ANALYSIS OF HUMAN EARLY FETAL FACIAL GROWTH AND JAW RELATIONSHIPS

by

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Abstract

Objectives: Abnormal jaw relationships can be a warning sign of the presence of congenital anomalies. During the late fetal period the jaw relationship is hypothesized to be stable allowing for detection of abnormal jaw position. This study aims to analyze growth of the jaws in 2D and 3D during the early fetal period in normal human conceptuses.

Methods: Lateral and frontal radiographs were available from a collection of 197 fetal specimens aged 10-20 weeks gestation, of these 26 specimens were scanned with micro-CT. Exclusion criteria was applied and a total of 14 linear and 5 angular measurements were made on digitized radiographs and micro-CT volume renderings. Linear regression models were used to analyze the relationship between the data collected and age in days of the specimens.

Results: Images comprising this study included 141 frontal radiographs, 120 lateral radiographs and 25 micro-CT scans. All linear measurements of the maxilla and mandible show a significantly positive association with increasing age in days. Age in days is a statistically significant ($P<0.001$) predictor of the size of the maxilla and mandible in all three planes of space. Both the maxilla and mandible increase more in width than length or height. Between 10-20 weeks, age in days is a significant ($P<0.001$) predictor of the jaw relationship. The radiographic data was divided into two groups, the jaw relationships during the 10-15 week period is significantly correlated with age ($P<0.001$). There is no correlation between jaw relationship and age during the 16-20 week period. From 10 to 15 weeks gestation the percent increase in size of all linear measurements is greater than during the 16-20 week period. Gender related growth rate differences are not observed.

Conclusions: During the early fetal period the maxilla and mandible grow more in width than height or length. Age in days is a strong predictor of maxillary and mandibular size in all three planes of space. Both the maxillary projection and the maxillary position relative to the mandibular position increase with increasing age especially in the 10 to 15 week timespan. The mandibular projection does not change with increasing age.

Preface

The author and research supervisor, Dr. Virginia M. Diewert, identified and designed this research project.

Human conceptuses were obtained from the embryo-pathology department at Women's Hospital in Vancouver for a study by Dr. Virginia M. Diewert between 1982 and 1988. Frontal and lateral radiographs were obtained after the routine examination of the conceptuses. Micro-CT scanning was performed in the Centre for High-Throughput Phenogenomics at the University of British Columbia, a facility supported by the Canada Foundation for Innovation, British Columbia Knowledge Development Foundation, and the UBC Faculty of Dentistry.

Analysis of the research data and preparation of the thesis was completed by the author.

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° = degrees

List of Abbreviations

2D: Two Dimension

3D: Three Dimension

ANB Angle: A point-Nasion-B point Angle

Al: Aluminum

BC: British Columbia

CT: Computed Tomography

kVp: peak kilovoltage

mA: milliampere

mm: millimeter

MNM Angle: Maxilla-Nasion-Mandibular angle

mS: milli-seconds

μA: micro Ampere

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I would like to thank my committee members, Dr. Joy Richman and Dr. Nancy Ford, for their support, encouragement and guidance of this project.

Dedication

To my mom and dad, Debra and Leland McFadden, who offer endless encouragement, support and unconditional love in whatever I choose to pursue.

To my husband, Daniel Newfield, who has always supported my goal to become an orthodontist. He has encouraged me during difficult times and celebrated my successes over the last 11 years. I am forever grateful of his patience, love and support. I am looking forward to our new adventures together.

Chapter 1: Introduction

1.1 Introduction of Growth and Development Studies

A thorough understanding of growth and development is imperative to understand the etiology of orthodontic problems, to be able to diagnose orthodontic problems, and to appropriately plan and carry out orthodontic treatment (Esenlik et al., 2014). Growth is a process that occurs throughout life from the time of fertilization all the way to adulthood at varying rates in different body tissues. Growth of the jaws, the maxilla and mandible, follows the general somatic growth trend and has been studied from its origins of embryonic development well into adulthood.

1.2 Postnatal Jaw Growth Studies

The majority of research regarding maxillary and mandibular growth focuses on the postnatal period. A large majority of the studies use longitudinal growth data collected from the 1920's to the 1960's at nine different centers in Canada and the United States. Together these nine collections have longitudinal growth data from 762 subjects ranging from 5 to 18 years old with some limited data from infancy to 60 years of age (AAOF, 2013).

1.2.1 Maxillary and Mandibular Growth Studies

The maxilla grows by two main mechanisms, passive displacement from the cranial base pushing the maxilla forward until approximately age seven and active growth at the maxillary sutures. These mechanisms cause the maxilla to grow down and forward out from under the cranium while at the same time undergoing bone remodeling. The mandible in contrast grows by surface apposition and remodeling, with bone apposition occurring on the posterior surface of the ramus and superior surface of the condyle which translates the mandible down and forward (Enlow and Hans, 1996).

Growth trends show that the maxilla and mandible follow the general somatic cephalocaudal growth gradient (Proffit, 2013). Growth of both the maxilla and mandible ceases first in width, followed by length then by height. Vertical growth of the height of the mandible has been documented to continue well into adulthood (Behrents, 1985). The patterns of growth of the maxilla and mandible are similar for males and females but gender differences in size, with males being larger, have been reported to emerge during adolescence, although a large overlap in size does occur (Broadbent et al., 1975).

It has been shown that the rate of growth of the mandible shows an adolescent growth spurt in both height and to a lesser extent length of the mandible. This occurs around the onset of puberty which happens on average at 14 years of age in males and 2 years earlier in females around 12 years of age (Woodside, 1968). This adolescent growth spurt of the mandible contributes to changes seen in the facial profile with increasing age.

1.2.2 Jaw Relationship Studies

Facial profile changes with age are evident when looking at a newborn baby with a convex facial profile and retrognathic mandible. The lack of a prominent mandible facilitates passage through the birth canal and subsequent feeding (Proffit, 2013). Postnatal changes in the jaw relationships have been studied longitudinally. A serial cephalometric study examining 30 subjects from 3 months of age until 18 year of age showed the convexity of both the soft tissue and underlying bony profile decreases as the mandible becomes positioned more forward

(Subtelny, 1959). From age 6 to 20 years the position of the maxilla relative to the anterior cranial base is approximately constant while the position of the mandible relative to the anterior cranial base shows angular increases (Ochoa and Nanda, 2004). Together these changes result in a decrease between the angle formed from a line from the frontonasal suture to the anterior edge of the maxilla and from the frontonasal suture to the anterior edge of the mandible called the ANB angle proposed by Steiner (Riolo, 1974; Steiner, 1953). Facial profiles show gender differences with females having a more convex profile than males (Nanda, 1971; Ochoa and Nanda, 2004).

1.3 Prenatal Period

The prenatal period can be divided into two stages: the embryonic period occurring from the time of implantation to 8 weeks gestation and the fetal period occurring from 8 weeks gestation to full term at 40 weeks gestation (Sperber and Guttmann, 2010).

The embryonic period is a time of rapid craniofacial development. During this time from implantation to 8 weeks gestation the three germ layers develop (ectoderm, endoderm and mesoderm), neural crest cells form and migrate, pharyngeal arches develop and facial prominences grow out and fuse. In the fetal period bone formation begins (Sperber and Guttmann, 2010).

1.3.1.1 Maxillary Development

The bones of the jaws form by intramembranous ossification or direct differentiation of neural crest derived mesenchyme into bone. The maxilla is a complex bone consisting of the paired maxillary bones and the paired palatine bones. The maxillary bone develops from four primary ossification sites, two of which are located where the infra-orbital nerve branches (Bush and Jiang, 2012). The first sign of ossification of the maxilla appears at Carnegie Stage 19, approximately 7 weeks of gestation. Two other primary ossification centers appear in the pre-maxilla on the lateral surfaces of the nasal capsule, however, these are short lived and rapidly fuse with the body of the maxilla (Wood et al., 1967). During the 8th week of gestation the palatine bones begin ossifying from primary centers located at the junction between the horizontal and perpendicular plates (O'Rahilly and Gardner, 1972; Sperber and Guttmann, 2010).

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1.3.1.2 Mandibular Development

The mandible is a single bone, derived from the first pharyngeal arch. Ossification begins at approximately Carnegie Stage 18 (O'Rahilly and Gardner, 1972), lateral to Meckel's cartilage where the inferior alveolar nerve and artery bifurcates (Lee et al., 2001). Ossification continues moving superior around the developing teeth as well as anteriorly and posteriorly to form the body and ramus of the mandible respectively. Secondary cartilages, the coronoid accessory and condylar cartilage appear between 10 to 14 weeks gestation to complete the formation of the mandible. The coronoid accessory cartilage becomes incorporated into the mandibular intramembranous bone to form the coronoid process before birth. The condylar cartilage appears by 10 weeks gestation to form the condyle; by 14 weeks gestation endochondral ossification of this cartilage also contributes to the mandibular formation (Lee et al., 2001; Sperber and Guttmann, 2010).

1.3.1.3 Embryonic Maxillary and Mandibular Growth

During the embryonic period while the maxilla and mandible are developing the head is positioned in a flexed position with the relatively small mandible located against the thorax (Diewert, 1983). From studies of sagittal and coronal sections of staged embryos it has been shown that during the late embryonic period the head elevates from the flexed position while the palatal shelves change their orientation from a vertical to horizontal position (Diewert, 1982). During these events the cartilaginous components of the midface and mandible grow very rapidly in length, almost two times as much as during the following fetal period, with Meckel's cartilage showing the greatest increase in length. This rapid increase in length positions Meckel's cartilage and the tongue farther forward. As these changes are occurring, the vertical height is also increasing more than the transverse width. As Meckel's cartilage grows out and down the tongue, which is attached via the genioglossus to the mandibular bone, moves inferiorly. The displacement of the tongue provides space for the palatal shelves to change their orientation in the space that was previously occupied by the tongue. As the palatal shelves change orientation growth occurs, which leads to contact in the midline (Diewert, 1982; Diewert, 1983). Gender differences in timing of palatal shelf elevation have been reported with males having palatal shelf reorientation occurring earlier than females (Burdi and Silvey, 1969).

1.3.1.4 Late Embryonic and Early Fetal Jaw Relationships

The fetal period begins at approximately 9 weeks gestation and lasts until 40 weeks gestation at which time the baby is born. By the beginning of this period the fetal face has a more human appearance and rapid facial growth is occurring (Humphrey, 1971). Jaw growth in the fetal period has been studied using two methods; autopsy data analyzed by direct measurements on dissected material, cephalometrically or histologically and in-vitro data obtained using both 2D and 3D ultrasound.

At the very end of the late embryonic period after fusing of the palatal shelves, the angle representing the anterior maxillary position relative to the anterior cranial base measured on histological sections, is reported to increase rapidly by more than 20 degrees from an average value of 61 degrees at 7 weeks gestation to 82 degrees at 9 weeks gestation (Diewert, 1985a). The average value reported at 9 weeks gestation is similar to average values reported postnatally with some hypothesizing that the facial form is established as early as the late embryonic period (Diewert, 1983).

Some studies have shown that during the late embryonic period the mandible becomes prognathic relative to the maxilla assuming a more forward position. This is commonly measured by the angle formed from a line from nasion (the junction between the nasal bone and frontal bone) to the most anterior edge of the maxilla and from nasion to the most anterior edge of the mandible, Steiner's ANB angle (Steiner, 1953). Average values reported showed that during palatal shelf reorientation the ANB angle decreases from an average of 10 degrees at 7 weeks gestation to -3 degrees at 9 weeks gestation as the mandible becomes positioned farther forward than the maxilla. This mandibular prognathism relative to the maxilla has been shown to be present until the early fetal period around 10 to 12 weeks after which it decreases (Burdi and Silvey, 1969; Diewert, 1983, 1985a, b; Humphrey, 1971). Other studies have also shown that the mandible gradually becomes more prognathic relative to the anterior cranial base with increasing age from 12 to 16 weeks gestation and is then stable until 40 weeks gestation (Lavelle, 1974; Levihn, 1967). One study found that prognathism of the mandible increases up to approximately 21 weeks gestation after which it is stable (Esenlik et al., 2014). The angular value of the mandibular position relative to the anterior cranial base has been shown to have a large variation from 50 to 80 degrees (Esenlik et al., 2014; Trenouth, 1981). It has been shown that the mandibular prognathism relative to the anterior cranial base increases at a slower rate than maxillary prognathism with only a 1.6 degrees per week average increase from 14 to 21 weeks gestation (Erdoglija, 1990). It may seem that a general consensus has been reached on the presence of temporary mandibular prognathism however there are conflicting data.

There are several studies that either fail to document mandibular prognathism or even find that the opposite, that the mandible is retrognathic at all times during the fetal period. Recent radiographic (Esenlik et al., 2014)and histologic (Radlanski et al., 2013) studies failed to find a temporary mandibular prognathic relationship during the late embryonic and early fetal period. Mandibular retrognathia has also been reported the fetal period (Birch, 1968). The evidence regarding the degree of retrognathia and whether the mandibular position is stable with increasing age is unclear. It has been shown that during the fetal period of 10 to 32 weeks gestation the mandible becomes more retrognathic relative to the anterior cranial base using radiographic images (Kvinnsland, 1971a). Other studies have shown the angular measurement of the prognathism of the mandible from the cranial base is stable during the fetal period (Ford, 1956).

Results from the late embryonic and early fetal facial studies have suggested that facial morphology might be established during this late embryonic time period where the cartilaginous components have rapid sagittal growth (Burdi and Silvey, 1969; Diewert, 1983; Johnston, 1974; Lavelle and Moore, 1970; Levihn, 1967). This is an important idea because any inhibition of growth during this time may lead to effects that cannot be reversed when bone takes over as the primary skeleton during the fetal period and these effects may be visible during the fetal period

(Diewert, 1985a). One of the aims of my study is to reinvestigate the relative position of the mandible with a larger sample of human fetuses using both 2D and 3D imaging.

1.3.2 Autopsy Data on Growth and Jaw Relationships

Some of the earliest studies on fetal growth using autopsy data demonstrated that the downward and forward patterns of facial growth observed postnatally are present prenatally as early as 12 weeks gestation (Burdi, 1965, 1969; Inoue, 1961; Levihn, 1967; Mestre, 1959).

All prenatal maxillary and mandibular growth studies demonstrate that the maxilla and mandible increase in size in all dimensions showing a linear relationship with increasing fetal age or increasing fetal size assessed most commonly using the crown-rump length (Burdi, 1965; Houpt, 1970; Johnston, 1974; Kvinnsland, 1971a, b). Means and standard deviations of the maxillary and mandibular sizes in different dimensions have been previously reported (Esenlik et al., 2014; Inoue, 1961; Malas et al., 2006).

Studies have compared the growth in different dimensions; length, height and width of the maxilla and mandible with contradictory results. When studying the absolute growth rates in two dimensions one study reported that from 12 to 40 weeks gestation, growth in the height of the maxilla and mandible occurs more than the growth in the sagittal length (Inoue, 1961). However, other studies have shown that during the same time period the maxilla and mandible increase more in sagittal length than in height (Houpt, 1970; Lavelle, 1974). There is yet another study which evaluated growth in three dimensions. Here it was concluded that from 12 to 19 weeks gestation the mandible grows more in width followed by length then height (Houpt, 1970). When looking at a longer period of time from 9 to 40 weeks gestation, an anatomic study on dissection material showed the height of the ramus grows the most, followed by body length then bigonial width (Malas et al., 2006). When studying relative growth rates while eliminating size as a factor, the maxilla and mandible appear to increase more in length, followed by width than height from 7 to 22 weeks gestation (Trenouth, 1991). Thus, additional studies are needed to determine which dimensions are undergoing the greatest to least amount of dimensional change during the fetal period.

When comparing the growth of the maxilla to the mandible the majority of studies have shown that the sagittal increases of the maxilla and mandible are at the same rate during the fetal period (Birch, 1968; Esenlik et al., 2014; Houpt, 1970; Johnston, 1974; Levihn, 1967; Radlanski et al., 2013). However, other studies using both radiographic (Inoue, 1961) and photographs of stained specimens (Radlanski et al., 2013) show the mandible grows at a faster rate than the maxilla in both the sagittal length and width.

The majority of the published research is in agreement that the growth of the maxilla and mandible during the fetal period is not occurring at the same rate in each dimension. Using finite element modeling, where size is eliminated and only shape is considered, it has also been shown that the maxilla and mandible undergo significant shape changes from 12 to 20 weeks gestation (Diewert et al., 1991). This uneven growth in different dimensions in each jaw may have an effect on the relationship of the jaws with respect to both the anterior cranial base and to each other.

The relative position of the maxilla to the anterior cranial base has also been measured in previous studies. This angle can be measured from the landmark sella, located in the developing anterior cranial base to nasion to the anterior edge of the maxilla. This relationship has been shown to be stable or change with age depending on the time frame investigated. Burdi (1965) photographs of sagittally sectioned samples, showed there is a stable angular relationship from 9 to 24 weeks gestation with no correlation of increasing projection of the maxilla from the anterior cranial base with increasing age. The mean of the maxillary angle relative to the anterior cranial base during the early fetal period has been reported to be within the range of the angles found postnatally (84 degrees) but a larger variation is reported during the fetal period with angles ranging from 60 to 103 degrees (Diewert, 1983; Radlanski et al., 2013). Other studies have found that the angular relationship is not stable during the early fetal period and that from 14 to 24 weeks gestation the angle of the maxilla relative to the anterior cranial base enlarges rapidly by 2.8 degrees per week changing from 61 degrees to 89 degrees (Erdoglija, 1990; Esenlik et al., 2014; Trenouth, 1981). Other studies have shown the maxillary angle increases from 12 to 16 weeks and then does not change from 16 weeks until 40 weeks gestation (Lavelle, 1974; Levihn, 1967). It has been hypothesized that the increase in the maxillary angle relative to the anterior cranial base is due to both the linear increase in size of the maxilla as well as it moving forward in position (Kvinnsland, 1971b).

When evaluating the relationship of the maxilla to the mandible by the ANB angle various results are reported depending on the time frame studied. It has been shown that the sagittal relationship of the maxilla to mandible changes during the early fetal period with the mandible becoming more retrognathic with age changing from a class III jaw relationship, where the mandible is positioned forward of the maxilla, to a class II jaw relationship, where the mandible is positioned behind the maxilla (Kvinnsland, 1971a; Lavelle, 1974; Trenouth, 1981; Trenouth, 1985a). However some studies have shown that this change in the maxillary to mandibular relationship ceases at approximately 18 to 20 weeks gestation with the jaw relationship remaining stable from that time until 40 weeks gestation (Esenlik et al., 2014; Koski, 1980; Levihn, 1967; Trenouth, 1981). Few studies have been able to show a statistically significant association of increasing jaw discrepancy with increasing age during the early fetal period due to small sample sizes and the large variation seen in the ANB angles reported which range from 4 to 28 degrees during the early fetal period of 10 to 20 weeks gestation (Radlanski et al., 2013).

When assessing if there are any periods of growth acceleration during the fetal period different results have been observed. In a study from 12 weeks gestation to birth it was shown that the fastest rate of craniofacial growth was from the 16^{th} to 20^{th} week gestation and this was faster than any other period of life (Levihn, 1967). In agreement with this study it was shown that the rates of facial growth were greater during the 3^{rd}, 4^{th} and 5^{th} month of gestation then slowed during the 6^{th} and 7^{th} months of gestation (Lavelle, 1974). However, Houpt (1970) studied the rates of growth from the 12^{th} to 19^{th} week of gestation and showed the rates of growth were constant with no evidence of acceleration during this period. When the period of most dynamic growth of the jaws is occurring should also be determined.

Studies evaluating gender differences in both size and growth rates of the maxilla and mandible during the fetal period have not shown statistically significant gender differences (Esenlik et al., 2014; Houpt, 1970; Inoue, 1961; Malas et al., 2006).

1.3.3 Ultrasound Data on Growth and Jaw Relationships

Normative data, both cross-sectional and longitudinal, on the average sizes of the maxillary length, mandibular length, mandibular ramus height and mandibular and maxillary transverse dimensions have been reported using both 2D and 3D ultrasound. These studies have also shown that a positive linear correlation exists between increased size of the maxillary and mandibular dimensions with increasing age gestation are correlated with increasing gestational age (Chaiyarach and Manotaya, 2012; Chitty et al., 1993; Goldstein et al., 2005; Hermann et al., 2015; Hermann et al., 2010; Otto and Platt, 1991; Roelfsema et al., 2006; Shyu et al., 2014; Tsai et al., 2004; Zalel et al., 2006). From 11 to 25 weeks gestation it has been shown that the length of the mandible increases 2x more than the height of the mandibular ramus(Hermann et al., 2010). A new study has shown that the rate of growth varies over the 11 to 24 week period with the growth rate of the maxilla decreasing with increasing gestational age. This is an important finding as it highlights that growth is occurring more rapidly during the early fetal period which provides more reason to screen for abnormal growth during the first trimester ultrasound (Hermann et al., 2015).

Fetal facial profiles during the second trimester until birth have been proposed as a screening tool to detect potential abnormalities. A challenge in using fetal facial profiles to screen for potential anomalies is the differences reported regarding the relationship of the maxillary and mandibular positions with increasing age. It has been shown that from 18 weeks to 34 weeks gestation the angles representing the protrusion of the maxilla and mandible from the anterior cranial base are stable and do not change with age (Roelfsema et al., 2006). From 16 weeks to 40 weeks gestation the relationship between the maxilla and mandible as measured by the ANB angle has been shown to be stable and does not change with age. The mean maxillary protrusion from the anterior cranial base measured 81.2 degrees; the mandibular protrusion from the anterior cranial base measured 66.7 degrees and the mean maxillary-nasion-mandibular angle (similar to the ANB angle), measured 13.53 degrees (Captier et al., 2011; de Jong-Pleij et al., 2011). However, other ultrasound studies have contradicted this showing that both the relationship of the maxilla to the anterior cranial base and the maxilla-nasion-mandibular angle have statistically significant associations with age. An increase in the maxilla to anterior cranial base angle shows a statistically significant but weak association with increasing gestation age from 16 to 39 weeks gestation (Captier et al., 2011). The maxilla-nasion-mandible angle from 14 weeks to 39 weeks gestation shows a statistically significant but weak association of decreasing maxilla-nasion-mandibular angle and increasing age (Ko et al., 2012). There currently lacks any ultrasound data on the jaw relationships prior to 14 weeks gestation.

In Canada it is recommended that all pregnant women receive an ultrasound screening in the second trimester between 18 to 22 weeks of gestation (SOGC, 2008). The current Canadian protocol for second trimester ultrasound screening provides information on the number of fetuses, gestational age assessed by biometry, and maternal and fetal anatomy. Although current standards do not include any subjective observations or objective measurement of the maxilla or mandible (Cargill et al., 2009), it would be valuable to use facial measurements to screen for other types of genetic and congenital anomalies.

1.4 Detection of Abnormalities Using Ultrasound

Approximately 5% of malocclusions treated by orthodontists can be attributed to a known cause such as a congenital craniofacial anomaly (Proffit, 2013). Children diagnosed with craniofacial anomalies often require complex medical management. Frequently, part of the management involves orthodontists. Many of the postnatal craniofacial malformations orthodontists treat in children have features also observable prenatally since the etiology of the malformations occur during the early stages of embryogenesis (Kjaer, 2010).

Severe craniofacial abnormalities are often a sign of a congenital or genetic disorder. A recent search on The Online Mendelian Inheritance of Man® found 83 potential genetic disorders associated with "maxillary hypoplasia", 273 potential genetic disorders associated with maxillary retrusion, 497 potential genetic disorders associated with micrognathia and 472 potential genetic disorders associated with "mandibular retrognathia" (OMIM®, 2015; Paladini, 2010). Ultrasound researchers have identified at least 13 possible syndromes associated with maxillary bone hypoplasia and at least 69 possible syndromes associated with micrognathia that can be diagnosed in utero (Goldstein et al., 2005; Paladini, 2010).

Due to the significant associations of jaw growth abnormalities with genetic syndromes there is interest in increasing the ability to detect jaw abnormalities in utero. The current detection rates and age at diagnosis of prenatal craniofacial abnormalities can be improved. For example, ultrasound detection of orofacial clefts was only made in 23% of all orofacial cleft cases (Russell et al., 2008) and the average age of diagnosis of micrognathia was 20 weeks gestation (Luedders et al., 2011; Vettraino et al., 2003). The importance of screening for abnormal jaw morphology has been emphasized and early detection of fetal anomalies is crucial. The first trimester ultrasound would be a good time to screen for potential fetal anomalies that can be associated with maxillary and mandibular pathology (Koo et al., 2014; Rotten and Levaillant, 2004; Zalel et al., 2006). Early detection of abnormalities is preferred because as the fetus ages it becomes more difficult to visualize the mandible on the ultrasound (Otto and Platt, 1991). It also allows more time for the family to prepare for potential additional findings and possible underlying syndromes while providing the health care team a chance to prepare to manage potential airway obstructions (Paladini, 2010). There is evidence that abnormalities of the maxilla, the mandible and fetal facial profile can be detected by 11 to 13 weeks gestation when the first trimester ultrasound would be completed (Borenstein et al., 2007; Lopez et al., 2010; Paladini, 2010).

Current ultrasound guidelines do not provide objective methods to detect craniofacial abnormalities. The need for objective methods such as size reference data to increase the sensitivity and reliability of detecting these abnormalities has been emphasized (Johnson and Sandy, 2003; Luedders et al., 2011; Nemec et al., 2014). Currently, the absence or reduced nasal bone length is a well-accepted adjunct method of screening for Trisomy 21 (Down Syndrome) in high risk pregnancies (Sepulveda et al., 2007). There is no current measurement that is well accepted as a method to detect abnormal maxillary and mandibular jaw relationships and over eleven different objective measures have been suggested in the ultrasound literature (Luedders et al., 2011). Measuring the lengths and widths of the maxilla and mandible to create reference indices for which individual values can be compared to has been completed and this data has been shown to help increase the detection of maxillary hypoplasia and micrognathia from 18 to 29 weeks gestation using both 2D and 3D ultrasound (Nemec et al., 2014; Neuschulz et al., 2015; Rotten et al., 2002; Zalel et al., 2006). Maxillary bone length has also been suggested as an adjunct measurement to nasal bone presence as a useful marker for Trisomy 21 (Cicero et al., 2004; Unsal et al., 2011). The Jaw Index is a measurement of the anterior-posterior length of the mandible in the midsagittal dimension divided by the biparietal diameter. This measurement was shown by Paladini et al. (1999) to increase the sensitivity to 100% and specificity to 98.1% for the diagnosis of micrognathia from 12 to 34 weeks gestation. More recently the fetal profile line which is a line that passes from the mid-sagittal of the most anterior portion of nasion to the mandible has been proposed as a way to measure frontal bossing and retrognathia based on its angulation from a true vertical position (de Jong-Pleij et al., 2012). An angular measurement of the relationship of the maxilla to nasion to the mandible (MNM angle) similar to the ANB angle used in orthodontic diagnosis was proposed as a way to detect profile anomalies in ultrasound images (de Jong-Pleij et al., 2011). This is the most commonly used method and it has an advantage over the Jaw Index because it can detect not only mandibular micrognathia and retrognathia but also maxillary hypoplasia and maxillary alveolar ridge interruption caused by a cleft lip and palate. The average value of the MNM angle measured in 2D ultrasounds is 13.53 degrees in a sample of 241 cross-sectional and 11 longitudinal normal fetuses. This value did not change with increasing age from 16 weeks until 30 weeks gestation. When measuring the MNM angle in pathologic cases on 2D ultrasound, 6 out of 8 cases with confirmed retrognathia had MNM angles above the 95^{th} percentile. In fetuses with orofacial clefts it was within normal ranges for cases with cleft lip and an intact alveolar ridge, above the 95^{th} percentile in 79% of cases with unilateral cleft lip with or without cleft palate. All cases of bilateral cleft lip and palate had MNM angles above the 95^{th} percentile. In one case of a Tessier 4 cleft which extends from the lip to the inner aspect of the lower border of the eyelid the MNM was above the 95^{th} percentile (de Jong-Pleij et al., 2013).

Ultrasound measurements have also been used to confirm Trisomy 21 between 14 to 38 weeks gestation. The MNM angle had a statistically significant reduced size in fetuses with a confirmed Trisomy 21 diagnosis but alone it was not a strong marker for Trisomy 21 (Vos et al., 2014). The MNM angle was also shown to detect Trisomy 18 (Edwards syndrome) when combined with other measures such as the fetal profile line and small nasal bone length (Vos et al., 2015). Thus in combination with other measures, especially chromosomal analysis, the MNM angle may provide information about the severity of the phenotype.

Due to the increased interest and potential for early fetal in-utero diagnosis of craniofacial anomalies more information regarding jaw growth and the jaw relationships during the early fetal period of 10 to 20 weeks gestation needs to be obtained. It is hoped that by understanding how normal growth occurs it will become easier to identify abnormal growth.

1.5 Aims of Study

The purpose of this study is to analyze the growth of the jaws in 2D and 3D during the early fetal period of 10 to 20 weeks of gestation in normal developing human conceptuses. My specific aims are to:

1) Quantify and compare the growth of the maxilla and mandible in all three planes of space.
2) Determine if there are changes in the jaw relationships with respect to age during this period.
3) Describe the changes in growth rates from 10 to 20 weeks gestation.
4) Determine if there are gender related growth rate differences.
5) To develop growth standards that could be used for prenatal diagnosis.

1.6 Hypotheses

1) The jaw size and relationships show age related changes during the fetal period.
2) Gender related facial growth rate differences are not present during the early fetal period.
3) Maxillary and mandibular size has a greater rate of increase during the first half (10-15 weeks gestation) of the early fetal period.

Chapter 2: Methods

2.1 Subjects

Fetal specimens used for this study were collected from therapeutic and spontaneous abortion material at the embryo-pathology department of Women's Hospital in Vancouver, BC between 1982-1988. At the time of autopsy all specimens were diagnosed as normally developing and in good condition with no evidence of dehydration, edema or maceration. Ethnicity is unknown but presumed to be largely European.

Data collected at the time of autopsy included: crown-rump length, crown-head length, head circumference, weight, gender and age in days and weeks post-fertilization. Age of the specimens was calculated by the embryo-pathologists based on established criteria using a combination of body parameters: crown-rump length, biparietal diameter, hand length, and femur length. Brains were removed as part of the pathological examination.

At the time of autopsy lateral and frontal radiographs were obtained using a Faxitron X-ray unit (Hewlett Packard, Arizona) using standardized imaging protocol and settings for kVp, mA and exposure time. Standardized head positioning methods with the head directly against the film were used with the medial plane parallel to the film for lateral exposures and the Frankfort horizontal perpendicular to the film for frontal radiographs (Diewert et al., 1991). Wire grid meshes were radiographed at known distances from the film to calculate the magnification factor of the radiographs. After the autopsy, 127 specimens were made available for the research study, they had been stored in 10% formalin and were available for selection for micro-computed tomography (micro-CT) scanning.

2.2 Data Collection and Measurements

2.2.1 2D Lateral and Frontal Radiographic Data Collection

A collection of lateral and frontal radiographs from 197 specimens age 8 to 20 weeks gestation was available for study. Exclusion criteria were established to select the most suitable radiographs to study. Radiographs were excluded if:

- Specimens were below 10 weeks of age or age information was not available.
- Parts of the maxilla or mandible were located outside the radiographic borders.
- Sufficiently out of focus or faulty exposure so landmarks could not be identified.
- Visible rotation of the head was evident: assessed by measuring the angulation of the bilateral condylion landmark from midsagittal menton. If the angle was more than 10 degrees the radiograph was excluded.
- Had insufficient calcification to identify the osseous landmarks.

141 frontal and 120 lateral radiographs were considered suitable for study. The radiographs were mounted on a light box and photographed at a constant distance of 11 mm from the film. A ruler was placed on the radiograph so that measurements could be calibrated. ImageJ software (version 1.47; NIH, Bethesda, MD) was used to measure the size of the maxilla and mandible in millimeters in all three planes of space and the angular jaw relationships on the radiographs. Twelve anatomic landmarks were selected that were easily identifiable and best represented the outlines of the bony maxilla and mandible in all three planes of space (Tables 2.1 & 2.2; Figures 2.1, 2.2, 2.3). When a bilateral landmark was evident on the lateral radiographs the midpoint between the right and left landmark was selected and used for measurements.

Table 2.1 Anatomic Landmarks on Lateral Radiographs (Jacobson et al., 2007)

Landmark	Abbreviation	Definition
Sella	S	Midpoint of the superior aspect of the developing post-sphenoid bone (Sherwood et al., 2001).
Nasion	Na	The most anterior inferior point of the frontal bone at the developing frontonasal suture.
Anterior Nasal Spine	ANS	The most anterior tip of the maxilla along the intermaxillary suture.
Posterior Nasal Spine	PNS	The most posterior tip of the horizontal plate of the palatine bone.
Menton	Me	The most anterior, inferior point of the lower border of the mandible.
Gonion	Go	Mid point of the contour of the ramus and body of the mandible.
Condylion	Co	The most posterior, superior point of the curvature of the developing condyle.
Orbitale	Or	The most inferior point of the orbital rim.

Table 2.2 Anatomic Landmarks on Frontal Radiographs (Jacobson et al., 2007)

Landmark	Abbreviation	Definition
Zygoma	Z	The most lateral point of the zygomatic process.
Jugale	J	The intersection between the maxillary tuberosity and zygomatic buttress.
Condylion	Co	The most superior, lateral point of the condyle.
Gonion	Go	The most lateral aspect of the midpoint between the ramus and body of the mandible.

Thirteen linear measurements were made to best represent the length, height and width of the maxilla and mandible (Tables 2.3 & 2.4; Figures 2.1 & 2.2). Five angular measurements and one calculated linear measurement were made to best represent the relationship of the jaws relative to the anterior cranial base and to each other (Table 2.5; Figure 2.3).

Table 2.3 Linear Measurements on 2D Lateral Radiographs

Measurement	Definition
Maxillary Length	The distance between ANS to PNS.
Maxillary Unit Length	The distance between Co to ANS.
Mandibular Body Length	The distance between Me to Go.
Mandibular Ramus Height	The distance between Go to Co.
Mandibular Unit Length	The distance between Co to Me.
Total Anterior Face Height	The distance between Na to Me.
Upper Anterior Height	The distance between Na to ANS.
Lower Anterior Face Height	The distance between ANS to Me.
Posterior Face Height	The distance between Or to Go.

Figure 2.1 Linear Measurements of Lateral Radiograph on a 15 Week Specimen

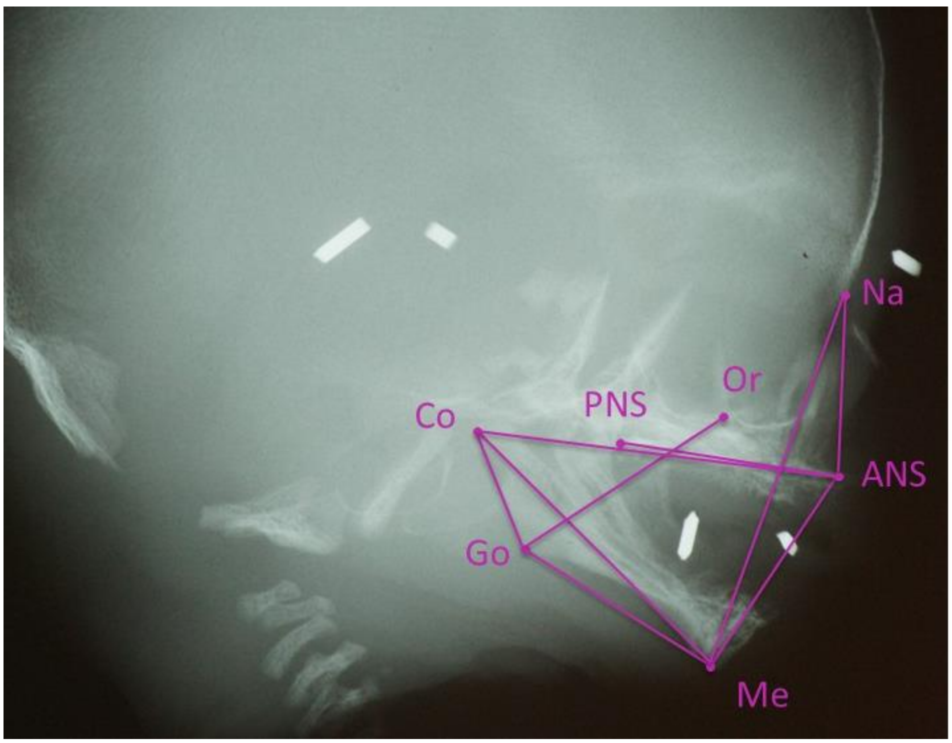


Table 2.4 Linear Measurements on Frontal Radiographs

Measurement	Definition
Bizygomatic Width	The distance between the right and left zygoma landmarks.
Bimaxillary Width	The distance between the right and left jugale landmarks.
Bicondylar Width	The distance between the right and left condylion landmarks.
Bigonial Width	The distance between the right and left gonion landmarks.

Figure 2.2 Linear Measurements of Frontal Radiograph on a 15 Week Specimen

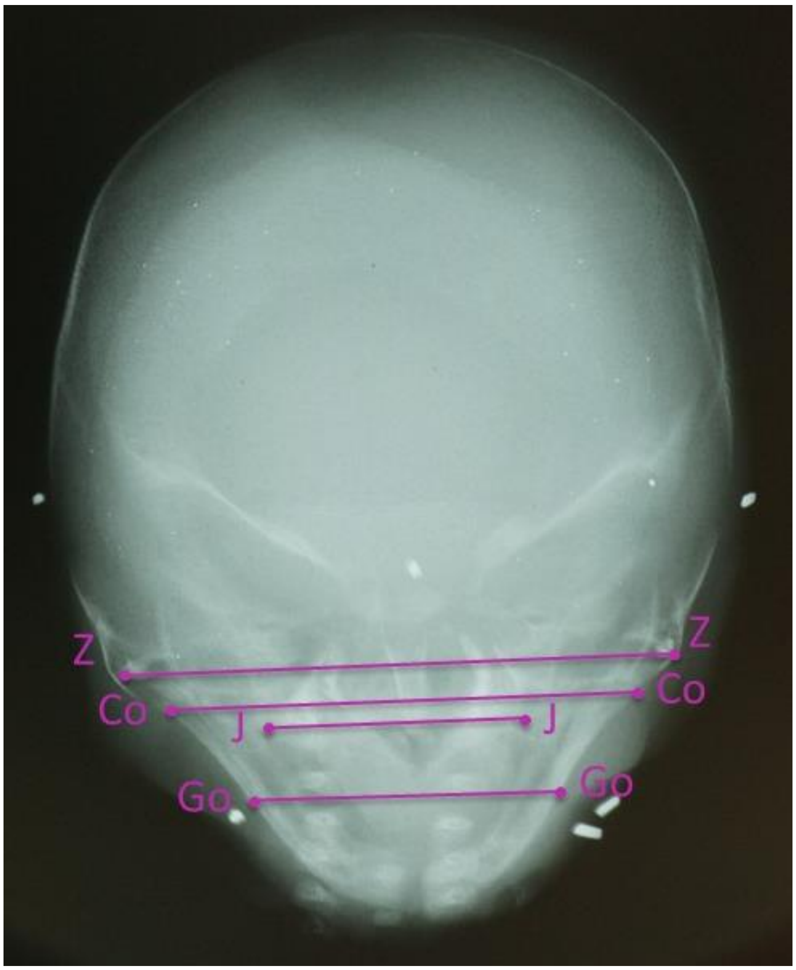
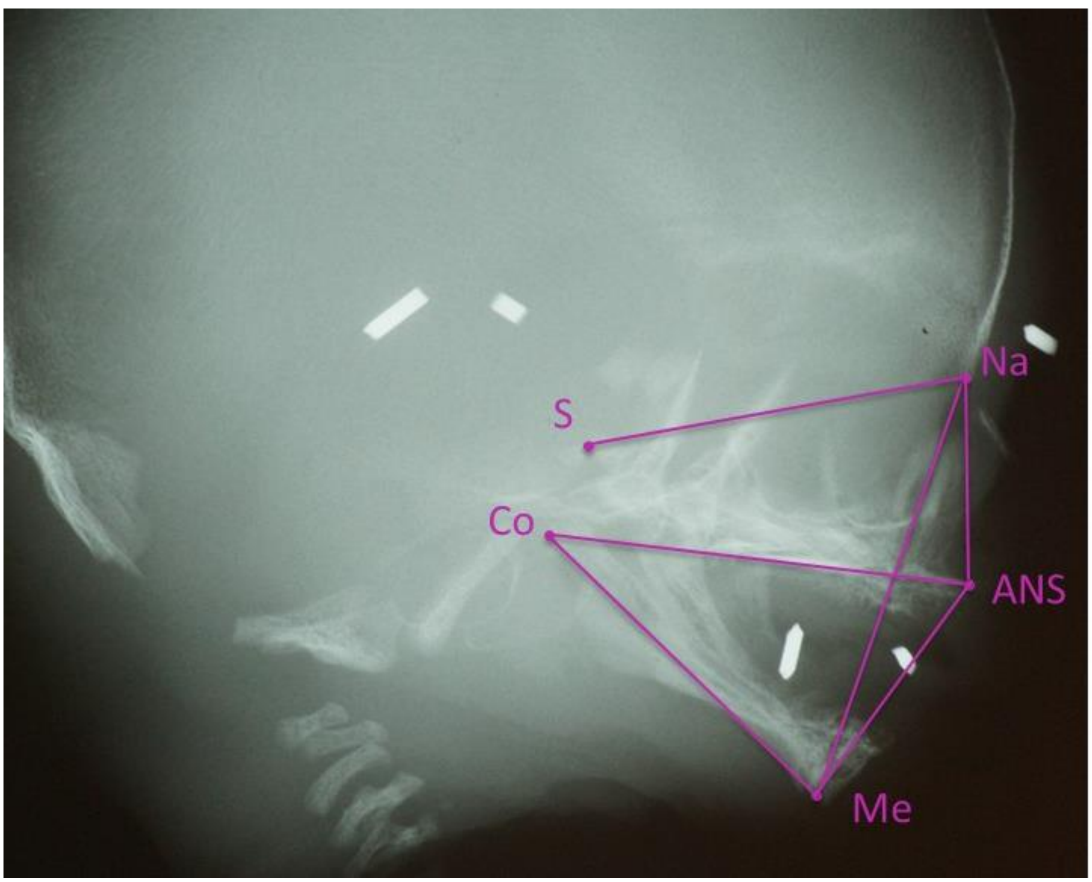


Table 2.5 Jaw Relationship Measurements on Lateral Radiographs (Steiner, 1953)

Measurement	Definition
Maxillary Protrusion Angle	The angle formed from the intersection of lines from S to Na and Na to ANS.
Mandibular Protrusion Angle	The angle formed from the intersection of lines from S to Na and Na to Me.
Maxillary-Nasion-Mandibular Angle	The angle formed from the intersection of lines from ANS to Na and Na to Me.
Facial Convexity Angle	The angle formed from the intersection of lines from Na to ANS and ANS to Me.
Maxillary to Mandibular Length Differential (Harvold, 1974)	The distance from Co to Me subtracted from Co to ANS. ((Co-ANS) - (Co-Me))
Mouth Opening Angle	The angle formed from the intersection of lines from ANS to Co and Co to Me.

Figure 2.3 Angular Jaw Relationship Analysis of a 15 Week Specimen



2.2.2 3D Micro-CT Data Collection

Due to the variable magnification on the lateral and frontal radiographs, the measurements were repeated using micro-CT data collected on the same specimens to confirm the radiographic findings. The specimens obtained for micro-CT scanning for this study were selected from the same collection of 127 specimens used in the original 2D radiographic study. They had been stored in 10% formalin since 1988. Specimens were examined and excluded if in unfavourable condition with visible distortions or macerations. Further selection was made to represent an early, middle and late specimen for each week of gestation from 10 to 20 weeks to accurately represent the growth trajectories. A total of twenty-six specimens were selected for micro-CT scanning.

Specimens were rinsed to remove the fixative and stored in plastic bags with a small amount of water to prevent dehydration. The micro-CT images were acquired with a Scanco Medical AG micro-CT100 machine (Brüttisellen, Switzerland) with an isotropic voxel size of 50 μm. The scan settings were set at 70 kVp, 200 μA with 500 mS integration time and a 0.5 mm aluminum filter. Micro-CT data was analyzed using Amira (version 5.6; Zuse Institute, Berlin, Germany). The micro-CT images were imported into Amira as multi-planar reconstructions and isosurfaces were created. Each specimen was reoriented to align the sagittal slice with the midsagittal plane and the transverse plane with the palatal plane to standardize the specimen position.

The twelve anatomic landmarks used for the 2D radiographic component were re-defined to be applied to the 3D data (Table 2.6). The same 19 measurements made on the lateral and frontal radiographs to represent the size of the maxilla and mandible in all three dimensions and the jaw relationships were repeated to measure the micro-CT data (Tables 2.7 & 2.8; Figures

2.4 & 2.5). The overlying isosurface was used to help guide the landmark selection, but final landmark placement and measurements were completed on the micro-CT raw data slices to ensure landmarks were appropriately located on the bony outline. Each measurement was made bilaterally on the right and left side. The right and left values were averaged and the averaged values were used for statistical analysis.

Table 2.6 Anatomic Landmarks on 3D Micro-CT (Jacobson et al., 2007)

Landmark	Abbreviation	Definition
Sella	S	The midpoint of the superior aspect of the developing post-sphenoid bone (Sherwood et al., 2001).
Nasion	Na	The most anterior, inferior and medial point of the frontal bone at the developing frontonasal suture.
Anterior Nasal Spine	ANS	The most anterior and medial tip of the maxilla along the intermaxillary suture.
Posterior Nasal Spine	PNS	The most posterior and medial tip of the horizontal plate of the palatine bone.
Menton	Me	The most anterior, inferior and medial point of the lower border of the mandible.
Gonion	Go	The most lateral mid-point of the contour of the ramus and body of the mandible.
Condylion	Co	The most posterior, superior and lateral point of the curvature of the developing condyle.
Orbitale	Or	The most inferior and lateral point of the orbital rim.
Zygoma	Z	The most lateral point of the zygomatic process.
Jugale	J	The most anterior aspect of the intersection between the maxillary tuberosity and zygomatic buttress.

Table 2.7 Linear Measurements on 3D Micro-CT

Measurement	Definition
Maxillary Length	The distance between ANS to PNS.
Maxillary Unit Length	The distance between Co to ANS.
Mandibular Body Length	The distance between Me to Go.
Mandibular Ramus Height	The distance between Go to Co.
Mandibular Unit Length	The distance between Co to Me.
Total Anterior Face Height	The distance between Na to Me
Maxillary Height	The distance between Na to ANS.
Lower Anterior Face Height	The distance between ANS to Me.
Posterior Face Height	The distance between Or to Go.
Bizygomatic Width	The distance between the right and left zygoma landmark.
Bimaxillary Width	The distance between the right and left jugale landmark.
Bicondylar Width	The distance between the right and left condylion landmark.
Bigonial Width	The distance between the right and left gonion landmark.

Table 2.8 Jaw Relationship Measurements on Micro-CT (Steiner, 1953)

Measurement	Definition
Maxillary Protrusion Angle	The angle formed from the intersection of a line from S to Na and Na to ANS.
Mandibular Protrusion Angle	The angle formed from the intersection of a line from S to Na and Na to Me.
Maxillary-Nasion-Mandibular Angle	The angle formed from the intersection of a line from ANS to Na and Na to Me.
Facial Convexity Angle	The angle formed from the intersection of a line from Na to ANS and ANS to Me.
Maxillary to Mandibular Length Differential (Harvold, 1974)	The distance from Co to Me subtracted from Co to ANS. ((Co-ANS)-(Co-Me))
Mouth Opening Angle	The angle formed from the intersection of a line from ANS to Co and Co to Me.

Figure 2.4 Lateral View of an Isosurface Created from Micro-CT Scan of a 15 Week Specimen

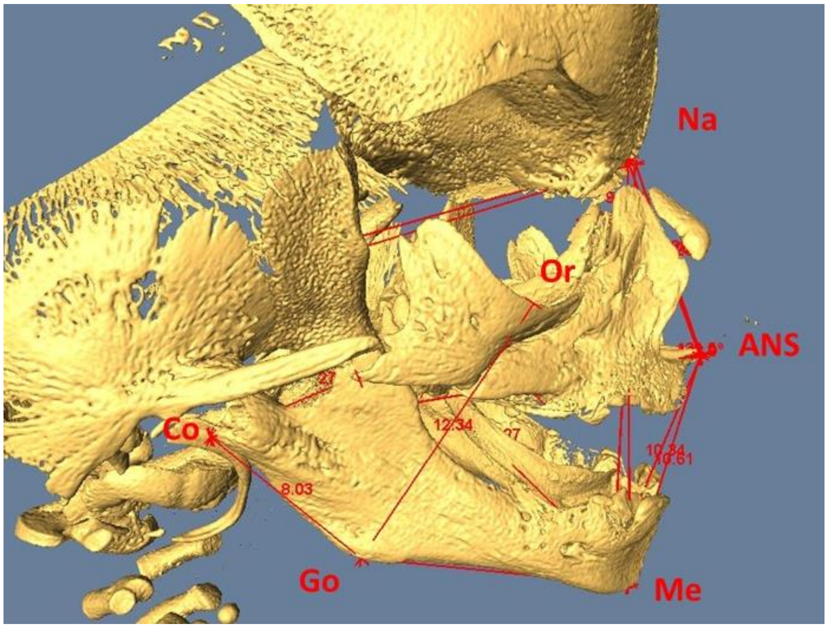
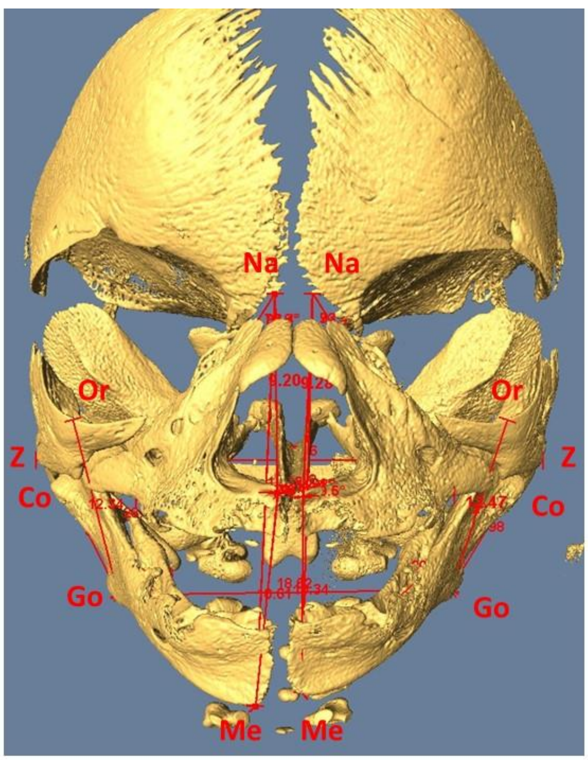


Figure 2.5 Frontal View of an Isosurface Created from Micro-CT Scan of a 15 Week Specimen



2.3 Reliability Testing

To determine the intra-examiner measurement reliability 20 lateral radiographs, 20 frontal radiographs and 10 micro-CT images were randomly selected and re-measured by the same examiner two months after the initial measurements were completed. The examiner re-measuring the radiographs and micro-CT images was blinded to the age of the specimen being measured. The measurements were recorded on a new Excel worksheet and the Dahlberg formula was used to quantify the measurement error (Dahlberg, 1940).

2.4 Statistical Analysis

Statistical analysis was completed using Excel (version 14.4.9; Microsoft Excel, Redmond, WA) and SPSS software (version 22; SPSS, Chicago, Ill).

Means and standard deviations were calculated for each measurement with the data pooled by age in week's gestation. This was completed for both the 2D radiographic and 3D micro-CT data to ensure the values reported were in the range of each other and what is reported in the literature.

Linear regression statistics were completed for each measurement with gestational age in days as the independent variable. The data was tested to ensure all assumptions of linear regression testing were met. The level of significance was established at P=0.001. Linear regression was used to achieve three objectives:

1) Establish growth rates for each jaw in each dimension.
2) To determine if there are changes in the jaw relationship with respect to age during the 10 to 20 week gestation period.
3) To determine whether gender related growth rate differences occur.

To assess for changes in jaw relationship with age the regression was first completed using the entire 2D radiographic and 3D micro-CT data set from 10 to 20 weeks gestation. The 2D radiographic sample was then divided into two groups; 10 to 15 weeks gestation and 16 to 20 weeks gestation and the regression was repeated again for each measurement in each group and differences between the two regression results were compared. This could not be completed for the micro-CT data set due to the small sample size.

Gender differences were examined by dividing the 2D radiographic sample into groups based on gender and linear regression was performed with each measurement for each group to assess for differences between the growth rates of males and females from 10 to 20 weeks gestation.

To assess if the rates of growth were constant over the 10 to 20 week gestation period the 2D radiographic data was pooled into 2-week interval groups and the percent change in size between two consecutive groups was calculated for each of the 13 linear measurements representing the growth of the maxilla and mandible.

Chapter 3: Results

3.1 Sample Distribution

3.1.1 2D Radiographic Sample Distribution

A subset of 141 frontal and 120 lateral radiographs were considered suitable to include for analysis in this study. The sample distribution of the specimens in each week of gestation and gender is shown below in Table 3.1 and 3.2. Two specimens of unknown gender were included in the overall analysis but removed when gender differences were assessed.

Table 3.1 Lateral Radiographic Sample Distribution

Age in Weeks Gestation	Number	Gender (Male, Female, Unknown)
10	2	1 M, 1 F
11	4	1 M, 3 F
12	18	11 M, 7 F
13	16	7 M, 9 F
14	16	10 M, 6 F
15	15	7 M, 8 F
16	9	6 M, 3 F
17	23	10 M, 13 F
18	6	3 M, 3 F
19	10	4 M, 4 F, 2 U
20	1	1 M
Total	120	61 M, 57 F, 2 U

Table 3.2 Frontal Radiographic Sample Distribution

Age in Weeks Gestation	Number	Gender (Male, Female, Unknown)
10	3	2 M, 1 F
11	5	1 M, 4 F
12	19	12 M, 7 F
13	20	9 M, 11 F
14	22	10 M, 12 F
15	12	5 M, 7 F
16	8	6 M, 2 F
17	33	16 M, 17 F
18	6	3 M, 3 F
19	12	4 M, 6 F, 2 U
20	1	1 M
Total	141	69 M, 70 F, 2 U

3.1.2 3D Micro-CT Sample Distribution

A total of 26 specimens were selected for micro-CT scanning. Due to the limited sample only one specimen was available to represent the 10-week period and no specimens in suitable condition were available to represent the 11 or 20-week age group. One specimen was excluded after micro-CT scanning due to loss of mineralization rendering it unable to be analyzed. The sample distribution of age in week's gestation and gender is shown in Table 3.3. All of the CT scanned fetal specimens had good quality 2D radiographs.

Table 3.3 Micro-CT Sample Distribution

Age in Weeks Gestation	Number	Gender (Male, Female)
10	1	1 M
11	0	-
12	3	1 M, 2 F
13	3	2 M, 1F
14	3	2 M, 1 F
15	3	1 M, 2 F
16	3	2 M, 1 F
17	3	1 M, 2 F
18	3	1 M, 2 F
19	3	2 M, 1 F
20	0	-
Total	25	13 M, 12 F

3.2 Assessment of Methods, Data Collection and Error

For the assessment of magnification on the lateral and frontal radiographs with the Faxitron X-ray unit, the images of the wire grid meshes at distances of 1 cm to 4 cm from the film revealed a magnification of 1.0% per cm (0.01 mm per mm distance from the film). Based on the best estimate the head widths range from 15 to 50 mm. On the lateral cephalometric radiograph the midsagittal landmarks would be located at 7.5 mm to 25 mm from the film. The maximum increase in the sagittal linear measurements based on these distances would be approximately 0.075 mm to 0.25 mm. The best estimate of head length showed values ranging from 20 to 60 mm. For the frontal radiographs, estimates of magnification would be approximately 0.3 mm if measured from midway of the head length. However, the absolute value would depend on the depth of each landmark from the film. These magnifications were small compared with the dimensional changes over the period studied, therefore were not expected to affect the results of the regression analyses.

The intra-examiner measurement error of the 2D linear measurements ranged from a minimum of 0.3 mm for the bigonial width to a maximum of 0.8 mm for the bicondylar width. The measurement error for the 2D angular measurements ranged from a minimum of 1.13° for the mandibular projection angle to a maximum of 2.17° for the maxillary projection angle. The measurement error for the 3D linear measurements ranged from a minimum of 0.05 mm for the bizygomatic width to a maximum of 0.27 mm for the mandibular ramus height. The measurement error for the 3D angular measurements ranged from a minimum of 0.30° for the mouth opening angle to a maximum of 0.67° for the facial convexity angle. The measurement error was small relative to the overall size increase and standard deviations during this period.

During the regression analysis 3 outliers were identified which raised the possibility that there was some measurement error. Indeed, 2 outliers showed a measurement recording error, they were re-measured and the correct value was recorded. The other outlier was removed from the data set and the regression analysis was completed again. However, the statistical significance, R^2 values, intercept and slope did not change with the outlier removed so the values reported include the outlier in the analysis.

3.3 Means and Standard Deviations

3.3.1 2D Radiographic Means and Standard Deviations

To ensure the radiographic measurements were accurate and not considerably affected by the magnification means and standard deviations were calculated for the 14 linear and 5 angular measurements grouped by age in week's gestation to compare with the current studies 3D results (Tables 3.4, 3.5 & 3.6). In the early specimens, week 10 and three of the four week 11 specimens, the sella landmark located in the developing anterior cranial base was not fully formed accounting for the lack of a mean and standard deviation for the maxillary and mandibular projection angles (S-Na-ANS, S-Na-Me) in Table 3.5.

Table 3.4 Lateral Radiographic Jaw Length and Height Means and Standard Deviations

Age (Week)	N	Na-ANS (mm)	Na-Me (mm)	ANS-PNS (mm)	Go-Me (mm)	Co-Go (mm)	Co-Me (mm)	Co-ANS (mm)	ANS-Me (mm)	Or-Go (mm)
10	2	5.22± 0.02	10.10± 0.01	5.81± 0.27	4.54± 0.47	3.67± 0.69	8.07± 0.35	8.73± 0.81	5.04± 0.05	4.65± 0.41
11	4	5.91± 0.29	12.05± 1.21	6.42± 0.47	5.88± 1.05	3.35± 0.74	8.87± 1.48	10.33± 0.71	6.15± 0.73	6.32± 0.47
12	18	6.97± 0.43	14.08± 1.11	7.56± 0.64	6.96± 0.52	4.07± 0.42	10.56± 0.72	11.64± 0.91	7.45± 0.90	7.62± 0.77
13	16	8.02± 0.69	15.35± 1.72	8.88± 0.76	8.15± 0.82	4.82± 0.70	12.41± 1.16	14.20± 1.38	7.85± 1.26	8.59± 1.02
14	16	9.39± 0.76	18.24± 1.70	10.47± 0.56	9.57± 0.76	5.57± 0.70	14.40± 1.29	16.14± 1.11	9.66± 1.16	9.85± 0.80
15	15	9.85± 0.74	19.67± 1.06	11.46± 0.66	10.27± 0.81	5.89± 0.56	15.38± 1.13	17.66± 1.17	10.94± 0.91	11.33± 0.70
16	9	11.33± 0.61	21.44± 0.99	12.13± 1.04	11.05± 0.89	6.36± 0.52	16.36± 1.05	19.06± 1.16	11.48± 0.89	12.46± 0.95
17	23	11.90± 0.84	23.13± 1.85	13.61± 0.70	12.46± 0.82	6.94± 0.51	18.24± 1.04	20.89± 1.02	12.58± 1.46	13.72± 0.87
18	6	11.90± 0.95	24.12± 1.87	14.01± 0.72	13.04± 0.91	6.70± 0.56	18.40± 1.21	20.77± 1.60	13.38± 1.27	14.51± 1.18
19	10	12.59± 0.87	25.64± 2.40	14.94± 0.53	14.03± 1.19	7.00± 0.58	19.94± 1.45	22.50± 0.87	14.53± 1.88	14.88± 1.08
20	1	13.33	27.69	15.19	14.20	6.71	20.29	23.69	16.20	14.62

Table 3.5 Lateral Radiographic Jaw Relationship Means and Standard Deviations

Age (Week)	N	S-Na-ANS°	S-Na-Me°	ANS-Na-Me°	Na-ANS-Me°	(Co-ANS) – (Co-Me) (mm)	ANS-Co-Me (°)
10	2	-	-	10.73 ± 3.01	158.00 ± 5.98	0.67 ± 0.46	34.71 ± 3.18
11	4	78.92	65.86	13.45 ± 0.90	151.72 ± 4.45	1.46 ± 0.84	36.08 ± 4.47
12	18	80.84 ± 4.39	66.28 ± 2.87	14.54 ± 2.58	151.82 ± 4.73	1.08 ± 0.44	38.84 ± 4.47
13	16	83.00 ± 4.70	67.61 ± 3.97	15.34 ± 3.71	149.43 ± 6.44	1.79 ± 0.74	33.45 ± 5.57
14	16	85.87 ± 4.18	67.68 ± 3.41	18.19 ± 3.15	145.22 ± 5.79	1.75 ± 0.99	36.12 ± 3.79
15	15	86.58 ± 2.42	66.96 ± 2.62	19.61 ± 2.49	142.79 ± 4.25	2.31 ± 0.80	37.92 ± 3.78
16	9	85.63 ± 3.50	66.13 ± 4.40	19.26 ± 2.34	141.98 ± 4.21	2.69 ± 1.08	36.79 ± 4.30
17	23	88.53 ± 2.58	68.55 ± 2.83	19.99 ± 1.79	141.61 ± 3.00	2.65 ± 0.78	36.67 ± 4.63
18	6	89.69 ± 2.77	70.10 ± 2.37	19.59 ± 3.04	143.44 ± 5.29	2.37 ± 0.44	39.39 ± 3.29
19	10	89.19 ± 2.05	68.47 ± 2.92	20.72 ± 3.45	141.55 ± 5.36	2.57 ± 1.02	39.44 ± 5.84
20	1	84.68	60.80	23.88	136.97	3.4	42.64

Table 3.6 Frontal Radiographic Jaw Width Means and Standard Deviations

Age (Week)	N	Z-Z (mm)	J-J (mm)	Co-Co (mm)	Go-Go (mm)
10	3	14.50 ± 0.70	9.09 ± 0.50	12.43 ± 0.45	8.53 ± 0.31
11	5	15.86 ± 2.97	10.31 ± 2.03	13.97 ± 2.57	9.70 ± 1.37
12	19	20.72 ± 2.66	11.96 ± 1.13	18.21 ± 2.22	12.32 ± 1.27
13	20	23.36 ± 2.72	13.68 ± 1.53	21.01 ± 2.51	13.85 ± 1.71
14	22	28.02 ± 1.86	14.91 ± 1.27	25.19 ± 1.97	16.85 ± 1.21
15	12	29.66 ± 2.17	15.12 ± 1.24	26.07 ± 2.57	17.80 ± 1.81
16	8	33.40 ± 1.77	15.76 ± 0.92	29.31 ± 1.87	19.89 ± 1.50
17	33	36.25 ± 1.95	22.10 ± 1.52	32.55 ± 2.00	22.03 ± 1.77
18	6	36.64 ± 1.63	24.05 ± 1.34	33.78 ± 2.46	22.77 ± 1.25
19	12	38.17 ± 1.84	24.77 ± 1.89	34.36 ± 1.74	24.33 ± 1.26
20	1	39.40	24.61	32.82	24.32

3.3.2 3D Micro-CT Means and Standard Deviations

The means and standard deviations were calculated for the 14 linear and 5 angular micro-CT measurements (Tables 3.7, 3.8 & 3.9). This was done to verify if the 2D measurements were within the range of what was obtained with the 3D measurements and also to provide new data that can be used to verify results of recent 3D ultrasound studies. Again, due to the early age of the specimen in the week 10 age group, the anterior cranial base is not fully ossified and no sella landmark could be located. Therefore the maxillary and mandibular projection angles were omitted (S-Na-ANS, S-Na-Me) (Table 3.9).

Table 3.7 3D Micro-CT Jaw Length and Height Means and Standard Deviations

Age (Week)	N	Na-ANS (mm)	Na-Me (mm)	ANS-PNS (mm)	Go-Me (mm)	Co-Go (mm)	Co-Me (mm)	Co-ANS (mm)	ANS-Me (mm)	Or-Go (mm)
10	1	5.11	11.18	5.70	6.35	3.93	10.00	10.77	6.37	6.64
11	0	-	-	-	-	-	-	-	-	-
12	3	6.98± 0.42	14.77± 0.90	9.09± 0.54	8.66± 0.75	5.56± 0.87	13.77± 1.48	15.87± 1.51	9.09± 0.54	9.16± 0.73
13	3	8.18± 0.78	16.13± 2.02	11.04± 0.66	10.72± 0.63	6.12± 0.47	16.29± 1.00	18.86± 1.05	9.38± 1.47	10.13± 0.85
14	3	8.75± 0.39	16.78± 0.86	10.90± 0.70	11.60± 0.60	6.98± 0.49	17.85± 0.84	19.94± 1.52	9.37± 0.86	11.29± 0.94
15	3	9.48± 0.25	18.42± 1.06	12.50± 0.53	13.05± 0.59	7.97± 0.04	20.26± 0.64	22.49± 0.56	10.15± 0.66	12.46± 0.39
16	3	10.22± 0.24	19.26± 0.52	13.21± 0.88	13.41± 0.89	8.49± 0.39	23.97± 1.30	23.97± 1.30	10.67± 0.35	13.20± 0.65
17	3	11.31± 0.71	23.30± 2.40	15.56± 0.70	15.11± 1.16	9.39± 0.23	23.79± 1.20	26.67± 1.02	14.12± 1.99	15.15± 0.10
18	3	11.25± 0.66	22.79± 0.58	14.94± 0.96	15.06± 0.99	8.78± 0.41	22.99± 1.21	26.14± 1.74	13.45± 0.60	15.03± 0.66
19	3	11.43± 0.63	23.65± 0.81	15.58± 0.31	16.10± 0.65	8.56± 0.48	23.91± 1.31	26.98± 0.68	14.53± 0.19	15.58± 0.61
20	0	-	-	-	-	-	-	-	-	-

Table 3.8 3D Micro-CT Jaw Width Means and Standard Deviations

Age (Week)	N	Z-Z (mm)	J-J (mm)	Co-Co (mm)	Go-Go (mm)
10	1	11.48	6.45	11.08	7.27
11	0	-	-	-	-
12	3	17.93 ± 3.56	8.99 ± 1.01	17.39 ± 3.27	11.13 ± 1.09
13	3	22.14 ± 1.44	11.30 ± 1.32	21.46 ± 1.56	14.41 ± 0.88
14	3	24.16 ± 2.72	13.31 ± 1.20	23.12 ± 3.01	15.53 ± 1.96
15	3	28.45 ± 1.48	15.54 ± 0.86	26.87 ± 0.68	18.06 ± 0.52
16	3	29.55 ± 2.77	15.87 ± 1.01	28.35 ± 1.68	18.31 ± 1.18
17	3	33.65 ± 1.98	17.49 ± 1.50	31.34 ± 1.81	21.47 ± 2.62
18	3	32.45 ± 1.42	16.94 ± 1.93	30.29 ± 2.23	20.57 ± 0.89
19	3	33.78 ± 1.73	18.27 ± 1.23	31.33 ± 1.44	22.08 ± 0.81
20	0	-	-	-	-

Table 3.9 3D Micro-CT Jaw Relationship Means and Standard Deviations

Age (Week)	N	S-Na-ANS (°)	S-Na-Me (°)	ANS-Na-Me (°)	Na-ANS-Me (°)	(Co-ANS)-(Co-Me) (mm)	ANS-Co-Me (°)
10	1	-	-	14.75	155.45	0.77	35.45
11	0	-	-	-	-	-	-
12	3	89.92 ± 1.56	63.95 ± 1.20	25.97 ± 1.18	133.95 ± 1.80	2.10 ± 0.84	34.73 ± 1.78
13	3	92.57 ± 3.60	67.82 ± 3.04	24.75 ± 1.35	133.53 ± 2.28	2.57 ± 0.24	29.63 ± 3.85
14	3	92.73 ± 4.47	70.17 ± 3.07	22.55 ± 3.57	136.18 ± 5.80	2.10 ± 0.76	27.88 ± 1.26
15	3	89.90 ± 4.13	68.42 ± 3.98	21.48 ± 2.74	138.03 ± 6.24	2.23 ± 0.97	27.00 ± 2.38
16	3	89.63 ± 1.86	66.55 ± 1.98	23.10 ± 0.26	134.42 ± 0.72	3.00 ± 0.37	26.42 ± 2.09
17	3	96.27 ± 3.00	70.02 ± 4.54	26.32 ± 3.01	132.40 ± 3.97	2.88 ± 0.18	31.82 ± 5.44
18	3	92.02 ± 3.42	67.48 ± 0.28	24.47 ± 3.36	134.87 ± 5.03	3.14 ± 0.63	30.95 ± 1.14
19	3	95.15 ± 0.69	67.65 ± 2.93	27.53 ± 2.69	131.13 ± 3.86	3.07 ± 0.68	32.45 ± 1.12
20	0	-	-	-	-	-	-

3.4 Maxillary and Mandibular Growth Results

3.4.1 2D Radiographic Results

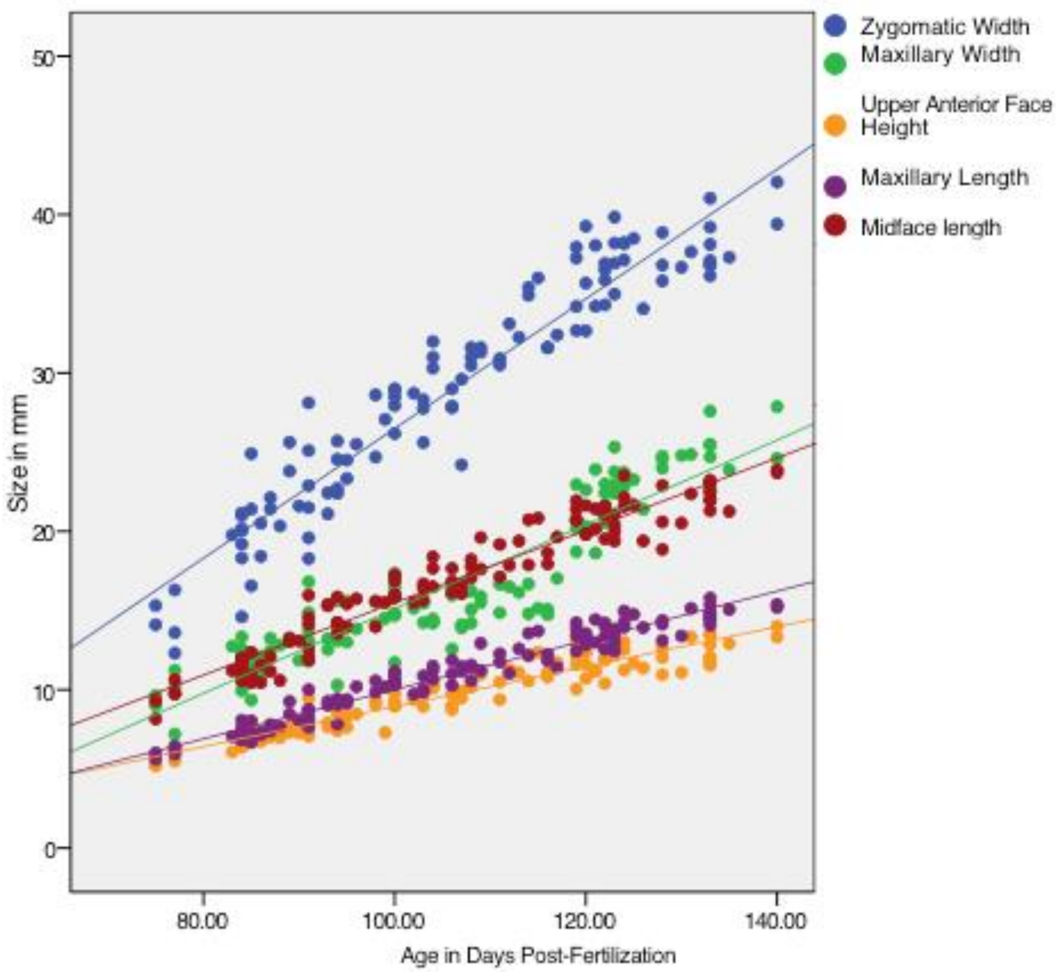
The linear regression analysis established that age in days post fertilization between 10 to 20 weeks gestation is a statistically significant predictor ($p<0.001$) for all 13 linear measurement representing the growth in length, height and width of the maxilla and mandible. Age in days post-fertilization accounted for the explained variability in the size of the maxilla and mandible ranging from 75.8% for the mandibular ramus height to 93.9% for the maxillary length (Table 3.10).

Table 3.10 2D Radiographic Maxillary and Mandibular Growth Linear Regression Results

Measurement	R^2	P	Slope
Maxillary Length (ANS-PNS)	0.939	<0.001	0.156
Upper Anterior Face Height (Na-ANS)	0.896	<0.001	0.125
Bimaxillary Width (J-J)	0.867	<0.001	0.272
Maxillary Unit Length (Co-ANS)	0.925	<0.001	0.229
Mandibular Body Length (Go-Me)	0.913	<0.001	0.147
Mandibular Ramus Height (Co-Go)	0.758	<0.001	0.066
Lower Anterior Face Height (ANS-Me)	0.844	<0.001	0.155
Bicondylar Width (Co-Co)	0.895	<0.001	0.372
Bigonial Width (Go-Go)	0.909	<0.001	0.264
Mandibular Unit Length (Co-Me)	0.917	<0.001	0.197
Total Anterior Face Height (ANS-Me)	0.900	<0.001	0.255
Posterior Face Height (Or-Go)	0.918	<0.001	0.164
Bizygomatic Width (Z-Z)	0.909	<0.001	0.407

To evaluate the absolute growth rates of the maxilla and mandible in different dimensions the slopes of the regression lines can be compared. It appears that the maxilla grows more in width (slope=0.272), followed by length (slope=0.156) followed by height (slope=0.125) (Table 3.10 & Figure 3.1).

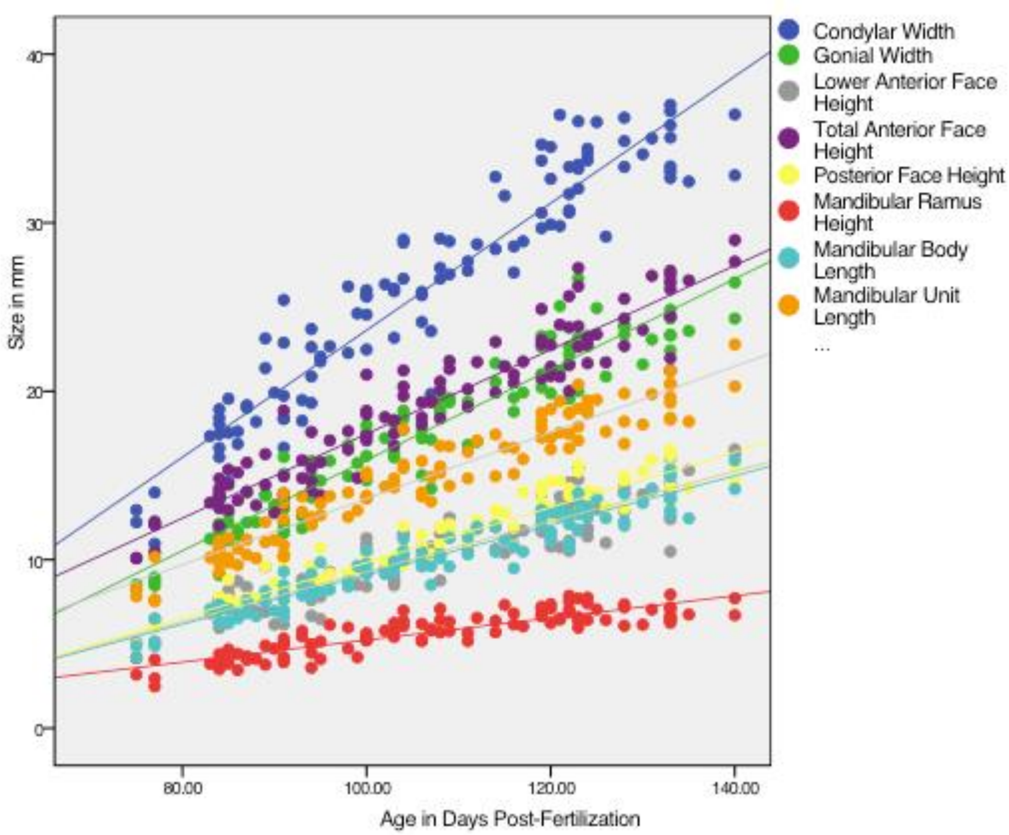
Figure 3.1 2D Radiographic Maxillary Growth Regression Slopes



The mandible shows similar trends with the largest increases seen in bicondylar width (slope=0.372) and bigonial width (slope=0.264) followed by the mandibular unit length (slope=0.197), mandibular body length (slope=0.147) and ramus height (slope=0.066) (Table

3.10 & Figure 3.2). The bizygomatic width appears to be growing the most rapidly of any measurement (slope=0.407). This is followed by the bicondylar width (slope=0.372) that appears to be growing more rapidly than the bimaxillary width (slope=0.272), which also grows more rapidly than the bigonial width (slope=0.264). The slopes show that growth in the anterior posterior dimension occurs more rapidly in the maxillary length (slope=0.156) and maxillary unit length (slope=0.229) than the mandibular body length (slope=0.147) and mandibular unit length (slope=0.197). The posterior face height (slope=0.164) and lower anterior face height (slope= 0.155) grow at a greater rate of increase than the upper anterior face height (slope=0.125) (Table 3.10).

Figure 3.2 2D Radiographic Mandibular Growth Regression Slopes



3.4.2 3D Micro-CT Results

The linear regression analysis established that age in days post fertilization between 10 to 20 weeks gestation is a statistically significant predictor ($p<0.001$) for all 13 linear measurement representing the growth in length, height and width of the maxilla and mandible in the micro-CT data. Age in days post-fertilization accounted for the explained variability in size of the maxilla and mandible ranging from 77.4% for the lower anterior face height to 94.2% for the posterior face height (Table 3.11).

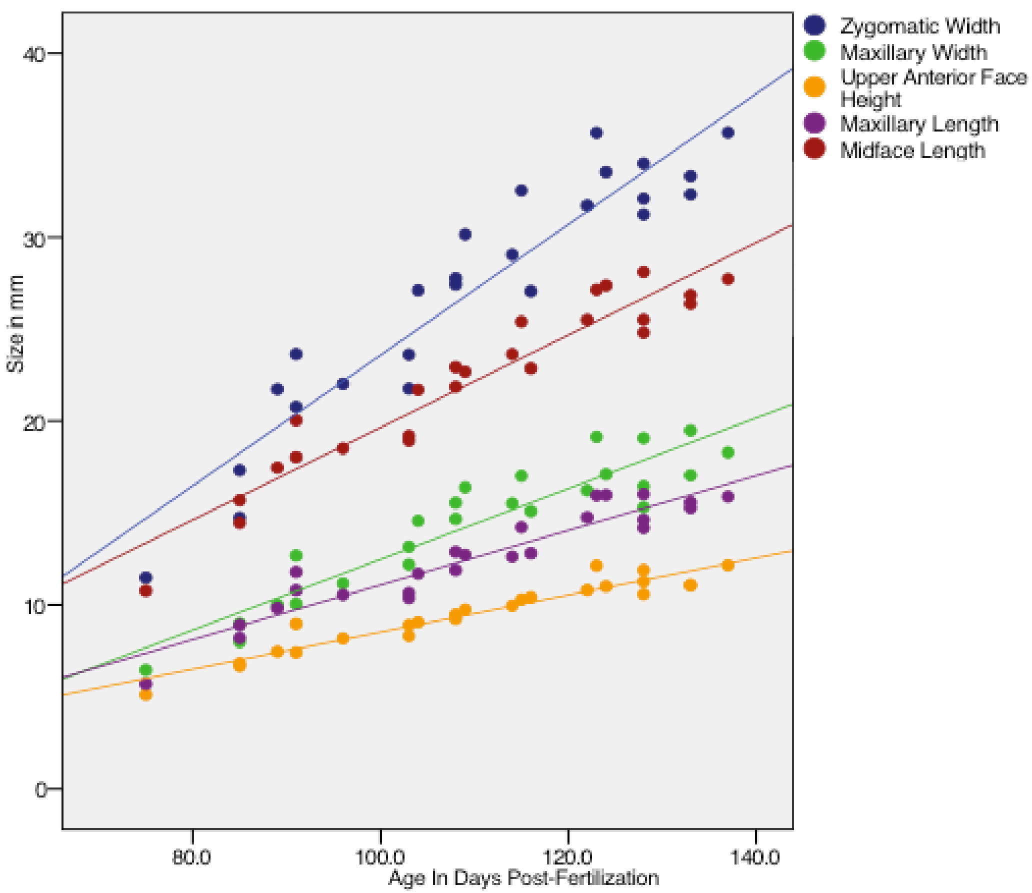
Table 3.11 3D Radiographic Maxillary and Mandibular Growth Linear Regression Results

Measurement	**R^2**	**P**	**Slope**
Maxillary Length (ANS-PNS)	0.884	<0.001	0.149
Upper Anterior Face Height (Na-ANS)	0.908	<0.001	0.101
Bimaxillary Width (J-J)	0.858	<0.001	0.192
Maxillary Unit Length (Co-ANS)	0.905	<0.001	0.251
Mandibular Body Length (Go-Me)	0.918	<0.001	0.153
Mandibular Ramus Height (Go-Co)	0.817	<0.001	0.080
Lower Anterior Face Height (ANS-Me)	0.774	<0.001	0.127
Bicondylar Width (Co-Co)	0.855	<0.001	0.315
Bigonial Width (Go-Go)	0.878	<0.001	0.230
Mandibular Unit Length (Co-Me)	0.912	<0.001	0.225
Total Anterior Face Height (ANS-Me)	0.879	<0.001	0.205
Posterior Face Height (Or-Go)	0.942	<0.001	0.147
Bizygomatic Width (Z-Z)	0.877	<0.001	0.355

When evaluating the slopes of the regression lines in the 3D data similar growth trends to the 2D results are apparent. The bizygomatic width increases the most rapidly (slope=0.355).

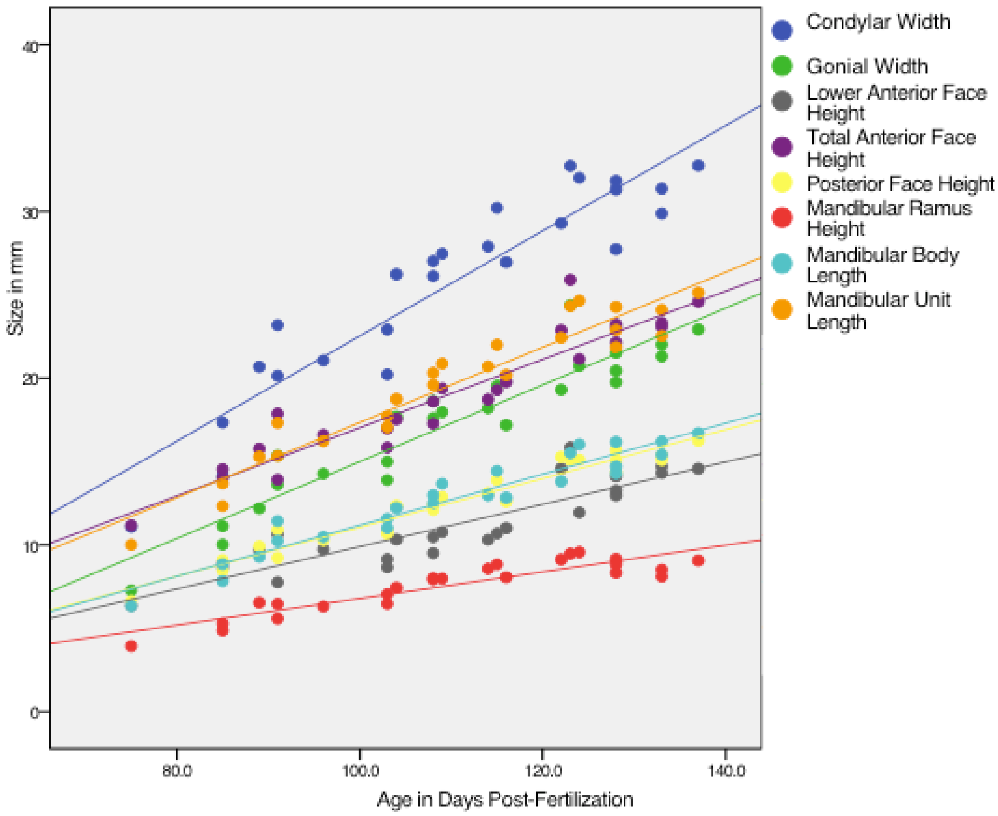
The maxilla grows more in width (slope=0.192), followed by length (slope=0.149) then height (slope=0.101) with the exception of the maxillary unit length (slope=0.251) having a higher slope than the bimaxillary width (slope=0.192) and maxillary length (slope=0.149). The maxillary unit length shows a higher slope than the bimaxillary width because the maxillary unit length measurement is a 3D measurement from condylion to the anterior nasal spine that incorporates both transverse and length dimension increases (Table 3.11, Figure 3.3).

Figure 3.3 3D Micro-CT Maxillary Growth Regression Slopes



The slopes of mandibular growth show similar trends as the maxillary growth with the slope showing greater increases in bicondylar (slope=0.315) and bigonial widths (slope=0.230), followed by increases in mandibular unit length (slope=0.251) and mandibular body length (slope=0.153). The smallest increases are for the lower anterior face height (slope=0.127) and mandibular ramus height (slope=0.080) (Table 3.11, Figure 3.4). There is contrast between the 3D results and the 2D results that show the lower anterior face height (slope=0.155) increases more rapidly than the mandibular body length (slope=0.147). The increase in width of the mandible, both bicondylar (slope=0.315) and bigonial (slope=0.230) occurs more rapidly than the increase in width of the maxilla (slope=0.192). The results of the 3D analysis are different than the 2D results that showed the maxillary width (slope=0.272) increased more rapidly than the bigonial width (slope=0.264). In the anterior posterior direction the slopes show that the mandibular body length (slope=0.153) increases more rapidly than the maxillary length (slope=0.149), which is opposite of the 2D results, but the maxillary unit length (slope=0.251) increases more rapidly than the mandibular unit length (slope=0.225) which is similar to the 2D results. The posterior face height (slope=0.147) and lower anterior face height (slope=0.127) increase more rapidly than the upper anterior face height (slope=0.101), which is similar to the 2D results (Table 3.11).

Figure 3.4 3D Micro-CT Mandibular Growth Regression Slopes



3.5 Jaw Relationship Results

3.5.1 2D Radiographic Results 10-20 Weeks Gestation

The linear regression analysis established that age in days post fertilization between 10 to 20 weeks gestation is a statistically significant predictor ($p<0.001$) of four of five measurements of jaw relationships. Age in days post-fertilization accounted for 36.9% of the explained variability of the maxillary protrusion angle, 41.9% of the explained variability of the maxillary-nasion-mandibular angle, 38.9% of the explained variability of the facial convexity angle and 29.8% of the explained variability of the maxillary to mandibular length differential.

Unexpectedly, age in days post-fertilization is not a statistically significant predictor of the mandibular protrusion angle (p=0.060) or the mouth-opening angle (p=0.099) as these values showed minimal increases with increasing age (Table 3.12). In contrast, the slopes show that with increasing age the maxillary projection angle increases. It is also apparent that with increasing age, the maxilla projects further ahead relative to the mandible as shown by the increasing maxillary-nasion-mandibular angle and the decreasing facial convexity angle (Table 3.12).

Table 3.12 2D Radiographic Jaw Relationship Results 10-20 Weeks Gestation

Measurement	R^2	P	Slope
Maxillary Protrusion Angle (S-Na-ANS)	0.369	<0.001	0.173
Mandibular Protrusion Angle (S-Na-Me)	0.022	0.060	0.037
Maxillary-Nasion-Mandibular Angle (ANS-Na-Me)	0.419	<0.001	0.142
Facial Convexity Angle (Na-ANS-Me)	0.389	<0.001	-0.237
Maxillary to Mandibular Length Differential ((Co-ANS)-(Co-Me))	0.298	<0.001	0.032
Mouth Opening Angle (ANS-Co-Me)	0.015	0.099	0.043

3.5.2 3D Micro-CT Results 10-20 Weeks Gestation

The linear regression analysis for the 3D data established that age in days post fertilization between 10 to 20 weeks gestation is a statistically significant predictor (p<0.001) of only one measurement of jaw relationship, the maxillary to mandibular length differential. Age in days post-fertilization accounted for 36.1% of the explained variability of the maxillary to mandibular length differential. Age is not a statistically significant predictor of the maxillary protrusion angle, the mandibular protrusion angle, the maxillary-nasion-mandibular angle, the facial convexity angle or mouth-opening angle. Age in days post-fertilization accounted for very little of the variability seen with these measurements ranging from 0.9% of the explained variability of the mandibular protrusion to 12.4% of the explained variability for the facial convexity angle (Table 3.13). Thus micro-CT data confirms the finding that mandibular protrusion does not change significantly over time in this group of fetuses.

Table 3.13 3D Micro-CT Jaw Relationship Results 10-20 Weeks Gestation

Measurement	R^2	P	Slope
Maxillary Protrusion Angle (S-Na-ANS)	0.077	0.101	0.074
Mandibular Protrusion Angle (S-Na-Me)	0.009	0.285	0.043
Maxillary-Nasion-Mandibular Angle (ANS-Na-Me)	0.097	0.071	0.072
Facial Convexity Angle (Na-ANS-Me)	0.124	0.047	-0.133
Maxillary to Mandibular Length Differential ((Co-ANS)-(Co-Me))	0.361	0.001	0.026
Mouth Opening Angle (ANS-Co-Me)	0.018	0.524	-0.028

3.5.3 Dividing the Sample Precisely Defines the Period of Jaw Relationship Changes

To further investigate if age related changes of the jaw relationship could be more precisely bracketed the 2D radiographic data was divided into two groups, 10 to 15 weeks gestation and 16 to 20 weeks gestation. The 3D micro-CT sample was too small to bin the data therefore only the 2D radiographic data was studied.

Linear regression analysis showed that age in days post fertilization between 10 to 15 weeks gestation is a statistically significant predictor of four of the five measurements of jaw relationship ($p<0.001$). Age in days accounted for 26.3% of the explained variability of the maxillary protrusion angle, 37.4% of the explained variability of the maxillary-nasion-mandibular angle, 34.6% of the explained variability of the facial convexity angle and 17.8% of the explained variability of the maxillary to mandibular length differential. Once again, age is not a statistically significant predictor of the mandibular protrusion angle ($p=0.441$), or the mouth-opening angle ($p=0.964$), these two values are stable and did not change with increasing age (Table 3.14).

Table 3.14 2D Radiographic Jaw Relationship Results 10-15 Weeks Gestation

Measurement	R^2	P	Slope
Maxillary Protrusion Angle (S-Na-ANS)	0.263	<0.001	0.286
Mandibular Protrusion Angle (S-Na-Me)	0.010	0.441	0.040
Maxillary-Nasion-Mandibular Angle (ANS-Na-Me)	0.374	<0.001	0.236
Facial Convexity Angle (Na-ANS-Me)	0.346	<0.001	-0.401
Maxillary to Mandibular Length Differential ((Co-ANS)-(Co-Me))	0.178	<0.001	0.038
Mouth Opening Angle (ANS-Co-Me)	0.000	0.964	0.003

From 16 to 20 weeks gestation, age in days post fertilization is not a statistically significant predictor of any measurement of jaw relationships and there is no association of increasing age with changes in any of the five jaw relationship measures (Table 3.15). Thus the major changes in jaw positions are occurring in the first 5 weeks rather than the last 5 weeks of the middle trimester.

Table 3.15 2D Radiographic Jaw Relationship Results 16-20 Weeks Gestation

Measurement	R^2	P	Slope
Maxillary Protrusion Angle (S-Na-ANS)	0.073	0.052	0.105
Mandibular Protrusion Angle (S-Na-Me)	0.006	0.571	0.035
Maxillary-Nasion-Mandibular Angle (ANS-Na-Me)	0.053	0.100	0.076
Facial Convexity Angle (Na-ANS-Me)	0.009	0.497	-0.052
Maxillary to Mandibular Length Differential ((Co-ANS)-(Co-Me))	0.004	0.669	-0.007
Mouth Opening Angle (ANS-Co-Me)	0.089	0.032	0.183

3.6 Rates of Jaw Growth

The percent increase of size was calculated for each of the 13 linear measurements representing growth of the maxilla and mandible in all three planes of space. The results showed the increase in size relative to the starting size is greater during the 10 to 15 week gestation period with values ranging from approximately 30% to 45% increase in size. The later half from 16 to 20 weeks gestation shows a smaller percentage increase in size of all measurements ranging from under 5% to 20%. An exception to this trend is seen with the bimaxillary width that shows an almost 40% increase in size from 14 to 17 weeks gestation (Figure 3.5). When investigating this exception the bizygomatic, bicondylar and bimaxillary width measurements were plotted to investigate differences in width increases (Figure 3.6).

Figure 3.5 Percent Increases in Maxillary and Mandibular Linear Dimensions

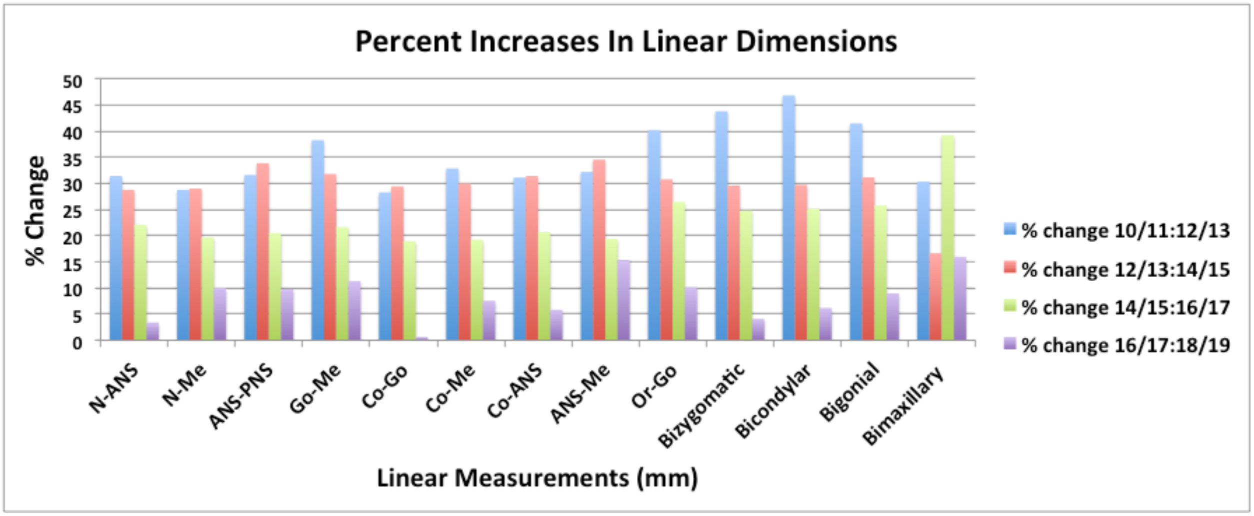
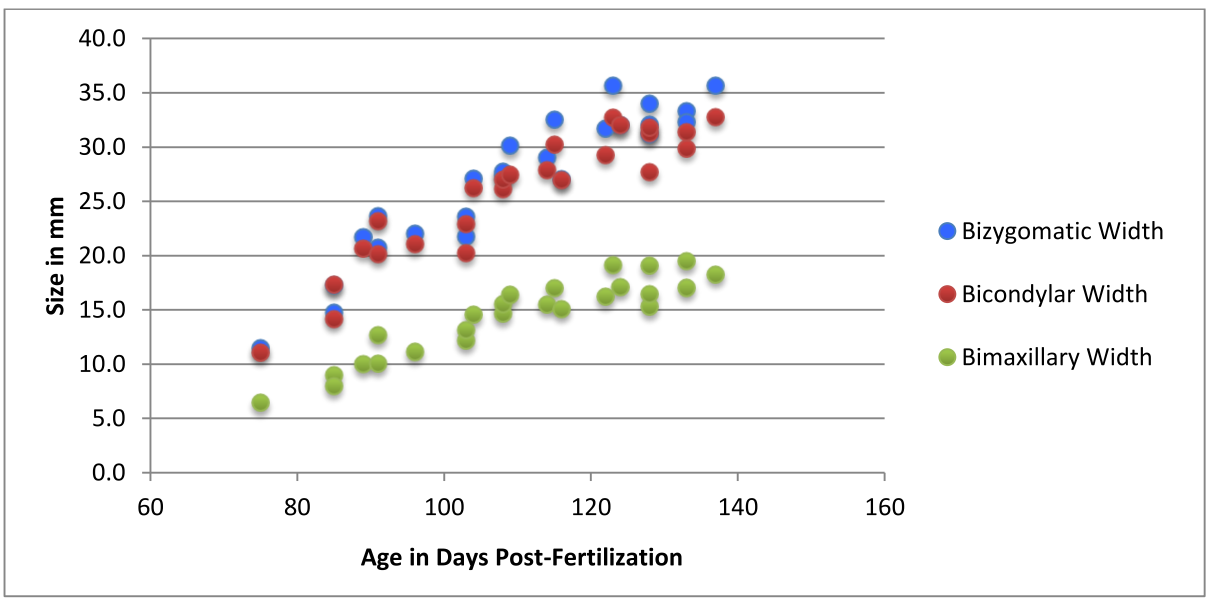


Figure 3.6 3D Micro-CT Measurements of Bizygomatic, Bicondylar, Bimaxillary Widths



3.7 Gender Differences

To investigate if there is growth rate differences between genders the sample was divided into two groups based on gender and linear regression analysis was completed for all 13 linear maxillary and mandibular dimension measurements and the 5 jaw relationship measurements (Table 3.16). Linear regression analysis showed that age in day's gestation is a statistically significant predictor ($p<0.001$) of all the linear measurements representing growth of the maxilla and mandible in all three dimensions for both males and females. The R^2 values and slopes are similar between the two genders for each linear measurement. When evaluating the linear regression results for the jaw relationship age in day's gestation is not a significant predictor of jaw relationship for the mandibular projection angle in males or females (p-value=0.521 & 0.068 respectively) or the mouth-opening angle in males or females (p-value=0.027 & 0.888 respectively). There appears to be a mild discrepancy between males and females with age accounting for 59.7% of the explained variability of the maxillary-nasion-mandibular mandibular angle in males and only 31.1% of the explained variability in females. This discrepancy was also seen with age in days accounting for 52.5% of the explained variability of facial convexity angle in males and only 31.8% of the explained variability in females.

Table 3.16 Gender Related Growth Rates and Jaw Relationship

	Male			Female		
Measurement	**R^2**	**P**	**Slope**	**R^2**	**P**	**Slope**
Maxillary Length	0.944	<0.001	0.156	0.932	<0.001	0.157
Upper Anterior Face Height	0.904	<0.001	0.127	0.882	<0.001	0.122
Bimaxillary Width	0.858	<0.001	0.277	0.871	<0.001	0.265
Maxillary Unit Length	0.942	<0.001	0.231	0.905	<0.001	0.231
Mandibular Body Length	0.911	<0.001	0.150	0.911	<0.001	0.141
Mandibular Ramus Height	0.742	<0.001	0.065	0.796	<0.001	0.069
Lower Anterior Face Height	0.857	<0.001	0.163	0.791	<0.001	0.138
Bicondylar Width	0.898	<0.001	0.370	0.892	<0.001	0.378
Bigonial Width	0.888	<0.001	0.261	0.926	<0.001	0.268
Mandibular Unit Length	0.909	<0.001	0.199	0.905	<0.001	0.192
Total Anterior Face Height	0.904	<0.001	0.263	0.874	<0.001	0.235
Posterior Face Height	0.907	<0.001	0.168	0.926	<0.001	0.162
Bizygomatic Width	0.920	<0.001	0.413	0.896	<0.001	0.405
Maxillary Projection Angle	0.438	<0.001	0.177	0.319	<0.001	0.176
Mandibular Projection Angle	0.007	0.521	0.017	0.063	0.068	0.060
Maxillary-Nasion-Mandibular Angle	0.597	<0.001	0.176	0.311	<0.001	0.120
Facial Convexity Angle	0.525	<0.001	-0.283	0.318	<0.001	-0.215
Maxillary to Mandibular Length Differential	0.308	<0.001	0.032	0.403	<0.001	0.038
Mouth Opening Angle	0.080	0.027	0.082	0.000	0.888	-0.005

Chapter 4: Discussion

4.1 2D Radiographic Data is in Agreement with Ultrasound Data

It is important to compare the 2D radiographic means with 2D ultrasound studies. It is reassuring to find that there is agreement in most values. From 11 to 14 weeks gestation the maxillary length in the current sample is 5-8 mm which is also reported by Shyu et al. (2014) and Chaiyarach and Manotaya (2012). At 14 weeks gestation the maxillary length in the current sample is only 1-2 mm longer than the maxillary length reported by Cicero et al. (2004) and Leung et al. (2006). Goldstein et al. (2005) studied the maxillary bone length from 14 to 40 weeks gestation. Our results for the 14-16 and 20-week groups were similar to the ultrasound data. There was more variability between the datasets in the 17 to 19 week gestation group, however this was very small and not more than 1 mm. Our study is possibly more accurate as we included 39 specimens from 17 to 19 weeks versus Goldstein et al. (2005) who only had 7 specimens from 17 to 19 weeks. Rotten et al. (2002) studied maxillary width using 2D ultrasound. The maxillary width was measured from the external aspect of the alveolar ridge at the height of the alveolus from 18 to 28 weeks gestation. On average our data was 2 mm larger in the specimens that were 18 to 20 weeks gestation. This variation is most likely explained by differences in landmark selection for the maxillary width measurements. The current study measured the maxillary width at the junction of the maxilla to the zygoma which may be a wider measurement than the one made at the developing alveolus.

Mandibular dimensions have been measured on at least 5 ultrasound studies. In general there is good agreement of the data from our study with the ultrasound data. In particular mandibular length is in good agreement between the 2D radiographs and the ultrasounds. In contrast some of the other measurements are different in the ultrasound images. Measurements on 2D ultrasounds from 11 to 13 weeks gestation show the mandibular body length increases from 5 mm to 8 mm (Shyu et al., 2014), which agrees with the mandibular body length measurements in the current study. Otto and Platt (1991) measured the total mandibular length from the temporomandibular joint to menton from 14 to 39 weeks gestation. Their reported values show an increase from 15 mm at 15 weeks gestation to 22 mm at 20 weeks gestation. Here we described a similar measurement, the Co-Me length that increased from 15 mm at 15 weeks gestation to 20 mm at 20 weeks gestation. Paladini et al. (1999) measured the length and width of the body of the mandible from 12 to 37 weeks gestation. The current studies reported values are in agreement at 12 weeks with the mandibular length measuring 5 mm and width measuring 8 mm. However, by 20 weeks of age our values were on average 2 mm larger for the mandibular length and 4 mm larger for the bigonial width. This may be explained by the magnification factor on the radiographs that would not be present in ultrasound. Zalel et al. (2006) measured the mandibular body length and width from 11 to 31 weeks gestation. From 11 to 20 weeks they reported a mandibular body length increase from 6 mm to 13.5 mm, this agrees with the averages reported in the current study. The bigonial width measured in the current study was larger than the mean reported by Zalel et al. (2006). Once again, the differences in values could be due to landmark selection. Zalel et al. (2006) measured from the inner aspect of the mandible while the current study measured from the outer aspect of the mandible. Rotten et al. (2002) measured mandibular widths from 18 to 28 weeks gestation from the outer edges of the alveolar ridges from 18 to 20 weeks the mandibular width was approximately 20-22 mm which is smaller than the current study’s findings of 22.77-24.32 mm for the bigonial width. Differences in landmark selection could account for this variation.

Esenlik et al. (2014) used radiographs to report on angular measurements of jaw relationships relating the maxilla and mandible to the anterior cranial base and to each other. They found the maxillary relationship to the anterior cranial base increased from 76.8 degrees to 84.6 degrees between 11 to 20 weeks gestation. The mandibular relationship relative to the anterior cranial base ranged from 64.1 degrees to 65.8 degrees between 11 to 20 weeks gestation. Our findings are in agreement with those measurements. The angular measurements relating the maxilla and mandible to each other were the ANB angle and facial angle. The ANB angle ranged from 12 degrees at 11 to 13 weeks gestation and 18.73 degrees at 17 to 20 weeks gestation. The facial angle ranged from 155.9 degrees at 11 to 12 weeks gestation and 147 degrees at 17 to 20 weeks gestation. The current study's angular measurement fell within the range for the reported measurements except for the one specimen at 20 weeks gestation which had a larger jaw discrepancy with the mandible being positioned farther back than the maxilla as shown by the reported maxillary-nasion-mandibular and facial convexity angle being outside the range reported by Esenlik et al. (2014). Captier et al. (2011) measured the jaw relationships on autopsy data ranging from 16 to 39 weeks gestation. The average maxillary and mandibular projections from the anterior cranial base were 81.8 degrees and 68.5 degrees respectively with the ANB angle measuring 4.7 degrees (± 2.2). The current study reported larger values for the maxillary projection and maxillary-nasion-mandibular angles and similar values for the mandibular projection angle. The difference in the maxillary projection and maxillary-nasion-mandibular angles could be due to landmark selection. To measure maxillary projection the current study used ANS as the selected landmark while Captier et al. (2011) used prosthion which is located at the inferior tip of the maxillary alveolus. To measure the maxillary to mandibular jaw relationship the current study used ANS and menton landmarks for the anterior maxillary and mandibular references, Captier et al. (2011) used prosthion and infradental the anterior edge of the mandibular alveolus. The maxillary-nasion-mandibular angle has been measured on ultrasounds with a mean value of 5.5 degrees from 14 to 25 weeks gestation (Ko et al., 2012). This is smaller than the current studies values but matches the findings of Captier et al. (2011). Again differences in reported values are likely due to differences in landmark selection. de Jong-Pleij et al. (2011) reported a mean maxillary-nasion-mandibular angle of 13.5 degrees with a large range of 8.9 to 19.5 degrees between 15 to 40 weeks gestation. The maxillary-nasion-mandibular angle results from the current study are within that reported range except for the 20-week specimen that shows a larger jaw discrepancy. In conclusion, the agreement of the current study's 2D values with previously published autopsy and ultrasound samples validates the use of this 2D sample to study jaw growth despite the small amount of magnification.

This study is the first to report fetal jaw size and relationships in 3D using micro-CTs. To validate the use of micro-CT data the values obtained can be compared to studies using autopsy data and 3D ultrasounds to ensure the measurements fall within the same range. Malas et al. (2006) used direct measurements on autopsy specimens to measure the mandibular length, ramus height, bicondylar and bigonial distances for specimens 9 to 40 weeks gestation. The current studies data was in the 3-10 mm range reported for the mandibular ramus height. The current studies mandibular body length was in the 10-19 mm range reported for the mandibular body length except for the 10 and 14 week specimens, which were smaller in the current sample. The current studies bicondylar and bigonial dimensions were smaller by about 8-10 mm than the bicondylar values of 19-41 mm and bigonial values of 16 – 33 mm. It is unclear if Malas et al. (2006) measured the transverse dimensions prior to or after dissecting the mandible from the cranium. Measurements made after dissection may result in inaccurate transverse dimensions since the mandible is not one stable bone, but rather two halves, during this period. Sample size may also have contributed to the difference, the Malas et al. (2006) sample was three times larger than the micro-CT sample.

There are not many studies using 3D ultrasound data since this is a relatively new imaging modality. Hermann et al. (2015) provided data on maxillary length measured on reconstructed 3D models from a 3D ultrasound. The mean maxillary length of 7-16 mm from 11 to 26 weeks gestation is similar to the current study. Hermann et al. (2010) also published 3D data on the mandibular dimension increases from 11 to 26 weeks gestation. The average mandibular base length (Go-Me) increased from 5.2 mm at 11 weeks gestation to 15 mm at 20 weeks gestation. The average mandibular ramus height increased from 2.7 mm at 11 weeks gestation to 6 mm at 20 weeks gestation. The average total mandibular length (Co-Me) increased from 7.7 mm at 11 weeks gestation to 20 mm at 20 weeks gestation. The current studies micro-CT findings fall within these ranges. Tsai et al. (2004) measured mandibular size on 3D rendered images of 3D ultrasound in 40 subjects from 13 to 35 weeks gestation. The average mandibular body length (Go to Me) from 16 to 22 weeks was 14.9 mm ± 4.1 and the micro-CT values are in agreement. Roelfsema et al. (2006) measured mandibular and maxillary dimensions on 3D models derived from 3D ultrasounds in an 18 to 34 week gestation sample. The average maxillary length was reported to be 16.5 mm at 18 weeks gestation, the maxillary length in our study is in agreement. The average mandibular body length (Go-Me) was 13.3 mm at 18 weeks gestation, the average mandibular body length from the micro-CT data was 2 mm larger, and differences are most likely due to how the measurement was performed. Roelfsema et al. (2006) measured the mandible length using the mid-sagittal distance instead of the true length of the body of the mandible. These values for mid-sagittal mandibular lengths are similar to the 2D radiographic value for mandibular body length.

Roelfsema et al. (2006) is the only study to report jaw relationship angles measured in 3D from 3D reconstructions of ultrasounds. They found the maxillary protrusion relative to the anterior cranial base to be 81.2 degrees at 18 weeks, which is smaller than the current study's reported average of 92 degrees. Differences could be due to landmark selection as well as due to the fine detail of the anterior nasal spine that can be detect on the micro-CT but is not as clearly seen on ultrasound. The mandibular protrusion angle relative to the anterior cranial base was 66.7 degrees at 18 weeks, which is similar to the micro-CT data mandibular projection angle average of 67.5 degrees.

After confirming the current studies measurements were within range of previously reported data it is evident that even though the sample size was small, the number of specimens used for the micro-CT data provided meaningful data on jaw growth and relationships during the early fetal period that correlated with the data obtained on living fetuses.

4.2 Discrepancies Between 2D and 3D Data

The largest discrepancy between the 2D radiographic data and 3D micro-CT data is the measurement of widths. The measurement of widths was consistently larger in the 2D sample than the 3D sample with the bizygomatic and bimaxillary width difference being larger than the difference for the bicondylar and bigonial widths. This was due to the distance between the film and the face when the specimens were radiographed. If the back of the head was placed directly against the film the structures towards the front of the face, the bizygomatic and bimaxillary widths, would be magnified more than those closer to the film, the bicondylar and bigonial widths. As the age of the specimens increased from 10 to 19 weeks gestation, the difference between the means and standard deviations of the 2D and 3D data becomes larger. With increasing age the size of the cranium also increases which positions the maxilla and mandible farther from the film than the smaller, younger specimens. As it is positioned farther from the film it increases the magnification of the radiograph that may affect the final measurement.

When comparing the 2D and 3D linear measurements, the 3D micro-CT means were larger for the mandibular body length, mandibular ramus height, mandibular unit length, maxillary unit length and posterior facial height. These differences can be explained because these measurements are not located in the mid-sagittal plane and therefore the 3D measurement incorporates a transverse component that can increase the overall measurement. Frequently the difference between the 2D and 3D measurements in postnatal studies is not statistically significant, but algorithms have been constructed to convert 2D measurements into 3D measurements (Gribel et al., 2011a; Gribel et al., 2011b). The 3D micro-CT maxillary length, maxillary projection angle, maxillary-nasion-mandibular angle and facial convexity angle were larger than the 2D values. This could be due to landmark selection; the anterior landmark for the maxillary length was the anterior nasal spine, which is a very fine projection of bone. Due to the small size the full anterior extension of this bone may not have been captured on the lateral cephalometric radiograph due to decreased x-ray absorption affecting the anterior contour of the maxilla and decreasing the anterior limit of the maxilla in 2D. The mouth-opening angle was on average larger in the 2D sample than the 3D sample, this discrepancy might be due to the effects of fixation on the samples used for micro-CT scanning. Formalin fixation cross-links proteins, leading to muscle fibers shortening (Chen et al., 2012). The specimens used for micro-CT scanning were stored in formalin for the last 27 years; this may have caused muscles to shrink potentially leading to the mouth closing.

4.3 Maxillary and Mandibular Growth

There are two discrepancies between the 2D and 3D width data to note. The difference in growth increases between the bimaxillary and bigonial widths and how overall the width measurements increase at a greater rate in the 2D sample than the 3D sample. Both of these discrepancies could be due to the effects of the magnification, exaggerating the increase in the transverse dimension over time. In the 2D data the maxillary length increases more than the mandibular body length, which is opposite of the 3D data. The unit length of the maxilla and mandible increases at a higher rate in the 3D than in 2D. These discrepancies between the 2D and 3D data are most likely due to the fact that 3D measurements incorporate transverse and anterior-posterior growth resulting in larger values seen for the 3D measurements. In both the 2D and 3D sample the posterior face height increases more than the lower anterior face height, which increases more than the upper anterior face height. The differences between the 2D and 3D data show how it may be important to create 2D or 3D ultrasound specific growth reference charts when assessing for maxillary and mandibular size anomalies.

Other studies using autopsy material have looked at fetal jaw growth in different dimensions. Houpt (1970) found that from 12 to 19 weeks gestation the mandibular width increased more than length followed by height and that the maxilla increased more in length followed by height. These results are in agreement with the current studies 2D and 3D findings. Lavelle and Moore (1970) found that from 16 to 27 weeks gestation the mandibular length increased more than height followed by width and the maxillary length increased more than the width. This is not in agreement with the current findings. Inoue (1961) found that over the 12 to 40 week period the growth in height occurs more than length and that the mandible grows more than the maxilla, this is not in agreement with the current study's findings. The difficulty in comparing growth in different dimensions during the fetal period is that growth my not occur uniformly in all dimensions throughout the entire fetal period. Published studies have analyzed fetal growth during different and often overlapping time frames; it may be that the jaws grow more in width during the early fetal period and then during the late fetal period they may increase more in length. Patterns like this are also seen postnatally with growth increases in width occurring earlier than length and growth in height occurring later in life (Behrents, 1985). Magnification effects may also explain the variation in findings as none of the previously published studies reported the amount of magnification present. Ultrasound studies have analyzed growth rates increases in maxillary and mandibular dimensions from 11 to 26 weeks gestation (Hermann et al., 2015; Hermann et al., 2010). It has been shown that the maxillary length increases at 1.72 mm/week, which is a greater increase than the mandibular body length increase of 1.2 mm/week. This faster rate of maxillary length growth than mandibular body length growth is shown in the current studies 2D results, as the maxillary length has a slope of 0.155 and the mandibular body length has a slope of 0.147. However, for the 3D micro-CT data the mandibular body length increases at a greater rate than the maxillary length. This discrepancy is due to how the measurements were made; if the mandibular body measurement is made as a mid-sagittal length the results will vary from measurements made following the true outline of the mandibular body in 3D because a transverse component is now also incorporated. The ultrasound data showed that the total mandibular length from condylion to mention increased at 1.7mm/week which is greater than the 1.2 mm/week increase in mandibular body length, and then 0.64 mm/week increase in the mandibular ramus height (Hermann et al., 2010). The slopes of both the 2D and 3D data confirm this finding. Being able to identify in which dimension the maxilla and mandible are growing the most during the early fetal period is important because it would be easier to identify growth abnormalities occurring in the dimension of most rapid growth. By knowing which dimensions are increasing at a greater rate it could help increase the sensitivity of detecting abnormal growth by comparing jaw dimension measurements to established normal growth curves.

4.4 Studying Shape Rather Than Linear Measurements

Another way to study growth rather than comparing slopes of absolute growth rates is to examine shape changes which are unrelated to size increases. Trenouth (1985b) emphasized that although the absolute growth could describe growth changes that were occurring it did not adequately show shape changes. Diewert et al. (1991) showed using finite element modeling that the maxilla and mandible had significant shape changes during the 12 to 20 week fetal period. When taking into account and adjusting for increasing size Trenouth (1991) showed that from 10 to 20 weeks gestation the fetal face shape changed more in length, followed by width, followed by height, which is not in agreement with the current study's findings.

4.5 Jaw Relationships are Correlated with Age for the 2D Data

When comparing the 2D radiographic and 3D micro-CT jaw relationship data from 10 to 20 weeks gestation differences in the ability of age to predict jaw relationships are seen. In the 2D radiographic sample gestational age in days is a significant predictor of increasing maxillary protrusion and a predictor for the increasing discrepancy between the maxilla and mandible as shown by the maxillary-nasion-mandibular angle, the facial convexity angle and the maxillary to mandibular length differential. In the 3D micro-CT data age is a significant predictor of only the maxillary to mandibular length differential. This discrepancy could be a result of the micro-CT sample being too small to show the statistically significant effects of age on jaw relationships.

The mandibular protrusion angle relative to the anterior cranial base did not change with increasing age in both the 2D and 3D data. This is important to consider when looking at how the maxillary to mandibular relationship changes during this time. Previous studies have shown the mandible has a temporary prognathic position in the early fetal period from 8 to 12 weeks gestation but some of these studies had a very small sample size (Burdi and Silvey, 1969; Diewert, 1983, 1985a, b; Humphrey, 1971). The appearance of this prognathic stage has been questioned since not all studies have shown this to be present (Esenlik et al., 2014; Radlanski et al., 2013). With the increased interest in using jaw relationship values to detect craniofacial abnormalities in utero the normal jaw relationship changes during the early fetal period are important to understand. In order to be able to detect what is abnormal there must be adequate data on what normal growth changes are occurring. Esenlik et al. (2014) showed the maxillary projection angle increased from 11 to 24 weeks gestation, the mandibular projection angle decreases by 2 degrees between 11 to 16 weeks gestation and that the maxillary to mandibular relationship changes with the mandible becoming more retrognathic from 11 to 24 weeks gestation as shown by the ANB and facial angle. However, the statistics lacked power due to a small sample size and the large overlapping standard deviation. The 2D radiographic regression results in the current study clarify the findings of Esenlik et al. (2014) that the maxilla becomes more prognathic with age and that the 2-degree difference in mandibular projection is not statistically associated with age changes. The finding of increased maxillary projection with increasing age is also in agreement with previously reported studies showing average increases of 1.6 degrees per week from 14 to 24 weeks gestation (Erdoglija, 1990). The current study did not have any samples showing a mandibular prognathic relationship but the 2D data did show that there was a change in the maxillary to mandibular relationship with the discrepancy between the maxilla and mandible becoming larger with increasing age from 10 to 20 weeks gestation. The current 2D results allude to the fact that the change in jaw relationship seen during this period may be due to the anterior positioning of the maxilla relative to a stable mandible which is in agreement with previously reported studies (Trenouth, 1981; Trenouth, 1985a).

There are two ways to investigate jaw relationship changes, using angular measurements of maxillary-nasion-mandibular and facial convexity angles or by looking at linear differences such as the maxillary to mandibular length differential. A difficulty in using a maxillary to mandibular angle such as the MNM or ANB angle is that the value can be affected by a number of factors such as the degree of mouth opening, the vertical distance of the maxillary or mandibular landmark from nasion or the horizontal position of nasion along the anterior cranial base (Jacobson, 1975; Radlanski et al., 2013). In fetal samples mouth opening cannot be standardized, as there are no teeth to allow the mandible to be positioned in occlusion, furthermore, fetal jaw movements such as mouth opening are present as early as 10 weeks gestation so the degree of mouth opening on ultrasound may affect the jaw angles measured (de Vries and Fong, 2006). No study to date has assessed the degree of mouth opening seen during the early fetal period. The current study found the degree of mouth opening to be uniform and age is not a predictor of mouth opening in this sample. Even with these limitations the maxillary-nasion-mandibular angle is most commonly used in ultrasound studies to assess jaw relationships. A difficulty in trying to compare studies looking at age related changes is the overlap in age ranges that are examined. The best data on the maxillary to mandibular relationship comes from a longitudinal ultrasound study that reported the maxillary-nasion-mandibular angle to be on average 13.5 degrees at 16 weeks gestation and not changing with increasing age until birth (de Jong-Pleij et al., 2011). This study was supported by another showing the jaw relationship did not change from 21 weeks gestation until birth (Captier et al., 2011). Both of these studies emphasized that there was a large variation in reported MNM angle values. The stability of the MNM angle from 16 weeks to gestation was supported by the current study that showed no age related increases of this angle from 16 to 20 weeks gestation. Another ultrasound study examining the same angle from 14 to 25 weeks gestation in an Asian sample showed a mean MNM angle of 5.5 degrees and that this value decreases with the jaw discrepancy becoming smaller from 14 to 39 weeks gestation (Ko et al., 2012). These reported values and age related findings are different from what has been previously reported and the current study. It has been suggested that the differences in the MNM angle seen prenatally may be attributed to ethnic differences between the different samples (Ko et al., 2012), as postnatal studies have shown ethnic differences in craniofacial form (Miyajima et al., 1996).

The other way to examine jaw relationships is to look at the differences in lengths measured from a common location. This method has been reported to be advantageous in looking at jaw discrepancies because it eliminates the potential effects of the position of nasion along the anterior cranial base or the vertical position of the anterior maxillary and mandibular landmarks from nasion (Harvold, 1974). This study highlighted the advantage of using this method as the reported values are not as variable as the maxillary-nasion-mandibular angle and it is the only value that age was a statistically significant predictor of in both the 2D and 3D samples.

Earlier studies show the maxillary-nasion-mandibular angle has age related changes up until 20 weeks gestation but was stable from then until gestation (Esenlik et al., 2014; Koski, 1980; Levihn, 1967; Trenouth, 1981). The current study's findings show changes in the jaw relationship are statistically significant with increasing age in the first half of the 10 to 15 week gestation period but not during the second half of the 16 to 20 week period. This supports that interpretation that the jaw relationship is stable from 16 weeks gestation until birth (de Jong-Pleij et al., 2011) and that the jaw relationships are established by the end of the 15th week gestation. This is an important finding because the values for the maxillary-nasion-mandibular angle currently used to assess for craniofacial abnormalities in the second trimester may not be valid in first trimester ultrasound screening (de Jong-Pleij et al., 2013; Vos et al., 2014; Vos et al., 2015).

4.6 Rates of Jaw Growth are Greater in the First 5 Weeks of the Middle Trimester

The current study demonstrated that not only did growth occur in different dimensions during the 10 to 20 week period but that it also occurred at different rates.

When comparing percent increases in size of the 13 linear measurements it was apparent that the increase in growth relative to overall size occurred more during the 10 to 15 week period with growth increments ranging from 30-45% increase in size, than during the latter half of 16 to 20 weeks period with growth increments ranging from under 5% to 20%. This finding is in agreement with Levihn (1967) and Lavelle (1974)who showed that the fastest rate of fetal growth occurred during the 4th to 5th months gestation (12 to 20 weeks) and 3rd, 4th and 5th months gestation respectively. Hermann et al. (2015) who showed that the maxillary length had an accelerated growth rate from 10 to 15 weeks gestation and then an almost constant growth rate of 10% from 16 to 26 weeks gestation. The accelerated early growth may be due to changes in the dominant growth mechanisms occurring during this time. During the late embryonic and early fetal period, before the maxilla and mandible have begun fully ossifying, the cartilage components of the midface and mandible are undergoing rapid growth. As the maxilla and mandible develop ossification spreads and the cartilage growth decreases while ossification takes over as the main growth mechanism (Diewert, 1982). This change may occur between 10-15 weeks and by 16 weeks ossification may be the main growth mechanism accounting for the smaller rates of increase seen. Houpt (1970) stated the growth rate was constant between 12 to 19 weeks gestation based on the linear regression slopes but this is not an accurate way to assess growth rates, as it does not consider the increase relative to the initial size. The bimaxillary value was the only value to show variation in timing of the growth acceleration. The percent increase in size was higher during the 14 to 17 weeks gestation at almost 40% than during the previous 10 to 13 weeks or 12 to 15 weeks gestation. This could be a result of the relatively small number of specimens in the 16-week period, only 8, relative to the 17-week period, 33 specimens. The results may have been skewed towards a greater rate of increase because there are more 17-week specimens and they are larger. However, if this were the case it would be expected that all width measurements would show the same trend. Another cause may be the rapid brain growth during this period. Trenouth (1984) hypothesized that brain growth can affect the midface due to the expansion of the temporal lobes affecting the position of the nasomaxillary segment. When our 3D data for bizygomatic, bicondylar and bimaxillary widths are plotted by age in days gestation it is evident that around day 108 which is approximately week 15 there appears to be a slight change in the bimaxillary slope which is not as apparent in the bizygomatic or bicondylar widths (Figure 3.6). This slight change may be a result of changes in brain development. From 10 to 27 weeks gestation the brain volume is differentially enlarging showing greater increases in the supratentorial region than in the infratentorial region (Jeffery and Spoor, 2002). Within this time frame neuronal maturation and organization in the developing fetal brain starts to occur around 16 weeks gestation, while neuronal migration decreases (Marin-Padilla, 1989). This development of different layers of the cerebral cortex in conjunction with the rapid growth in the supratentorial region may influence the growth of the midface and be a contributing factor to the larger percent increase seen in the bimaxillary width during the 14^{th} to 17^{th} week gestation.

An accelerated growth period from 10 to 15 weeks gestation further emphasizes the importance of screening for craniofacial abnormalities at an early stage as it is easier to identify abnormal growth during a period of more rapid increases in size.

4.7 Gender Differences

Linear regressions were completed for each measurement by gender using the 2D radiographic data to determine whether similar growth trends are seen between genders. For all linear measurements age is a statistically significant predictor of size for both males and females, with the slopes and R^2 values reported being similar for both the male and female samples. The jaw relationship angles showed some variation between males and females. The statistical significance for all measures of jaw relationships is similar for males and females. However, in male's, age in days accounted for more of the explained variability of the maxillary-nasion-mandibular and facial convexity angles compared to females. This difference may be attributed to random variation in sample distribution and the fact that there is a large variation in jaw relationships reported (Captier et al., 2011). The similar growth trends seen for both males and females show that gender differences in jaw growth are not present during the early fetal period, and this is in agreement with previously published fetal jaw growth studies (Esenlik et al., 2014; Houpt, 1970; Inoue, 1961; Malas et al., 2006).

Chapter 5: Conclusions and Recommendations for Future Studies

5.1 Conclusions

From 10 to 20 weeks gestation both the 2D radiographic and 3D micro-CT data support the trends that the maxilla and mandible are rapidly increasing in size in all three dimensions showing greater rates of growth in width followed by length then height.

The 2D radiographic data shows there is a statistically significant ($P<0.001$) association of the maxillary projection angle and the maxillary-nasion-mandibular angle increasing with age in days gestation while the facial convexity angle decreases with age in days gestation. Age in days gestation is a moderate predictor of these measurements. However, the 2D angular measurements were not in agreement with the 3D micro-CT sample. A future study should increase the number of specimens studied with micro-CT. The cephalometric and micro-CT data both show that the maxillary to mandibular length differential shows little change with age and age in days gestation is a statistically significant ($p<0.001$) but weak predictor of the measurement. Both the 2D and 3D data support the finding that the mandibular projection angle does not change with increasing age and age in days is not a significant predictor of the mandibular projection. This was the first study to quantify the degree of mouth opening and found that age in day's gestation is not a predictor of mouth opening and the value does not change significantly with increasing age. From 10 to 15 weeks gestation age is a significant predictor of the maxillary projection, maxillary-nasion-mandibular angle, facial convexity angle and the maxillary to mandibular length differential and these values increase, or decrease for facial convexity angle, with increasing age. From 16 to 20 weeks gestation age is not a statistically significant predictor of any jaw relationship measurement and the jaw relationships are stable and do not change with increasing age.

From 10 to 15 weeks gestation there is a greater rate of change in the percent increase in size ranging from approximately 30% to 45% increases for all linear measurements representing the growth of the maxilla and mandible in all three dimensions. This rate slows down with a smaller percent increase in size from under 5% to 20% occurring from 16 to 20 weeks gestation.

Growth related gender differences are not evident in this sample. This means that during the early fetal period or middle trimester, ultrasound studies can use one set of standards for both males and females.

5.2 Strengths

The strength of this study is that it provides normative data regarding the average maxillary and mandibular size in different dimensions, maxillary and mandibular growth rates in different dimensions and the jaw relationships during the early fetal period of 10 to 20 weeks gestation. The current ultrasound literature does not have data for all the dimensions of the maxilla and mandible or jaw relationships during this early fetal age range. This is valuable information to contribute as it both supports and provides new data for ultrasound use which may help increase the ability to detect craniofacial abnormalities during the early fetal period. This is also the first study to report absolute measurements and growth data on maxillary and mandibular sizes in 3D using micro-CT. This is valuable as it provides new standard values which can be compared to those obtained on 3D ultrasounds of which its use is increasing (Salem et al., 2014). This study also highlights the importance of using separate growth charts when comparing values measured on 2D versus 3D ultrasounds as the growth charts may be different for measurements not located in the mid-sagittal plane such as the mandibular body length. This study reinforces the findings that the jaw relationship changes are occurring from 10 to 15 weeks gestation and are then stable from 16 to 20 weeks gestation, and that the maxillary to mandibular length differential may be a more suitable means to detect jaw discrepancies than the MNM angle. This is important information for the ultrasound community as they begin to detect craniofacial abnormalities during the first trimester ultrasound scan as this information may guide how they choose to measure jaw discrepancies.

5.3 Limitations

A limitation of this study is that it is a cross-sectional study. However cross-sectional population-based studies are the standard for establishing typical growth curves. Moreover, our data is in agreement with the imaging of living babies in utero thus it is relevant to clinical practice.

The limitation of the 2D radiographic data is the magnification factor that was not adjusted for. Although the magnification in the current study was determined to be a minor amount this may have potentially affected the growth results by exaggerating the increase in width that would have been magnified more than the increase in sagittal measurements. Instead of adjusting each measurement for the magnification the micro-CT data was used in an attempt to confirm the 2D radiographic findings. The limitation of the micro-CT data was that the sample size was small, only 25 specimens compared to the over 100-sample size for the radiographic data. This may have led to the micro-CT results for jaw relationships being statistically insignificant when there was just not a large enough sample size to show statistical significance. The small sample size may also have affected the results of the 2D radiographic data when it was divided into the 10 to 15 weeks and 16 to 20 weeks gestation groups.

This study lacked information regarding the ethnicities of the specimens included in the sample. As mentioned there are postnatal ethnic differences in craniofacial patterns and some believe these ethnic differences may be evident as early as the prenatal period (Miyajima et al., 1996; Shyu et al., 2014). The potential for ethnic variability at this early stage may have an effect on the current studies results, especially the differences in the averages of the jaw relationship results.

5.4 Future Research Recommendations and Clinical Relevance

Future studies on the maxillary and mandibular fetal morphology should be done using 3D morphometrics to adequately describe the changes in morphology of the maxilla and mandible rather than comparing slopes of growth in different dimensions. This will allow for a more powerful statistical analysis to determine exactly if and how the morphology of the bones is changing during this period of growth.

Further studies on fetal maxillary and mandibular growth should be completed using ultrasound technology. Ultrasound data is more clinically relevant as it is more applicable when trying to detect abnormal growth. There is a need for the ultrasound literature to more clearly define and standardize the measurements being using to quantify maxillary and mandibular growth and jaw relationships. Standardizing measurements will allow for a more accurate creation and comparison of normal growth curves for which abnormal specimens can be compared against. The maxillary-nasion-mandibular angle measurement does have its limitations. In light of the findings in this study that show the maxillary-nasion-mandibular angle to have a large variability it might be advantageous to look at the maxillary to mandibular length differential as a measurement of jaw discrepancy. If the maxillary-nasion-mandibular angle is used a measurement approximating the degree of mouth opening should also be obtained to ensure the jaw relationship measurement is not affected by mouth opening.

The use of ultrasound is also advantageous in that it allows growth studies to be longitudinal as it can follow growth of subjects who receive both first and second trimester ultrasounds. Future studies should aim to gather data on the same subjects from the first and second trimester ultrasound screenings. Future studies should also make an attempt to identify the ethnicities of the sample being studied to determine if ethnic variation in craniofacial features does occur prenatally and if so, ethnic specific growth standards should be created.

In summary, this work will be very useful in clinical genetic practice. In all chromosomal or other genetic abnormalities, the genotype does not completely correlate with the phenotype, even when a specific mutation is identified. This variance in expressivity is due to epigenetic effects. Thus analyzing the individual in more detail using jaw measurements would really help to prepare the parents for the level of care that would be needed after birth.

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