risks for a Down syndrome pregnancy equaled or exceeded the 1:270 threshold risk. Of 51141 women aged under 35, 1050 (2.1%) were offered an amniocentesis which identified 10 of an approximated 48 (21%) fetuses with trisomy 21 as well as four cases of trisomy 18.

2.8. Down Syndrome Risk Tables Based on MSAFP and Age

The corroboration of the retrospective studies for MSAFP screening of Down syndrome by the prospective data initiated the construction of risk tables that combine both maternal age and MSAFP risk estimates for the purpose of counselling pregnant women whose risks are at or above specified cut-off values. Hershey et al (1986) were one of the first groups to construct such a table. Using a total of 165 cases of trisomy 21 from five studies, including their own data, Hershey et al applied Bayes' theorem for each maternal age to derive an estimate of the risk of Down syndrome using both age and MSAFP values. They suggest offering an amniocentesis to all women whose risk for a fetus with trisomy 21, based on her age and MSAFP value, is greater than the risk of a 35 year old woman, determined by her age alone (in their study this threshold risk was taken to be 1:365). As a refinement, the authors suggest adjusting the MSAFP values for maternal weights less than 90 lbs or greater than 160 lbs.

Palomaki and Haddow (1987a) agree with the risk table constructed by Hershey et al but emphasize that this risk table presents cumulative rather than individual risk estimates. They explain how this suggests that all women aged 33 with a MSAFP value less than or equal to 1 MoM will be offered an amniocentesis because the cumulative risk of having a fetus with trisomy 21 for such women is 1:347 which is greater than the threshold risk of 1:365. The fact is, a 33

year old woman with a MSAFP value of 1.0 will have a significantly lower individual risk (approximately 1:820) than a 33 year old woman with a MSAFP value of 0.4 whose risk is approximately 1:160. Using a cumulative risk table one would be referring a large number of women for amniocentesis when their individual risk estimates would not warrant such an approach.

Palomaki and Haddow (1987b) published their risk table based on the derivation of an individual woman's likelihood ratio for Down syndrome on the basis of her MSAFP measurement and age. The likelihood ratio is the proportion of trisomy 21 pregnancies with a particular MSAFP level divided by the proportion of unaffected pregnancies with the same level. The calculation of individualized odds can be assigned by combining the maternal age odds from published studies with the likelihood ratios calculated by Palomaki and Haddow (1987b). They cite an example of a 32 year old woman with a MSAFP MoM of 0.50. The woman's maternal age odds in the second trimester is 1:563 (from published studies). This odds ratio is then multiplied by the calculated likelihood ratio (2.37) corresponding to the MSAFP result, yielding a combined odds of 2.37:563 or 1:238. Not unlike the table published by Hershey et al, Palomaki and Haddow stress that the number of cases of Down syndrome used to construct such a table is very small and the need to update risk estimates with new cases will be imperative.

Cuckle et al (1987) calculated their risk tables using the same method of odds calculations as Palomaki and Haddow (1987b). However, separate estimates of likelihood odds based on MSAFP values were calculated using (1) the time since the first day of the last menstrual period and (2) an ultrasound biparietal diameter measurement. In each case this was done with and without adjusting MSAFP levels for maternal weight. Furthermore, they constructed six different MSAFP screening policies with cut-off levels chosen so that an amniocentesis would be offered when the risk of Down syndrome for an individual woman, based on the above criteria, equaled or exceeded risk levels set at 1:100, 1:150, 1:200, 1:250, 1:300, and 1:350 respectively for the six policies.

Once again, it was shown that the policy of combining maternal age and MSAFP measurements for the screening of Down syndrome is more efficient than screening on the basis of maternal age alone (Cuckle et al, 1987). However, Cuckle et al are concerned with the difficulty of altering the existing clinical practice of offering an amniocentesis to women based strictly on their age. Women over the age of 35 with a positive MSAFP result that does not include them in the "at risk group" may feel unjustified in not being offered an amniocentesis under the new screening policy. Cuckle et al (1987) suggest that such women can still be offered an amniocentesis while the new policy is being implemented and eventually expectations will change, and the problem diminish.

Tabor et al (1987) also created risk estimates of pregnant women carrying fetuses with trisomy 21 based on their age and MSAFP measurements. They developed an iso-risk curve which reveals, for women of all ages, which combinations of maternal age and MSAFP value yield a Down syndrome risk equal to or greater than 1:400 (the risk of fetal trisomy 21 based solely on the maternal age risk of a 35 year old women). Their policy would refer 9.4% of pregnant women in their study for an amniocentesis and would detect 53% of the fetuses with Down syndrome. However, this high detection rate is partly due to a low median MSAFP value (0.64 MoM) determined in 86 pregnancies affected with Down syndrome.

The aforementioned risk tables were shown by Hook (1988) to fluctuate in the 35-year-equivalent-risk values used to place women in the "at risk or not" categories. For instance at age 39, the 35-year-equivalent-risk value ranged from 1.12 MoM (Tabor et al, 1987) to 1.9 MoM (Palomaki and Haddow, 1987). Hook (1988) pointed out that according to these MSAFP values the proportion of 39 year old women that would be given risk figures below this threshold would vary from 7.1% to 38% respectively. Although this variation does not seem to affect the risk values for women less than 35 years of age, Hook suggests that extreme caution be used before employing any of the above risk tables for older women.

If one were to use the risk tables to counsel women concerning the risk of Down syndrome, one has the option of

employing either a cautious or conservative approach. The risk table provided by Tabor et al (1987) predicts the greatest increase in risk for younger women, and therefore, offers a cautious approach to counselling. On the other hand, if one were counselling conservatively, one would access the risk table of Palomaki and Haddow (1987b) which offers risk values predicted according to the smallest changes from risk values based on maternal age alone.

2.9. Maternal Serum Levels of Unconjugated Oestriol

The suggestion that a reduction in synthesis or secretion by the fetal liver is responsible for reduced levels of amniotic fluid and maternal serum AFP in Down syndrome pregnancies (Cuckle et al, 1986) prompted Canick et al (1988) to investigate whether other fetal liver products might also be reduced. Their study focussed on measuring maternal serum levels of unconjugated oestriol in both unaffected and Down syndrome pregnancies. Unconjugated oestriol is a steroid product of the placental unit synthesized in part by the fetal liver (Siiteri and MacDonald, 1966). A previous study had reported that total oestriol excretion in the third trimester was lower in Down syndrome pregnancies than in unaffected pregnancies (Jorgensen and Trolle, 1972).

Maternal serum unconjugated oestriol was measured in 22 pregnancies associated with Down syndrome and 110 unaffected pregnancies (five controls for each case of Down syndrome) matched for gestation, smoking habits, maternal weight, length

of freezer storage of samples, and maternal age (Canick et al, 1988). The median unconjugated oestriol MoM for the Down syndrome pregnancies was 0.79, a statistically significant decrease compared to the unaffected pregnancies ($p<0.05$).

Wald et al (1988a) reported on unconjugated serum oestriol levels in a further 77 pregnancies associated with Down syndrome and 385 unaffected control pregnancies. Once again, statistically significant reductions were detected in the unconjugated oestriol levels in serum from mothers bearing Down syndrome fetuses compared to the control pregnancies. The median level for the affected pregnancies was 73% of that in the controls ($p<0.001$).

Based on these findings, the authors were interested in determining whether maternal serum levels of unconjugated oestriol, like MSAFP and maternal age, could be incorporated into an antenatal program that screens for Down syndrome. Maternal serum levels of unconjugated oestriol were not found to be dependent on maternal age or MSAFP levels, therefore, they can be used independently or alongside the other screening variables in determining the relative risk a pregnant women has in bearing a fetus with Down syndrome (Wald, 1988a).

The employment of unconjugated oestriol to a Down syndrome screening program was found to be more efficient than the use of age and AFP alone. For instance, Wald et al determined that a screening practice based on maternal age and

MSAFP would detect 31% of the Down syndrome pregnancies by screening 3.8% of all pregnancies (based on a cut-off risk of 1:250). A screening practice based on maternal age and unconjugated oestriol would detect 41% of the Down syndrome population by screening 5.2% of all pregnancies. However, a Down syndrome screening policy based on all three variables would identify 45% of the Down syndrome pregnancies by screening 5.2% of the population of pregnant women. Furthermore, to achieve the same detection rate (45%) using age and MSAFP levels alone, a 1:430 risk cut-off level would be required which would yield a false positive rate of 9.8%. It is therefore, apparent that the implementation of a screening program using the three screening variables would require less women to undergo an amniocentesis with more Down syndrome pregnancies being detected than a screening program based on MSAFP and age alone.

2.10. Maternal Serum Levels of Human Chorionic Gonadotropin

Previous reports of an association between low levels of human chorionic gonadotropin (hCG) and pregnancies resulting in spontaneous abortions (Brody and Carlstrom, 1965) prompted Bogart et al (1987) to evaluate the possibility that protein levels of hCG in maternal serum were associated with chromosomal abnormalities. Maternal serum levels of hCG were measured in 25 pregnancies with chromosomal anomalies (17 with Down syndrome) and 74 chromosomally normal pregnancies. Due to the finding that there is no significant difference in mean

hCG concentration from 18 weeks of gestation to term (Braunstein et al, 1976), the median value for all normal controls was used for hCG MoM calculations. Of the 25 pregnancies with chromosomal abnormalities 14 (56%) had maternal serum hCG levels ≥ 2.5 MoM, while only 1 of 74 (1.35%) normal pregnancies had maternal serum levels ≥ 2.5 MoM. Focussing only on pregnancies with trisomy 21, 11 of 17 (65%) cases had maternal serum hCG levels ≥ 2.5 MoM.

Wald et al (1988b) furthered the above study by determining maternal serum hCG levels in 77 Down syndrome pregnancies and 385 unaffected pregnancies matched for maternal age, gestational age, and duration of storage of the serum sample. The median hCG concentration in the Down syndrome pregnancies was 2.04 MoM, a statistically significant increase compared to the unaffected pregnancies ($p<0.001$).

Once again, the group of Wald et al (1988b) ascertained the efficiency of using a biochemical marker, maternal serum levels of hCG, in the screening for pregnancies associated with Down syndrome. They determined that because none of the screening variables previously discussed are strongly correlated with one another, each can provide an independent measure of risk. The greatest Down syndrome detection rate was accomplished using various cut-off levels for each of the three biochemical variables along with maternal age risk estimates. The best combination (using a threshold risk of 1:250) yielded a detection rate of 60% with a false positive

rate of 5%. Such a policy, if adopted generally, would reduce the number of Down syndrome births in the United Kingdom from 900 to approximately 350 a year (Wald et al, 1988b).

The cost effectiveness of an antenatal screening program for Down syndrome using serum levels of MSAFP, unconjugated oestriol, and hCG was also evaluated by Wald et al (1988b). They explain that at any level of detection, the extra cost of the biochemical assays would be significantly less than the cost of the amniocentesis and karyotypes that would be needed to obtain the same detection rate. They provide an example which explains that in order to achieve a 60% detection rate in 1000 pregnancies screened using age and the biochemical assays, 48 (47 unaffected and approximately one affected) would require an amniocentesis compared with 201 (200 affected and approximately one affected) using age and MSAFP levels alone. The revenue saved by performing 153 less amniocenteses would pay for approximately 5000 hCG and unconjugated oestriol assays (the cost of the MSAFP assay is not included because most centres routinely perform the test to screen for neural tube defects).

2.11. Future Directions

The efforts put forward in the determination of a woman's risk of carrying a Down syndrome fetus based on her age, and serum levels of MSAFP, hCG, and unconjugated oestriol are preliminary. Large international collaborative studies are presently underway in order to determine whether the screening

policies proposed by Wald et al (1988b) will retain their efficacy when applied in a prospective fashion.

Studies are also underway to evaluate the possibility of employing the aforementioned screening policy during the first trimester. Preliminary data from four studies (based on 22 cases) (Brambati et al, 1986) (Barkai et al, 1987) (Cuckle et al, 1988) (Wald and Cuckle, in press) have revealed that MSAFP is also significantly lower in Down syndrome pregnancies in the first trimester compared to age matched controls. One study did not support such a finding (Scioscia et al, 1987). Furthermore, Cuckle et al (1988) reported that serum unconjugated oestriol, but not hCG, may also be useful in the first trimester screening for Down syndrome. A large international multicentre study is in the process of collecting data on approximately 100 affected and several thousand unaffected pregnancies. It will become apparent upon the publication of the prospective studies as to whether prenatal diagnostic centres will be able to put to use the newly proposed screening policies.

3. Sonographic Screening for Down Syndrome

The increasing use of ultrasound in the second trimester has prompted the search for sonographic signs that will aid in differentiating the fetus with Down syndrome from the normal fetus. Recognition of these sonographic signs at an early enough stage in gestation would render the woman at risk eligible for an amniocentesis and thus, upon determination of the fetal karyotype, offer her important options concerning the management of her pregnancy. The following is a list of some of the more promising sonographic markers that have been associated with Down syndrome.

3.1. Duodenal Atresia

One sonographic sign that has the potential to identify a large proportion of Down syndrome fetuses is the presence of duodenal atresia. Approximately 30% of livebirths affected with duodenal atresia also have Down syndrome (Fonkalsrud et al, 1969; Potts and Darstin, 1986). This frequency should be higher in the second and third trimesters of pregnancy due to the large number of Down syndrome fetuses that are spontaneously aborted before birth (Hook et al, 1983).

Loveday et al (1975) were the first to describe ultrasound B-scan appearances of duodenal atresia in utero. Two fluid filled levels were noted, one in the stomach, the other in the distended duodenum proximal to the atretic

segment. These images make up the classical "double bubble" appearance that is characteristic of duodenal atresia.

There is some question as to whether the prenatal diagnosis of duodenal atresia can be made early enough in gestation so as to permit elective termination of a fetus with trisomy 21. The average gestational age of 12 cases of duodenal atresia and one case of ileal atresia, detected antenatally, was 33.7 weeks (Miro and Bard, 1988). The earliest antenatal diagnosis in this study was made at 27 weeks. Other reports confirm the diagnosis of duodenal atresia at 22 weeks (Romero et al, 1988) and 22.7 weeks (Balcar et al, 1984).

The difficulty in diagnosing duodenal atresia in the second trimester may be a result of the fetus not swallowing a sufficient amount of fluid to cause dilatation of the stomach at the time of the sonogram (Nelson et al, 1982). At 28 weeks of gestation the normal fetus swallows almost three times the amount of amniotic fluid in 24 hours as does a fetus at 16 weeks of gestation (Pritchard, 1966). At approximately 29 weeks the volume of amniotic fluid swallowed by the fetus with duodenal atresia may exceed the resorptive capacity of the gut and result in the dilatation of the fetal stomach (Nelson et al, 1982).

A diagnosis of Down syndrome in the third trimester is at too late a stage to offer elective pregnancy termination. However, the prenatal diagnosis of both duodenal atresia and

Down syndrome in the third trimester may benefit the parents by knowing in advance that they have a chromosomally abnormal fetus (Romero et al, 1988). Furthermore, the prenatal diagnosis of a chromosomally normal infant with duodenal atresia may alter the neonatal morbidity by decreasing the delay before corrective surgery and thus decreasing the chances of metabolic complications associated with the disorder (Miro and Bard, 1988; Romero et al, 1988).

With the improvement in echographic equipment and expertise of the sonographer it is not inconceivable to think that in the near future the diagnosis of duodenal atresia will be consistently made at a time in gestation that would allow a mother to undergo an amniocentesis to reveal the karyotype of her fetus and still have the opportunity to alter the obstetric management upon the diagnosis of a Down syndrome fetus (Miro and Bard, 1988). However, the benefit of routine screening of every pregnant woman for duodenal atresia has not yet been demonstrated (Barss, 1985). Similar prospective studies to those performed for MSAFP screening for Down syndrome should be carried out in order to determine the relative risk a pregnant woman has in carrying a Down syndrome fetus when that fetus is affected with duodenal atresia. Determination of the sensitivity, specificity, and positive predictive value is crucial before routine screening for Down syndrome by sonographic means can be administered.

3.2. Congenital Heart Defects

A second sonographic sign associated with Down syndrome involves the presence of congenital heart defects. The incidence of congenital heart defects in neonates with trisomy 21 is approximated at 50% (Nora and Nora, 1978; Copel et al, 1986). Some of the more common of these lesions are persistent atrioventricular canal, ventricular and atrial septal defects, tetralogy of Fallot, and patent ductus arteriosus (Balcar et al, 1984). The four-chamber view of the heart has recently been proposed as a sensitive (92%) screening method for the detection of congenital heart disease (Copel et al, 1987) as has the practice of Doppler echocardiography (Yagel et al, 1988; Shenker et al, 1988).

Copel et al (1988), using echocardiographic techniques, prenatally diagnosed 34 fetuses with congenital heart defects of which 11 (32%) were aneuploid. Two cases of congenital heart defects were detected in a total of 12 (17%) trisomy 21 fetuses. In the first case, a complete atrioventricular septal defect was diagnosed at 24 weeks of gestation and in the second case a ventricular septal defect and transposition of the great arteries were diagnosed in a fetus at 18 weeks of gestation. The small number of trisomy 21 fetuses sampled may explain the difference in the incidence of congenital heart defects in this study compared to others in the literature.

Copel et al (1987) suggest that fetal echocardiographic screening using the four-chamber view should be included as a

part of all routine obstetric ultrasound examinations. Furthermore, the identification of congenital heart defects in second trimester fetuses with trisomy 21, as well as other aneuploidies, have led some to propose that karyotypic analysis should be performed on all fetuses with congenital heart defects (Copel et al, 1988). At this time it is premature to offer women whose fetuses have congenital heart defects an amniocentesis without further prospective studies to determine the actual risk of Down syndrome once a congenital heart defect is diagnosed.

3.3. Increase in Nuchal Skin Thickness

A third sonographic marker associated with Down syndrome involves the detection of an increase in skin or soft tissue thickening at the back of the neck. Hall (1966) reported that excess skin at the back of the neck was present in 80% of the Down syndrome neonates examined. Benacerraf et al (1985a, b; 1987a) set out to determine whether the sonographic marker in question was present in second trimester fetuses.

To measure the soft tissue thickness behind the occiput, transverse-view scans of the fetal head were obtained at the level needed to measure the biparietal diameter. The plane of section was then angled downward to include the occiput at the level of the cerebellum. A nuchal fold of 6 mm or more was considered abnormal (Benacerraf et al, 1987a).

In their original report, Benacerraf et al (1985a), retrospectively examined the association between increased

nuchal skin thickening and Down syndrome. Of 904 amniocenteses performed for genetic evaluation, six cases of Down syndrome (trisomy 21) were diagnosed karyotypically. Of these six cases, two (33%) had excess skin or soft tissue at the back of the neck. One other instance of increased nuchal skin thickening was detected in a karyotypically normal fetus, representing a false positive rate of 0.1%.

In order to further test the sensitivity and specificity of this sonographic sign, a large prospective study was performed consisting of routine views of the neck area (Benacerraf et al, 1987a). A total of 3825 consecutive fetuses aged between 15 and 20 weeks, undergoing an amniocentesis because of maternal age, low MSAFP, or other risk factors for Down syndrome, revealed 21 cases of Down syndrome. Of these 21 cases of Down syndrome, 9 (43%) displayed an abnormally thickened nuchal fold. Three of these nine cases with increased nuchal fold thickening also had other abnormalities: one had generalized hydrops and a cardiac abnormality; one had multiple congenital abnormalities (tetraploidy 21); and one had hydrocephalus. The remaining six cases had the increased occipital skin thickening as the only abnormality detected. There were four false positive findings among the remaining 3804 fetuses analyzed (0.1%). One of the false positives had a 5p+ karyotype.

In their studies, Benacerraf et al (1987a) have shown that a nuchal skin thickening of 6 mm or more has a

sensitivity of 43% and a specificity of 99.9% in the detection of Down syndrome pregnancies. The positive predictive value is 69%. Although the sonographic sign of abnormally thickened nuchal skin in the second trimester is not sufficient for a definitive diagnosis of Down syndrome, it has been successfully shown that this marker could be useful in identifying among younger women, those who are at an increased risk of bearing a Down syndrome conceptus, thereby making them candidates for amniocentesis.

Toi et al (1987) also examined the association between excess nuchal skin and Down syndrome. They studied 11 Down syndrome abortuses whose menstrual ages ranged from 18 to 23 weeks. Specific attention was placed on examining any external soft tissue abnormalities in the region of the neck. Only two instances (18%) of abnormally thickened nuchal skin were detected.

Toi et al (1987) also disclosed that increased nuchal skin thickening was not found to be as specific for Down syndrome as was originally determined by Benacerraf et al (1987a), and Benacerraf and Frigoletto (1987). Of 28 cases of normal fetuses between the menstrual ages of 18.5 and 25.5 weeks, six (21%) had nuchal skin thickening greater than 5 mm (range: 6 to 12 mm). Benacerraf and Frigoletto (1987) reported that the width of the nuchal fold in 303 consecutively normal fetuses was consistently between 1 and 5 mm regardless of gestational age. Such a drastic difference

in the specificity of increased nuchal skin thickening in the detection of Down syndrome fetuses between the results of Benacerraf et al and Toi et al must be due to differences in the methods used to measure nuchal tissue. Toi et al state that they were able to deliberately create an image of a thickened nuchal fold by varying the angle of the plane of section used to measure the amount of skin at the nape in seven karyotypically normal fetuses. It appears that the two groups have different criteria when measuring sonographically determined nuchal skin.

The discrepancy in specificity between the two groups can be further explained by the gestational age differences in the fetuses studied. Toi et al measured nuchal tissue in the late second and early third trimesters, whereas Benacerraf et al surveyed mid-second trimester fetuses. Fetuses in the third trimester can have what appears to be a thickened nuchal fold when in a deflexed attitude. This apparent increase in nuchal skin may be the result of such a position effect and would thereby explain the increases in nuchal tissue observed by Toi et al (Benacerraf, 1987b).

Comments by Toi et al (1987) also focused on the high positive predictive value (69%) found in Benacerraf et al's studies. The incidence of Down syndrome was extremely high (1:155) in one of their sample populations (Benacerraf et al, 1985b). The general population has an incidence of Down syndrome of 1:660, and it is even lower in women under 30

years of age (1:1500). A lower incidence will not affect the stable properties of sensitivity and specificity but will result in a lower positive predictive value (Toi et al, 1987). Toi et al calculated the expected performance of the sonographic test in the lower risk populations and concluded that a positive test would predict Down syndrome in only 20% of fetuses. This means that 80% of the pregnancies that would undergo an amniocentesis because of a sonographically determined thickened nuchal fold would result in a normal karyotype. Although these results may seem poor, they still fare better than the maternal age screening programs commonly in practice.

A recent report by Rodis et al (1988) suggests that the redundart skin of the fetal neck may in fact represent early cystic hygromas that have resolved in utero before 16 weeks of gestation. Cystic hygromas are associated with chromosomal aneuploidies, particularly with Turner syndrome and to a lesser degree with Down syndrome (Pearce et al, 1984). Whether they are the cause of the webbed neck or excess nuchal skin observed in these two anomalies is still an unresolved question. Benacerraf et al (1985a) point out, however, that cystic hygromas differ readily from nuchal skin thickening. Cystic hygromas are known to have a masslike consistency, whereas, nuchal skin thickening is symmetrical and flat rather than protuberant and cystic. In any case, all occurrences of sonographically detected cystic hygromas should be followed up

by an amniocentesis because of their strong association with Turner syndrome. Any additional cases of Down syndrome diagnosed due to the occurrence of a cystic hygroma will be a welcome bonus.

3.4. Nonimmune Hydrops Fetalis

This condition is characterized sonographically by the appearance of fluid accumulation in serous cavities (ascites, pleural effusion, pericardial effusion) and/or edema of soft tissue in the absence of a fetomaternal blood group incompatibility (Vintzileos et al, 1987). Numerous maternal, fetal, and placental problems are known to cause the condition, however, the majority of cases are still considered idiopathic. The more common of the known causes include fetal cardiac anomalies (20%), chromosomal disorders (16%) (including trisomies 21, 18, and 13, Turner syndrome, triploidy, etc.), malformation syndromes (11%), and the twin-twin transfusion syndrome (10%) (Vintzileos et al, 1987).

The association between nonimmune hydrops fetalis and Down syndrome has not been as well documented as with the previously mentioned sonographic markers, most probably due to the failure in obtaining fetal karyotypes when the condition presented itself (Fujimoto et al, 1983). Mahoney et al (1984) identified two occurrences of trisomy 21 out of 27 cases of nonimmune hydrops fetalis (7.4%). In another series Holzgreve et al (1984) found two cases of trisomy 21 out of 50 cases of hydrops (4%). Other findings of Down syndrome fetuses with

hydrops have been reported by Fujimoto et al (1983) (six cases), Perlin et al (1981) (one out of eight cases), and Hutchison et al (1982) (two out of 61 cases).

Chromosome analysis is commonly performed when a prenatal finding of nonimmune hydrops fetalis occurs because of previous reports of an association with trisomy 18 as well as Turner syndrome. The fortuitous finding of cases with trisomy 21 is an added bonus.

3.5. Ratio of Biparietal Diameter to Femur Length

The aforementioned sonographic markers, whose use in the exposure of fetuses at risk for Down syndrome has been revealed above, all share a certain technical difficulty in their ability to be detected. For this reason, highly trained sonographers with years of experience would be required to screen hundreds of fetuses in order to achieve any level of success in the screening of fetuses at risk for Down syndrome. Lockwood et al (1987) have proposed a simple, readily reproducible method of sonographic screening to differentiate Down syndrome from normal fetuses. This new sonographic sign involves the use of standard ultrasound biometry, in the form of biparietal diameter (bpd) and femur length measurements, with the premise being that Down syndrome fetuses have increased bpd/femur length ratios when compared to normal fetuses.

Lockwood et al (1987) retrospectively evaluated the bpd, femur length, and bpd/femur length ratio in 55 fetuses with

Down syndrome and 544 control fetuses at two medical centres. All fetuses were between 15 and 23 weeks of gestation. Pregnant patients were referred for an amniocentesis for reasons including advanced maternal age, abnormal MSAFP concentrations, and a family history of genetic disorders. The bpd was measured with electronic calipers from the outer edge of the skull to the inner edge of the other side of the skull, across the thalami. Femur lengths were also measured with electronic calipers upon visualization of the femur from the greater trochanter to the end of the ossified shaft, perpendicular to the long axis of the bone. Data from the two medical centres had to be analyzed independently due to apparent differences in the methodology of femur length measurements.

The bpd/femur length ratio was consistently higher in the Down syndrome population for all gestational ages studied at both medical centres. This finding was due to shorter femur lengths in Down syndrome fetuses and not because of differences in bpd measurements. No statistically significant differences were detected between cases and controls for mean gestational age, cephalic index, or bpd. This finding is consistent with that of Perry et al (1984), who also found no significant differences in the cephalic index between second trimester Down syndrome fetuses and controls. The bpd/femur length ratios were however, found to be better predictors of Down syndrome fetuses than femur length alone. The bpd/femur

length ratio was also noted to decrease with gestational age in the control groups from both centres.

In one of the medical centres, comprising a sample population from New Haven, 18 out of the 35 Down syndrome fetuses (51%) had bpd/femur length ratios 1.5 standard deviations (SD) above the mean of the control population for a given gestational age. Of the 349 control cases evaluated, 26 were also above 1.5 SD of the mean, for an overall false positive rate of 7%. Similarly, in the other medical centre, comprising a Boston population, 14 out of the 20 Down syndrome fetuses (70%) had bpd/femur length ratios 1.5 SD above the mean. The false positive rate was 5% (9/195).

A Down syndrome screening policy based on selecting women whose fetuses have a bpd/femur length ratio that is 1.5 SD above the control population mean (New Haven), for an amniocentesis, given a general population incidence for Down syndrome of 1/710 in the second trimester, would identify one case of Down syndrome out of 103. This screening policy has a greater positive predictive value for a general population than do maternal age screening policies for high risk (1/270) populations. In a high risk population (women $\geq$ 35 years of age) the positive predictive value of the bpd/femur length ratio is 1/37 (Lockwood et al, 1987).

The use of a sonographic marker that relies on standard biometric analyses has great potential in the screening of fetuses at risk for Down syndrome. Both bpd and femur length

measurements are recorded during a routine ultrasound examination in the second trimester. For this reason, neither sophisticated training nor expensive equipment are required to obtain the necessary parameters in order to differentiate between Down syndrome and normal fetuses (Lockwood et al, 1987). Furthermore, a screening policy based on bpd/femur length ratios has a potentially higher sensitivity, specificity, and positive predictive value than does a policy based on low MSAFP concentrations combined with maternal age risk estimates.

3.6. Combining Several Sonographic Signs

The echographic screening for pregnancies affected with Down syndrome could be greatly improved by detecting several sonographic markers that are characteristic albeit nonspecific for Down syndrome. "The physical diagnosis of Down syndrome is an impressionistic one based on the sum of numerous abnormalities, none of which is specific to or unique in Down syndrome" (Shapiro, 1983). This, along with the fact that there exists a wide range of variability in each of the physical and developmental characteristics of Down syndrome (Levinson et al, 1955), make a clinical diagnosis based on any single characteristic very difficult. Therefore, the physical identification of Down syndrome must be based on the occurrence of a large number of nonspecific abnormalities (Lee, 1972; Smith and Berg, 1976; Rex and Preus, 1982; Shapiro, 1983).

An example of such an application is found in a paper by Benacerraf et al (1987c). They have combined the sonographic findings of a thickened nuchal fold (>5 mm) and shortened femur length (as a function of the biparietal diameter) to form a sonographic screening policy that is 75% sensitive and 98% specific in the detection of fetuses at risk for Down syndrome. When combined with other congenital abnormalities (atrioventricular canal defects, meconium peritonitis, hydrops, cystic hygroma, and hydrocephalus) the sensitivity for the detection of Down syndrome rose to 82% without a subsequent loss in specificity. The positive predictive value was determined for three different risk groups and all fared much better than maternal age or MSAFP screening policies.

Balcar et al (1984) were also aware of the necessity to detect combinations of defects associated with Down syndrome in order to make a clinical diagnosis. They state that the combination of cardiac and gastrointestinal abnormalities are not specific for Down syndrome, but are also present in the VATER association, asplenia, de Lange, trisomy 13, trisomy 18, triploidy, and partial trisomy 10q syndromes (Smith, 1982). Nonetheless, they suggest that the particular combination of duodenal atresia and common atrioventricular canal is unique to Down syndrome. They go on to report the sonographic detection of a fetus at 22 weeks of gestation with duodenal atresia, an incomplete interventricular septum, and absence of the interatrial septum. Based on this diagnosis and the

association of the above defects with Down syndrome, the parents elected to terminate the pregnancy. Amniocentesis performed before prostaglandin infusion, resulted in a 47,XY +21 karyotype.

3.7. Future Directions-Risk Scoring

In order to more accurately classify a pregnancy at risk for Down syndrome, a method of quantifying the risk status of a fetus should be developed. Numerical values could be assigned for each of the various factors associated with Down syndrome. The risk score would be statistically related to the probability that a fetus has Down syndrome. Therefore, if the total risk score exceeds some preset risk score, then the fetus in question will be eligible to undergo an amniocentesis to determine its karyotype. For example, a pregnant woman of a particular age with a MSAFP measurement of a particular concentration, is bearing a fetus with a sonographically detected duodenal obstruction. Based on these three criteria the woman would be assigned a particular risk score which when converted to a probability, would determine her risk of bearing a fetus with Down syndrome. In this way, a risk score could be obtained for every fetus observed by an obstetrician with access to this system.

The Problem Oriented Perinatal Risk Assessment System (POPRAS) is a risk scoring strategy that is in current practice to identify pregnancies at risk for perinatal demise (Hobel and Merkatz, 1985). Risk scores for selected variables

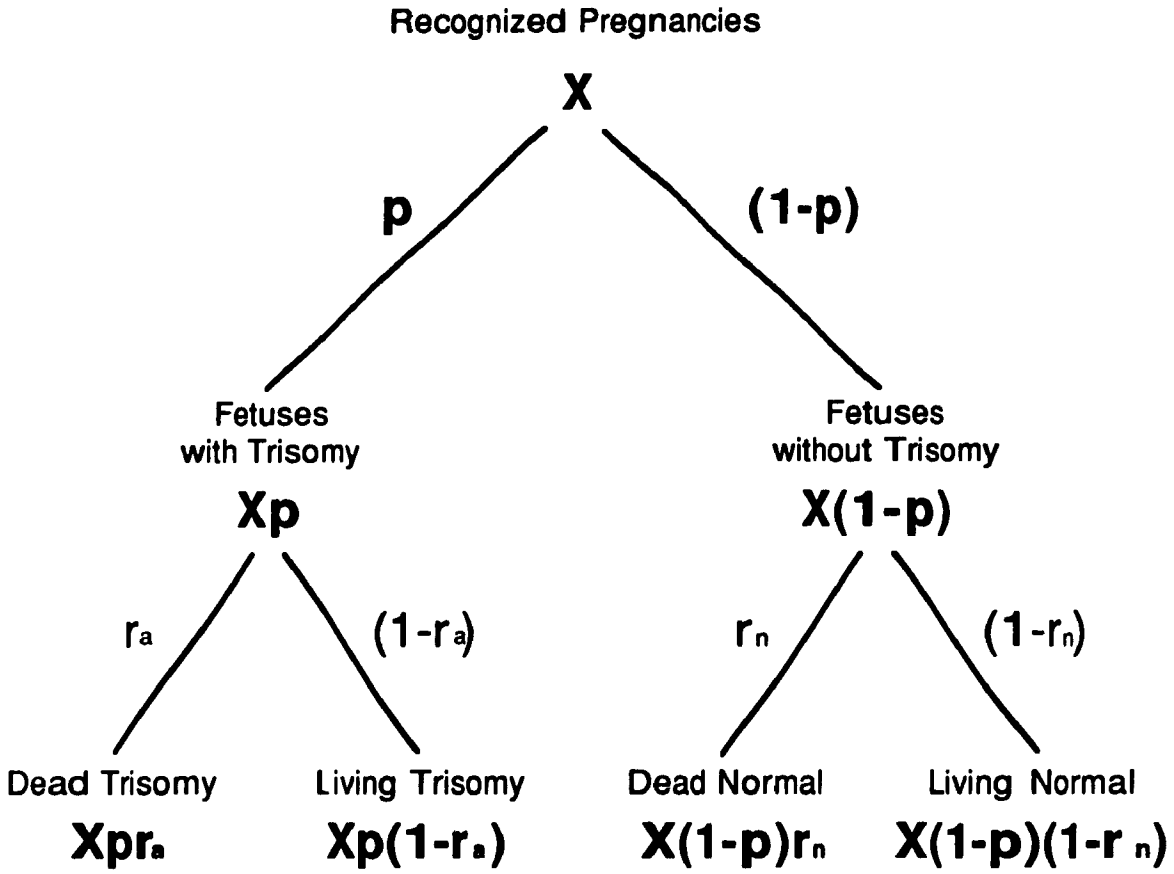
are calculated using a stepwise logistic multiple regression analysis. This function enables the compilation of the probability of a poor perinatal outcome conditional on a number of risk factors (Hobel and Merkatz, 1985). It is not inconceivable to establish a comparable risk scoring strategy for pregnancies associated with Down syndrome. In fact, Boué et al (1980) designed an exemplary table which had the potential to determine risk scores for pregnancies associated with Down syndrome. Their table was developed to determine risks for Down syndrome fetuses early in gestation, but relied on some factors that did not differ significantly between normal and Down syndrome fetuses.

Section II. Experimentation

1. INTRODUCTION

The survival of the products of fertilization through embryonic and fetal development until birth is dependent upon several factors: firstly, the genetic makeup of the conceptus; secondly, the maternal environment; and thirdly, interactions between the two. When a pregnancy fails much of the primary focus is directed towards the abortus to reveal a possible cause for such a failure, and with good reason for between 40% and 60% of all first trimester abortions are due to fetal chromosomal abnormalities of various types (Boué et al, 1985). Occasionally, however, the same chromosomally aberrant fetus will not result in a spontaneous abortion. This may suggest that the survival of such a fetus is dependent on more than just its genetic complement. The fact that it has not been selectively eliminated supports the possibility that its survival is partially determined by either maternal attributes or an interaction between fetal and maternal attributes. The experiments discussed in the following pages concentrate on analyzing the factors controlling the survival of aneuploid fetuses in the mouse.

Stein et al (1975) proposed a simple model depicting the distribution, among spontaneous abortions and births, of conceptuses with and without anomalies, such as aneuploidy (Figure 1). A recognized conceptus designated as X, has a probability p, of suffering from some kind of aneuploidy, such as trisomy. The expected number of fetuses (X) with trisomy



Xp = expected number of fetuses with trisomy

r_a = probability that a trisomic fetus will abort spontaneously

r_n = probability that a normal fetus will abort spontaneously

$$F = \text{Observed Rate of Trisomy} = \frac{\text{Living Trisomy}}{\text{Total Living}} = \frac{Xp(1-r_a)}{Xp(1-r_a)+X(1-p)(1-r_n)}$$

Figure 1: DISTRIBUTION AMONG LIVING AND DEAD, OF FETUSES WITH AND WITHOUT TRISOMY ***(Modified from Stein et al, 1975).***

is equal to Xp, and the expected number of fetuses without trisomy is equal to X(1-p). Fetuses with trisomy have a probability r_a of being spontaneously aborted, and a probability of $1-r_a$ for being born. The probability of a normal fetus, without trisomy, being spontaneously aborted is equal to r_n, and the probability of a normal fetus being born is $1-r_n$.

From this model it can be inferred that the prevalence of trisomy at a particular time in gestation is dependent on only two factors: (1) the incidence of that defect at conception (Xp); and (2) the probability that such an anomalous conceptus will survive until the time of observation, which is represented as $F\ [Xp(1-r_a)/Xp(1-r_a) + X(1-p)(1-r_n)]$ (Figure 1). By regulating the incidence of aneuploidy at conception, it is possible to systematically analyze the factors controlling the survival of an aneuploid conceptus (Vekemans and Trasler, 1987).

1.1. The Mouse as a Model

A mouse model for the study of aneuploidy has been particularly useful for these types of studies. A trisomic state can be experimentally induced for virtually any of the 20 pairs of acrocentric mouse chromosomes (Gropp, 1982; Epstein, 1986). The generation of specific types of aneuploidy is dependent on mouse strains carrying different Robertsonian translocations.

The breeding scheme utilized in producing trisomic animals involves the mating of parental mice each homozygous for a particular Robertsonian translocation which have a chromosome arm in common (Figure 2). The offspring produced from such a mating will be heterozygous for two Robertsonian translocations with partial homology for one of their arms. As a consequence, when gametes from the offspring partake in the first meiotic prophase a quadrivalent is formed which often leads to the nondisjunction of the Robertsonian translocation chromosomes depending on their mode of segregation during the first meiotic anaphase (Figure 3).

Alternate segregation of the chromosomes will give rise to balanced gametes and zygotes when fertilized by a wildtype gamete. Adjacent I segregation will give rise to gametes that are either hypo or hyperhaploid for the chromosome arm in common on the Robertsonian translocation chromosomes. Upon fertilization with wildtype gametes, both monosomic and trisomic zygotes will be formed. Finally, adjacent II segregation of the metaphase chromosomes will give rise to gametes that when fertilized by wildtype gametes, produce zygotes that are both monosomic and trisomic for the particular chromosome arms used for the Robertsonian translocation chromosomes. Neither the fertilized products of the adjacent II segregants, nor the monosomic embryos survive past the implantation stage of gestation. Therefore, only the diploid and trisomic conceptuses were available for study.

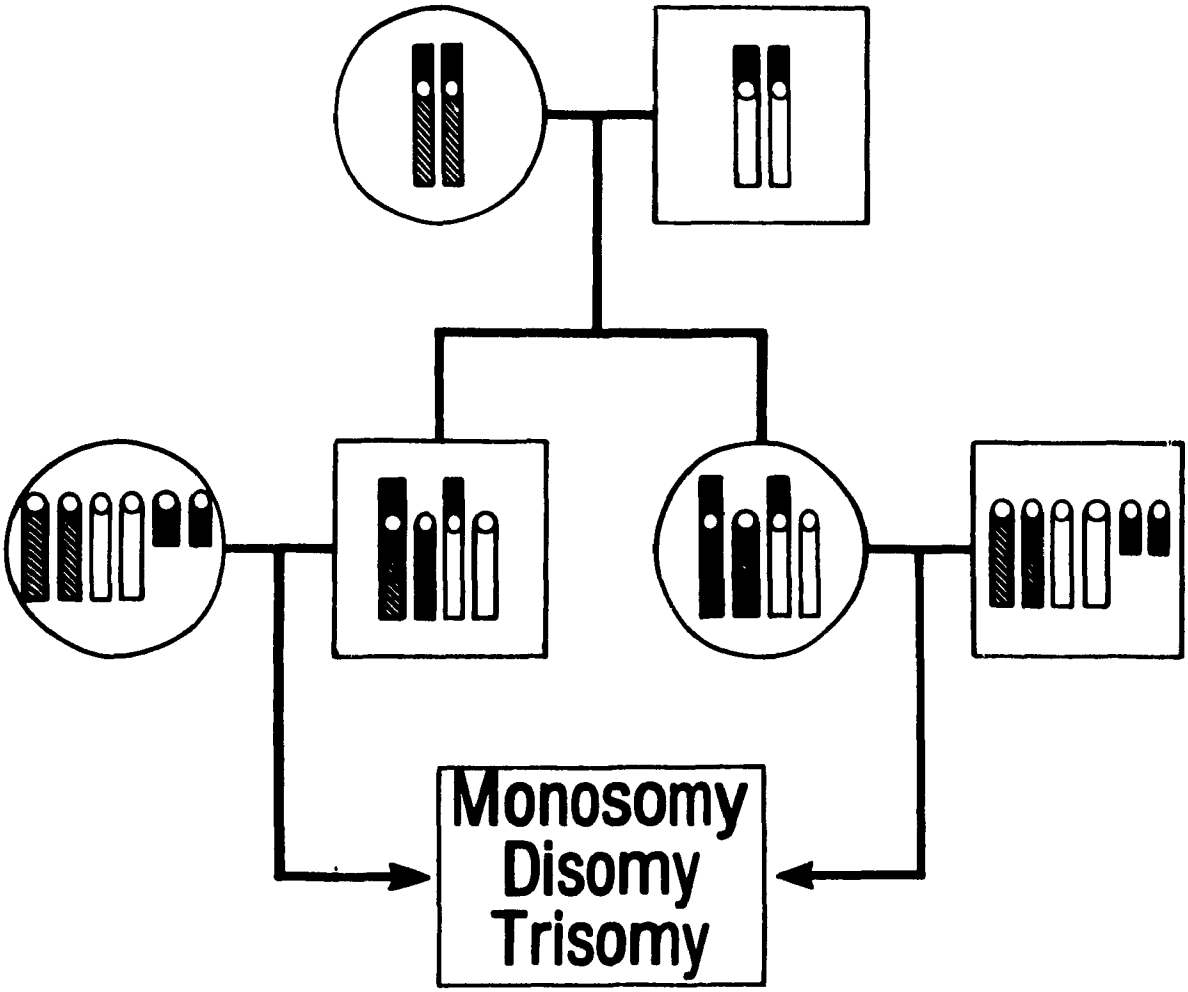


Figure 2: Breeding Scheme for the Production of Mice Heterozygous for Two Robertsonian Translocations with Monobrachial Homology.

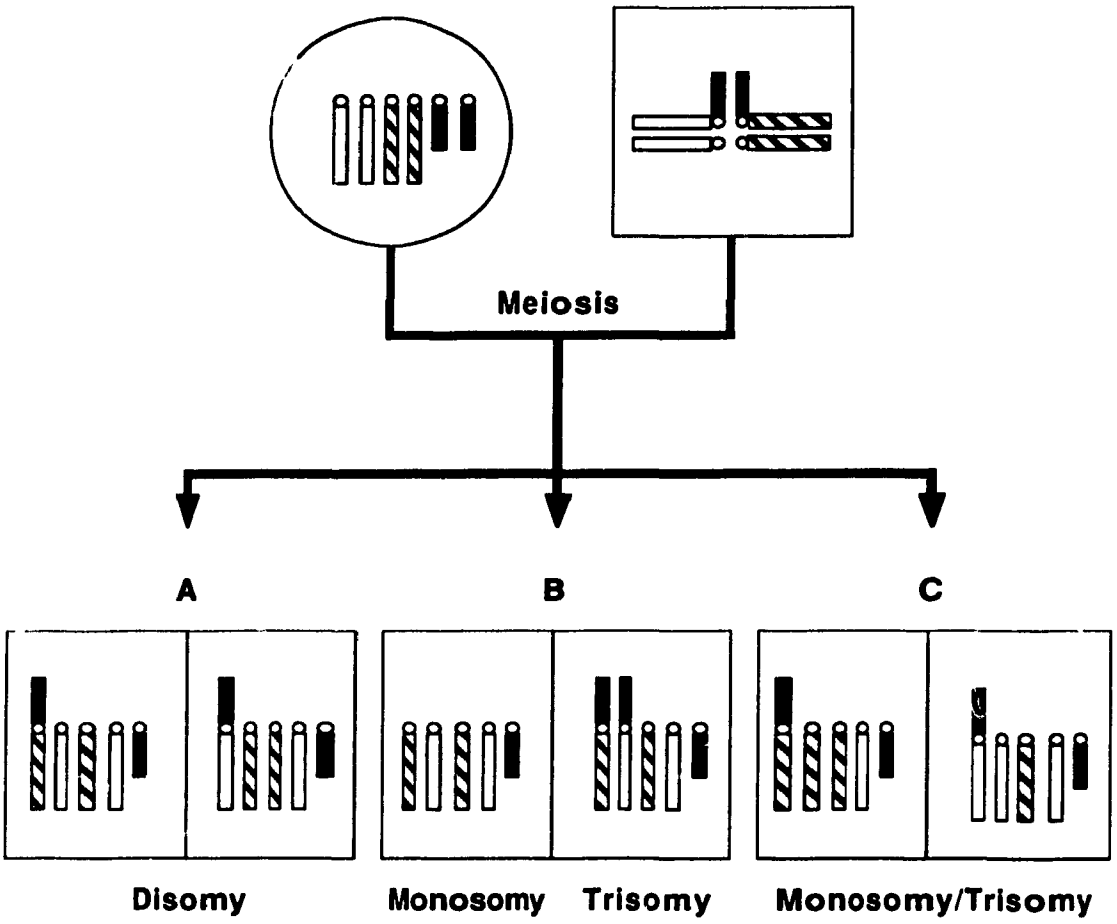


Figure 3: Scheme of chromosome disjunction in the progeny from a cross between a male mouse heterozygous for two Robertsonian translocations with monobrachial homology and a wildtype female mouse. Meiotic segregation of the Robertsonian translocation chromosomes are depicted by: A) Alternate segregation; B) Adjacent I segregation; C) Adjacent II segregation.

(Modified with permission from C. Jacob)

1.2. The Proposal

By following the aforementioned breeding scheme for the production of aneuploid conceptuses, Vekemans and Trasler (1987) investigated the possibility that the survival of fetuses trisomic for chromosome 19 in the mouse was under genetic control. They disclosed that the prevalence of living trisomy 19 fetuses on day 15 of gestation varied significantly with the genetic background of the female parent.

What remained to be answered by this model was whether the genetic factors which control the survival of trisomy 19 conceptuses are specific for the type of aneuploidy in question, or whether they act through a common mechanism for all types of aneuploidy. In responding to this question, it was decided to investigate the factors controlling the survival of another one of the murine trisomies, in this case, trisomy 16. If genetic factors are implicated in the survival of murine trisomy 16 conceptuses, then comparisons can be made between the maternal strains that select against, or promote the survival of both murine trisomies 16 and 19.

Murine trisomy 16 was selected as a representative model for aneuploidy in this study because of its ability to survive close to term, and the fact that it is one of the best studied of the mouse trisomies. Others consider murine trisomy 16 to be a model for Down syndrome in humans due to the homology of several genes on murine chromosome 16 and the "Down syndrome region" on human chromosome 21 (Epstein et al, 1985; Reeves et

al, 1986; Reeves et al, 1987). It must be noted however, that regions of human chromosomes 3, 7, 16, and 22 also share some homology with mouse chromosome 16. Murine trisomy 16 is hereby used as a representative model of aneuploidy, and should not be considered as a mouse model for Down syndrome.

2. Materials and Methods

2.1. Mice: Source and Maintenance

The study of genetic influences on biological systems necessitates the strict control of all environmental variables. In order to distinguish the genetic effects from those due to the surrounding environment, the mice used for experimental purposes in this study were maintained in a constant environment. Their housing, diet, light cycle, and temperature were all sustained with minor variation.

The inbred mouse strains used in this study (C57BL/6J, SWV, and C3H/HeJ) were all from colonies kept in our laboratory in the Department of Biology at McGill University. The mouse strains homozygous for the Robertsonian translocations, Rb(6.16)24 lub and Rb(16.17)8 Lub, were purchased from Muriel T. Davisson, Jackson Laboratory, Bar Harbor, Maine. All mice used for experiments were housed in plastic cages with wood chip bedding and fed a diet of Purina Lab Chow and acid water ad libitum. A cycle of five and one half hours of light and eighteen and one half hours of darkness was maintained in a separate room from the breeding stocks at a constant temperature of 24 ±1°C.

2.2. Breeding scheme

Female mice homozygous for the Rb(6.16)24 lub translocation chromosome were mated to male mice homozygous for the Rb(16.17)8 lub translocation chromosome. The

offspring produced from this mating are heterozygous for the two Robertsonian translocations with partial homology for one of their arms (Figure 2). The male Rb(6.16)/Rb(16.17) double heterozygotes were then crossed to virgin females of the C57BL/6J, SWV, and C3H/HeJ inbred strains of mice. Reciprocal crosses were performed by crossing virgin female Rb(6.16)/Rb(16.17) double heterozygotes with males of the same three inbred mouse strains. All females were controlled for maternal age by experimenting exclusively on mice no older than six months (182 days) of age.

Males were put in a cage of one to four females in the morning. At the end of the dark cycle the males were removed and the females were checked for the presence of a vaginal plug. If a plug was found it was recorded as day 0 of gestation and the mouse was weighed to the nearest 0.1 g. Fourteen days later the mouse was weighed again and palpated. If determined to be pregnant, the mouse was isolated for dissection the following day. Pregnant females were killed by cervical dislocation on the fifteenth day of gestation. The uterus was dissected out and the position of all implants (live fetuses, dead fetuses, and moles) was recorded. Only healthy mothers possessing litters with three or more live fetuses were included in the experimental data. The ovaries were also extracted in order to count the number of corpora lutea.

2.3. Cytogenetics

Fetuses were dissected from the uterus and underwent a hepatectomy for cytogenetic analysis. The remaining fetal tissue and placenta were fixed in Bouin's solution for one week and then transferred to 70% ethanol for permanent storage. The fetal liver was put into a petri dish with 2-3 ml of minimum essential medium (Flow) containing 15% fetal calf serum and colcemid (Gibco) (final concentration = 0.1ug/ml) for 2.5 hours in an incubator set at 37°C and a CO_2 concentration of 5%. Upon removal of the medium, 2-3 ml of a 0.5% KCl solution was added to the petri dish and returned to the incubator for 45 minutes. The KCl solution was replaced with 2-3 ml of a fresh cold fixative (3 methanol : 1 acetic acid) and stored overnight at 4°C. The following morning the fixative was removed and 0.5-1 ml of a 60% acetic acid solution was added for 4 minutes. The fetal tissue was then minced using a pasteur pipette and dropped onto two slides on an inclined slide warmer at 40°C.

One slide from each fetus was stained with a 4% Giemsa (Gibco) solution. The second slide was C-banded for sex determination. Banding of the constitutive heterochromatin (C-banding) was performed by immersing slides in: 1) 1N HCl at room temperature for 10 minutes;
2) 0.3N $Ba(OH)_2$ at 37°C (time is according to the age of the slide); 3) 2SSC at 60°C for 20 minutes; and 4) 4% Giemsa for 10 minutes. Slides were rinsed in distilled water between

each step.

Sex determination was carried out by screening both C-banded and solid giemsa stained metaphase spreads. When C-banded the mouse Y chromosome stains fainter than the characteristic dark staining observed around the centromeric region (Figure 4). The mouse Y chromosome can also be identified when stained with solid Giemsa. It is the smallest chromosome in the murine genome and can be differentiated from the next to smallest chromosome, 19, due to the presence of a secondary constriction on chromosome 19.

2.4. Identification of Trisomic Fetuses

Slides were screened on a Leitz compound microscope. Three to five chromosome spreads were counted for each fetus. Trisomic fetuses were represented by a chromosome complement containing 38 acrocentric and two metacentric (Robertsonian translocations) chromosomes (41 chromosome arms in total). Euploid fetuses were represented, cytogenetically, by 38 acrocentric chromosomes and only one metacentric chromosome (40 chromosome arms in total).

Trisomic fetuses were also readily identified on day 15 of gestation upon the presentation of pronounced edema and a hygroma of the back of the neck (Figure 5). These morphologic markers possessed a high sensitivity (89%) in the identification of fetuses with trisomy 16. They were eventually used in addition to cytogenetic analysis in order to characterize each fetus.

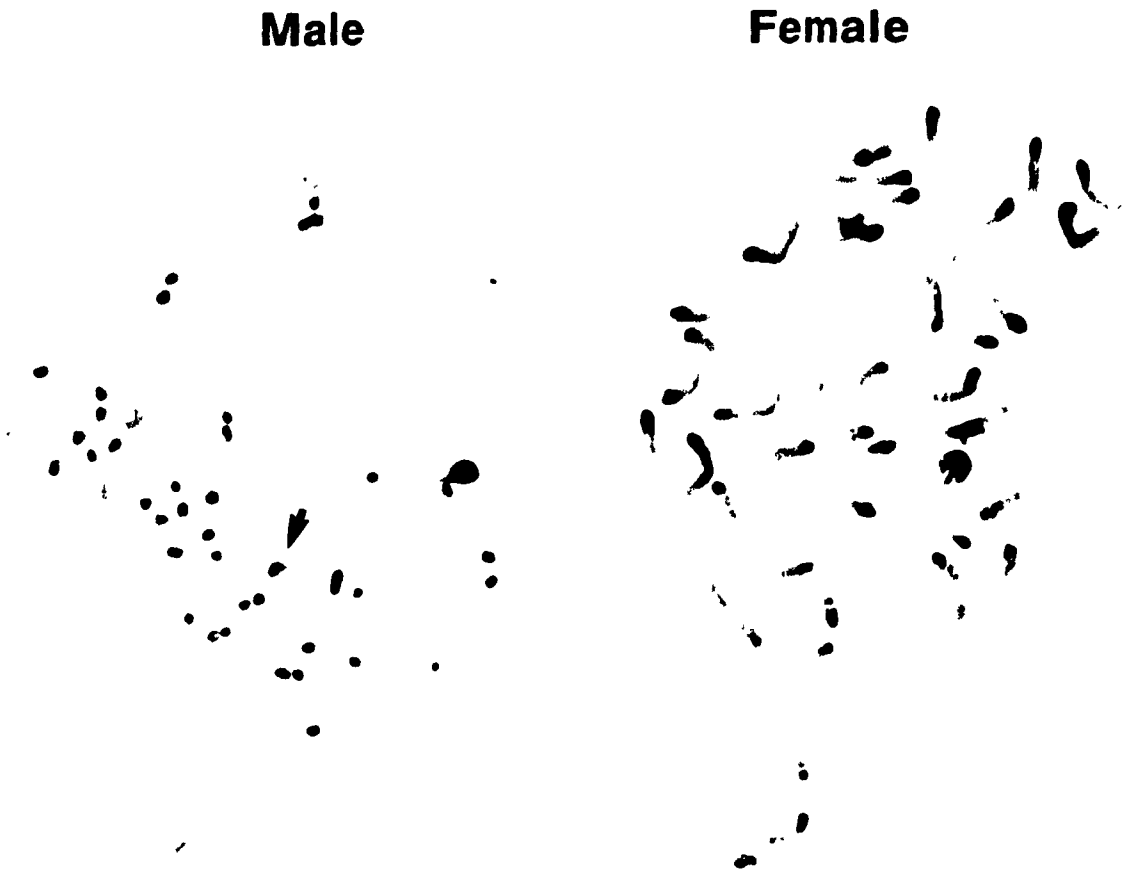


Figure 4: C-Banded Metaphase Chromosomes from Male and Female Trisomy 16 Fetuses (The arrow depicts the Y chromosome.)

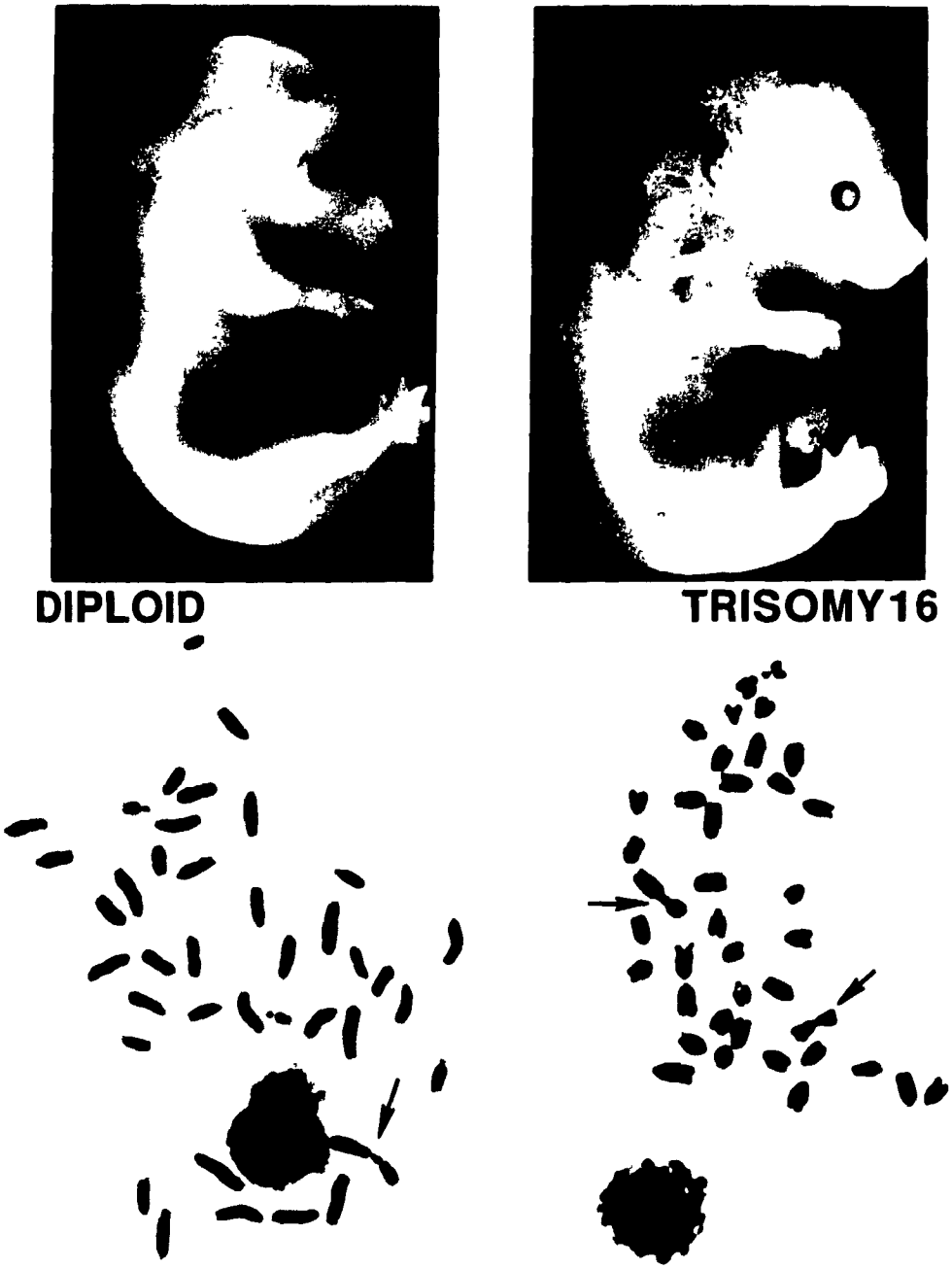


Figure 5 : Diploid and trisomy 16 fetuses on day 15 of gestation with corresponding metaphase chromosomes. Arrows point to Robertsonian translocations: two in trisomy 16; one in the diploid fetus.

3. Results

3.1. Cytogenetic Versus Morphologic Analysis

In determining the prevalence of trisomy 16 mouse fetuses on day 15 of gestation in various genetically unrelated female strains of mouse, common cytogenetic practices were carried out to determine the chromosomal complement of each fetus. However, success has been achieved in diagnosing trisomy 16 fetuses on day 15 of gestation by observing the manifestations of the developmental dyshomeostasis of the compromised fetus. Between days 14 and 16 of gestation trisomy 16 fetuses display a generalized transient edema and hygroma of the back of the neck (Miyabara et al, 1982; Gearhart et al, 1986) (Figure 5). These morphologic characteristics were tested for their ability to differentiate the trisomic fetus from its normal littermate (Table 1). The positive and negative predictive values of diagnosing both trisomy 16 and diploid fetuses by these morphologic signs were each determined to be 97%. Due to the great efficacy of this diagnostic tool it was used in addition to the reliable but cumbersome task of determining the fetal karyotype by cytogenetic analysis. In all cases, slides were prepared of the fetal chromosomes.

3.2. Prevalence of Trisomy 16

The prevalence of living trisomy 16 fetuses on day 15 of gestation was found to vary significantly with the genetic background of the female parent (Table 2). The frequency of

		Cytogenetics	
		Trisomic	**Normal**
Morphologic Analysis	**Positive:** Hygroma and Edema Present	39 (a)	1 (b)
	Negative: Hygroma and Edema Absent	5 (c)	189 (d)

Sensitivity=a/(a+c)= 88.6%
Specificity=d/(b+d)= 99.5%
Positive predictive value= a/a+b= 97%
Negative predictive value=d/c+d= 97%

Table 1: Fourfold table demonstrating the efficacy of morphology in the differentiation between diploid and trisomy 16 fetuses on day 15 of gestation

Cross		Fetuses Examined		Frequency of Trisomy (%)	
Female	Male	Diploid	Trisomic		
C57BL/6J	Rb(6.16)/(16.17)	127	16	11	p<0.05
C3H/HeJ	Rb(6.16)/(16.17)	116	31	21	
SWV	Rb(6 16)/(16.17)	157	39	20	

Table 2: Prevalence of Trisomy 16 on Day 15 of Gestation in Genetically Different Female Mice Mated to Rb(6.16)\Rb(16.17) Males.

Cross		Fetuses Examined		Frequency of Trisomy (%)	
Female	Male	Diploid	Trisomic		
Rb(6.16)/(16.17)	C57BL/6J	132	14	10	p>0.25
Rb(6.16)/(16.17)	C3H/HeJ	132	21	15	
Rb(6 16)/(16.17)	SWV	130	23	14	

Table 3: Prevalence of Trisomy 16 on Day 15 of Gestation in Rb(6.16)/Rb(16.17) Females Mated to Genetically Different Male Mice.

trisomy 16 was significantly lower ($p<0.05$, G-test of independence) (Sokal and Rohlf, 1981) in the C57BL/6J female (11%) compared to both the SWV (20%) and C3H/HeJ (21%) females.

A total of 486 fetuses from the three crosses were examined (400 diploid; 86 trisomic), with approximately 150 fetuses from each cross. The total number per cross was decided upon prior to experimentation because this is the number required to establish a significant difference ($p<0.05$) between 10% and 20% as was documented when trisomy 19 fetuses were examined (Vekemans and Trasler, 1987).

In order to determine whether the observed strain differences were due to maternal or fetal factors, reciprocal crosses were performed. A total of 452 fetuses from the three reciprocal crosses were examined (394 diploid; 58 trisomic), with approximately 150 fetuses from each cross. Females doubly heterozygous for the Rb(6.16)/Rb(16.17) Robertsonian translocations were crossed to males of the C57BL/6J, SWV, and C3H/HeJ strains of inbred mice. No significant differences in the prevalence of living trisomy 16 fetuses were detected in these reciprocal crosses (10%, 14%, and 15% respectively) (Table 3) ($p>0.25$, G-test of independence). Furthermore, a correlation between the frequencies of trisomy (arcsine) between the forward and reciprocal crosses proved not to be statistically significant ($p=0.31$). Therefore, maternal factors residing perhaps in the uterine environment or

contributing to fetal development are responsible for the observed strain differences.

3.3. Factors That May Influence the Prevalence of Trisomy 16

A number of interpretations offer potentially valid explanations for why there are strain differences in the prevalence of fetuses with trisomy 16. In order to evaluate the extent that the prevalence of trisomy 16 depends statistically on various independent variables, a step-wise regression procedure was performed. For these analyses, the Statview 512+^{TM} (BrainPower, 1986) program was run on a Macintosh SE personal computer. The prevalence of trisomy 16 (%) was transformed to arcsine. Zero values were transformed using 1/4n, where n is the number of live fetuses per litter (Zar, 1984).

The independent variables incorporated into the step-wise regression included maternal weight on the day of fertilization, maternal age, the number of eggs shed per pregnant female (represented as corpora lutea), as well as the number of implants per pregnant female. The procedure was run for each of the three crosses in Table 2. From Table 4, it can be concluded that none of the aforementioned independent variables approached statistical significance, thus suggesting that they had no influence in whole or in part on the prevalence of trisomy 16 fetuses.

Variables	Female strains					
	C57BL/6J		C3H/HeJ		SWV	
	F-value	p-value	F-value	p-value	F-value	p-value
Maternal weight at day zero of gestation	0 92	>0.5	0.54	>0.5	2.8	>0.2
Maternal age	0 93	>0.5	0 40	>0.5	0.17	>0.5
Corpora Lutea	0 13	>0 5	0.16	>0 5	0.63	>0.5
Implants	0.57	>0.5	0.11	>0.5	2.0	>0.2

Table 4: Statistical Dependence of Four Independent Variables on the Prevalence of Trisomy 16 in Three Female Strains of Mice.

In order to test the possibility that the prevalence of trisomy 16 is conditional on a heterogeneous group of corpora lutea, the corpora lutea were ranked in ascending order and divided in half into "high' and "low" subgroups. For each strain, the corresponding frequencies of trisomy were compared between the "high" and "low" subgroups (Table 5). Once again, a G-test of independence disclosed that no significant differences exist in the prevalence of trisomy 16 between litters with a large or small number of corpora lutea.

Furthermore, statistically significant correlations were not found between the mean number of corpora lutea (for each strain tested) and the prevalence of trisomy 16 ($r=0.42$, $p=0.72$, d.f.=1), as well as the mean number of implants and prevalence of trisomy 16 ($r=0.30$, $p=0.81$, d.f.=1) (Table 6). These findings further confirm that the number of eggs shed and the number of implants have no bearing on the prevalence of trisomy 16 fetuses on day 15 of gestation.

3.4. Fetal Loss

The fetal loss was examined in terms of both preimplantation and postimplantation loss. Preimplantation death was determined by dividing the number of nonimplanted embryos (corpora lutea minus implants) by the number of eggs shed (corpora lutea). Postimplantation death was determined by dividing the number of moles (deciduomata) and late deaths by the number of implants.

Female	Mean number of corpora lutea in high group	Mean number of corpora lutea in low group	Prevalence of Trisomy 16 in high group (%)	Prevalence of Trisomy 16 in low group (%)	Statistical level of significance (p)
C57BL/6J	128 (15)*	99 (15)	12 3	10.0	>0.75
C3H/HeJ	127 (14)	105 (15)	20.3	22.1	>0.75
SWV	154 (13)	135 (14)	23.3	17.0	>0.25

* The number in the parenthesis refers to the number of litters examined.

Table 5: Comparison between the Prevalence of Trisomy 16 Fetuses In Litters with High and Low Ranking Corpora Lutea.

Female	Number of Litters	Corpora lutea per litter	Implants per litter	Moles per litter
C57BL/6J	30	7.57	6.70	1.80
C3H/HeJ	29	8.00	6.69	1.53
SWV	27	10.70	9.11	1.37

Table 6: Corpora Lutea/Litter, Implants/Litter, and Moles/Litter in Three Strains of Female Mice Mated to Rb(6.16)/Rb(16.17) Males.

A) Preimplantation Loss

The three strains examined displayed no statistically significant differences in their frequency of preimplantation loss (Figure 6a). Furthermore, no significant correlation exists between the prevalence of trisomy 16 (arcsine) and the preimplantation loss ($r=0.91$, $p=0.28$, d.f.=1).

B) Postimplantation Loss

Figure 6b reveals that females of the C57BL/6J strain have a significantly higher postimplantation loss (29%) compared to females of the SWV strain (20%) ($p<0.05$, G-test of independence). Females of the C3H/HeJ strain however, have a postimplantation loss midway between the two other strains (24%). A correlation analysis between the number of moles/litter and the prevalence of trisomy 16 fetuses (arcsin) is not regarded as statistically significant ($r=-0.87$, $p<0.5$) (Figure 7).

3.5. Sex ratio

Table 7 displays the gender distribution of both trisomic and euploid fetuses from SWV and C57BL/6J females. Progeny from neither of these two strains showed any apparent deviations in their sex ratios from the expected 1:1 ratio, or from each other ($p>0.5$, G-test of independence).

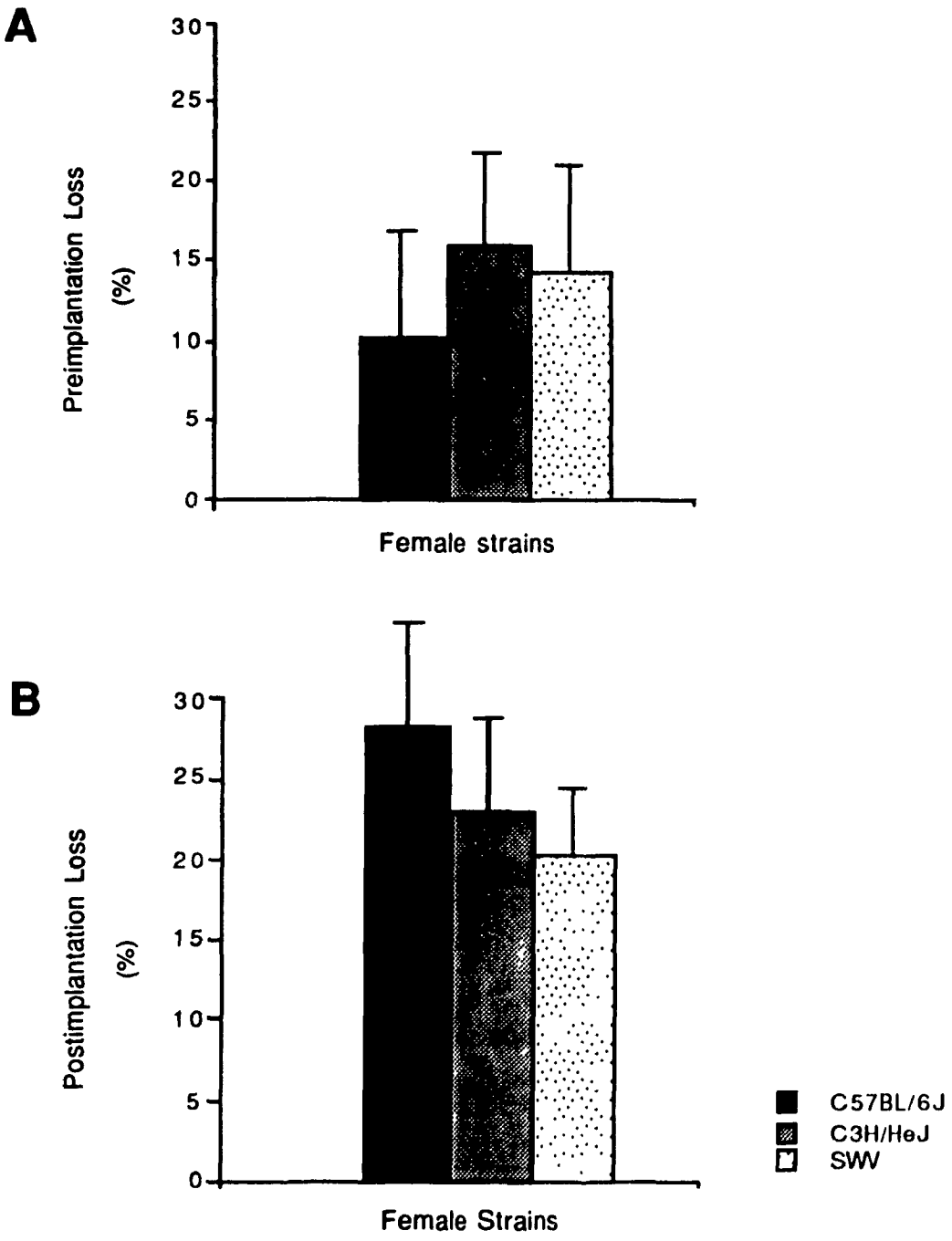


Figure 6: Mean Pre (A) and Post (B) Implantation Losses (with 95% Confidence Limits) in Three Strains of Female Mice Mated to Rb(6.16)/Rb(16.17) Males.

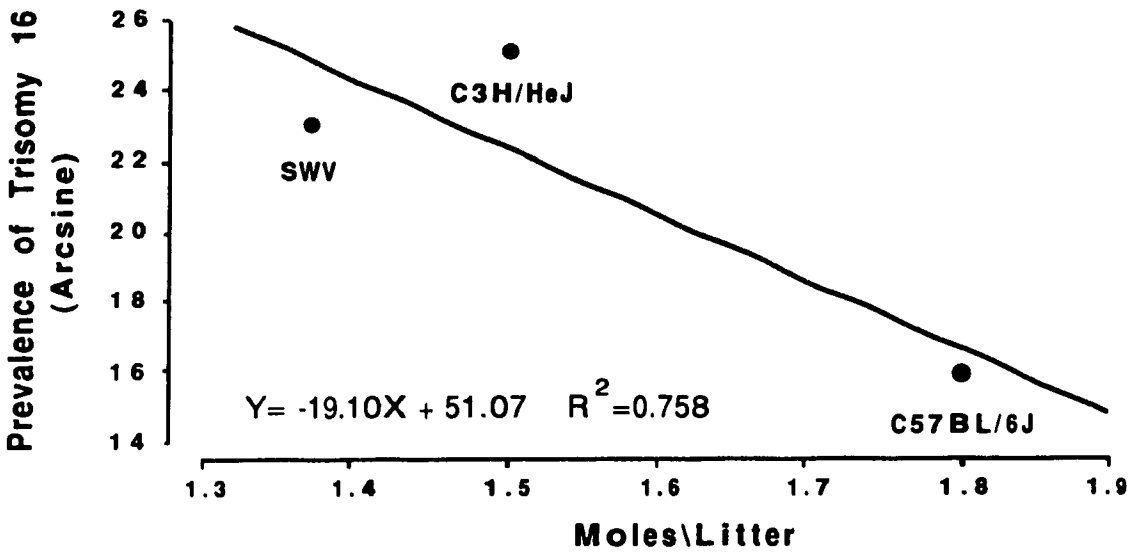


Figure 7: **Correlation Between the Prevalence of Trisomy 16 (Arcsine) and the Number of Moles/Litter in Three Female Strains of Mice Crossed to Rb(6.16)/Rb(16.17) Males.**

	Cross		Gender of Fetuses		
	Dam	Sire	Male	Female	
Diploid	SWV	Rb(6.16)/(16.17)	31	42	p>0.5
	C57BL/6J	Rb(6.16)/(16.17)	27	30	
Trisomy 16	SWV	Rb(6.16)/(16.17)	15	16	p>0.5
	C57BL/6J	Rb(6.16)/(16.17)	9	7	

Table 7: Gender Distribution of Diploid and Trisomy 16 Fetuses on Day 15 of Gestation

3.6. Reciprocal Crosses

The reciprocal crosses were analyzed for differences in their prevalence of trisomy, corpora lutea, implants, preimplantation loss, and postimplantation loss. The maternal strain used in the reciprocal crosses is kept constant. Thus, it would be expected that no significant differences be found in any of the aforementioned variables due to the fact that they are each influenced by the same maternal background.

Interestingly enough, a statistically significant difference was detected in one of the variables tested. Female Rb(6.16)/(16.17) mice crossed to SWV males gave rise to a significantly lower number of postimplantation losses than did females from the other two reciprocal crosses ($p<0.005$) (Figure 8b). Nonetheless, a correlation between the prevalence of trisomy (arcsine) and the postimplantation loss proved to be statistically insignificant ($p=0.25$). There were no other significant variations in any of the other parameters examined in the reciprocal crosses.

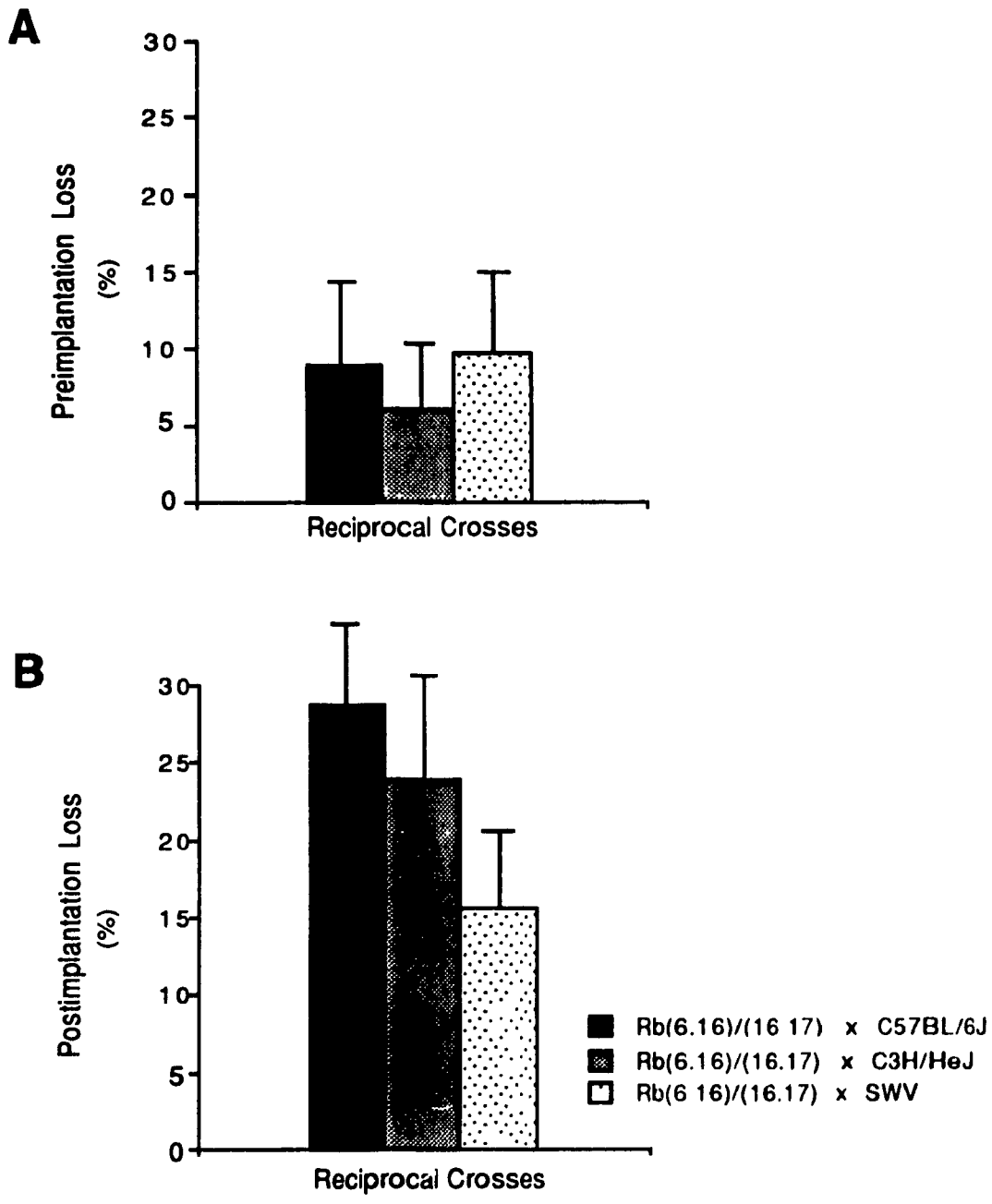


Figure 8: Mean Pre (A) and Post (B) Implantation Losses (with 95% Confidence Limits) in Rb(6.16)/Rb(16.17) Female Mice Mated to Genetically Unrelated Males.

4. DISCUSSION

In the current investigation, it is evident that the genetic background of the female parent plays a significant role in determining the prevalence of mouse fetuses with trisomy 16 on day 15 of gestation.

It is necessary to reiterate that the prevalence of trisomy at a given time in gestation is dependent on its incidence, as well as the probability that the anomalous conceptus will survive until the time of observation (Stein et al, 1975). The importance of supplying genetically equivalent sperm complements to each of the females tested is a crucial factor in order to compare rates of survival of trisomic fetuses. It is imperative that the incidence of trisomy 16 at conception be equivalent for each mouse strain tested. By mating the same strain of male mouse, doubly heterozygous for the Rb(6.16) and Rb(16.17) translocations, to genetically unrelated female strains of mouse, one is ensured that the proportion of hyperhaploid sperm supplied by these males will remain consistent throughout the experiments (Zackowski and Martin-Deleon, 1988). Furthermore, Zackowski and Martin-Deleon (1988) have confirmed that the different types of chromosomally abnormal sperm produced by the Rb(6.16)/Rb(16.17) males are fully capable of fertilization. This opportune finding negates the possibility that prezygotic selection could have been acting on the hyperhaploid sperm. The theory of prezygotic selection against human sperm with an

abnormal chromosomal complement has also been disproven by Martin (1988).

Upon assurance that the incidence of trisomy at conception was constant, efforts were made to identify potential interpretations for why strain differences were observed in the prevalence of trisomy 16 fetuses (Table 2). A variety of factors could have an influence on the prevalence of trisomy at a particular stage in gestation (postzygotic selection). For instance, the number of corpora lutea, which is a measure of the number of oocytes shed, can potentially be associated with postzygotic selection of trisomic conceptuses. It is possible that the greater the number of oocytes shed, the greater the competition for implantation sites among the fertilized eggs. If this scenario is conceivable, then one could assume that the competition for implantation sites would benefit the euploid rather than the aneuploid embryos.

A second factor that may influence the prevalence of trisomy 16 fetuses and thereby encourage postzygotic selection of trisomy 16 conceptuses, is maternal age. The upper limits of maternal age were controlled for by limiting the age of an experimental mouse to less than or equal to six months. However, there were no limitations on how young an experimental mouse could be. It is possible that young mice are not as efficient as older mice in eliminating trisomic conceptuses, thereby possessing a lower probability of spontaneously aborting a trisomic conceptus. Polani (1983)

used this hypothesis to explain why in humans younger mothers had a higher prevalence of Down syndrome livebirths compared to older mothers.

A third factor, maternal weight on the day of fertilization, has potential to alter the prevalence of trisomy 16 fetuses. Maternal weight on the day of fertilization is strongly correlated to maternal age for each of the strains examined, nonetheless, it is possible that a lighter mouse may not be as physiologically apt to maintain a trisomic conceptus as would a larger mouse, thereby possessing a greater r_a.

Finally, the number of implants, which is strongly correlated to the number of corpora lutea (for each strain examined), may influence the postzygotic selection against postimplantation embryos. As a result of selective competition for nutrients and space, Debrot and Epstein (1986) proposed that trisomic and tetrasomic embryos would be at a proliferative disadvantage in a crowded situation.

In order to test whether any of the above variables do indeed have an effect on the prevalence of trisomy, a step-wise regression analysis was performed. As can be observed in Table 4, none of the aforementioned variables influence at all or in part the prevalence of trisomy 16 fetuses.

A further analysis was carried out in order to determine whether heterogeneity exists in the distribution of corpora lutea and if the prevalence of trisomy 16 is correlated with

one of the heterogeneous groups. The number of corpora lutea, for each strain, was ranked into high and low groups and the prevalence of trisomy was compared between the two groups. No statistically significant differences were found (Table 5) confirming once again that the number of corpora lutea per litter has no significant influence on the prevalence of fetuses with trisomy 16 on day 15 of gestation.

If the incidence of trisomy 16 at fertilization is considered constant and there is no apparent influence common to the three female strains tested that can explain tneir differences in the prevalence of trisomy 16, then some form of postzygotic selection against trisomy 16 fetuses must be occurring. However, in order for a postzygotic selection theory to be proven, it must be shown that selective elimination of trisomy 16 fetuses is actually undertaken.

The preferential loss of trisomy 16 conceptuses could be occurring either before or after implantation. Significant differences in the preimplantation loss were not detected in the first set of crosses (Figure 6a). It must be noted that because preimplantation loss is a measure of the nonimplanted oocytes (fertilized or not) divided by the total number of oocytes shed (corpora lutea), there is room for error in the prediction of the preimplantation loss, and therefore, possible misinterpretations of the results. For instance, in this study, fertilized eggs cannot be differentiated from unfertilized eggs. Furthermore, the true number of corpora

lutea is somewhat difficult to establish without microscopic examination of the ovary itself. The preimplantation loss in this study should therefore be considered as merely an estimate of the true preimplantation loss.

In studies carried out on 11 autosomal trisomies in the mouse, one of which was trisomy 16, it was determined that trisomic embryos undergo normal cleavage and blastulation as well as possess mean cell counts equivalent to their diploid littermates (Dyban and Baranov, 1987). Earlier studies agree with the above findings that trisomic embryos can survive past the preimplantation stage of development (Ford 1971, Gropp 1973, Epstein and Travis 1979, Debrot and Epstein 1986). The preimplantation loss in this study, although limited in accuracy, is nonetheless consistant with other experiments.

If postzygotic selection of fetuses with trisomy 16 is occurring, then one would expect an inverse correlation between postimplantation loss and prevalence of trisomy 16 on day 15 of gestation. The postimplantation loss is a heterogeneous composite of moles (deciduomata) and late deaths. A mole is the product of a maternal response by the uterine tissue to an implanting egg which later dies (Bateman, 1984). The growth of the deciduum is autonomous until the eleventh day of pregnancy. If at this time the implanted egg is dead it will be recognized as a mole. A conceptus dying subsequent to this developmental stage is regarded as a late death. Discrimination as to whether the deciduum encompasses

a mole which constituted a fertilized diploid or aneuploid egg is difficult if not impossible to determine at day 15 of gestation. Therefore, moles, which in this study comprise at least 90% of the postimplantation loss at day 15 of gestation, are also considered a heterogeneous group.

The negative correlation in this study between the postimplantation loss and the prevalence of trisomy 16 on day 15 of gestation is not a statistically significant one, but is, albeit weak, in the appropriate biological direction (Figure 7). Furthermore, C57BL/6J females were found to have a statistically significant increase in their postimplantation loss compared to SWV females (Figure 6b). These two mouse strains have a low (11%) and high (20%) prevalence of trisomy 16 respectively. Females of the C3H/HeJ strain were found to have a postimplantation loss intermediate to the other two strains, even though they had a high (21%) prevalence of trisomy 16 (Figure 6b).

The lack of statistical significance in the correlation between postimplantation loss and prevalence of trisomy 16 may be due to the heterogeneity in the composition of the fetal loss refered to above. It may be that the C3H/HeJ females have a greater background rate of spontaneous loss of diploid compared to trisomy 16 conceptuses, than do the other two females examined. In fact, Rohrborn (1968) confirmed this hypothesis. He found that C3H females bred to C3H males have a postimplantation loss of 12.3%. As inbred mice have a

considerably low rate of spontaneous aneuploidy (Ford, 1971), the majority of this fetal loss can be considered to be composed of diploid conceptuses. The background rate of spontaneous loss in the C3H female is slightly greater than the rate of spontaneous loss in the C57BL/6J (10%) (Juriloff, 1978) and SWV (10%) (Biddle, 1975) strains of mouse.

Due to the heterogeneous nature of postimplantation losses and strain differences in the background rates of spontaneous abortions of diploid conceptuses, some means of standardizing the fetal loss is necessary. Stein et al (1975) proposed a simple model of the distribution, among spontaneous abortions and births, of conceptuses with and without anomalies such as trisomy (Figure 1). From this model it can be inferred that the prevalence of trisomy at a particular time in gestation (F) is equal to the expected number of living trisomic conceptuses divided by the total number of living conceptuses, both trisomic and diploid [$F= xp(1-r_a)/xp(1-r_a) + x(1-p)(1-r_n)$] [x= number of pregnancies; p= probability of trisomy; r_a= the probability that a trisomic fetus will be spontaneously aborted; and r_n= the probability that a normal (diploid) fetus will be spontaneously aborted].

Using this equation, one is able to determine r_a for each of the female strains of mouse examined. Some assumptions, however, will have to be made. Firstly, the incidence of trisomy 16 at conception will have to be approximated. Vekemans and Trasler (1987) have predicted that the incidence

of hyperhaploid sperm in the Rb(5.19) 1Wh/Rb(9.19) 163H double hetrozygote male is 20%. Their determination of this incidence was based on experiments performed by White et al (1972) who measured the frequency of balanced and unbalanced gametes in a male mouse doubly heterozygous for the Rb(5.19) 1Wh and Rb(9.19) 163H translocation chromosomes. A comparable incidence of trisomy 16 at conception was determined by Zackowski and Martin-Deleon (1988). They examined first cleavage stage conceptuses produced by mating females to the same doubly heterozygous males used in the present study.

The second assumption that must be made regards the use of r_n. The spontaneous rate of loss of diploid conceptuses in F_1 matings of C57BL/6J, SWV, and C3H/HeJ mice has already been stated to be 10%, 10%, and 12% respectively. Although the relative differences in spontaneous loss of diploid conceptuses between the female strains of mice examined may remain constant in the present study, the exact proportions may differ due to the fact that Rb(6.16)/Rb(16.17) male mice are used in the matings.

The rate of spontaneous loss (r_n) is known to be influenced by the prevalence of aneuploidy. For instance, an increase in r_n has been noticed in human twin pregnancies when one of the twins is aneuploid and the other diploid (Vekemans, personal communication). If this holds true for mice, then the actual number of moles in this study will presumably be greater than expected due to the presence of trisomic fetuses

in the majority of litters. Judging from the results in Table 6, this appears to be the case.

The rate of loss of trisomy 16 conceptuses (r_a) can be determined by implementing the appropriate values into the above equation. Due to the uncertainty of r_n, both conservative and liberal estimates of this value were used for each strain. The conservative value of r_n is taken from previously cited studies on inbred strains of mice. The more liberal estimate of r_n is based on values from the present study. Table 8 reveals the estimates of r_n and r_a for each of the three mouse strains analyzed.

When applying a conservative estimate of r_n to the Stein et al equation, an r_a of 56%, 6%, and 10% were revealed for females of the C57BL/6J, C3H/HeJ, and SWV mouse strains, respectively. When employing a more liberal estimate of r_n, r_a values equalling 63%, 18%, and 23% were disclosed for the above strains, respectively.

To summarize, even though a statistically significant linear correlation between postimplantation loss and prevalence of trisomy 16 on day 15 of gestation was not observed, a substantial difference in the frequency of selection against trisomy 16 fetuses (r_a) by the three strains of mice examined was detected. The C57BL/6J female had a low prevalence of trisomy 16 on day 15 of gestation as a result of a high (56%-63%) rate of spontaneous loss of trisomy 16 fetuses. On the other hand, the C3H/HeJ and SWV females both

Female	Prevalence of Trisomy 16 (%)	r_n (%) (conservative)	r_n (%) (liberal)	r_a (%) (range)
C57BL/6J	11	10	25	56-63
C3H/HeJ	21	12	29	6-18
SWV	20	10	23	10-23

Table 8: Values of r_n and r_a for Three Strains of Female Mice Mated to Rb(6.16)/Rb(16.17) Males.

had a high prevalence of trisomy 16 on day 15 of gestation as a result of their poor ability to selectively eliminate their aneuploid fetuses (r_a ranges from 6%-18% and 10%-23% respectively). Furthermore, because no differences in the prevalence of trisomy 16 were observed in the reciprocal crosses (Table 3) the ability to selectively eliminate fetuses with trisomy 16 must be a maternal attribute.

Although there were no differences in the prevalence of trisomy 16 in the reciprocal crosses, differences were apparent in the postimplantation loss (Figure 8b). Female Rb(6.16)/Rb(16.17) mice mated to SWV males displayed a significant decrease in their postimplantation loss compared to the other two reciprocal crosses. However, because the prevalence of trisomy 16 in this cross did not differ from the other females, the postimplantation loss differences in this cross are possibly due to a decrease in the number of blighted ova that would normally have implanted. Unfortunately, because the incidence of trisomy 16 in oocytes is unknown, r_a cannot be predicted for the Rb(6.16)/(16.17) female.

Bacchus et al (1987) have also noticed an inverse correlation between the prevalence of trisomy 16 and the postimplantation loss. However, comparisons between the present data and their own is difficult due to their use of superovulated females. Superovulated female mice add an uncontrolled variable to the model which may further stress

the aforementioned maternal screening process.

A 1984 study investigated the prevalence of trisomy 16 and the associated malformations resulting from the extra chromosome (Miyabara et al, 1984). Miyabara determined that the prevalence of trisomy 16 at various days of gestation after day 15 was greater in the C3H/He female strain compared to the C57BL/6 female strain. However, even though these results confer with those from the present study, they must be regarded as speculative due to their small sample size analyzed.

Miyabara et al (1984) also stated that the type and prevalence of cardiovascular anomalies in mouse conceptuses with trisomy 16 are dependent, to some extent, on the female strain employed for crossing. This statement is based on the finding that certain cardiovascular anomalies, particularly persistent common atrioventricular canal defects, occur to a greater degree in trisomy 16 fetuses arising from the C57BL/6 maternal strain compared to the C3H/He maternal strain. Unfortunately, the reciprocal crosses were not performed in order to determine whether in fact this seemingly direct relationship between the presence of a cardiac malformation and the survival of the trisomy 16 conceptuses is dependent on the genetic background of the maternal parent.

A similar relationship is apparent with trisomy 12 conceptuses. Trisomy 12 conceptuses were produced by crossing double metacentric chromosome carrying males (Rb5Bnr/Rb9Bnr)

to both BALB/c and C57BL/6J inbred strains of female mice (cited in Epstein, 1986). Trisomy 12 conceptuses produced on a BALB/c maternal background rarely survived beyond day 15 of gestation and had a 90% occurrence of exencephaly. By contrast, when trisomy 12 conceptuses were produced on a C57BL/6J maternal background some of the trisomy 12 progeny survived until term and the occurence of exencephaly was only 60%.

Another example of the role that the genetic background has in determining the type of defects observed in aneuploid conceptuses was noted by Beechey and Searle (1988). They observed that a number of their trisomy 15 embryos sustained open neural tubes. However, when the trisomy 15 embryos were produced on a different maternal background the defective neural tubes were not apparent (cited by Dyban and Baranov, 1987).

The genetic control of the survival of aneuploid conceptuses is not restricted to trisomies. Magnuson et al (1985) reported that the lethal period for monosomy 19 embryos begins earlier when the maternal background is contributed by ICR or BALB/c females compared to C57BL/6J females. Furthermore, Biddle (1986) reported a decrease in the prevalence of monosomy X females on a SWV, compared to a C3H/HeJ, maternal background.

Perhaps the most compelling evidence in support of the idea that the survival of a trisomic fetus is genetically

controlled by a maternal response comes from the work done by Vekemans and Trasler (1987). They investigated the survival of trisomy 19 fetuses on day 15 of gestation. Fetuses with trisomy 19 were produced by crossing males heterozygous for the Rb(5.19) 163H and Rb(9.19) T1WH translocation chromosomes with females of genetically unrelated strains of mice.

Once again, it was determined that the prevalence of trisomic fetuses varied significantly with the genetic background of the female parent. An inverse relationship ($p=0.1$) between the fetal loss and the frequency of trisomy 19 was also detected, thus suggesting that selective elimination of conceptuses with trisomy 19 was occurring. Furthermore, it appeared that the frequencies of trisomy clumped into high (20%) and low (10%) groups. A rankit analysis confirmed that the two groups did indeed fit a bimodal distribution which suggested that a very small number of genes were responsible for this selection mechanism (Vekemans, 1989). Upon examination of the prevalence of trisomy 19 on day 15 of gestation in the reciprocal crosses, no significant differences were revealed, thus indicating once again that the survival of trisomy 19 fetuses is genetically controlled by the female parent.

If the maternal response that selects against fetuses with trisomy 19 (or promotes their survival) is exhibited by the same mother in response to a fetus with trisomy 16, then one could hypothesize that the maternal control of both

trisomies is one and the same. That is, a mother who is able to select or promote the survival of trisomy 19 fetuses will exhibit the same response when that fetus is trisomic for chromosome 16. According to Table 9, this hypothesis is invalidated by the present data. Mothers of the C57BL/6J strain of mouse were able to select against trisomy 16 fetuses but not trisomy 19 fetuses. On the other hand, when SWV mothers were employed the inverse was observed. Mothers of the C3H/HeJ strain were not able to select against conceptuses with either trisomies 16 or 19. It thus appears that the maternal ability to selectively eliminate a trisomic fetus is specific for the triplicated chromosome.

In studying the morphology and chromosomal complement of human spontaneous abortions, Byrne et al (1985) determined that those chromosome anomalies that do not survive until term are more likely to cease intra-uterine development at an earlier stage than those which do survive to term. Murine trisomies 16 and 19 are both compatible with survival until term (Gropp, 1982), however, their development is prematurely interrupted depending on the particular genetic background of the female parent. In addition to this point, six of ten trisomy 21 abortuses studied by Creasy et al (1976) demonstrated no observable malformations that could explain their demise. The inability of these trisomy 21 conceptuses to survive can be explained by the proposed maternal ability that selectively eliminates them.

Female	Frequency of Trisomy	
	16	19
C57BL/6J	LOW	HIGH
SWV	HIGH	LOW
C3H/HeJ	HIGH	HIGH

Table 9: Comparison of the Frequency of Survival for Murine Trisomies 16 and 19 in Females of Different Strains of Mice

The elucidation of the actual number and chromosome location of the major genes involved in the maternal response to trisomy 16 fetuses will occur through the use of recombinant inbred strains of mice. Recombinant inbred strains of mice are the result of randomly mated pairs of mice in an F_2 generation of a cross between two existing inbred strains and perpetuated for 20 or more generations by brother-sister matings (Heiniger and Dorey, 1980). The resulting genetic consequence of this breeding system is that each recombinant inbred strain is equally likely to be homozygous for the alternate alleles of autosomal loci at which the progenitor strains differed (Heiniger and Dorey, 1980). Each of the progenitor strains have been typed for a variety of biochemical markers. Therefore, linkage between the trait of interest and one or several of the biochemical markers will be detected upon analyzation of the segregation of the trait of interest with one or several of the markers.

Presently, implementation of 11 recombinant inbred strains resulting from the C57BL/6J X C3H/HeJ progenitors, with over 220 typed loci, is being undertaken in our laboratory (Demczuk, personal communication). The two progenitor strains have a low and high prevalence of trisomy 16 respectively. Studies are also underway to identify progenitor strains in order to identify the gene(s) involved in the maternal response against trisomy 19 fetuses. Once these tasks have been accomplished, further comparisons will

determine whether in fact the same gene(s) are involved in selecting against specific trisomic fetuses in the mouse. Using the knowledge that homologous chromosomal regions are conserved between mouse and man, efforts will then be made to identify the location of the human homologue to this mouse gene. The necessary population studies will eventually follow.

Sex Ratio

As a result of the selective elimination of trisomy 16 conceptuses in the C57BL/6J strain of mouse, questions were raised as to whether differential selection on the basis of sex was occurring as well. Indeed differences in the sex ratio of human trisomic abortuses, conceptuses, and livebirths have been reported. For instance, males exceeded females in clinically recognized trisomy 21 livebirths (Bernheim et al, 1979; Lindsten et al, 1981;). Appropriately enough, females were found in a greater prominence in trisomy 21 abortuses (in most but not all studies) (Carr and Gedeon, 1978; Kajii et al, 1980; Lauritsen 1976; Warburton et al, 1980; Hassold et al, 1983). On the other hand, males were more pronounced in trisomy 18 conceptuses (Therkelsen et al, 1973).

In the present study the sex ratio of trisomy 16 fetuses on day 15 of gestation was not found to deviate from the sex ratio of their normal (diploid) littermates (G-test of independence, $p>0.5$) (Table 7). Furthermore, the sex ratio of trisomy 16 fetuses derived from the C57BL/6J mother was not

statistically different from the trisomy 16 progeny arising from the SWV mothers ($p>0.5$) (Table 7). Such findings refute the possibility that differential selection of trisomy 16 conceptuses is sex dependent.

Miyabara et al (1984) found that the sex ratio in the trisomy 16 progeny from three different female inbred strains was elevated, albeit not to statistical significance. Unfortunately, the authors did not compare the sex ratios of the trisomy 16 conceptuses with the sex ratio of the normal littermates. Nonetheless, based on these and other morphologic findings the authors proposed that murine trisomy 16 is a good animal model for human trisomy 21. However, their finding of an increased sex ratio in trisomy 16 fetuses do not concord with human data. A total of 64 individuals with translocation Down syndrome revealed a sex ratio of 1.06 (cited in Hassold et al, 1983). Interpretations as to why the sex ratio is higher in Down syndrome resulting from three copies of free chromosome 21, as compared to translocation Down syndrome, are scarce.

5. Conclusion

Presently, efforts to identify conceptuses at risk for autosomal trisomies have focussed on identifying in such conceptuses, factors that are directly or indirectly associated with the chromosomal anomaly. Biochemical and sonographic markers are the tools along with maternal age that are being used to screen for various aneuploidies. However, little effort has been directed towards recognizing factors in the mother that may predispose her to carry an aneuploid conceptus to term. The experimental data presented in this manuscript is an initial attempt to identify, in the mother, genetic factors that control the survival of aneuploid fetuses.

Data utilizing a mouse model for aneuploidy have revealed that the genetic background of the female parent plays a significant role in determining the prevalence of fetuses with trisomy 16 on day 15 of gestation. It has been determined that the mouse strain with a lower prevalence of trisomy 16 has a higher probability of having such a fetus result in a spontaneous abortion than survive to the time of observation (day 15 of gestation). Furthermore, this maternal ability that selectively eliminates, or protects the survival of, trisomy 16 fetuses, appears to be specific for the particular trisomy in question. That is, the genetic control of the survival of trisomy 16 fetuses does not appear to be the same for trisomy 19 fetuses.

Studies are currently underway that will attempt to identify the genetic factors controlling the survival of trisomy 16 fetuses. If such efforts are successful, it is hoped that extrapolations from the mouse model to humans will allow for the identification in the population, of individuals at risk for giving birth to an aneuploid offspring.

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