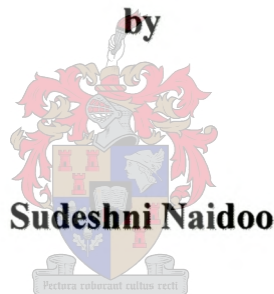# Fetal Alcohol Syndrome in the Western Cape: Craniofacial and Oral Manifestations – a case control study

by

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DECLARATION

I, the undersigned, hereby do declare that the work contained in this dissertation is my own original work and that it have not previously in its entirety or in part submitted it at any university for a degree.

SUDESHNI NAIDOO   **DATE**

The work presented in this dissertation was undertaken in the Department of Community Dentistry, University of Stellenbosch, Tygerberg, Cape Town

ABSTRACT

Introduction: Fetal alcohol syndrome (FAS) consists of multi-system abnormalities and is caused by the excessive intake of alcohol during pregnancy. The teratogenic effect of alcohol on the human fetus has now been established beyond reasonable doubt and FAS is the most important human teratogenic condition known today. The syndrome, first described by Lemoine in1968 in the French literature and in the English literature by Jones and Smith in 1973, has since been corroborated by numerous animal and human studies.

This study has grown out of several epidemiological, prenatal and infant studies in areas of the Western Cape that are currently being undertaken by the Foundation for Alcohol Related Research (FARR). Preliminary data from studies in Wellington have confirmed that a significant proportion of school-entry children have FAS. The prevalence of FAS in this community exceeds that for Down syndrome by a factor of 30 times. The frequency of FAS in high-risk populations of the Western Cape is the highest reported anywhere in the world. With this background, and the paucity of FAS literature related to dentistry, the aim of this study was to determine the craniofacial and oral manifestations of FAS in a sample of school-going children in the Western Cape.

Methodology: This study is a descriptive, case-control, cross-sectional study using a random cluster sampling method. On the day of examination, children were weighed, and their height and head circumference were measured. They then had photographs and radiographs taken, followed by an oral examination. For each child, the following information was recorded on the data capture sheet: date of birth, gender, head circumference, weight and height, enamel opacities, dental fluorosis, plaque index, gingival bleeding index, dentition status, oral mucosal lesions and dentofacial anomalies.

Results: The total sample of 90 children with diagnosed FAS and 90 controls, were matched for age, gender and social class. There were no significant age differences between the two groups (p=0.3363) and the mean ages were 8.9 and 9.1 for the FAS and control groups respectively.

Head circumference (HC) differed significantly between the two groups (p<0.0001) and the three photographic diagnostic measurements were all influenced by head circumference. The prevalence of enamel opacities between FAS and controls was not significantly different and averaged around 15% for both groups. The opacities were found largely in the maxillary central incisor and lower first molar teeth. More than three quarters of both the cases and the controls demonstrated the presence of plaque and almost two thirds demonstrated gingival bleeding on probing. FAS patients had statistically significantly (p<0.001) more dentofacial anomalies than the controls. The mean dmft score for the FAS sample was slightly higher, though not significantly different from that of the controls and the decayed component (d) made up the largest part of the index in both groups. None of the FAS children had any missing or filled teeth, and in the case of the controls these were also rarely found. Thirty nine children (21.67%) of the total sample were caries-free.

Discussion: This study represents one of the largest sample sizes documenting the craniofacial and oral and dental manifestations of the FAS to date. Forty two per cent of the FAS sample manifested growth retardation and this was statistically significant (p<0.0001) when compared to their controls. Analysis of the face using anthropometry supports many of the previous clinical descriptions of the effects of neonatal alcohol exposure and offers some new perspectives on the FAS facial phenotype. The characteristic dysmorphic facial features found included ptosis of the upper eyelids, epicanthic folds, short upturned nose, thin vermillion border of the upper lip and a smooth philtrum.

Overall the analysis of the caries data for this study in respect of differences between cases and controls was found to be unremarkable. The lack of difference in the primary and permanent dentitions between the cases and controls could have been anticipated in this population due to the high prevalence of dental caries among children from the Western Cape. The FAS children showed significantly lower dental ages when compared to the controls. Dental maturation has previously been shown to be mildly, but consistently, delayed in children with delayed development and therefore this is a not surprising finding for the FAS children in this study.

Differences between skeletal age and chronological age were noted for both boys and girls, but as a whole, in the present study groups (FAS and controls) showed little variation in skeletal development.

Measurements related to the face height and mandibular size appear to be the most important in distinguishing the FAS children from the controls. Most (5 out of 8) of the discriminating linear measurements studied lie in the front of the skull area. Most of the discriminating measurements are vertical measurements and only two of the measurements are lines between soft tissue points. When comparing the photographic analyses of the facial features versus the cephalometric assessments; the four facial features most typical of a FAS child had a Positive Predictive Value (PPV) of 92% and a Negative Predictive Value (NPV) of 90% and the eight linear measurements from the cephalometric analyses had a PPV of 92% and a NPV of 95%. One can therefore conclude that the external facial features are probably more reliable in discriminating between the two groups than the cephalometric measures. For further analyses, other models where a single angular measurement explains a combination of linear measurements need to be investigated. This might further improve the discriminating abilities of the cephalometric measurements as a whole.

Conclusions: This study has shown the importance of the oral and craniofacial features of FAS. FAS can no longer be viewed as just a rare and peculiar childhood disorder. Awareness and recognition of children with FAS is important so that they can be correctly diagnosed and referred appropriately. Prevention of the secondary disabilities and most importantly, the prevention of FAS in subsequent programmes can be planned. The dentist who treats children with FAS must recognise that such patients might be emotionally and mentally handicapped and may make treatment difficult and there may be a need for the child to be treated with behaviour modification and/or premedication before restorative treatment. The dentist should also be aware of the need for an accurate medical history, and possible medical consultations, before treatment can be undertaken safely.

OPSOMMING

Fetale alkoholsindroom (FAS) bestaan uit multisisteem abnormaliteite en word veroorsaak deur oormatige inname van alkohol tydens swangerskap. Die teratogeniese uitwerking van alkohol op die menslike fetus word nie meer betwyfel nie en FAS is die belangrikste menslike teratogeniese toestand tans bekend. Die sindroom, soos aanvanklik deur Jones en Smith in 1973 beskryf, is sedertdien deur vele studies op mens en dier bevestig.

Hierdie studie het gegroei uit vele epidemiologiese-, prenatale- en kleuterstudies in dele van die Weskaap wat tans onderneem word deur die Stigting vir Alkoholverwante Navorsing. Voorlopige data van die studies in Wellington bevestig dat 'n betekenisvolle deel van skoolbeginners FAS het. Die prevalensie van FAS in hierdie gemeenskap oortref dié van Down se sindroom met 'n faktor van 30. Die frekwensie van FAS in die Weskaap is die hoogste wat in die wêreld gerapporteer is. Met hierdie agtergrond, en die skaarste aan FAS literatuur wat op tandheelkunde betrekking het, was die doel van hierdie studie om die kraniofasiale en mondmanifestasies van fetale alkoholsindroom in 'n monster van skoolkinders in die Weskaap te ondersoek.

Metodologie: Hierdie studie was 'n beskrywende, gevallebeheerde deursneestudie waarin 'n lukrake gebondelde monstermetode gebruik is. Op die dag van die ondersoek is die kinders geweeg en hulle lengte en kopomtrek bepaal. Hierna is foto's en x-straalopnames geneem, gevolg deur 'n mondondersoek. Die volgende inligting is vir elke kind aangeteken: geboortedatum, geslag, kopomtrek, massa en lengte, glasuur-opasiteite, tandfluorose, plaakindeks, gingivale bloedingsindeks, gebitstatus, mukosale letsels en dentofasiale anomalieë.

Resultate: Die totale monster, bestaande uit 90 kinders met gediagnoseerde fetale alkoholsindroom en 90 bypassende kontroles, is vergelyk ten opsigte van ouderdom, geslag en sosiale klas. Daar was geen betekenisvolle ouderdomsverskille tussen die twee groepe nie (p-=0.3363). Kopomtrek het betekenisvol tussen die twee groepe verskil (p<0.0001), en die drie fotografiese diagnostiese afmetings is almal beïnvloed deur kopomtrek.

Die prevalensie van glasuur-opasiteite tussen die FAS- en kontrolegroep was nie betekenisvol nie en het rondom 15% vir beide gewissel. Die opasiteite is hoofsaaklik gesien in maksillêre sentrale snytande en mandibulêre eerste molare. Meer as driekwart van beide groepe het plaak getoon, en byna tweederdes het gingivale bloeding met sondering gehad. Die gevallegroep het statisties betekenisvol meer (p<0.001) dentofasiale anomalieë getoon. Die gemiddelde dmft telling vir die FAS groep was effens hoër, alhoewel nie betekenisvol nie, as die kontrolegroep, en die "delayed" (vertraagde erupsie) komponent (d) het die grootste deel van die indeks uitgemaak in beide groepe. Geen van die FAS kinders het enige afwesige tande (m) of herstelde tande (f) gehad nie, soos ook gevind in die kontrolegroep. Nege-en-dertig kinders (21.67%) van die totale monster was kariesvry.

Bespreking: Hierdie studie verteenwoordig een van die grootste monstergroottes tot op datum waarin ondersoek ingestel is na die kraniofasiale en mond- en tandmanifestasies van die fetale alkoholsindroom. Twee-en-veertig persent van die FAS monster het vertraagde groei getoon en dit was statisties-betekenisvol (p<0.0001) vergeleke met die kontrolegroep. Antropometriese analise van die gesig steun die vele kliniese beskrywings van neonatale blootstelling aan alkohol, en bied ook nuwe perspektiewe op die FAS gesigsfenotipe. Die kenmerkende dismorfiese gesigseienskappe wat gevind word, sluit ptose van die boonste ooglede, epikantusvoue, kort opgedraaide neus, dun vermiljoen rand van die bolip en 'n gladde filtrum in.

In die geheel was die analise van die karies data ten opsigte van verskille tussen gevalle en kontroles onopvallend. Die afwesigheid van 'n verskil in die primêre en sekondêre gebitte in die gevalle en kontroles kon in hierdie bevolking verwag gewees het as gevolg van die hoë voorkoms van tandkaries onder kinders in die Weskaap. Die FAS kinders het betekenisvol-laer gebitouderdomme gehad as die kontrolegroep. Gebitmaturasie is in geringe maar deurlopende mate vertraag in kinders met vertraagde ontwikkelings, soos voorheen al getoon, en is daarom nie verbasend vir die FAS kinders in hierdie studie nie. Verskille tussen skeletale ouderdom en chronologiese ouderdom is gevind in beide seuns en dogters, maar in die geheel het dié huidige groepe (FAS en kontroles) min variasie in skeletale ontwikkeling getoon.

Dit wil voorkom of afmetings wat verband hou met die gesigshoogte en grootte van die mandibula die belangrikste is om FAS kinders van die kontrolegroep te onderskei. Meeste (5 uit 8) van die diskriminerende lineêre afmetings wat bestudeer is, lê op die voorkant van die skedel. Die meeste is vertikale afmetings, terwyl slegs twee lyne tussen sagte weefsel punte. Waneer die fotografiese analises van die gesigseienskappe vergelyk word met die sefalometriese waarnemings, word gevind dat die vier gesigseienskappe tipies van 'n FAS kind 'n Positiewe Voorspelbare Waarde (PVW) van 92% en 'n Negatiewe Voorspelbare Waarde (NVW) van 90% het, en die agt lineêre afmetings vanaf die sefalometriese analise 'n PVW van 92% en 'n NPV van 95% het. Daar kan dus afgelei word dat die eksterne gesigseienskappe waarskynlik meer betroubaar is om te onderskei tussen die twee groepe. Vir verdere analise behoort ander modelle waar 'n enkel hoekige afmeting 'n kombinasie van lineêre afmetings verduidelik, ondersoek te word. Dit mag die diskriminerende vermoëns van sefalometriese afmetings in die geheel verder bevorder.

Gevolgtrekking: Hierdie studie het die belang van orale en kraniofasiale eienskappe van FAS getoon. Die toestand kan nie langer as 'n seldsame en eienaardige aandoening van kinders beskou word nie, en bewustheid en herkenning van fetale alkoholsindroom pasiënte is belangrik sodat hulle korrek gediagnoseer en op gepaste wyse verwys kan word. Die tandarts wat FAS pasiënte behandel, moet besef dat sulke pasiënte emosioneel en geestelik belemmer mag wees en dus hantering en behandeling bemoeilik. Daar mag 'n behoefte ontstaan vir gedragsmodifikasie en/of premedikasie voor herstellende behandeling. Verder moet die tandarts bewus wees van die behoefte aan 'n akkurate mediese geskiedenis, en moontlik konsultasie met 'n geneesheer, voor behandeling veilig ingestel kan word.

DEDICATION

This thesis is dedicated

to

my parents

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Glossary

Apoptosis: A type of cell death that occurs in individual, damaged cells and during which cells break down in a form of cell suicide as a result of biological processes within the cell; also called programmed cell death (because it occurs during normal development).

Astrocyte: A type of *glia* cell.

Cerebellum: As area at the base of the brain involved in the maintenance of posture, balance, and coordination.

Corpus callosum: A bundle of nerve fibers connecting the brain's hemispheres.
Cortex (or cerebral cortex): The outer layer of gray matter covering the surface of the forebrain that contains the brain regions controlling sensory, motor, perceptual, emotional, and higher cognitive processes.

Differentiation: The process through which unspecialised embryonic cells or tissues are modified and altered to achieve specific physical forms, physiological functions, and chemical properties.

Enzyme: A protein that facilitates chemical reactions.
Excitatory neurotoxin: Any substance that damages nerve cells by causing excessive nerve cell activity.

Free radicals: Highly reactive molecules that cannot exist in a free state for a prolonged period and which can cause cell damage; include reactive oxygen species.

Gene expression: The processes through which the genetic information contained within a gene on the DNA is converted into a gene product (e.g., a protein).

Glia cell: A type of cell in the central nervous system that serves diverse support functions for the development and function of the nerve cells.

Glucose: A type of sugar molecule that is the main energy source for most cells and which is needed to form the building blocks of DNA, fat molecules, and certain hormones.

Mitochondria: *Organelles* that generate energy for the cell"s metabolic processes; also help maintain calcium levels in nerve cells.

Necrosis: A type of cell death that occurs in groups of cells in response to disease or injury in which cells swell and rupture, typically resulting in inflammation.

Organelle: A functional component of a cell; each organelle has its own membrane and specialized function.

Oxidative stress: The end result of an imbalance between *free radicals* and substances that scavenge those reactive species (i.e., antioxidants); can lead to cell damage.

Pathogenesis: The source or cause of an illness or abnormal condition.

Phenotype: The observable characteristics (e.g., physical appearance or behavior) of an organism.

Receptor: A protein molecule on the surface of a cell that interacts with a specific chemical messenger, such as a neurotransmitter or hormone.

Retinoic acid: A chemical derived from retinal (i.e., vitamin A) that is necessary for normal embryonic development, including the development of a certain cell type, the cranial neural crest cells, into head an facial structures.

Teratogenic: That which causes abnormalities during development.

List of Abbreviations

ACh	Acetylcholine
ADH	Alcohol dehydrogenase
AMP	Adenosine monophosphate
ARBD	Alcohol related birth defect
BAL	Blood alcohol level
BDNF	Brain-derived neurotrophic factor
CNCC	Cranial neural crest cells
CNS	Central nervous system
CRF	Corticotropin-releasing factor
DA	Dopamine
EGF	Epidermal growth factor
ERK	Extracellualr protein kinase
FAE	Fetal alcohol effect
FAS	Fetal alcohol syndrome
FAAR	Foundation for alcohol related research
GABA	Gamma-amino butyric acid
GH	Growth hormone
HC	Head circumference
HNE	4-Hydroxynonenal
HPA	Hypothalamic-pituitary-adrenal axis
ICD	Inner canthal distance
IGF	Insulin-like growth factors
IPD	Inter pupillary distance
LTP	Long-term potentiation
mRNA	Messenger ribonucleic acid
MAP	Mitogen activated protein
MBP	Myelin basic protein
MEOS	Microsomal ethanol oxidising system
MNP	Medial nasal prominence

NCAM	Neural cell adhesion molecule
NCC	Neural crest cells
NGF	Nerve growth factor
NMDA	N-methyl-D-aspartate
NOS	Nitric oxide synthase
NPV	Negative predictive value
PFL	Palpebral fissure length
PKC	Protein kinase
PPV	Positive predictive value
RA	Retinoic Acid
SD	Standard deviation
SEM	Scanning electron microscope
VP	Vasopressin

Jan Steen (1625-1679)

The Effects of Intemperance

National Gallery, London

The painting is an allegorical warning of the evils of alcohol, although the mood is one of amused indulgent disapproval, rather than puritanical revulsion. The woman slumped on the left, whose pocket is being picked by a boy and above whose head is a basket of unused domestic utensils, illustrates the proverb "Wine is a Mocker"; the boy on her left who throws roses before swine refers to a second proverb – to throw pearls before swine; and the children who foolishly feed the cat, a third. In Steen's world children are often used to point out the foolishness of their elders.

PART ONE

Chapter 1: Introduction and Overview of the Thesis

The potential adverse effect of maternal alcoholism on the developing fetus has been referred to in early Greek and Roman mythology (Jones and Smith, 1973). In a Carthagian ritual, the bridal couple was forbidden to drink wine on their wedding night so that defective children might not be conceived - Judges 13:7 states "Behold, thou shalt conceive, and bear a son; and now drink no wine or strong drink........." (Haggard and Jellinek, 1942). In 1834, a British House of Commons select committee investigating drunkenness, reported that infants born to alcoholic mothers had a starved, shrivelled and imperfect look (Jones and Smith, 1973, 1975). The first documented observations appeared in 1849 with the publication of the essay by Carpenter entitled " The Use and Abuse of Alcoholic Liquors in Health and Disease" (Librizzi, 1982). In 1900, Sullivan (1900) recorded increased abortion and still birth rates among chronic alcoholic mothers in a Liverpool prison and an increased frequency of epilepsy in their surviving offspring. Various investigators have since then reported increased frequency of abnormalities and decreased birth weight of surviving children born to chronic alcoholic mothers (Ladraque, 1901; Roe, 1944).

Until about 20 years ago, the dangers of maternal alcohol consumption to the developing fetus were generally dismissed. Reports linking maternal alcohol use and fetal growth deficiency emerged from France in the late 1950s (Lecomte 1950; Christiaens, Mizon and Delmarle, 1960). Then in 1968, Lemoine and colleagues described growth deficit, mental retardation and an unusually high rate of birth defects in 127 children born to alcoholic mothers. In addition, Ulleland (1972) found pre- and postnatal growth deficiency and developmental abnormalities in 8 out of 12 children born to alcoholic mothers.

Based in part on these reports, Jones and Smith (1973) introduced the term "fetal alcohol syndrome" (FAS) to describe a pattern of abnormalities (distinctive craniofacial disorders and a pattern of altered growth and morphogenesis) found in some children prenatally exposed to alcohol.

Their definition was significant as it clearly delineated a clinically recognisable syndrome that was distinct from all other patterns of congenital malformation and was seen exclusively in offspring of mothers who drank large amounts of alcohol during pregnancy. Fetal alcohol syndrome (FAS) consists of multi-system abnormalities and is caused by the excessive intake of alcohol during pregnancy. The teratogenic effect of alcohol on humans has now been established beyond reasonable doubt and FAS is the most important human teratogenic condition known today. The incidence of FAS varies from nation to nation and is dependent upon a population's alcohol consumption (Carones et al, 1992). It occurs with a birth prevalence of between 0.22 – 0.33 per 1000 live births in the US (Abel and Sokol, 1991; Abel and Sokol 1987), and an average of 0.97 per 1 000 for the rest of the developed world (Abel, 1995), which closely approximates to the frequency of more familiar dysmorphic conditions like Down syndrome.

Various methods, including both active and passive case ascertainment, have been used to determine the prevalence of FAS. Information for estimating FAS prevalence comes from birth records, registries, clinic-based studies and population-based initiatives (Institute of Medicine, 1996). Because of the wide variety of methods used, comparison of prevalences and epidemiological characteristics of FAS between populations is often of little value. For example, virtually all active case ascertainment studies have been carried out among American Indians, while passive case ascertainment has been predominantly used in mainstream North American and European populations (May, 1996).

The syndrome, first described by Lemoine in1968 in the French literature and in the English literature by Jones and Smith in 1973, has since been corroborated by numerous animal and human studies. During the ensuing few years, more than 100 patients with FAS were reported. The earliest descriptions concentrated on the most severely affected patients, in whom the syndrome was most clearly recognisable. It soon became apparent, however, that the diagnosis of FAS could encompass children with varying degrees of growth failure and mental deficiency and some did not display all of the physical abnormalities originally considered essential for the diagnosis.

Many investigators have catalogued, quantified and refined these hallmarks of FAS over the years and have established that the most consistent consequences of heavy maternal drinking during pregnancy are prenatal and postnatal growth deficiency and brain and craniofacial abnormalities (Hanson et al, 1976, 1978; Chernoff, 1977; Clarren and Smith, 1978; Clarren et al, 1978; Smith, 1980; Streissguth et al, 1980; Sulik et al, 1986; Kotch and Sulik, 1992; Coles, 1994; Weston et al, 1994).

When the term 'fetal alcohol syndrome' was first coined, it was used to describe and diagnose individuals whose phenotype represented the most severely affected. Since that time, it has become apparent that the effects of prenatal exposure on the fetus can be broad-based and not limited to the original descriptions of FAS (Zajac and Abel, 1992; Aase, 1994; Stratton et al, 1996; Mattson et al, 1998). Studies have shown that the effects of prenatal alcohol exposure fall along a continuum from extreme (perinatal fetal demise) to more subtle anomalies (behavioural problems) and that FAS represents the severe end of the continuum in the phenotypic expression of prenatal alcohol exposure (Streissguth et al, 1980; Blakley, 1988; Aase, 1994; Stratton et al, 1996; Sowell et al, 2001). Even though fetal alcohol syndrome can be diagnosed without confirmation of heavy maternal drinking (Institute of Medicine, 1996), a detailed maternal history is very desirable to confirm the nature of gestational drinking and to document social circumstances, particularly in cases where dysmorphology is less consistent (Aase, 1994).

The typical characteristics of FAS are (i) facial abnormalities including microcephaly, narrow forehead, micrognathia, maxillary hypoplasia, flat midface, narrow palpebral fissures, short and small nose, long upper lip with a narrow vermillion border, diminished or absent philtrum and epicanthal folds, (ii) central nervous system dysfunction with mental retardation ranging from mild to severe, (iii) growth deficiency as in lower weight and height at birth persisting into the postnatal period and (iv) various cardiovascular and skeletal abnormalities (Jones and Smith, 1973). Miller, Israel and Cuttom (1981) reported opthalmological findings like myopia, microphthalmia, ptosis, strabismus and blepharophimosis. Many other organ abnormalities have been reported including cleft lip and palate, renal malformations and deafness.

1.1   A profile of alcohol use in South Africa

Alcohol plays a prominent role in the cultural, social and economic life of South Africa (Parry and Bennetts, 1998). However, there is increasing evidence that the misuse of alcohol threatens not only public health, but also economic and social development. Alcohol abuse has an enormous negative impact on public health, and while part of the solution to the abuse of alcohol will come from macro-level development (indirect strategies) like improvement of literacy and the provision of educational opportunities, by far the most important will be the policy process aimed at directly addressing alcohol-related problems (Parry and Bennetts, 1998).

1.2   A brief history of alcohol in South Africa

South Africa's apartheid legacy runs through its history of alcohol use. Conflict has long surrounded the production, distribution and use of alcohol. In traditional African society the use of alcoholic drinks was regulated. Drinking did not occur on a daily basis. People did not drink alone or just for the sake of drinking. Rather, drinking served a communal and ceremonial function. After colonisation, the British unsuccessfully prohibited the use of alcohol by Africans in an attempt to prevent what they saw as social decay and disorder encouraged by its use. Laws were promulgated making it illegal for Black Africans to produce and obtain liquor, and these laws were only repealed as late as 1962 (Parry and Bennetts, 1998).

Paradoxically, alcohol was also seen as a means of establishing and maintaining economic and social control, particularly on the farms, mines and urban industry. In the Cape, employers on wine and other farms, and in the emerging diamond and gold mines to the north, used alcohol to attract and retain workers from rural areas (Parry and Bennetts, 1998). Many people of the Western Cape are involved in growing grapes and producing wine, and this has influenced the modal regional drinking patterns. Wine was distributed among labourers and consumed daily as partial payment for labour, a custom referred to as the "dop" system.

This tradition became an institutionalised element of farming practice in the Cape and an important element of the social control exercised over indigenous peoples of the region (Scully, 1992, Levine, 2001). Evidence indicates that although the "tot" or "dop" system no longer operates as widely or with such intensity as originally introduced under colonial agriculture, about 5% of farms in the Western and Northern Cape provinces still practise it today. It takes many forms, including partial payment in lieu of wages and the provision of alcohol to supplement wages both during the week and over the weekends (London et al, 1998a, te Water Naude et al, 1998). Furthermore, increased availability of inexpensive commercial wine, beer, and liquor today in shebeens (illegal bars) and carry-out sources has exacerbated problems of heavy drinking. Weekend binge drinking is a major form of recreation among subsegments of the population (Parry and Bennetts, 1998; Parry 1999).

The "dop" system has been outlawed by at least 2 legislative acts, but residual patterns of regular and heavy alcohol consumption by workers remain today as its legacy in Western Cape society (Whittaker, 1987; London, 1999; London et al, 1998b, Crome and Glass, 2000). Alcohol abuse remains one of the major challenges facing the health services in the Western Cape and elsewhere (London, 1999; Parry 1999). The adverse health and development impacts of the dop system and alcohol abuse are substantial among the rural farming communities in the Cape, where alcohol-related trauma, exceptionally high rates of TB, child and adult malnutrition and the FAS are common (London et al, 1998b, Levine, 2001).

Drinking during pregnancy has been reported to be frequent in parts of the Western Cape, even among prenatal clinic patients. In one study, 34% of prenatal patients in the large metropolitan areas of Cape Town reported drinking during pregnancy (Croxford and Viljoen, 1999). In the Cape rural areas 46% to 51% reported drinking during pregnancy (Croxford, 1998). In recent years there has been growing concern about the number of FAS cases in certain parts of South Africa (NIAAA, 1996, 1998).

1.3 The rationale for this study

Children with FAS are often profoundly handicapped: their intellectual and behavioural development is impaired, they have learning disabilities and memory deficits, and need constant support from their communities. Despite the extent of this problem, the effects of FAS continue to go unrecognized by many health professionals. Although there have been numerous reports of FAS in the medical literature since 1973, little attention has been given to it in the dental literature. This is in spite of the fact the craniofacial deformities form an important part of the syndrome complex. The primary purpose of this study was to document the oral, dental and craniofacial findings of a group of Western Cape school-going children with FAS and compare them with matched controls.

The importance of the orofacial and cranial manifestations of the FAS is well established, but there is still an enormous need for further research, particularly epidemiological research, in order to understand and deal with it better. This study arose out of the need for epidemiological data and out of academic interest. Despite the wealth of information related to the medical aspects of FAS, it was clear from a review of the literature that there is a paucity of literature in the dental field. This thesis presents findings from a case-control study of the orofacial and cranial manifestations associated with FAS. The central tenet deals with the importance, diagnosis and recognition of the manifestations associated with FAS.

This report may serve to alert the practising dentist or medical practitioner to the ravages and primary clinical manifestations of the FAS. Awareness and recognition of children with FAS are important so that they can be correctly diagnosed and managed appropriately. Understanding the many disturbances of growth and development which result from the widespread use of alcohol during pregnancy obviously warrants attention. In addition, dentistry has much to offer in helping determine true aetiologic factors in disturbances that affect growth and development of teeth, bones and various soft tissues of the oral cavity. This is best accomplished by having a thorough knowledge of the disease processes involved.

Despite the fact that the focus of the present study is on the oral and craniofacial manifestations of FAS, it needs to be contextualized within the broader understanding of alcohol abuse as a serious vice affecting all strata of society. The government has recently showed political commitment to addressing alcohol abuse and the challenge now is to translate policies into reality. The data arising from this study may be utilized to increase the awareness of health care workers to the fetal effects of alcohol abuse and assist them to identify and diagnose children timeously.

1.4   Structure of the report

Following an introduction and background, the literature review is divided into three chapters. The first of these (Chapter Two) discusses the principles of normal and abnormal embryogenesis, a very complex and finely orchestrated process. The second (Chapter Three) reviews the existing literature related to the pathogenesis of the FAS with a specific focus on the cellular and molecular bases for alcohol's teratogenic effects and the third (Chapter Four) reviews literature related to the epidemiology of FAS and discusses the clinical issues associated with diagnosis and recording. This is followed by a synopsis of the research design and methodology (Chapter Five). As a consequence of the number of the different variables that have been recorded, the results are presented in four parts (Chapter 6-9). Each of these chapters begins with a background and introduction to the examination, an overview of the methodology and statistical analysis used, the results, followed by a discussion of the results, and some concluding remarks. Chapter Six discusses the demography of the sample, followed by the anthropometric findings and the general clinical examination (including the minor anomalies). The photographic analysis of the facial characteristics will be described and discussed. In Chapter Seven the intra-oral examination, describing the hard tissues, oral health status and the soft tissues is reported and discussed. In Chapter 8 the findings of the dental and skeletal age assessment will be discussed. The results of the dental and skeletal age assessment will be presented and discussed. Chapter 9 documents the cephalometric examination results while Chapter 10 will provide a discussion of the overall findings and the author's recommendations are suggested.

Review of the Literature

The objective of the literature review is to reveal the relationship between acute and chronic maternal alcohol abuse during pregnancy and the damage to the child *in utero* that results in the FAS and with a specific focus on the craniofacial and oral aspects of the syndrome. The first section of the review (Chapter 2) describes only the most basic principles of embryology as related to the pathogenesis of the FAS and includes details of the structural development of the orofacial structures. The second section of the review summarises the pathogenesis and neuropathological findings (Chapter 3) and reflects the fundamental importance of teratogenic alcohol effects on the developing brain. The exact mechanism of alcohol damage to the fetus is still unknown. The third section (Chapter 4) is concerned with clinical issues and introduces the general problem of alcohol teratogenicity in humans. It presents a detailed description of the clinical symptoms of FAS and a discussion on the epidemiology of maternal alcohol abuse, both globally and locally.

Chapter 2:   Principles of Normal and Abnormal Embryogenesis

2.1   Introduction

Normal embryogenesis is an exceedingly complex and finely orchestrated process whose molecular and biochemical basis is still poorly understood. The subject of dysmorphology relates to aberrant structural development occurring primarily before birth. This chapter of the literature review discusses only the most basic principles of embryology as related to development of the FAS. Further details of the structural development of the orofacial structures are provided in a later section.

2.2   Developmental timing

Prenatal development may be conveniently divided into three time periods: the implantation stage, extending from the time of fertilisation of the egg to the end of the third week of gestation; the embryonic stage, beginning from the beginning of week 3 to the end of week 7, and the fetal stage, from week 8 until birth (Aase, 1990).

During the implantation stage, rapid cell proliferation leads to formation of the hollow blastocyst, within which develops the embryonic plate. The amniotic cavity appears and primitive circulatory connections with the placenta are established. Early cell differentiation begins late in this period, with somites demarcated in the mesodermal layer, and longitudinal folds of neuro-ectoderm indicating the site of the future brain and spinal cord. The embryonic stage is the time of primary tissue differentiation and the formation of definitive organs. Neural tissues undergo rapid proliferation, closure of the neural tube and flexion of its anterior segments to form the divisions of the developing brain. The heart begins to beat, allowing blood to circulate through the newly formed vascular system even before the cardiac structures are fully formed. Primordia of the facial structure, limbs and internal organs appear and gradually assume their final shapes and positions.

By the beginning of the fetal stage, a distinctly human form has been established, and the differentiation of all organ systems is complete. The remainder of gestation is primarily a period of growth in size with progressive enlargement of skeletal structures, muscle and especially the brain.

2.3   Embryologic mechanisms

Early embryonic development involves distinct phenomena by which cells form various tissues, organs and body structures. Simple cell proliferation occurs at different rates in different parts of the body, both before and after differentiation into specific tissues. Some cell types, such as melanocytes, undergo migration in relation to their neighbours, eventually arriving at locations far removed from their sites of origin. Programmed cell death is an important factor in the formation of many structures, as in the separation of the digits of the hand. Fusion between adjacent tissues is also an important mechanism in the formation of such structures as the upper lip and heart. There is now clear evidence that many of these developmental processes are brought about by induction by neighbouring cells.

Tissues of different types seem to communicate chemically with each other, across intervening membranes, and the ensuing "dialogue" appears to be crucial to normal embryonic development. All of these developmental processes normally take place in a specific sequence peculiar to each tissue or structure, and the timing may be quite critical. For this reason, even a brief delay in cell migration or cell death may result in abnormal formation of the structure in question and of the adjoining tissues. Sometimes, a seemingly minor interruption of the intricate orchestration of development produces a cascade of developmental abnormalities. If a structure has reached a certain stage of development before malformation occurred, the time of appearance of the defect can often be estimated with considerable accuracy. Once a tissue or structure has reached a certain stage of differentiation, its eventual form becomes established, and this stage seems to be reached quite early in embryogenesis.

2.4   The neural crest

The early development of the head is dependent on the formation of two series of segmented structures, the rhombomeres of the hindbrain and the branchial arch series. The initial formation of these two systems is closely linked, as the principal source of the branchial arch mesenchyme is the neural crest. The subsequent development of the two systems maintains a close level of integration, as specific spatial relationships between skeletal, muscle and neural elements arising from the same axial level are established (Hunt et al, 1998). The neural crest arises from neuroepithelial cells along the lateral border of the neural plate. Neural crest cells (NCC) migrate from the neural tube to peripheral locations throughout the body. NCC differentiate into many types of adult cells such as sensory and autonomic neurons, Schwann cells and pigment-producing cells. Cells from part of the cranial neural crest also differentiate into bone, cartilage, dentine, dermal fibroblasts and several regions of the heart and the great vessels. The control of differentiation of NCC seems to be diverse, with some cells being determined before they migrate and others responding to environmental cues along their path of migration (Newgreen and Erikson, 1986; Morris-Kay and Tan, 1987).
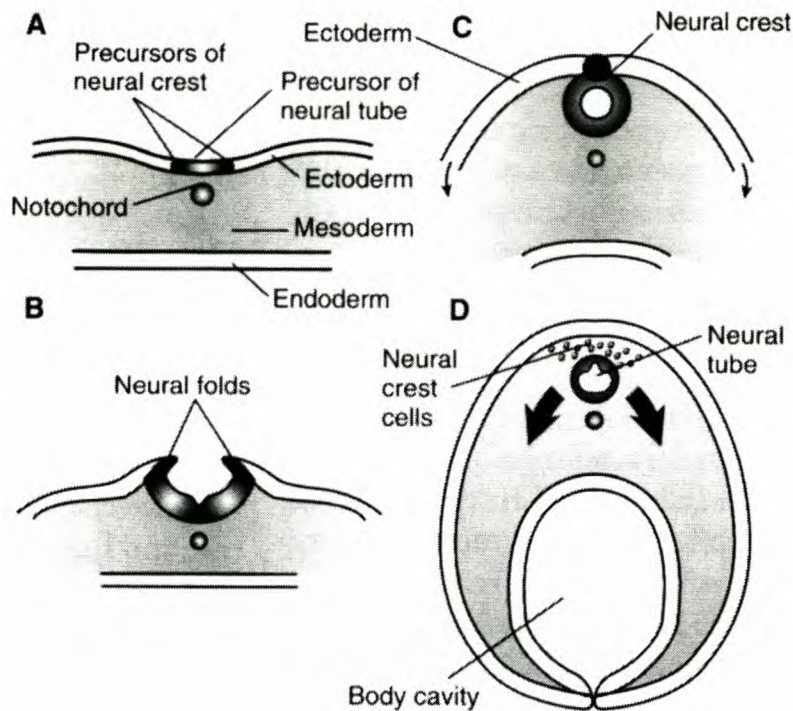
Figure 1: Cross-sectional view - Formation of the neural tube

Early in an embryo's development, a strip of specialised cells, the notochord (A), induces cells of the ectoderm directly above it to become the primitive nervous system (ie. neuroepithelium). The neuroepithelium then wrinkles and folds over (B). As the tips of the folds fuse together, a hollow tube (ie. the neural tube) forms (C) – this is the precursor of the brain and spinal cord. Meanwhile the ectoderm and endoderm continue to curve around and fuse beneath the embryo to form the body cavity. Cells originating from the fused tips of the neuroectoderm (ie. neural crest cells) migrate to various locations throughout the embryo, where they will initiate the development of diverse body structures (D). (Adapted from Goodlett and Horn, 2001)

The cranial neural crest is a major component of the cephalic end of the embryo. Comparative anatomical and developmental research suggests that the neural crest may represent the major morphological substrate for the evolution of the vetebrate head. Cells of the cranial neural crest form many tissues of the facial region (Noden, 1986; Osumi-Yamashita and Eto, 1990) and are patterned with level-specific instructions (Hunt et al, 1998).

2.5   Development of the head and neck

A detailed knowledge of human fetal craniofacial growth is necessary for understanding the mechanisms underlying the growth process and how altered growth patterns may give rise to specific craniofacial anomalies. Morphogenesis and changes in the size and shape of the craniofacial complex are dramatic during the first two trimesters of human development (Diewert and Lozanoff, 1993 a, b). Craniofacial growth during the third trimester is complex, and growth in overall size slows considerably (Plavcan and German, 1995).

The early craniofacial region arises from the rostral portions of the neural tube, the notochord, and the pharynx which is surrounded by a series of aortic arches. Between these structures and the overlying ectoderm are large masses of neural crest and mesodermally derived mesenchyme. A number of these components show evidence of anatomical segmentation or segmental patterns of gene expression (Sperber, 1989). Early cranial development is characterized by a number of migrations and displacements of cells and tissues. The neural crest is the first tissue to exhibit massive migratory behaviour. Migrations of segmental groups of neural crest cells provide the mesenchyme for much of the facial region. The musculature of the craniofacial region is derived from the somitomeric mesoderm or the occipital somites (Sperber, 1989). The connective tissue component of the facial musculature is of neural crest origin. According to present understanding the neural crest cells, which constitute the bulk of the mesenchyme of the primordia, become endowed with specific morphogenetic information before they migrate into the facial primordia. The nature of this information and the way it is used remain poorly understood. Specific patterns of both retinoic acid receptors and retinoic acid-binding proteins in these structures are important in both normal morphogenesis and in retinoic acid-induced anomalies of the facial region (Gans and Northcutt, 1983).

The pharyngeal (branchial) region is organised around paired mesenchymal pharyngeal arches, which alternate with endodermally lined pharyngeal pouches and ectodermally lined branchial grooves. The face and lower jaw arise from an unpaired frontonasal prominence and the paired nasomedial, maxillary and mandibular process. Through differential growth and fusion, nasomedial processes form the upper jaw and lip, and the frontonasal prominence forms the upper part of the face. The expanding mandibular processes merge to form the lower jaw and lip. A nasolacrimal groove between the nasomedial and maxillary processes ultimately becomes canalized to form the nasolacrimal duct, which connects the orbit with the nasal cavity (Sperber, 1989).

The palate arises from the fusion of an unpaired median palatine process and paired lateral palatine processes, the former forming the primary palate and the latter the secondary palate. The olfactory apparatus begins as a pair of thickened ectodermal placodes. As these sink to form the nasal pits, they are surrounded by horseshoe-shaped nasomedial and nasolateral processes. The former form the bridge and septum of the nose, and the nasolateral processes form the alae of the nose (Sperber, 1989).

Teeth form from interactions between oral ectoderm (dental lamina) and neural crest mesenchyme. A developing tooth is first a tooth bud, which then passes through a cap and bell stage. Late in the bell stage, ectodermal cells (ameloblasts) of the epithelial enamel organ begin to form enamel. After the amelobalsts induce differentiation of the odontoblasts from neural crest-derived ectomesenchyme in the dental pulp region of the dental follicle, the odontoblasts produce dentine. Precursors of the permanent teeth form dental primordia along with the more advanced primary teeth (Lumsden, 1988; Kollar, 1981).

Malformations, such as cleft lip and cleft palate represent the persistence of the structural arrangements that are normal for early embryonic stages. Others, such as hypertelorism, result from growth disturbances in the frontonasal process (Mazzola, 1976). The relationship in humans between maternal alcohol exposure and clefts remains unclear (Munger et al, 1996).

2.6   Abnormal embryogenesis

Given the complexity of normal embryonic development, it may seem surprising that it takes place as well as it does. Failure or inadequate completion of any one of the embryonic functions as outlined briefly above can give rise to malformation of any tissue or structure.

The nature of the resulting abnormality depends on the tissue involved, the exact developmental mechanism at fault, and the time at which it occurs. During the implantation stage, most aberrations severe enough to damage the embryo at all cause spontaneous abortion.

Maternal alcohol ingestion during pregnancy causes general growth restriction and craniofacial, neurological (calvarial and vertebral) and cardiac defects in the offspring – an association of systems consistent with impact on developing neural crest cells (Johnston and Bronsky, 1995; Siebert, Graham and MacDonald, 1985). It has been recognised for over 30 years that ethanol at teratogenic concentrations causes excessive cell death. The critical period seems to be gastrulation and mesoderm formation (Brannigan and Burke, 1982; Sulik, Johnston and Webb, 1981; Webster et al, 1983), which occurs in humans shortly after implantation.

Abnormal developmental mechanisms acting during the embryonic stage may cause a whole gamut of structural abnormalities, since organ formation is in progress. Inadequate cellular proliferation usually results in deficiencies of body structure, ranging from absence of a limb to mild diminution of the size of the external ear. Abnormal or incomplete differentiation of cells into mature tissues may cause widespread anomalies of a single organ system. Failure of cellular induction is probably responsible for abnormalities like biliary atresia, while aberrant cell migration plays a part in certain defects of the face. Inadequate cell death may lead to problems such as syndactyly and incomplete tissue fusion is involved in various forms of cleft lip and palate (Sperber and Machin, 1994; Sulik, Cook and Webster, 1988).

2.6.1 Effects of ethanol on craniofacial development

Gastrulation is the embryonic phase of development in which the embryo takes the form of a "hollow ball" of cells. It is an embryonic phase common to all animal species including man. Among the events occurring during gastrulation is the formation of a structure called the embryonic disc, which later differentiates to give rise to parts of the brain as well as features of the midface. During gastrulation the mesoderm is formed primarily from the posterior midline (primitive streak) of the embryonic disc. Newly formed mesodermal cells migrate anteriorly between the ectoderm and endoderm to the region subjacent to the prospective neural plate (brain). After the initial migration of the mesodermal cells from the primitive streak, the notochord is formed.

Mesoderm formation in mammals requires extremely rapid rates of cell proliferation and with the onset of gastrulation, proliferation accelerates rapidly (Snow, 1977). As gastrulation-stage embryos do not yet have a functional placenta, teratogenic agents, like alcohol, are capable of acting independently of a placental circulation. Although, the pathogenic mechanism of the teratogenicity of alcohol remains unknown, recent studies have shown a correlation between morphological abnormalities, suppressed rates of cell division and DNA synthesis (Pennington et al, 1983; Priscott, 1982; Dreosti et al, 1981)

Acute maternal exposure to alcohol during gastrulation produces a spectrum of associated defects. Scanning electron microscope (SEM) analysis of presomite embryos examined as early as 8 to 12 hours after the initial maternal alcohol exposure shows abnormal narrowing of the anterior aspect of the embryonic disc. Examination of the embryos at sequentially later stages suggests that this narrowing occurs primarily at the expense of the midline (Sulik and Johnston, 1983). As the primordia of the olfactory, lens and otic placodes are positioned at the periphery of the embryonic disc, narrowing of its most anterior aspect would bring the placodes abnormally close to the midline.

Too close apposition of the placodes provides little space for the normal development of the medial nasal prominences (MNP), the tissue from which the tip of the nose and the philtrum are derived. The lowest part of the MNP, from which the philtrum of the lip forms, appears to be more markedly deficient than the upper part from which the tip of the nose will develop. In FAS, the perioral region is susceptible to abnormalities of all types and of varying degrees of severity. The MNP deficiency results in the characteristic perioral appearance seen in FAS: absence of the lateral ridges of the philtrum produces a flat or smooth philtrum which may be associated with a long upper lip and loss of the usual cupid's bow configuration of the vermillion. The upper vermillion is often turned under, producing a narrow (thin) carmine margin.

As the anterior portion of the hard palate and alveolar ridge in which the upper incisors form are also MNP derivatives, rudimentary or malpositioned upper incisors have been described (Sulik and Johnston, 1983). The size of the skull is almost entirely dependent on the volume of its contents. Brain growth causes the calvarium to enlarge in a passive manner. Holoprosencephaly relates to the fact that as the forebrain decreases, there is a tendency for the first three ventricles to fail to separate, resulting in a "single cavity" forebrain (holoprosencephaly) (Gorlin et al, 1990).

De Myer et al (1964) and De Myer (1975) speculated that facial midline anomalies are pathognomic of brain malformation, based on their work with mouse models, Sulik and Johnston (1983) speculated that the principal craniofacial features of FAS appear to be a mild expression of one type of holoprosencephaly. These features include reduced or missing mid-line components of the brain (Sulik et al, 1984), and face (Sulik and Johnston, 1983; Sulik et al, 1983). SEM analyses of brain morphogenesis in embryos have shown ventro-medial forebrain deficiencies of varying degrees of severity resulting in microcephaly and/or microencephaly. If these speculations are correct, it is possible that the face may "predict" the brain in children prenatally exposed to alcohol (Moore et al, 2002).

Some studies have shown that children prenatally exposed to heavy levels of alcohol have an increased risk of cognitive impairment despite the absence of the classic FAS facial gestalt (Mattson et al, 1997, 1998, 1999; Autto-Rämö, 2000; Clark et al, 2000; Sowell et al, 2001). Astley et al (1999) reported a strong correlation between alcohol-induced craniofacial anomalies and cognitive impairments in the nonhuman primate, which they suggested as evidence that the midline facial anomalies that occur secondary to prenatal alcohol exposure may be sensitive indicators to brain dysfunction. More recently, Astley and Clarren (2001), using their newly developed four-digit diagnostic code, reported a strong correlation between the severity of facial disruption and the extent of brain dysfunction in FAS patients.

Accompanying the brain defects are ocular manifestations, including microphthalmia, abnormal retinal vascular patterns, ptosis, strabismus, corneal opacities as well as short palpebrae fissures (Sulik et al, 1983; Miller et al, 1981; Strömland, 1981). Ptosis of the eyelids (Figure 2) is caused by a weakened levator palpebral muscle, allowing the lid to sag downwards, partly covering the pupil. Strabismus refers to any deviation of the optic axis of the eyes from parallel in any direction of gaze.

**Figure 2: Bilateral ptosis in a FAS child
(author's photograph)**

The face is embryonically complex, made up of contributions from the neural crest, branchial arches and mesodermal tissues. In general, it is built up from paired lateral structures of branchial arch origin that fuse at the midline. These are sheathed by neural crest cells that migrate from their original positions over the first six to seven somites, coursing both laterally around the sides of the face and centrally over the forehead. Facial malformations appear to be multifactorial in origin, involving genetic susceptibility and environmental causes (Friede, 1995; Aase, 1990).

A hypoplastic midface is caused by inadequate growth of the central maxilla. Malformations of the lower face and jaw are related to hypoplasia of the first pharyngeal arches. Micrognathia, the most common deformational abnormality of the lower face, involves the mandible. The flattened appearance of the nose is caused by a short columella that pulls down the nasal tip and distorts the nares (Aase, 1990) (Figure 3).

Figure 3: Face of child with FAS, showing short nose, long middle part of upper lip without a central groove and a thin upper lip (author's photograph)

Preauricular pits and tags occur in 0.5-1% of individuals, with a wide variance in frequency among different racial groups. They are thought to be remnants of early embryonic branchial cleft or arch structures. In FAS, overdevelopment of the root of the helix produces a railroad track configuration of the ear lobe (Figure 4).
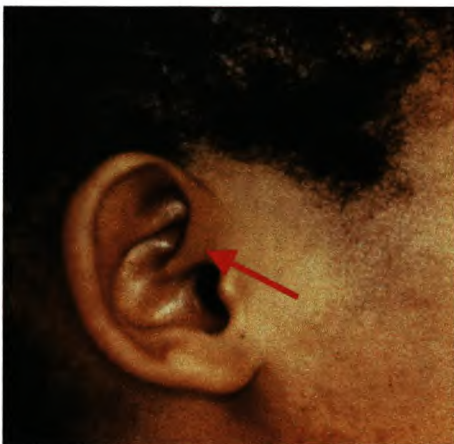
Figure 4: Prominent helical root (railroad track ear) in child with FAS (author's photograph)

2.7.   Embryogenesis

The central nervous system is derived from embryonic ectoderm, specifically the neural plate. Early in pregnancy, the brain separates into three principal subdivisions: forebrain, midbrain and hindbrain. The forebrain eventually divides into the cerebral hemispheres. The hindbrain subdivides into the rostral metencephalon and more caudal myelencephalon, which is continuous with the spinal cord. The metencephalon gives rise to the pons and the cerebellum, and the myelencephalon develops into the medulla oblongata.

While these major subdivisions are taking place, the neural tube undergoes complex histogenesis. Initially it is composed of a single layer of columnar cells, the neural epithelium. After some initial multiplication and migration, the tube begins to close. The neural epithelium gives rise to cells that eventually become neurons. The neuroblasts, having already migrated to the mantle layer of the neural tube, rapidly increase in number. After completion of proliferation and migration of neurons, the glial cells begin to proliferate and increase the mass of the brain. In general, neuron proliferation is completed by the end of the second trimester, whereas glial proliferation peaks during the third trimester. Myelination takes place primarily during the third trimester, but also continues postnatally.

2.7.1   Effects of ethanol on the fetal brain

Ethanol is a potent teratogen for the developing nervous system and causes a variety of abnormal neurological effects in children born of alcoholic mothers, including mental retardation, loss of motor control, visual impairment and reduced myelination. Since mental retardation is a hallmark of FAS and abnormal neuronal differentiation is characteristic of mental retardation (Marin-Padilla, 1975), it is not surprising to find anatomic and histologic abnormalities among the adverse effects of ethanol on the human fetal brain (Peiffer et al, 1979; Clarren, 1978; Jones and Smith, 1973). Delayed neuronal maturation has been found in rats exposed to ethanol *in utero* (Hammer and Scheibel, 1981). Impaired maturation of Purkinje cells has been reported (Volk et al 1981).

Diaz and Samson (1980) found that ethanol exposure during the glial cell proliferation phase of rat brain growth resulted in a 19% reduction in total brain weight. In an *in-vitro* model of neuronal-glial interaction, the peripheral glia, Schwann cells, were reduced in numbers by ethanol exposure (Johnson and Smith, 2002). In studying the early phases of brain development, Sulik et al (1981) found histologic abnormalities in the neural ectoderm within 24 hours of ethanol exposure. They concluded that the decreased development of the neural plate and its derivatives accounted for the craniofacial malformations seen in FAS. A disrupted neural plate may cause absence of the corpus callosum (Peiffer et al, 1979; Clarren et al, 1978; Jones and Smith, 1973); absence of the anterior commissure (Peiffer et al, 1979; Clarren et al, 1978), enlarged or fused ventricles (Peiffer et al, 1979; Clarren et al, 1978; Jones and Smith, 1973) and aberrant neuronal and glial cell migration (Peiffer et al, 1979; Clarren et al, 1978; Jones and Smith, 1973).

In addition to normal cell migration, neuronal fibres may also be disturbed (West et al, 1981). The aforementioned studies indicate that there are several critical periods during brain development when ethanol may have an adverse effect. Specifically it appears that ethanol can alter both the early phase of brain embryogenesis as well as the latter phases of brain growth, including glial cell proliferation and migration.

2.7.2   Neurochemistry

Prenatal exposure to ethanol alters neonatal neurochemistry. Brain protein synthesis, neurotransmitter production, nucleotide production and nerve myelination are all affected by *in utero* alcohol exposure. Protein synthesis in fetal rat brain ribosomes has been found to be decreased by prenatal exposure to ethanol (Rawat, 1975). In addition to alterations in protein synthesis, prenatal ethanol exposure affects neurotransmitter synthesis and storage (Ellis et al, 1978). In rats prenatally exposed to ethanol, myelination in the cerebral cortex is delayed (Jacobsen, Rich and Tobsky, 1978) and changes in myelination have been detected biochemically (Druse and Hofteig, 1977). Chronic prenatal ethanol exposure in the rat has also been shown to decrease brain DNA content in the newborn (Henderson et al, 1979). Sialic acid incorporation into synaptosomal glycoproteins has been found to be impaired by ethanol (Stibler et al, 1983).

2.8   Concluding remarks to Chapter 2

Prenatal ethanol exposure in both humans and animals results in diminished fetal brain growth. In addition, studies have demonstrated disrupted brain embryogenesis and altered neurochemistry consequential to maternal ethanol ingestion. The mechanisms by which ethanol is teratogenic are probably manifold, including direct fetotoxicity of ethanol, and poor maternal nutrition secondary to placentotoxicity. The preponderance of evidence indicates that human maternal ethanol abuse, without concomitant factors, is detrimental to fetal brain growth and development and often leads to impaired mental and behavioural functioning.

Chapter 3: Pathogenesis of FAS - Cellular and Molecular Basis for Alcohol's Teratogenic Effects

3.1   Introduction

Many factors play a role in the development of FAS. Among these are the stage of development of the fetus at the time of its exposure to alcohol, the frequency and quantity of maternal alcohol consumption during pregnancy, the timing of alcohol intake during gestation, the nutritional status of the mother and her intake of other drugs, the genetic background of the mother and of the fetus, and the mother's overall state of health. Part 2 of this literature review discusses the pathogenesis of FAS. Key mechanisms discussed are: fetal hypoxia, excessive production of certain prostaglandins, and a direct effect of ethanol on developing cells, especially those of the central nervous system, altering net protein synthesis, neuronal membrane composition and/ or neuronal process formation, and production of neurotrophic factors needed for cell growth and interaction. These putative abnormalities are not of course mutually exclusive and in fact may contribute in varying degrees to alcohol-induced fetal deficits (Michaelis, 1990; Schenker et al, 1990).

3.2   Stages in fetal development

Human gestation is divided into three major periods: the implantation, the embryonic sband the fetal stage. It is during the embryonic period that malformations are most readily produced by various drugs introduced directly into the maternal blood stream or administered through the maternal diet. Chemical or physical agents that produce fetal malformations are called teratogens, from *teraton*, the Greek word for monster. Most teratogens show selectivities for certain organs based on the timing of exposure of the embryo to the teratogen, the dose of the teratogen taken by the mother, and the sensitivity of the dividing cells of each primordial organ to the teratogen's effects (Larsson, 1973).

Organs and limbs of a developing embryo are formed from collections of specialised cells. Exposure of an embryo to a teratogen during the period when primordial cells of an organ are actively proliferating may have devastating effects on the formation of that organ. Malformations of the head and face are the most common malformations in FAS and are correlated with exposure to alcohol during the embryonic stage and with the dose of alcohol consumed by the mother (Jones et al, 1974; Ernhart et al, 1987). The brain is most commonly malformed. Embryonic events at the cellular level that are potential targets of alcohol-induced disruption are summarised in Figure 5. The first stage of events is cell division and proliferation. The second stage is cell growth and differentiation, by which cells become specialised in structure and function. The final stage is the migration of maturing cells to their ultimate locations in the developing embryo, where they remain and adhere to the surrounding matrix of cells. Nutritional, hormonal, genetic and cellular factors direct each of the above stages. Alcohol can affect many of these factors, thereby influencing organ formation and growth (Figure 5).
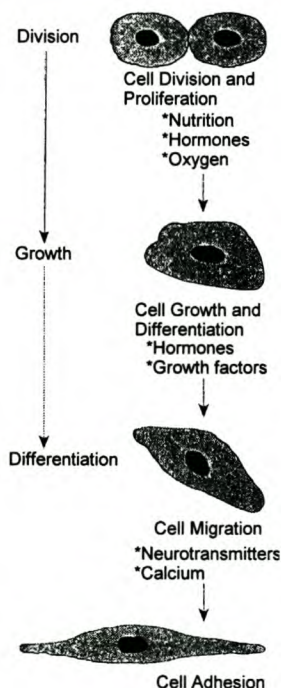
Figure 5: Cellular events that are potential agents of alcohol-induced disruption. Nutritional, hormonal, and cellular factors direct each stage of events (Michaelis and Michaelis, 1994)

3.3   Mechanisms of alcohol's embryonic effects

The fetotoxic effects of maternal ethanol consumption have been documented for over two decades, yet the mechanisms underlying this devastating phenomenon remain uncertain. The wide variety of cellular and biochemical effects of ethanol on fetal tissues is itself a puzzle and it may strongly suggest that fetotoxic responses to ethanol reflect a multifactorial setting and that alcohol adversely affects a multitude of cellular functions critical for growth of the developing organism (Abel and Hannigan, 1995; Dreosti, 1993) including inhibition of protein and DNA synthesis (Dreosti et al, 1981)

Four distinct mechanisms have been postulated: (i) at the time of conception and during the first weeks of development, ethanol may act as a cytotoxic or mutagenic agent, causing either cell death or subsequent lethal chromosomal aberrations; (ii) during the period roughly 4-10 weeks after conception, ethanol acts cytotoxically, causing excessive cell death in the CNS and abnormalities in nerve cell migration, either from cell damage or due to loss of guiding or 'marker' cells; (iii) later in pregnancy, from 8-10 weeks onwards, ethanol is thought to again disrupt or delay cell migration and development; (iv) Alcohol interferes in various ways with neurotransmitter production in the CNS, leading to neuroendocrine abnormalities, including an effect on the hypothalamus which leads to suppression of growth hormone release (Pratt, 1984). These hypotheses are summarised in Table 1 below.

Table 1: Four possible mechanisms by which ethanol damages the human CNS before birth

Timing	Possible mechanism	End-result
1 Shortly after conception	Cell death or chromosomal errors	Early spontaneous abortion
2 About 4-10 weeks	Cytotoxic effects: cell loss and abnormal cell migration	Regional agenesis, heterotopias, microencephaly and structural disruption
3 From about 8-10 weeks onwards	Delays in neuronal transmission leading to abnormal synapse formation	Behavioural difficulties
4 After the first few weeks	Action on hypothalamus to suppress release of growth hormone	General growth deficit

3.3.1 Ethanol associated hypoxia, oxygen effects and free radical formation

Since cellular oxygenation is critical for all physiological processes, any factors which reduce cellular oxygenation can be expected to affect living organisms adversely, especially those in an embryological or fetal state of development.

Decreased fetal oxygenation can arise as a result of many factors, such as decreased blood flow to tissues, decreased oxygen content in the blood, decreased oxygen saturation of red blood cells, and impaired dissociation of oxygen from red blood cells, all of which are associated with maternal alcohol ingestion. Since hepatic metabolism of alcohol removes considerable amounts of oxygen from blood (Ugarte and Valenzuela, 1971), the result is reduced availability of oxygen for other tissues. During pregnancy, such alcohol-related decreases in tissue oxygenation are manifested in placental hyperplasia (Fisher et al, 1985; Sanchis and Guerri, 1986). Decreased placental oxygenation may in turn affect energy-dependent placental transport of nutrients to the fetus (Fisher et al, 1981, 1984, 1986).

In animal studies, high blood alcohol levels (BALs) may also induce a transient collapse of umbilical arteries (Mukherjee and Hodgen, 1982), thereby depriving the fetus of blood flow altogether. Spasms in human uterine tissue at low alcohol concentrations may also occur (Savoy-Moore et al, 1989). In addition to decreasing blood flow to tissues, alcohol may also decrease availability of oxygen to tissues because it impairs the unloading of oxygen from red blood cells by acidifying blood (Yang et al, 1986).

In FAS, several neurotrophic factors are capable of mitigating neurotoxicity associated with ethanol, hypoglycaemia and hypoxia (Mitchell et al, 1998). Diminished oxygen delivery to fetal tissues during maternal drinking may result either from local release of prostaglandins or from the direct release action of alcohol on blood vessels of the placenta and umbilical cord.

Alcohol has been shown to constrict these blood vessels (Mukerjee and Hodgen, 1982), by inducing release of substances (endothelians) produced by cells lining the inner walls of the blood vessels (Tsuji et al, 1992). Increased production and release of these substances can markedly diminish blood circulation, inducing hypoxia that may delay cell proliferation, growth or migration. Following exposure of the fetus to alcohol during early gestation, the primordial cells that will form part of the nervous system and those that will form part of the tubules of the kidney show signs of damage (Gage and Sulik, 1991; Petkov et al, 1992; Assadi and Zajac 1992). Some of these changes are similar to those observed in cells injured by hypoxia in adult animal tissues (Siesjo, 1991). Though these studies may be conjecture, hypoxia may therefore be the common mechanism that brings about developmental delays and malformations in several types of tissues. Alcohol-related hypoxia can lead to intrauterine growth retardation via several different but related mechanisms. Since cell proliferation and differentiation are dependent on protein synthesis, decreased substrate availability could lead to intrauterine growth retardation (Fisher et al, 1984; Fisher, 1988). The nutrient substrate may initially be less than optimal because alcohol abusing mothers consume less than the required amounts of nutrients for support of normal fetal growth, and because alcohol itself may interfere with gastrointestinal absorption of these nutrients in blood. Decreased substrate availability could arise from alcohol-related impairment of essential amino acids (Henderson et al, 1982; Fisher et al, 1985), glucose (Snyder et al, 1986) or vitamins and minerals (Schenker et al, 1992) due to a combination of decreased placental blood flow (Falconer, 1990) and impairment of oxygen-dependent Na^+K^+-ATPase membrane transport processes (Fisher et al, 1986).

Impairment of membrane transport processes may not only reduce placental transport of nutrients, it may also inhibit protein synthesis throughout the body, as shown in *in vitro* studies and models that circumvent placental transport, such as chicks and embryos grown in culture (Brown et al, 1979; Dreosti et al, 1981; Pennington et al, 1983). The resulting growth retardation could also result in minor physical anomalies and developmental delays by altering the timing and interplay between different organ systems (Kennedy, 1984).

An organ that is particularly sensitive to hypoxia and that is most frequently damaged by alcohol abuse by the pregnant mother is the brain (Figure 6). The most common brain abnormalities in FAS are a decrease in the overall size of the brain and a diminution in the thickness of the brain cortex.

Nerve cells are dependent on a continuous supply of oxygen and glucose to meet their energy needs. When nerve cells are stressed by decreased oxygen and glucose supplies, as may happen in FAS, they release large quantities of glutamic acid (Choi, 1992). Glutamic acid is an amino acid that functions as a neurotransmitter. It may in turn cause excessive electrical activity that may produce swelling and damage to the nerve cells, primarily through excessive entry into the cells of sodium and calcium.

Cerebrum
Largest portion of the brain, including the cerebral hemispheres (cerebral cortex and basal ganglia); involved in controlling consciousness and voluntary processes

Corpus Callosum
A bundle of fibres connecting the brain's hemispheres

Hippocampus
Part of the limbic system, which is involved in emotional aspects of behaviour; also plays a role in memory

Basal Ganglia
A group of structures lying deep in the brain involved in movement and cognition

Cerebellum
Involved in maintenance of posture, balance, and coordination

Cortex
Outer layer of grey matter covering the surface of the cerebrum and the cerebellum

Neocortex
Outer portion of the cerebral cortex that contains the most structurally complex brain tissue

Diencephalon
- **Septal area** - related to the limbic system, which is involved in emotional aspects of survival behavior
- **Thalamus** - a communication centre that relays information to the cerebral cortex
- **Hypothalamus** - important in maintaining the body's internal environment, or homeostasis, through the receipt of sensory and chemical input

Figure 6: Areas of the brain that can be damaged *in utero* by maternal alcohol consumption (Mattson, Jernigan and Ripley, 1994).

As Michaelis (1990) has argued, alcohol's impact on the developing brain can also be understood in terms of hypoxia. In the adult brain episodes of hypoxia have their greatest impact on pyramidal neurons in the CA_1 area of the hippocampus and on Purkinje cells in the cerebellum (Sowell et al, 1996; Auer et al, 1989; Stoltenberg-Didinger and Spohr, 1983; Jorgensen and Diemer, 1982). These are the same areas that are most affected by gestational and neonatal alcohol exposure in animals (Dewey and West, 1985; Barnes and Walker, 1981).

The hippocampus may be more vulnerable because it is richly vascularized and high concentrations of excitatory amino acid neurotransmitters such as glutamate and aspartate, and these transmitters are released selectively during periods of hypoxia (Butters et al, 2002; Tsai and Coyle, 1998; Reynolds and Brien, 1996; Bosley et al, 1983). Hypoxia directly induces down-regulation of glutamate receptors in the fetal rat brain (Kater et al, 1988), an effect that also occurs in brains exposed prenatally to alcohol (Farr et al, 1988). Excess release of L-glutamate can have neurotoxic effects on post-synaptic glutamate receptors, especially *N*-methyl-*D*-asparate-sensitive glutamate receptors (NMDA sites) (Hughes et al, 1998; Michaelis, 1990; Rothman and Olney, 1987). The excessive, sustained neuroexcitation is believed ultimately to become neurotoxic as a result of subsequent release of lipid peroxides (Riley and Behrman, 1991) and fatty acids such as prostaglandins, and accumulation of calcium (Ca^{2+}) which ultimately results in cell death (Diaz-Granados et al, 1997; Spuhler-Phillips et al, 1997).

Hypoxia also causes the formation of free radicals. Alcohol itself also induces free radical formation in some embryonic cells (Davis et al, 1990). Alcohol's teratogenic effects may also arise as a result of damage to cells through the generation of free oxygen radicals such as superoxide anion (O_3^-), hydroxyl radical (OH^-), and highly potent hydrogen peroxide (H_2O_2). Such oxygen radicals are continuously generated in the course of normal cellular respiration when electrons escape from their carriers and are taken up by oxygen. These molecular entities are highly reactive within cells and cause peroxidation of cell membranes (Riley and Behrman, 1991). Free radicals can damage the cell surface, allowing calcium to leak into and accumulate in the cells (Kukreja and Hess, 1992).

The abnormal accumulation of calcium in nerve cells may cause them to release neurotransmitters, which may have toxic effects of their own on some cells. To protect themselves from oxygen-related free radical formation, cells normally rely on various antioxidants and enzymes (eg. vitamins C, E , glutathione), but these resources can be depleted by alcohol and by reduced nutrient supply. One of these enzyme systems, superoxide dismutase, uses zinc as a co-factor. Zinc is also an essential trace element for fetal growth and a co-factor in alcohol dehydrogenase, the enzyme involved in metabolism of alcohol. Decreased zinc levels are commonly associated with alcoholism (McClain and Su, 1983) and have frequently been implicated in FAS (Flynn et al, 1981; Halmesmaki et al, 1985; Assadi and Ziai, 1986).

Tanaka et al (1981, 1982) observed low zinc levels and hypoglycaemia in rats with FAS. Alcohol may also impair placental transport of zinc and fetal uptake of zinc (Grishan et al, 1982; Zidenberg-Cherr et al, 1988). When alcohol is administered in combination with a low zinc diet, the combination has a much greater impact on fetal weight reduction than either alcohol or zinc deficiency alone (Keppen et al, 1985). Zinc deficiency has been found to promote membrane lipid perioxidation (Dreosti, 1984).

Associations between ethanol-induced cranial neural crest cell (NCC) damage in mammalian embryos and subsequent malformations of FAS have been documented (Chen and Sulik, 1996; Rovasio and Battiato, 1996). The vulnerability of NCCs to this teratogen may result, at least in part, from their sensitivity to free radical damage. The similarity between the susceptibility of NCCs to ethanol and their susceptibility to exogenous free radicals in concert with the free radical scavenger-mediated amelioration of ethanol and exogenous free radical-induced NCC death strongly suggests that free radicals play a significant role in ethanol-induced NCC death (Chen and Sulik, 1996).

In addition to undernutrition, smoking not only directly increases the body's burden of free radicals, but it also depletes cellular reserves of antioxidants and zinc and contributes to primary undernutrition by reducing food consumption, especially intake of antioxidants such as vitamins A, C and E, and zinc. As a result, the body's cellular defence mechanisms against free oxygen radicals are further weakened by smoking, increasing vulnerability to alcohol-generated radicals. Ultimately, all fetal damage will involve perturbations in cellular growth, differentiation, proliferation, migration and/or regulation. The absence of a specific pathognomonic feature or, conversely the number of ubiquitous anomalies associated with FAS, implies alcohol acts through a relatively general mechanism or one which has wide-ranging impact. Abel and Hannigan (1996) have hypothesised that two of these mechanisms are hypoxia and free radical formation, reasons for which are described below.

3.3.2 An integrative hypothesis: risk and mechanisms

Abel and Hannigan (1996) hypothesise that there is a link between the differential susceptibility to FAS/alcohol-related birth defects (ARBDs) among certain populations and the hypoxia/free radical mechanism of alcohol teratogenesis. They contend that "permissive" socio-behavioural factors such as binge drinking, smoking, as well as other factors, such as poverty, are major risk factors for FAS. Dose for dose, binge drinking, for example, creates higher toxic peak blood alcohol levels (BALs) than sustained levels of alcohol consumption. Poverty is a risk factor because it is associated with decreased nutritional intake, stress and increased exposure to environmental pollutants. These 'permissive' factors lead to internal biological conditions which are 'provocative' in that they provoke cellular changes that exacerbate alcohol's toxic effects. These provocative changes include altered blood flow, decreased reserves of antioxidants, generation of stress hormones, and increase in free radicals. The cellular hypoxia from decreased blood flow, stress-related respiratory alkalosis, and/or from smoking (which independently decreases uterine blood flow and blood oxygenation) combine with alcohol-induced cellular hypoxia in a synergistic manner.

When an undefined critical level of hypoxia is exceeded, cellular changes occur which manifest themselves in various aspects of FAS/ARBDs such as altered facial morphology or low birth weight. Increased free radical formation likewise contributes to cell damage and may account for the physical as well as behavioural damage associated with FAS/ARBDs.

3.4 Prostaglandins

Prostaglandins are a family of complex derivatives of polyunsaturated fatty acids, especially arachidonic acid, whose normal function is to modulate certain cellular functions in the body. These compounds are biologically potent and have a hormone-like action in that they can effect functions in tissues distant from the site of their production. It has been shown that prostaglandins may be mediators of at least some of the teratogenic actions of ethanol in both mouse and human tissue model systems (Randall et al, 1996). Prostaglandin activity is markedly increased during fetal exposure to alcohol (Randall et al, 1996; Schenker et al, 1990; Anton et al, 1990). These chemicals have powerful effects and their overproduction may be responsible for tissue hypoxia brought about by prostaglandin-induced constriction of blood vessels. Lack of oxygen functions as a trigger for cells in different tissues, such as the heart, to convert more arachidonic acid into prostaglandin (Kukreja and Hess, 1992). This process would aggravate the pre-existing hypoxia and, in the fetus exposed to alcohol, could lead to further tissue damage and growth retardation.

3.5 Direct effect of ethanol on developing cells

There is still no comprehensive understanding of the genesis of the most prominent feature in FAS, ie. the fetal growth retardation and the microcephaly. Whether the growth retardation of the brain is due to lack of trophic factors or to increased or upregulated cell death during development has not been elucidated. Apoptosis, ie. genetically determined cell death, plays a major role in determining the final size of organs.

It has been reported that the FAS/Apo (apoptosis)-1 receptor is transiently expressed in the developing brain cortex during the peak period of naturally occurring apoptotic cell death and maximum sensitivity to alcohol (Cheema et al, 2000; Ikonomidou et al, 2000). In addition, ethanol is found to induce a susceptibility to apoptotic signals at low doses by regulating the expression of mRNAs for cytotoxic receptors such as FAS/Apo (apoptosis)-1 in the developing cerebral cortex. Furthermore, ethanol alters the uptake of critical nutrients such as glucose and amino acids and causes changes in several kinase-mediated signal transduction pathways that regulate these biochemical processes (Shibley and Pennington, 1997).

In humans, microcephaly is a predominant feature of FAS (Yagle and Costa, 1999). An analogous measure, microencephaly (small brain for body size) can be used for evaluating the detrimental effects of the differential timing of alcohol exposure on brain development in animal model systems (Maier et al, 1999). Ethanol is a potent inhibitor of muscarinic receptor-mediated proliferation of glial cells. Glial proliferation has been suggested as a major target of ethanol neurotoxicity during development, leading to microencephaly (Miller, 1996a; Riley et al, 1995). Mattson et al (1996) demonstrated significant reductions in the cerebral vault, basal ganglia and diencephalon in children with FAS. In addition, they found that the volume of the cerebellar vault was smaller than that of the controls.

When basal ganglia were divided into the caudate and lenticular nuclei, both of these regions were significantly reduced in children with FAS. Finally, when the overall reduction in brain size was controlled, the proportional volume of the basal ganglia, and more specifically, the caudate nucleus was reduced in the children with FAS.

3.6   Impaired cell signalling, migration and adhesion

Many studies now suggest that neurotransmission, which is the mechanism by which neurons communicate, is particularly sensitive to ethanol. Neurotransmission is a complex process, and there are many different sites at which ethanol could potentially act to modify neurotransmission (Figure 7). Prenatal exposure to moderate quantities of ethanol causes profound and long-lasting deficits in the cellular signalling mechanisms associated with activity-dependent synaptic plasticity and memory formation (Buckley, Savage and Caldwell, 2002; Allan et al, 1997; Sutherland et al, 1997).

One of the most severe consequences of maternal alcohol consumption during pregnancy is the damage to the developing central nervous system, by causing substantial neuronal loss in multiple brain regions (McAlhany et al, 2000; Costa and Guizzetti, 1999). Mental retardation and microcephaly are among the most significant central nervous system dysfunctions due to *in utero* exposure to alcohol. During brain development, certain types of cells are programmed to move to precise locations so that they can serve a specific role in the overall co-ordinated activity of the brain. In many children with FAS, these nerve cells have failed to migrate to their appropriate sites (Kotkoskie and Norton, 1988; Miller, 1993). In addition, Davis et al (1999) found that ethanol-induced changes in growth factor signal transduction contribute to the teratogenic effects of ethanol in the developing brain. Ethanol was found to decrease extracellular signal-related protein kinase (ERK) activation *in vivo* and decreased nuclear translocation of brain-derived neurotrophic factor (BDNF)-stimulated ERK *in situ*. BDNF and nerve growth factor have been shown to protect against ischaemia/hypoglycaemia caused by ethanol and Mitchell et al (1999) demonstrated marked growth factor neuroprotection against a myriad of conditions encountered by developing ethanol-exposed fetuses.
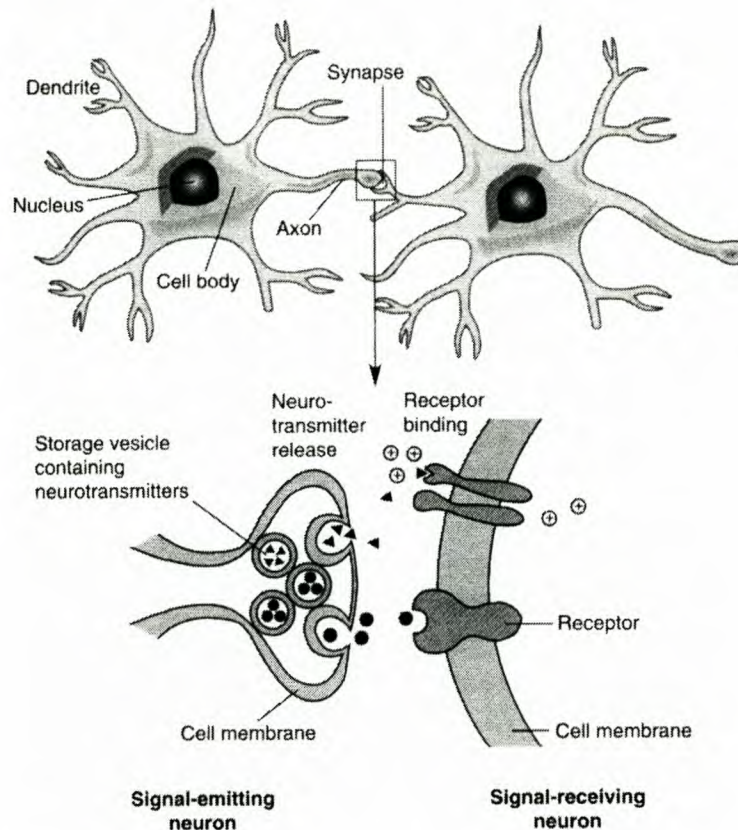
Figure 7: Structural features of a typical nerve cell (neuron) and synapse

This schematic drawing depicts the major components of a typical neuron, including the cell body with the nucleus; the dendrites that receive signals from other neurons; and the axon, which relays nerve signals to other neuron at a synapse. When a nerve signal reaches a synapse, it causes the release of chemical messengers (neurotransmitters) from storage vesicles. The neurotransmitters travel across a minute gap between the cells and interact with the protein molecule receptors located in the membrane surrounding the signal-receiving neuron. This interaction causes biochemical reactions that result in the generation, or prevention, of a new nerve signal, depending on the type of neuron, neurotransmitter, and/or receptor involved (Goodlett and Horn, 2001).

Ye and Tao (2002) found that ethanol and protein kinase C share the same pathway to suppress glycine receptors and their observations suggest that activation of protein kinase C contributes to ethanol-induced inhibition of glycine receptors. Gestational exposure to ethanol inhibits insulin-stimulated neuronal viability, mitochondrial function, Calcein AM retention (membrane integrity), and GAPDH expression, and increased dihydrorosamine fluorescence (oxidative stress) and pro-apoptotis gene expression (p53, Fas-receptor and Fas-ligand).

De la Monte and Wands (2002) found that insulin-stimulated CNS survival mechanisms are significantly impaired by chronic gestational exposure to ethanol, and that the abnormalities in insulin signalling mechanisms persist in the early postnatal period, which is critical for brain development.

Mahadev and Vemuri (1999) reported differences in tyrosine-specific protein kinase activity and in phosphotyrosine-containing proteins observed during pre- and postnatal ethanol exposure may reflect specific functional defects in the cerebral cortex which could possibly underlie the mechanism contributing to FAS. In addition, there were selective changes in protein kinase C isoforms and phosphorylation of endogenous substrate proteins in rat cerebral cortex during pre- and postnatal ethanol exposure (Mahadev and Vemuri, 1998).

Very complex molecules in the local environment surrounding maturing cells promote appropriate cell movement and adhesion. Alcohol interferes with the cellular response to these molecules (Gatalica and Damjanov, 1991). If the maturing cells do not detect the subtle gradients of these chemicals, then they will not initiate migration or will fail to complete migration correctly (Miller, 1993).

Gruol and Parsons (1996) found that chronic alcohol reduces calcium signalling elicited by glutamate receptor stimulation in developing cerebellar neurons. The incorrect migration of some nerve cells within the brain may also be linked to abnormalities in calcium regulation and the release of glutamic acid described earlier (Komuro and Rakic, 1992, 1993).

Ethanol has been shown to cause alterations of both neuronal and glial cells, including cell loss, and changes in their migration and maturation. Costa and Guizzetti (1999) have proposed that one of the potential targets for the developmental neurotoxicity of ethanol may be represented by the signal transduction systems activated by cholinergic muscarinic receptors. Ethanol inhibits second messenger systems activated by various G-protein-coupled receptors, including certain subtypes of muscarinic receptors.

Although the role of muscarinic receptors in brain development have not been fully elucidated, two potentially relevant effects have been discovered in the past few years. By activating muscarinic receptors coupled to phospholipid metabolism, acetylcholine can induce proliferation of glial cells, and act as a tropic factor in developing neurons by preventing apoptotic cell death. Ethanol has been shown to inhibit both actions of acetylcholine *in vitro.* These effects may lead to a decrease in glial cells and a loss of neurons, which have been observed following *in vivo* ethanol exposure. This may be the basis of microcephaly and cognitive disturbances in children with FAS (Costa and Guizzetti, 1999).

L1 cell adhesion molecule is critical for the development of the central nervous system. Mental retardation, hydrocephalus and agenesis of the corpus callosum are observed both in FAS and in children with mutations in the gene for the cell adhesion molecule L1 (Johnson, Hill and Bowie, 1984; Bearer and Buck, 2002). Ethanol inhibits L1-mediated cell adhesion in transfected fibroblasts, neural cells and cerebellar granule cells (Li and Napper, 2002). There appears to be striking structural specificity for the effects of L1 of a variety of straight, branched and cyclic alcohols, suggesting a ligand-receptor interaction (Zhang et al, 2002; Wilkemeyer et al, 2002). Ramanathan et al (1996) found that ethanol potently and completely inhibited L1-mediated adhesion in fibroblast cells and they surmised that because L1 plays a role in both neural development and learning, ethanol inhibition of L1-mediated cell-cell interactions could contribute to FAS memory disorders. Ozer et al (2000) found that alcohol exposure affects the level of myelin basic protein (MBP) expression, consequently causing a reduction in brain myelination that may lead to neuronal dysfunction. Although there is a view that neural cell adhesion molecule (NCAM) is involved both directly and indirectly in neuronal cell migration, Ozer et al (2000) speculate that alcohol neuroembryotoxicity uncouples this relationship and that other adhesion molecules, such as laminin, may be responsible.

The hippocampus and dentate gyrus possess a relatively simple cytological organization, each consisting of a single type of neuron – the pyramidal neuron in the hippocampus and the granule cell in the dentate gyrus. Both cell layers are tightly packed and spatially isolated. Each region has a simple dendritic organisation and is characterized by afferents which are segregated into specific laminae along the dendrites, allowing detection of subtle changes in afferent organisation. Prenatal exposure to ethanol affects the hippocampus, producing developmental delays (Davis and Smith, 1981) and long-lasting alterations to dendrites (Abel et al, 1983) as well as loss of pyramidal cells (Barnes and Walker, 1981; Walker et al, 1980). Furthermore, pre- and postnatal exposure to ethanol induces changes in the number and shape of the dendritic spines from hippocampal CA1 pyramidal neurons with a predominance of spines with long, thin pedicles on cortical pyramidal cells. The reduced numbers and abnormal geometry of spines suggest that an abnormal maturation of nerve cells occurs (Lopez-Tejero et al, 1986; Schapiro et al, 1984) as a consequence of chronic ethanol consumption during pregnancy.

Because normal maturation of dendritic spines on cortical neurons is characterized by the progressive appearance of mushroom-shaped and stubby spines (Purpura, 1975; Marin-Padilla, 1972), the predominance of a primitive character characterized by spines with thin, long pedicles has been interpreted to be the result of an abnormal development of the dendritic spine pattern on cortical pyramidal neurons (West et al, 1984). Chronic alcohol ingestion may alter the electrical excitability and thereafter the firing pattern of the neuronal cells (Tarelo-Acuna et al, 2000). Miller et al (1999) found an increase in the rostral corpus collosum suggesting that parietal and frontal cortices are particularly susceptible to ethanol. The altered callosal connectivity may be a component of the structural abnormalities that underlie executive processing problems associated with FAS. The activation of phospholipase D (PLD) is a common response to mitogenic stimuli in various cell types. As PLD-mediated signalling is known to be disrupted in the presence of ethanol, Kotter and Klein (1999) tested whether PLD is involved in the ethanol-induced inhibition of cell proliferation in rat cortical primary astrocytes. They concluded that the antiproliferative effects of ethanol in glial cells are due to the disruption of the PLD signalling pathway.

Nitric oxide is a novel messenger that is involved in neuronal cell-cell communication and seems to play a neurotrophic role in normal brain development. Chronic prenatal exposure can produce CNS teratogenesis, in which one of the targets is as mentioned above, the hippocampus, by decreasing nitric oxide synthase (NOS) in the fetal hippocampus, and this is correlated with restricted growth of this brain region (Kimura et al, 1996).

3.6.1 Ethanol interactions with neurotransmitter transporters

Another site at which ethanol has been reported to act is at the transporter molecules that transport neurotransmitters from the extracellular space of the brain to the intracellular environment; this mechanism is responsible for inactivating the cations of many of the neurotransmitters. Adenosine transporters have been reported to be inhibited by ethanol (Krauss et al, 1993) and as a consequence, extracellular adenosine levels in the brain rise. There is evidence that some of these effects of ethanol are mediated via this increase in adenosine. Transporters for the neurotransmitters norepinephrine (Lin et al, 1997), dopamine (Shen at al, 1999; Wang et al, 1997) and serotonin (Zafar et al, 2000; Alexi and Azmitia, 1991) have been reported to be affected by ethanol. This suggests that these transporters, which are all related members of the same gene family, might be important sites of ethanol action.

The development of the serotonin (5-HT) system is markedly impaired by *in utero* ethanol exposure. There is a significant and long-lasting reduction in the density of 5-HT neurons, a lower concentration of 5-HT and a decreased concentration of 5-HT reuptake sites. *In vitro* studies also detect an early vulnerability of 5-HT and other rhombencephalic neurons (Manteuffel, 2002). Exposure of alcohol to the developing fetus has been shown to cause distinct effects on serotonin transporter (5-HTT) binding sites in the brains of developing male and female rats depending on the age and gender of the offspring. Alcohol exposure increased 5-HTT binding sites in the cortical and hippocampal layers, lateral nucleus of the amygdala and the dorsal raphe nucleus, but decreased 5-HTT binding sites in the medial nucleus of amygdala, dorsomedial and ventromedial nuclei of the hypothalamus (Alexi and Azmitia, 1991).

The alcohol exposure decreased 5-HTT binding sites temporarily in the ventromedial nucleus of the hypothalamus in the 21 day old female rat; this effect was found to disappear by day 40. In contrast, the alcohol exposure increased 5-HTT binding sites in the lateral nucleus of the amygdala in the brain in the adult animal, suggesting that ethanol exposure *in utero* may alter serotonin transmission in discrete brain regions permanently (Zafar et al, 2000).

Prenatal alcohol exposure has been demonstrated to reduce dopamine (DA) neurotransmission in the forebrain area, which could be contributed by altered electrical activity in midbrain DA neurons. Shen et al (1999) demonstrated that prenatal ethanol exposure induced a long-lasting reduction in the activity of midbrain DA neurons in adult animals. The effect was not the result of cell loss but possible changes in the electrical properties of DA neurons. The decreased electrical activity in midbrain DA neurons could contribute to the prenatal ethanol exposure-induced reduction in DA content and metabolites observed in previous studies and the attention/hyperactivity problems reported in children with FAS.

3.6.2   Ethanol interactions with neurotransmitter receptors (postsynaptic effects)

Probably the most important sites of ethanol action that have been discovered to date are the protein molecules that are the receptors for neurotransmitters. There are two basic subtypes of post-synaptic receptors: ligand-gated ion channels and metabotropic receptors.

Ligand-gated ion channels are multimeric proteins that form small pores in the lipid bilayer. These receptors produce fast postsynaptic responses. Some of the receptors in this category are the gamma-amino butyric acid type A ($GABA_A$) receptor and the related glycine receptor, nicotinic cholinergic receptors, receptors for ATP and several families of glutamate receptors (AMPA, kainite and N-methyl-D-aspartate (NMDA) receptors).

The so-called metabotropic receptors are not ion channels, but instead induce biochemical events in the postsynaptic cell that alter its function; these might include biochemical activation of kinases or the release of intracellular Ca^{2+} and the initiation of Ca^{2+} dependent processes in these cells (Isenberg et al, 2002).

Subunit expression of $GABA_A$ receptors at the cell surface is important for receptor function and involves a highly regulated process of synthesis, assembly, endocytosis and recycling to the membrane. Chronic ethanol exposure alters the expression of various subunits of $GABA_A$ receptors: $GABA_A$ receptor α1-subunit peptide levels are reduced and α4-subunit peptides are increased in the cerebral cortex (Kumar et al, 2002).

Neuronal nicotinic acetylcholine receptors (nnAChRs) are more sensitive to ethanol modulation than most other receptors. They are ubiquitously distributed in the brain and are known to modulate the release of various neurotransmitters including GABA and dopamine. Ethanol modulates these nnAChRs by an increase in both the channel open probability and the activation of low affinity Ach binding site (Zuo, Yeh and Narahashi, 2002).

The serotonin 5-HT$_3$ receptor is a channel that is not related to the $GABA_A$ receptor, but whose function is also enhanced by ethanol (Lovinger and White, 1991; Machu and Harris, 1994; Zhou et al, 1998). The glycine receptor is closely related to the $GABA_A$ receptor, and also shows enhancement (Aguayo and Pancetti, 1994; Mascia et al 1996; Mihic, 1999). Nicotinic receptors also belong to the same gene family and the function of some of these receptors is enhanced by ethanol (Wu et al, 1994; Aistrup et al, 1999; Narahashi et al, 1999).

A subclass of glutamate-activated ion channels called N-methyl-D-aspartate (NMDA) receptors are antagonized by ethanol. These effects are non-competitive ie., they cannot be overcome by higher concentrations of glutamate, which suggests that ethanol is not simply interfering with the binding of glutamate to its receptor site (Lovinger, White and Weight, 1990).

During development of the CNS, the function of the NMDA receptors change from a generally protective or neurotrophic role to one in which increased activity can result in excitotoxicity. Each of the major subtypes of glutamate receptors (AMPA, kainate, NMDA) are inhibited by ethanol (Crump et al, 2001). Although there is a range of sensitivities for each of these receptors, synaptic responses mediated by kainite (Weiner et al, 1999) and NMDA (Lovinger et al, 1990) receptors are generally more sensitive to the inhibitory effects of ethanol than are the AMPA receptors (Weiner et al, 1999), which mediate most excitatory transmissions in the brain. The NMDA receptors play a particularly important role in ethanol-related damage during development with acute exposure suppressing NMDA receptor activity and chronic exposure producing compensatory oversensitivity or up-regulation of receptors (Barron, Littleton and Prendergast, 2002). NMDA receptors are subject to dynamic activity-dependent trafficking between synaptic and non-synaptic locations within hippocampal dendrites. Such adaptations in response to depressed synaptic activity may contribute to the development of chronic tolerance to ethanol.

Chronic exposure to ethanol induces an increase in the NMDA receptor clustering and synaptic localisation in the primary hippocampal neurons (Carpenter-Hyland, O'Donnell and Chandler 2002; Crump, Dillman and Craig, 2001). In addition, ethanol modulates the phosphorylation of the NMDA receptor complex, thereby inducing inhibition of NMDA receptor function (Ferrani-Kile, Velasquez and Leslie, 2002).

Some subtypes of nicotinic receptors (such as the $\alpha 7$ subtype of this receptor) are inhibited by ethanol, not enhanced (Aistrup et al, 1999). Although there is evidence that some effects of ethanol on ligand-gated channels are direct, there are also indications that many of the effects of ethanol with ligand-gated ion channels may be indirectly mediated. Ethanol has also been proposed to act by changing the levels of endogenous allosteric modulators of the $GABA_A$ receptor (Morrow et al, 1999; Van Doren et al, 2000).

3.6.3 Interaction of ethanol with metabotropic synapses

The second major class of synapses, those with metabotropic receptors, represent a second set of potential targets for ethanol action. The evidence to support specific interactions of ethanol with these kinds of receptors is not well developed as it is for the ligand-gated ion channels. These receptors also present multiple targets for ethanol: the receptor itself, and elements of the transduction mechanisms used by these receptors, which might include G proteins (Mitchell and Litman, 2002), kinases and other cellular proteins. Ethanol can also affect the activity of a variety of other ancillary proteins that play a critical role in synaptic transmission; Ca^{2+} channels are one good example of this type of protein that seems to be an important site of ethanol action (Mullikin-Kilpatrick et al, 1995).

3.6.4 Conclusions regarding the cellular mechanisms of ethanol action

Older concepts concerning the mechanisms underlying ethanol action suggested that ethanol had a generalised, non-specific effect on many cellular targets, and that these effects were mediated by an ability of ethanol to disrupt lipid membranes. More recent evidence now suggests that this is not the case. Synaptic transmission appears to be one mechanism in the brain that is quite sensitive to the effects of ethanol. However, there are wide differences in the sensitivities of the different synapses to ethanol, and there appear to be certain synapses that are highly sensitive to intoxicating concentrations of ethanol.

Post-synaptic receptors appear to be one of the major sites of action of ethanol. Although some of the effects of ethanol may be indirect, there is accumulating evidence to suggest that ethanol can interact directly with some of these receptors, most probably at hydrophobic sites of limited dimensions that can accommodate ethanol and similar alkanols, but exclude alcohols that exceed a certain critical molecular weight. Because of the rapid explosion of potential targets for ethanol action, it seems likely that multiple effects of ethanol are important.

3.7   Neuronal process formation, membrane composition and neuronal cell loss

Assuming that pre-and postnatal exposure to alcohol can produce neuronal cell loss, aberrant neuronal migration, abnormalities in axonal growth and innervation, decreases in dendritic arborization and spine development, as well as an overall decrease in brain weight, another common molecular event underlying these observations comes into play: the role of intracellular calcium ions (Ca^{2+}) in the growth cone region of developing neurons. It is hypothesized that the regulation of Ca^{2+} entry into the growth cone plays an important role in neuronal process elongation. Ethanol can affect intracellular Ca^{2+} levels in mature cells both through direct action on Ca^{2+} transport systems and channels and through receptor-regulated plasma membrane conductances, such as the NMDA receptor channels and intracellular release sites activated by inositol triphosphate (Michaelis, 1990).

Many of these responses can be conceptually connected to effects on membrane structure and function – studies document ethanol effects on fetal cell replication, membrane transport systems, membrane fluidity, Na^{+}-K^{+} pump expression, and EGF receptor expression (Henderson et al, 1999). Recent studies (Edwards, Monk and Chen, 2002; Henderson et al, 1999; Mitchell et al, 1999;Carter and Wands, 1985) have provided evidence that oxidative stress may be one mechanism by which ethanol produces these membrane-related events. Oxidative stress is generally caused by toxic oxygen free radicals that initiate lipid peroxidation. Henderson et al (1999) observed ethanol-induced oxidative stress in cultured fetal rat hepatocytes, which showed morphological and biochemical signs of mitochondrial damage. Ethanol increased H_2O_2, as well as O^{2-} lipid peroxidisation products, along with signs of membrane damage. Ethanol was found to inhibit activities of mitochondrial respiratory chain components (a potential source of enhanced levels of H_2O_2 and O^{2-}) and this could be reversed by antioxidant treatment. The low levels of antioxidants in fetal tissues and an exaggerated response of fetal mitochondria to prooxidant stimulation *in vitro,* suggest that fetal cells are strongly predisposed to oxidative stress (Henderson et al, 1999).

Recent studies have also suggested that fetal tissues are likewise prone to the formation and subsequent accumulation of at least one toxic lipid perioxidation product, 4-hydroxynonenal (HNE), generated within mitochondria (Geurri, 1998; Savage et al, 1998). Importantly, HNE is a potent inducer of apoptosis in neurons. Ramachandran et al (2002) and Chen et al (2002) showed that maternal ethanol consumption can elicit a cascade of events in the fetal brain *in utero* which culminates in apopototic cell death that is mediated at a mitochondrial level. The increase in the mitochondrial content of HNE, and the rapid increase of reactive oxygen species following ethanol exposure support the role for oxidative stress and HNE in the proapoptotic responses to ethanol. Additionally, the low antioxidant defences in fetal tissues and accumulation of toxic aldehyde products of lipid perioxidation predisposes the fetus to oxidative damage.

Marin-Garcia et al (1996) found a significant decrease in mitochondrial ATP synthase activity in both the brain and liver, as well as a decrease in complex III activity in the liver of neonatal rats whose mothers were alcohol-fed during pregnancy. Prenatal ethanol exposure has been associated with long-standing cognitive, intellectual and behavioural impairments in children. Prenatal exposure to ethanol affects many crucial neurochemical and cellular components of the developing brain. Ethanol interferes with all stages of brain development, and the severity of the damage depends on the amount of ethanol intake and level of exposure. Experimental observations also indicate that the toxic effects of ethanol are not uniform: some brain regions are more affected than others and, even within a given region, some cell populations are more vulnerable than others (Guerri, 1998). The neocortex, the hippocampus, and cerebellum are the regions in which the neurotoxic effects of ethanol have been associated with behavioural deficits. Previous studies suggest that these deficits are, in part, linked to neurochemical abnormalities that reduce the ability to sustain long-term potentiation (LTP) in hippocampal formation in adult offspring (Sutherland et al, 1997). Savage et al (1998) found that prenatal ethanol exposure produces long-lasting deficits in the neurochemical mechanisms responsible for activity-dependent potentiation of amino acid transmitter release without affecting the synaptic machinery responsible for amino acid uptake, storage and release.

However, Tanaka (1998) found that one of the most vulnerable structures in the rat fetus exposed to ethanol *in utero* was the synaptic formation in the hippocampus. The consistent dysmorphogenesis of synapses during early brain development may be associated with the functional impairment of the CNS in FAS.

At a cellular level, ethanol disrupts basic developmental processes, including interference with division, proliferation, cell growth, differentiation and the migration of maturing cells. Alterations in astroglia development and in neuronal-glial interactions may also influence the development of the nervous system. Astroglial cells control and regulate the homeostasis in the extracellular environment and have the ability to sense and modulate synaptic activity. The astroglial cells are highly interconnected through gap-junctions, which are intracellular channels that define a direct pathway for bi-directional signalling. Adermark, Olsson and Hansson (2002) found that acute ethanol exposure effects astroglial gap-junction communication. Ethanol exposure alters several functions of astroglial cells, including protein trafficking, in which the actin cytoskeleton and its regulators, Rho protein, play an important role.

Gausch et al (2002) demonstrated that ethanol markedly affects actin organisation and that this effect is mediated by RhoA signalling pathways. In addition, they suggest that the ethanol-induced actin ring reorganization might be an initial signal of the apoptotic process. An impairment of several neurotransmitter systems and/or their receptors, as well as changes in the endocrine environment during brain development, are also important factors involved with behavioural dysfunctions observed after prenatal ethanol exposure (Guerri, 1998).

The deleterious effects of ethanol on the developing human brain are poorly understood. Recent studies have shown that specific brain areas, such as the basal ganglia, the corpus callosum and parts of the cerebellum might be especially susceptible to alcohol's teratogenic effects (Roebuck et al, 1998). Ikonomidou et al (2000) have reported that ethanol, acting by a dual mechanism, triggers widespread apoptotic neurodegeneration in the developing rat forebrain.

Vulnerability co-incides with synaptogenesis, which in humans extends from the sixth month of gestation to several years after birth. During this period transient ethanol exposure can delete millions of neurons from the developing brain. This could serve to explain the reduced brain mass and neurobehavioural disturbances associated with FAS. Animal studies modelling FAS have demonstrated that developmental exposure to alcohol exposure is associated with decreased weight and significant neuronal loss in multiple regions of the developing brain (Ashwell and Zhang, 1996). Maier et al (1999) found that gross regions of the brain are differentially vulnerable to an alcohol insult during the first two trimesters.

Neuronal cell loss is one of the most debilitating effects of fetal ethanol exposure (Lou et al, 1997). Animal models have clearly established that ethanol exposure can deplete neurons in the developing nervous system. However, the mechanism by which ethanol reduces cell number is unclear and the effects on the neuronal precursors are not homogenous, some cells are more susceptible to ethanol toxicity than others (Lou and Miller, 1997; Miller, 1996b). Lou et al (1999) showed that in the developing nervous system, ethanol may limit the number of proliferating neuronal precursor cells by two mechanisms: ethanol-induced cell death and by causing cell cycle delay, thereby lengthening the doubling time of cells.

Miller (1995) reported that not only does ethanol directly depress the proliferation of neuronal precursors, but also causes the death of neurons during the period of synaptogenesis. McAlhany et al (2000) found that ethanol exposure during neural development induced a dose-related increase in both apoptosis and necrosis. Low concentrations of ethanol specifically increased DNA fragmentation, while all concentrations increased phosphatidylserine translocation, suggesting that ethanol induction is not a unitary process. Furthermore, only higher concentrations of ethanol induced necrosis. Ethanol specifically induced phosphorylation of c-jun N-terminal-kinase (JNK), a mitogen-activated protein (MAP) kinase selectively associated with apoptosis. In contrast, it did not alter phosphorylation of another MAP kinase, the extracellular signal-related kinases (ERK) that mediate cell survival. Thus, ethanol activated specific intracellular cell death-associated pathways and induced cell death (McAlhany et al, 2000).

Hsiao et al (1999) found that early postnatal ethanol exposure inhibits maturation of $GABA_A$ receptors in developing medial septum/diagonal band (MS/DB) neurons, suggesting that these receptors may represent a target for ethanol related to FAS, and could contribute to its associated neurobehavioural deficits. In addition, early postnatal ethanol exposure during the brain growth spurt disrupts the expected developmental pattern of $GABA_A$ receptor development across the brain. Whether these changes are due to cellular damage, delayed gene expression or post-translation modification need to be determined (Hsiao et al, 1998).

The cerebral cortex is a target site of ethanol teratogenesis. L-glutamate is a major excitatory neurotransmitter that plays an important neurotrophic role in brain development. Optimal function of the glutamate neuronal system is required for normal brain development; over-activation could lead to excitotoxic-induced neuronal injury, whereas under-activation could delay/restrict brain development (Reynolds et al, 1995). Because the blood-brain barrier is incomplete in the developing fetus, an elevation of fetal serum glutamate could expose the immature, growing brain to potentially toxic levels of extracellular glutamate (Karl et al, 1995).

Recent evidence suggests that ethanol abuse produces its diverse effects on the brain to a substantial degree by disrupting the function of the major excitatory neurotransmitter, glutamate. Ethanol inhibits NMDA receptor, which mediates the post-synaptic excitatory effects of glutamate. Ethanol's inhibition of NMDA receptors in the fetal brain is likely to contribute to the CNS manifestations of FAS (Tsai and Coyle, 1998).

FAS is characterised by numerous nervous system anomalies with the developing hippocampus being highly vulnerable (Mitchell, Paiva and Heaton, 1999). The brain regions of the hippocampus and cerebellum are particularly vulnerable to alcohol's inhibitory effects on protein synthesis (Peters and Steele, 1982).

Prenatal exposure to alcohol can result in abnormal hippocampal development and function (Berman and Hannigan, 2000) and ethanol exposure during the brain growth spurt will deplete cerebellar neurons (Dohrman et al, 1997; Webb et al, 1996; Bonthius et al, 1996). The mechanisms underlying this neuronal loss remain elusive. Nerve growth factor (NGF) is a neurotrophin that promotes cell survival in various brain areas, and there is evidence that NGF may play a role in the developing cerebellum and can ameliorate the neurotoxic effects of ethanol (Webb et al, 1996).

The early neonatal period of rats is one of enhanced vulnerability to cerebellar Purkinje cell loss associated with binge-like alcohol exposure, with a prominent sensitive period during the first neonatal week coinciding with the early stages of dendritic growth (Li et al, 2002; Lee at al, 2002; Eskue et al, 2002; Goodlett and Eilers, 1997). Regionally specific Purkinje cell death may occur in humans prenatally exposed to alcohol (Sowell et al, 1996). Dohrman et al (1997) found that ethanol exposure in neonatal rats reduced expression of NGF receptors on Purkinje cell dendrites and this could interfere with neurotrophic support of Purkinje cells by reducing levels of available NGF receptor. The hippocampus is the key area for human intellectual function and memory formation. FAS is a major cause of mental retardation. Children with FAS have mild to severe defects in these areas, with many of them exhibiting low intellectual functioning, others appear to have attention-deficit disorder (Shaywitz et al, 1980). Many of the children exhibit poor coordination of fine muscle movements. Some cognitive and learning disabilities may be related to hippocampal damage produced during fetal alcohol exposure (Bellinger et al, 1999; Heaton et al, 1995a; 1995b), and some movement disorders from damage to the cerebellum. \

3.8 Local growth factors

In addition to nutritional and hormonal factors, whose effects are widespread, various chemical substances regulate cell development locally in the region where they are produced. Some of these substances affect cell proliferation, growth and differentiation, whereas others modulate cell migration and adhesion.

There is evidence that continued exposure of the fetus to alcohol may interfere with the function of at least some of these chemicals. Growth factors acting through surface receptors on cells powerfully stimulate the transcription of the genetic material and the synthesis of proteins. This leads to a growth of cells as well as to differentiation, such as the branching of nerve cells or development of contractile fibres in muscle cells.

The CNS is particularly susceptible to ethanol toxicity. In fact, heavy gestational ethanol consumption is one of the leading known causes of mental retardation in the Western world (Lou and Miller, 1998). Ethanol exposure disrupts the proliferation of glial and neuronal precursors in the developing CNS. Depending upon cell population and blood ethanol concentration, ethanol can either inhibit or stimulate cell proliferation. Two features of cell proliferation that are affected by ethanol exposure are the growth fraction (the proportion of cells that are actively cycling) and the cell cycle kinetics, particularly the length of the G1 phase of the cell cycle.

Cell proliferation in the developing CNS reflects the action of positive (mitogenic growth factors) and negative (antiproliferative factors) regulators. Increasing evidence shows that ethanol interferes with the action of growth factors. The inhibitory effects of ethanol on cell proliferation may result from interference with mitogenic growth factors (eg. bFGF, EGF, PDGF, IGF-1, IGF-II). Conversely, the stimulatory effects of ethanol may result from the interference with growth inhibitory factors (eg. TGF). Interestingly, both *in vivo* and *in vitro* studies show that proliferating neural cells display differential sensitivity to ethanol (Krishna and Phillips, 1994). This differential sensitivity correlates with their response to mitogenic growth factors; that is, cells that are actively regulated by mitogenic growth factors are much more susceptible to ethanol than cells that are less or unresponsive to such factors (Lou and Miller, 1998).

Ethanol interference with growth factor action could occur at three levels: ligand production, receptor expression and/or signal transduction. Thus, ethanol-induced alterations of the developing CNS that characterise FAS apparently result from alterations in the regulatory action of growth factors (Lou and Miller, 1998).

Insulin-like growth factors I and II (IGF-I and IGF-II) and IGF-binding proteins are important modulators of fetal growth. IGF-I is a key regulator of fetal growth and development (Singh et al, 1994). Most studies on maternal ethanol exposure have revealed reduced circulating IGF-I levels in the fetus (Halmesmaki et al, 1989; Sonntag and Boyd, 1989; Breese and D'Costa, 1993). IGF-I has been shown to increase alcohol dehydrogenase activity in hepatocytes by increasing the production of this enzyme at a pre-translational level (Mezey et al, 1990). Thus, the alcohol-induced decrease in IGF-I levels could in turn decrease hepatic alcohol dehydrogenase activity, leading to impaired hepatic metabolism and clearance of alcohol – a vicious circle.

The findings of reduced serum concentration of IGF-I in the offspring of alcohol-fed animals points to a direct interaction of alcohol with the growth-hormone-releasing hormone/growth hormone/insulin-like growth axis factor that is independent of indirect effects via alcohol-induced maternal malnutrition (Breese et al, 2002; Singh et al, 1994). Mauceri et al (1993) found that circulating levels of IGF-II and specific binding proteins are altered in growth-retarded fetuses exposed to ethanol. Alterations in the expression and levels of IGFs and IGF binding proteins may contribute to fetal growth deficiency (Mauceri et al, 1993).

Ethanol has been found to inhibit brain growth and reduce CNS 3',5'- cyclic adenosine monophosphate (AMP) with an associated decrease in binding of cAMP by the regulatory subunit as a result of ethanol exposure. Furthermore there is a specific loss of phosphorylation of the regulatory subunit by kinase catalytic subunit as a result of ethanol exposure. As the tissue content of cAMP and the degree of regulatory subunit phosphorylation are important parameters for the regulation of protein kinase A activity, it is hypothesized that these alterations may be the biochemical transformations that underlie ethanol-induced growth suppression (Pennington, 1990). Protein kinase C (PKC) regulates the cell cycle and has been linked to growth.

McIntyre et al (1999) examined the effect of ethanol on PKC isoform expression in the developing chick brain and found that ethanol exposure decreased head weight in chickens at day 5 in a dose-dependent manner and a decreased brain weight at day 7 and 10. In addition, they found that only specific PKC isoforms are developmentally expressed in the embryonic chick brain and that ethanol may inhibit the expression of those PKC isoforms that are developmentally regulated.

Dow and Riopelle (1985) were the first to suggest that alcohol could diminish the effect of growth factors on nerve cells. Since then, studies have demonstrated reduced synthesis and release of a nerve growth-promoting factor in newborns exposed to alcohol during gestation (Heaton et al, 1992). Diminished protein synthesis by a newborn or fetus exposed in the uterus to alcohol has been repeatedly documented, especially in the brain (Tewari et al, 1992).

In the adult nervous system, neurotransmitters mediate cellular communication within neuronal circuits. In developing tissues and primitive organism, neurotransmitters subserve growth regulatory and morphogenic functions. Accumulated evidence suggests that acetylcholine (ACh), released from growing axons, regulates growth, differentiation, and plasticity of the developing nervous system neurons (Lauder and Schambra, 1999).

ACh and other muscarinic agonists stimulate the proliferation of rat cortical astrocytes by activating muscarinic cholinergic receptors. Proliferation of glial cells induced by muscarinic agonists is especially sensitive to the inhibitory effect of ethanol. This action may be relevant to its developmental neurotoxicity, particularly microencephaly (Guizzetti and Costa, 1996). In addition, to intrinsic cholinergic neurons, the cerebral cortex and hippocampus receive extensive innervation from cholinergic neurons in the basal forebrain, beginning prenatally and continuing throughout the period of active growth and synaptogenesis. Acute exposure of ethanol in early gestation (which prevents formation of basal forebrain cholinergic neurons) or neonatal lesioning of basal forebrain cholinergic neurons, significantly compromised cortical development and produced persistent impairment of cognitive functions.

Therefore, it can be surmised that exposure to environmental neurotoxins like alcohol, that affect cholinergic systems may seriously compromise brain development and have long-lasting morphologic, neurochemical and functioning consequences (Lauder and Shambra, 1999). However, few animal experiments are undertaken in chronically exposed subjects, making extrapolation of findings from animal studies to humans, difficult.

3.9   Effects on the developing immune system

Children exposed to alcohol *in utero* suffer from growth and mental retardation, physical abnormalities and immune dysfunction. Ethanol consumption results in significant changes in the immune system of experimental animals and humans (Cook, 1998; Szabo, 1999). Jerrells and Weinberg (1998) have shown that *in utero* exposure to ethanol results in alterations in the immune system of the offspring that persist into adult life. In addition, they demonstrated long-term adverse effects of prenatal ethanol exposure on T-cell responses to mitogens, and provided further evidence that deficits seem to be more robust in male than female offspring. B cell lymphopoiesis was found to be delayed in mice exposed to alcohol *in utero* and it is thought that the alcohol exposure disrupts the ability of B-lineage intermediates to progress along the developmental pathway to maturity, thereby leaving the animal immunocompromised at birth (Biber et al, 1998). *In utero* alcohol exposure can also result in abnormalities of B cell development and may initiate an early stage of B cell development (Moscatello et al, 1999). Svinarich et al (1998) found that human first trimester trophoblasts express high levels of cytokines when cultured in the presence of ethanol. Trophoblasts may therefore be an important exogenous source of cytokines for the fetus, and altered cytokine levels during early gestation may have an adverse effect on the development of the fetal immune system.

3.10   Nutritional factors

One of the mechanisms by which FAS occurs is the direct toxicity of ethanol on the developing fetus. However, many chronic alcoholics are also chronically malnourished. In addition, alcohol may be toxic to the placenta as well as the fetus. Since the placenta is the major conduit for the supply of nutrition to the fetus, as well as the producer of hormones that mobilize maternal nutritional stores, ethanol-associated placentotoxicity could result from "selective" fetal malnutrition, independently of the mother's nutritional status (Fisher, 1985).

Several nutrients are essential for fetal growth and normal development of the brain. Vitamins, especially folate, and nutrients, such as essential amino acids, are required for normal embryogenesis. Furthermore, the placenta produces hormones responsible for mobilization of maternal fat and glucose stores to provide adequate energy calories for the fetus (Munro, 1980). Normal growth and development during the gestational period requires the transfer of a constant supply of amino acids and glucose from the mother to the placenta. This placental transfer of essential amino acids is an energy-dependent, membrane-associated active transport process (Miller and Berndt, 1975).

Several studies have shown that alcohol directly inhibits the transport of both amino acids and glucose (Schenker et al, 1989; Snyder et al, 1986; Fisher et al, 1984). In experimental animals, the alcohol-exposed fetus suffers from selective amino acid deficiencies (Lin et al, 1990). Rat embryos exposed to alcohol *in vitro* exhibited marked growth retardation, decreased glucose metabolism and diminished protein synthesis (Snyder et al, 1992). Thus, alcohol ingested by the mother deprives fetal tissues of the energy sources and materials needed for cell proliferation, growth and differentiation and might not only lead to growth retardation, but could also compound any toxic effects of ethanol on embryogenesis. Some organs of the developing fetus, such as the brain, are relatively protected during early periods of development against damage caused by malnutrition, unless the malnutrition reaches severe levels (Dobbing, 1974).

This points to the timing of exposure of the fetal organs to alcohol; for example, Bonthius and West (1990) described the damaging effects of binge alcohol intake on different populations of nerve cells during later stages of development of the brain.

In addition to amino acids, other nutrients are essential for normal fetal growth and development. Specifically, zinc is required for normal DNA synthesis, as well as for the normal functioning of many enzyme systems. Nutritional deficiencies reported in either alcoholic mothers or their infants include those of trace metals such as zinc and vitamins. Another nutrient essential to the synthesis of new DNA is folic acid and folic acid deficiencies during gestation produce malformations in the fetus. Folate is accumulated by the placenta and released into the fetal circulation at concentrations greater than that of the maternal blood.

The placenta has a specific receptor for binding folate and Fisher et al (1985) have demonstrated a decrease in placental folate receptor activity in the placentas of animals exposed to ethanol prior to and throughout pregnancy. There is evidence of decreased transfer of a form of vitamin B_6 from an alcoholic mother to her fetus through the placenta (Schenker et al, 1992). In addition, a possible defect in the metabolism of folic acid has been identified in some of the tissues exposed to alcohol during gestation (Lin et al, 1992).

Together with the effects on transport of essential nutrients, ethanol also has placentotoxic effects. In the rat, ethanol causes a decrease in placental protein synthesis, specifically placental lactogen (Wunderlich et al, 1979), an important factor in the mobilization of maternal nutrient stores. Miller et al (1995) found decreased amounts of iron, transferrin and ferritin in three CNS regions in rat brain after *in utero* ethanol exposure. Ethanol induced alterations in iron homeostasis that persisted into adulthood. The net result was that the timely delivery and bioavailability of iron was compromised by ethanol exposure. The defects in iron regulation are permanent and may underlie ethanol-induced abnormalities in iron-dependent growth processes such as myelination.

Recent research has focussed on the interaction of ethanol and vitamin A in models to explore if an interaction between these two compounds might potentially be the mechanism for FAS. The rationale for this include the known facts that: (1) in adults, ethanol ingestion alters vitamin A metabolism and tissue distribution; (2) there are many phenotypic similarities between FAS and malformations of both vitamin A toxicity and deficiency; (3) the vitamin A metabolite, retinoic acid (RA), is a potent mediator in embryogenesis and differentiation (Zachman and Grummer, 1998; Grummer and Zachman, 1995). There is no evidence of vitamin A deficiency in the liver or blood of fetuses from experimental animals fed alcohol through the gestational period, but instead, vitamin A appears to accumulate in the liver (Leichter et al, 1991), suggesting that the vitamin is not being metabolised normally. This may be significant because vitamin A is metabolised to RA, which, in developing cells functions as a chemical signal for the activation of DNA transcription. Therefore, the lack of RA may be responsible for some of the developmental delays and craniofacial malformations seen in FAS (Yin et al, 1999; Ang et al, 1996; Duester, 1991; Pullarkat, 1991).

RA is known to act as a signalling molecule during embryonic development, but little is known about the regulation of RA synthesis from retinol. The rate-limiting step in RA synthesis is the oxidation of retinol, a reaction that can be catalysed by alcohol dehydrogenase (ADH). Ethanol is also a substrate for ADH, and high levels of ethanol inhibit ADH-catalysed retinol oxidation (Deltour et al, 1996). One interaction that could possibly alter fetal development is that the synthesis of RA from retinol, catalysed by alcohol dehydrogenase, might be competitively inhibited by ethanol leading to RA deficiency (Han et al, 1998). Napoli (1999) found that ethanol competes directly with retinal dehydrogenation to impair RA biosynthesis.

RETINOIC ACID'S POSSIBLE LINK TO FAS'S DISTINCT FACIAL FEATURES
Alcohol's teratogenic effects on the development of craniofacial structures may involve a reduced production of the compound retinoic acid during a narrow period of early embryonic development. Derived from vitamin A, retinoic acid is essential for the normal development of various tissues and organs in vertebrates, including the development of neural crest cells into craniofacial features. Retinoic acid acts through specific receptors, some of which turn on or turn off the genes that regulate the timing, coordination, and sequencing of various steps in the development of craniofacial features as well as of certain brain regions. Severe vitamin A deficiency of insufficient retinoic acid formation can produce birth defects, and alcohol can prevent of reduce the production of retinoic acid in the brain. This disruption in the control and timing of retinoic acid-mediated gene regulation may be a key component in alcohol's harmful effects on the fetus.

3.10.1 Maternal nutritional factors and oral health

The formation of the dentition is subject to nutritional and metabolic influences during the prenatal period (Larson, 1964). Disturbances, when they occur during the formation of the dental structures, are registered permanently as enamel and dentine defects and may render teeth less resistant to dental disease (Wei, 1974). Optimal maternal nutrition is required for optimal calcification of the primary dentition *in utero* (DePaola and Alfano, 1977). Severe and prolonged dietary disturbances during the critical periods of dentinogenesis and amelogenesis produce alterations in the morphology, chemical composition, cellular structure, eruption timing and appearance of the teeth (Dreizen, 1972).

In man, the complex multiphasic process of odontogenesis begins as early as the sixth week of embryonic life in a sequence consisting of initiation, proliferation, histodifferentiation and morphodifferentiation followed by calcification (which is evident as early as the fourth month *in utero*). Metabolic disturbances resulting from severe maternal vitamin A deficiency may cause defective formation of the teeth (Shaw and Sweeney, 1973). The dental structures are particularly susceptible during certain critical periods in development (Larson, 1964). DePaola (1978) defines a critical period as "that time in the development of an organ system that is marked by rapid synthesis and accretion of protein and equates with the intense hyperplastic growth phase".

Disturbances during these critical periods result in inevitable damage not only to the dental structures, but the maxillary and mandibular jaw bones, salivary glands, oral epithelium and craniofacial structures (DePaola and Jordan, 1978). Severe linear hypoplasia of the teeth has been positively correlated with maternal malnutrition and protein and vitamin A deficiency (Glick, 1978; Winter, 1976). Maternal protein deficiency affects the biochemical composition and impairs function of salivary glands (Menaker and Navia, 1973; 1974). Because final genetic expression if influenced by nutrition, infection and environmental factors, craniofacial development may also be affected by these influences (DePaola, Miller and Drummond, 1973); some mechanisms are described below (see 3.12).

3.11 Hormonal factors

The processes of cell proliferation and differentiation are controlled by many internal stimuli, both chemical and physical emanating from the milieu of the developing tissues. The production and release of hormones from both the maternal and fetal glands and from the placenta influence the formation and development of tissues as diverse as the brain and palate. For example, in experimental animals exposed *in utero* to alcohol, there is a decrease in blood and brain concentrations of corticosteroid hormones during the newborn period (Kakihana et al, 1980; Taylor et al, 1982). These hormones regulate various aspects of metabolism and influence the organism's response to stress. The deficiency of corticosteroid hormone production leads to deficits in the response of the newborn to stress. There are also deficits in the synthesis of sex steroid hormones. These hormone deficits may lead to the abnormal development of the brain, especially those specialised regions of the brain that differ between male and female and that control sex-related behaviour (Rudeen, 1992).

Some of the developmental defects characteristic of congenital or experimental hypothyroidism are also observed in children prenatally exposed to ethanol, suggesting that a subset of neurological defects attributable to ethanol exposure are produced by interfering with thyroid hormone action.

Scott et al (1998) found that ethanol may interfere with thyroid hormone action during fetal brain development by selectively reducing the mRNA encoding alpha-1 thyroid hormone receptor in fetal rat brain. Thyroid hormone deficiencies during pregnancy have also been identified in fetuses born to human alcoholic mothers (Hernandez et al, 1992); these deficiencies may have a deleterious effect on the development of some tissues, in particular the brain (Hannigan and Bellisario, 1990). In the cerebellum, derangements in the maturation and migration of nerve cells to their appropriate locations caused by thyroid hormone deficiencies are similar to those observed when experimental animals are exposed to alcohol at or shortly after birth (Kornguth et al, 1979).

Hormones and other chemical messengers exert their effects on cells by binding specific receptor proteins on the cell surface or inside cells. The receptors for steroid hormones, thyroid hormones and RA are all members of a specific family of receptor proteins that regulate transcription of DNA in a cell (Evans, 1988). Under the influence of appropriate hormones or other chemical messengers, these receptors can alter the rate of synthesis of proteins, thereby regulating the growth and differentiation of a cell. In addition, both thyroid hormone and RA regulate another hormone, growth hormone (Bedo et al, 1989).

Exposure to ethanol *in utero* has been found to alter the growth hormone (GH)/growth factor axis and retard growth. GH release is regulated by the interaction of the hypothalamic hormones somatostatin (somatotropin release inhibiting factor – SRIF) and GF- releasing factor (GRF). Communication between these two factors occurs at both the hypothalamic and pituitary levels. A relative reduction in growth hormone formation and release may contribute significantly to growth retardation in human FAS (Conway et al, 1997; Conway and Swain, 1997).

There are a variety of mechanisms through which alcohol alters the activity of the hypothalamic-pituitary-adrenal (HPA) axis. In adult rats, acute ethanol treatment increases plasma ACTH and corticosteroid levels primarily by stimulating the release of corticotropin-releasing factor (CRF) and possibly vasopressin (VP) from nerve terminals in the median eminence. Increased CRF gene transcription in the hypothalamus may also be important. The HPA axis remains activated during chronic ethanol exposure. Changes in the responsiveness of hypothalamic neurons, a phenomenon itself dependent in part on a number of intermediate secretagogues, as well as decreased pituitary responsiveness to VP, all play a role. Finally, the activity of the HPA axis is influenced by exposure to ethanol during embryonic development, with mature offspring showing hyporesponsiveness to many stimuli. These altered responses appear to be caused in part by changes in the synthesis/release of CRF, possibly under the influence of nitric oxide. CRF, VP, ACTH, and corticosteroids are important regulators of the immune system, behaviour, metabolic pathways and reproductive parameters. Alcohol may therefore influence such functions through the pathological secretion of these hormones (Rivier, 1996).

3.12   Mechanisms of craniofacial disturbances

Technical advances are radically altering our concepts of normal prenatal craniofacial development. These include concepts of germ layer formation, the establishment of the initial head plan in the neural plate, and the manner in which head segmentation is controlled by regulatory (homeobox) gene activity in neuromeres and their derived neural crest cells. There is also a much better appreciation of ways in which new cell associations are established. For example, the associations are achieved by neural crest cells primarily through cell migration and subsequent cell interactions that regulate induction, growth, programmed and cell death. These interactions are mediated primarily by two groups of regulatory molecules: growth factors (FGF, TGF alpha) and the steroid/thyroid/retinoic acid superfamily. Much progress has been made on mechanisms involving the final differentiation of skeletal tissues. Molecular genetics and animal models for human malformations are providing insights into abnormal development. A mouse model for FAS demonstrates a mid-line anterior neural plate deficiency which leads to olfactory placodes being positioned too close to the midline, and other secondary changes. Work on animal models for the retinoic acid system (RAS) shows that there is major involvement of neural crest cells (Johnston and Bronsky, 1995).

FAS is characterised by numerous craniofacial and neuronal anomalies. The type and severity of the defects may be related to the time and dose of maternal ethanol exposure. Animal and epidemiological studies suggest that the craniofacial malformations result from exposure to high levels of alcohol during the first trimester, more specifically, during the first two months of gestation (Ernhart et al, 1987; Graham et al, 1988; Day et al, 1989).

Indeed, Ernhart et al (1987) report a dose-response relation between craniofacial anomalies in children and maternal alcohol intake (28-85g per day) around the time of conception, and found that greater dysmorphia was associated with lower intellectual development.

Ethanol administered during presomitic stages results in the typical FAS craniofacial phenotype and is accompanied by loss of cranial neural crest cells (CNCCs) through ethanol-induced cell death. Cartwright and Smith (1995) found that there are distinct developmental windows during which the CNCCs may be particularly susceptible to ethanol-induced cell death. Ethanol exposure seems to affect specific events adversely during neural crest development. The timing of embryonic ethanol exposure relative to CNCC development could account, in part, for heterogeneous craniofacial defects observed in FAS.

Endogenous retinoic acid has been observed in vertebrate embryos as early as gastrulation, but the mechanism controlling spatiotemporal synthesis of this important regulatory molecule remains unknown. Some members of the alcohol dehydrogenase family catalyse retinol oxidation, the rate-limiting step in RA synthesis (Ang et al, 1996). During neuralation, RA and class IV ADH mRNA were colocalized in the craniofacial region, trunk and forelimb bud. Class IV ADH mRNA was detected in cranial neural crest cells and craniofacial mesenchyme. The spatiotemporal expression pattern and enzymatic properties of class IV ADH are consistent with a crucial function of RA synthesis during embryogenesis. In addition, the finding of endogenous RA and class IV ADH mRNA in the craniofacial region has implications for the mechanism of FAS (Ang et al, 1996).

An underlying assumption in many mechanistic studies of prenatal alcohol exposure is that cell death contributes to the pathology of alcohol exposure. This linkage is logical given that the organs and structure affected by alcohol often exhibit striking levels of cell death within precursor populations in response to a physiologically relevant alcohol exposure. However, alcohol's effects upon the embryo are complex. Debalak, Su and Smith (2002) tested the relevance of this assumption on chick embryo models for alcohol-induced facial dysmorphology and found that cell death contributed greatly to the facial dysmorphology, but argued further that alcohol was likely to target events in addition to apoptosis, namely, the sonic hedgehog pathway and neural crest regeneration.

Maternal ethanol intake has a significant deleterious effect on craniofacial development (Haselbeck and Deuster, 1998). Hernandez-Guerrero et al (1998) hypothesize that the reduction in cephalometric dimensions found in their study could be a manifestation of disorganised neural and mesenchymal development. The ability of both acute and chronic exposures to elicit cell death within specific embryonic and adult tissues is believed to partly underlie ethanol's pathogenicity; however, the mechanism underlying this cell death is unknown. Cartwright et al (1998) partially characterised the mechanism of ethanol-induced neural crest cell death in a chick embryo model of FAS. *In situ* DNA end-labelling demonstrated this cell death was apoptotic and occurred at low embryonic ethanol levels. Regardless of the initial exposure time, this apoptosis always appeared at a distinct developmental time point simultaneous with the normal deletion of a cranial neural crest subset. This suggested that ethanol might act through aberrant activation of the endogenous death pathway. Apoptotic deletion of cranial neural crest could partially explain the craniofacial deficits characteristic of FAS (Cartwright et al, 1998).

Several forms of cell perturbations have been associated with ethanol ingestion during pregnancy. In FAS diminished maxillofacial development and inhibition of cell regulation *in vivo* and *in vitro* have been described (Maier et al, 1999; Shibley and Pennington, 1997; Gerhart, 1988). Epidermal growth factor (EGF) stimulated maxillofacial growth, DNA synthesis, and it is a potent mitogen for a number of cell types. EGF exerts its effect on cells through binding to a specific cell surface receptor which leads to the activation of a tyrosine kinase intracellular part of the receptor. Carpenter and Cohen (1976) and Hernandez-Guerrero et al (1996) examined the inhibitory effect of alcohol on EGF in the mouse dental follicle in the offspring of alcoholic mothers using immunochemistry. They found (i) significant differences in mandible weight after parturition; (ii) the tooth germs in the offspring of ethanol treated mice were morphometrically smaller than those of the controls; (iii) immunoexpression of EGF in the mandibular first molar of the control group was strong and homogenous while in the experimental group the expression was light and heterogenous. They concluded that maternal alcoholism reduces EGF in offspring. Alcohol use during pregnancy may be a cause of isolated cleft lip with or without cleft palate (Munger et al, 1996).

3.13   Concluding remarks to Chapter 3

Many crucial biochemical and cellular events are affected by exposure of the fetus to alcohol during gestation. As can be seen from this part of the literature review, it is too early to speculate whether alcohol's effects on molecular or cellular function are more significant. Rather, alcohol can influence development via a number of direct and indirect mechanisms. Alcohol can alter the proliferation, migration, differentiation and cell survival of neuronal cells. Alcohol can also disrupt the development of glial cells, leading to alterations in cell signalling and myelination. Alcohol may act on the cell membrane. For example, alcohol can disturb membrane fluidity, which can affect cell adhesion, migration and cell communication. Prenatal alcohol can also have effects on glutamate receptors and GABA receptors, and act on intracellular messengers. For example, alcohol can decrease or increase intracellular calcium; an optimal level of intracellular calcium is necessary for normal outgrowth of neuronal fibers. Yet, despite this multitude of possible mechanisms, not all neuronal cell populations are equally affected by prenatal alcohol. One of the challenges for alcohol researchers is to determine why some cells are resistant whereas others are relatively vulnerable to prenatal alcohol.

Chapter 4: Epidemiology and Clinical Issues

4.1 Introduction

The extent and pattern of alcohol exposure necessary to produce the effects causing FAS are still under investigation. Epidemiological studies of mothers who drink during pregnancy have identified traits that are strongly associated with FAS births. Women who were older, multigravidas, not currently married and who smoked cigarettes and used other drugs were more likely to have children with FAS (Sokol et al, 1980). Later studies identified a greater risk among women characterised by advanced maternal age, high parity, low socio-economic status and with severe drinking patterns (Abel, 1995; Abel and Hannigan, 1995; Darrow et al. 1992).

Some of the contributory factors include variable maternal factors that may have a protective effect, specific maternal, fetal or genetic vulnerability factors that make a particular fetus more susceptible to the effects of alcohol exposure *in utero,* and fetal and embryonic damage may be enhanced by other risk factors that work synergistically with alcohol. Varying susceptibility may also be related to differences in the amount and pattern of alcohol ingestion, peak blood alcohol levels attained, the duration of the fetus's exposure, and variations in timing of exposure during critical periods of development when the fetus is most susceptible to the teratogenic effects of alcohol. Part 3 of the literature review reports on the epidemiology, examines these various factors and considers their implications for the occurrence of FAS.

4.2   Epidemiology of FAS

Lemoine et al (1968) in France and independently Jones et al (1973) in the United States were the first to report on alcohol embryotoxicity in humans. Lemoine et al (1968) reported on the characteristics of 127 children of alcoholic parents. They noted a recognizable craniofacial pattern: microcephaly, a short and upturned nose, small lips and retrognathia.

The facial anomalies were 'typical during the first two years….and changed with age.' Most of their patients were underweight and exhibited marked pre and postnatal growth retardation, mental retardation and various malformations such as heart defects, microphthalmia, cleft palate, hip dislocation and visceral anomalies. Jones et al (1974) described children who were underweight with growth retardation and microcephaly. All showed motor and mental developmental delay. Characteristic dysmorphic features included ptosis of the upper lids, epicanthic folds, short upturned nose, small, thin vermillion of upper lip and retrognathia. Furthermore, some children suffered from congenital heart defect, anomalous palmar creases and limited supination. After these publications, numerous case reports appeared and confirmed the clinical picture of FAS (Egeland et al, 1998). Many investigators have since refined, catalogued and quantified the hallmarks of FAS. Abel (1995) has calculated an average frequency of FAS in the "Western World" at 0.97 per 1000.

Kaminski et al (1981) in a prospective epidemiological study observed no correlation between maternal alcohol consumption and major malformations in the offspring. But they found increased frequencies of prematurity and still births, as well as decreased birth weights. Autto-Rämö and Granström (1991) in Finland, examined 53 children at one and a half years of age who had various durations of prenatal exposure to alcohol. The mothers either drank moderately (28-150g per week) or heavily (more than 150g per week) during the first trimester (group I) or heavily during the first and second trimester (group II), or heavily throughout the entire pregnancy (group III). There were no developmental differences between group I children and non-exposed controls. The children of group II and III showed significantly lower scores in language and total mental assessment.

The number of developmentally delayed children increased with increasing duration of prenatal alcohol exposure. In group II there was one child with FAS, whereas in group III, five of the 19 children (38%) were diagnosed with FAS.

A few reports have emanated from the United Kingdom. Beattie et al (1983) reported on a series of 40 affected children whose mothers were all addicted to alcohol. Plant (1985) carried out a prospective study on 1012 new-borns. Ninety-two per cent of the mothers drank alcohol during pregnancy, but mostly in moderate amounts. Despite a sophisticated study methodology, Plant failed to observe a single case of FAS. However, this study is suspect as the ability to diagnose fetal alcohol syndrome in the newborn is tenuous at best.

Sulaiman et al (1988) examined 952 consecutive primigravidas in Scotland and correlated maternal alcohol intake with perinatal outcome. There was no negative influence on the newborn if the mothers consumed less than 14g alcohol per day during pregnancy. Among the newborns of mothers who had consumed 14-17g daily, there was an insignificant decrease in birth weight and head circumference. This decrease in birth weight and head circumference, in addition to the decrease in length and Apgar scores, was significant among the newborns of mothers who drank more than 17g per day (without an upper limit).

In Germany, the first large series (68 cases) was reported by Majewski et al (1976). Since then more than 600 cases have been observed in three centres in Germany. The Berlin study group have concentrated on the electroencephalographic abnormalities (Spohr et at, 1979), paediatric neurological, psychological and psychiatric findings (Nestler et al, 1981; Spohr and Steinhausen, 1984), and long-term follow up (Spohr et al, 1993; Steinhausen et al, 1994). Löser first examined the type and frequency of congenital heart defects (Löser and Majewski, 1977; Löser, 1987) and later included somatic and psychiatric anomalies as well as long-term follow up findings (Löser, 1995; Löser et al, 1992). Majewski (1981, 1993) reported on the dysmorphic features, growth data and pathogenesis.

In a South African community in the Western Cape Province, May et al (2000) reported that the community-wide, age-specific rate was 39.2% per 1000 children aged 6 and 7 years. The only other paper to report a higher rate of FAS was from Canada in a study on a "highly disrupted" American Indian reserve with high unemployment (Robinson, Conry and Conry, 1987). Using active case ascertainment methods they reported a rate of 120 children younger than 19 years per 1000 with FAS.

4.3   Metabolism of alcohol

Alcohol is rapidly absorbed into the body and metabolized. However, the appearance of alcohol in the blood is not related in a simple way to the amount consumed, because some is metabolized in the stomach by the zinc-containing enzyme alcohol dehydrogenase (ADH) before absorption.

BLOOD VESSELS
- carry newly absorbed alcohol to liver

Blood concentration depends on:
- alcohol intake
- stomach ADH activity
- proportion of fat in the body
- rate of liver oxidation

LIVER
Oxidation of alcohol

FACTORS AFFECTING RATE OF ALCOHOL METABOLISM

Antacids and other drugs
Heavy alcohol consumption
Female sex
High fat consumption
Ethnic origin
Genetic background

ADH
ADH activity
STOMACH

ADH
Alcohol dehydrogenase converts alcohol into acetaldehyde

Figure 8: The fate of alcohol in the human body (Gurr, 1996)

This oxidation process is so rapid that the level of acetaldehyde in the human body normally does not become notably elevated during the oxidation of alcohol. These reactions take place in the liver and upper gastro-intestinal tract (Figure 8). The fetal liver does not contain the enzyme alcohol dehydrogenase (Pikkarainen and Räthä, 1968). The quantitative role of ADH in alcohol metabolism is controversial. Factors that reduce the activity of stomach ADH tend to result in greater than normal rise in blood alcohol concentration in response to alcohol consumption. Women have less gastric ADH activity than men, and there are marked ethnic variations. Once absorbed, alcohol quickly spreads into all tissues and fluids in proportion to their relative content of water. The greater the proportion of fat in the body the greater the rise in concentration in the blood. There is no plasma protein binding for alcohol. The same dose of alcohol per unit of body weight can produce different blood alcohol concentrations in different individuals because of the large variations in proportions of fat and water in the body and the low lipid:water partition coefficient of ethanol. Women generally have a smaller volume of distribution for ethanol than men because of their higher percentage of body fat. Women will have higher peak blood ethanol levels than men when given the same dose of ethanol as gram per kilogram body weight but no differences occur when given the same dose per litre of body water.

The principal route by which the liver metabolizes alcohol is via the enzyme ADH (Figure 9, a), although at least three other pathways have been described (Figure 9, b-d) (Gurr, 1996). The second important pathway for alcohol metabolism is via the microsomal ethanol oxidising system (MEOS). The activity of this enzyme system is not normally detected in liver cells but is "induced" in response to exposure of the membranes of the endoplasmic reticulum to alcohol – the presence of alcohol sends signals to the cell to produce more enzyme protein to catalyse the oxidation of alcohol. In alcoholism, where exposure to alcohol is more or less continuous, MEOS is permanently induced.
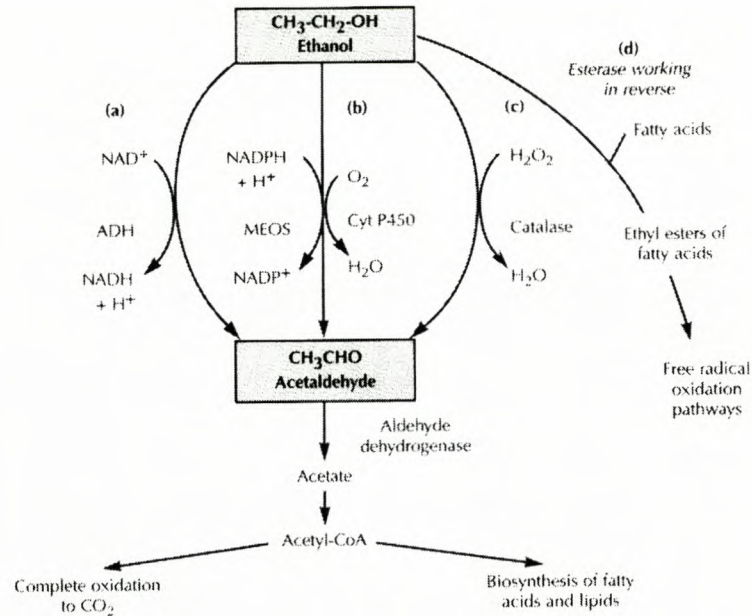
Figure 9: Metabolic pathways for the disposal of alcohol (Gurr, 1996)

The third pathway, which is probably a minor one, is indirectly via the enzyme catalase, which is ubiquitous in cells. If alcohol is present, the breakdown of hydrogen peroxide by catalyse can result in the oxidation of alcohol. Acetaldehyde is the first product of the metabolism of alcohol by each of these pathways. Because acetaldehyde is toxic, it is normally cleared rapidly by conversion into acetate and then into acetyl CoA in the mitochondria. Therefore, acetaldehyde dehydrogenase, which disposes of acetaldehyde, is a key enzyme in alcohol metabolism and detoxification (Gurr, 1996).

4.3.1   Physiology of alcohol during pregnancy

4.3.1.1 Absorption of alcohol

Absorption of alcohol across biological membranes occurs rapidly through a process of passive diffusion throughout the gastro-intestinal tract. Therefore, the higher the concentration of ethanol, the greater is the resulting concentration gradient, and the more rapid is the absorption.

Rapid removal of ethanol from the site of absorption by an efficient blood flow will help to maintain the concentration gradient and thereby promote absorption. The rate of absorption is related to the amount of absolute alcohol in the beverage ingested. Other factors are the motility of the gastro-intestinal tract, the vascularity of the mucous membranes and the concentrations and distribution of water in the various organs. During pregnancy there is usually a delay in gastric emptying time and decreased intestinal motility. It is therefore expected that pregnant women will have lower peak alcohol levels, but these are sustained longer as a result of delayed absorption (Newman and Correy, 1980).

4.3.1.2 Distribution of alcohol

Alcohol is distributed at a diffusion equilibrium according to the water content of the various body compartments. Alcohol readily crosses the placenta and reaches concentrations in the placenta equivalent to those in the maternal circulation. The pregnant uterus and fetus add considerably to the distribution of alcohol because of an approximated water increase of six litres in all compartments. The effects of alcohol will vary with changes in the water concentration during pregnancy. In early pregnancy the fetal water concentration is high, in advancing pregnancy, maternal water retention increases (Newman and Correy, 1980).

4.3.1.3 Elimination of alcohol

As described above, alcohol is metabolized mainly by the enzyme ADH and the formation of intermediary acetaldehydes. Peak alcohol concentrations depend on the enzymatic facility available and research has shown that fetuses of mothers with lesser ADH capacity were more severely affected (Sulik, Johnston and Webb, 1981).

4.3.1.4 Alcohol in the fetus

The fetal blood alcohol concentration falls at half the rate of maternal concentration. By comparing the elimination of alcohol in adults, small-for-dates and preterm infants, it can be computed that the fetus develops its own alcohol metabolism only after maturation of liver enzymes, which occurs during the second half of pregnancy. It is assumed that the fetus is at much greater risk for alcohol effects during the first half of pregnancy (Newman and Correy, 1980).

4.4   Critical period, thresholds, specificity and vulnerability

4.4.1   Critical periods

In the first half of the last century, the placenta was considered a natural barrier capable of protecting the developing child from exposure to harmful agents, including disease and toxic chemicals. When researchers discovered during the 1950s and 1960s that certain diseases such as rubella, and some environmental agents, such as mercury, were teratogenic (Scialli, 1992), concern focused on the first 3 months of pregnancy, the first trimester. This stage was considered the risky period – when exposure might lead to birth defects – whereas exposure during the later part of pregnancy was not considered dangerous. A possible explanation for this view involves the kinds of defects that generally appear if exposure to a teratogen occurs early in pregnancy.

The first trimester is the critical period of organogenesis, when the major organs form. Exposure during this time, and particularly during the first two months – the embyronic period – can result in dysmorphia (Cohen, 1990). The effects of teratogenic exposure during the second and third trimesters, which include growth retardation and neurological defects (Scialli, 1992), are not as obvious. By the 1980s however, research revealed that these ideas may not be completely accurate (Fabro et al, 1984). Many chemicals and organisms are capable of crossing the placental barrier.
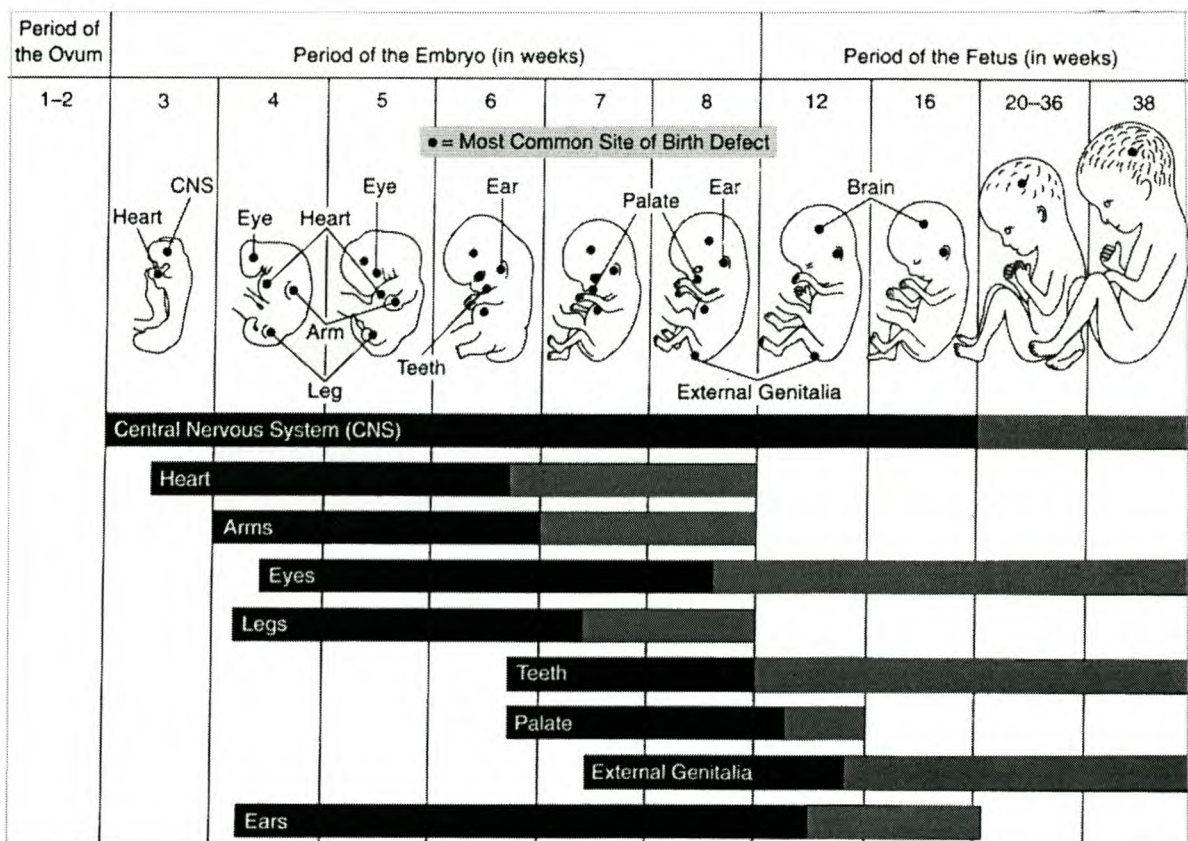
Figure 10: Vulnerability of the fetus to defects during different periods of development

The black portion represents the most sensitive periods of development, during which teratogenic effects on the sites listed would result in major structural abnormalities in the child. The grey portion of the bars represents periods of development during which physiological defects and minor structural abnormalities would occur. Adapted from Moore (1993).

Inherent in the idea of a critical period of pregnancy is that the embryo or fetus is vulnerable during specific times when undergoing particular developmental processes (Figure 10). The fetus is susceptible to alcohol's toxicity throughout its development, but structural anomalies arise primarily when exposure occurs during "critical periods" of development. This period-sensitive vulnerability can be pinpointed when a specific process in development is affected only during these windows of time. These periods are well documented. The first trimester, especially the period between the second and eighth week of gestation, is the time when bodily organs and appendages are forming (Wilson, 1973).

This period of organogenesis is highly susceptible to perturbation by teratogens. Exposure prior to or after this period may have other effects on the conceptus, but these may not be readily observable malformations. Since development of different organs occurs at different times during this period of organogenesis, unless exposure to alcohol always occurs at the same moment during development, there will be considerable variation in both kind and severity of malformations that may arise in conjunction with prenatal alcohol exposure. In addition, very high exposure to a teratogen can cause malformations prior to or following the time of greatest susceptibility (Wilson, 1973). Intrauterine growth retardation from alcohol occurs primarily when exposure occurs during the third trimester, whereas functional damage can occur at any time during development once organs have formed. In other words, maternal alcoholism can result in damage to the developing fetus, which does not necessarily manifest itself in the form of structural defects or FAS, because drinking has not occurred regularly throughout pregnancy. Whereas FAS will not occur unless drinking has occurred during all three trimesters of pregnancy, individual anomalies may occur as a result of drinking (usually at relatively high levels) during more discrete periods. Thus, one reason why FAS occurs at relatively low incidence among alcoholic women (Abel, 1995) is that many of these women appear to restrict their drinking to a particular period of pregnancy. However, few longitudinal studies have been performed to confirm or refute this.

4.4.2   Insight from animal studies

Despite inherent limitations, investigations of timing of alcohol exposure in animal models have led to many important insights into physical anomalies in humans. Sulik and Johnson (1983) established that exposure in mice around day 7 of gestation produces facial dysmorphia and correlated brain alterations equivalent to that seen in children with FAS. Clarren et al (1988), showed that facial malformations occurred between gestational days 20-32 and that early exposure was much more damaging to growth and behaviour than later in pregnancy, even if a larger dose was given at a later time. From animal studies, it is clear that early exposure (first trimester) produces significant dysmorphia and neurological damage; however, there are also effects on the nervous system as a result of later exposure only.

Miller (1992) investigated exposure during the second half of gestation (equivalent to the second trimester in humans) and found brain weight reduction a consistent finding in this period and in the early postnatal period (equivalent to the third trimester in humans). During the second half of gestation, nerve cells in the neocortex are generated and migrate to the appropriate brain regions. Alcohol exposure appears to affect the timing and pattern of nerve cell generation, both delaying the process and altering the number of cells that are produced. In addition, cell migration patterns are altered so that unusual cell malformations can be observed in many areas in the brain including the hippocampus, cerebellum, sensory nucleus and neocortex (Miller, 1992). Third trimester effects have been studied in a rat model by West and Goodlett (1990). Exposure to alcohol during this period leads to reduction in brain weight and head circumference, presumably associated with alterations in brain structure and function. The number of cells in certain regions of the hippocampus (learning and memory) and cerebellum (motor ability) are reduced.

4.4.3   Timing of alcohol effects in humans

4.4.3.1 Physical effects

Because facial dysmorphia occurs during the embryonic period, craniofacial anomalies in humans are probably associated with drinking during this initial stage of pregnancy (Coles et al, 1985). In statistical studies of craniofacial anomalies, Ernhart et al (1987) report that a relationship between these anomalies and the first trimester exposure was evident. The anomalies were also related to later intellectual development in that greater dysmorphia was associated with lower IQs. In their longitudinal studies, Graham et al (1988) found minor physical anomalies in alcohol-exposed children, present at birth and also at age 4 years, that were related to heavy drinking in the preconceptual period rather than midpregnancy. Day et al (1989) found that physical anomalies observed in infants were associated with heavy drinking in the first two months of pregnancy only. In addition, older mothers who were drinking heavily gave birth to children with higher dysmorphology than younger mothers who drank heavily (Kalberg et al, 2002).

4.4.3.2 Effects on growth

Growth is usually measured by weight, head circumference and height. In contrast to the relationship observed with facial dysmorphia, effects on growth appear to be related to exposure later in pregnancy. Data suggests that exposure continuing throughout pregnancy produces fetal growth deficiencies that can be observed at birth. When alcohol is discontinued by the beginning of the second trimester, children of drinkers may approach the growth of children of nondrinkers (Coles et al, 1985; Rosett et al, 1980), whereas Day et al (191) showed that exposure to alcohol during the second and third trimesters were linked to decreases in height, weight and head circumference. Despite the extensive research effects on prenatal alcohol exposure, information about timing of exposure as it relates to the fetus's vulnerability to particular effects on behaviours remains limited.

Animal studies and epidemiological studies strongly suggest that the facial malformation characteristic of FAS results from exposure during the first trimester and, more specifically, during the first two months of gestation. The relationship becomes less clear in the examination of growth retardation. It appears that both early exposure (during the first two months of pregnancy) and exposure during the third trimester affect growth. Specifically, effects on head circumference – and brain growth, appear to be the most consistent and permanent outcomes of exposure during these two periods. It is also clear from data that although a mother's heavy alcohol use in pregnancy is potentially damaging to the fetus, her stopping use is likely to have a beneficial outcome even on many of the functions (eg. growth and behaviour) that were affected by earlier drinking.

No absolutely safe level of alcohol consumption has yet been established and the critical period during pregnancy is still unknown. It has been shown that the nutritional status during the third trimester of pregnancy can have its greatest effect on fetal brain development (Zeman and Stambrough, 1969).

Ethanol is known to cross the placental barrier and has been found in mother's milk (Vorherr, 1974). Ethanol can act upon milk ejection by inhibiting oxytocin release (Cobo, 1973). Consequently, undernutrition of the child is a distinct possibility in heavy drinkers who nurse their infants. Thus, any effects on prenatal exposure to alcohol may be compounded by the alcoholic mother who breastfeeds her infant (Kesaniemi, 1974, Catz and Giacolo, 1972). However, the fact that FAS does not occur in every pregnancy characterised by alcoholism is important from a research perspective. Identifying the time of gestation when the fetus is most susceptible to anatomical derangements secondary to alcohol exposure may be useful from an epidemiological standpoint, but for purposes of preventive perinatal medicine, it is negligible. One would not advise a patient to avoid alcohol intake only during organogenesis. If advice is given to abstain, it is given for the duration of the pregnancy. Since there is no "safe" time to drink, there is no advantage from a prevention standpoint in advising a patient that drinking later in pregnancy is less dangerous than drinking during early pregnancy.

4.4.4 Amount and pattern of drinking

Advice regarding a "safe" time to drink is, however, not the same as saying there is no "safe" amount to drink. Thresholds exist for all teratogens, below which they do not produce adverse effects (Wilson, 1973). Majewski et al (1976) have suggested that chronicity of alcoholism increases the likelihood of FAS, but no upper level of consumption has been established that allows for prediction of definite damage to the fetus nor a lower level that excludes the possibility of fetal harm. However, Noble et al (1997) suggested that 3oz/89ml ethanol or six drinks per day constituted a "significant risk" for FAS with nearly 50% of the infants exposed experiencing alterations in a variety of physiological and morphological features.

Kline et al (1980) observed that the risk of abortion was doubled if 30ml or 1oz of alcohol was consumed twice weekly. Ouelette et al (1977) and Rosenlicht et al (1979) found that the risk of anomalies doubled with the daily consumption of 45ml alcohol. In the absence of reliable information as to what these thresholds are, most physicians counsel their patients to be on the "safe side" and not to drink at all. One of the reasons for this controversy is that FAS has only been reliably linked to "heavy" drinking during gestation. In part, this confusion stems from the terms such as "heavy", "moderate" or "social" drinking in relation to FAS, which may have different meanings to those who issue warnings and those who receive them (Abel and Kruger, 1995). It has been suggested that instead of relying on these vague terms, identifying the number of drinks (thresholds) that places a fetus at risk for FAS would be of more value if only from an information/communication point of view. Determining the point at which drinking begins to be dangerous for the developing fetus is itself problematic because drinking behaviour varies greatly. In addition, conclusions about harmful levels to a fetus cannot be based on experimental paradigms in which pregnant women are given graded amounts of alcohol, as is possible when comparable studies are conducted on animals. Instead, researchers must rely on what their subjects tell them they have consumed, and these self-reports can be related to outcomes. Since the self-reported information is subject to distortion due to denial, underreporting or inadvertent inaccuracy, relationships are often tenuous and conclusions always tentative.

To complicate matters further, the extent of underreporting of substance abuse in general may vary considerably according to the gestational period for which the information is collected, and in some cases consumption may be at least three times higher than what is reported to researchers (Day et al, 1993; Dicker and Leighton, 1994).

Underreporting may also vary according to socio-economic class, race, cultural acceptance or criticism of certain drinking practices, and general lifestyle factors, all of which may affect what individuals self-report about their drinking behaviour. For example, both abstention rates and rates of heavier drinking tend to be higher among pregnant African-Americans and Native Americans compared with Caucasians (Day et al, 1993; Faden et al, 1994). As a result, people in cultures where drinking is not commonplace, and excessive drinking is socially censured, may be motivated to underreport to a much greater extent than cultures where drinking is accepted.

Underreporting means that threshold levels based on estimated dose-response relationships should be considered "guidelines" rather than hard and fast cut-off points between "safe" and "dangerous" levels of consumption. Another potential variable that must be considered in addition to the amount of alcohol ingested, is the pattern of ingestion. In most cases thresholds are based on dose-response studies that collect data in terms of average amount of alcohol consumed per day. However, several studies in humans and animals have found that binge drinking is more deleterious than continuous drinking even though the same amount of alcohol may be consumed over a given period of time (Alleback and Olsen, 1998; West et al, 1990; Streissguth et al, 1989). These studies indicate that it is the maximum peak blood alcohol concentration that is obtained, rather than the total daily or weekly ingestion, that determines whether the exposure is deleterious. One of the reasons why some women are less likely than others to give birth to children with FAS is therefore possibly related to regular consumption of alcohol compared with sporadic binges.

An often repeated question has been whether alcohol could be demonstrated to be the specific teratogenic agent in FAS. Could fetal damage be due to a combination of malnutritional and environmental factors that are part of the alcoholic lifestyle? The scope of this research precludes full discussion of the extensive animal studies that have been completed and that demonstrate the teratogenic properties of alcohol in a wide variety of species. In humans, FAS has not been found in non-drinking, malnourished populations.

Alcohol remains the only common environmental agent consumed by women bearing children with FAS. Thresholds may also be affected by concurrent use of other drugs which act synergistically with alcohol to affect fetal development. Women who abuse alcohol frequently abuse tobacco and drugs, have poor health and may be malnourished. Any of these factors could result in birth defects on their own. Clarifying the issue of whether alcohol abuse specifically causes a particular constellation of birth defects is difficult to achieve in human research; determining the threshold of these effects is even more difficult

4.4.5 Individual and social factors

Previous studies have left many questions unanswered about the epidemiology of FAS. Epidemiological, clinical and laboratory studies all indicate that major risk factors for FAS are associated with the mother's individual characteristics and her social milieu. Specific traits such as advancing maternal age, high gravidity and parity, early age at onset of regular drinking, length of drinking career and quantity, frequency and timing of maternal drinking during gestation partially explain the prevalence of FAS (Croxford et al, 2002; May, 1995; Abel and Hannigan, 1995; Pierog et al, 1979). Furthermore, socio-economic status is a major risk factor (May et al, 2000; Abel, 1995; Bingol et al, 1987).

There are several well-known characteristics associated with drinking among women. Caucasian women are more likely to drink prior to and during early pregnancy than African-American women (Streissguth et al, 1991a), but heavy drinking during pregnancy tends to be more common among African-Americans and Native Americans (Russel, 1989; Day et al, 1993; Faden et al, 1994). Marital status is also related to drinking behaviour, with unmarried women more likely to drink than married women (Serdula et al, 1991). Specifically with regard to pregnancy, drinking in early pregnancy tends to occur more commonly among less educated women (Streissguth et al, 1991b). Older pregnant women are more likely to drink than younger pregnant women (Faden et al, 1994; National Institute on Drug Abuse, 1994), and women who are heavy smokers are often heavy drinkers as well (Serdula et al, 1991; Faden et al, 1994).

Heavy drinkers are more likely to associate with men who are also heavy drinkers or use illicit drugs (Day et al, 1993), and are less likely to seek and obtain prenatal care (Day et al, 1993; Faden et al, 1994). All these factors can interact with alcohol to affect fetal development (Abel and Hannigan, 1996). In addition, there are numerous other predictive factors related to drinking behaviour which become stronger with higher levels of alcohol consumption, such as unemployment, divorce/separation, depression, anxiety, low self-esteem, eating disorders and abusive spousal relationships (Gomberg and Nirenberg, 1993; Galanter 1996; Wilsnack 1995a, b). These interpersonal and socio-cultural factors are relevant for identifying women potentially at risk for abusive drinking.

In a study comparing over 23 countries (including UK, USA, Canada, France, Germany and Japan), in terms of their average annual growth rate in the amount of alcohol consumed per person, South Africa was ranked highest with a growth rate of 2%. From 1978-1994 there was a 150% increase in the per capita consumption of alcoholic beverages in South Africa. In South Africa, certain groups are particularly at risk for alcohol-related problems: males of all races, African people in townships or informal settlements, workers in certain occupations, young people of all races (Parry, 1999). The risk factors include poverty, urbanisation, boredom and community perceptions and peer pressure (males and teenagers being more susceptible).

More than 30% of women in the Western Cape reported risky drinking (> 3 drinks per day) during the weekends (Parry, 1999). Viljoen et al (2002) and Croxford and Viljoen (1999) found a high prevalence of alcohol and tobacco use among pregnant women in the poorer communities of the Western Cape. Nearly a quarter of the sample reported alcohol intake sufficient to put their unborn child at high risk for FAS. These heavy drinkers followed a pattern of binge drinking over weekends and showed a marked preference for beer. Combined alcohol and tobacco use was reported in 30% of the sample.

4.4.6   Genetic susceptibilities

Although it is clear that ethanol is directly or indirectly responsible for the features of FAS, it is noteworthy that there may also be a genetic influence. Studies in animals, using inbred strains, have likewise indicated that genetic differences can affect vulnerability to the effects of alcohol exposure during pregnancy (Chernoff, 1980). Chernoff (1980) has suggested that the incidence of fetal malformations in mice may be related to the animal's capacity to metabolize alcohol. Fetal abnormalities and maternal blood alcohol levels were inversely proportional to maternal alcohol dehydrogenase activity.

On the other hand, induction of the microsomal ethanol-oxidizing system was associated with increased fetal abnormalities. Randall and Taylor (1979) found that the blood ethanol level of the mother is a more important variable than the amount of ethanol actually ingested. Such studies suggest that a genetic predisposition in humans, possibly related to the ability to metabolize ethanol, could be related to the teratogenic effects of alcohol consumption during pregnancy.

Reports on human twins with different severities of alcohol related birth defects (ARBDs) (Christoffel and Salafsky, 1975) implies a genetic difference in fetal susceptibility to alcohol. Whereas women may differ genetically in terms of enzyme polymorphisms they may have for metabolising alcohol, this would not explain the differential susceptibility of twins to maternal alcohol consumption, although differences in metabolic activity may explain why some offspring of women who drank heavily during pregnancy are severely affected while others are not. In respect of the latter possibility, Sokol et al (1986) compared 25 cases of FAS with 50 non-FAS control infants in a "synthetic" case-control study to examine possible factors related to FAS. They found that in addition to drinking more, the mothers of the FAS cases were characterised by increased age, increased frequency of black race, higher gravidity and higher parity. In the presence of four factors: number of drinking days, Michigan Alcoholism Screening Test (MAST) positivity, parity and race – the chance of having a FAS-affected offspring rose to 82.5%.

In addition to maternal and fetal genetic variables, the question of mutagenesis, or an acquired genetic defect resulting from prenatal ethanol exposure has been raised (Church and Holloway, 1983). The possibility of an acquired mutagenesis has implications for understanding the genetics of alcoholism. Recent evidence concurs that genetic background is an important modulator of alcohol's effects on the developing fetus and results also suggest that embryos have a varying capacity to repair and recover from earlier neural crest losses (Cavieres and Smith, 2000). Heaton et al (1999) found that the cell death repressor gene Bc1-2 can protect neurons from ethanol neurotoxicity and that modulation of cell death effector or repressor gene products may play a significant role in the development of ethanol neurotoxicity.

4.5   Risk factors

While alcohol is clearly the etiological cause of FAS, Abel and Hannigan (1996), believe that permissive and provocative factors effect its expression. By "permissive", they refer to predisposing behavioural, social and environmental factors, such as patterns of alcohol consumption, socio-economic status and poor nutrition, that initiate "provocative" conditions that contribute to FAS. Provocative factors are those biological conditions that create an internal milieu that increases fetal vulnerability to alcohol at a cellular level.

4.5.1   Permissive factors

Clinical, epidemiological and experimental studies consistently point to permissive factors as contributing to the occurrence of FAS. The three major permissive risk factors in FAS are the pattern of alcohol consumption, poverty and smoking behaviour.

4.5.1.1 Pattern of alcohol consumption

Experiments in neonatal rats have found that the threshold blood alcohol level (BAL) for abnormal brain development is about 150 mg/dl (Pierce and West, 1986, Bonthius et al, 1988). This BAL would result from ingestion of about 7 "drinks" in a 2 hour period by a 63.5kg woman.

Since exceeding the critical threshold depends not so much on alcohol consumption as on the way it is consumed, teratogenicity is dependent on patterns of consumption. Whereas the average consumption per week might be the same, the higher BALs associated with weekend drinking compared with lower sustained drinking would be a risk factor for FAS (Pierce and West, 1986, Bonthius et al, 1988). Summarising drinking patterns in terms of drinks or amount of absolute alcohol per week during pregnancy is not meaningful, given that BAL is a critical factor; a much more meaningful measure is consumption during drinking episodes. Since peak BAL is a key risk factor for FAS, group differences in drinking behaviour may explain group differences in the incidence of FAS.

In general, there are stronger relationships between indicators of problem drinking and deleterious effects in offspring than between maternal alcohol consumption levels and offspring outcomes (Majewski, 1981; Russel et al, 1991). A positive Michigan Alcohol Screening Test (MAST) score, indicative of problem drinking and, by inference, high levels of drinking, is a major predicator for FAS (Sokol et al, 1986) and the main factor contributing to formulas relating low levels of alcohol consumption to adverse fetal outcome (Ernhart et al, 1985).

Racial differences in susceptibility to alcohol have been proposed as the reason for the higher incidence in FAS among African-Americans compared with Caucasians (Sokol et al, 1986, 1989). Although some researchers have found that individuals may have some undefined fetal genotype that may render them more susceptible to alcohol's teratogenic effects (Christoffel and Salafsky, 1975; Sanatolaya et al, 1978), Abel and Hannigan (1996) were unable to show any biological conditions causing the large disparities in the incidence of FAS among racial groups.

On the other hand there are data indicating racial differences in drinking patterns. For example, African-American alcoholic women drink more heavily on week-ends compared with Caucasian alcoholic women, who are more likely to drink constantly (Dawkins and Harper, 1983). Epidemiological data suggesting that African–American women have a lower threshold for FAS than Caucasians are based entirely on self-report data, and are subject to underestimation, especially among problem drinkers (Ernhart et al, 1988).

In a recent Western Cape study, women reported most drinking as binge drinking and most alcohol was purchased on weekends because this was frequently the only time the women had the means to purchase it (May et al, 2000).

4.5.1.2 Poverty

A second major permissive factor contributing to FAS is poverty, which is a global indicator for poor maternal nutrition and health and increased stress (marital instability, unemployment, decreased access to prenatal care) any of which can independently affect pregnancy adversely (Feinleib, 1989). The poor nutritional status associated with alcohol consumption may act synergistically with alcohol on the fetus (Dreosti, 1993). Despite the use of animal models to unravel the role of nutritional factors in ARBDs, questions remain about the role of secondary nutritional effects (like reduced protein levels) in fetal growth and development (Wunderlich et al, 1979, Kennedy, 1984). Poverty is associated with stress, which results in adrenal release of corticosterone and epinephrine, and poor living conditions, which increases exposure to environmental pollutants. Prenatal stress can produce many effects associated with perinatal alcohol exposure (Pasamanick and Lilienfield, 1955; Weller et al, 1988; Ernhart 1992; Edwards et al, 1994).

4.5.1.3 Smoking

The combination of alcohol ingestion and smoking is of particular interest because a large per centage of alcoholics are also smokers. It is uncommon to find women who abuse alcohol only. Statistical techniques (like multiple regression) can be used to separate influences of individual factors in complex systems, but important interactive effects between alcohol and other drugs can occur, leading to additive and/or synergistic teratogenic effects.

The combination of maternal alcohol consumption and cigarette smoking increases the risk for low birth weight, smaller head circumference, learning difficulties, and febrile convulsions compared to alcohol abuse alone (Wright et al, 1983; Halmesmaki, 1988; Cassano et al, 1990; Olsen et al, 1991; Cornelius, Goldschmidt and Day, 2002; Knopik et al, 2002). However, while prenatal nicotine and ethanol co-exposure act as significant early developmental teratogens, Birru, Vaglenova and Breese (2002) have hypothesized that a competitive effect of these drugs for neuronal targets may lead to adaptive and compensatory consequences in the systems responsible for learning and memory behaviours.

In addition, recent data have suggested that smoking may play a causal role in the association between heavy drinking and smoking by reducing the sensitivity to the intoxicating effects of alcohol (Rohrbaugh et al, 2002). Africa's tobacco consumption levels are similar to those in the USA in the 1920s and very few women smoke. In 1990, about 580 cigarettes per adult per year were sold in Africa, compared with the global average of 1 660. However, in middle-income countries like South Africa and Mauritius, smoking-related diseases are a common and important health problem. About 47% of men and 12% of women currently smoke in South Africa. In the Western Cape, lung cancer death rates increased by 100% among men and 300% among women over the period 1968-1988. Prenatal surveys in and around the Western Cape reported that 42.8% drank alcohol and 45.6% smoked during pregnancy (Croxford and Viljoen, 1999).

4.5.2 Concluding remarks to risk factor involvement

Prenatal alcohol exposure can result in FAS and other physical and behavioural anomalies. Alcohol is clearly an aetiological factor in FAS/ARBDs. However, permissive factors must also contribute to the occurrence of these anomalies because only a small proportion of children exposed prenatally to even high levels of alcohol are born with FAS. Among these permissive factors are patterns of alcohol consumption, low socio-economic status, smoking and as yet unspecified maternal constitutional factors. Several possible mechanisms have been proposed for alcohol's teratogenic effects and it is unlikely that there is any single causative process.

Defining alcohol teratogenicity in the human with the precision desirable for offering patients rational advice and for clinical risk assessment and management is difficult. First the drinking of alcoholic beverages is a complex human behaviour, difficult to ascertain accurately or to represent quantitatively. Second, the pattern of ARBDs in individuals is not invariant. Different collections of anomalies are a result of variations in times of exposure during gestation, number and patterning of drinks consumed, additional risk factors such as poverty, diet and smoking, and individual genotypic susceptibilities. In the light of all these differences in defining a valid "threshold", it is tempting to advise patients that the only safe course to follow with regard to drinking during pregnancy is that of total abstention.

But this advice reflects as much the clinician's frustration as it does a concern for protecting every pregnancy from the potentially harmful effects of alcohol. Under these circumstances it is very likely that the "science" of medicine may have to recognise the "art" that enables an experienced physician to counsel one patient differently from another, depending on the physician's awareness of the patient's risk factors for ARBDs and her past and current drinking behaviour.

4.6 Clinical features and diagnosis of the fetal alcohol syndrome

In numerous case reports and in all the larger studies cited in Section 4.2, a similar and recognizable pattern of mental and growth retardation, facial dysmorphias and malformations in children of alcohol addicted mothers was observed. Jones et al (1973) defined three major categories of abnormality in children with FAS:

(i) Slow growth both before and after birth, involving height, weight and head circumference

(ii) Deficient intellectual and social performance and muscular co-ordination

(iii) A consistent pattern of minor structural anomalies of the face, together with more variable involvement of the limbs and heart.

With some modification, these criteria still form the basis for the clinical diagnosis of FAS. No single symptom is specific for FAS. Evidence of abnormality in all three areas is enough to exclude most other birth defect syndromes. The ascertainment of cases and the clinical description vary from author to author. Some studies reported only on severely affected children, while in others only milder forms were examined. In the latter, internal malformations are rare or lacking. Therefore, a direct comparison of the frequencies of clinical symptoms in the different studies is not meaningful. In summary the clinical features of FAS are: (i) marked prenatal and postnatal growth deficiency (ii) CNS dysfunction (iii) facial characteristics (iv) major organ system malformation. The general findings include retardation of growth (microencephaly and hypoplastic genitalia), height and weight.

4.6.1 Growth Abnormalities

Children with FAS commonly have a low birth weight and remain small for their age (Jones et al, 1974). The intrauterine growth retardation results in weights, lengths and head circumferences below the tenth per centile. The occurrence of compensatory (catch-up) growth had been debated. Jones et al (1973) reported the absence of linear and head circumference compensatory growth, and Hanson et al (1976) concurred with this view.

Jackson and Hussain (1990) showed that compensatory growth in stature, weight and head circumference can take place in some circumstances. They also showed that there was a delay in bone age. This was in disagreement with Clarren and Smith (1978) who stated that bone age is usually normal in FAS. Hanson et al (1976) reported that postnatal weight gain was poor, becoming underweight for length; however, the Jackson and Hussain (1990) study suggested that there is a long-term tendency to gain weight more than stature. Current evidence suggests that girls with FAS in adolescence may become obese, but all othe parameters remain below the 10th centile throughout life (Streissguth et al, 1991b). Slow growth in head circumference indicates slow brain growth. Microcephaly, resulting from abnormal glial migrations and diminished white matter is often the earliest sign of CNS involvement.

4.6.2 Physical abnormalities

The pattern of physical abnormalities in FAS includes subtle abnormalities in the face, an increasing frequency of malformations, congenital cardiac defects (especially atrial and ventricular septal defects), anomalies of the urinary tract and genitals. Abnormalities of the limbs and joints include deformities in the small joints of the hand.
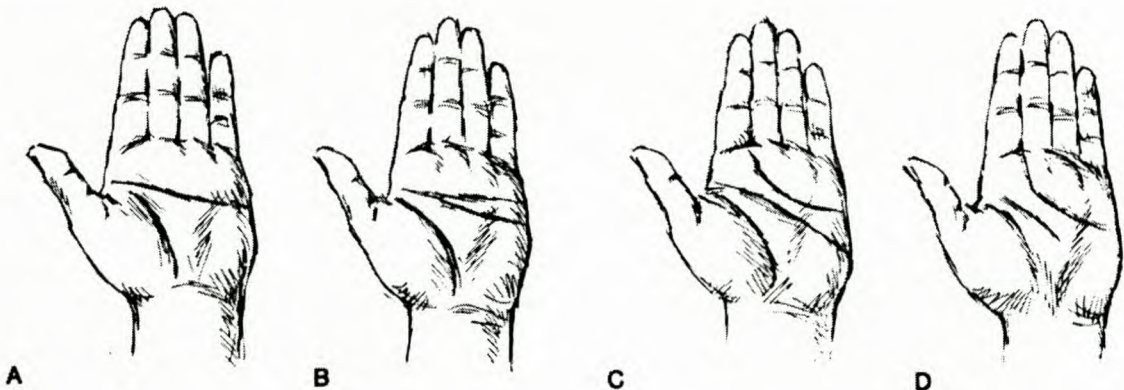
Figure 11: Normal variation in palmar crease patterns.
(A) Single transverse distal palmar crease. (B) Fused distal and proximal transverse creases. (C) Sydney line (extension of the proximal transverse crease to the extreme medial edge of the palm. (D) Abrupt angulation of the distal transverse crease, existing between the index and middle fingers (hockeystick crease). Adapted from Aase, 1990.

In the hands, palmar crease patterns appear to be altered (Popich and Smith, 1970). Figure 11 shows the normal variation in palmar crease patterns. In FAS children, a hockey stick crease – where the distal transverse crease runs quite horizontally across the postaxial palm and then abruptly angles distally to exit between the index and middle finger is often seen (Figure 12).

A single transverse crease (simian crease) is seen unilaterally in 4% of normal population and bilaterally in about 1%. Its only implication is that the second and third metacarpal bones may be slightly shorter than usual and closer in length than the fourth and fifth. This will slightly alter the relative planes of flexion of the fingers and require only a single palmar flexion crease to accommodate them.

The common malformations of the fingers include clinodactyly and camptodactyly, as well as incomplete rotation of the elbow. Clinodactyly (Figure 13) is the curving of a finger to one side, usually toward the midline, in the plane of the palm. Involvement of the fifth finger is most common and is caused by varying degrees of hypoplasia of the middle phalanx, which changes the planes of joint movement in the finger. Camptodactyly occurs when the proximal interphalangeal joints are held in a partially flexed position and cannot be straightened, sometimes with thickened connective tissue on the volar surface. The fifth finger is most commonly affected, but others may be involved as well, usually bilaterally and symmetrically.

Figure 12: Hockey stick crease

Figure 13: Short 5th fingers and clinodactyly

Table 2: Facial Characteristics in fetal alcohol syndrome		
	Characteristic facial feature	**Associated features**
Eyes	Short palpebral fissures	Myopia, clinical microphthalmia, ptosis, strabismus
Nose	Short and upturned in early childhood, hypoplastic philtrum	Flat nasal bridge, epicanthal folds
Maxilla	Flattened	
Mouth	Thinned upper vermillion of lip	Prominent lateral palatine ridges, cleft lip with/without cleft palate, small teeth
Mandible		Retrognathia in infancy, micrognathia or relative prognathia in adolescents
Ears		Posterior rotation, abnormal concha

Oral and facial findings have become the most critical clinical variable in recognizing and diagnosing FAS. Although the overall facial appearance is the single most helpful clue in the diagnosis of FAS (Table 2), each specific facial abnormality represents a minor anomaly or a variant of normal and thus may escape notice unless specifically looked for by the diagnostician. A unique clustering of morphogenic abnormalities produces the characteristic appearance. The following facial features are characteristic: a flat mid face, short palpebral fissures, a flat nasal bridge, with an upturned nasal tip and hypoplastic philtrum with a thin upper vermillion border (Figure 14) (Clarren, 1982; Wood, 1977; Clarren 1981). One of the most distinctive and consistent signs of FAS is found in the eyelids. Affected children often appear to have widely spaced eyes (Figure 14), although measurements reveal that the eyes are normally situated. This discrepancy is caused by the short palpebral fissures (eye openings). That is, the distance between the inner and outer corners of each eye is shortened, making the eyes appear smaller and further apart than normal. Drooping of one or both eyelids is also frequently seen in FAS (Figure 2).

Figure 14: Typical FAS facial features: widely spaced eyes, long smooth philtrum, thin upper lip, upturned nose, rounded forehead (author's photograph)

Slow growth in the centre of the face is another hallmark of FAS (Johnson et al, 1996). This slow growth produces a hypoplastic midface; the area between the eyes and mouth may seem flattened or depressed and the bridge of the nose is often low. Riekman (1984) found that the flat midface is not skeletal in origin but is rather a result of the short palpebral fissures, a broad nasal base and the absence of a philtrum. Slow nasal growth in the outward direction, away from the plane of the face, may leave a crescent-shaped fold of the skin covering the inner corner of the eye. If the nose grows slowly in length, from its attachment between the eyebrows to the tip, the nostrils often point forward as well as downward.

Another subtle but characteristic facial feature in children with FAS is found in the philtrum, the area between the nose and the mouth. This region is normally characterised by a vertical midline groove, bordered by two vertical ridges of skin. Where the groove meets the vermillion margin of the upper lip, an indentation is seen, producing a "cupid's bow" configuration of the lip. The classic FAS face shows a long, smooth philtrum without the ridges and with a smoothly arched upper lip margin (Figure 15).

Figure 15: Long smooth philtrum

Other facial anomalies may also be present and these may vary in degree of severity, consisting of micrognathia (Clarren and Smith, 1978), occasional cleft lip and/or palate, and small teeth with faulty enamel (Wood and Turner, 1981).

4.6.3   Dental development

There are no studies that have examined the issue of dental development in FAS children: whether or not there is a delay in development and/or eruption in FAS children. The concept of physiological age is based upon the degree of maturation of different tissue systems. Several biological ages have been developed: skeletal age, morphological age, secondary sex character age and dental age. These criteria can be applied separately or together to assess the degree of physiological maturity of the growing child (Demirjian, Goldstein and Tanner, 1973). Dental age is of particular interest to the orthodontist planning the treatment of different types of malocclusions in relation to maxillo-facial growth. In paediatric endocrinopathies, the diagnosis and results of treatment may sometimes be better evaluated if dental age is assessed together with other maturity indicators. Until recently, clinical eruption has been the only criterion used for dental maturity or dental age. The timing and eruption of permanent teeth in relation to race and gender have been studied by several authors (Knott and Meredith, 1966; Houpt et al, 1967).

Gingival emergence, which is often erroneously called eruption, represents only one stage in the continuous process of dental eruption or migration to the occlusal level. Emergence may be influenced by local factors: ankylosis, early or delayed exfoliation of the deciduous tooth, impaction or crowding of the permanent tooth (McDonald, 1969). In contrast, Sapoka and Demirjian (1971) found that the formation rate of permanent teeth is not affected by the premature loss of deciduous teeth. Furthermore, if clinical emergence is used as a criterion for dental age assessment, it can only be applied up to the age of 30 months (completion of deciduous dentition) and after the age of 6 years (eruption of the first permanent molar) until the age of 14 years.

Different definitions of formation and "eruption" have been used. Visible emergence usually occurs when root formation is about three quarters completed, but quite large departures from this rule have been observed. Furthermore, the association between emergence and formation varies between different teeth (Demirjian, 1986).

Liliequist and Lundberg (1971) investigated a scoring system for maturity and Fanning and Brown (1971) used a multivariate analysis approach. These studies have led to the conclusion that tooth formation is a more reliable indicator of dental maturity than gingival emergence or "eruption". In order to study dental formation, different developmental stages have been defined by several authors (Nolla 1960; Fanning 1961; Moorrees et al, 1963). These stages have usually been marked by recognizable tooth shapes, from the beginning of calcification through to the final mature form. Useful stages must be easily recognizable, and such that a tooth always passes through the same stages in every individual. Since the stages are indicators of maturity and not of size they cannot be defined by any absolute length measurements. Demirjian et al (1973) developed a method of estimating overall dental maturity or dental age, by a quantity based on the stages observed in each tooth. This problem occurs in defining any other kind of maturity, for example, skeletal maturity based on the hands and wrist. Two general approaches have been advocated. One of these, the Atlas approach (Greulich and Pyle, 1959) sets out a typical "profile" of stages at each of a series of ages over the age range being studied.

Any new set of ratings is then compared with these profiles until the best matching one is found, and the corresponding age then becomes the estimate for skeletal or dental age. The other approach (Tanner, Whitehouse and Healy, 1962) is to give each bone or tooth a score depending on its stage. The scores on all the teeth are then added together to give a total maturity score which can be converted directly to dental age using an appropriate table of standards. Demirjian et al (1973) adopted the method described by Tanner et al (1973) for their study.

The development of the dentition is an integral part of craniofacial growth even though it is little associated with other maturational processes. Dental maturation has been shown to be mildly but consistently delayed in small for gestational age children, but to a lesser degree than skeletal maturation (Garn et al, 1965; Keller et al, 1970) and the degree of reduction in stature exceeds the degree of delay in tooth formation (Ito et al, 1993). To the best of the author's knowledge there has been no previous study examining dental maturation in FAS children.

4.6.4   Intellectual and behavioural abnormalities

The first reports of FAS documented delayed intellectual and behavioural development in all children studied. A variety of different learning and behaviour problems appears at different ages. Children with FAS tend to be handicapped, with an IQ between 60 and 75, and frequently experience additional difficulties with eating, motor co-ordination and speech. The child's natural behavioural progression seems to be one of irritability in infancy, hyperactivity with a short attention span and poor short-term memory during childhood and decreasing sociability as adults (Landesman-Dwyer, Ragozin and Little, 1981).

4.7   Concluding remarks to Chapter 4

In summary, the current criteria for the diagnosis of FAS depend on recognition of a consistent pattern of minor, often subtle physical anomalies; generalised but disproportionate growth retardation; and non-specific developmental and behavioural aberration. Some of these characteristics change with time, and their degrees of severity may vary among individuals. Underdiagnosis usually occurs when a complete pattern of abnormalities cannot be substantiated, often because of the patient's age, racial background or familial characteristics. Overdiagnosis may result from too much emphasis on maternal drinking history, the presence of non-specific abnormalities or failure to recognize a different but similar congenital disorder. Some of these problems are discussed briefly below.

Changes in features with age: Recently, it has become clear that many of the critical diagnostic features of FAS change as the child grows older. The diagnosis is the most difficult in newborns and adults. For example, relative mid-facial hypoplasia and a short nose with a low nasal bridge are normal features of many newborn babies; however, if these features persist into the second year of life and beyond, they may be diagnostically helpful. Conversely, continued slow growth of the face and nose through adolescence compensates for the earlier midfacial hypoplasia and obscures the typical facial appearance of individuals with FAS as they pass into adult life.

Influence of racial and familial traits: Because most of the clinical features of FAS are not discrete abnormalities but fall somewhere along a continuum, it is important to consider the normal variation of features in the patient's racial group or family. For example, a moderate degree of midfacial hypoplasia and a flattened nasal bridge are normal characteristics of many of the Cape Coloured groups and should be considered during examination of children from this group. Height growth, some facial features and even creases on the palms may be influenced so much by hereditary factors that the signs of FAS are obscured or mimicked.

Chapter 5: Research Design and Methodology

5.1   Introduction, aim and objectives

This study arose out of the need for epidemiological data and out of academic interest. Despite the wealth of information related to the medical aspects of FAS, it was clear from a review of the literature that there is a paucity of literature in the dental field. In order to initiate this study, meetings and liaison with experts in the field, groups and established structures that work with FAS were held. These included the Foundation for Alcohol-related Research (FARR) at the University of Cape Town, the Departments of Paediatrics and Community Health at both the University of Stellenbosch and Cape Town, the Medical Research Council's Alcohol Abuse Programme, and the Fetal Alcohol Syndrome Support Association.

This chapter presents the aims and objectives of the study, the study design, sampling, matching and inclusion criteria. In addition, the survey method, development and piloting of the data capture sheet, data entry and method of analysis are described.

In South Africa, medical research into FAS has so far been limited to a few reports, none of which are oral or dental related (Beyers and Moosa, 1978; Jaffer, Nelson and Beighton, 1981; Palmer, 1985; van Rensburg, 1985). No epidemiological data have been assimilated on the subject. Anecdotal experiences of many paediatricians and medical practitioners, particularly in the Western Cape, are that FAS is a common diagnosis and cause of mental retardation. Audits of the genetic clinics and outreach programmes in the Western Cape over the past 5 years have consistently revealed a diagnosis of FAS in 1 in 10 referrals. Similarly, in genetic screening programmes of several institutions for the mentally handicapped, FAS was confirmed as the primary diagnosis in 10-25% of children in the school-going age group.

This study has grown out of several epidemiological, prenatal and infant studies in areas of the Western Cape that are being currently undertaken by the Foundation for Alcohol Related Research (FARR). Preliminary data from studies in Wellington have confirmed that a significant proportion of school-entry children have FAS. The prevalence of FAS in this community exceeds that for Down syndrome by a factor of 30 times. The frequency of FAS in the Western Cape is the highest reported anywhere in the world (Viljoen, 1999). With this background, and the paucity of literature related to dentistry, the aim of this study was to determine the craniofacial and oral manifestations of FAS in a sample of school-going children in the Western Cape.

The objectives of the study were to determine:
*the oral hygiene status
*the dental caries status
**craniofacial measurements and relationships (total head size, head-body weight ratio, upper or middle craniofacial asymmetry)
**facial anomalies: microcephaly, palpebral fissure length, nose, midface, philtrum, vermillion
*dental anomalies: fused teeth, congenitally absent, microdontia, hypodontia, anodontia, enamel anomalies
*dental development in relation to chronological age
*skeletal development in relation to chronological age
*the prevalence of oral mucosal lesions
**the presence cleft lips, cleft palates
**occlusal and other orthodontic parameters: cuspid relationships, Angles relationships, crossbites (posterior, anterior, single, multiple tooth)
**cephalometric parameters: cranial base, midface, mandible, incisor relationships and the integumental profile
**minor anomalies: palmar creases, dermal patterns, clinodactyly, camptodactyly

* **represents new data**
** **represents more extensive data, case-control study**

5.2   Study design

This study was a descriptive, case-control, cross-sectional study using a random cluster sampling method. The study consisted of thorough oral examination, impressions for study casts, and radiographic assessment. In addition, a structured data capture sheet was used to collect data. A cross-sectional study design was chosen as it studies outcomes without prior knowledge of predictors (Newman et al, 1988). Data were collected on a single occasion to describe and provide prevalence data for outcome and predictor variables and identify associations between the two. Large amounts of data can be collected and networks and links can be examined quickly to reveal cross-sectional associations.

5.3   Selection of study population

The population included all children in Grade 1 from 12 of the 13 primary schools in Wellington. These children are similar in social and economic character to many others in the Western Cape. The Wellington community had a population of 45225 (35364 urban and 9861 rural) in 1996, the vast majority of whom are classified in terms of the previous Population Registration Act in South Africa (according to the population/ethnic group) as "Coloured". The sample size was based on an estimated prevalence of 10% for FAS and a confidence limit of 3-15%. The precision of an estimate if affected by sample size and study design. In this study the sample size was determined so as to ensure precision and validity. The sampling strategy selected when the study was designed also improved the degree of precision obtained. A control group was required to provide a yardstick of the background disease in the population and blinding was used to reduce measurement bias (Sitthi-amorn and Poshyachinda, 1993).

5.4   **Inclusion criteria:** Children diagnosed with fetal alcohol syndrome

5.5   Diagnosis and screening of FAS cases

The diagnosis, by active case ascertainment, of the FAS cases was done as part of an initiative funded by United States and South African sources to establish the prevalence of FAS in the Wellington community in the Western Cape. FAS had been diagnosed previously in South Africa but not in an explicit epidemiologic study.

A study committee of the Institute of Medicine recently endorsed active case ascertainment as the most accurate method for epidemiological studies (Institute of Medicine, 1996). In the active case ascertainment survey, there was no attempt made to aggregate the individual traits of prenatal alcohol exposure into lesser, nonsyndrome diagnoses commonly referred to as fetal alcohol effects, alcohol-related birth defects, or alcohol-related neurodevelopmental deficits. Only fetal alcohol syndrome (or not fetal alcohol syndrome), the most accurate and rigorous diagnosis, was used (May et al, 2000). Specific FAS diagnostic components of the Institute of Medicine (1996) were used for the initial screening: (i) facial and other dysmorphology, (ii) diminished structural growth for age, (ii) developmental (intelligence and social skills) delay, and, when possible (iv) confirmation of maternal alcohol consumption. Data for each of these components were independently collected, quantified, and analysed.

5.5.1   Establishing a 2-tier screening through preliminary physical and dysmorphology assessment (May et al, 2000).

Dysmorphology, growth, and developmental data for children were collected by means of a 2-tier screening method after normative data were assessed for this particular population. Four 2-person teams, 1 expert dysmorphologist and 1 South African physician trained in FAS diagnosis worked independently but simultaneously and used standardized assessment criteria to examine all children in sub-A (first-grade) classrooms. The author was part of the group who was trained and calibrated in FAS diagnosis. Twelve of the 13 elementary schools in the community (n=992 sub-A children) were assessed. The one school consisting of 80 children refused to participate.

The low mobility of the local population ensures that the vast majority of the study children were born locally. Children from the community who were in special schools for the developmentally delayed were also examined. Two cases of FAS from this group were confirmed via diagnostic methods similar to those described below.

The screening of schoolchildren proceeded as follows. First, a complete dysmorphology examination was given to each of the initial 406 schoolchildren from classrooms in 6 of the rural and urban schools to gauge both local normative growth parameters and possible FAS dysmorphology relative to US National Centre for Health Statistics charts. Second, data for these 406 children were analysed. All the children with suspected classic FAS had height, weight and occipitofrontal circumference measurements below the 10th centile for 1 of the 3 measures.

Third, with local parameters assessed, cut-off points were set for implementing the 2-tier screening system. Fourth, all of the 586 children in sub-A classrooms in the remaining 6 schools received tier I screening (height, weight, and occipitofrontal circumference). Children whose measurements were below the 10th centile on occipitofrontal circumference or on both height and weight were referred for the complete examination (tier II) by the dysmorphology teams. Finally 220 of the remaining children met these criteria and were referred for complete examinations. Therefore, 626 children (63%) received full dysmorphology examinations.

Every child receiving the complete screen (tier II) was examined by 2 of the physician teams. Each 2-member team examined and measured the child's occipitofrontal circumference, palpebral fissure length, philtrum length, inner and outer canthal distance, and other indicators such as abnormalities in joints, heart function, and palmar creases. Findings were recorded on child data forms, and physicians in each team verified each other's finding. All physicians were 'blinded' from any prior knowledge of the child or mother. Once seen by 1 team, the child was directed to another 'blinded' team who repeated the examination and measurements as a reliability check.

Mean differences between dysmorphologists' measurements for the first 25 children were checked and were insignificant for key measures: inner canthal distance (0.22 cm) interpupillary distance (0.29 cm), and palpebral fissure length (0.04 cm). Interrater reliability was later checked for 194 matched pairs with the square root of the Pearson product moment correlation (r). Results were 0.91 for inner canthal distance, 0.85 for interpupillary distance, and 0.84 for philtrum measurements.

5.5.2   Complete diagnostic sequence

After the dysmorphology examination had been completed by 2 teams, a child was assigned a preliminary diagnosis of 'not FAS', 'deferred', or 'FAS' based on the qualified FAS checklist and all clinical findings. Children with a deferred diagnosis had the appearance and some anomalies of FAS with growth delay, but developmental test and maternal interview data were definitely required for a final diagnosis. Only those with the classic FAS phenotype and measurements well below the fifth centile on all measures received a preliminary fetal alcohol syndrome diagnosis. All children with an initial or deferred diagnosis of FAS then underwent developmental testing and prenatal risk assessment.

5.6   Matching

Matching means that controls are selected which have certain characteristics in common with the cases. The characteristics, or variables, are those that would confound the effect of the putative risk factor(s). For this study, it was done on a one-to-one basis where each case was matched for age, gender, school (rural or urban) and class in school. The major advantage of matching is to cancel out the confounding effects of competing variables and, of course, to guarantee the comparability of cases and controls in that regard. It also guarantees that sufficient numbers will be available for the categories of interest (WHO, 1992). Once subjects were identified, a control subject was selected by matching for gender, age and class. Identical developmental and life skills testing were performed on subjects and control subjects with the Griffiths Intelligence and Development Test, a standard test translated into Afrikaans and used throughout South Africa.

5.7   Enhancement of validity of case-control studies

Validity of the study is increased:

(i)   if the cases are representative of all cases in a particular setting;

(ii)   if the controls are similar to the cases in respect of the risk factors other than the study factor;

(iii)   if cases and controls are truly selected independently of exposure status;

(iv)   if the sources of bias are mitigated or at least shown to have affected the results.

5.8   Maternal data

The mothers of the control children became the maternal controls. Structured maternal interviews, investigated drinking patterns before, during and after the index pregnancy, socio-economic status indicators; demographic variables; and other risk factors in the social context. Questions extracted from prenatal risk factor questionnaires from the United States were rewritten for South Africa, locally relevant questions were added, questions were pilot tested with 6 local subjects, and adaptations were made.

The protocols used drinking questions that were designed to elicit accurate reporting of both "free" alcohol supplied as part of their work compensation, as was the custom in this province, and alcoholic beverages purchased. Photographs of standard beer and wine containers sold locally were shown to the respondents so that quantity, frequency, and variability of drinking could be assessed in standard ethanol units. All interviews were administered in Afrikaans in the field by a public health nurse.

5.9   Instrument

A structured data capture sheet was the method chosen for collecting the data in this study (Appendix 1), the purpose of which was to collect factual data for measurement and analysis. The data capture sheet was designed to ensure that it suited the aims and objectives of the study, was clear, simple, unambiguous, minimized potential errors from the researcher and coder and enabled efficient, meaningful analysis of the acquired data.

5.9.1   Development of the data capture sheet

Planning of the data capture sheet began in January 1998. It was developed from discussions with colleagues and experts in the field from Tygerberg Hospital Paediatric and Neurology departments, the Foundation for Alcohol-related Research (FARR) and the Department of Occupational Medicine at the University of Cape Town.

Formulation of the parameters that required to be measured and the generation of a draft data capture sheet took about a year, after a thorough review of the literature and of existing data capture sheets. For the purpose of this study, the data gathered was divided into four sections: (i) demographic - age and gender of the child;(ii) anthropometric measurements - height, weight and head circumference; (iii) clinical examination - ears, eyes, mouth, hands and (iv) intra-oral examination - caries and periodontal status, occlusal relationships, the presence of dentofacial anomalies, bruxism or other oral habits.

5.9.2   Piloting the data capture sheet

A pilot study was carried out in 1998 as part of the DOPSTOP project (Te Water Naude et al, 1998). Fifty four farms in Stellenbosch were randomly selected to participate. A questionnaire was administered to all farm workers over the age of 16 years. Questions related to demography, employment, medical history, health service utilization, women's health and risk behaviour were elicited.

In addition, anthropometric measurements were taken, blood samples were taken for chemistry and serological tests. Forty two per cent of mothers reported drinking during pregnancy and the FAS prevalence was found to be 2.5%.

A pilot study was conducted on the DOPSTOP sample to test the data capture sheets and examination procedures in terms of acceptability, practicability and relevance. This facilitated the identification of problems with the data collection and examination procedures which could then be solved prior to commencement of the main study. The children diagnosed with FAS (n=12) from the DOPSTOP sample, were used to pilot the data capture sheet that was used for this study.

The pilot study was carried out to:

(i)   test the suitability of the method of collecting the data
(ii)   test how long each examination took to complete
(iii)   check the adequacy of the data capture sheet
(iv)   check that all the parameter measurements were clear and unambiguous
(v)   ensure that no major item had been omitted and
(vi)   remove any items that did not yield usable data.

5.9.3   Preparation of the final draft

After the pilot study, irrelevant and problematic items were identified and consequently deleted or reformulated. This resulted in important improvements to the data capture sheet and a general increase in the efficiency of the enquiry. A final draft of the data capture sheet with 18 items was then printed and used for the final study. Its design and construction took 18 months to complete.

5.10   Data collection

5.10.1 Clinical examination and documentation

On the days of the examinations, children with signed, informed consent forms (Appendix 3), were collected from school and bussed to the School of Oral Health Sciences, Tygerberg where the examination took place. The examiner was blind as to whether the child was a case or a control and every child had an identical examination. Children were weighed, and their height and head circumference were measured. To minimise variations in these anthropometric measurements the same researcher measured all the children using the same instruments. They then had photographs (4.14) and radiographs (4.15) taken, followed by a thorough oral examination in a fully equipped dental examination room.

For each child, the following information was recorded on the data capture sheet: Record number, name, date of birth, gender, head circumference, weight and height, enamel opacities, dental fluorosis, plaque index, gingival bleeding index, dentition status and treatment need (DMFS/dmfs), oral mucosal lesions and dentofacial anomalies.

5.10.2   Validity and reliability

The author was the only investigator involved in the gathering and interpretation of the data (except for the intra-oral examination of the hard tissues), thereby assuring the standardised recording of all the information presented. To ensure validation of the data capture sheets, the instrument was subjected to a test-retest procedure (by repeatedly administering the scales to the same sample within a short period).

5.10.3   Anthropometry

Growth assessment is the single measurement that best defines health and nutritional status. Various anthropmetric indices were used to assess growth status. Height, weight and head circumference were measured and assessed against head circumference and weight-versus-height charts. Height-for-age portrays performance in terms of linear growth and essentially measures long-term growth faltering; weight-for-height reflects body proportion or the harmony of growth and weight-for-age represents a convenient synthesis of both linear growth and body proportion (WHO, 1986).

5.10.3.1   Head circumference

The growth of the head is used to gauge the growth of the brain. Head circumference is related to intracranial volume and is used to estimate the rate of brain growth. A head circumference more than two standard deviations below the mean is often associated with neurological impairment and resultant mental retardation (Brandt, 1981; Pryor and Thelander, 1968; O'Connell et al, 1965). A soft measuring tape is used for determining the tangential linear distances taken along the skin surface between two landmarks (eg. head circumference).

In this study, the maximal head circumference was measured where the tape was applied firmly over the glabella and supra-orbital ridges anteriorly and around that part of the occiput posteriorly as described. The child was instructed to stand sideways to the examiner with the head held straight and eyes looking forward. The tape measure was opened and passed around the head from left to right. The free and fixed ends were then transferred to the opposite hands so that the tape passes completely around the head and crosses in front of the examiner. Using the middle finger of the left hand, the examiner then pressed the loose tape to the forehead of the child to determine the most anterior part of the head. Having done this, the tape was pulled tighter and the procedure repeated with the middle finger of the right hand to determine the posterior part of the occiput. Once determined, the tape was pulled firmly to compress the hair and the measurement was read to the last completed centimetre unit.

5.10.3.2 Weight

A calibrated beam balanced scale with non-detachable metric weights was used to weigh each child. The child, lightly dressed with no socks and shoes, was instructed to stand still and straight but not rigid, with feet together. The greater of the two counter weights was then moved until the nearest 10kg point below the child's weight was determined. The smaller counter weight was then moved down the scale to the nearest 100g mark below the point of overbalance was reached and this was recorded as the real weight. This procedure was necessary to determine weight to the last completed unit.

5.10.3.3 Standing height

Height was measured by an anthropometer following standard procedures. The child was instructed to stand with their feet together, and without socks and shoes, with back and heels against the upright bar. The shoulders were checked to be in a relaxed position. A horizontal bar, attached to the vertical bar, was brought down snugly to make contact with the vertex of the child's head and the greatest height measurement was recorded to the nearest centimetre.

5.11 Extra-oral examination

The most common facial anomalies observed in FAS include short palpebral fissures, a smooth philtrum and a thinner upper lip (Jones and Smith, 1973; Hanson, Jones and Smith, 1976). In its most pronounced form, the face in FAS can be an important aid in diagnosis when coupled with other key features such as developmental delay and documented prenatal exposure to alcohol (Jones and Smith, 1973; Hanson, Jones and Smith, 1976; Clarren and Smith, 1978).

Existing methods of diagnosis based on clinical assessment or photographic analysis are effective when the facial disruption is obvious (Astley and Clarren, 1995; 1996). Craniofacial anthropometry is the gold standard in medical measurements (Farkas, 1994, 1996).

There are multiple descriptions of this technique (Farkas 1981; Ward, 1989; Kolar and Salter, 1996; Hall et al, 1987, Moore et al, 2001). In this study the facial features of inner canthal distance, inter pupillary distance, palpebral fissure lengths, philtrum smoothness and upper lip thickness were recorded from the standardized photographs (Astley and Clarren, 1996; Astley et al, 1992; Clarren et al, 1987) (Section 5.15). All measurements were made using sliding calipers. The sliding caliper measures the linear projective distances between two landmarks in the same plane or in neighbouring planes (eg. palpebral fissure length). All measurements were taken by one examiner. To decrease intra-measurement error, each measurement was taken twice, with a third measurement taken if the first and second measurements differed by >2 mm. In instances where the third measurement was required the measurement (first or second) that was closest to the third measurement was used for data analysis.

5.11.1 Eye region

The following measurements were carried out from the standardized photographs (Figure 16):

(i)   Inner-canthal distance (yellow): the distance between the right and left inner canthi (en-en), measured from the innermost corner of each eye, in a straight line.

(ii)   Inter-pupillary distance (blue): distance measured between the centre of each pupil.

(iii)   Palpebral fissure length (green): the distance between the endocanthion and the exocanthion of right and left eyes.

Figure 16: Facial measurements (author's photograph)

5.11.2 Mouth region

The phenotypic expressions of philtrum smoothness and upper lip thinness were recorded on 5-point Likert scales from the standardized photographs (Figure 17).

Figure 17: Philtrum smoothness: area between the upper lip and subnasion, with the focus on presence of midline vertical furrow bordered by two vertical ridges.
5-point Likert ordinal scale as follows: 1 deeply furrowed, 2 somewhat furrowed, 3 mid range, 4 somewhat smooth, 5 very smooth
Upper lip thinness: the upper lip demarcation by its vermillion border.
5-point Likert ordinal scale as follows: 1 very thick, 2 somewhat thick, 3 mid range, 4 somewhat thin, 5 very thin
(Adapted from Astley and Clarren, 1996)

5.12   Intra-oral: hard and soft tissues examination

Examinations were carried out in the dental clinic with the patient seated and normal examination light. The instruments used consisted of a number 4 plane dental mirror, a specially designed WHO community periodontal index probe with a 0.5-mm ball tip and with a black band between 3.5 and 5.5 mm and rings at 8.5 and 11.5 mm from the ball tip, 75mm square four-layered gauze swabs and cotton wool rolls. The marking widths, calibration and tine diameter of the probes used were standardized (Van der Zee, Davies and Newman, 1991).

5.12.1 Enamel opacities

The modified developmental defects of enamel (DDE) index was used to describe any enamel opacities that were present (WHO, 1997). Enamel abnormalities were classified into one of three types on the basis of their appearance. They varied in their extent, position on the tooth surface and distribution within the dentition. The clinical examination was carried out as follows:

Six index teeth (16, 11, 26, 36, 31, 46) were examined on the buccal surfaces only. If any index tooth was missing, the item was not coded. Buccal surfaces, ie. from the incisal edges or the cuspal points to the gingival and from the mesial to the distal embrasure, were inspected visually for defects; areas such as hypoplastic pits were checked with a periodontal probe to confirm diagnosis. Any gross plaque or food deposits were removed and the teeth were examined under wet conditions. If there was any doubt about the presence of an abnormality, the tooth surface was scored "normal" (code 0). Any abnormality that was not readily classified into one of the three basic types was scored as "other defects" (code 4). If more than two thirds of a tooth surface was heavily restored, badly decayed or fractured, it was not examined (code 9).

5.12.1.1 Codes and criteria

0   Normal

1   Demarcated opacity. In enamel of normal thickness and with an intact surface, there was an alteration in the translucency of the enamel, variable in degree. It was demarcated from the adjacent normal enamel with a distinct and clear boundary and was white, creamy, yellow or brown in colour

2   Diffuse opacity. An abnormality altering the translucency of the enamel, variable in degree, and white in colour. There was no clear boundary between the adjacent normal enamel and the opacity could have been linear or patchy or have had a confluent distribution.

3   Hypoplasia. A defect involving the surface of the enamel and associated with a localized reduction in the thickness of the enamel. It occurred in the form of (a) pits – single or multiple, shallow or deep, scattered or in rows arranged horizontally across the tooth surface; (b) grooves – single or multiple, narrow or wide; or (c) partial or complete absence of enamel over a considerable area of dentine. The affected area may have been translucent or opaque.

4   Other defects

5   Demarcated or diffuse opacities

6   Demarcated opacity and hypoplasia

7   Diffuse opacity and hypoplasia

8   All three conditions

9   Not recorded

5.12.2 Dental fluorosis

Fluorotic lesions are usually bilaterally symmetrical and tend to show a horizontal striated pattern across the tooth. The premolars and second molars are most frequently affected, followed by the upper incisors. The mandibular incisors are least affected.

Dean's index criteria was used (Dean, 1942). The recording was made on the basis of the two teeth that were most affected. If the two teeth were not equally affected, the score for the less affected of the two was recorded. When teeth were scored, the examiner started at the higher end of the index i.e. "severe" and each score was eliminated until the present condition was arrived at. If there was any doubt, the lower score was given.

5.12.2.1   Codes and criteria:

0   Normal

1   Questionable. The enamel showed slight aberrations from the translucency of normal enamel, which ranged from a few white flecks to occasional spots.

2   Very mild. Small, opaque, paper-white areas scattered irregularly over the tooth but involved less than 25% of the labial tooth surface.

3   Mild. The white opacity of the enamel of the teeth was more extensive than for code 2, but covered less than 50% of the tooth surface.

4   Moderate. The enamel surfaces of the tooth showed marked wear and brown stain was frequently a disfiguring feature.

5   Severe. The enamel surfaces were badly affected and hypoplasia was so marked that the general form of the tooth was affected. There were pitted and worn areas and brown stains were widespread; the teeth often had a corroded appearance.

8   Excluded (eg. crowned tooth)

9   Not recorded

5.12.3 Plaque index (Pl I)

The plaque index of Silness and Löe (1964) was used to assess the thickness of plaque at the gingival area. It is sensitive to small changes, can be transformed easily to record the presence or absence of plaque and is widely used in epidemiological research (Greene, 1990). It was selected as a precise and reliable index.

The data from this index can also be summarised as the number of surfaces per individual with a given score. Plaque scores were recorded for the distal, buccal, mesial and lingual surfaces of 6 selected teeth. The teeth were air dried and examined visually using an opertaing light in a dental chair, a plane mouth mirror and a periodontal probe. Bacterial plaque on the cervical third of the tooth was evaluated. The probe was used to test the surface when no plaque was visible. The probe was passed across the cervical third of the tooth surface and near the entrance to the sulcus. When no plaque adhered to the probe tip, the area was scored 0. When plaque adhered, a score of 1 was assigned. Plaque on the surface of calculus and on dental restorations in the cervical third were also included in the evaluation.

5.12.3.1 Codes and criteria

0   No plaque

1   A film of plaque adhering to the free gingival margin and the adjacent area of the tooth.

2   Moderate accumulation of soft deposits within the gingival pocket that could be seen with the naked eye or on the tooth and gingival margin.

3   Abundance of soft matter within the gingival pocket and/or on the tooth and the gingival margin.

Scoring Pl I was done for the individual, by adding the scores for each tooth and dividing by 6. The Pl I ranges from 0 to 3 (0 - excellent; 0.1-0.9 - good; 1.0-1.9 - fair; 2.0-3.0 – poor).

5.12.4 Gingival bleeding index (GBI)

Bleeding on gentle probing is an early sign of gingival inflammation and precedes colour changes and enlargement of the gingival tissues. The gingival bleeding index of Carter and Barnes (1974) was used to record the presence or absence of gingival inflammation as determined by bleeding from inter-proximal gingival sulci.

The teeth were dried and examined visually using an operating light, a plane mouth mirror and a periodontal probe. The probe was run along the soft tissue wall near the entrance to the gingival sulcus to evaluate bleeding. Thirty seconds was allowed for re-inspection of an area that did not show bleeding immediately. Bleeding was recorded as present or absent. Bleeding indicates the presence of disease. No attempt was made to quantify the severity of bleeding as "no bleeding" implied healthy gingivae.

The following index teeth were examined:

17 16	11	26 27
47 46	31	36 37

The two molars in each posterior sextant were paired for recording and, if one was missing, no replacement was used. Sextants with fewer than two teeth which were not indicated for extraction, were excluded. If only one tooth was remaining in the sextant, it was included with the recording of the adjacent sextant in the same arch. If no index teeth or tooth was present in a sextant qualifying for examination, all the remaining teeth in the sextant were examined and the highest score was recorded for the sextant. When the central incisors, 11 and 31 were missing, adjacent centrals 21 and 41 were recorded.

5.12.5 Dentition status and treatment need – DMFS/dmfs

The decayed, missing and filled tooth surfaces DMFS (Klein, Palmer and Knutson, 1938) and the dmfs (Gruebbel, 1944) indices were used to determine dental caries experience, past and present, by recording tooth surfaces involved. Third molars were not counted. A systematic approach to the assessment of the dentition status and treatment was adopted using the WHO Guidelines to Oral Health Surveys (1997).

It is common to record the subdivisions separately so that they may be compared independently. This reveals information on the change in the ratio of the components which may be related to such factors as availability and accessibility of treatment. The examination proceeded in an orderly manner from one tooth or tooth space to the adjacent tooth or tooth space. A tooth was considered present in the mouth when any part of it was visible. For the posterior teeth 5 surfaces were examined: buccal, lingual, mesial, distal and occlusal and for the anterior teeth 4 surfaces were evaluated: buccal, lingual, mesial and distal. During the main study, calibration exercises were conducted to monitor and maintain examiner consistency. Individuals were randomly selected for re-examination.

5.12.5.1 Codes and criteria

0   **Sound crown.** A crown was recorded as sound if it showed no evidence of treated or untreated clinical caries. In addition, a crown with the following defects was also coded as sound: white or chalky spots, discoloured or rough spots that were not soft to touch with the metal CPI probe, stained pits or fissures in the enamel that did not have visual signs of undermined enamel, or softening of the floor or walls detectable with a CPI probe, dark, shiny, hard, pitted areas of enamel in a tooth showing signs of moderate to severe fluorosis, lesions that, on the basis of distribution or history, appeared to be due to abrasion.

Sound root. A root was recorded as sound when it was exposed and showed no evidence of treated or untreated clinical caries. (Unexposed roots are coded as 8)

1   **Decayed crown.** Caries was recorded as present when a lesion in a pit or fissure, or on a smooth tooth surface, had an unmistakable cavity, undermined enamel, or a detectable softened floor or wall. A tooth with a temporary filling, or one which is sealed (code 6) but also decayed, was also included in this category. The CPI probe was used to confirm visual evidence of caries on the occlusal, buccal and lingual surfaces. Where any doubt existed, caries was not recorded as being present.

Decayed root. Caries was recorded as present when a lesion felt soft or leathery to probing with the CPI probe. If the root caries was discrete from the crown and required a separate treatment, it was recorded as root caries. For single carious lesions affecting both the crown and the root, the likely site of origin of the lesion was recorded as decayed. When it was not possible to judge the site of origin, both the crown and the root were recorded as decayed.

2   **Filled crown, with decay**. A crown was considered filled, with decay, if it had one or more permanent restorations and one or more areas that were decayed.

Filled root, with decay. A root was considered filled, with decay, if it had one or more permanent restorations and one or more areas that were decayed. For any restoration involving both the crown and the root with secondary caries, the most likely site of the primary lesion was recorded as filled, with decay. When it was not possible to judge the site of origin of the primary carious lesion, both the crown and the root were recorded as filled, with decay.

3   **Filled crown, no decay**. A crown was considered filled, without decay, when one or more permanent restorations were present and there was no caries anywhere on the crown. A tooth that had been crowned because of previous decay was recorded in this category. A tooth that had been crowned for other reasons (e.g. a bridge abutment), was coded as 7.

Filled root, no decay. A root was considered filled, without decay, when one or more permanent restorations were present and there was no caries anywhere on the root. For any restoration involving both crown and root, the most likely site of the primary lesion was recorded as filled.

4   **Missing tooth, as a result of caries**. This code was used for teeth that had been extracted because of caries and was recorded under coronal status. The root status of a tooth that had been scored as missing because of caries was coded as 7 or 9.

5   **Tooth missing, for any other reason**. This code was used for teeth judged to be absent congenitally, or extracted for orthodontic reasons, periodontal disease, trauma, etc. Root status of a tooth that had been as scored a missing because of caries was coded as 7 or 9.

6   **Fissure sealant**. This code was used for teeth in which a fissure sealant had been placed on the occlusal surface. If a tooth with a sealant had decay it was coded as 1.

7   **Bridge abutment, crown, veneer**. This code was used under coronal status to indicate that a tooth formed part of a fixed bridge i.e. is a bridge abutment. It was also used for crowns placed for reasons other than for caries and for veneers or laminate covering the labial surface of a tooth on which there was no evidence of caries or a restoration. Missing teeth replaced by bridge pontics were coded 4 or 5 under coronal status, while root status was scored 9.

8   **Unerupted crown**. This code was used for a tooth space with an unerupted permanent tooth. Teeth scored as unerupted were excluded from all calculation concerning dental caries. This category does not include congenitally missing teeth, or teeth lost as a result of trauma, etc.

T   **Trauma (fracture)**. A crown was scored as fractured when some of its surface was missing as a result of trauma and there was no evidence of caries.

9   **Not recorded**. This code was used for any tooth that could not be examined for any reason. It was used under root status to indicate either that the tooth had been extracted or that calculus was present to such an extent that a root examination was not possible.

5.12.6 Oral mucosa

5.12.6.1 Examination procedure

An examination of the oral mucosa and soft tissues in and around the mouth was made on every subject. Any abnormalities of the mucosa or of the gingiva were recorded on the chart on the data capture sheet (Roed-Petersen and Renstrup, 1969). In addition, a full description of the lesion's size, shape, type and anatomical site was documented. If the cause of the lesion was obvious it was noted.

The examination was thorough and systematic and was performed in the following sequence:

(i)   Labial mucosa and labial sulci (upper and lower)

(ii)   Labial part of the commisures and buccal mucosa (right and left)

(iii)   Tongue (dorsal and ventral surfaces, margins)

(iv)   Alveolar ridges/gingiva (upper and lower)

(v)   Floor of the mouth

(vi)   Hard and soft palate

Two mouth mirrors were used to retract the tissues. The following procedure was used and the following codes were used to record the absence, presence or suspected presence of the condition: The lips were examined with the mouth closed and open. The colour, texture and any surface abnormalities of the vermillion border were noted. The mandible vestibule was examined visually with the mouth partially opened. The colour and any swelling of the vestibular mucosa was observed. The maxillary vestibule and fraenulum with the mouth partially opened was examined. Using the plane mouth mirrors as retractors and the mouth wide open, the entire buccal mucosa extending from the commisures and back to the anterior tonsillar pillar was examined. Any changes in pigmentation, colour, texture and mobility of the mucosa were noted. Alveolar ridges were examined from all sides (bucally, palatally, lingually).

With the tongue at rest and the mouth partially opened the dorsum of the tongue was inspected for any swelling, ulceration, coating or variation in colour or texture. The patient was then asked to protrude the tongue and the examiner noted any abnormality of mobility. The margins of the tongue were inspected with the aid of the mouth mirrors and then the ventral surface was observed. While the tongue was still elevated, the floor of the mouth was inspected for swellings or any other abnormalities. With the mouth wide open and the subject's head tilted backwards, the base of the tongue was gently depressed. The hard palate was inspected first followed by the soft palate. Any mucosal or facial tissues that seemed to be abnormal, as well as the submandibular and cervical lymph nodes, were palpated.

5.12.6.2 Topographical classification of oral mucosa

Table 3: Oral mucosal lesions codes

CODE	CONDITION	CODE	LOCATION
1	No abnormal condition	11	Upper lip
2	Angular cheilitis	12	Lower lip
3	Commisural pits	13	Mucosa of upper lip
4	Traumatic lesions	14	Mucosa of lower lip
5	Geographic tongue	15	Mucosa around corner of mouth on r side
6	Dentoalveolar abscess	16	Mucosa around corner of mouth on l side
7	Herpes labialis	17	Cheek mucosa on r side of patient
8	Fissured tongue	18	Cheek mucosa on l side of patient
9	Ulcerations	19	Mucosa of upper jaw, bet lip/cheek & gum
10	Herpetic gingivostomatitis	20	Mucosa of upper jaw, bet lip/cheek & gum
11	ANUG	21	Mucosa of gums of upper teeth
12	Verruca vulgaris	22	Mucosa of gums of lower teeth
13	Papilloma	23	Top surface of tongue
14	Focal epithelial hyperplasia	24	Sides of tongue
15	Melanotic hyperpigmentation	25	Undersurface of tongue
16	Candidiasis	26	Mucosa bet under surface of tongue & gums of lower teeth
17	Other	27	Mucosa of hard palate
		28	Mucosa of soft palate

Table 4: Criteria used for diagnosis of oral mucosal lesions

Lesion	Criteria
1. White non-adherent lesions	
(a) Candidiasis: acute pseudomembraneous	Creamy white patches, wipeable leaving red patches
(b) Aspirin Burn	White necrotic epithelium; Will slough off/rub off to reveal ulcer underneath
2. White or white/red adherent lesions	
Local aetiology suspected	
(a) Frictional white lesion	Whitish or greyish patch on the mucosa which cannot be rubbed off
(b) Cheek & lip biting	Oral mucosa shows a rough, grey-white, macerated surface with irregular, flaky desquamation. Lesion is located self-infliction by chewing is possible.
Local aetiology not present/known	
(a) Leukoplakia	- A white patch, or plaque, that cannot be wiped off and cannot be characterized clinically or pathologically as any other disease - Varies from small circumscribed areas to extensive lesions involving a large area of the mucosa - Surface appearance is variable; - May be smooth or wrinkled - Smooth-surfaced but traversed by small cracks - Nodular or speckled - Colour can be white, whitish-yellow or grey **Recommended subdivisions:** - *homogeneous*: lesions that are uniformly white with smooth or corrugated surface - *non-homogeneous*: lesions in which part is white and part appears reddened. Three types have been described: - Erythroleukoplakia (erosive leukoplakia) – white lesion that includes red areas - Nodular leukoplakia – lesion with slightly raised white areas (granules or nodules) - Verrucous leukoplakia – exophytic lesion with irregular sharps or blunt projections.
(b) **Candidiasis, chronic hyperplastic**	- Adherent white plaque, may be flat or elevated - May incorporate erythematous areas
(c) **Red lesions**	
Candidiasis, acute atrophic Erythroplakia	- Bright red atrophic patches on mucosa - burning or itching sensation is common, palate is a common location; bright red patches or plaques of mucosa that cannot be characterized clinically or pathologically as any other condition

Lesion	Criteria
3. Perioral conditions (a) Actinic keratosis (actinic elastosis or actinic cheilitis)	- Vermillion border poorly defined (loss of distinction between vermillion border, labial mucosa and skin - Border may have localized crust formation and/or a whitish colour, common in patients with outdoor occupations
(b) Angular cheilitis	- Bilateral folds in the skin of the labial commissures, surface tissue appears wrinkled, fissured or cracked - No tendency to bleeding, although a crusted exudate may be present - Mucosal surface of commissures are usually not involved
4. Tongue lesions **(a) Fissured tongue**	- Shallow or deep fissures on the dorsum of the tongue; most common pattern is a marked central fissure, from which smaller fissures radiate laterally - Food debris may accumulate in fissures and result in inflammation - Often association with geographic tongue
(b) Geographic tongue	- Localized absence of filiform papillae - Affected areas are irregularly shaped - Areas change location over time
(c) Hairy tongue	- Overgrowth of filiform papillae in which they become elongated or thickened - Colour of tongue varies from white to yellow or greenish, but is most commonly brown or black
(d) Median rhomboid glossitis	- Deep red or white ovoid area devoid of tongue papillae - Located in the central dorsum of the tongue near the foramen caecum - Sometimes demarcated from surrounding mucosa by a furrow
(e) Hairy leukoplakia	- Flat white lesion which cannot be rubbed off - Corrugated surface appearance - Usually found on the lateral borders of the tongue
5. Ulcers (a) ANUG	"Punched-out" papillae - Pseudomembranous exudate - Bleeding upon slight palpation - Pain, distinctive oral odour
(b) Herpetic gingivostomatitis (primary herpes)	- Severe gingival inflammation - Whitish, serofibrinous exudate - Vesicles and/or shallow ulcers; pain, malaise, fever
(c) Herpes labialis (secondary herpes)	- Clusters of vesicles or crusts - Found on vermillion border - Duration: less than three weeks - History of recurrence
(d) Recurrent aphthous ulceration	- Well-defined, greyish-white ulcer(s) - Ulcers surrounded by red halo - Pain; duration: 10-21 days; history of recurrence

Lesion	Criteria
(e) Ulcer (non-specific)	- Traumatic ulcers - Idiopathic ulcers - Toothbrushing-induced ulcers
6. Elevated lesions (a) Gingival hyperplasia	- Enlarged gingiva and interdental papillae - Papillae may be stippled or glazed in appearance - Usually presents as a generalized condition
(b) Mucocele	- Well-defined, fluid-filled swelling - Normal pink or bluish colour - Commonly found on labial mucosa and floor of the mouth (ranula)
(c) Focal epithelial hyperplasia (Hick's disease)	- Multiple circumscribed soft elevations - Whitish to normal coloration - Primary seen in American Indians and Eskimos
(d) Papilloma	- Exophytic growth, usually pedunculated - Verrucous, "cauliflower-like' surface - White or greyish colour is characteristic
(e) Verruca vulgaris	- Sessile or pedunculated lesion(s) - Papillomatous surface; common locations are the labial commissure & gingiva
(f) Tumour (non-specific)	Kaposi's sarcoma - Oral tumour(s) presenting with bluish or reddish macules in early stages - Lesions later become darker, elevated and sometimes lobular - The palate and gingiva are the common intraoral locations
7. Pigmented lesions (a) Amalgam tattoo	- Asymptomatic pigmented area, non-elevated - Bluish, blackish or slate-grey colour - Borders are usually poorly defined - Mucosal surface appears normal
(b) Naevus	- Well-circumscribed flat or elevated area - Pigmented with melanin - Colour range from blue to brown or black - Cannot be classified as due to exogenous pigmentation

5.13   Dentofacial anomalies

5.13.1 Crowding in the incisal segments

Both the upper and lower incisal segments were examined for crowding. Crowding in the incisal segment was the condition in which the available space between the right and left canine teeth was insufficient to accommodate all four incisors in normal alignment. Crowding in the incisal segments was recorded as follows:

0   No crowding

1   One segment crowded

2   Two segments crowded

5.13.2 Spacing in the incisal segments

Both the upper and lower incisal segments were examined for spacing. When measured in the incisal segment, spacing was the condition in which the amount of space available between the right and left canine teeth exceeded that required to accommodate all four incisors in normal alignment. If one or more incisor teeth had proximal surfaces without any inter-dental contact, the segment was recorded as having space. Spacing in the incisal segments was recorded as follows:

0   No spacing

1   One segment spaced

2   Two segments spaced

5.13.3 Diastema

A midline diastema was defined as the space, in millimetres, between two permanent maxillary incisors at the normal position of the contact points. This measurement was made at a level between mesial surfaces of the central incisors and was recorded to the nearest whole millimetre.

5.13.4 Anterior maxillary overjet

This was the measurement of the horizontal relation of the incisors made with the teeth in centric occlusion. The distance from the labial-incisal edge of the most prominent upper incisor to the labial surface of the corresponding lower incisor was measured with the CPI probe parallel to the occlusal plane. The largest maxillary overjet was recorded to the nearest whole millimetre. If the incisors occlude edge to edge, the score was zero.

5.13.5 Anterior mandibular overjet

Mandibular overjet was recorded when any lower incisor protruded anteriorly or labially to the opposing upper incisor ie. as in crossbite. The largest mandibular overjet (mandibular protrusion), or crossbite, was recorded to the nearest whole millimetre. The measurement was the same for the anterior maxillary overjet.

5.13.6 Vertical anterior openbite

If there was a lack of vertical overlap between any of the opposing pairs of incisors (openbite), the amount of the openbite was estimated using a CPI probe. The largest openbite was recorded to the nearest whole millimetre.

5.13.7 Anterior-posterior molar relationship

This assessment was based on the relation of the permanent upper and lower first molars. When the assessment could not be based on the first molars, the relations of the permanent canines and premolars was assessed. The right and left sides were assessed with the teeth in occlusion and only the largest deviation from the normal molar relation was recorded. The following codes were used:

1   Normal
2   Half cusp. The lower first molar was half a cusp mesial or distal to its normal relation
3   Full cusp. The lower first molar was one cusp or more mesial or distal to its normal relation.

5.14   Photographic examination

Photographs for clinical records and correlation with clinical examination were taken. In addition, photographs may be used as baseline information from which progression and change of the craniofacial manifestations can be monitored. Photographs of teeth which were clinically diagnosed to have any tooth anomalies were also taken.

All photographs were taken in the same room, against the same background and with the same camera. Photographs were taken of the full face and profile. Two views were taken with the mandible in the rest position: the right lateral view and the frontal view. The lateral view was necessary to allow assessment of the profile. The frontal view can reveal and record asymmetries (Bengel, 1985).

All the photographs were taken with a Nikon 401 camera, with a 105mm lens at an F-stop of F8 and a shutter speed of 125th of a second. The focal length was preset at 1.4 metres. Two external studio flashes were used for extra light.

Subjects were photographed with a name card with a 2cm line, held at the focus plane. This line/measurement was later used as a guideline for the printing of life-size photographs. Ilford FP4 plus black and white film was used. Films were processed with Ilford Ilfotec HC film developer at a dilution of 1:15 at 20 degrees centigrade for three and a half minutes. Photographic prints were made on Ilford Multigrade IV paper.

All photographs were taken with the camera in the vertical position and at the patient's eye level. Particular importance was given to the choice and positioning of the background and lighting. The background was homogeneous ie. unstructured and non-reflective. The lighting direction and distance of its source from the background was chosen so that the shadow of the head fell outside the field of the view of the camera.

All photographs were standardized so as to allow direct comparisons (Bengel, 1985). This was achieved by standardizing the conditions that were used. This was related not only to the photographic apparatus, but also to the framing and lighting. With regard to the apparatus, the most critical item was the lens and the two parameters of importance were the scale of reproduction and the clear working distance. The scale of reproduction is the ratio of the image size to subject size. The clear working distance is the distance from the subject to the front of the lens. The scale of reproduction together with the focal length of the lens determined the clear working distance.

Photographs had to meet the following criteria for inclusion into the study: (i) the camera was aligned in the Frankfurt horizontal plane with minimal left-to-right rotation; (ii) the subject had a relaxed facial expression with the eyes fully opened and the lips gently closed, and (iii) the image had adequate exposure and focus to allow accurate measurement of the facial features (Farkas, 1994).

5.15   Radiographic examination

Cephalometric, panoramic and hand radiographs were taken.

5.15.1 Cephalometric radiographs

(i)   Describe dimensional relationships of the cranio- facial components to establish facial type

(ii)   Identify, classify and localize skeletal and dental abnormalities, jaw and dentition malrelationships. This provides information on malocclusion and skeletal classification.

(iii)   Identify cranial base abnormalities and facial asymmetry

(iv)   Analyse angular and dimensional relationships of the craniofacial components - this gives an indication of the extent of dentofacial growth

A standard lateral cephalometric radiograph of each subject was taken using a cephalostat in the Division of Maxillofacial Radiology at the University of Stellenbosch (Cranex TOME Ceph machine, Sonerex Orion Corporation), with an object-to-film distance of 15cm, 8 mAs exposure (0.8 sec 10 mA current) and a 66-70 kVp. The cephalometric radiographs were developed using an Agfa CP-GL Medical xray film green, 18 x 24cm. Each subject was asked to keep his/her teeth in centric occlusion, with the lips at rest, for each exposure. All radiographs were taken by the same operator.

One hundred and eighty standardized cephalometric lateral head radiographs, of the fetal alcohol syndromes patients and controls were digitized and a total of 37 skeletodental and integumental landmarks were located on each radiograph (Figure 18). This was done using a computer programme Quick Ceph Image ^{TM} (Orthodontic Processing, Chula Vista, California). The programme is registered as an orthodontic diagnostic package and allows on-screen digitization of computed landmarks on the xray once it has been captured.

All the on-screen cephalometric measurements were taken twice by the author and no significant (p>0.05) intra-examiner error was found. In addition, measurements were compared to hand measurements to verify the accuracy. These data were then exported into a spreadsheet version that was loaded onto a PC for statistical analysis. The standard cephalometric radiographs were analysed using Bolton standards of dentofacial development growth data (Broadbent et al, 1975, Bolton, 1962). The basic units of analysis used were angles and distances in millimetres (lines). The tables (Table 5 and 6) below define some of the most important cephalometric landmarks used (see Figure 18).
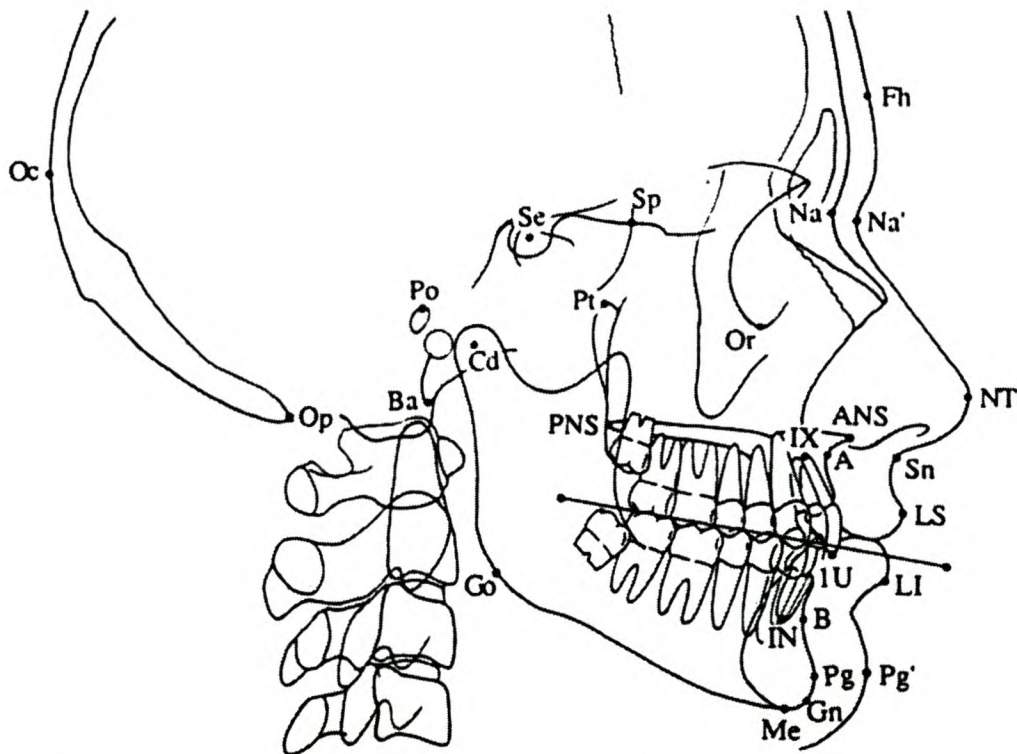
Figure 18: Schematic figure identifying the lateral cephalometric landmarks used in the study. (Gir, Aksharanugraha and Harris, 1989; Frias, Wilson and King, 1982)

S, sella; N, nasion; Or, orbitale; ANS, anterior nasal spine; A, point A; IX, UI1 root tip;UI1, UI1 incisal tip; LI1, LI1 incisal tip; IN, LI1 root tip; B, B point; Pg, pogonion; Gn, gnathion; Me, menton; Go, gonion; Ba, basion; Op, opisthion; Oc, occipitale; Po, anatomic porion; Cd, centre of condylion; Pt, pterygoid point; Sp, sphenoid point; PNS, posterior nasal spine; ANS, anterior nasal spine; Fh, supraorbital prominence of forehead; Na', soft tissue nasion; Sn, subnasale; LS, labrale superius; LI, labrale inferius; Pg', soft tissue pogonion.

Table 5: Definition of cephalometric landmarks

Lateral Skeletal Landmarks		
A	A point, subspinale	Signifies the apical base or junction of maxillary basal bone with alveolar bone. It lies anterior to the upper central incisor apex and is the deepest midline point in the curved bony outline from the base of the alveolar process of the maxilla
ANS	Anterior nasal spine	The tip of spinous process of the maxilla forming the most anterior projection of the floor of the nasal cavity in the midsagittal plane
Ar	Articulare	The point of intersection of the inferior surface of the cranial base with the posterior margin of the border of the ascending ramus
B	B Point, supramentale	Signifies apical base or the junction of mandibular basal bone with alveolar bone. Lies near the apex of the lower incisor, the most posterior point between Pog and the crest of the labial plate
Ba	Basion	The lowest point on anterior medial margin of the foramen magnum in the midsagittal plane
Bo	Bolton Point	The intersection of the occipital condyle and the outline of the foramen magnum; the deepest point on the curve posterior to the occipital condyle which lies on the atlas
Co	Condylion, Cd	The most posterior and superior point on the outline of the mandibular condyle
Gn	Gnathion	The most anterior-inferior point on the contour of the bony chin
Go	Gonion	The centre of the inferior contour of the angle of the mandible or the most posterior and inferior point on the angle of the mandible. Formed by the junction of the ramus with the lower border of the mandibular body on its posterior inferior aspect
Id	Infradentale	The alveolar rim of the mandible; the highest, most anterior point on the alveolar process in the midsagittal plane, between the mandibular central incisors
Me	Menton	The most inferior point on the outline of the mandibular symphysis in the midsagittal plane
Na	Nasion	The most anterior point of the junction of the frontal and nasal bones
O	Point O	The intersection or point of convergence of the parallel to supraorbital line, the palatal plane, occlusal plane and mandibular plane

Lateral Skeletal Landmarks Cont'd		
Or	Orbitale	The lowest point on the averaged inferior borders of the bony orbits
Pog	Pogonion	The most anterior point on the symphysis of the mandible in the midsagittal plane, the bony chin prominence
Po	Porion	The midpoint of the upper contour of the external auditory meatus
PNS	Posterior nasal spine	The most posterior point on the spine of the palatine bone of the hard palate
Pr	Prosthion	The alveolar rim of the maxilla: the lowest most anterior point on the alveolar of the premaxilla, in the midsagittal plane, between the maxillary central incisors
S	Sella, Sella turcica	The centre of the pituitary cavity
So	Sphenooccipital synchondrosis	The junction between the occipital bone and the basisphenoid bones
Xi	Xi point	Represents the centre of the ramus

Dental		
L1	Mandibular central incisor	The most labial point on the crown of the mandibular central incisor
L6	Mandibular first molar	The tip of the mesiobuccal cusp of the mandibular first permanent molar
U1	Maxillary central incisor	The most labial point on the crown of the maxillary central incisor
U6	Maxillary first molar	The tip of the mesiobuccal cusp of the maxillary first permanent molar
Soft Tissue		
G	Glabella	The most anterior soft tissue point of the frontal bone
Me'	Soft tissue menton	The point of the lower contour of the chin opposite to the hard tissue menton
Pog'	Soft tissue pogonion	The most anterior soft tissue point of the chin
Pr, Pn	Pronasale	The tip of the nose
Sn	Subnasale	The point at which the nasal septum merges mesially with the upper cutaneous lip in the midsagittal plain.
St'	Stomiom	The central point of the interlabial gap

Table 6: Definition of common cephalometric planes and angles

Sagittal Skeletal Landmarks		
APog	A point-Pogonion	A sagittal reference relating the inclination and anterior-posterior position of upper and lower incisors to the jaws irrespective of the cranial base (Ricketts)
ArGo	Articulare-Gonion	Measurement of the length of the ramus
BaN	Basion-Nasion	Represents the cranial base (Ricketts)
BoN	Boltonpoint-Nasion	**Bolton Plane:** Represents the cranial base
CdGo	Condylion-Gonion	Measurement of the length of the ramus
FH	Porion-Orbitale	**Frankfort Horizontal:** Represents the cranial base (Downs)
GoGn	Gonion-Gnathion	**Mandibular Plane:** Represents the mandibular position (Steiner)
MeGo	Menton-Gonion	Represents the extent of the mandibular base
NPog	Nasion-Pogonion	**Facial Plane:** A sagittal reference plane
NA	Nasion-A	A sagittal reference line for the maxilla's anterior-posterior position (Steiner)
NB	Nasion-B	A sagittal reference line representing anterior-posterior position of the mandible (Steiner)
PP	ANS-PNS	**Palatal Plane:** Represents the palatal plane or angle of the maxilla
SN	Sella-Nasion	Anteroposterior extent of the anterior cranial base. Cranial base in midsagittal plane

5.15.1.1   Clinical use and measurements

The skull can be considered to be composed of five major structural components, including the cranium, the cranial base, the skeletal maxilla and mandible and the maxillary and mandibular dentition. All cephalometric analysis describes the interrelationship of these parts or may describe the form of the individual unit. These relationships may be described in both the vertical and horizontal planes. The following variables were used (Frias et al, 1982, Gir et al, 1989) for assessment:

5.15.1.2   Cranial base

Basion-sella length

Basion-nasion length

Sella-nasion length

Sphenoid-nasion length

Basion-sella-nasion angle

Selle-nasion FH angle

5.16.1.3   Midface

Nasion-ANS length

Sella-nasion-A angle

Nasion-A to FH angle

Pt-vertical/nasion length (projected parallel to Frankfort horizontal)

Pt-vertical/A length (projected parallel to Frankfort horizontal)

PNS $\perp$ FH length

Pterygoid-PNS length

Palatal plane to FH angle

Many reports have suggested from visual assessment that FAS children exhibit midface insufficiency (Jones et al, 1973; Clarren and Smith, 1978; Hanson, Jones and Smith, 1976; Streissguth, Clarren and Jones, 1985; Spohr and Steinhausen, 1987). Midface height (N-ANS) and anteroposterior position relative to the cranial base (SNA, PTV-A, NA-FH) and facial angle (N-A to FH) were measured.

5.15.1.4 Size and shape of mandible and incisor relationships

Since reports have commented that mandibular retrognathia is a sequela of FAS, the morphology of the mandible was examined in detail. Several parameters were assessed: anteroposterior and sagittal size and position of the mandible and its components, sagittal heights of the lower third of the face, and tooth positions and angulations. The following points and measurements were analysed:

5.15.1.4.1 Mandible

Nasion-menton length

ANS-menton length

Sella-nasion-B point angle

Sella-nasion-pogonion angle

Y axis angle (Downs)

Condylion-gnathion length

Condylion-gonion length

Gonion-pogonion length

Sella-gonion length

Condylion-gnathion-menton angle

Gonion-menton to FH angle

AOBO difference (mm)

5.15.1.4.2 Incisor relationships

Interincisal angle (UI-LI)

UI1 to FH angle

LI1 to gonion-menton angle

5.15.1.4.3 Size and shape of the facial and integumental profile (Genecov et al, 1990)

Nasion'-subnasale-pogonion angle

Forehead-nasion'-NT angle

NT $\perp$ nasion-pogonion distance

Nasion'-NT-subnasale angle

NT-subnasale-LS angle

Subnasale'-LS length

LI-pogonion' length

LS $\perp$ nasion'-pogonion distance

LI $\perp$ nasion'-pogonion distance

5.16.2 The extraoral panorex radiograph

The extraoral panorex (GE Panelipse II) with a 96 mAs exposure (24 sec, 4 mA current) and a 66-70 kVp was used to evaluate dental development to determine the developmental stage of the seven left permanent mandibular teeth. The panorex films were developed using a Konica Medical Film (MG-SR) 127 x 307cm.

5.16.2.1 Assessment of dental maturity

Dental maturity can be determined by the stage of tooth eruption or the stage of tooth formation. The latter is proposed as a more reliable criterion for determining dental maturation (Fanning, 1962; Nolla, 1960; Hotz, Boulanger and Weisshaupt, 1959).

From several investigations (Nolla, 1960; Demirjian et al, 1973; Demisch and Wartmann, 1956), the tooth calcification of homologous teeth was found to be symmetrical; therefore, only left mandibular teeth in the panoramic radiographs were examined. In the case of any missing left mandibular teeth, the right teeth corresponding to the missing teeth were substituted.

The maxillary posterior teeth were omitted from the study because superimposition of calcified structures in this area resulted in inaccurate assessment of the stage of development of these teeth. There are a number of standard scales for rating the tooth calcification stage (Nolla, 1960; Garn and Lewis, 1957; Morreess, Fanning and Hunt, 1963; Fanning and Brown, 1971). In the present study, dental maturation was assessed from panoramic radiographs using Demirjian's seven teeth system (Demirjian, Goldstein and Tanner, 1973). This method was chosen as the most precise and accurate evaluation of dental age (Hägg and Matsson, 1985; Staaf, Mörestad and Welander, 1991; Cameron, 1993) because its criteria consist of distinct details based on shape criteria and proportion of root length, using the relative value to crown height rather than on absolute length. Foreshortened or elongated projections of developing teeth will not affect the reliability of the assessment.

Tooth formation was divided into eight stages and criteria for the stages were given for each tooth separately. Each stage of the seven teeth was given a score according to a statistical model. Standards were given for each sex separately. The sum of the scores for the seven teeth were transferred to a dental age (Demirjian et al, 1973; Demirjian et al, 1985). The orthopantomograms were rated by a single examiner. At the end of each day, five randomly selected radiographs were reassessed, and the results compared. Disagreement occurred in no more than 5% of films and was never more than one stage. Neither a systematic nor a significant method error was found.

5.16.2.1.1 Assigning the ratings

1. The mandibular permanent teeth were rated in the following order: second molar, first molar, second premolar, first premolar, canine, lateral incisor, central incisor.

2. All teeth were rated on a scale A to H (Figure 20). The rating was assigned by following carefully the written criteria for each stage, and by comparing the tooth with the diagrams and radiograph pictures given in Figure 20. The illustrations were used only as an aid and not as the sole source of comparison.

    For each stage there were one, two or three written criteria marked a), b) or c). If only one criterion was given this had to be met for the stage to be taken as reached; if two criteria were given, then it was deemed sufficient if the first one of them met the stage to be recorded as reached; if three criteria were given, the first two of them had to be met for the stage to be considered reached. At each stage, in addition to the criteria for that stage, the criteria for the previous stage had to be satisfied. In borderline cases, the earlier stage was always assigned.

3. There were no absolute measurements taken. A pair of dividers was used to compare the relative length (crown : root). To determine apex closure stages no magnifying glass was necessary. The rating was made with the naked eye.

4. The crown height was defined as being maximum distance between the highest tip of the cusps and the cemento-enamel junction. When the buccal and lingual cusps were not at the same level, the midpoint between them was considered to be the highest point.
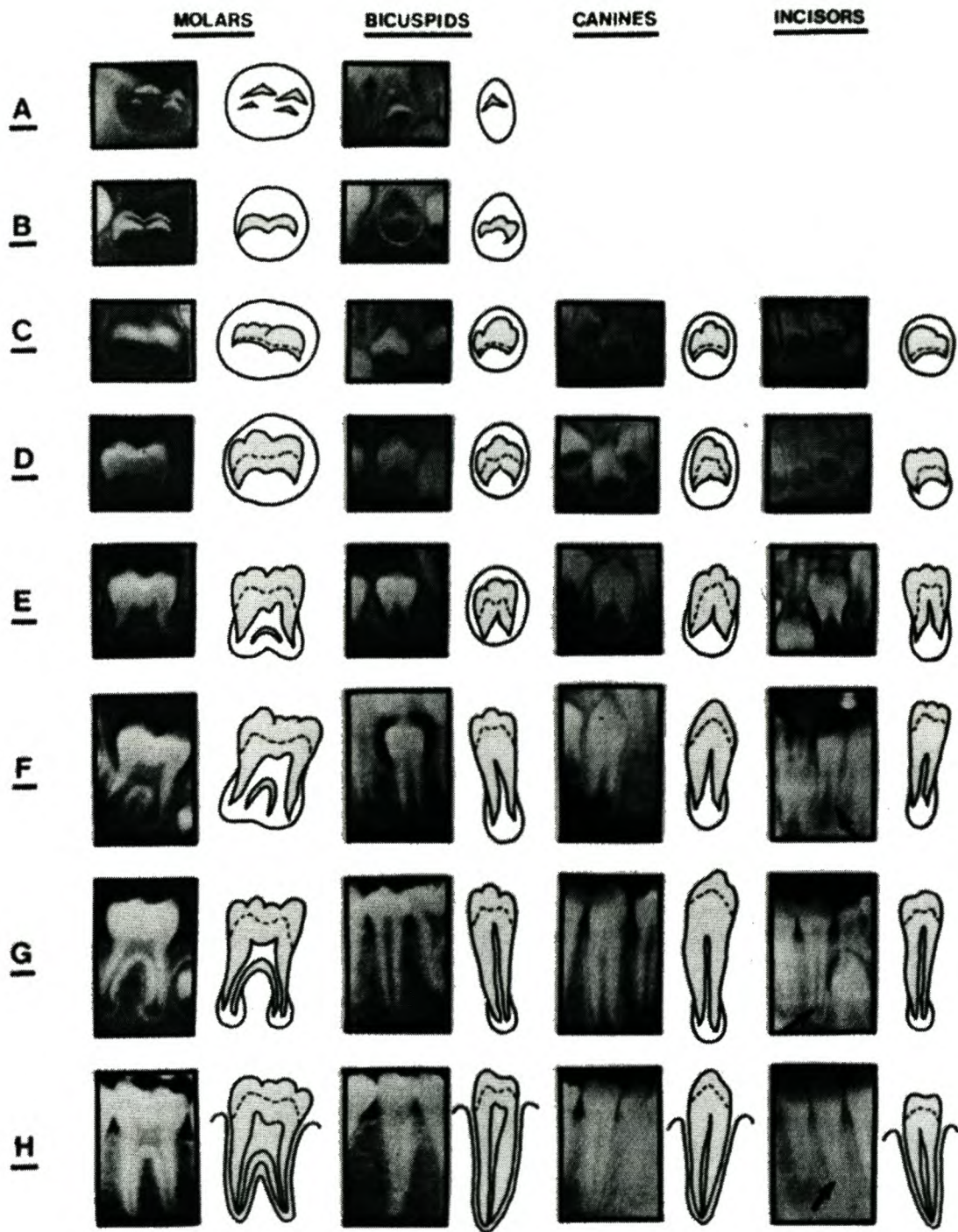
Figure 19: Developmental stages of the permanent dentition (Demirjian et al, 1973)

5.16.2.1.2   Dental formation stages

If there was no sign of calcification, the rating 0 was given. The crypt formation was not taken into consideration. The dental formation stages are elaborated in Table 7.

Table 7: Description of dental formation stages

Stage	Description
A	In both uniradicular and multiradicular teeth, a beginning of calcification was seen at the superior level of the crypt in the form of an inverted cone or cones. There is no fusion of these calcified points.
B	Fusion of the calcified points formed one or several cusps which united to give a regularly outline occlusal surface.
C	a. Enamel formation was complete at the occlusal surface. Its extension and convergence towards the cervical region was seen. b. The beginning of a dentinal deposit was seen. c. The outline of the pulp chamber had a curved shape at the occlusal border.
D	a. The crown formation was completed down to the cemento-enamel junction. b. The superior border of the pulp chamber in the uniradicular teeth had a definite curved form, being concave towards the cervical region. The projection of the pulp horns, if they were present, gave an outline shaped like an umbrella top. In molars, the pulp chamber had a trapezoid form. c. Beginning of root formation was seen in the form of a spicule.
E	**Uniradicular teeth:** a. The walls of the pulp chamber now formed straight lines, whose continuity was broken by the presence of the pulp horn, which was larger than in the previous stage. b. The root length was less than the crown height **Molars:** a. Initial formation of the radicular bifurcation was seen in the form of either a calcified point or a semi-lunar shape. b. The root length was still less than the crown height
F	**Uniradicular teeth:** a. The walls of the pulp chamber now formed a more or less isosceles triangle. The apex ended in a funnel shape. b. The root length was equal to or greater than the crown height. **Molars:** a. The calcified region of the bifurcation had developed further down from its semi-lunar stage to give the roots a more definite and distinct outline with funnel shaped outlines. b. The root length was equal to or greater than the crown height.
G	a. The walls of the root canal were now parallel and its apical end was still partially open (distal root in molars).
H	a. The apical end of root canal was completely closed (Distal root in molars). b. The periodontal membrane had a uniform width around the root and the apex.

5.16.2.1.3   Using the scoring system

1.   Each tooth had a rating, assessed by the procedure described above.

2.   This was converted into a score using self-weighted scores for the dental stages for boys and girls.

3.   The scores for all seven teeth were added together to give the *maturity score*.

4.   The maturity score was then converted directly into a dental age by using a specific table (Demirjian et al, 1973) that was constructed by means.

All of the assessments were made on an illuminated viewing box in the dark room by two examiners (SN, GN). The interpretations of the panoramic radiographs were discussed until agreement was reached. Exact chronological ages were verified by reference to the patient's birth date. To test reproducibility of the assessments of dental age, the same investigators reevaluated randomly selected the panoramic radiographs of 10 of the same male and 10 of the same female subjects 6 weeks after the first evaluation. The differences between double interpretations were statistically tested.

5.16.3   The hand-wrist radiographs

5.16.3.1   Assessment of skeletal age

The methods most widely used to determine skeletal age are those of Tanner et al (1975) and Greulich and Pyle (1959). Studies have compared the two methods and found minor, insignificant differences between them (King et al, 1994; Cole, Webb and Cole, 1988; Milner, Levick and Kay, 1986). However, the Greulich and Pyle method appeared to be less time consuming and tedious and is therefore the preferred method. The *Atlas* of Greulich and Pyle (1959) comprises a large series of standard anteroposterior radiographs of the hand. Each radiograph is assigned to a specific age in years and months and the patient's skeletal age is determined by comparing his or her radiograph with the standard in the *Atlas* for the appropriate gender. In this study, each hand-wrist radiograph for the cases and controls was assigned a skeletal age by comparing it with the standard plates in the *Radiographic Atlas of Skeletal Development of the Hand and Wrist* (Greulich and Pyle, 1959).

5.16.3.2   Examination procedure

1. The radiograph of the child was compared with the standard of the same gender and nearest chronological age in the *Atlas*

2. The radiograph was then compared with the adjacent standards, both older and younger than the one that was of the nearest chronological age

3. The standard which superficially appeared to resemble it most closely was then selected for more detailed comparison

4. The more detailed comparison of the individual bones and the epiphysis visible in them was then made; "maturity indicators"[1] also provided a basis for detailed assessment of the hand radiograph

5. The bones were examined in a regular sequence for all the radiographs: examination began at the distal ends of the radius and ulna, proceeded to the carpals, then to the metacarpals, and then to the phalanges. In addition, the carpals too, were studied in a regular sequence – in the order in which they usually appear – capitate, hamate, triquetral, lunate, scaphoid, trapezium, trapezoid and pisiform (Figure 20).

6. When an individual bone on the radiograph being assessed was in the same stage of development as the corresponding bone in the standard selected for the detailed comparison, then it was given the skeletal age that had been assigned to that bone in the standard. If it appeared to be either less or more advanced than its counterpart in that standard, it was compared with the same bone in adjacent standards.

7. The skeletal age assigned to it was that which was given to it in the standard to the corresponding bone that showed the same degree of development.

[1]Footnote: As individual centres of ossification grow and develop from their first appearance as tiny irregularly shaped single, or occasionally multiple bony nodules until they attain their definitive size and shape, they pass through a regular series of changes in form that characterise successive stages in their progress toward maturity. In a radiograph of the hand and wrist only some of these changes in form are visible, but even the silhouettes of the various developing ossification centres serve to distinguish stages of their maturation. These provide the basis for assessing the developmental status of individual bones and are referred to as "maturity indicators".

Figure 20: Radiograph showing individual carpals and epiphyses, numbered approximately in the order in which their ossification begins (Greulich and Pyle, 1959).

All of the assessments were made on an illuminated viewing box in the darkroom by two examiners (SN, GN). The interpretations of the hand-wrist radiographs were discussed until agreement was reached. Exact chronological ages were verified by reference to the patient's birth date.

To test reproducibility of the assessments of dental age, the same investigators reevaluated randomly selected hand-wrist radiographs of 10 of the same male and 10 of the same female subjects 6 weeks after the first evaluation. The differences between double interpretations were statistically tested.

If the level of agreement between the examinations did not meet the recommended minimum level, the examiners reviewed the interpretation criteria and additional calibration examinations were conducted until an acceptable consistency was achieved. Agreements were in the range of 85-90%.

5.17   Standardization and calibration

The objectives of the standardization and calibration exercises are to:

(i)   ensure uniform interpretation, understanding and application of the criteria for the various diseases and conditions that were observed and recorded.

(ii)   ensure that each examiner could examine consistently to a standard.

(iii)   minimize variations between different examiners.

5.17.1 Intra-examiner calibration

For the extra-oral examination there was only a single examiner. Consistency was determined by examining a randomly selected group of 10 patients twice, on successive days. These patients possessed, collectively, the full range of conditions expected to be assessed in the main study.

By comparing the results of the two examinations, the examiner was able to obtain an estimate of the extent and nature of the diagnostic errors. If the level of agreement between the examinations did not meet the recommended minimum level, the examiner reviewed the interpretation criteria and additional calibration examinations were conducted until an acceptable consistency was achieved. Agreements were in the range of 85-90%.

5.17.2 Inter-examiner calibration

For the intra-oral examination (hard tissue), there were two examiners (SN, UMEC). These examiners were calibrated and by consensus made the final diagnosis for each tooth. Consistency was determined by examining a randomly selected group of 10 patients twice, on successive days. By comparing the results of the two examinations, the examiners were able to obtain an estimate of the extent and nature of the diagnostic errors.

If the level of agreement between the examinations did not meet the recommended minimum level, the examiners reviewed the interpretation criteria and additional calibration examinations were conducted until an acceptable consistency is achieved. Agreements were in the range of 85-90%.

5.18   Statistical methods used in data analysis

Following the literature review, the author selected the relevant statitistical tests required and the data was processed and analysed by the Biostatistics Unit of the Medical Research Council, South Africa using Statistical Analyses Software (SAS) Version 8.2. Several statistical tests were carried out to determine statistical significant differences between the FAS subjects and the controls. The level of significance was set at 0.05. Interpretation of all the statistical data was done by the author. The various statistical methods used are described in the chapters that present the results as it is hoped that it will enhance the discussion related to the findings.

5.19   PROCEDURES

5.19.1 Establishing contacts

Access to the participants of the study was made initially by letter to the participating school principals and parents (Appendix 2). An introduction of the researcher, the basic aims and objectives of the study, what participating in the study would involve and how long the examination would take were explained. It was emphasized that strict confidentiality would be maintained at all times and that the results of the study would be presented in a manner that ensured anonymity. Once signed informed consent (Appendix 3) was received for each child, arrangements were made for the clinical examinations to be carried out at a time convenient to the participants and schools. Children were collected from the schools and brought to School for Oral health Sciences, Faculty of Health Sciences at Tygerberg Hospital.

5.19.2 Ethical considerations

The protocol was submitted to the Ethics Committee of the University of Stellenbosch for ethical approval (Approval was granted – Appendix 4). Informed consent was obtained from the principal of each participating school and the parents or guardians of the child. It was emphasized that strict confidentiality would be maintained at all times.

Specific interventions for children with FAS was planned with the Avalon Treatment Centre, Department of Genetics at University of Cape Town and the Departments of Community Dentistry at the University of Stellenbosch and the University of the Western Cape and their outreach clinics. Where subjects were found to have conditions requiring treatment, they were referred to the appropriate oral health or specialist services.

Each participant received an individual oral health report within 3 months of completing of the survey, with appropriate advice. The individual report could be taken to their private dental practitioner or alternatively treatment was offered at the dental school or the nearest dental clinic. Written informed consent was obtained from the parents of the children whose photographs are used in this report.

5.20   Concluding remarks to Chapter 6

This chapter described the selection of the study population which included the diagnosis and screening of the FAS cases. A sample consisting of 90 cases with diagnosed FAS and 90 controls were selected for this study. The instrument developed to collect the data and the piloting of it is described in this chapter. The instrument chosen was a focused, structured data capture sheet in four parts. Following consultation with experts in the field and a thorough literature review this was considered to be the most appropriate method of obtaining the information required which was to determine the oral, dental and facial manifestations of children with FAS in a case-control study design.

The chapter describes in detail the methods and codes used to elicit the measurements required both for the extra-oral and the intra-oral examination. In addition, the statistical methods used in the data analysis are described.

PART TWO: RESULTS AND DISCUSSION

This study involved clinical, radiological and photographic measurements of 90 children with FAS and 90 matched controls. Owing to the number of different variables that have been examined and in the ensuing results chapters, the findings will be presented in four parts. Each chapter will give a background and introduction to the examination, an overview of the methodology and statistical analysis used, the results followed by a discussion and concluding remarks.

Chapter 6 discusses the demography of the sample, followed by the anthropometric findings (height, weight and head circumference). The general clinical examination (including the minor anomalies will be described), the photographic analysis of the facial characteristics (palpebral fissure length, inter-pupillary distance and inner-canthal distance as well as two categorical variables, smoothness of the philtrum and thinness of the lip) will be described and discussed.

Chapter 7 discusses the intra-oral examination, describing the hard tissues (enamel opacities, dentofacial anomalies, caries status), oral health status (plaque and gingival states), and soft tissues (oral mucosal lesions).

In Chapter 8 the findings of the panoramic and wrist radiographic findings will be discussed as well, pursuant of the dental and skeletal age assessments.

Chapter 9 documents results of the cephalometric examination (cranial base, midface, mandible and incisor relationships and the size and shape of facial and integumental profile).

Chapter 10 will provide a discussion of the overall findings and the final recommendations.

Chapter 6: Demography, Athropometry, General Clinical Features and Photographic Examination

6.1 Introduction

The examinations in this part of the study involve the general clinical examination and the photographic measurements of 90 children with FAS and 90 matched controls. The methods and statistical analyses used are described. The demography of the sample is presented followed by the anthropometric findings (height, weight and head circumference), the general clinical examination (including the minor anomalies), and the results of the photographic analysis of the facial characteristics (palpebral fissure length, inter-pupillary distance and inner canthal distance, as well as two categorical variables, smoothness of the philtrum and thinness of the lip).

6.2 Statistical Methods

Various anthropometric indices were used to assess growth status. Height, weight and head circumference were measured and assessed against head circumference and weight-versus-height charts. Height-for-age portrays performance in terms of linear growth and essentially measures long-term growth faltering. Weight-for-height reflects body proportion or the harmony of growth and weight-for-age represents a convenient synthesis of both linear growth and body proportion.

Because the anthropometric data were from children of different ages and genders, all measurements were standardized to allow for comparisons. Standardization was performed by converting all the data to "standard" or Z scores. This process was accomplished in the usual manner by subtracting the population mean from the individual measurement value and dividing by the population standard deviation (SD). Descriptive analysis involved computing the mean, SD, minimum, maximum and the range of the clinical and radiographic parameters.

6.2.1 Continuous data

Continuous data were compared by the Student's t-test. The conditions to be fulfilled before the Student's t-test can be used are listed below (Mould, 1998a):

(i)   The observations must be independent in order to avoid bias

(ii) The observations must be drawn from normal populations

(iii) These normal populations must have the same variance

(iv) The variables involved must have been measured in an interval scale, so that it is possible to do arithmetic operations

When faced with small numbers many authors use the more robust non-parametric Mann-Whitney U test which does not require the data to be analysed to follow any specific distribution (Mould, 1998b). In most cases the Student's t-test was used, but where there was uncertainty about the distribution of the data the Mann-Whitney U test was also applied.

6.2.2 Categorical data

The chi-squared (x^2) test for determining association of the categorical variables and bivariate analysis using the Student's t-test was performed to compare categorical variables (such as age, gender etc).

6.2.3 Multivariate vs. univariate analysis

Multivariate as well as bivariate analysis using the Students t-test was performed to compare categorical variables (such as age, gender, etc). Univariate, bivariate and multiple logistic regression analysis and relative risk were utilized to establish whether an association exists between case, the controls and the variables tested. The main advantage of multivariate analyses is that several variables can be studied simultaneously with correlations that reflect the extent of the relationship among three or more variables.

Analysis of variance (ANOVA) is used to compare several variables but in this study multiple regression analyses were used because it provided a mathematical model of the linear relationship between a dependent and two or more independent variables (Weintraub et al, 1985).

6.2.4   Analysis of the facial measurements

Logistic regression was used for the statistical analysis of the facial measurements taken from the standardized photographs that were used to identify the subset/cluster of facial measurements that best differentiated subjects with and without FAS. This was because the discriminant function approach in the estimation of the logistic coefficient is based on the assumption that the independent variables are multivariate normal in each of the outcome groups. However, the estimations could be biased when normality does not hold. Five of the facial variables were entered in the logistic regression model. Student's t-test and the x^2 test, were used to test for significance. Unless stated otherwise, the probability level of $p < 0.0005$ was accepted to indicate statistical significance. As cases and control were matched by gender and age, a conditional logistic regression analysis for matched case-control studies, based on Cox Proportional Hazards Model (Schlesselman, 1982), was also performed because the author wanted to investigate the relationship between the outcome (case or control) and the set of prognostic factors. The Wald test was used for testing the individual variables.

6.3   Results

6.3.1   Demography

The sample of 90 cases (children diagnosed with FAS) and 90 controls were matched for age, gender and social class (Table 8). The mean age of the cases was 8.9 years with the controls slightly older at 9.1 years. This was not significant (p=0.3363) and the age ranges for both the groups were the same ie. 6.4 years for both the case and the controls (Figure 21). There was an equal gender distribution in both the cases and controls with 45 males and 45 females in each group, as could be expected as a result of sample matching.
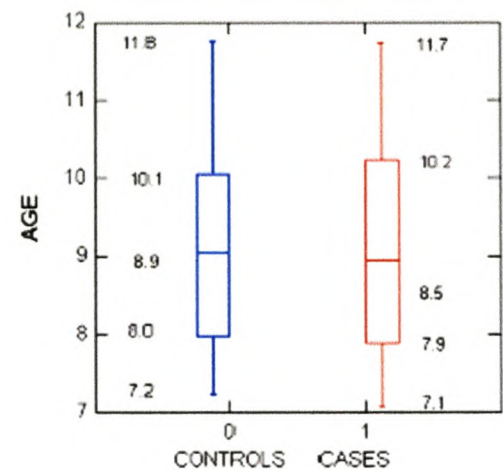
Figure 21: Age profiles

Table 8: Demographic profile: FAS and controls

Characteristic	Control (n=90)	FAS (n=90)
Age (years)		
Mean (SD)	9.1 (1.29)	8.9 (1.29)
Minimum-maximum	7.23-11.75	7.07-11.74
Gender		
Girls n (%)	45 (50)	45 (50)

6.3.2   Anthropometry

The results of the anthropometric measurements in respect of weight, height and head circumference showed significantly lower values for the FAS children when compared to the controls (Table 9). The weight-for-age ratio of the FAS cases (21.53) was significantly lower than for the controls (26.88) at a probability level of $p<0.001$.

Similarly, the height-for-age of the FAS subjects (121.11) was lower than that of the controls (126.78). The head circumference differed significantly between the two groups (p<0.0001). The mean head circumference of the FAS children was 49.03 cm and 51.53 cm for the controls (Figure 22). Table 10 shows statistically significantly differences (p<0.0001) for both weight-for-age z-scores (WAZ) and height-for-age z-scores (HAZ) between the FAS children and the controls.

Table 9: Descriptive statistics of anthropometric measurements: mean (SD)

Variable	Controls (n=90)	FAS (n=90)	Student's T-test (p-value)
Weight (kg)	26.88 (SD 5.35)	21.53 (SD 3.25)	<0.0001
Height (cm)	126.79 (SD 2.53)	121.11(SD 5.62)	<0.0001
Head circumference (cm)	51.53 (SD 1.29)	49.03 (SD 1.26)	<0.0001
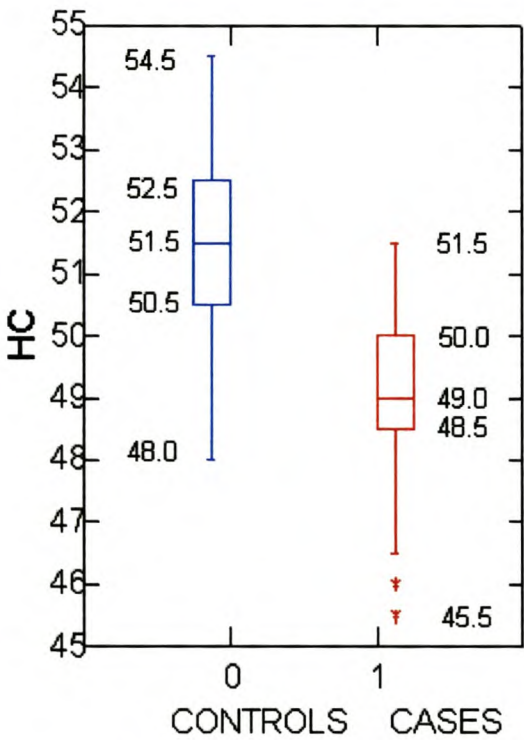
Figure 22: Head circumference (cm) (p<0.0001)

Table 10: Height-for-age z-score (HAZ), Weight-for-age z-score (WAZ) in FAS and control subjects

Characteristic	Control (n=90)	FAS (n=90)
WAZ (mean)	-0.519 (SD 0.874)	-1.637 (SD 0.685)
p value	<0.0001	
HAZ (mean)	-0.957 (SD 0.977)	-1.775 (SD 0.807)
p value	<0.0001	

Tables 11 and 12 show the Pearson Correlations of the paired differences between head circumference and weight for the entire group and for the cases and controls respectively. There was a significant correlation of the paired differences between the head circumference and weight for the two groups (r=0.3229, p=0.0019) (Table 11) and between the FAS (r=0.4736, p<0.0001) and control children (r=0.3153, p=0.0025) (Table 12).

Table 11: Pearson Correlation of paired differences between head circumference (HC) and weight (wt)

Variable	N	Mean	SD	Median	Minimum	Maximum
Dif_wt	180	-5.344	5.288	-4.750	-28.500	6.500
Dif_hc	180	-2.500	1.689	-2.500	-8.000	0
	dif_hc					
Dif_wt (r)	0.3229					
	<.0019					

Table 12: Pearson Correlation of FAS and control head circumference (HC) and weight

FAS						
Variable	**N**	**Mean**	**SD**	**Median**	**Minimum**	**Maximum**
Weight	90	21.533	3.250	21.000	16.000	31.000
HC	90	49.033	1.258	49.000	45.500	51.500
	HC					
Weight	0.4736					
p value	<0.0001					
CONTROL						
Variable	**N**	**Mean**	**SD**	**Median**	**Minimum**	**Maximum**
Weight	90	26.878	5.355	25.000	20.000	49.500
HC	90	51.533	1.293	51.500	48.000	54.500
	HC					
Weight	0.3153					
p value	0.0025					

6.3.3 General clinical examination

The pattern of physical abnormalities in the FAS, as reported in the literature (see section 4.7.2), includes subtle abnormalities of the face, an increasing frequency of malformations, congenital cardiac defects (especially atrial and ventricular septal defects), anomalies of the urinary tract and genitals and psychomotor disturbances (fine motor dysfunctions and poor standardized performance tasks). Abnormalities of the limbs and joints include deformities in the small joints of the hand. In the hands, palmar crease patterns appear to be altered. In FAS children, a hockey stick crease – where the distal transverse crease runs quite horizontally across the postaxial palm and then abruptly angles distally to exit between the index and middle finger is often seen (Figure 12).

The minor anomalies that were found in the total sample (n=180) are shown in Table 13. The common malformations of the fingers include clinodactyly and camptodactyly, as well as incomplete rotation of the elbow, causing limited supination. All the malformations of the hands were found to be statistically significantly different between the FAS and the control children.

Clinodactyly (Figure 13) is the curving of a finger to one side, usually toward the midline, in the plane of the palm. Camptodactyly occurs when the proximal interphalangeal joints are held in a partially flexed position, sometimes with thickened connective tissue on the volar surface. The fifth finger is most commonly affected, but others may be involved as well, usually bilaterally and symmetrically. In addition, significantly more FAS children manifested growth retardation and some structural abnormality ($p < 0.0001$).

Table 13: Clinical findings – minor anomalies

Characteristic	Control (n=90) n (%)	FAS (n=90) n (%)	p value
Hands			
Clinodactyly	5 (5.6)	36 (40.0)	< 0.0001
Camptodactyly	0 (0)	23 (25.6)	< 0.0001
Limited supination	0 (0)	12 (13.3)	0.0003
Anomalous palmar creases	5 (5.6)	48 (53.3)	< 0.0001
Hypoplasia: terminal phalanges	2 (2.2)	15 (16.7)	0.0009
Other abnormalities			
Growth deficiency	1 (1.1)	76 (84.4)	< 0.0001
Structural abnormality	5 (5.6	80 (88.9)	< 0.0001

6.3.4 Photographic analysis: facial characteristics

6.3.4.1 Background

The FAS facial phenotype is characterized by a cluster of minor facial anomalies that include short palpeberal fissures (PFL), larger inner canthal (ICD) and inter-pupillary distance (IPD), a smooth philtrum and a thin upper lip (Clarren and Smith, 1978). Since the facial anomalies occur on a continuum and not as discrete traits, the facial component of the diagnostic criterion based on clinical assessment and/or photographic analysis of facial features is most effective when the facial disruption is obvious (Astley and Clarren, 1995, 1996). Use of the facial component in the diagnosis becomes much more elusive when less severe effects of prenatal alcohol exposure are expressed.

The most common facial anomalies observed in FAS include short palpebral fissures, a smooth philtrum and a thinner upper lip. In its most pronounced form, the face in FAS can be an important aid in diagnosis when coupled with other key features such as developmental delay and documented prenatal exposure to alcohol. The intention of this part of the study was to use existing methods of diagnosis based on clinical assessment and photographic analysis to identify an ideal minimum cluster of FAS facial features that may add additional discriminating power to assist with the diagnosis of FAS. In this study the facial features, inner canthal distance, inter-pupillary distance, palpebral fissure lengths, philtrum smoothness and upper lip thickness were recorded from the standardized photographs.

All measurements were made on the standardized photographs using sliding calipers by one examiner. To decrease intra-measurement error, each measurement was taken twice, with a third measurement taken if the first and second measurements differed by >2 mm. In instances where the third measurement was required the measurement (first or second) that was closest to the third measurement was used for data analysis. The sample of 90 cases and 90 controls had been previously matched for age, gender, social class at the time the photographs were taken.

6.3.4.2 Photographic Measurement Results

The variation of phenotypic expression across the 90 cases with FAS, relative to the 90 matched controls, is shown in Table 14.

Table 14: Summary of facial features of FAS and controls

Characteristic	Control (n=90)	FAS (n=90)	p value
	n (%)	n (%)	
Face:			
Hypoplastic midface	2 (2.2)	39 (43.3)	< .0001
Hypolastic mandible	0 (0)	2 (2.2)	0.4972
Hypoplastic maxilla	0 (0)	0 (0)	-
Ears:			
Posterior rotation	0 (0)	2 (2.2)	0.4972
Abnormal concha	0 (0)	5 (5.6)	0.0590
Preauricular pits	0 (0)	3 (3.3)	0.2458
Other: malformed etc.	0 (0)	7 (7.8)	0.0138
Eyes:			
Inner canthal distance (mm)	2.92 (SD 0.25)	2.88 (SD 0.24)	0.3040
Inter-pupillary distance (mm)	5.31 (SD 0.36)	5.06 (SD 0.28)	0.8065
Short palpebral fissures (mm)	2.64 (SD 0.17)	2.41 (SD 0.12)	< .0001
Strabismus	0 (0)	8 (8.8)	0.0066
Ptosis	0 (0)	11 (12.2)	0.0006
Epicanthal folds	9 (5)	55 (61.2)	< .0001
Nose:			
Flattened, low nasal bridge	5 (5.6)	48 (53.4)	< .0001
Short upturned nose	0 (0)	3 (3.4)	0.2458
Lips:			
Long philtrum	0 (0)	28 (31.2)	< .0001
Smooth philtrum	5 (5.6)	60 (66.6)	< .0001
Narrow, thin vermillion	0 (0)	40 (44.4)	< .0001
Mouth:			
Cleft lip	0 (0)	0 (0)	-
High, arched palate	0 (0)	3 (3.4)	0.2458

In order to identify an ideal minimum cluster of FAS facial features that may add additional diagnostic discriminating power the following diagnostic measurements were considered: palpebral fissure length (PFL), inter-pupillary distance (IPD) and inner canthal distance (ICD) as well as two categorical variables (Table 15), smoothness of the philtrum and thinness of the lip, both ranked on a 5-Point Likert Scale (Figure 16).

Philtrum smoothness refers to the area between the upper lip and subnasion and the examination focused on the presence or absence of the midline vertical furrow bordered by the two vertical ridges. The upper lip thinness was a measurement of the upper lip demarcated by its vermillion. The phenotypic expressions of philtrum smoothness and upper lip thinness were recorded on 5-point Likert ordinal scales which had the advantage of being technically simple. Figure 18 in the methodology chapter, shows pictorial examples of the 5-point Likert ordinal scales used to rank philtrum smoothness and upper lip thinness.

Table 15: Contrasts in facial features between FAS & controls

Predictor & outcome variables	Controls (n=90)	FAS (n=90)	p value
Palpebral fissure length/inner canthal distance ratio			**< 0.0001**
Mean (SD)	0.86 (0.07)	0.77 (0.07)	
Minimum-maximum	0.68-1.00	0.58-0.95	
Philtrum 5-point Likert scale	**n=90**	**n=90**	
deeply furrowed	8 (8.8)	0 (0)	
somewhat furrowed	24 (26.6)	2 (2.2)	
mid range	31 (34.4)	13 (14.4)	
somewhat smooth	26 (28.8)	15 (16.6)	
very smooth	1 (1.1)	60 (66.6)	
Upper lip 5-point Likert scale	**n=90**	**n=90**	**< 0.0001**
very thick	42 (46.6)	2 (2.2)	
somewhat thick	23 (25.6)	2 (2.2)	
mid range	18 (20.0)	42 (46.6)	
somewhat thin	7 (7.8)	34 (37.8)	
very thin	0 (0)	10 (11.2)	

As seen in the bar graphs below (Figure 23) the FAS children had smooth philtrums and very thin upper lips whereas children in the control groups had furrowed philtrums and thicker upper lips.
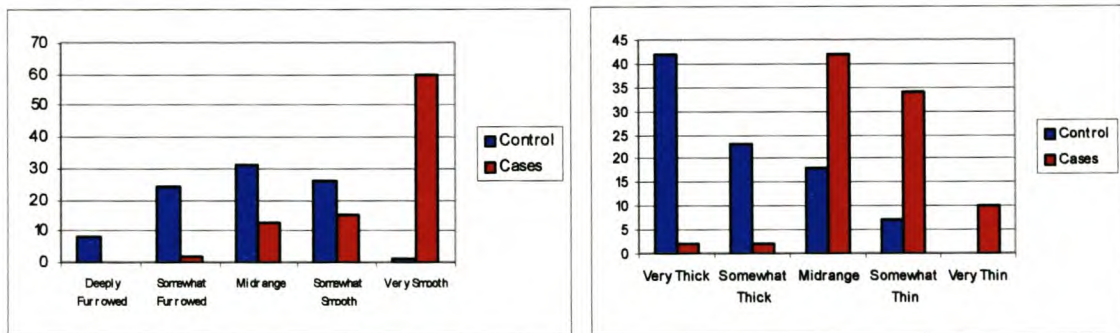
Figure 23: Bar graphs showing philtrum and lip smoothness, based on the Likert ordinal scale data shown in Table 15.

All three diagnostic measurements were influenced by age, but because abnormal diagnostic measurements become less pronounced as the child gets older, these measurements were standardized for age (PFL_st, IPD_st, ICD_st). It was for this reason that age was not included as a variable in the model, as it is already accommodated in the model using standardized variables. Both IPD and ICD were highly correlated (Table 16), but only one of the two was required for the model and ICD was chosen as it was less correlated with both HC and PFL than IPD, which was more highly correlated with both these variables. IPD_st was therefore not entered into the model.

To graphically show correlation coefficients, scatter diagrams are used in deciding whether any relationship existed between two variables, where the values of one variable are plotted against the other variable. If there is a strong correlation, there is an obvious straight-line relationship apparent with little "scatter", while the weaker the relationship the more the scatter increased.

As seen from the scatter plot matrix (Figure 24) the spread and distribution of PFL_st for the two groups are different, especially when one compares the HC_st and ICD_st where the spread and distribution in both groups were found to be similar.
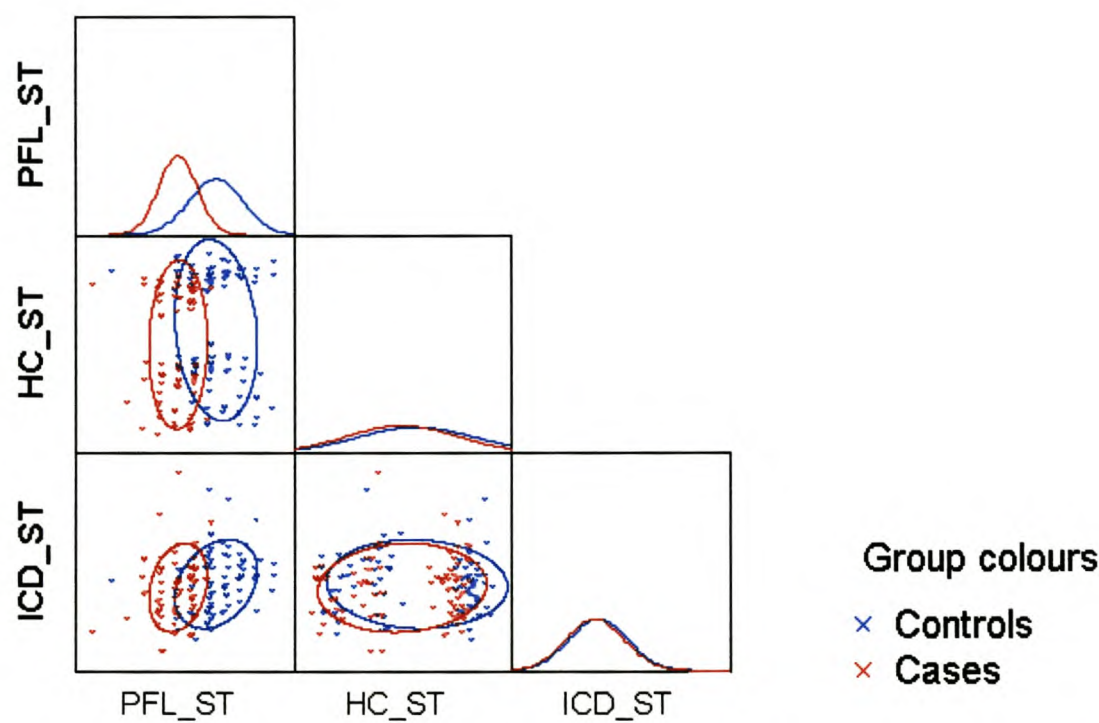
Figure 24: Scatterplot distribution of PFL_st palpebral fissure length (PFL_st), head circumference (HC_st) and inner canthal distance (ICD_st)

Table 16: Spearman Correlations: FAS and controls

Case	PFL_st	IPD_st	ICD_st	HC_st	Philtrum	Lip
Control						
PFL_st		0.5172	0.2591	0.1032	-0.0450	0.1091
IPD_st	0.6737		0.7678	0.1240	0.1714	0.1712
ICD_st	0.3798	0.7607		0.0601	0.1796.	0.1087
HC_st	-0.1151	-0.0447	-0.0521		0.1125	0.0817
Philtrum	-0.0685	-0.0814	-0.0859	-0.0260		0.4717

With regard to the statistical method used, it was irrelevant as to whether a logistic regression or discriminant analyses was used as the results would have been the same, however logistic regression was preferable. The reason for this choice is that the discriminant function approach in the estimation of the logistic coefficient is based on the assumption that the independent variables are multivariate normal in each of the sample groups. However, the estimations could be biased when normality does not hold.

Table 17: Logistic Regression: final model

	Parameter estimates	**Standard errors**	**p-value**
PFL-st*	43.21	9.22	<0.0001
HC-st	5.51	2.27	0.0150
Philtrum**	-1.49	0.44	0.0010
Lip**	-0.94	0.38	0.0134

The five variables (PFL_st, ICD_st, HC_st, lip thinness and philthrum smoothness) were entered in the logistic regression model. The final model (Table 17) excluded ICD_st and had an $r^2 = 0.6165$, indicating that this model explains 61.65% of the variation in the data. According to the Student's t-test, PFL_st differed significantly (p<0.0001) between the two groups (*) and according to the x^2 test, both philtrum and lip thinness were dependent on the group to which the child belonged (p<0.0001) (**).

As cases and control were matched for gender and age, a conditional logistic regression analysis for matched case-control studies (based on Cox Proportional Hazards Model) was also performed to investigate the relationship between the outcome (case or control) and the set of prognostic factors (PFL_st, ICD_st, HC_st, philtrum, lip). Based on the Wald test for individual variables, PFL_st, HC_st, philtrum and lip thinness were statistically different. These results were exactly the same as those produced by standard logistic regression.

From this part of the study, we were able to conclude that it was possible to identify a minimum cluster of facial features to assist with the diagnosis of FAS children and these features were the palpebral fissure length, head circumference, a smooth philtrum and a thin upper lip.

6.4   Discussion

This study focuses on the craniofacial, and oral and dental manifestations of the FAS. A total of 90 FAS cases and 90 controls were matched for age, gender and social class and formed the sample of this study (Table 8). There were no significant age differences between the two groups (p=0.3363) and the age ranges for both the groups were the same. The mean age of the cases was 8.9 years with the controls slightly older at 9.1 years. Owing to the selection and matching, there was an equal gender distribution across the cases and controls with 45 males and 45 females in each group with a matched pair.

6.4.1   Anthropometry

Forty two per cent of the FAS sample manifested growth retardation and this was statistically significant (p<0.0001) when compared to their controls (Table 12). Children with FAS commonly have a low birth weight and remain small for their age. This study confirms the findings of Jones et al (1974) that the intrauterine growth retardation results in weights, lengths and head circumferences below the tenth per centile. The occurrence of compensatory (catch-up) growth has been debated. Jones et al (1973) reported the absence of linear and head circumference compensatory growth, and Hanson et al (1976) concurred with this view. However, Lemoine et al (1968) suggested that it might improve in later childhood. Jackson and Hussain (1990) showed that compensatory growth in stature, weight and head circumference can take place in some circumstances. They also showed that there was a delay in bone age. This was in disagreement with Clarren and Smith (1978) who stated that bone age is usually normal in FAS. Hanson et al (1976) reported that postnatal weight gain was poor, becoming underweight for length; however, the Jackson and Hussain (1990) study suggested that there is a long-term tendency to gain weight more than stature. Adipose tissue is especially reduced.

Based on the anthropometry results shown in Table 9 (page 152), the FAS children weighed significantly less than the controls, were shorter and had significantly smaller head circumferences. This corresponds well to what is found in the literature (Majewski, 1996; Spohr et al, 1993; Streissguth et al, 1991a). Low weight-for-age indicates a state of acute malnutrition of a short- term nature while low height-for-age indicates stunted growth which reflects a process of failure to reach linear growth potential as a result of sub-optimal health and/or nutrition conditions. On a population basis, high levels of stunting are associated with poor socio-economic conditions and the increased risk of frequent and early exposure to adverse conditions such as illness and/or inappropriate feeding practices (WHO, 1997); however, the matching for gender and age in this study would have negated these factors.

Head circumference differed significantly between the two groups (p<0.0001) (Table 9) and the three photographic diagnostic measurements (palpebral fissure length, inter-pupillary distance and inner canthal distance) were all influenced by head circumference (Figure 24).

Results from this study corroborate previous observations that individuals diagnosed with FAS exhibit, on average, small heads (microcephaly), small faces, short palpebral fissures and a hypoplastic face (Lemoine et al, 1968; Jones and Smith 1973; Hanson et al, 1976; Clarren and Smith, 1978; Korányi et al, 1981; Abel, 1984; Vitéz et al, 1984; Day et al, 1989; Jackson and Hussain, 1990; Astley et al, 1992; Aase, 1994; Astley and Clarren, 1995, 1996). This study also confirms that the effects of prenatal alcohol exposure on the face fall along a continuum instead of occurring as discrete traits.

Slow growth in head circumference usually indicates slow brain growth (Astley and Clarren, 2001, Majewski, 1996). Microcephaly, resulting from abnormal glial migrations and diminished white matter is often the earliest sign of central nervous system involvement. The enlargement of head circumference and corresponding brain weight is an important goal of medical care in children with FAS.

In the present study, there was a statically significant correlation between weight and head circumference for both the cases and the controls. Controls were heavier and had markedly larger head circumferences when compared to the FAS children. Majewski (1996) studied the influence of weight on head circumference in FAS patients and found that heavier children had markedly larger head circumferences, reflecting possible correlation between weight and brain growth. However, those differences were nearly the same as at birth, and the catch-up growth of head circumference in heavier children was not observed to be greater than in underweight children and so he concluded that his patients with the greater weight did not have better brain growth. Nevertheless, it is recommended that head circumference should be increased by early and high-calorie feeding especially in the first six months of life. Increased head circumference parallels increased brain growth (Brandt, 1981) and probably parallels improved mental development as a result of increase in myelinization and growth of dendrites and synapses.

6.4.2 General clinical features

Facial anomalies such as maxillary hypoplasia, smooth philthrums, ears and thin upper lips are commonly reported in the FAS literature (Abel, 1984). In this study, forty four per cent of the FAS sample manifested some form of structural abnormality and this was statistically significant (p<0.0001) when compared to their controls (Table 13).

6.4.2.1 Craniofacial dysmorphology

It has been suggested that the length of the nose is related to the length of the philtrum (Aase, 1990). The length of the face is determined by the maxilla. Since only the nose and the philtrum occupy this area of the central face and growth of both is determined by the same bone, it is thought that a change in length of one usually results in a compensatory change in length of the other. Therefore in most cases, a short nose results in a long philtrum and vice versa (Aase, 1990).

The present study concurred with the many clinical descriptions indicating that FAS individuals have widely-spaced eyes and a long philtrum (Clarren and Smith, 1978; Abel, 1984; Vitéz et al, 1984; Day et al, 1989; Jackson and Hussain, 1990; Aase, 1994). In fact, FAS children in this study had statistically significantly longer philtrums than their controls (Table 16). Although the craniofacial dysmorphological features in growing FAS children changes its distinctive expression, a number of facial symptoms persist in adolescence and remain useful diagnostic features after puberty: short palpebral fissures, smooth philtrum and in particular, a thin upper lip and a small head circumference (Streissguth et al. 1991b; Spohr, 1996).

6.4.2.2 Infrequent malformations and fluctuating asymmetry

In addition to the characteristic dysmorphic facial features, FAS children were found to have anomalous palmar creases and limited supination (Table 13). In bilaterally symmetrical structures, antimeres should ideally develop as mirror images of one another, largely because the genetic information for both sides is the same (Potter and Nance, 1976). The degree of phenotypic similarity reached by paired organs is governed by the epigenetic processes that operate during morphogenesis (Alberch, 1982; Katz, 1982; Cheverud, 1984). Failure of this system in the face of environmental disturbances (referred to as "developmental noise") results in fluctuating asymmetry, the magnitude of which may be used as a measure of individual developmental stability (van Valen, 1962).

Assessment of fluctuating asymmetry has long been utilized to estimate the degree of environmental or genetic stress in the developing embryo (Hoyme, 1993; Kieser and Groeneveld, 1988). In the practice of dysmorphology, fluctuating asymmetry has not been commonly utilized as a marker for assessing abnormal human morphogenesis. Rather the presence or absence of minor anomalies is utilized to assess the degree of developmental instability in the embryo.

A minor anomaly is defined as an unusual morphologic feature found in less than 45% of the general population, which is of no serious medical significance to the affected individual (Jones, 1988). They may be *malformations* (structural defects arising from intrinsically abnormal developmental processes), *deformations* (abnormal structures resulting from non-disruptive mechanical forces applied to a once normally formed part, or *disruptions* (structural defects) arising from destruction of a once normally formed part) (Spranger et al, 1982). Minor anomalies have been used clinically in dysmorphology in a variety of ways: firstly, some minor anomalies may be the external 'red flags' indicating the presence of specific occult major anomalies; secondly, patterns of minor anomalies constitute the majority of features by which multiple malformation syndromes occur and lastly, three or more minor anomalies may be a non-specific indicator of an occult major anomaly (Hoyme, 1993).

A variety of ear malformations in the FAS children were found in this study (Table 14). Church and Gerkin (1988) and Church et al (1997) found high rates of peripheral and central hearing disorders in FAS children. These rates exceeded those reported for the general paediatric population (Coles et al, 1991), but were similar to and even exceeded the rates observed in other groups of children with craniofacial anomalies (Downs et al, 1981). The anthropometric configuration of the face in a child with FAS results in a "shallow angle to the eustachian tubes", discouraging middle ear drainage. Hence, these may be conductive hearing loss due to effusion or infection and sensorineural hearing loss (due to direct teratogenic damage) in children with FAS.

Other types of hearing disorders associated with prenatal alcohol exposure include delayed maturation of the peripheral and brainstem auditory pathways and a high incidence of intermittent conductive hearing loss (Streissguth et al, 1985). FAS patients frequently suffer from various language and speech disorders, such as poor receptive and expressive language skills, slurred and monotonous speech, articulation and fluency problems (Streissguth et al, 1985). Central and peripheral hearing disorders, as well as dentofacial defects and mental impairment, may contribute to these language and speech disorders. In this study, FAS children had statistically significantly more dentofacial anomalies than the control children.

Abnormalities of the limbs and joints are seen much more frequently in children with FAS than the general population and this is shown in the present study where findings related to the infrequent malformations and anomalies for example, clinodactyly, camptodactyly and abnormal palmar creases were all statistically significant when compared with the controls (Table 13, page 155). These findings were also reported by Aase (1994).

Abnormalities of facial structures with embryonic origins in the cranial neural crest appear to share common causal pathways, including the teratogenic effects of ethanol. Craniofacial malformations and clefts are well known in animal models of ethanol-induced teratogenesis. Kotch and Sulik (1992) observed a pattern of excessive cell death at the rim of the anterior neural plate in mouse embryos within 12hr of ethanol exposure and have proposed that early insult to selected cell populations may account for some of the human malformations.

Clefts are likely to have multiple genetic and environmental causes. Evidence of gene-environment interactions in the aetiology of clefts have emerged (Hwang et al, 1995), and it is possible that maternal alcohol exposure interacts with genetic or environmental factors. It has been proposed that ethanol acts as a competitive inhibitor of ADH-catalysed retinal oxidation in embryonic tissues, resulting in disruptions in the distribution of retinoic acid needed to specify spatial patterns (Duester, 1991; Duester et al, 1991).

The relationship in humans between maternal alcohol exposure and clefts remains unclear. Niebyl et al (1985) found no association between maternal alcohol use and clefts, but their study had limited statistical power with only 59 cases. However, it has been documented that alcohol use during pregnancy may be a cause of isolated cleft lip with or without cleft palate. Werler et al (1991) found a threefold elevated risk of cleft lip with or without cleft palate in infants whose mothers reported an average of five or more drinks per drinking day; this exposure level is not easily interpreted and few of the mothers were in the highest level of exposure.

Munger et al (1996) reported that maternal alcohol use during pregnancy was found to be associated with an increased risk of isolated cleft lip without or without cleft palate. When compared to women who did not drink alcohol during pregnancy, the relative odds of isolated offspring having a cleft lip with or without cleft palate rose with increasing level of maternal drinking as follows: 1-3 drinks per month, 1.5; 4-10 drinks per month, 3.1 and more than 10 drinks per month, 4.7 (chi-squared test for trend, p=0.003). Adjustment for maternal smoking, vitamin use, education and household income did not substantially alter these results. No significant association was found between alcohol use and isolated cleft palate or clefts in children with multiple birth defects. Clefts appear not to be a major feature of the FAS, although they may occasionally be present, but clefts may be part of the broader spectrum of the more common and less severe fetal effects of maternal alcohol use (Majewski, 1996).

Despite the fact that in this present study no children were seen with orofacial cleft birth defects possibly because of the small sample size, but in view of the graded increase in the risk of isolated cleft lip with or without cleft palate with increasing amounts of alcohol consumed (Munger et al, 1996), it would be prudent to consider that there may be no safe level of alcohol consumption during pregnancy.

Women in their reproductive years face a dilemma regarding their decision to drink alcohol or not because the susceptible period for many of the teratogenic effects of alcohol may be during the first few weeks of pregnancy, before many women realize they are pregnant. The hazards of alcohol consumption should be frankly discussed with all women who may become pregnant; this information makes a strong case for the expansion of preconceptual counselling.

6.4.3 Photographic analysis

Craniofacial anthropometry has been used to assess abnormal craniofacial variation in five craniofacial syndromes (Ward et al, 2000), as well as to describe the facial phenotype in conditions such as Apert syndrome (Farkas et al, 1985), Down syndrome (Allanson et al, 1993) and Treacher-Collins syndrome (Kolar et al, 1985). Craniofacial anthropometry has also been used in the clinical setting as a diagnostic aid (Goldstein et al, 1988) and as a means of objectifying clinical descriptions of individual patients (Ward and Goldstein, 1987).

A key factor to the success of this part of the study was the quality of the photographic images. All the photographs were taken by a professional photographer using a handheld camera. Obtaining a quality photograph does not require sophisticated equipment or expertise. One need only focus on four elements: proper alignment, proper exposure, focal length and a relaxed facial expression (Farkas, 1994). Recording facial measurements indirectly from a photograph rather than directly from the face has several advantages. By capturing a photographic image, all three key facial features can be recorded on objective, continuous scales. This approach maximises measurement accuracy and precision.

Photographic analysis also has the following advantages: (i) photographic images are inexpensive, (ii) professional expertise or sophisticated equipment is not needed for collection (iii) photographs can be stored and analysed with complete anonymity by cropping the images, (iv) data can be transferred electronically for centralised analysis, maximizing consistency of interpretation; and (v) population based surveillance of more representative segments of the population could be achieved because a broad age range can be accurately assessed and surveillance need not be restricted to hospital or research institutions, because of the ease with which data can be collected. Analysis of the face using anthropometry both supports many of the previous clinical descriptions of the effects of neonatal alcohol exposure and offers some new perspectives on the FAS facial phenotype. Characteristic dysmorphic facial features found included ptosis of eyelids, epicanthic folds, short upturned nose, thin vermillion border of the upper lip and a smooth philtrum.

No two individuals with FAS necessarily had identical facial features; all however, have the overall gestalt. To define gestalt, we took a multivariate approach. The discriminant analysis used in this study accomplished this by identifying both the ideal minimum number of features and the magnitude of expression of each feature that most accurately differentiated individuals with and without the facial gestalt.

The discriminant analysis between the FAS and the control group (Table 17) corroborates previous observations (Astley and Clarren, 1995, 1996; Moore et al, 2001; 2002) which identified short palpebral fissures, smooth philtrum and thin upper lip as the minimum cluster of features needed to define the phenotype and differentiate individuals with highest accuracy. The pattern profile of the eye measurements for the FAS group (Table 14, page 157) supports earlier findings. FAS individuals appear to have widely spaced eyes (Abel, 1984; Clarren et al, 1987; Astley et al, 1992; Abel et al, 1993; Aase, 1994; Astley and Clarren, 1995, 1996; Strömland et al, 1999), but this is really due to the shortened palpebral fissures.

This finding is confirmed by Strömland et al (1999) and Moore et al (2002), that it is not the eyes that are abnormally widely spaced, but rather that the palpebral fissures do not extend out as far and thus the biocular breadth is reduced. The clinical impression of widely spaced eyes may be further enhanced by the fact that a near normal-sized interocular breadth is present in individuals with small heads and face, adding to the impression of a large distance between the eyes in relation to the rest of the face.

In this study, the FAS group had a high degree of phenotypic variability which was greater than that found in the control group. Such variability supports what is known about the complexity of ethanol toxicity as suggested by results derived from experimental animal and human studies. These studies have shown that the susceptibility to FAS or other alcohol-related birth defects depends on fetal and maternal factors such as genetic predisposition, degree, pattern and timing of alcohol exposure, and indirect maternal effects such as poor nutrition and other drug use (see Chapter 4).

Some researchers have found acute maternal alcohol exposure in mice will induce different craniofacial anomalies in offspring depending on the gestational day of alcohol exposure and the period of exposure (Randall and Taylor, 1979; Webster et al, 1980, 1983; Sulik et al, 1981, 1984, 1986, 1988; Sulik and Johnston, 1983; Blakley, 1988; Kotch and Sulik, 1992). Several other researchers (Coles et al, 1985; Ernhart et al, 1987; Rostand et al, 1990; Autto-Rämö et al, 1992) found a correlation between the amount of alcohol consumed during the first trimester of pregnancy and the severity and quantity of craniofacial anomalies. Therefore results of animal and human studies suggest that the greater variability found in the FAS group as compared to the control group, as is reported in this study, may in part be explained by the timing and degree of prenatal alcohol exposure. A difference of a few weeks in the timing of exposure could produce variations in sizes, shapes and proportion traits such as the philtrum, palpebral fissures and midface.

The search for the underlying mechanism(s) responsible for the craniofacial anomalies associated with prenatal alcohol exposure has been complicated by the variability of expression of the craniofacial features involved, the many factors that may play a role in alcohol's teratogenic effects, and the likely involvement of numerous dysfunctional neurochemical and biochemical processes.

While results from this study support the presence of great variability in the craniofacial phenotype of individuals affected by prenatal alcohol exposure, evidence presented here suggests that the resulting phenotype may be identified. This study supports the use of anthropometry for expansion of the measurements used to determine the FAS phenotype. In each child with intrauterine and postnatal growth retardation, microcephaly and a dysmorphic face, the tentative diagnosis of FAS is the most probable, because FAS is rather frequent, and all other types of intrauterine growth retardation are rare or very rare (except trisomy 18).

However, some researchers have called for finding a substitute for using the FAS face as an indicator of fetal-alcohol-affected individuals as there are too many non-FAS individuals affected by prenatal exposure that go unidentified (Sampson et al, 2000). There is evidence to show that the problem may not be in the use of the face in identifying these individuals, but rather in thedifferent types of methods used to assess the face for dysmorphology (Astley and Clarren, 1996).

While the face may not be the best source for diagnosing alcohol-affected individuals, it can serve as a way of identifying children with high risk of having alcohol effects and therefore can be used as a quick and easy screening tool. Once these children have been identified, it will then be possible to use the more sophisticated diagnostic methods as described by Astley and Clarren (1997; 2000), and more sophisticated analyses to define the extent of the effects from alcohol exposure as suggested by Sampson et al (2000).

Surveillance generally uses methods distinguished by their practicality, uniformity and their rapidity of use. To date, effective methods for FAS surveillance do not exist. Passive surveillance such as hospital-based birth defects registries examine only the medical record, not the patient, and focus on an age group when FAS is known to be misdiagnosed and under-reported (Little et al, 1990). Passive surveillance has resulted in marked underestimation of FAS prevalence. In contrast, active surveillance relies on the direct collection of data from patients and, results in more valid and reliable data and in estimated rates of FAS that are an order of magnitude higher than those estimated from passive surveillance (Little et al, 1990). The results of this study demonstrated that facial photographs have the potential for serving as a highly efficient, reproducible and potentially highly accurate active surveillance tool.

6.5   Concluding remarks

This chapter described the demographic, anthropometric, and general clinical findings including the results of the photographic examination. By study design there were no significant age differences between the two groups in the sample. The results of the anthropometric measurements in respect of weight, height and head circumference all showed significantly lower values for the FAS children when compared to the controls.

Almost all of the physical abnormalities described in children with the FAS were seen in this study. These abnormalities included deformities in the small joints of the hand, altered palmar creases and malformation of the ears.

While using the face to identify alcohol-affected individuals will not determine the neuroanatomical and neurobehavioral deficits, the data from this study suggest that craniofacial anthropometry still has a place in the field to quickly and reliably identify children with subtle alcohol effects. This study has shown by statistical analysis that an ideal minimum cluster (palpebral fissure length, head circumference, a smooth philtrum and a thin upper lip) of clinician-friendly measurements may be utilized more broadly in the diagnostic field to differentiate individuals with the highest accuracy.

Chapter 7: Oral Health Status - Soft and Hard Tissues

7.1 Introduction

This part of the study examined the soft and hard tissues of the mouth and determined the oral health status by examining the amount of plaque and gingival bleeding in each child. With regard to the hard tissues, enamel opacities, tooth anomalies, dentofacial anomalies and the dental caries status were assessed. The results and discussion are presented in this chapter.

7.2 Results

7.2.1 Plaque scores

More than three quarters of both the cases and the controls demonstrated the presence of plaque (Table 18). There was no significant difference between case and control.

Table 18: Plaque (present or absent)

	Yes n (%)	No n (%)	Total n (%)
FAS	81 (90)	9 (10)	90
Control	76 (84.4)	14 (15.6)	90
Total	157 (87)	23 (13)	180

7.2.2 Gingival bleeding

Almost two thirds of both case and control groups had gingival bleeding on probing (Table 19). There was no significant difference between the two groups.

Table 19: Frequency of gingival bleeding

	Yes n (%)	No n (%)	Total n (%)
FAS	63 (70)	27 (30)	90
Control	59 (65.5)	31 (34.5)	90
Total	122 (68)	58 (32)	180

7.2.3 Oral mucosal lesions

The prevalence of oral mucosal lesions between FAS and controls was not statistically significant and ranged from 12% for the control group to 21% for the cases (Table 20). Overall, 16.6% of the sample had one or more oral lesion. The lesions seen were dentoavleolar abscesses largely related to carious teeth and traumatic lesions. Other lesions included ulcers (herpetic and aphthous) and angular cheilitis.

Table 20: Oral soft tissue lesions (present or absent)

Feature	Absent	Present	Total
	n (%)	n (%)	
Control	79 (87.8)	11 (12.2)	90
Case	71 (78.9)	19 (21.1)	90
Overall prevalence	16.6%		

7.2.4 Enamel opacities

The modified developmental defects of enamel (DDE) index was used to describe the enamel opacities (WHO, 1997). Enamel abnormalities were classified into one of three types on the basis of their appearance. They varied in their extent, position on the tooth surface and distribution within the dentition. The prevalence of enamel opacities between FAS and controls was not significant and was approximately 15% for both groups (Table 21). The opacities were seen largely in the maxillary central incisor and lower first molar teeth. Most of the opacities were demarcated, diffuse or of the hypoplastic variety. There was no significant difference between the cases and the controls.

Table 21: Frequency of enamel opacities

	Yes	No	Total
	n (%)	n (%)	n (%)
FAS	14 (15.5)	76 (84.5)	90
Control	15 (16.6)	75 (83.4)	90
Total	29 (16)	151 (84)	180

7.2.5 Dentofacial anomalies

Dentofacial anomalies were investigated in respect of crowding of incisors, spaces between incisors, presence of diastemas, maxillary overjets, mandibular overjets and openbites (Table 22). The cases had statistically significantly (p<0.001) more dentofacial anomalies than the controls and these occurred most commonly as crowding of the incisors, maxillary overjet and openbite.

Table 22: x^2 frequency of dentofacial anomalies

Feature	Crowded incisors	Spaced incisors	Diastema	Maxillary overjet	Mandibular overjet	Openbite
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Control (n=90)	1 (16.7)	2 (66.7)	1 (33.3)	6 (33.3)	5 (62.5)	7 (31.2)
Case (n=90)	5 (83.3)	1 (33.3)	2 (66.7)	12 (66.7)	3 (37.5)	15 (68.8)
Total	6 (100)	3 (100)	3 (100)	18 (100)	8 (100)	22 (100)
p value	<0.0001					

7.2.6 Tooth and eruption disturbances

Congenitally absent teeth were seen in five of the FAS children and two of the controls (Table 23). Of the five FAS children, four had congenitally absent premolars and one a congenitally missing lateral incisor. The control children had a second premolar and first permanent molar absent. In addition to the missing teeth, three FAS children presented with a transposed central incisor (21), "peg laterals" and taurodontic mandibular first molars. There were no significant differences between the cases and the controls.

Table 23: Tooth disturbances

Feature	Disturbance present	Absent teeth	Absent laterals	Absent premolars	Absent molars	Other
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Control (n=90)	2 (20)	2 (29)	0 (0)	1 (20)	1 (100)	0 (0)
Case (n=90)	8 (80)	5 (71)	1 (100)	4 (80)	0 (0)	3 (100)
Total	10	7 (100)	1 (100)	5 (100)	1 (100)	3 (100)

7.2.7   Caries status

7.2.7.1 Caries experience

To determine caries status, the DMFT index was used. This index scored the number of decayed, missing and filled teeth (dmft index) and the Decayed, Missing and Filled teeth (DMFT index) for the primary and permanent dentition respectively (WHO, 1997). It is common to record the subdivisions of the indices separately so that they may be compared independently. This may reveal information on the change in the ratio of the components which may be related to such factors as availability of and attitude to treatment.

A summary of the overall caries status of the cases and controls is shown in Table 24. Thirty nine children (21.67%) of the total sample were caries-free. In the following tables, DMFT and dmft refer to the permanent and the primary dentition caries status respectively.

Table 24: Summary of caries status

Characteristic	Control (n=90)	FAS (n=90)	
dmft	Mean (SD)	Mean (SD)	p value
d	1.56 (1.84)	1.79 (1.93)	0.4085
m	0.02 (0.14)	0	0.1585
f	0.06 (0.43)	0	0.2272
dmft	1.63 (1.91)	1.79 (1.93)	0.5880
DMFT	Mean (SD)	Mean (SD)	p value
D	0.90 (1.37)	0.71 (1.06)	0.3020
M	0.03 (0.23)	0.01 (0.10)	0.4140
F	0.01 (0.10)	0.04 (0.42)	0.4686
DMFT	0.94 (1.40)	0.77 (1.1)	0.3454

The mean dmft score for the FAS sample was slightly more, though not significantly different, than that of the controls and the decayed component (d) made up the largest part of the index in both groups (Table 25). None of the FAS children had any missing teeth nor filled teeth, and in the case of the controls this was also rarely found.

Table 25: Mean dmft index of 90 cases and 90 controls

	decayed		missing		filled		dmft score	
	FAS	Con	FAS	Con	FAS	Con	FAS	Con
Mean	1.79	1.56	0	0.02	0	0.06	1.79	1.63
SD	1.93	1.84	0	0.15	0	0.43	1.93	1.91
Min Score	0	0	0	0	0	0	0	0
Max Score	7	9	0	1	0	4	7	9

The mean DMFT score for the FAS sample was slightly lower, and not significantly different from than that of the controls and the decayed component (D) made up the largest part of the index in both groups (Table 26). Missing and filled teeth were rarely found in both groups.

Table 26: Mean DMFT index of 90 cases and 90 controls

	Decayed		Missing		Filled		DMFT Score	
	FAS	Con	FAS	Con	FAS	Con	FAS	Con
Mean	0.71	0.9	0.01	0.03	0.04	0.01	0.77	0.94
SD	1.06	1.37	0.11	0.23	0.42	0.11	1.11	1.39
Min Score	0	0	0	0	0	0	0	0
Max Score	3	7	1	2	4	1	4	7

7.2.7.2 Caries experience according to age

The dmf scores for both case and controls, at various age intervals, are summarized in Table 27. From this table it can be seen that there is no discernable difference between the dmft of the cases and controls for the various age groups.

Table 27: Mean dmft index of 90 cases and 90 controls in age frequencies

	decayed		missing		filled		dmft score	
Age	FAS	Con	FAS	Con	FAS	Con	FAS	Con
7 < 8	2.6	2.0	0	0.70	0	0.13	2.60	2.20
p value	0.18		0.16		0.32		0.40	
8 < 9	2.05	2.57	0	0	0	0.05	2.05	2.62
p value	0.33		-		0.32		0.30	
9 <10	1.87	1.53	0	0	0	0	1.87	1.53
p value	0.53		-		-		0.52	
10<11	0.56	0.17	0	0	0	0	0.56	0.17
p value	0.14		-		-		0.14	
11<12	0.33	0	0	0	0	0	0.33	0
p value	0.36		-		-		0.36	

The mean DMFT scores at various age intervals are summarised in Table 28. From this it can be seen, as expected, that there is a gradual increase in the DMFT score with increasing age. The trend for the DMFT scores for the controls are higher and increasingly so with age, when compared to the cases, though it is not statistically significant.

Table 28: Mean DMFT index of 90 cases and 90 controls in age frequencies

	Decayed		Missing		Filled		DMFT score	
Age	FAS	Con	FAS	Con	FAS	Con	FAS	Con
7 < 8	0.43	0.43	0	0	0	0	0.43	0.43
p value	1.00		-		-		1.00	
8 < 9	0.57	0.67	0	0.10	0	0	0.57	0.76
p value	0.77		0.32		-		0.57	
9 <10	0.93	1.27	0	0	0	0	0.93	1.27
p value	0.46		-		-		0.46	
10<11	1.06	1.06	0	0.06	0.22	0	1.28	1.11
p value	1.00		0.33		0.33		0.75	
11<12	1.00	2.67	0.17	0	0	0.17	1.17	2.83
p value	0.24		0.36		0.36		0.24	

7.2.7.3 Caries experience according to gender

Table 29: Mean dmft index of 90 cases and 90 controls according to gender

	decayed		missing		filled		dmft score	
	FAS	Con	FAS	Con	FAS	Con	FAS	Con
Male	2.18	1.53	0	0	0	0.11	2.18	1.64
Female	1.40	1.58	0	0.04	0	0	1.40	1.62

The dmft score for the FAS males was 2.18 compared to 1.64 of the controls and 1.40 for the FAS females compared to 1.62 for their controls. The mean decayed teeth values made up the greatest proportion of this score in both sexes and the mean for FAS males was higher (2.18) than that for the FAS females (1.40) (Table 29). Both the missing and filled scores were zero for both the FAS males and females and were consequently disregarded for further comparisons. The differences between the d and dmft between the males and females were not found to be statistically significant (Table 30).

Table 30: dmft -Wilcoxon's 2 Sample Test

Gender Variable	d	dmft
Males: FAS vs Control	0.1933	0.2911
Female: FAS vs Control	0.9625	0.9251
FAS: Male vs Female	0.0691	0.0691

Table 31: Mean DMFT index of 90 cases and 90 controls according to gender

	Decayed		Missing		Filled		DMFT score	
	FAS	Con	FAS	Con	FAS	Con	FAS	Con
Male	0.60	1.07	0	0.04	0	0	0.60	1.11
Female	0.82	0.73	0.02	0.02	0.09	0.02	0.93	0.78

The DMFT score for the FAS males was 0.60 compared to 1.11 of the controls and 0.93 for the FAS females compared to 0.78 for their controls (Table 31). The differences between the DMFT, the D, M and the F components were not found to be statistically significant (Table 31), for cases and controls for males and females.

The mean decayed teeth values made up the greatest proportion of this score in both sexes and the D component in the FAS females was slightly higher (0.82) than that of the FAS males (0.60) and the female controls (0.73). Both the missing and filled scores were zero for the FAS males. The differences between the D and DMFT between the males and females were not found to be statistically significant (Table 32).

Table 32: DMFT -Wilcoxon's 2 Sample Test

Gender Variable	D	DMFT
Males: FAS vs Control	0.1148	0.0945
Female: FAS vs Control	0.6125	0.4157
FAS: Male vs Female	0.4013	0.2048

7.3   Discussion

7.3.1   Plaque scores and gingival bleeding

The levels of plaque and gingival bleeding were high and this was not an unexpected finding, since these children come from backgrounds where oral hygiene (toothbrushing) is not a high priority. It was surmised that due to the behavioural and neurological problems that children with FAS have, poor oral hygiene may have been a reflection of this; however, there is a threshold level beyond which one cannot discriminate.

7.3.2   Oral soft tissue lesions

The results of this examination showed that 16.6% of the children had one or more soft tissue lesions (Table 20). This figure is considerably lower than the figure (32.9%) reported by Arendorf and van der Ross (1996), who examined 1051 black pre-school children, aged between 18 and 80 months, in and around Cape Town. In this study, there was no difference between the FAS group and the controls. The dentoalveolar abscess was the most common lesion seen. Other lesions seen included angular cheilitis, herpetic and aphthous ulcerations.

7.3.3   Enamel opacities

The more premature the birth and the lower the birth weight, the higher the tendency for the child to from systemic derangements which can affect dental development adversely (Pimlott et al, 1985; KimSeow, Humphreys and Tudehope, 1978). Enamel hypoplasia is increasingly recognized as a biomarker of a range of systemic disturbances including intra-uterine malnutrition, maternal stressors and infectious diseases in the first years of life (Matee et al, 1994; Hargreaves et al, 1989, Pimlott et al, 1985). Various reports have documented enamel hypoplasia in children with FAS (Nelson et al, 1990; Glick, 1978; Winter, 1976).

The prevalence of enamel opacities in this study, ranged between 15 and16% for both groups. This concurs with what was reported by Hargreaves et al (1989) in a general population of children from the four ethnic groups (White, Indian, Coloured and Black), where hypoplasia ranged from 8.2-19.7%. "Coloured" children had a high prevalence of 16%. In the present study, the opacities were seen largely in the maxillary central incisors and lower first molar teeth. This too concurs with Hargreaves et al (1989) where maxillary central incisors showed the most enamel defects. Pimlott et al (1985) and Dummer et al (1986) described a much higher involvement of the maxillary than of the mandibular permanent teeth; but no satisfactory explanation has been reported.

7.3.4 Caries status

In this study the DMFT index was utilized because only an expression of caries experience in general was required. The method of examination and the criteria used for the index are in accordance with those of the World Health Organization (1997) and have been fully described in Chapter 6.

Overall the analysis of the caries data for this study in respect of differences between cases and controls was found to be unremarkable. The lack of difference in the primary and permanent dentitions between the cases and controls could have been anticipated in this population because of the high prevalence of dental caries among children from the Western Cape (DOH, 2002). The observed decline of caries experience in primary teeth with increasing age can be attributed to exfoliation of teeth. The dmft was relatively low, with a predominance of the decayed (d) component for both cases and controls. Together with an almost complete absence of any missing or filled teeth, this may be ascribed to the lack of access to care and possibly perceptions about oral health, and dietary as well as oral hygiene habits.

The DMFT overall for the controls (0.94) was higher, though not significant, than the cases (0.77). As expected, for both cases and controls there was an increase in DMFT with increasing age. However, the increments were larger for the controls. This is possibly due to delayed eruption of the permanent dentition in the FAS children, as shown by the difference in dental age between the cases and the controls (See Chapter 8: Tables 40 and 41).

It is difficult to explain the differences in dmft/DMFT between males and females, both in cases and controls. The dmft for the females was lower than that of the males. The reverse is true for the DMFT. This could be explained possibly by the early exfoliation of primary teeth and earlier eruption of permanent teeth in females compared to males (Demirjian et al, 1985).

Direct comparison of dmft/DMFT scores of this study with similar population groups in South Africa is problematic because of the lack of detail of sample selection, age frequencies and methods expressing caries experience in these studies. Generally, the dmft/DMFT scores were lower or similar to those found in comparable groups in the Western Cape (Hartshorne et al, 1993). In this study, the dmft scores for both the case and control were lower than 3 for the lowest age category (7-8 years) and much lower than the dmft obtained for Western Cape (5.78 SD 4.31). It should be noted however, that the data reflected in the Provincial sample only refers to 6 year olds. For the mean DMFT, the controls (2.83) and the Provincial (2.2 SD 2.54) 12 year old sample are similar.

7.3.5 Dentofacial anomalies

Prior observations on the dentofacial anomalies of FAS have included the presence of crossbite and overbite (Barnett and Shusterman, 1985; Webb et al, 1988), hypoplastic teeth and a high incidence of caries (Majewski and Goecke, 1982; Steinhausen and Spohr, 1986; Wood, 1977); delayed dentition and hypoplastic enamel (Jackson and Hussain, 1990). In this study, the cases had statistically significantly more dentofacial anomalies than the controls. The most frequently occurring anomalies were crowded incisors, maxillary overjet and openbites. When the author explored the possible reasons for these conditions with the children nearly all reported that they engaged in a thumb-sucking habit. In thumbsuckers the bases of the maxillae are on average anteriorly displaced and longer in comparison with controls. The incidence of malocclusion is also higher for thumbsuckers. The most common effects of a thumbsucking habit on the dentition are open bite, narrow upper arch and a crossbite (Linder-Aronson and Rølling, 1981).

Church et al (1997) observed in FAS patients high incidences of dental crowding, crossbite, overjet, delayed dentition, absent and displaced teeth and malaligned and rotated teeth. This could have been due to the fact that their patients were recruited from an Oral Cleft Centre at the Children's Hospital in Michigan and that 52% had some form of cleft palate. However, this was not the case in a study by Riekman (1984) who found anomalous dental conditions between the two groups (cases and controls) to be similar. In addition, the incidence of crossbite was also unremarkable.

Dentofacial anomalies were found to be more frequent in FAS patients, yet they often go unheralded and undescribed. These anomalies, because of their pronounced and prevalent nature, are important not only because they can influence speech, but because they may require orthodontics and plastic surgery and can aid with FAS diagnosis. The relationship between hearing, speech, language and dentofacial disorders in FAS patients need to be better documented and described, so as to aid diagnosis and treatment.

7.4   Concluding remarks

The findings from this chapter that described the health status of the hard and soft tissues of the mouth were not as noteworthy as was expected. This could be ascribed to the fact that the most vulnerable times for most of the developmental defects associated with the developing teeth occur during the first year of life (Hargreaves et al, 1989).

Chapter 8: Dental and Skeletal Age Assessment

8.1 Background

Dental maturity was determined by assessing the stage of tooth formation. This method is a reliable criterion for determining dental maturation (Fanning, 1962; Nolla, 1960; Hotz, Boulanger and Weisshaupt, 1959). In the present study, dental maturation was assessed from panoramic radiographs using Demirjian's seven teeth system (Demirjian, Goldstein and Tanner 1973) expressed as dental age in months. This method was chosen as the most precise and accurate evaluation of dental age (Hägg and Matsson, Staaf, Mörestad and Welander, 1991; Cameron, 1993) because its criteria consists of distinct details based on dental maturation and apexogenesis and proportion of root length. As expected, there were significant gender differences between both groups (cases and controls), for the dental and skeletal age assessments. Therefore, the results of this part of the study will be presented separately for the boys and girls.

8.2 Methods

Descriptive statistics were obtained by calculating the means and standard deviations of the chronological ages and dental ages for the stages of calcification. The Spearman rank order correlation coefficient was applied to measure the associations between skeletal maturity indicators, dental age, height, weight and head circumference. The Wilcoxon rank sum test was used as a non-parametric analogue to the paired-difference t-tests. Correlation coefficients were used to measure the degree of correlation between dental age, skeletal age and anthropometric measurements. The correlation coefficient varies between +1 and −1, the sign indicating whether the correlation is positive or negative. The nearer the correlation coefficient to +/-1, the stronger the correlation is said to be and the stronger the relationship between the two variables is. The nearer to zero a correlation coefficient is, the weaker the relationship between the two variables, until at zero there is no linear relationship.

To graphically show correlation coefficients, scatter diagrams were used in deciding whether any relationship existed between two variables, where the values of one variable are plotted against the other variable. If there is a strong correlation, there was an obvious straight-line relationship apparent with little "scatter", while the weaker the relationship the more the scatter increased.

8.3   Results
8.3.1   Dental age

Figure 25 below is a graph depicting the entire sample's dental age against chronological age in months. The line drawn through the graph represents where chronological age is the same as dental age. As can be seen, most of the controls (blue points) fall above the line indicating that their dental age is greater than their chronological age.
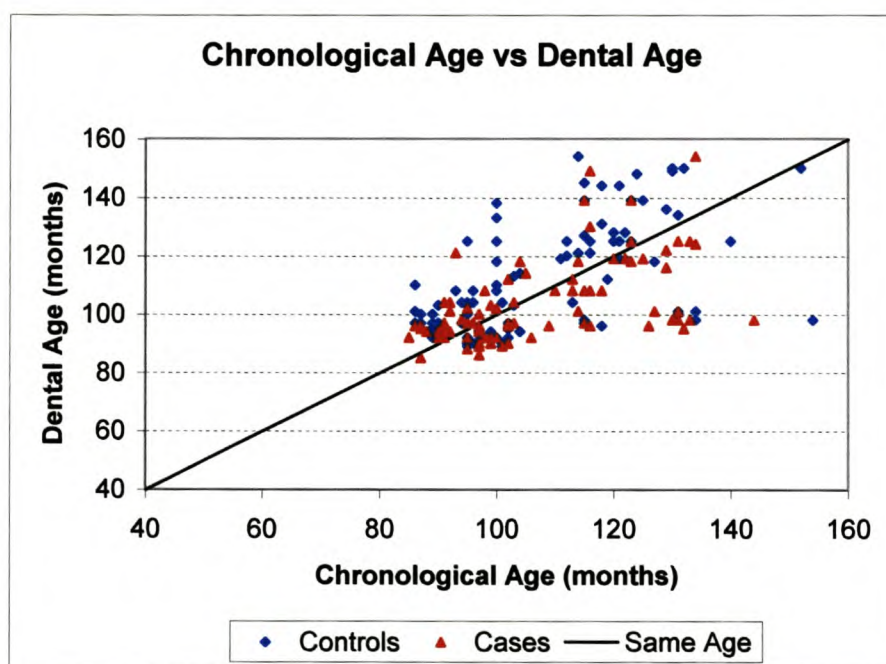
Figure 25: Chronological age vs dental age in months for entire sample (n=180)

The mean chronological age, the dental age and the dental delay score* of the boy cases and controls is shown in Table 33. It can be seen that for the boys, there are significant group differences for dental age vs chronological age between the cases and the controls (p = 0.027).

Table 33: Chronological age, dental age and dental delay score* in years (boys)

Years	FAS boys				Control boys			
	Mean	SD	Min	Max	Mean	SD	Min	Max
Chronological age	8.93	1.27	7.07	11.74	9.17	1.24	7.23	11.75
Dental age	9.10	1.24	7.50	12.80	9.96	1.47	7.60	12.80
Dental delay score*	0.17				0.79			
p value	0.027							

*Dental delay score = dental age – chronological age

The mean chronological age, the dental age and the dental delay score* of the girl cases and controls are shown in Table 34. It can be seen that for the girls, there is only a marginally significant group difference for dental age vs chronological age ($p = 0.0827$).

Table 34: Chronological age, dental age and dental delay score in years: Girls

Years	FAS girls				Control girls			
	Mean	SD	Min	Max	Mean	SD	Min	Max
Chronological age	8.94	1.32	7.27	11.1	9.06	1.34	7.37	11.3
Dental age	8.33	1.08	7.10	11.6	8.79	1.43	7.40	12.3
Dental delay score	-0.61				-0.27			
p value	0.0827							

*Dental delay score = dental age – chronological age

Using the Wilcoxon scores (rank sums) for dental age in boys (Table 35) it can be seen that there are significant intra-group differences in dental age, whereas for the girls (Table 36), there is only a marginally significant group difference.

Table 35: Group differences in dental age between cases and controls in years (boys)

Wilcoxon scores (rank sums)		
Boys (n=90)	Mean (years)	p value
Case (n=45)	9.10	0.0027
Control (n=45)	9.96	

Table 36: Group differences in dental age between cases and controls in years (girls)

Wilcoxon scores (rank sums)		
Girls (n=90)	Mean (years)	p value
Case (n=45)	8.32	0.0827
Control (n=45)	8.79	

From the tables and figures above, it can be seen that the dental age measurements done consistently show lower values for the FAS children when compared to the controls (Table 33 and 34). The dental age of the FAS boy cases (9.10 years) was significantly lower than for the controls (9.96 years) at a probability level of p=0.0027. Similarly, the FAS girl cases (8.32 years) dental age was lower than that of the controls (8.79 years).

8.3.2 Skeletal age

Figure 26 below is a graph depicting the entire sample's skeletal age against chronological age in months. The line drawn through the graph represents where chronological age is the same as skeletal age. As can be seen, most of the points for the FAS cases fall below the line indicating a retardation of skeletal age compared with chronological age.

Figure 26: Chronological age vs skeletal age in months for entire sample (n=180)

The mean chronological age, the skeletal age and the skeletal delay score* of the boy cases and controls are shown in Table 37 below. It can be seen that for the boys, there are significant group differences for skeletal age vs chronological age (p < 0.0001).

Table 37: Chronological age, skeletal age and skeletal delay score* in months (boys)

Months	FAS				Control			
	Mean	SD	Min	Max	Mean	SD	Min	Max
Chronological age	107.1	14.58	85.0	134.0	108.7	15.85	86.0	152.0
Skeletal age	84.5	14.69	48.0	120.0	99.2	15.65	72.0	132.0
Skeletal delay score	-22.6				-9.5			
p value	<0.0001							
*Skeletal delay score = skeletal age - chronological age								

Figure 27: Chronological age vs skeletal age in months (boys)

Figure 27 above is a graphic representation depicting the boy's skeletal age against chronological age in months. The line drawn through the graph represents where chronological age is the same as skeletal age. As can be seen, all the cases (red points) and most of the controls (blue points) fall below the line indicating that their skeletal age is lower than their chronological age.

The mean chronological age, the skeletal age and the skeletal delay score* of the girl cases and controls are shown in Table 38. It can be seen that for the girls, there are significant group differences for skeletal age vs chronological age (p < 0.0009).

Table 38: Chronological age, skeletal age and skeletal delay score* in months (girls)

Months	FAS				Control			
	Mean	SD	Min	Max	Mean	SD	Min	Max
Chronological age	107.6	15.97	86.0	144.0	108.7	16.18	86.0	154.0
Skeletal age	98.9	12.99	69.0	120.0	108.0	14.18	82.0	144.0
Skeletal delay score	-8.7				-0.7			
p value	0.0009							

*Skeletal delay score = skeletal age - chronological age

Using the Wilcoxon scores (rank sums) for skeletal age in boys (Table 39) and girls (Table 40) it can be seen that there were significant intra-group differences for skeletal age.

Table 39: Group differences in skeletal age: cases and controls in months (boys)

Wilcoxon scores (rank sums)		
Boys (n=90)	Mean	p value
Case (n=45)	84.5	<0.0001
Control (n=45)	99.2	

Table 40: Group differences in skeletal age: cases and controls in months (girls)

Wilcoxon scores (rank sums)		
Girls (n=90)	Mean	p value
Case (n=45)	98.9	0.0009
Control (n=45)	108.0	

From the tables and figures above, it can be seen that the dental age measurements done consistently show lower values for the FAS children when compared to the controls (Table 37 and 38). The skeletal age of the FAS boy cases (84.5 months) was significantly lower than for the controls (99.2 months) at a probability level of $p<0.0001$. Similarly, the FAS girl cases (98.9) skeletal age was lower than that of the controls (108 months).

9.3.3 Correlation of dental and skeletal age with anthropometric measures

When dental age (D_Age) was compared to height and weight in the control boys, there were relatively low, though significant, associations between dental age and height (r=0.5517, p value <0.0001) and dental age and weight (r=0.5882, p value <0.0001). For the FAS boys, higher and significant associations were found between dental age and height (r=0.7163, p value < 0.0001) and dental age and weight (r=0.6530, p value < 0.0001). There was no association between dental age and head circumference (HC) in the boys (Table 41).

Table 41: Spearman correlations coefficients*: case and control (boys)

	Sk_age	D_Age	Ch_Age	HC	Height	Weight
Sk_Age		0.7784	0.8764	0.0078	0.4438	0.4889
D_Age	0.5040		0.8846	0.0835	0.5517	0.5882
Ch_Age	0.5221	0.8117		-0.0255	0.4861	0.5641
HC	0.0454	0.1497	0.1342		0.1970	0.1432
Height	0.6015	0.7163	0.7213	0.3742		0.5970
Weight	0.5248	0.6530	0.6250	0.5245	0.8677	

● **FAS**   ● **Controls**
*The correlation coefficient ranges from –1 (perfect negative correlation) to 1 (perfect positive correlation), with 0 indicating no correlation.
Sk_Age=skeletal age, D_Age= dental age, Ch_Age= chronological age, HC= head circumference

Skeletal age in control boys, was not significantly associated with height (r=0.4438, p value=0.0023) and weight (r=0.4889, p value=0.0007), but for the FAS boys, relatively low, but significant associations were found between skeletal age and height (r=0.6015, p value<0.0001) and skeletal age and weight (r=0.5248, p value =0.0002). In Table 42, for both the case and control boys, dental age showed the highest degree of correlation with chronological age (FAS r=0.8117; controls r=0.8846) and the lowest degree of correlation for the FAS boys occurred with skeletal age (r=0.5040) and control boys with height (r=0.4861). Height and weight for both groups had the lowest correlation with skeletal age (Table 42).

Table 42: Highest and lowest correlation for boys

Boys	FAS		Control	
	Highest Correlation	Lowest correlation	Highest correlation	Lowest correlation
Sk_Age	Height	D_Age	Ch_Age	Height
D_Age	Ch_Age	Sk_Age	Ch_Age	Height
Ch_Age	D_Age	Sk_Age	D_Age	Height
Height	Weight	Sk_Age	Weight	Sk_Age
Weight	Height	Sk_Age	Height	Sk_Age

Sk_Age=skeletal age, D_Age= dental age, Ch_Age= chronological age

When dental age was compared to the height and weight in the control girls, there were low associations between dental age and height (r=0.3620, p value = 0.0146) and between dental age and weight (r=0.3129, p value = 0.0364). For the FAS girls, slightly higher associations were found between dental age and height (r=0.5080, p value = 0.0004) and dental age and weight (r=0.4426, p value = 0.0023). There was no association between dental age and head circumference in the girls (Table 43).

Table 43: Spearman Correlations Coefficients: case and control (girls)

	Sk_age	D_Age	Ch_Age	HC	Height	Weight
Sk_Age		0.3516	0.8397	0.2305	0.5079	0.4666
D_Age	0.6705		0.5690	-0.0779	0.3620	0.3129
Ch_Age	0.8317	0.5869		0.1305	0.5796	0.4978
HC	0.0803	-0.0823	0.0561		0.3267	0.4861
Height	0.6809	0.5080	0.7102	0.2352		0.6280
Weight	0.5983	0.4426	0.5947	0.3723	0.8719	

● FAS   ● Controls

*The correlation coefficient ranges from –1 (perfect negative correlation) to
1 (perfect positive correlation), with 0 indicating no correlation.
Sk_Age=skeletal age, D_Age= dental age, Ch_Age= chronological age, HC= head circumference

Table 44: Highest and lowest correlation for Girls

Girls	FAS		Control	
	Highest Correlation	**Lowest correlation**	**Highest correlation**	**Lowest correlation**
Sk_Age	Ch_Age	Weight	Ch_Age	D_Age
D_Age	Sk_Age	Weight	Ch_Age	Weight
Ch_Age	Sk_Age	D_Age	Sk_Age	Weight
Height	Weight	D_Age	Weight	D_Age
Weight	Height	D_Age	Height	D_Age

Sk_Age=skeletal age, D_Age= dental age, Ch_Age= chronological age

Skeletal age in control girls, was not significantly associated with height ($r=0.5079$, p value=0.0004) and weight ($r=0.4666$, p value=0.0014), but for the FAS girls, relatively higher and significant associations were found between skeletal age and height ($r=0.6809$, p value <0.0001) and skeletal age and weight ($r=0.5983$, p value <0.0001).

In Table 44, for the control girls, dental age showed the highest degree of correlation with chronological age ($r=0.56903$) and the lowest degree of correlation with weight (0.3129). For the FAS girls dental age was most highly correlated with skeletal age ($r=0.5869$) and the lowest correlation occurred with weight ($r=0.4426$). Height and weight for both groups had the lowest correlation with dental age.

8.4   Discussion

8.4.1   Dental development

Human growth shows considerable variation in respect of the chronological age at which individual children reach similar developmental events. As such, the developmental status of a child is best estimated relative to the specific stages of physiologic maturity. In practice we may distinguish among four physiologic or developmental indices: somatic, skeletal, dental and sexual maturity. Theoretically, strong relationships among indices imply concordance of controlling mechanisms.

Valid associations also provide a means of prediction, allowing judgments to be based on a single examination. It is generally agreed that some markers of maturity can indicate whether a person is advanced or delayed as a whole (Demirjian et al, 1985).

In humans, the complex multiphasic process of tooth development begins during the second month of prenatal life, and completion of the cellular phase of amelogenesis is a prerequisite for tooth eruption. Studies in experimental animals indicate that severe and prolonged cellular disturbances during the critical period of dentinogenesis and amelogenesis produce alterations in the morphology, chemical composition, cellular structure, eruption timing and gross appearance of teeth (Shaw 1967, Dreizen, 1972), thus suggesting that the dentition is not exempt from the retardation in growth and development resulting from cellular insults.

Considerable variations in the development of children of the same chronological age have led to the concept of physiological age. Physiological age is the registry of the rate of progress towards maturity that can be estimated by somatic, sexual, skeletal and dental maturity. Somatic maturity is recognised by the annual growth increments in height and weight (Krogman, 1968). Somatic growth is considered to be the result of multiple factors, jointly acting at specific times during the growing period.

One of these factors is endrocrinological factors which play an important role in normal growth. Growth hormone (GH), insulin-like growth factor 1 (IGH-1) as well as the thyroid and steroid hormones are all crucial for development during early childhood (Demirjian et al, 1973).

The capacity of tissue to accept and utilize hormones is dependent upon factors via appropriate receptors as well as the amount of GH binding protein (GHBP) and IGF-1 binding protein (IGFBP) that are important for hormonal transport, and consequently their tissue uptake that results in growth activity (Demirjian and Goldstein, 1976). Insulin-like growth factors I and II (IGF-I and IGF-II) and IGF-binding proteins are important modulators of fetal growth. IGF-I is a key regulator of fetal growth and development (Singh et al, 1994).

Most studies on maternal ethanol exposure have revealed reduced circulating IGF-I levels in the fetus (Halmesmaki et al, 1989; Sonntag and Boyd, 1989; Breese and D'Costa, 1993). When these hormones, factors, receptors and binding proteins are insufficiently produced, or are not correctly co-ordinated, various disturbances of growth may occur. It is generally assumed that orofacial development follows the predominant growth pattern of the body, which is controlled by the same endocrine system (Demirjian et al, 1973). Independently from each other, different examiners have reported delayed dental maturity in children with or without impaired hormonal status, as part of a syndrome (Pelsmaekers et al, 1997; Nykänen et al, 1998; Hägg and Matsson, 1985 and Loevy, 1983). Several forms of cell perturbations have been associated with ethanol ingestion during pregnancy. Diminished maxillofacial development and inhibition of cell regulation *in vivo* and *in vitro* have been described in children presenting with FAS (Maier et al, 1999; Shibley and Pennington, 1997).

8.4.2   Tooth anomalies

In view of the various growth and structural deficiencies seen in the FAS children it was anticipated that tooth anomalies might also be seen in this population. Varying degrees of anodontia (congenitally absent teeth, "peg laterals", transposition and taurodontism) were seen in eight of the FAS children and two of the controls, but these were not significant. Numerical variations of teeth are considerably less common in the primary than permanent dentition (Peck et al, 1998). Many workers (Seow and Lai, 1989; Bjerklin et al, 1992; Baccetti, 1998, Peck et al, 1998) have reported significant associations among different types of dental anomalies, for example with tooth agenesis, peg-shaped incisors, aplasia of molars etc. but this was not seen in the present study. In this study, four of the five cases of missing teeth were related to the second premolar in both arches. Because hypodontia has been observed most frequently in the upper lateral incisors, third molars and second premolars, this has been spoken of as a phylogenetic reduction in the human dentition, presumably associated with an evolutionary tendency for shortened jaws (Nakata, 1995).

8.4.3 Dental maturity

Dental maturity, also expressed as dental age, is an indicator of the biological maturity of a growing child. Dental age can be based on dental emergence or on the stages of tooth formation. The latter method is better, as tooth emergence is of short duration determined by the time of appearance of the tooth in the mouth (Demirjian and Levesque, 1980), and is altered by local factors such as lack of space (Moorrees et al, 1963) and systemic factors such as nutritional status (Infante and Owen, 1973; Thomson and Billewicz, 1968). As tooth formation is a continuous morphogenic process, a sequence of developmental stages can be defined on the basis of progress of mineralisation, as seen on radiographs. Mineralization of teeth is less affected by variations in nutritional and endocrine status than other growth parameters (Garn et al, 1965a; 1965b), and dental age has been found to correlate well with chronological age (Lewis and Garn 1960, Demirjian et al, 1985).

Several methods for the determination of dental development from radiographs have been described, and standards for determining dental age have been established (Moorrees et al, 1963; Gustafson and Koch, 1974; Demirjian, 1986; Smith, 1991; Mörnstad, Staaf and Welander, 1994; Nykänen et al, 1998). The methods basically define the stages of mineralization of teeth observed in radiographs and code them according to previously determined codes. In addition to stages observed in the radiographs, Gustafson and Koch (1974) considered the time of eruption in their tooth development assessment. Mörnstad et al (1994) measured the crown height, apex width and root length of the teeth observed in the radiographs. Most methods make use of panoramic radiographs for the assessment, although Moorrees et al (1963) made use of periapical radiographs.

The aim of this part of the study was to assess the development of dental maturity in terms of chronological age versus dental age, from panoramic radiographs, using the widely accepted Demirjian 7-tooth method (Demirjian et al, 1973). One of the reasons for the widespread acceptance of this method is that the maturity scoring system that it creates is universal in application. We hypothesized that as growth is delayed in FAS children, there should be a concomitant dental maturity delay.

The completion of root development in single-rooted conical second molars seems to occur considerably later than in molars with two roots (Krckmanova et al, 1999), and this was taken into account when the information provided by the maturity scores was interpreted. The Demirjian scoring system is not ideal for the early developmental stages of developing teeth. The canine, for instance, gets score zero when its development has not begun and also when the crown is almost complete (Stage C – see Methods Section Figure 20). The developmental stages of the incisors and the first molar were the easiest to estimate, and those of the second premolar and the second molar the most difficult. Thus the prediction of tooth formation stages is most difficult in teeth which begin their development late. The literature has shown that the most accurate estimates to determine dental age are obtained in children under 10 years of age, the reason being that the shorter stages take place at an earlier age (Krckmanova et al, 1999; Hägg, and Matsson, 1985).

In this respect, the results of this study correspond with earlier findings, as shown by the dental maturity curves of Demirjian and Goldstein (1976), or the graphic method designed by Moorrees et al (1963), which indicated that late-forming teeth are more variable than early-forming teeth. Hägg and Matsson (1985) and Haaviko (1974) found high correlations (r=0.7-0.9 and r=0.93 respectively), between true and estimated age in children under 10 years of age, regardless of the method used. This was confirmed in a recent study by Bolaños et al (2000).

8.4.3.1 Dental age: FAS vs control

The development of the dentition is an integral part of craniofacial growth, even though it is only marginally related to other maturation processes. Several authors have concluded that the correlation between dental maturity and physical development is low (Anderson et al, 1975; Hägg and Taranger, 1982; Demirjian et al, 1985). Dental maturation has been shown to be mildly but consistently delayed in patients with delayed development (Garn et al, 1965a; Keller et al, 1970; Pirinen, 1995), but to a lesser degree than skeletal maturation. The results of these earlier studies are difficult to interpret because of a lack of controls. In the present study, there is a marked delay in dental development in the FAS group as compared with the control group.

The FAS children showed significantly lower dental ages when compared to the controls (Tables 35 and 36 - page 189). Since dental maturation has previously been shown to be delayed in children with delayed development (Pirinen, 1995; Keller et al, 1970; Garn et al, 1965b; Garn et al 1959) this is a not surprising finding for the FAS children in this study. The mean dental age indicated that the FAS boys and control boys mature earlier than the FAS girls and control girls. Differences between dental age and chronological age were noted for both boys and girls, but as a whole the control groups and the FAS groups showed little variation in dental development.

8.4.3.2 Dental age vs chronological age

A review of the correlation coefficients in this study indicated that for all the children except the FAS girls, dental age was most highly correlated with chronological age than any other variable (Tables 42 and 44). This observation indicates that chronological age is the best single predictor of dental maturity. Approximately one half of the variability in dental age may be accounted for by chronological age variability. It appears that dental development is unrelated to other developmental systems. It is subject to less variation in relation to chronological age and appears to be controlled independently.

The results compare favourably with previously published reports (Reventlid, Mornstad and Teivens, 1996; Lewis, 1991; Demirjian et al, 1985, Harris and Nortjé, 1984; Nortjé, 1983) in that the degree of association was closer between dental age and chronological age than between dental age and skeletal age. Dental maturation appears to be a distinct process tightly linked to chronological age, and independent of general growth and skeletal age (Van Erum et al, 1998, Eid et al, 2002).

8.4.3.3 Dental age vs height, weight and head circumference

Height and weight are the physical manifestations of growth and development which are probably the most utilized measures in assessments of growth and development. Body weight is probably the best index of nutrition and growth as it summarizes all increments in size (Stuart and Meredith, 1946). The different stages of the life cycle exhibit different rates and trends in growth. When evaluating growth and development by means of physical measurements, many factors which introduce variability into the growth trends and rates need to be considered.

Many investigators (Harris et al, 1993; Filipsson and Hall, 1976; Garn et al, 1965a, b; Green, 1961; Gray and Lamons, 1959; Spier, 1958; Talmers, 1952) have studied the relationship between the dentition and various aspects influencing growth and development. The findings of these investigators, in general, support the theoretical contention that positive relationships exist in varying degrees between the maturation of various tissue systems, whereas other investigators found low correlations between dental development and body growth (Demirjian et al, 1985; Patterson et al, 1984; Anderson, Thompson and Popovich, 1975).

In the present study, forty two per cent of the FAS children manifested growth retardation (p<0.0001) as compared to their controls (Table 11, page 153). Low birth weight infants are susceptible to several developmental problems which can cause potentially long-lasting effects that slow growth during infancy and childhood. Children with FAS commonly have a low birth weight and remain small for their age (Jones et al, 1974). The intrauterine growth retardation results in weight, length and head circumferences below the tenth per centile.

Table 33: Chronological age, dental age and dental delay score* in years (boys)

Years	FAS boys				Control boys			
	Mean	SD	Min	Max	Mean	SD	Min	Max
Chronological age	8.93	1.27	7.07	11.74	9.17	1.24	7.23	11.75
Dental age	9.10	1.24	7.50	12.80	9.96	1.47	7.60	12.80
Dental delay score*	0.17				0.79			
p value	0.027							

*Dental delay score = dental age – chronological age

The mean chronological age, the dental age and the dental delay score* of the girl cases and controls are shown in Table 34. It can be seen that for the girls, there is only a marginally significant group difference for dental age vs chronological age (p = 0.0827).

Table 34: Chronological age, dental age and dental delay score in years: Girls

Years	FAS girls				Control girls			
	Mean	SD	Min	Max	Mean	SD	Min	Max
Chronological age	8.94	1.32	7.27	11.1	9.06	1.34	7.37	11.3
Dental age	8.33	1.08	7.10	11.6	8.79	1.43	7.40	12.3
Dental delay score	-0.61				-0.27			
p value	0.0827							

*Dental delay score = dental age – chronological age

Using the Wilcoxon scores (rank sums) for dental age in boys (Table 35) it can be seen that there are significant intra-group differences in dental age, whereas for the girls (Table 36), there is only a marginally significant group difference.

Table 35: Group differences in dental age between cases and controls in years (boys)

Wilcoxon scores (rank sums)		
Boys (n=90)	Mean (years)	p value
Case (n=45)	9.10	0.0027
Control (n=45)	9.96	

Table 36: Group differences in dental age between cases and controls in years (girls)

Wilcoxon scores (rank sums)		
Girls (n=90)	Mean (years)	p value
Case (n=45)	8.32	0.0827
Control (n=45)	8.79	

From the tables and figures above, it can be seen that the dental age measurements done consistently show lower values for the FAS children when compared to the controls (Table 33 and 34). The dental age of the FAS boy cases (9.10 years) was significantly lower than for the controls (9.96 years) at a probability level of p=0.0027. Similarly, the FAS girl cases (8.32 years) dental age was lower than that of the controls (8.79 years).

8.3.2 Skeletal age

Figure 26 below is a graph depicting the entire sample's skeletal age against chronological age in months. The line drawn through the graph represents where chronological age is the same as skeletal age. As can be seen, most of the points for the FAS cases fall below the line indicating a retardation of skeletal age compared with chronological age.

Figure 26: Chronological age vs skeletal age in months for entire sample (n=180)

The mean chronological age, the skeletal age and the skeletal delay score* of the boy cases and controls are shown in Table 37 below. It can be seen that for the boys, there are significant group differences for skeletal age vs chronological age (p < 0.0001).

Table 37: Chronological age, skeletal age and skeletal delay score* in months (boys)

Months	FAS				Control			
	Mean	SD	Min	Max	Mean	SD	Min	Max
Chronological age	107.1	14.58	85.0	134.0	108.7	15.85	86.0	152.0
Skeletal age	84.5	14.69	48.0	120.0	99.2	15.65	72.0	132.0
Skeletal delay score	-22.6				-9.5			
p value	<0.0001							
*Skeletal delay score = skeletal age - chronological age								

Figure 27: Chronological age vs skeletal age in months (boys)

Figure 27 above is a graphic representation depicting the boy's skeletal age against chronological age in months. The line drawn through the graph represents where chronological age is the same as skeletal age. As can be seen, all the cases (red points) and most of the controls (blue points) fall below the line indicating that their skeletal age is lower than their chronological age.

The mean chronological age, the skeletal age and the skeletal delay score* of the girl cases and controls are shown in Table 38. It can be seen that for the girls, there are significant group differences for skeletal age vs chronological age (p < 0.0009).

Table 38: Chronological age, skeletal age and skeletal delay score* in months (girls)

Months	FAS				Control			
	Mean	SD	Min	Max	Mean	SD	Min	Max
Chronological age	107.6	15.97	86.0	144.0	108.7	16.18	86.0	154.0
Skeletal age	98.9	12.99	69.0	120.0	108.0	14.18	82.0	144.0
Skeletal delay score	-8.7				-0.7			
p value	0.0009							

*Skeletal delay score = skeletal age - chronological age

Using the Wilcoxon scores (rank sums) for skeletal age in boys (Table 39) and girls (Table 40) it can be seen that there were significant intra-group differences for skeletal age.

Table 39: Group differences in skeletal age: cases and controls in months (boys)

Wilcoxon scores (rank sums)		
Boys (n=90)	Mean	p value
Case (n=45)	84.5	<0.0001
Control (n=45)	99.2	

Table 40: Group differences in skeletal age: cases and controls in months (girls)

Wilcoxon scores (rank sums)		
Girls (n=90)	Mean	p value
Case (n=45)	98.9	0.0009
Control (n=45)	108.0	

From the tables and figures above, it can be seen that the dental age measurements done consistently show lower values for the FAS children when compared to the controls (Table 37 and 38). The skeletal age of the FAS boy cases (84.5 months) was significantly lower than for the controls (99.2 months) at a probability level of p<0.0001. Similarly, the FAS girl cases (98.9) skeletal age was lower than that of the controls (108 months).

9.3.3   Correlation of dental and skeletal age with anthropometric measures

When dental age (D_Age) was compared to height and weight in the control boys, there were relatively low, though significant, associations between dental age and height (r=0.5517, p value <0.0001) and dental age and weight (r=0.5882, p value <0.0001). For the FAS boys, higher and significant associations were found between dental age and height (r=0.7163, p value < 0.0001) and dental age and weight (r=0.6530, p value < 0.0001). There was no association between dental age and head circumference (HC) in the boys (Table 41).

Table 41: Spearman correlations coefficients*: case and control (boys)

	Sk_age	D_Age	Ch_Age	HC	Height	Weight
Sk_Age		0.7784	0.8764	0.0078	0.4438	0.4889
D_Age	0.5040		0.8846	0.0835	0.5517	0.5882
Ch_Age	0.5221	0.8117		-0.0255	0.4861	0.5641
HC	0.0454	0.1497	0.1342		0.1970	0.1432
Height	0.6015	0.7163	0.7213	0.3742		0.5970
Weight	0.5248	0.6530	0.6250	0.5245	0.8677	

● **FAS**   ● **Controls**
***The correlation coefficient ranges from –1 (perfect negative correlation) to
1 (perfect positive correlation), with 0 indicating no correlation.
Sk_Age=skeletal age, D_Age= dental age, Ch_Age= chronological age, HC= head circumference**

Skeletal age in control boys, was not significantly associated with height (r=0.4438, p value=0.0023) and weight (r=0.4889, p value=0.0007), but for the FAS boys, relatively low, but significant associations were found between skeletal age and height (r=0.6015, p value<0.0001) and skeletal age and weight (r=0.5248, p value =0.0002). In Table 42, for both the case and control boys, dental age showed the highest degree of correlation with chronological age (FAS r=0.8117; controls r=0.8846) and the lowest degree of correlation for the FAS boys occurred with skeletal age (r=0.5040) and control boys with height (r=0.4861). Height and weight for both groups had the lowest correlation with skeletal age (Table 42).

Table 42: Highest and lowest correlation for boys

Boys	FAS		Control	
	Highest Correlation	Lowest correlation	Highest correlation	Lowest correlation
Sk_Age	Height	D_Age	Ch_Age	Height
D_Age	Ch_Age	Sk_Age	Ch_Age	Height
Ch_Age	D_Age	Sk_Age	D_Age	Height
Height	Weight	Sk_Age	Weight	Sk_Age
Weight	Height	Sk_Age	Height	Sk_Age

Sk_Age=skeletal age, D_Age= dental age, Ch_Age= chronological age

When dental age was compared to the height and weight in the control girls, there were low associations between dental age and height (r=0.3620, p value = 0.0146) and between dental age and weight (r=0.3129, p value = 0.0364). For the FAS girls, slightly higher associations were found between dental age and height (r=0.5080, p value = 0.0004) and dental age and weight (r=0.4426, p value = 0.0023). There was no association between dental age and head circumference in the girls (Table 43).

Table 43: Spearman Correlations Coefficients: case and control (girls)

	Sk_age	D_Age	Ch_Age	HC	Height	Weight
Sk_Age		0.3516	0.8397	0.2305	0.5079	0.4666
D_Age	0.6705		0.5690	-0.0779	0.3620	0.3129
Ch_Age	0.8317	0.5869		0.1305	0.5796	0.4978
HC	0.0803	-0.0823	0.0561		0.3267	0.4861
Height	0.6809	0.5080	0.7102	0.2352		0.6280
Weight	0.5983	0.4426	0.5947	0.3723	0.8719	

• FAS   • Controls
*The correlation coefficient ranges from –1 (perfect negative correlation) to
1 (perfect positive correlation), with 0 indicating no correlation.
Sk_Age=skeletal age, D_Age= dental age, Ch_Age= chronological age, HC= head circumference

Table 44: Highest and lowest correlation for Girls

Girls	FAS		Control	
	Highest Correlation	**Lowest correlation**	**Highest correlation**	**Lowest correlation**
Sk_Age	Ch_Age	Weight	Ch_Age	D_Age
D_Age	Sk_Age	Weight	Ch_Age	Weight
Ch_Age	Sk_Age	D_Age	Sk_Age	Weight
Height	Weight	D_Age	Weight	D_Age
Weight	Height	D_Age	Height	D_Age

Sk_Age=skeletal age, D_Age= dental age, Ch_Age= chronological age

Skeletal age in control girls, was not significantly associated with height (r=0.5079, p value=0.0004) and weight (r=0.4666, p value=0.0014), but for the FAS girls, relatively higher and significant associations were found between skeletal age and height (r=0.6809, p value <0.0001) and skeletal age and weight (r=0.5983, p value <0.0001).

In Table 44, for the control girls, dental age showed the highest degree of correlation with chronological age (r=0.56903) and the lowest degree of correlation with weight (0.3129). For the FAS girls dental age was most highly correlated with skeletal age (r=0.5869) and the lowest correlation occurred with weight (r=0.4426). Height and weight for both groups had the lowest correlation with dental age.

8.4   Discussion

8.4.1   Dental development

Human growth shows considerable variation in respect of the chronological age at which individual children reach similar developmental events. As such, the developmental status of a child is best estimated relative to the specific stages of physiologic maturity. In practice we may distinguish among four physiologic or developmental indices: somatic, skeletal, dental and sexual maturity. Theoretically, strong relationships among indices imply concordance of controlling mechanisms.

Valid associations also provide a means of prediction, allowing judgments to be based on a single examination. It is generally agreed that some markers of maturity can indicate whether a person is advanced or delayed as a whole (Demirjian et al, 1985).

In humans, the complex multiphasic process of tooth development begins during the second month of prenatal life, and completion of the cellular phase of amelogenesis is a prerequisite for tooth eruption. Studies in experimental animals indicate that severe and prolonged cellular disturbances during the critical period of dentinogenesis and amelogenesis produce alterations in the morphology, chemical composition, cellular structure, eruption timing and gross appearance of teeth (Shaw 1967, Dreizen, 1972), thus suggesting that the dentition is not exempt from the retardation in growth and development resulting from cellular insults.

Considerable variations in the development of children of the same chronological age have led to the concept of physiological age. Physiological age is the registry of the rate of progress towards maturity that can be estimated by somatic, sexual, skeletal and dental maturity. Somatic maturity is recognised by the annual growth increments in height and weight (Krogman, 1968). Somatic growth is considered to be the result of multiple factors, jointly acting at specific times during the growing period.

One of these factors is endocrinological factors which play an important role in normal growth. Growth hormone (GH), insulin-like growth factor 1 (IGH-1) as well as the thyroid and steroid hormones are all crucial for development during early childhood (Demirjian et al, 1973).

The capacity of tissue to accept and utilize hormones is dependent upon factors via appropriate receptors as well as the amount of GH binding protein (GHBP) and IGF-1 binding protein (IGFBP) that are important for hormonal transport, and consequently their tissue uptake that results in growth activity (Demirjian and Goldstein, 1976). Insulin-like growth factors I and II (IGF-I and IGF-II) and IGF-binding proteins are important modulators of fetal growth. IGF-I is a key regulator of fetal growth and development (Singh et al, 1994).

Most studies on maternal ethanol exposure have revealed reduced circulating IGF-I levels in the fetus (Halmesmaki et al, 1989; Sonntag and Boyd, 1989; Breese and D'Costa, 1993). When these hormones, factors, receptors and binding proteins are insufficiently produced, or are not correctly co-ordinated, various disturbances of growth may occur. It is generally assumed that orofacial development follows the predominant growth pattern of the body, which is controlled by the same endocrine system (Demirjian et al, 1973). Independently from each other, different examiners have reported delayed dental maturity in children with or without impaired hormonal status, as part of a syndrome (Pelsmaekers et al, 1997; Nykänen et al, 1998; Hägg and Matsson, 1985 and Loevy, 1983). Several forms of cell perturbations have been associated with ethanol ingestion during pregnancy. Diminished maxillofacial development and inhibition of cell regulation *in vivo* and *in vitro* have been described in children presenting with FAS (Maier et al, 1999; Shibley and Pennington, 1997).

8.4.2 Tooth anomalies

In view of the various growth and structural deficiencies seen in the FAS children it was anticipated that tooth anomalies might also be seen in this population. Varying degrees of anodontia (congenitally absent teeth, "peg laterals", transposition and taurodontism) were seen in eight of the FAS children and two of the controls, but these were not significant. Numerical variations of teeth are considerably less common in the primary than permanent dentition (Peck et al, 1998). Many workers (Seow and Lai, 1989; Bjerklin et al, 1992; Baccetti, 1998, Peck et al, 1998) have reported significant associations among different types of dental anomalies, for example with tooth agenesis, peg-shaped incisors, aplasia of molars etc. but this was not seen in the present study. In this study, four of the five cases of missing teeth were related to the second premolar in both arches. Because hypodontia has been observed most frequently in the upper lateral incisors, third molars and second premolars, this has been spoken of as a phylogenetic reduction in the human dentition, presumably associated with an evolutionary tendency for shortened jaws (Nakata, 1995).

8.4.3   Dental maturity

Dental maturity, also expressed as dental age, is an indicator of the biological maturity of a growing child. Dental age can be based on dental emergence or on the stages of tooth formation. The latter method is better, as tooth emergence is of short duration determined by the time of appearance of the tooth in the mouth (Demirjian and Levesque, 1980), and is altered by local factors such as lack of space (Moorrees et al, 1963) and systemic factors such as nutritional status (Infante and Owen, 1973; Thomson and Billewicz, 1968). As tooth formation is a continuous morphogenic process, a sequence of developmental stages can be defined on the basis of progress of mineralisation, as seen on radiographs. Mineralization of teeth is less affected by variations in nutritional and endocrine status than other growth parameters (Garn et al, 1965a; 1965b), and dental age has been found to correlate well with chronological age (Lewis and Garn 1960, Demirjian et al, 1985).

Several methods for the determination of dental development from radiographs have been described, and standards for determining dental age have been established (Moorrees et al, 1963; Gustafson and Koch, 1974; Demirjian, 1986; Smith, 1991; Mörnstad, Staaf and Welander, 1994; Nykänen et al, 1998). The methods basically define the stages of mineralization of teeth observed in radiographs and code them according to previously determined codes. In addition to stages observed in the radiographs, Gustafson and Koch (1974) considered the time of eruption in their tooth development assessment. Mörnstad et al (1994) measured the crown height, apex width and root length of the teeth observed in the radiographs. Most methods make use of panoramic radiographs for the assessment, although Moorrees et al (1963) made use of periapical radiographs.

The aim of this part of the study was to assess the development of dental maturity in terms of chronological age versus dental age, from panoramic radiographs, using the widely accepted Demirjian 7-tooth method (Demirjian et al, 1973). One of the reasons for the widespread acceptance of this method is that the maturity scoring system that it creates is universal in application. We hypothesized that as growth is delayed in FAS children, there should be a concomitant dental maturity delay.

The completion of root development in single-rooted conical second molars seems to occur considerably later than in molars with two roots (Krckmanova et al, 1999), and this was taken into account when the information provided by the maturity scores was interpreted. The Demirjian scoring system is not ideal for the early developmental stages of developing teeth. The canine, for instance, gets score zero when its development has not begun and also when the crown is almost complete (Stage C – see Methods Section Figure 20). The developmental stages of the incisors and the first molar were the easiest to estimate, and those of the second premolar and the second molar the most difficult. Thus the prediction of tooth formation stages is most difficult in teeth which begin their development late. The literature has shown that the most accurate estimates to determine dental age are obtained in children under 10 years of age, the reason being that the shorter stages take place at an earlier age (Krckmanova et al, 1999; Hägg, and Matsson, 1985).

In this respect, the results of this study correspond with earlier findings, as shown by the dental maturity curves of Demirjian and Goldstein (1976), or the graphic method designed by Moorrees et al (1963), which indicated that late-forming teeth are more variable than early-forming teeth. Hägg and Matsson (1985) and Haaviko (1974) found high correlations (r=0.7-0.9 and r=0.93 respectively), between true and estimated age in children under 10 years of age, regardless of the method used. This was confirmed in a recent study by Bolaños et al (2000).

8.4.3.1 Dental age: FAS vs control

The development of the dentition is an integral part of craniofacial growth, even though it is only marginally related to other maturation processes. Several authors have concluded that the correlation between dental maturity and physical development is low (Anderson et al, 1975; Hägg and Taranger, 1982; Demirjian et al, 1985). Dental maturation has been shown to be mildly but consistently delayed in patients with delayed development (Garn et al, 1965a; Keller et al, 1970; Pirinen, 1995), but to a lesser degree than skeletal maturation. The results of these earlier studies are difficult to interpret because of a lack of controls. In the present study, there is a marked delay in dental development in the FAS group as compared with the control group.

The FAS children showed significantly lower dental ages when compared to the controls (Tables 35 and 36 - page 189). Since dental maturation has previously been shown to be delayed in children with delayed development (Pirinen, 1995; Keller et al, 1970; Garn et al, 1965b; Garn et al 1959) this is a not surprising finding for the FAS children in this study. The mean dental age indicated that the FAS boys and control boys mature earlier than the FAS girls and control girls. Differences between dental age and chronological age were noted for both boys and girls, but as a whole the control groups and the FAS groups showed little variation in dental development.

8.4.3.2 Dental age vs chronological age

A review of the correlation coefficients in this study indicated that for all the children except the FAS girls, dental age was most highly correlated with chronological age than any other variable (Tables 42 and 44). This observation indicates that chronological age is the best single predictor of dental maturity. Approximately one half of the variability in dental age may be accounted for by chronological age variability. It appears that dental development is unrelated to other developmental systems. It is subject to less variation in relation to chronological age and appears to be controlled independently.

The results compare favourably with previously published reports (Reventlid, Mornstad and Teivens, 1996; Lewis, 1991; Demirjian et al, 1985, Harris and Nortjé, 1984; Nortjé, 1983) in that the degree of association was closer between dental age and chronological age than between dental age and skeletal age. Dental maturation appears to be a distinct process tightly linked to chronological age, and independent of general growth and skeletal age (Van Erum et al, 1998, Eid et al, 2002).

8.4.3.3 Dental age vs height, weight and head circumference

Height and weight are the physical manifestations of growth and development which are probably the most utilized measures in assessments of growth and development. Body weight is probably the best index of nutrition and growth as it summarizes all increments in size (Stuart and Meredith, 1946). The different stages of the life cycle exhibit different rates and trends in growth. When evaluating growth and development by means of physical measurements, many factors which introduce variability into the growth trends and rates need to be considered.

Many investigators (Harris et al, 1993; Filipsson and Hall, 1976; Garn et al, 1965a, b; Green, 1961; Gray and Lamons, 1959; Spier, 1958; Talmers, 1952) have studied the relationship between the dentition and various aspects influencing growth and development. The findings of these investigators, in general, support the theoretical contention that positive relationships exist in varying degrees between the maturation of various tissue systems, whereas other investigators found low correlations between dental development and body growth (Demirjian et al, 1985; Patterson et al, 1984; Anderson, Thompson and Popovich, 1975).

In the present study, forty two per cent of the FAS children manifested growth retardation (p<0.0001) as compared to their controls (Table 11, page 153). Low birth weight infants are susceptible to several developmental problems which can cause potentially long-lasting effects that slow growth during infancy and childhood. Children with FAS commonly have a low birth weight and remain small for their age (Jones et al, 1974). The intrauterine growth retardation results in weight, length and head circumferences below the tenth per centile.

In this study, there was high correlation between height and weight for the FAS children (boys r=0.8677, girls r=0.8719). More than two-thirds of the differences in weight can be accounted for on the basis of their differences in height. Overall, there were significant differences, albeit a low association, when dental age was compared to height in the cases and the controls. Children with the lowest height-for-age centiles were the most dentally delayed which may suggest that height status would improve as dental age caught up with chronological age.

Older children were more delayed in their dental development possibly as there is a proportionately greater opportunity for dental age to diverge from chronological age as children grow older. Similarly, there were significant differences (p<0.0001), albeit a low association when dental age was compared to weight in the cases and the controls. This finding indicates that for a given age, children who weigh more or less than the average, had larger or smaller dental ages respectively. There was no association between dental age and head circumference in both the girls and boys.

8.4.4 Skeletal maturity

The radiographic study of the hand and wrist is one of the most useful single procedures available for determining the developmental status of children. A single radiograph of a child's hand can provide useful information as: (i) it affords an objective measure of the amount of progress a child has made towards attaining physical maturity; that is, it enables one to determine the child's developmental status and to compare this with that of others of the same gender and age; (ii) it makes it possible to distinguish the poorly from the adequately mineralized skeleton, this providing additional information to the clinical evaluation of nutritional status; (iii) it reveals imbalances in skeletal development, and often enables one to infer when those imbalances were initiated and (iv) it discloses episodes of interrupted growth that provide a generic record of past illness and trauma (Greulich and Pyle, 1959).

In this study, the skeletal maturity assessment was based on the system described in the *Radiographic Atlas of Skeletal Development of the Hand and Wrist* (Greulich and Pyle, 1959). This technique offers an organised and relatively simple approach to determine the level of maturation. The system uses only 14 anatomical sites located on the radius, ulna, carpals and phalanges, most of which exhibit consistency in the time of onset of ossification. This method is used by nearly 80% of paediatricians to determine skeletal age (Buckler, 1993). Many investigators have found significant correlation among the maturation stages derived from hand-wrist radiographs, changes in height and facial growth (Fishman 1982; Silveira et al, 1992; Hunter, 1966). The developmental regularity of the bones of the hand and the wrist is evident before and after birth. In the embryo it manifests itself in the fixed order in which mesenchymal analgen of the carpals become transformed into cartilage. There is a comparable regularity in the order of chondrification of the other skeletal elements of the hand and wrist, as well as the primary centres of ossification. After birth, there is considerable regularity too, in the order in which the carpals and epiphyses begin to ossify.

8.4.4.1 Skeletal age: FAS vs control

In the present study the mean age of skeletal maturity, indicated that the FAS girls mature earlier than the FAS boys, but the control boys matured earlier than the control girls (Tables 39 and 40-page 192). This finding differs from the basic findings of several reports of children in the general population that girls mature earlier than boys (Fishman, 1982; Hägg and Taranger, 1982; Grave and Brown, 1976, Björk & Helm, 1972). In this study, differences between skeletal age and chronological age were noted for both boys and girls, but as a whole, the present study groups (FAS and controls) showed little variation in skeletal development.

Cole et al (1988) have explained that there are three sources of discrepancy between skeletal age and chronological age: natural variations between individuals in their rates of skeletal maturation, systematic error inherent in the method used to assess skeletal age and variation between the various examiners.

In the present study, it is possible that the first two sources may have influenced the discrepancy between skeletal and chronological ages. Examiner error was probably least likely, since the skeletal age assessment was performed by two examiners simultaneously, and the reproducibility test showed a very strong coefficient of reliability (r=0.99) between double assessments. The natural variation in skeletal age between the subjects whose radiographs were used to set the standard plates in the *Atlas* (Greulich and Pyle, 1959) and the sample in this study may be in part associated with environmental factors and racial differences, the sample in the A*tlas* having been derived from a white, north European ancestry. Systematic error of the A*tlas* method might be derived from widely spaced standards. Some hand-wrist radiographs were not exactly comparable to the standard plates.

8.4.4.2 Skeletal age vs chronological age

All the children in the sample had skeletal ages significantly lower than their chronological age (Tables 43 and 44). The present study shows a significant mean reduction in the skeletal age of this sample when compared with the group used to compile the *Atlas* of Greulich and Pyle (1959) in the 1950s. In recent years, several studies have been published on the relevance of this *Atlas* to different populations in the 50 years since it was published. Lewis et al (2002) showed a significant mean reduction in the skeletal ages of a sample of Malawian children when compared with the sample from the *Atlas*. Van Rijn et al (2001) and Groell et al (1999) found the Gruelich and Pyle *Atlas* to be still applicable in Dutch Caucasian and central European children respectively. Mora et al (2001) found significant differences in skeletal maturation between American children of European and African descent. Rikhasor et al (1999) showed that Pakistani children of both genders were a few months in advance of their skeletal development compared with the *Atlas* until puberty, but fell behind post-puberty. Loder et al (1993) investigated black and white children in the geographical area from which the *Atlas* originated and found minor changes, however both the Loder et al (1993) and Ontell et al (1996) studies comparing skeletal and chronological ages in healthy children were limited by the lack of using age-adjusted normal standards for height and weight to verify normal growth in subjects.

The cause of the markedly reduced skeletal age in this present study is not clear. It is tempting to blame poor nutrition; however we are unable to show a relationship between undernutrition and the degree of skeletal delay. Two other studies of delay in skeletal development by Fleshman (2000) and Mackay (1952) also suggested poor nutrition as being the cause of skeletal delay, but they were unable to show statistically significant support for this hypothesis. It is likely that factors other than nutrition are involved that retard skeletal age, for example the prevalence of chronic diseases, such as tuberculosis and diarrhoea. We did not specifically determine the prevalence of chronic diseases in the sample. It would have been of interest to reassess them from that point of view, especially with regard to the FAS children who presented with the greatest skeletal delay.

The findings of a reduced skeletal age in this study, may imply that care should be exercised when using the atlas of Greulich and Pyle (1959), and it may become necessary to develop a new bone age *Atlas* for sub Saharan Africa, addressing the diversity of ethnic groups that would enhance our ability to determine skeletal maturation with accuracy, reliability and consistency.

8.4.4.3 Skeletal age vs height, weight and head circumference

With regard to skeletal age and the anthropometric measures, the correlations did not fall into any obvious pattern. However, for the FAS children there appears to be an indication of an association between skeletal age, height and weight. One might hypothesize that the factors which control skeletal growth and development may also be important in determining height and weight. There was no association between skeletal age and head circumference in both the girls and boys of both the FAS and control cohorts.

8.4.5   Inter-relationships: dental and skeletal age

Several studies have found a positive correlation between skeletal, somatic and sexual maturity (Demirjian et al, 1985; Houston, 1980; Onat and Numan-Cebeci, 1976; Marshall, 1974). However, regarding the correlation between dental and skeletal maturity, some authors (Sahin Saglam and Gazilerli, 2002; Krailassiri et al, 2002; Koshy and Tandon, 1998; Mappes et al, 1992; Demirjian et al, 1985) found these events to be independent, while others (Lamons and Gray, 1958; Green, 1961) found a positive but low correlation (r=0.77 and r=0.46 respectively). Gleiser and Hunt (1955) verified that a delay in ossification of the bones of the hand and wrist often co-incides with a lag of tooth formation. Sahin Saglam and Gazilerli (2002) concluded from their study that the relationship between dental age and skeletal age is not sufficient for dental age to be used as a substitute for skeletal maturity. This study has shown that a positive but low correlation exists between dental and skeletal age (Table 41 and 43). Others have tried to relate the dental eruption stages with pubertal events and found a low correlation (r=0.35; Hägg and Taranger, 1982), since the onset of dental eruption presents a large variability and is under great environmental influences (Demirjian, 1986). The age group of the sample in the present study did not warrant any comparison in this regard.

8.5   Concluding remarks

In this chapter, the nature of the inter-relationships among chronological age, dental age, skeletal age and height and weight was investigated. The purpose was to highlight the value of various physical indices of the overall developmental status and nature of the relationships among these indices between the cases and the controls. In summary, this study provides evidence of a positive association between dental age, skeletal age and height and weight in both the FAS children and the controls. From the correlation coefficients it is evident that there is a relationship between dental and skeletal development. Though statistically significant, the degree of association found was low. The data suggest that both dental and skeletal ages may be a reflection of general somatic growth. There was no association found with head circumference.

Garn, Lewis and Kerewsky (1965a) have previously demonstrated that taller children are slightly advanced in respect of their dental ages. In addition, Gyulavari (1966) and Lee, Low and Chang (1965) have demonstrated significant positive associations between dental age and skeletal maturation. Therefore the finding that taller children were more advanced in their dental ages than the shorter children, was not unexpected. The associations between dental age and skeletal age and height could imply that genetic factors may cause premature eruption in children of greater stature for their age. Taller parents may produce taller children that are advanced in dental and skeletal age. The opposite would be expected from children of shorter parents (Ito et al, 1993).

In this study, there appeared to be no obvious gender difference in the significance of any association found between dental age, skeletal weight, height, weight and head circumference. The lack of an association with head circumference and dental and skeletal age was not surprising, as previous literature has shown that in general, head circumference correlates better with weight and height, than with dental or skeletal age.

Height and weight have been related to nutritional status (Abidoye and Ihebuzor, 2001; Fleshman, 2000; Hailu and Tessema, 1997) and as children with severe malnutrition are known to have a shorter statures, weigh less, have reduced head circumferences and brain development (Rosso, Hormazabal and Winnick, 1970; Winnick and Rosso, 1969), the association between the extremes in skeletal age and height, weight and head circumference, suggests that skeletal age may be an adjunct for identifying children with retarded development as a result of serious nutritional deficiencies.

Cognisance should be taken of the fact that the growth of individuals is often irregular, when any norms of development based on central tendencies and variabilities of healthy children are applied. Some aspects of growth and development for healthy children may show a shifting pattern of growth, that is, a shift from high through average to low, and back to average again, when comparing a child with his or her maturing age group.

Therefore, correlation of these aspects of growth and development will not often show the degree of correlation which theoretically exists between different areas of growth and development. It should be pointed out that specifically chosen evaluations (panoramic and hand-wrist radiographs) in various areas of growth and development were obtained for this part of the study. A more complete appraisal of the entire skeleton rather than the bones from the hand-wrist radiograph alone, and the evaluation of the entire dentition, rather than just the mandibular teeth, might improve the correlation between the variables.

This part of the study attempted to determine the relationship of dental and skeletal age to several measures of growth between the FAS cohort and the control children. The results indicate that both dental and skeletal ages are significantly related to child growth and perhaps nutritional status. These findings, however, need to be confirmed via additional longitudinal studies.

Chapter 9: Cephalometric Analysis

9.1   Background

The skull can be considered to be composed of five major structural components, including the cranium, the cranial base, the maxilla and mandible and the maxillary and mandibular dentitions. All cephalometric analyses describe the interrelationship of these parts and may describe the form of the individual unit. These relationships may be described both in the vertical and horizontal sagittal planes.

Twenty linear measurements and 17 angular measurements for each of the 180 children in the sample were examined. The 37 variables measured from each lateral cephalometric radiograph were formulated to assess the size, shape and relative position of three craniofacial complexes: (i) the cranial base, (ii) midface and (iii) mandible. In addition, nine variables were computed to compare the shapes of the soft-tissue profile. Linear and angular measurements were used to describe the relationship of these structural units. Univariate and multivariate statistical techniques were used to identify measurements which best discriminated between the cases and the controls.

Linear measurement measures the distance between two landmarks, which can be compared directly to the comparative norms. The disadvantage of this measurement is that the size of the individual is not taken into account. Age and size weighting is therefore important with all linear measurements. Angular measurement measures the angles formed between constructed planes. As angles are a ratio-based measurement, they inherently incorporate the effect of size when comparisons are made (Rakosi, 1982). None of the angular measurements except for the palatal plane to Frankfort Horizontal (FH) angle revealed any significant differences between the cases and controls. However, several linear measurements in the FAS children were found to be significantly different from the controls, especially those related to the cranial base, midface and mandible.

Statistical analysis was confined to correlations between the linear measurements, as it was found that they contributed greatly to distinguishing and describing the FAS children.

9.2   Methods

Descriptive analysis involved computing the mean, standard deviation (SD), minimum, maximum and the range of the cephalometric parameters. Both univariate and multivariate techniques were used in the analyses. Spearman correlation coefficients were calculated between age and each of the cephalometric measurements to get an indication as to which measurements had significant age associations. The influence age had over this growth period for each cephalometric measurement was further investigated by plotting Altman centile curves (calculated from the absolute residuals) against age for each of these measurements (Altman, 1993).

Correlation gives strength to a relationship (if any) which exists between two variables; what it does not do, however, is to express such relationship quantitatively. Regression defines the nature of a relationship between two (or more) variables. It is concerned with defining mathematically a linear relationship between a particular dependent variable and one or more independent variables. Regression is a more powerful tool than correlation, although the two are closely related mathematically and correlations are a useful aid in interpreting regression analysis.

Multivariate analysis moves away from the idea of looking at one variable or the relationship between one variable and another. Rather it is concerned with grouping or the classification of particular variables. The multivariate techniques that were used in this study were Stepwise Logistic Regression (backward) and Principal Component Analysis. The Principal Component Analysis is a technique designed to reduce a matrix of correlated variables to a smaller set of uncorrelated linear combinations. Thus, the first component will account for the greatest amount of variation and the second, uncorrelated with the first, will account for the next greatest amount of variation.

Both methods were used to select a subset of measurements with the highest discriminating qualities (discriminating between the two groups). For the logistic regression a prevalence of 10% and probability level of 5% were used. A biplot (graphical representation of multivariate information in a data matrix) was constructed to describe the multivariation of the data, as analysed with the above-mentioned techniques (Johnson and Wicheren, 1998).

9.3   Results

Figure 28 shows all the linear measurements that were taken from the cephalometric radiograph for each child. As can be seen, some lines are connected to the soft tissue points, whereas other connect hard tissue points, and other points are positioned where two intersecting lines meet.
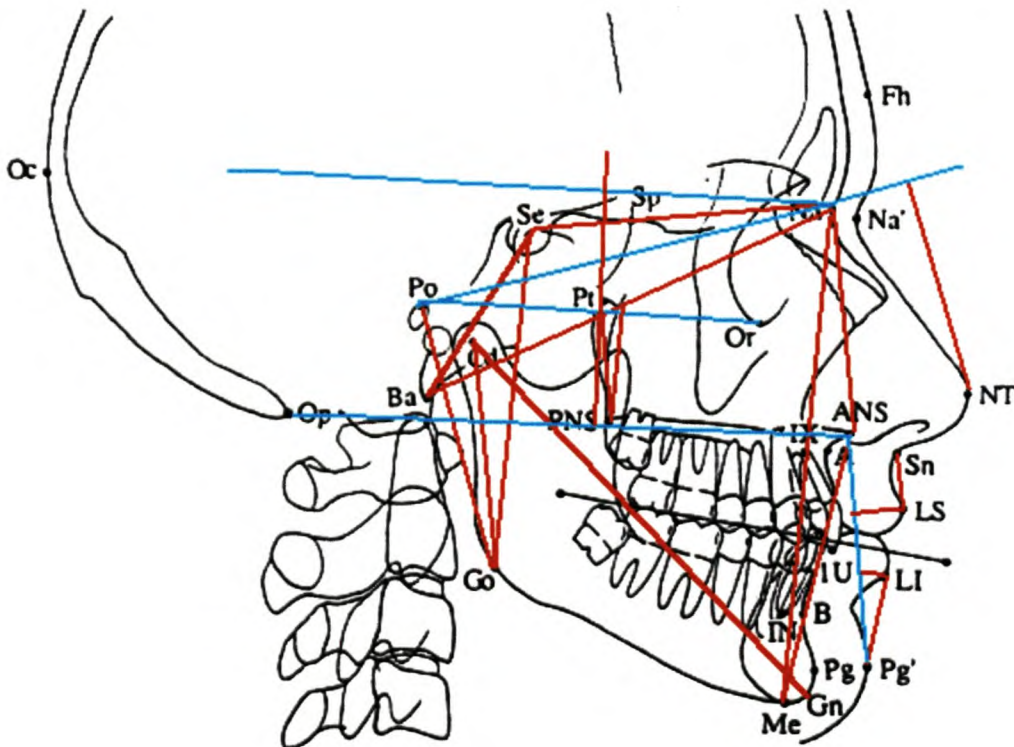
Figure 28: Linear measurements taken from the cephalometric radiographs (Refer to Tables 5 and 6 in the Methods chapter)

9.3.1 Age – an important parameter

Age played an important role in the analyses of this part of the results, as age and growth of the skull are closely related. As seen in Figure 29, both groups had relatively wide age ranges that do not differ between the two groups, but which tended to accentuate the growth taking place over time. However, there were no age differences between the two groups, as their profiles and age ranges were the same.
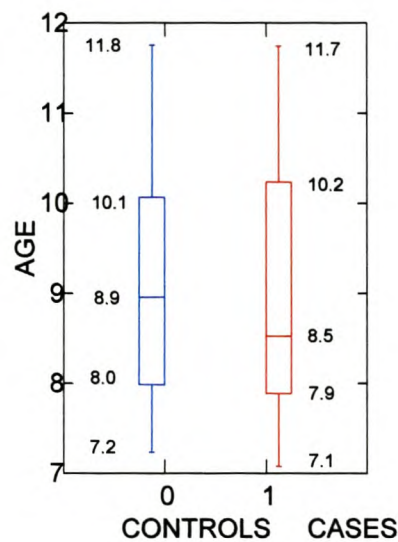
Figure 29: Age ranges

Table 45 shows the Spearman correlations between age and all the linear measurements. Seven measurements, shown in red, BaS – posterior cranial base length, BaNa – total cranial base length, PtVertA, HAxisGn, GoPo - mandibular corpus length, SGo – posterior total face height, PNSFH - midface height had significant age associations. Figure 30 gives an indication as to where these significant linear measurements are situated.

Table 45: Spearman correlations between age and all the linear measurements for the entire sample (n=180)

	BaS	BaNa	SNa	NaANS	PTVertNa
AGE	0.4599	0.5210	0.3700	0.3028	0.3042
	PtVertA	**PtPNS**	**NaMe**	**ANSMe**	**HAxisGn**
AGE	0.6373	0.3776	0.3692	0.2902	0.5870
	HAxisGo	**GoPo**	**SGo**	**AOBO**	**NtNaPo**
AGE	0.3626	0.5383	0.4473	-0.1319	0.3647
	SubnslSup	**LInfPo**	**LSupNaPo**	**LInfNaPo**	**PNSFH**
AGE	0.3296	0.1949	0.2556	0.3547	0.4015
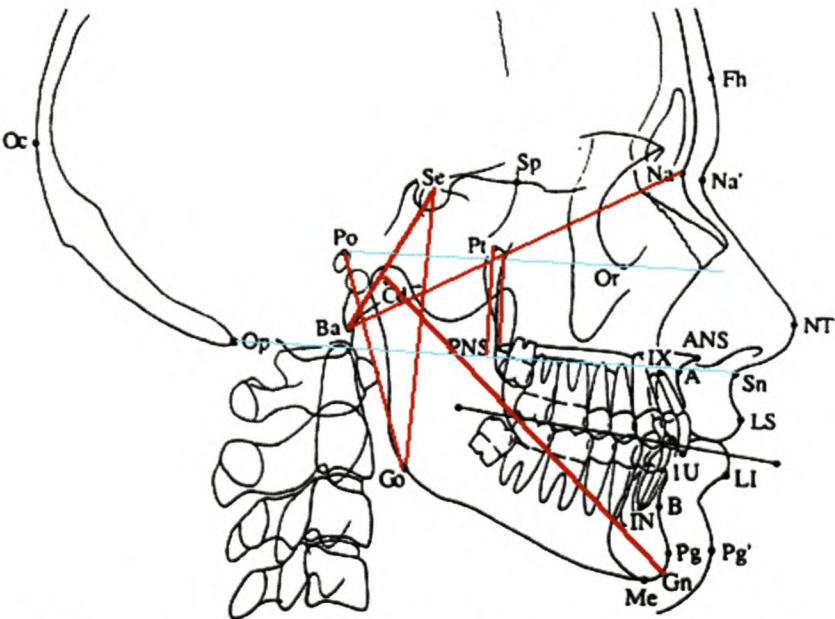
Figure 30: Location of all the significant linear measurements from the entire sample (n=180)

9.3.2 Investigating growth over time

In order to investigate the influence of age over this period of growth for each of the linear measurements, the control group (as an indication of normal children) was used to calculate 95% and 80% tolerance lines. Altman centile curves were used, where the absolute residuals were plotted against age (Altman, 1993). Figure 31 shows this for the Basion-Nasion (BaN) length.
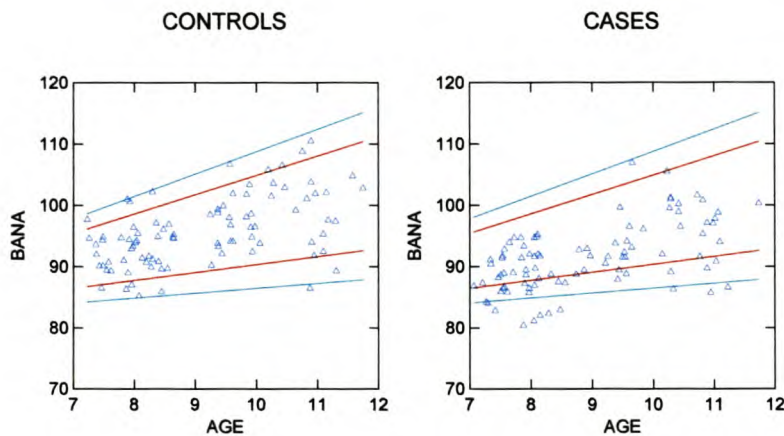
Figure 31: Altman centile curves – BaN length

These expected normal tolerance lines for growth over time as calculated on the control/normal group (left in Figure 31) were then directly applied to the FAS group (right in Figure 31). In blue are the 95% tolerance lines, but because these showed very little discrimination in the FAS group, 80% tolerance lines (red) were also calculated. These lines show much better discrimination in the FAS group, only 11% of the FAS cases fell outside the 95% tolerance lines (blue), but this increased to 30% when the 80% tolerance lines were used.

As this scenario existed for most of the linear measurements, a single measurement as a discriminator could not be used. Most of the FAS children fell within the same limits as those set up by the control/normal children, therefore a large overlap existed between the two groups. Moving on from this univariate approach, a more multivariate approach was used in order to seek better discrimination between the two groups.

The screening methods used to select those measurements which best discriminate between the two groups were i) Principal Components Analysis and ii) Stepwise Logistic Regression. The results from both these methods were compared and as the results overlapped, a final set of eight linear measurements were selected as those with the highest discriminating qualities. They were: NaANS, PtVertN, NMe, SupNaPo, ANSMe, PtPNS, LinfPo and HAxisGn (Figure 32). As can be seen in Figure 30 most of these 8 measurements are located in the anterior skull area, and are mostly vertical measurements. The one diagonal measurement (HAxisGn) was also one of the linear measurements that is significantly influenced by age.
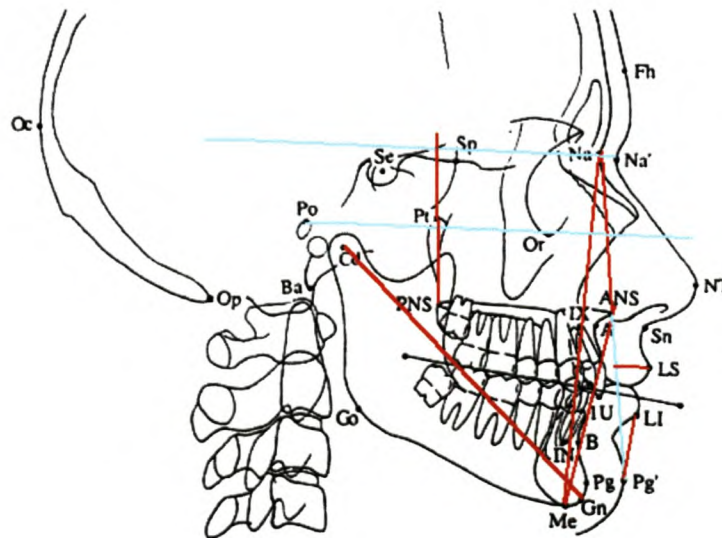
Figure 32: Location of the 8 linear measurements with the highest discriminating qualities

9.3.3 Biplot of principal components

A biplot was constructed using Principal Component Analyses to describe the multidimensional variation of the data (Figure 33). A biplot is a graphical representation of multivariate information in a data matrix (Johnson and Wicheren, 1998). In Figure 33, the cases are displayed in red triangles and the controls in blue, each with their own confidence ellipse (default - 68%) centered at the sample means of the x and y variables.
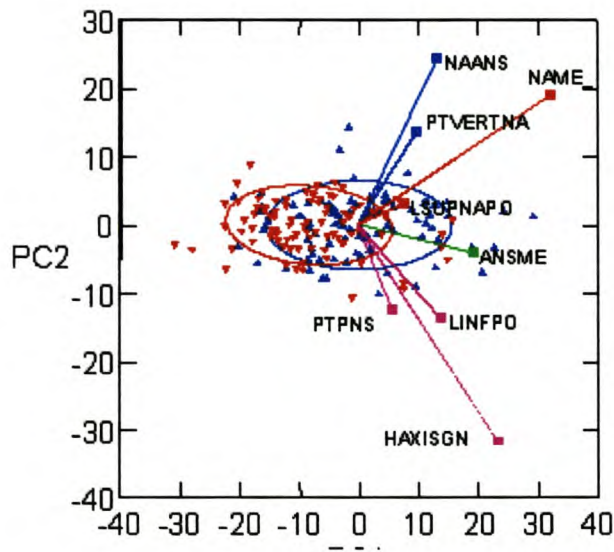
Figure 33: Biplot using Principal Component Analysis

The huge overlap between the two groups that was referred to earlier can be clearly seen. In this overlapping area, 29% of diagnoses are false positives and 28% are false negatives.

In Figure 34, the eight measurements identified by the Principal Component Analyses are the lines drawn in blue, red, green and pink. Those that lie in the same direction (same colour) are highly correlated i.e. NaANS & PtVertN; NaMe & SupNaPo; PtPNS & LInfPo and HAxisGn. Note that ANSMe is on its own (separate direction). These coloured lines correspond with the lines on the right of the picture in Figure 33. From Figures 33 and 34, it appears as if ANSMe (mandible height), HAxisGn (mandible length) and NaANS (upper anterior face height) are the three most important measurements in discriminating between normal and FAS children.
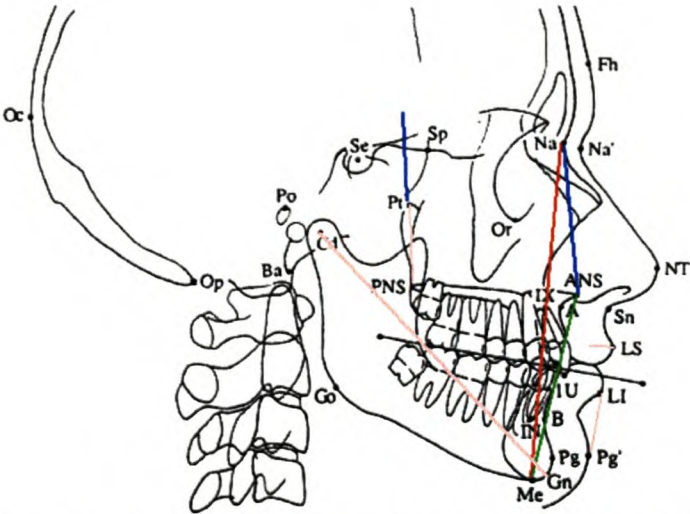
Figure 34: Location of the 8 linear measurements identified with Principal Component Analysis

9.3.4 Individual component analysis

9.3.4.1 Cranial base

In this study, there were five variables measuring cranial base size and angulation (Table 46). The sella-nasion plane serves as a relatively stable base from which to assess changes in the dentofacial complex. It parallels the anterior cranial fossa, which is completed quite early and is a valid and stable reference plane (Graber, 1969).

Table 46: Variables used to assess size and shape of the cranial base

Variable	Sample Mean		Paired t-test
	FAS	Controls	
Basion-sella length (BaS)	40.88	42.59	<0.01
Basion-nasion length (BaN)	91.37	95.54	<0.01
Sella-nasion length (SNa)	60.16	63.12	<0.01
Basion-sella-nasion angle	128.74^0	128.44^0	0.69
Sella-nasion FH angle	9.75^0	9.60^0	0.77

All lengths in mm
BaNa = Total cranial base length   **BaS = Posterior cranial base length**
SNa = Anterior cranial base length

Although the angulation of the cranial base was not statistically significantly different, all of the linear measures were statistically significantly different. The decreased S-Na length represents shortening of the anterior cranial base. The lengths of the basion-nasion, basion-sella and sella-nasion are shorter than normal.

9.3.4.2 Midface

Many reports have suggested from visual assessment that FAS children exhibit midface insufficiency (Jones et al., 1973; Clarren and Smith, 1978; Hanson, Jones and Smith, 1976; Streissguth, Clarren and Jones; 1985; Spohr and Steinhausen, 1984). In this study, midface height both by visual assessment and linear measurement (Na-ANS) is significantly different in the FAS children compared with the controls (Table 47). This differs from the study by Gir et al (1989) who reported that midface height (Na-ANS) and the anteroposterior position relative to the cranial base (SNA, PtVert-A, Na-FH) and the facial angle (Na-A to FH) were virtually identical in the FAS and control series. However, what concurs with their study appears to be the deviant cant of the palatal plane in the FAS children.

The palatal plane is rotated downwards posteriorly, which decreases the posterior midface height (Pt-PNS, PNS $\perp$ FH) and increases the angle of the palate with the Frankfort horizontal; PNS $\perp$ FH and palatal plane to FH angle being statistically significant. This localized displacement thus appears to affect the sagittal position of the anterior (vertical) limit of the palate as seen with the Na-ANS height. Table 48 shows the variables that were used to assess size and shape of the midface. Note that for all the vertical linear measurements $p < 0.01$.

Table 47: Variables used to assess size and shape of the midface

Variable	Sample Mean		Paired t-test
	FAS	Controls	
Nasion-ANS length	39.84	42.69	<0.01
Sella-nasion-A angle	94.66^0	95.06^0	0.55
Nasion-A to FH angle	95.09^0	94.54^0	0.24
Pt-vertical/nasion length	50.06	52.86	<0.01
Pt-vertical/A length	51.54	54.24	<0.01
PNS ⊥ FH length	24.84	25.98	<0.01
Pterygoid-PNS length	22.84	23.18	0.37
Palatal plane to FH angle	6.08^0	4.72^0	<0.01

All lengths in mm

9.3.4.3 Mandible

Since previous reports have commented on the retrognathia of the mandible in children with FAS, the morphology and relationships of the mandible were examined in detail (Table 48). Several parameters were assessed: anteroposterior and sagittal size and position of the mandible and its components, sagittal heights of the lower third of the face and tooth positions and angulations. The Y-axis (the line joining the sella and gnathion) is used to indicate the chin point on the face, the direction of mandibular growth or the relative retrusion or protrusion of the mandible. SNB represents the anteroposterior position of the mandible.

The resulting picture is of a mandible that is in normal anteroposterior and sagittal position. The FAS mandible appears to be undersized and indeed the corpus length (Go-Pg) is significantly shorter than that of the controls. The effective mandibular length is measured from condylion to gnathion (Co-Gn). Relative component sizes appear to differ significantly in the FAS cases: the FAS mandible has a shorter corpus (p<0.01) a shorter ramus (p<0.01) but a slightly larger gonial angle. Sella-Gonion is the distance from the centre of the cranial base to the gonial angle of the mandible and represents posterior facial height The significant decrease in the ANS-Me and Se-Go lengths reflects shortened anterior and posterior facial heights respectively.

Table 48: Variables used to assess size and shape of mandible

Variable	Sample Mean		Paired t-test
	FAS	**Controls**	
Mandible			
Nasion-menton length	98.21	103.22	<0.01
ANS-menton length	59.67	61.81	<0.01
Sella-nasion-B point angle	79.92^0	79.92^0	1.00
Sella-nasion-pogonion angle	78.36^0	78.64^0	0.63
Y axis angle (S-Gn)	93.76^0	93.07^0	0.20
Condylion-gnathion length	94.56	99.40	<0.01
Condylion-gonion length	37.20	40.15	<0.01
Gonion-pogonion length	65.55	68.61	<0.01
Sella-gonion length	58.25	62.04	<0.01
Condylion-gon-menton-angle	125.59^0	124.53^0	0.27
Gonion-menton to FH angle	29.47^0	28.72^0	0.28
AOBO difference (mm)	3.77	3.86	0.78

9.3.4.4 Incisor relationships

There were no significant differences between tooth positions and angulations (Table 49).

Table 49: Variables used to assess incisor relationships

Variable	Sample Mean		Paired t-test
	FAS	**Controls**	
Incisor relationships			
Interincisal angle	123.07^0	122.21^0	0.51
UI 1 to FH angle	115.07^0	114.98^0	0.93
LI 1 to gonion-menton angle	92.38^0	94.10^0	0.04

9.3.4.5 Integumental profile

Unlike previous reports from the literature, "frontal bossing", an increased prominence of the forehead, was not seen in the present study. Similarly the other soft-tissue parameters of the nose, lips and facial proportions (Table 50) are very similar in the two groups, the one exception being the LI-pogonion' length, which is significantly different (p<0.01), which could be due to the shorter ramus seen in the FAS cases.

Table 50: Variables used to assess size and shape of facial and integumental profile

Variable	Sample Mean		Paired t-test
	FAS	Controls	
Nasion'-subnasale-pogonion angle	155.00^0	155.87^0	0.31
Forehead-nasion'-NT angle	141.97^0	138.64^0	0.08
NT $\perp$ nasion-pogonion distance	22.29	23.19	0.01
Nasion'-NT-subnasale angle	119.74^0	118.94^0	0.43
NT-subnasale-LS angle	46.66^0	47.60^0	0.50
Subnasale'-LS length	17.09	17.11	0.97
LI-pogonion' length	22.80	25.45	<0.01
LS $\perp$ nasion-pogonion distance	12.34	12.36	0.96
LI $\perp$ nasion-pogonion distance	10.17	10.47	0.38

9.4   Discussion

Cephalometric examination provides a valuable tool: by relating various parts of the face, the jaws and the dentition, information is obtained that aids diagnosis and classification. Cephalographs provide a quantitative medium for describing the dentofacial pattern at the time when the radiograph was taken. It does not present information on the quality of growth and development.

In spite of the considerable interest in FAS, few studies have attempted to quantify the craniofacial features of FAS (Frias, Wilson and King, 1982; Riekman, 1984, Gir, Aksharanugraha and Harris, 1989; Jackson and Hussain, 1990). In this study, Spearman correlations between age and the linear measurements, found seven measurements (BaS – posterior cranial base length, BaNa – total cranial base length, PtVertA, HAxisGn, GoPo - mandibular corpus length, SGo – posterior total face height, PNSFH - midface) with significant age associations. Measurements related to the face height and mandibular size appear to be the most important in distinguishing the FAS children from the controls. The lower posterior face height and shorter mandibles seen in the FAS children were also found in other studies that examined children with growth hormone deficiency (Spiegel et al, 1971; Pirenen et al, 1994) and children born small for gestational age and with catch-up growth failure (Van Erum, 1997).

With regard to the positioning of the linear measurements:

- Most (5 out of 8) of the discriminating measurements lie in the front of the skull area.
- Most of the discriminating measurements are vertical measurements, with one a horizontal and one diagonal measurement.
- Only two of the measurements are lines between soft tissue points.

When comparing the photographic analyses of the facial features versus the cephalometric assessments:

- The four facial features most typical of a FAS child had a positive predictive value (PPV) of 92% and a negative predictive value (NPV) of 90%.
- The eight linear measurements from the cephalometric analyses had a PPV of 92% and an NPV of 95%.

At this stage one can therefore conclude that the external facial features are probably more reliable in discriminating between the two groups.

Midface insufficiency (hypoplasia) is a commonly cited feature of FAS. Hanson, Jones and Smith (1976) place the frequency of visually assessed "midface hypoplasia" at 65%. The cephalometric analysis of this study showed that the frontal prominence, in combination with the below-average corpus length of the mandible, gives an impression of a small midface. In addition, the soft tissue features, such as the short palpebral fissures and thin upper lip with an indistinct philtrum, further exaggerate the perception of a hypoplastic mandible. In this study, facial depth measurements show that there are deficits in the upper, middle and lower face, with the midface being most severely affected (Table 48, page 219). This finding partially supports clinical reports of a hypoplastic midface (Korányi et al, 1981; Frias et al, 1982; Vitéz et al, 1984; Clarren et al, 1987; Gir et al, 1989; Jackson and Hussain, 1990, Astley et al, 1992, Astley and Clarren, 1995).

However, it is noteworthy that no quantitative documentation of midface hypoplasia is available; indeed, previous cephalometric studies of FAS children found midface heights to be unremarkable and true midface hypoplasia could not be demonstrated (Gir, Aksharanugraha and Harris, 1989; Frias, Wilson and King, 1982). Instead, Frias et al (1982) suggested that an abnormal positioning of the maxilla due to restricted forward growth of the face, which in turn was due to abnormal brain growth and shortening of the anterior cranial base, gave the appearance of midface hypoplasia. In addition, Riekman (1984) previously came to the same conclusion that the apparently flat midface in FAS is not of skeletal origin, but rather due to the absence of a well-defined philtrum, a broad nasal base, and short palpebral fissures. Gir et al (1989) suggested that over-development of the upper third of the face (frontal bossing) and lower third of the face (elongated mandibular corpus) produced the impression of a small midface. The anthropometric data in this study show that there is generalized reduction in all facial depths, and the midface measurements tend to be more reduced than the upper and lower facial depths.

During growth the mandible is spatially relocated away from the cranial base, its size and shape being modified by the eruption of the dentoalveolar structures and extensive resorption and deposition of bone (Knott, 1971). The direction of growth may be horizontal, horizontal/vertical or vertical, but entirely horizontal or entirely vertical growth seems to be rare (Björk, 1969). Since previous reports have commented on the retrognathia of the mandible in children with FAS, several parameters related to the morphology and relationships of the mandible were examined in detail (Table 49, page 219). All vertical measurements of the face (anterior total face height (Na-Me), upper anterior face height (Na –ANS), lower anterior face height (ANS- Me) and the mandibular ramus height (Cd-Go)) were all statistically significantly different (p<0.01) for the FAS group when compared with the controls. In the FAS children, with both the midface height and the lower facial height reduced, this causes a greater deficit in total face height.

Moore et al (2002) found that midface height and nasal bridge length appear to be similarly affected by alcohol exposure. This finding contradicts those of Clarren et al (1987), who argued that on average FAS children have a long midface relative to length of nose. Per clinical description of FAS individuals, these individuals tend to have a small, upturned nose; however, the clinical descriptions do not describe shortening of the midface (Streissguth et al, 1980; Abel, 1984; Spohr et al, 1994; Stratton et al, 1996).

None of the angular measurements except for the palatal plane to FH angle revealed any significant differences between the cases and controls. This appears to indicate that the relationship between the various structures in the face (based on angular measurements) are not affected as much as the distances between certain landmarks, concurring with observations seen by Frias et al (1982) in their cephalometric analysis of FAS subjects and Moore et al (2002) in their anthropometric analysis of the FAS face. Several linear measurements in the FAS children were found to be significantly different from the controls, especially those related to the cranial base, midface and mandible. Thus from the present study, it seems that growth retardation is most pronounced in craniofacial components with a high relative and absolute growth potential in childhood, and concurs with a previous study carried out by Buschang et al (1983).

The literature has shown that alcoholic mothers weigh less than control mothers during pregnancy (Hernandez-Guerrero et al, 1998; Croxford et al, 2002). This may then cause offspring to have smaller skull and facial structures. As alcohol also affects the development of the brain, this will also contribute to reduced skull growth and expansion (Padmanabhan and Muawad, 1985). In humans, this is important since brain growth begins shortly after organogenesis, reaches a maximum rate around birth and continues during the first few years of life. Linear measurements of the jaws and skulls of the FAS children in this study were significantly affected, as was also described in a study by Hernandez-Guerrero et al (1998). The underlying mechanism(s) that give rise to these effects cannot be fully explained by the present research. Prenatal alcohol exposure is complicated by interactions at the membrane levels of developing cells, neurochemical and biochemical processes needed for normal growth.

Although the anatomy of the cranial base is complex, its structures and relationships are important in cephalometric analysis: both jaws are related to it and therefore variations in its size and form can affect jaw relationships. The literature has demonstrated that FAS children have smaller calvaria (Jones et al, 1973; Clarren and Smith, 1978; Clarren et al, 1978; Streissguth, Herman and Smith, 1978) and as mentioned in the literature review, microcephaly is regarded as one of the cardinal features of children with FAS (Jones et al, 1973; Jones and Smith, 1973). Although the angulation of the cranial base was not statistically significantly different, all of the linear measures were reduced and statistically significantly different for the FAS children in this study (Table 46, page 216). The decreased Se-Na length represents shortening of the anterior cranial base.

Analysis of the cranial base linear dimensions (Table 47, page 218) reveals particular underdevelopment of the posterior cranial base. The posterior cranial base comprises the basiocciput and the posterior part of the basisphenoid which are united at the speno-occipital synchondrosis, an important centre for cartilaginous growth. The posterior cranial base growth is mainly due to growth at the spheno-occipital synchondrosis (Ford, 1958), which is the only cranial base synchondrosis to remain active after 7 years of age, usually remaining active until 16 years of age in males and 13 years of age in females (Konie, 1957).

SBa (sella basion) and NBa (nasion basion) were statistically significantly reduced in FAS children and this could be explained by a primary defect in the cranial base resulting from a premature closure of the spheno-occipital and spheno-ethmoidal synchondrosis (Burdi et al, 1986). Growth at this centre displaces the anterior cranial structures, to which the upper facial skeleton is related. Korkhaus (1957) reported that the premature fusion of the cranial base synchondrosis is related to underdevelopment of the nasomaxillary complex and this may therefore account for the nasomaxillary hypoplasia in FAS.

Based on Moss's hypothesis of functional matrices (Moss and Salentijn, 1969), it can be postulated that the abnormalities described in the present study are a direct consequence of abnormal brain growth, which leads to the early closure of the spheno-ethmoidal synchondrosis and to shortening of the anterior cranial base. This in turn produces an inhibition in the forward growth or advance of the midface and results in the clinically apparent deficiency of the midface and occasionally a relative mandibular prognathism.

The incisor relationships were determined primarily by angular measurements related to the positioning of the incisors (Table 49, page 219). The most commonly used angular measurements are the interincisal angle, the long axis of the lower incisor to the Frankfort plane horizontal. Various authors have placed the mean interincisal angle between 125° and135°. Larger angles result from very upright incisors and are frequently associated with deep overbites. Small angles occur in dental protrusion. The ideal angle between the lower incisor and the mandibular plane angle is 90°, while the upper incisor angulation relative to the Frankfort horizontal plane has a mean value of 110°. Larger angles usually indicate maxillary incisor protrusion. In this study, the incisor relationships in both the cases and the controls were unremarkable.

9.5   Conclusions

This chapter described and discussed the findings from the cephalometric examination. This study has shown that measurements related to the face height and mandibular size appear to be the most important features when distinguishing FAS children. Overall, the FAS children in the present study presented with a vertically and horizontally underdeveloped maxilla, together with features of a long face syndrome with a large gonial angle and a short ramus in relation to the total face height. There was also a tendency for the development of an anterior open bite, which appears to be compensated for by an increase in the anterior alveolar process to bring the incisor teeth into occlusion. The latter adaptation occurred mainly in the mandible. This study demonstrates that the growth retardation in the FAS children not only affects their statural height but also their craniofacial growth.

Chapter 10: General Discussion and Recommendations

This study developed from several epidemiological, prenatal and infant studies that are being carried out in the Western Cape. Preliminary data have shown that the frequency of the fetal alcohol syndrome in the Western Cape is the highest reported anywhere in the world (Viljoen, 1999). It was with this background and the paucity of literature in this field in relation to dentistry, that this study was carried out. It is the largest study documenting oral and craniofacial features to date.

The validity of the study was dependent upon the accurate diagnosis of the FAS children and this has been more than adequately described in the methodology section. It is hoped that the findings of this study will provide an opportunity for health care workers to gain insight into the nature of the epidemiology and pathogenesis of FAS. This study, the first in South Africa, provides data that contribute substantially to the current knowledge of the oral and craniofacial manifestations of FAS.

There were no significant age differences between the two groups in the sample and the results of the anthropometric measurements in respect of weight, height and head circumference showed significantly lower values for the FAS children when compared to the controls. Almost all of the physical abnormalities described in children with FAS were seen in this study. Some of these abnormalities included derformities in the small joints of the hand, altered palmar creases and malformation of the ears.

The most prominent findings include the confirmation of the importance of the characteristic facial features in diagnosis and the cephalometric study confirming the anthropological findings. Knowledge of the clinical features of FAS will assist health professionals identify affected children who otherwise might not receive the best available health care.

In addition, the "myths" that have been generated in the FAS literature regarding oral and dental health over these past 40 years have been dispelled – for example, that FAS children have a higher incidence of cleft palate, high arched palates and defective teeth – the results of this study do not show this. The findings of this study are remarkably similar to the few studies carried out abroad that have documented orofacial manifestations.

The data from this study suggest that craniofacial anthropometry still has a place in the field to quickly and reliably identify children with subtle alcohol effects. An ideal minimum cluster (palpebral fissure length, head circumference, a smooth philtrum and a thin upper lip) of clinician-friendly measurements has been described and it may be utilized more broadly in the diagnostic field to differentiate individuals with the highest accuracy.

The oral health status of the hard and soft tissues were not as noteworthy as was expected and this could be ascribed to the fact that the most vulnerable time for most of the developmental defects associated with the developing teeth occurs during the first year of life (Hargreaves et al, 1989). The relationship of dental and skeletal age to several measures of growth between the FAS cohort and the control children indicate that both dental and skeletal ages are significantly related to child growth and perhaps nutritional status. These findings, however, need to be confirmed via additional longitudinal studies.

The inter-relationships among chronological age, dental age, skeletal age and height and weight were investigated. The purpose was to highlight the value of various physical indices of the overall developmental status and nature of the relationships among these indices between the cases and the controls. This study provides evidence of a positive association between dental age, skeletal age and height and weight in both the FAS group and the controls. The data suggest that both dental and skeletal ages may be a reflection of general somatic growth. There was no association found with head circumference.

Results from the cephalometric examination in this study have shown that measurements related to the face height and mandibular size appear to be the most important features when distinguishing FAS children. Overall, the FAS children in the present study presented with a vertically and horizontally underdeveloped maxilla, together with features of a long face syndrome with a large gonial angle and a short ramus in relation to the total face height. There was also a tendency for the development of an anterior open bite, which appears to be compensated for by an increase in the anterior alveolar process to bring the incisor teeth into occlusion. The latter adaptation occurred mainly in the mandible. This study demonstrates that the growth retardation in the FAS children not only affects their statural height but also their craniofacial growth.

FAS can no longer be viewed as just a rare and peculiar childhood disorder. It should be realized that the long-term perspective of FAS in many cases implies a developmental disability beginning at birth and reaching from infancy to adulthood. The spectrum of multiple handicaps changes during different age periods and, currently, recent research is beginning to unravel and identify the magnitude of physical, mental and psychological problems that will emerge in adult FAS. There is a need for large-scale, longitudinal standardized studies to elucidate the finer aspects of the onslaught of alcohol on the development of the craniofacial and oro-dental apparatus of the developing fetus. These studies would need to incorporate biological and genetic variables that exacerbate fetal damage, that could not be included in the present study.

Recommendations

It has been shown from the results of this study that there are two areas of particular interest to the dentist: the craniofacial anomalies and the medical problems that may affect the dental management of these patients. The awareness and recognition of patients with fetal alcohol syndrome is important so that they can be correctly diagnosed and referred appropriately. They are likely to have maxillary hypoplasia and micrognathia. Any of these may necessitate follow up for malocclusion resulting from skeletal disharmonies. Among the medical problems are deficiencies in growth and intelligence.

Because of the various congenital cardiac defects (especially atrial and ventricular valvular defects), the patient's medical practitioner needs to be consulted on the patient's susceptibility to subacute bacterial endocarditis and the need for prophylaxis prior to dental treatment. Since the children have decreased intelligence, there may be behaviour management problems that are best treated with behaviour management and/or premedication.

Understanding the many disturbances of craniofacial growth which result from the widespread use of alcohol during pregnancy obviously warrants attention. In addition, dentistry has much to offer in the determination of true aetiologic factors in disturbances that effect growth and development of teeth, the facial skeleton and soft tissues of the oral cavity. Research in the coming decade should be directed at:

(i)   identifying objective biological and genetic markers for alcohol abuse in women and fetal risk for FAS in women;

(ii)   clarifying currently identified and additional risk factors contributing to or permitting the varied expression of FAS;

(iii)   improving the understanding of the craniofacial mechanisms of alcohol teratogenesis and developing methods for the prevention, treatment and amelioration of FAS.

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Appendix 1: FAS Oral Health Status Data Capture Sheet

1.   **Record Number:** …………………………
2.   **Date of birth** (YY/MTH/DAY):

3.   **Name:** …………………………………………………………
4.   **Gender:**…………………

5.   **Extra-Oral Examination:**

Feature	Yes	No	Code
Normal extra oral appearance			
FACE/JAW: Hypoplastic mid-face			
Retrognathic, hypoplastic mandible			
Flattened, hypoplastic maxilla			
EARS: Posterior rotation			
Abnormal concha			
Pre-auricular pits			
Other:			
EYES: Short palpebral fissures			
Strabismus			
Ptosis			
Epicanthal folds			
Micro-opthalmia			
Myopia			
NOSE: Flattened, low nasal bridge			
Short, upturned nose			
Anterior nares			
LIPS: Long philthrum			
Smooth philthrum			
Narrow, thin upper vermillion			
MOUTH: Cleft lip			
Cleft palate			
Cleft lip & palate			
High, narrow, arched palate			
HANDS: Clinodactyly			
Camptodactyly			
Limited supination			
Palm creases			
Short fifth fingers			
HEART: Mumur			
Valve/septal defect			
Other heart defect:			

6. Physical Measurements

Age:		Code
Height	Height %	
Weight	Weight %	
OFC	OFC %	
ICD	ICD %	
IPD	IPD %	
PFL	PFL %	

7. **Fluorosis:** (0 = normal, 1 = questionable, 2 = very mild, 3 = mild, 4 = moderate and 5 = severe, 8 = excluded, 9 = not recorded):

8. **Tooth anomalies, Specify**...

9. Enamel Opacities/Hypoplasia

```
12   11   21   22
 |   |    |    |
 |   |    |    |
 |   |    |    |
46                36
```

0 = normal
1 = demarcated opacity
2 = diffuse opacity
3 = hypoplasia
4 = other defects
5 = demarcated and diffuse opacities
6 = demarcated opacity and hypoplasia
7 = diffuse opacity and hypoplasia
8 = all three conditions
9 = not recorded

10. Tooth size, shape, presence

Feature	Yes	No	Code
Normal			
Microdontia			
Oligodontia (few teeth)			
Anodontia (no teeth)			
Microdontia & Oligodontia			
Other, specify...........................			

11. Oral Mucosa:

CONDITION		LOCATION
No abnormal condition	0	Vermilion border
Angular cheilitis	1	Commissures
Commissural pits	2	Lips
Traumatic lesions	3	Sulci
Geographic Tongue	4	Buccal mucosa
Ulceration (aphthous, herpetic, traumatic)	5	Floor of the mouth
Acute necrotizing gingivitis	6	Tongue
Candidiasis	7	Hard or soft palate
Dentoalveolar abscess	8	Alveolar ridges
Herpes labialis		
Fissured tongue		
Other:………………………………………..		

CONDITION	LOCATION

12. Plaque Index:

0 = none
1 = < 1/3
2 = > 1/3
9 = Not recorded

13. Tooth Surface Loss:

	15/55	14/54	13/53	12/52	11/51	21/61	22/62	23/63	24/64	25/65
B										
O/I										
P										

	45/75	44/74	43/73	42/72	41/71	31/81	32/82	33/83	34/84	35/85
B										
O/I										
L										

14.   Dental Caries and Treatment Need - Children

	S	O	B	L	M	D		S	O	B	L	M	D	
17							37							
16							36							
15/55							35/75							
14/54							34/74							
13/53							33/73							
12/52							32/72							
11/51							31/71							
21/61							41/81							
22/62							42/82							
23/63							43/83							
24/64							44/84							
25/65							45/85							
26							46							
27							47							

PERMANENT
0=sound
1=decayed
2=filled with decay
3=filled no decay
4=missing due to caries
5=missing for other reasons
6=sealant
T=trauma
8=unerupted tooth
9=excluded tooth

PRIMARY
A
B
C
D
E

F
T

TREATMENT
0=none
P=preventive
F=fissure sealant
1=one surface filling
2=two surface filling
5=pulp care
6=extraction
7=need for other care
9=not recorded

dmft: **DMFT:**   **dmfs:**   **DMFS:**

d	m	f	dmft	D	M	F	DMFT	d	m	f	dmfs	D	M	F	DMFS

15. Extra-Oral Examination

0 = Normal extra-oral appearance
1 = Ulceration, sores, erosions, fissures (head, neck, limbs)
2 = Ulceration, sores, erosions, fissures (nose, cheeks, chin)
3 = Ulceration, sores, erosions, fissures (commisures)
4 = Ulceration, sores, erosions, fissures (vermillion border)
5 = Cancrum oris
6 = Abnormalities of upper and lower lips
7 = Enlarged lymph nodes (head, neck)
8 = Other swellings of face and jaws
9 = Not recorded

Notes:

....
....
....
....

16. Non-cavitated carious lesions

	S	O	B	L	M	D			S	O	B	L	M	D	
16								36							
15/55								35/75							
14/54								34/74							
13/53								33/73							
12/52								32/72							
11/51								31/71							
21/61								41/81							
22/62								42/82							
23/63								43/83							
24/64								44/84							
25/65								45/85							
26								46							

17. Teeth – Present (1)/ Absent (2)

Perm		Prim		Perm		Prim	
17				37			
16				36			
15		55		35		75	
14		54		34		74	
13		53		33		73	
12		52		32		72	
11		51		31		71	
21		61		41		81	
22		62		42		82	
23		63		43		83	
24		64		44		84	
25		65		45		85	
26				46			
27				47			

18. Dento facial anomalies

Feature	Yes	No	Code
SPACE: Crowding in incisal segments			
Spacing in incisal segments			
Diastema (mm)			
OCCLUSION: Anterior maxillary overjet			
Anterior mandibular overjet			
Vertical anterior open-bite			
Antero-posterior molar relation			
OTHER:			

Appendix 2

UNIVERSITEIT VAN STELLENBOSCH
UNIVERSITY OF STELLENBOSCH

INFORMATION SHEET FOR PRINCIPALS, PARENTS & CARERS

Fetal Alcohol Syndrome and Oral health

Dr Sudeshni Naidoo of the Department of Community Dentistry at University of Stellenbosch, Tygerberg Hospital is carrying out research into the oral health of children with fetal alcohol syndrome (FAS). This will be done by examining the teeth, gums and facial features of children with FAS against the teeth, gums and facial features of children without FAS. This research has not been done before, is important and will be of great value as it may shed light on the type of oral and dental problems found in children with FAS, how many children have problems, what causes them, how to prevent and how to treat them.

Participation in the study will involve:

i)   completion of a short questionnaire;
ii)   photographs taken of the face;
iii)   having x-ray photographs taken of the teeth; and
iv)   having an oral examination of the mouth

The whole process should take about half an hour. There are no risks involved if your child participates in this study and there should be no more discomfort than for a routine dental check up examination.

All information gathered as part of the study will be treated as strictly confidential. No one will have access to this information except the researcher. Neither your name nor anything that identifies you will be used in any reports of this study. All information collected will be maintained and stored in such a way as to keep it as confidential as possible. If you have any questions or queries regarding the study please contact Dr Sudeshni Naidoo (Tel: 021-937 3148 work; 021-686 2720 home).

The information above has been explained to me by ....…………
and I understand what will be required if my child takes part in the study. My questions concerning this study have been answered by him/her. I agree to allow my child to participate in the research being undertaken by Dr Sudeshni Naidoo. I understand that at any time I may withdraw my child from this study without giving a reason and without it affecting my child's normal care, management or schooling.

Signed: ....…..   Date   ....

Print name:…………………………………..

Principal/Witness/Information provider:..………

Appendix 3

UNIVERSITEIT VAN STELLENBOSCH
UNIVERSITY OF STELLENBOSCH

CONSENT FOR ORAL EXAMINATION

January 2000

Dear Parent of ……………………………………………………..,

We are from the Department of Community Dentistry at the University of Stellenbosch. As you have know from the information sheet that was provided to you, we are interested in examining your child's face, mouth and teeth to look for any problems. We are doing this to see if there are ways in which we can prevent any problems or help with any problems they may have.

The procedure will take about 15-20 minutes. We may take photographs and x-rays. We will only take photographs of your teeth and no-one will be able to see your face on the photographs. There are no risks in participating and there should be no more discomfort than in a routine dental check up examination. All information gathered in the study will be treated as strictly confidential. No one will have access to this information except the researcher. Neither your name nor anything that identifies you will be used in any reports of this study. All information collected will be maintained and stored in such a way as to keep it as confidential as possible.

Your child will be picked up from school during the months of March, April, May and will be taken to the Tygerberg Hospital Dental Clinic for the mouth examination. The children will be given sandwiches, biscuits, tea/coffee and juice and will also receive free toothbrushes and toothpaste.

If you would like your child to take part in the study, please sign the bottom of this letter. Please contact Dr S Naidoo on telephone number at work 021-937 3148 or at home on 021-686 2720 if you would like any more information about the study.

Thank you for your co-operation

Yours sincerely

Dr Sudeshni Naidoo

I understand what will be required of my child to take part in the study. I agree to allow my child to participate in the research being undertaken by Dr Sudeshni Naidoo. I understand that at any time I may withdraw my child from this study without giving a reason and without affecting his/her normal care, management or schooling.

Name: ....…………..   ....………..
(print in block letters)   (Signature)

Date: ....…….   Witness: …………………………….

Appendix 4

UNIVERSITEIT VAN STELLENBOSCH
UNIVERSITY OF STELLENBOSCH

21 June 1999

Dr S Naidoo
Department of Community Dentistry

Dear Dr Naidoo

RESEARCH PROJECT: **"FETAL ALCOHOL SYNDROME IN THE WESTERN CAPE: CRANIOFACIAL & ORAL MANIFESTATIONS**
PROJECT NUMBER: **99/059**

It is a pleasure to inform you that Subcommittee C of the Research Committee have approved the above-mentioned project on 6th May 1999, including the all ethical aspects involved.

This project is therefore now registered and you can proceed with the work. Please quote the above-mentioned project number in all further correspondence.

Patients participating in a research project at Tygerberg Hospital will not be treated free of charge as the Provincial Administration of the Western Cape does not support such research financially.

Due to heavy workloads the nursing corps of Tygerberg Hospital cannot offer comprehensive nursing care in research projects. It may therefore be expected of a research worker to arrange for private nursing care.

Yours faithfully

CJ VAN TONDER
for DEPUTY REGISTRAT (TYGERBERG CAMPUS)

CJVT/ev