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Analysis of Spheno-Occipital Synchondrosis (SOS) Fusion in a Contemporary Southern Nevada Subadult Hispanic Population Using Archival Cone-Beam Computerized Tomography (CBCT) Images

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ANALYSIS OF SPHENO-OCCIPITAL SYNCHONDROSIS (SOS) FUSION IN A CONTEMPORARY SOUTHERN NEVADA SUBADULT HISPANIC POPULATION USING ARCHIVAL CONE-BEAM COMPUTERIZED TOMOGRAPHY (CBCT) IMAGES

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Abstract

Analysis of Spheno-Occipital Synchondrosis (SOS) Fusion in a Contemporary Southern Nevada Subadult Hispanic Population Using Archival Cone-Beam Computerized Tomography (CBCT) Images

By

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This study employs the three-dimensional visualization capability of cone-beam computed tomography (CBCT) to investigate the relationship between chronological age and timing of fusion of the spheno-occipital synchondrosis in a male/female subadult Hispanic population of Southern Nevada. The sample includes cross-sectional data of 374 orthodontic patients (166 males and 208 females) aged 8-20 years. The SOS is scored by a four-stage scoring system as completely open (stage 0), less than half fused (stage 1), more than half fused (stage 2), or completely fused (stage 3) as visualized in the midsagittal plane in the CBCT image. The relationship between SOS fusion stage and chronological age for both males and females is investigated for utility in age estimation efforts of living persons.

Mean ages for fusion stage 0, stage 1, stage 2, and stage 3 were 10.90, 12.66, 13.87, and 16.35 years in males, and 9.86, 10.54, 11.42, and 14.84 in females, respectively. Spearman correlation analysis showed a significantly strong positive correlation between age and SOS fusion stage for the entire population (Spearman's rho = 0.719, $p < 0.001$). Results from linear regression analysis showed males have a strong positive correlation between age and fusion stage ($r=0.847$, $p <0.001$). Differences in mean age were statistically significant for all fusion stages in males. Females have a moderately strong positive correlation between age and fusion stage ($r=0.643$, $p <0.001$). Differences in mean age were only statistically significant between fusion stages 0 & 3, 1 & 3, and 2 & 3 for females.

There is a significant positive relationship between SOS fusion and chronological age in subadult Hispanic males and females of Southern Nevada. Results from this research suggest that SOS fusion stage could be used as a subadult age estimation technique for Hispanic males of Southern Nevada, but not for females. Ultimately, SOS fusion stage should be used in conjunction with other skeletal or dental indices for age estimation efforts in this population and not as a stand-alone age indicator.

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I would like to thank my research team, Drs. Edward Herschaft, Robert Danforth, Brian Chrzan, and Debra Martin, for their guidance and support with this project. Gratitude is also extended to Dr. Marcia Ditmeyer for her expertise and assistance with the statistical analysis. I would also like to recognize and thank Amy Pam for her help with image acquisition, and research assistants, Michael Hartmann and Derek Stutz, DMD class of 2020, for their dedication and time devoted to image preparation. Another sincere thank you to Dr. Haley Buchanan for her time and assistance with interobserver analysis, but more importantly, for her friendship.

A very special acknowledgement is given to my husband and daughters, Eric, Jaycie, and Colbie Baker, for providing relentless support and encouragement in the pursuit of my passion. The foundation of my entire being is grounded in the support from my family. Without them, none of this would have even been possible.

Dedication

To my husband, Eric,

You inspire me to be more open-minded and altruistic. You are my foundation.

To my daughters, Jaycie and Colbie,

May you always find the strength and courage to pursue your dreams.

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Chapter 1: Introduction

Age estimation is recognized as one of the fundamental principles of forensic anthropology. Forensic age estimation has been a particular point of interest in recent years due to an increasing number of unidentified human remains, criminal prosecution, global increase in immigration, and a rising number of cases requiring legal age determination for living individuals with no legal proof of date of birth (Krishan & Kanchan, 2013; Prasad et al., 2003). Accurate age estimation of subadult populations is of particular importance, especially considering the adult versus subadult legal ramifications in criminal prosecution and immigration rights. All together, these circumstances underscore the importance of age estimation not only for the obvious forensic analysis in post-mortem applications but also particularly in living persons.

Age itself tends to be the most valuable and accurate biological parameter in subadult forensic anthropology cases and various skeletal and dental indices can be utilized for its investigation (Franklin, 2010). For juveniles and adolescents, age estimation efforts include a variety of well-documented and predictable developmental markers in the immature skeleton (Scheuer and Black, 2000). According to Franklin, 2010, three of the most common methods to estimate subadult age are based on the following criteria:

- Degree of dental development, specifically tooth formation and eruption
- Metric analysis of long bones
- Assessment of ossification centers, including their size and timing of appearance as well as fusion degree relative to full maturity.

One of the ossification centers that can be utilized as a developmental marker for age estimation is the spheno-occipital synchondrosis (SOS). The SOS is a growth center of the cranial base that matures/ossifies into adolescence and is therefore of particular utility in forensic anthropology when assessing the ages of subadult populations (Krishan & Kanchan, 2013).

The first to recognize the paucity of information regarding the timing of closure of individual growth sites of the cranial base, including the SOS, was Ford in 1958 (Ford, 1958). He evaluated SOS closure in skeletonized specimens of humans and reported that complete fusion of the SOS takes place around 17-25 years of age (Ford, 1958). In his article "Growth of the human cranial base" he declared that studying the components of the cranial base is becoming increasingly important with the "…. widespread use of radiographic cephalometry" (Ford, 1958). Successive efforts to utilize radiographic imaging techniques to study the normal timing of SOS closure began with Irwin in 1960 through the use of midsagittal tomography (Irwin, 1960). Contrary to Ford, Irwin observed that complete fusion was generally achieved by the age of 18 (Irwin, 1960). Efforts to further interpret SOS closure relative to chronological age progressed with Powell and Brodie in 1963 and Konie in 1964. These researchers recognized that early human anatomy textbooks reporting complete SOS fusion between 18-25 years of age were invalid. Actually, SOS fusion generally occurred much earlier and did not necessarily reflect a transition to legal adulthood (Powell and Brodie, 1963; Konie, 1964).

More contemporary texts and anthropological literature continue to propose that fusion is related to maturational events, such as growth spurts and hormonal fluctuations that occur during adolescence (Scheuer & Black, 2000). Thus, the earlier accepted SOS fusion interval of 18-25 years of age is likely overestimated (Scheuer & Black, 2000). The initial historical overestimation of SOS fusion age is also supported by very recent assessments of global populations where complete fusion is reported to occur as early as a mean age of 16.4 years in an American male population studied by Alhazmi et al. and 13.8 years in an Australian female population studied by Lottering et al. (Alhazmi et al., 2017; Lottering et al., 2015). Variability in age at complete fusion among different populations does in fact reinforce the idea that originally accepted SOS fusion timing is outdated. Thus, this study is performed to further explore the concept that SOS fusion occurs during adolescence and that there is variability in SOS closure among global populations.

The aim of this project is to use archival cone-beam computed tomography (CBCT) scans acquired from a sample of modern subadult Hispanic orthodontic patients from the University of Nevada - Las Vegas School of Dental Medicine (UNLV SDM) in order to evaluate a single age estimation technique utilizing the spheno-occipital synchondrosis. This CBCT analysis is the first to examine the timing of SOS closure in a contemporary subadult Hispanic population of the United States. The morphological age estimation method developed in this research will help to advance knowledge regarding the formulation of ethnic specific age estimation standards. Thus, the collection of valid skeletal developmental markers that can be utilized by forensic anthropologists, forensic odontologists, and medico-legal personnel for a variety of objectives will be improved and enhanced.

Research Questions and Hypotheses

1. Does the degree of fusion of the spheno-occipital synchondrosis, as visualized on midsagittal CBCT images, correlate with chronological age in a male/female subadult Hispanic population of Southern Nevada?

   Hypothesis: The degree of spheno-occipital synchondrosis fusion correlates with chronological age and is statistically significant ($p < 0.05$).

   Null Hypothesis: There is no statistically significant correlation between degree of spheno-occipital synchondrosis fusion and chronological age for this population.

2. Does mean age for each SOS fusion stage, as visualized on midsagittal CBCT images, demonstrate sexual dimorphism in a male/female subadult Hispanic population of Southern Nevada?

   Hypothesis: Mean age for each SOS fusion stage is sexually dimorphic with females demonstrating earlier mean age for each fusion stage.

   Null Hypothesis: Mean age for each fusion stage is not sexually dimorphic for this population.

3. Do subadult Hispanic males of Southern Nevada demonstrate a statistically significant ($p < 0.05$) relationship between mean age and fusion stage? If so, between which stages do statistically significant differences in mean age exist?

   Hypothesis: Males have a positive correlation between age and fusion stage and demonstrate statistically significant differences ($p < 0.05$) in mean ages between all fusion stages.

   Null Hypothesis: Males demonstrate no statistically significant ($p < 0.05$) correlation between age and fusion stage.

4. Do subadult Hispanic females of Southern Nevada demonstrate a statistically significant ($p < 0.05$) relationship between mean age and fusion stage? If so, between which stages do statistically significant differences exist?

   Hypothesis: Females have a positive correlation between age and fusion stage and demonstrate statistically significant differences ($p < 0.05$) in mean ages between all fusion stages.

   Null Hypothesis: Females demonstrate no statistically significant ($p < 0.05$) correlation between age and fusion stage.

5. Does a male/female subadult Hispanic population of Southern Nevada differ from other global populations in age when SOS is completely fused compared to the results from investigations using techniques other than CBCT analysis?

   Hypothesis: Male/Female subadult Hispanics of Southern Nevada observationally demonstrate variable age at complete SOS fusion compared to the results of:

   - Macroscopic studies by Coqueugniot and Weaver, 2007, Shirley and Jantz, 2011, Mahon et al., 2017,
   - Conventional radiographic study by Powell and Brodie, 1963,
   - Conventional CT studies by Madeline and Elster, 1995, Okamoto et al., 1996, El-Sheikh and Ramadan, 2006, Bassed et al., 2010, Franklin and Flavel, 2014, Can et al., 2014, Lottering et al., 2015.

   All of which utilize techniques other than CBCT analysis for their study of various global populations.

   Null Hypothesis: There is no observational difference between a male/female Hispanic subadult population of Southern Nevada and the results of the following studies utilizing techniques other than CBCT analysis in age when the SOS is completely fused:

- Macroscopic studies by Coqueugniot and Weaver, 2007, Shirley and Jantz, 2011, Mahon et al., 2017,
- Conventional radiographic study by Powell and Brodie, 1963,
- Conventional CT studies by Madeline and Elster, 1995, Okamoto et al., 1996, El-Sheikh and Ramadan, 2006, Bassed et al., 2010, Franklin and Flavel, 2014, Can et al., 2014, Lottering et al., 2015.

6. Do results in mean age for each SOS fusion stage differ between this CBCT analysis of a male/female subadult Hispanic population of Southern Nevada compared to results obtained from the CBCT analysis of a modern American population reported in the literature by Alhazmi et al., 2017?

   Hypothesis: Using CBCT technology, male/female subadult Hispanics of Southern Nevada observationally demonstrate different mean ages for each SOS fusion stage compared to the male/female modern American population studied by Alhazmi et al., 2017.

   Null Hypothesis: Using CBCT technology, there is no observational difference between a male/female subadult Hispanic population of Southern Nevada and the CBCT study of a male/female modern American population studied by Alhazmi et al., 2017 in mean age of each SOS fusion stage.

Development of the Cranial Base

The human embryonic skull is composed of pre-formed cartilage that is replaced by bone during embryogenesis (Scott, 1958). Beginning during the third month in utero and continuing during the first year after birth, blood vascular elements start to infiltrate various areas of the cartilaginous skull leading to the progressive osteogenesis of primary ossification centers (Figure 2.1) of the cranial base bones (Scott, 1958). Multiple secondary ossification centers develop during infancy and fuse with the primary ossification centers to form the basioccipital, basisphenoid, presphenoid, and ethmoid bones (Figure 2.1) (Scott, 1958).

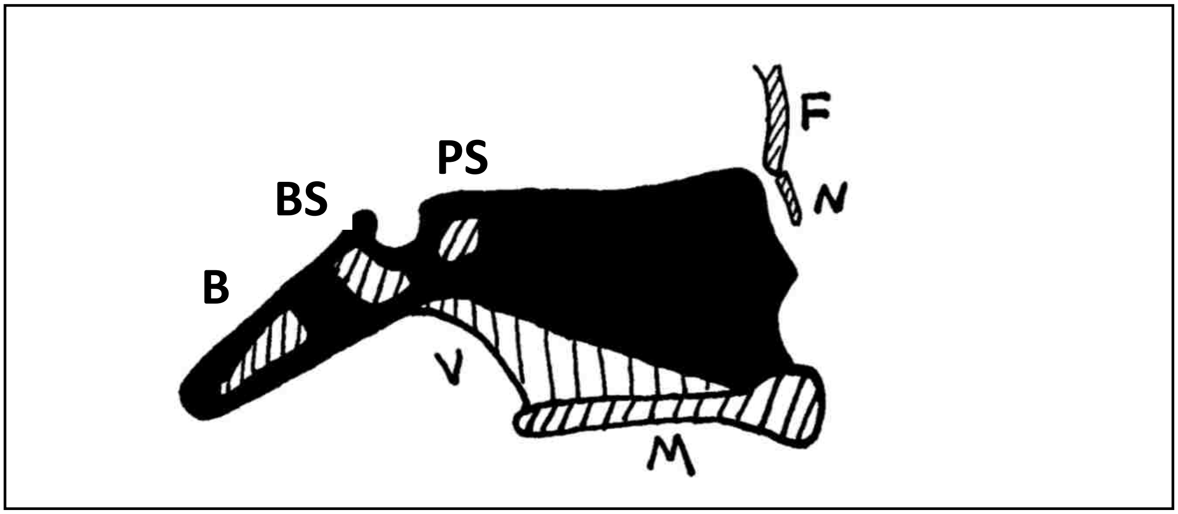


Figure 2.1 *Midsagittal View of Fetal Cartilaginous Skull. Solid fill represents cartilage, hashed fill represents primary ossification centers of the basioccipital (B), basisphenoid (BS), presphenoid (PS), frontal (F), nasal (N), maxillary (M), and vomer (V) bones. Refer to Appendix A for original, unmodified figure* (Scott, 1958)

Bands of cartilage, otherwise known as synchondroses, remain between these ossified bones of the cranial base and represent active growth centers until completely fused during development. These cranial base synchondroses are exceptionally important in the prenatal and postnatal growth of the craniofacial skeleton. Timing of the ossification and fusion of the cranial base synchondroses can be investigated and exploited as markers of skeletal development and maturity.

Midline Cartilaginous Synchondroses of the Cranial Base

The three primary growth-related midline cranial base synchondroses (Figure 2.2) that separate the bones of the cranial base are:

1. Intersphenoid synchondrosis
2. Spheno-ethmoidal synchondrosis
3. Spheno-occipital synchondrosis

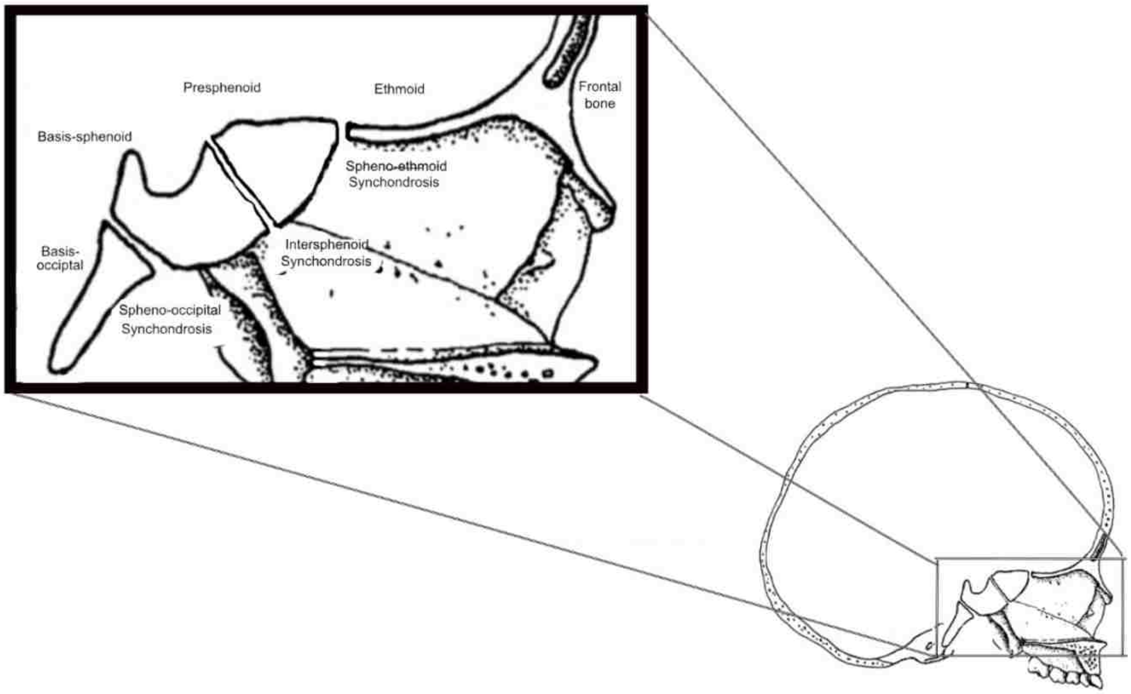


Figure 2.2 *Midsagittal View of the Cranial Base Synchondroses. Refer to Appendix B for original, unmodified figure* (Melsen, 1969)

The intersphenoid synchondrosis (ISS) separates the presphenoid and basisphenoid bones until fused. It fuses near the time birth and therefore does not contribute significantly to the postnatal growth of the cranial base (Madeline & Elster, 1995).

The spheno-ethmoidal synchondrosis (SES) separates the presphenoid and ethmoid bones until fused. Endochondral ossification here contributes to growth of the cranial base through approximately seven years of age in humans (Scott, 1958).

The spheno-occipital synchondrosis (SOS) separates the basisphenoid and basioccipital bones and is located inferior to the hypophyseal fossa and superoanterior to the foramen magnum (Bassed et al., 2010). It represents a major postnatal craniofacial growth mechanism as it is the last of the cranial base synchondroses to ossify and terminate growth (Scott, 1958). Morphologically, the SOS resembles a two-sided epiphyseal cartilage and fusion commences on the endocranial surface and proceeds ectocranially until fusion is complete (Scott, 1958; Powell & Brodie, 1963). There is, however, still considerable differences reported in the literature regarding the timing of fusion of the SOS in different global populations.

Visualization Techniques for the Spheno-Occipital Synchondrosis

Visualization of SOS fusion has been performed with a variety of techniques, the most common including direction inspection on dry bone samples (macroscopic), conventional radiographic imaging, conventional computed tomography scans (CT), and the more technologically advanced cone-beam computed tomography scans (CBCT) (Coqueugniot & Weaver, 2007; Shirley & Jantz, 2011; Mahon et al., 2017; Powell & Brodie, 1963; Madeline & Elster, 1995; Okamoto et al., 1996; El-Sheikh & Ramadan, 2006; Bassed et al., 2010; Franklin & Flavel, 2014; Can et al., 2014; Lottering et al., 2015; Sinanoglu et al., 2016; Alhazmi et al., 2017). Macroscopic visualization is necessarily limited to post-mortem analysis only. Age estimates of living individuals, however, have to be more accurate than of decedents. Therefore, the range of suitable visualization techniques is limited to conventional radiographic imaging or conventional CT or CBCT scans (Schmeling et al., 2007).

Conventional two-dimensional radiographic imaging is both inexpensive and attainable and has historically been useful for determining the closure of the SOS. Two-dimensional imaging has the significant disadvantages of superimposition, magnification, and low resolution. These limitations introduce error with structure identification which limits the use of conventional radiographic technique in visualization, particularly of midline structures like the SOS.

Compared to two-dimensional radiographic imaging, the use of conventional CT scans is reported to be more accurate and offers an appropriate, reliable, and more representative source of contemporary population-specific data (Franklin & Flavel, 2014; Can et al., 2014; Bassed et al., 2010). Conventional CT scans offer the advantages of three-dimensional assessment and high-resolution of the region of interest. The reported disadvantages of conventional CT scan investigations, however, include cost, attainability, and radiation exposure (Can et al., 2014). The comparatively recent advent of cone-beam computed tomography utilizes similar technology to conventional CT but reduces radiation exposure and cost. Therefore, this methodology offers accurate visualization and analysis of midline cranial base structures for use in assessment of subadult forensic age estimation.

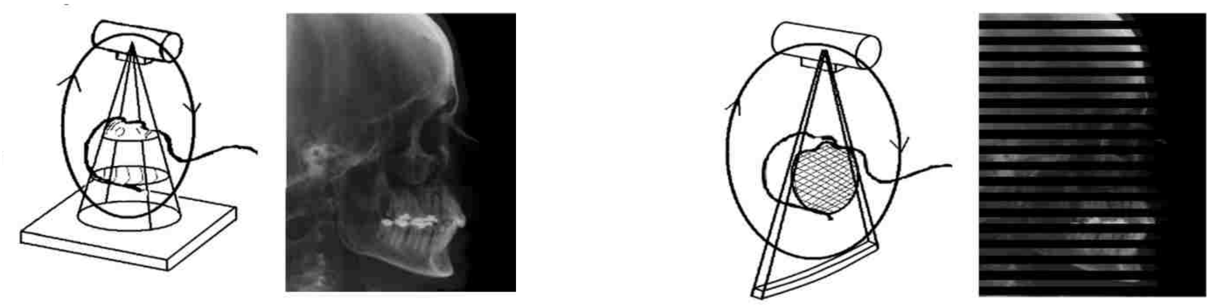
Principles of Conventional Computed Tomography (CT) and Cone-Beam Computed Tomography (CBCT)

In general, computed tomography is a three-dimensional imaging modality that is advantageous when compared to conventional two-dimensional radiography; primarily because it eliminates superimposition and structure magnification. Additionally, technological advantages of CBCT compared to conventional medical computed tomography (CT) include the following:

- It maintains high-resolution images
- It affords the patient a considerable dose reduction
- It is less costly.

The first commercially available CBCT unit was introduced in Europe in 1999 and in the United States in 2001 (Scarfe et al., 2012; Miles & Danforth, 2007). For the advantages discussed previously, CBCT technology continues to gain popularity in forensic science and dental medicine; particularly maxillofacial surgery, implantology, endodontics, periodontics, and orthodontics.

CBCT image acquisition and technique are unique to conventional medical CT in several ways. Primarily, CBCT imaging utilizes a cone-shaped beam from the radiation source (the tube), whereas conventional CT uses a fan shaped x-ray beam (Figure 2.3).



Cone-Beam CT

Fan Beam CT

Figure 2.3. *Comparison of Cone-Beam CT and Fan Beam CT (Farman & Scarfe, 2009)*

In conventional CT, the patient is positioned in the scanner unit in a supine posture with the tube on one side and the detector on the other (Scarfe et al., 2012). The patient is moved a known distance, usually between 1 mm to 1 cm, as the X-ray tube and detector scan across the patient (Scarfe et al., 2012). The fan shaped beam progressively scans the patient to acquire a series of axial plane slices that are subsequently stacked to re-create the three-dimensional structures being imaged (Mah & Hatcher, 2004). This technique results in high-resolution images at the expense of significant radiation doses. For example, a conventional CT scan for a maxillary implant site evaluation can expose a patient to as much as 2,100 microsieverts (Miles & Danforth, 2007). This is the approximate dose equivalent to 375 panoramic images in one sitting (Miles & Danforth, 2007). It is because of this high radiation dose that conventional medical CT is not a reasonable radiologic methodology for use in dental medical practice where radiographic analyses are conducted on a more regular basis.

However, CBCT scanners utilize a cone-shaped beam and an image sensor system to provide multiple transmission images that are directly integrated to re-create volumetric information (Abramovitch & Rice, 2014). The patient is positioned in an upright seated or standing posture while the CBCT unit scans the patient in a circular path around the vertical axis of the head. Multiple exposures are taken at fixed intervals along the rotational arc with each exposure creating a basis image (Abramovitch & Rice, 2014). A complete set of basis images are collected upon completion of the rotational scan and then combined to form the "projection data" or image (Abramovitch & Rice, 2014).

The direct formation of volumetric information allows for a *single* 180-degree rotation of the X-ray source, quite contrary to conventional medical CT. The latter technique creates *multiple* slices must be stacked to configure the image (Mah & Hatcher, 2004). A typical radiation exposure from a CBCT unit ranges from 40-500 microsieverts; the approximate dose equivalent to as few as four to six panoramic radiographs (Miles & Danforth, 2007). Thus, CBCT image acquisition with a single rotation sweep allows for radiation dose reduction without compromise to image resolution.

Image reconstruction with conventional CT and CBCT scanners utilizes volume elements or volume cells, also known as *voxels*. A voxel is a small cuboidal structure that represents volumetric data and is an advanced three-dimensional version of the two-dimensional picture element known as a *pixel*. Voxel sizes are inversely related to image resolution in that smaller voxel sizes yield images of higher resolution. Voxel sizes are specific to the radiographic machine parameters and vary per manufacturer.

However, conventional CT and CBCT differ in voxel dimensions. In conventional CT the voxels are *non-isotropic* rectangular cubes, where two of the sides are equal but the third side has selectable width ranging from 1.0 mm to ≥1.0 cm (Miles & Danforth, 2007). Conversely, CBCT scanners use *isotropic* square cubes, where all three sides have equal dimensions of a routine range within 0.07 mm to 0.25 mm. These figures are *at least* four times smaller than a *non-isotropic* conventional CT voxel on each side (Farman & Scarfe, 2009). This notable reduction in voxel size with CBCT scanners yields significant improvement in image resolution with CBCT technology compared to conventional CT. The high-resolution and comparatively lower radiation dose afforded by CBCT allows for unique application and provides a high degree of image accuracy with superior visualization of craniofacial anatomy, including midline structures like the spheno-occipital synchondrosis.

Morphological Scoring of Spheno-Occipital Synchondrosis Fusion

Review of the literature yielded numerous articles that utilize various visualization and imaging techniques in order to classify the morphological fusion pattern of the SOS. These articles can be very broadly categorized by the scoring scheme utilized to assign a fusion stage to the developing synchondrosis:

- Three-stages of fusion
- Four-stages of fusion
- Five-stages of fusion
- Six-stages of fusion

All scoring systems distinguish the endocranial to ectocranial fusion progression but differ in the extent of detail used to describe the morphological closure pattern.

The three-stage scoring system is the most simplified and is commonly used in macroscopic bone analyses (Shirley & Jantz, 2011; Coqueugniot & Weaver, 2007; Mahon et al., 2017). This method involves the survey of either the endocranial or ectocranial border. Studies involving skeletonized remains most commonly score the synchondrosis from the ectocranial surface whereas those utilizing cadavers routinely score from the endocranial surface.

Complete fusion in an ectocranial assessment yields a completely fused synchondrosis given that fusion proceeds from the endocranial border and terminates on the ectocranial border. Assignment of complete fusion in an endocranial assay, however, does not yield a completely fused synchondrosis and does not indicate to what degree the synchondrosis is fused. The incongruence between which border is scored during analysis can affect the age estimation results; especially if applied in an improper context.

Caution must be exercised when evaluating and comparing the results of macroscopic studies as evidenced by the slight but critical variability in the use of the three-stage scoring scheme. For consistency, only relevant macroscopic studies that involve analysis of the ectocranial border will be reviewed for this project. Ectocranial border assessment offers the ability to compare the results of this study with those of other designs utilizing different visualization methods but to identify complete fusion of the synchondrosis.

The first stage in the three-stage scheme analyzing the ectocranial surface represents unfused or completely open synchondrosis. The second stage identifies fusion activity as the gap begins to fill with bone but has some form of discontinuity between the edges, thereby classifying stage two as closing, fusing, or partially united. The third and final stage is assigned to a completely filled gap thereby classifying stage three as closed, fused, or completely united. Fusion stage assignment via macroscopic analysis furthermore lacks the imaging acuity afforded by radiographic techniques and presents difficulty in the ability to identify discrete fusion edges or cortication of fusion borders when only viewed visually.

Conversely, radiographic-specific analyses employ four through six-stage scoring schemes to assess SOS fusion status. Powell and Brodie initially introduced a six-stage scoring scheme in their early laminographic investigation of SOS closure (Powell & Brodie, 1963). The six-stages describe progressive closure as percentages (completely open, superior border closed, superior one-fourth closed, superior one-half closed, superior three-fourths closed, complete obliteration) (Powell & Brodie, 1963).

Initial conventional CT examinations were performed by Madeline and Elster in 1995. They introduced a five-stage scoring system in order to provide CT standards to describe the pattern of SOS closure (Madeline & Elster, 1995):

1. Margins are clearly separated.
2. Clear separation noted along most sections, but some areas are indistinct and suggestive of bony bridging.
3. Area of fusion noted across a portion of the SOS.
4. Complete fusion with remnant sclerotic margins.
5. Complete closure with no apparent vestige remaining.

Bassed et al. in 2010 also introduced and employed a five-stage scoring scheme. They refined Powell and Brodie's original six-stage scheme to specifically incorporate a new growth stage in order to account for the ability to visualize a fusion scar with high-resolution CT images (Powell & Brodie, 1963; Bassed et al., 2010):

1. Completely open or unfused.
2. Fusion of superior border, patency of remainder of synchondrosis.
3. Half the length is closed.
4. Complete fusion, site is still visible by way of fusion scar.
5. Complete obliteration of site with appearance of normal bone throughout.

Though similar in initial and final stage description, the five-stage scoring schemes differ significantly in determination of intermediate stages of fusion progression. This would become particularly important when comparing results among age estimation studies that utilize different morphological approaches. However, consistent between Madeline and Elster and Bassed et al. five-stage formats is that scoring is limited to midsagittal plane (Madeline & Elster, 1995; Bassed et al., 2010).

A four-stage system was proposed by Franklin and Flavel in 2014 (Franklin & Flavel, 2014). They modified the five-stage scheme from Bassed et al. in order to decrease subjectivity;

especially in cases where an individual could be classified as being between two stages (Bassed et al., 2010; Franklin & Flavel, 2014):

1. Unfused: completely open with no bone present in gap.
2. Fusing endocranially: no more than half the length fused.
3. Fusing ectocranially: greater than half length fused, ectocranial border unfused.
4. Complete fusion: appearance of normal bone throughout, a fusion scar may be present.

Also similar to the five-stage scheme, scoring with this technique is limited to midsagittal plane.

A more morphologically detailed six-stage scoring scheme was recently introduced and advocated by Lottering et al. in an investigation using conventional CT (Lottering et al., 2015). This development was based on integration of morphological fusion features such as ossification nodules (Lottering et al., 2015). Unique to the Lottering et al. six-stage scheme is the evaluation of SOS fusion in the axial plane in addition to the midsagittal plane. This six-stage scheme incorporates both the axial and midsagittal views to identify central and peripheral ossification nodules that mature into bony bridges; characterizing fusion stages three through five in this scheme (Lottering et al., 2015). Stage six is used to classify complete fusion from the endocranial through the ectocranial border:

1. Open: absence of calcification in the joint space.
2. Commencing fusion: fusion has commenced on one or both of the lateral margins
3. Appearance of median ossification nodule: an isolated midsagittal hyper dense nodule. Fusion is evident on both right and left lateral margins. Bony bridging is not evident on median sagittal slice.
4. Active fusion: high density of ossification nodules in joint space. Bony bridging between ossification nodule and adjacent borders evident. Less than half the length of the synchondrosis length is fused.
5. Advanced fusion: greater than half the length of the synchondrosis is fused. Complete bony bridging of the ectocranial border is not evident. Ossification nodules

demonstrate integration with adjacent bony margins as indicated by diminished radiopacity of the nodules.

6. Complete fusion: no radiolucent notches/remnants along the endo- and ectocranial margins. A vestige may be present and should be examined for radiolucent gaps.

Lottering et al. particularly advocated the utilization of this extended six-stage scoring system for morphological accuracy and to reduce the amount of overlap between consecutive stages. (Lottering et al., 2015).

As evidenced by the variability in fusion scoring systems, one can only accurately compare intermediate fusion stages of identical description between different studies. It would be invalid, for example, to compare Stage 2 across all studies that employ different scoring schemes. Fusion stage assignments vary among different studies since they depend on the visualization method employed. Additionally, specific to macroscopic studies, fusion stage is governed by whether the endocranial or ectocranial border is scored.

Statistical Analyses in Age Estimation with Spheno-Occipital Synchondrosis Fusion

Various statistical approaches have been employed by different researchers for age estimation from closure of the SOS. This could also play an important role regarding the observed variability in time of synchondrosis closure across population studies.

A majority of the previous studies evaluated the correlation between stages of SOS fusion and chronologic age using Spearman's correlation ratio (Alhazmi et al., 2017; Mahon et al., 2017; Sinanoglu et al., 2016; Franklin & Flavel, 2014; Can et al., 2014; Shirley & Jantz, 2011). Regression parameters were utilized to predict average age at each closure stage for both sexes taking age as the dependent variable and stage of SOS closure as the independent variable (Alhazmi et al., 2017; Sinanoglu et al., 2016; Can et al., 2014).

However, critics of these statistical methods suggest that scoring method, sample size, and age range are important limitations of studies investigating age estimation from SOS closure (Shirley & Jantz, 2011). Franklin and Flavel and Shirley and Jantz both investigated the relationship between age and fusion stage utilizing Spearman correlation but employed transition analysis probability distribution to determine the average age individuals transition between the stages (Franklin & Flavel, 2014; Shirley & Jantz, 2011).

Lottering et al. also used the Bayesian statistical approach to model the transition from one development stage to another (Lottering et al., 2015). They reported that the advantage of this statistical approach is that it is less disposed to problems presented by developmental outliers and sample size constraints than age ranges obtained by percentile methods (Lottering et al., 2015).

Age Estimation using Spheno-Occipital Synchondrosis Fusion with Macroscopic Evaluation of the Ectocranial Border

A review of the literature found three relevant contemporary macroscopic studies that examined the relationship between chronological age and SOS fusion in various global populations. On the basis of direct inspection of 162 skeletonized remains in an American population, Shirley and Jantz reported a strong positive correlation between age and fusion stage for both males and females. This study utilized maximum likelihood estimates from transition analysis to predict age ranges for unfused, fusing, and fused ectocranial surface of the SOS (Shirley & Jantz, 2011). Shirley and Jantz reported that the 95% prediction intervals can be used to assign age when ectocranial fusion of the SOS is the sole parameter being evaluated (Shirley & Jantz, 2011). With this analysis they concluded that males transition from closing to closed SOS at 17.4 years of age and females at 13.7 years (Shirley & Jantz, 2011).

In an effort to develop aging standards for a Portuguese population, Coqueugniot and Weaver focused on the maturation of the infracranial skeleton (Coqueugniot & Weaver, 2007). Additionally, they explored and presented data on the closure of the SOS via inspection of the ectocranial surface from an identified human skeletal collection of 137 individuals (Coqueugniot & Weaver, 2007). Although no clear discussion was provided regarding statistical analysis, they presented age ranges for each of the three developmental stages (unfused, fusing, and fused). They reported the youngest age in which complete SOS fusion was observed as 19 years and 14 years for males and females, respectively (Coqueugniot & Weaver, 2007).

A South African Black sample of 147 skeletons was more recently studied by Mahon et al. (Mahon et al., 2017). Reported results indicated that complete fusion of the SOS occurred slightly later in this population compared to those of other studies. Additionally, unlike previous reports, they found no significant differences between males and females for the age at which ectocranial fusion commences. Results in this study generated a mean age of 16.7(±1.2) years in males and 16.2(±2.9) years in females (Mahon et al., 2017). Correlation between age and fusion stage was determined to be strong for males and moderate for females. In both male and female populations, complete SOS fusion was noted in 100% of all individuals >20 years of age (Mahon et al., 2017).

Although direct inspection methods are inherently used in postmortem applications and are of particular value in forensic contexts for human identification, results from macroscopic analyses are not the most comparable or applicable when considering age estimation of the living. However, even though there is a lack of visual acuity in identifying bony changes in gross anatomical samples, the results of macroscopic studies serve to advance understanding of growth and development of the human skeleton (Coqueugniot & Weaver, 2007). Macroscopic analyses can be used to establish standards in forensic postmortem settings for age at death determination, especially in cases of unidentified human remains.

Age Estimation using Spheno-Occipital Synchondrosis Fusion with Conventional Radiographic Technique

One of the initial robust radiographic analyses of the SOS was performed by Powell and Brodie on 398 midsagittal laminagrams of an American population in 1963 (Powell & Brodie, 1963). Prior to this radiographic analysis, findings on human cranial growth and development were primarily derived from dried skull material. Their principal goal was to appraise the timing of SOS closure and study growth and development of the posterior cranial base. They reported age at SOS closure in American males between 13-16 years, and between 11-14 years in American females (Powell & Brodie, 1963).

This research was the first to describe sex differences in the timing of SOS closure. Prior research conducted by Irwin in 1960 utilized midsagittal tomography but did not divide the study population according to sex (Irwin, 1960). Instead, Irwin reported SOS closure age as a mean for the entire male and female population and therefore results are not relevant to contemporary knowledge of age estimation efforts given the currently understood sexual dimorphic trends.

A radiographic examination of the SOS utilizing cephalometric laminagraphy was also performed by Konie in 1964 (Konie, 1964). The purpose of this research was to investigate the correlation between the maturation of the hand and wrist bones and SOS closure in an American population. Therefore, it did not directly report statistics for age estimation efforts utilizing the SOS (Konie, 1964). Konie did, however, report a positive correlation between SOS closure and maturation of the hand and wrist bones. That progression from initial ossification of the synchondrosis to its complete obliteration required more time in males than females (Konie, 1964).

Conventional radiographic analysis has played an important role in the evolution of understanding the development of the spheno-occipital synchondrosis. Nevertheless, conventional radiographic techniques are increasingly less applicable in the appraisal of cranial midline structures. The availability and improved visualization capacity of three-dimensional imaging techniques has contributed to this fact.

Age Estimation using Spheno-Occipital Synchondrosis Fusion with Conventional Computed Tomography Evaluation

Madeline and Elster were among the first to employ conventional CT images in evaluation of SOS closure in the human chondrocranium (Madeline & Elster, 1995). The purpose of their research was to chronicle the development of human skull sutures and synchondroses throughout childhood and provide CT standards for timing and pattern of closure. They examined 189 images of an American population and found that the SOS remained partially open into the teenage years. They reported that the 95th percentile, in which 95% of the examined spheno-occipital synchondroses attained complete fusion, was 18 years of age in males and 16 years in females (Madeline & Elster, 1995).

In 1996, Okamoto et al. used CT analysis to study SOS development in a Japanese population of 253 individuals (Okamoto et al., 1996). Unlike other CT studies, they did not classify SOS fusion according to stage, but only assessed three points of fusion (Madeline & Elster, 1995; El-Sheikh & Ramadan, 2006; Can et al., 2014; Bassed et al., 2010; Franklin & Flavel, 2014; Lottering et al., 2015):

- Persistence of SOS
- Location and time of appearance of ossification centers
- Vestige of SOS after fusion.

They were among the first to describe that ossification centers were seen to develop initially within the SOS. Fusion was reported in males by age 13 and in females by age 12. There was no persistence of open SOS after the age of 13 in either sex. Even though statistical analyses were not reported for this study, Okamoto et al. were the first to evaluate a Japanese population and observe complete fusion at this early age (Okamoto et al., 1996).

A sample of 207 CTs from an Arabian Gulf population was investigated by El-Sheikh and Ramadan in 2006 in an attempt to establish a local standard for age of SOS fusion (El-Sheikh & Ramadan, 2006). In the 2006 proceedings of the American Academy of Forensic Science meeting, their abstract reported that complete closure of the SOS was observed as early as 12 years in males and 11 years in females. Complete closure was observed in all males >18 years and females >16 years (El-Sheikh & Ramadan, 2006).

The usefulness of SOS fusion degree for age estimation in a Turkish population was examined by Can et al. in 2014 (Can et al., 2014). They evaluated CT scans of 638 subjects and reported mean age of complete fusion, with a scar still visible, as 20.34 (±3.4) years and 18.17 (±3.5) years for males and females, respectively (Can et al., 2014).

Several regional intrapopulation CT analyses of modern Australians have been performed by Bassed et al., Franklin and Flavel, and Lottering et al. (Bassed et al., 2010; Franklin & Flavel, 2014; Lottering et al., 2015). Populations from the states of Victoria, Queensland, and Western Australia were evaluated. Bassed et al. studied Victorian Australians and observed that closure was complete by the age of 17 years in both males and females (Bassed et al., 2010). Franklin and Flavel, however, evaluated Western Australians and reported that, on average, complete fusion occurred at 19.83 (±2.94) years in males and 18.62 (±3.55) years in females (Franklin & Flavel, 2014). In their study, Lottering et al. reported that Queensland, Australian males >16.3

years of age and females ≥13.8 years old achieved complete fusion (Lottering et al., 2015). Using Bayesian transition analysis, they also reported that males are most likely to transition to complete fusion at 15.6 years and females at 13.1 years (Lottering et al., 2015). Comparing the results of these studies of modern Australians, Lottering et al. proposed that their inconsistency suggests intrapopulation variation among the major geographic regions of Australia (Lottering et al., 2015).

Age Estimation using Spheno-Occipital Synchondrosis Fusion with Cone-Beam Computed Tomography

CBCT technology has become increasingly popular in recent years in the analysis of SOS fusion. Review of the literature yielded four contemporary articles that examine SOS fusion with CBCT.

Sinanoglu et al. were the first to utilize CBCT images to investigate the relationship between SOS fusion and chronological age (Sinanoglu et al., 2016). They evaluated 238 CBCT images (90 males and 148 females between 7-25 years old) for SOS fusion in a Turkish population (Sinanoglu et al, 2016). Complete fusion of the SOS at mean ages of 20.02 years (±4.10) and 18.21 years (±3.77) for males and females, respectively was reported (Sinanoglu et al., 2016). The mean age for totally open SOS was 10 years of age for both sexes.

In a recent CBCT study performed in an American population, Alhazmi et al. evaluated 741 CBCT images (361 males and 380 females between 6-20 years old) and found that the SOS was completely fused at mean ages of 16.41 years (±1.40) and 15.25 years (±1.89) for males and females, respectively (Alhazmi et al., 2017). Mean ages for complete fusion in this study were lower than those of the Turkish population evaluated by Sinanoglu et al. (Sinanoglu et al., 2016).

Although their primary aim was not to investigate the relationship between SOS fusion and chronological age, two other studies were reviewed. Kocasarac et al. performed a combination study with panoramic and lateral cephalometric radiography with CBCT for a Turkish population (Kocasarac et al., 2017). The aim of the study was to evaluate skeletal maturity from individual assessment of 3rd molar mineralization, cervical vertebrae maturation, and SOS fusion. As part of their constituent analysis, resultant descriptive statistics for SOS fusion stages and chronological age indicated a mean age for complete SOS fusion of 18.92 years (±3.90) and 18.02 years (±3.63) for males and females, respectively (Kocasarac et al., 2017).

Fernandez-Perez et al. used CBCT images of an American population to correlate SOS fusion to cervical vertebrae maturational stage and found a strong significant correlation ($r=0.89$) (Fernandez-Perez et al., 2016). Age estimation using SOS fusion was not the principal aim of their study. Therefore, they did not report ages at SOS fusion stage. However, this study does serve as a guide for future CBCT and SOS research in that SOS fusion could be used to indicate skeletal maturation status. They concluded that SOS fusion stage is a reasonable indicator of growth maturation (Fernandez-Perez et al., 2016). This is obviously of particular value in dental medicine for future orthodontic and dental implant treatment planning.

Chapter 3: Methodology

The following protocol, #1104233-1, entitled "Analysis of spheno-occipital synchondrosis (SOS) fusion in a contemporary Southern Nevada subadult Hispanic population using archival cone-beam computerized tomography (CBCT) images" was reviewed by the Office of Research Integrity at UNLV, and deemed exempt from IRB review (Appendix C).

Sampling Protocol

This study retrospectively examined 374 anonymized CBCT images (166 males and 208 females) with an age range of 8-20 years. The CBCT images of self-identified Hispanic patients were isolated from the digital patient database of the Department of Orthodontics, University of Nevada - Las Vegas School of Dental Medicine (UNLV SDM). All scans were taken by trained radiology technicians with the CBCT machine (CB MercuRay, Hitachi Medical Corp) between 2010 and 2018 on pre-, active, and post-orthodontic patients. Scans were made using a large field of view, with a matrix of 512x512, voxel size of 0.38mm^3,100 kV, 15mA, and exposure time of 10 seconds.

All images were de-identified to create a database of Hispanic anonymized images. Pertinent scan details including race, sex, date of birth, and date of CBCT exposure were recorded in a Microsoft Excel spreadsheet. The exclusion parameters applied to the Hispanic database included:

- Patients with a medical history significant for developmental and/or syndromic disorders affecting craniofacial development (e.g.: cleft lip and/or cleft palate, Down, Treacher Collins, Crouzon, Apert, and Pfeiffer syndromes)
- All CBCT scans with distortion, movement artifact, or abnormal morphologies resulting from pathology or trauma.

Image data was stored in Digital Imaging and Communications in Medicine (DICOM) format on a UNLV SDM server with password protected access.

Visualization and Scoring of Spheno-Occipital Synchondrosis Fusion

Volumetric renderings of CBCT scans were converted from DICOM to INV format and analyzed with InvivoDental version 5.4.1 software (Anatomage, San Jose, CA). The 3D reconstruction was visualized in a dark room on a 24-inch screen monitor with display resolution of 1920x1080 pixels. Following the method used in Alhazmi et al., axial and coronal views were used to orient the cranium in a standardized position (Figure 3.1) (Alhazmi et al., 2017). This allowed for assessment of the fusion status in the midsagittal plane. The image settings were adjusted on the software to *mild sharpen* and 1mm slice thickness was chosen.

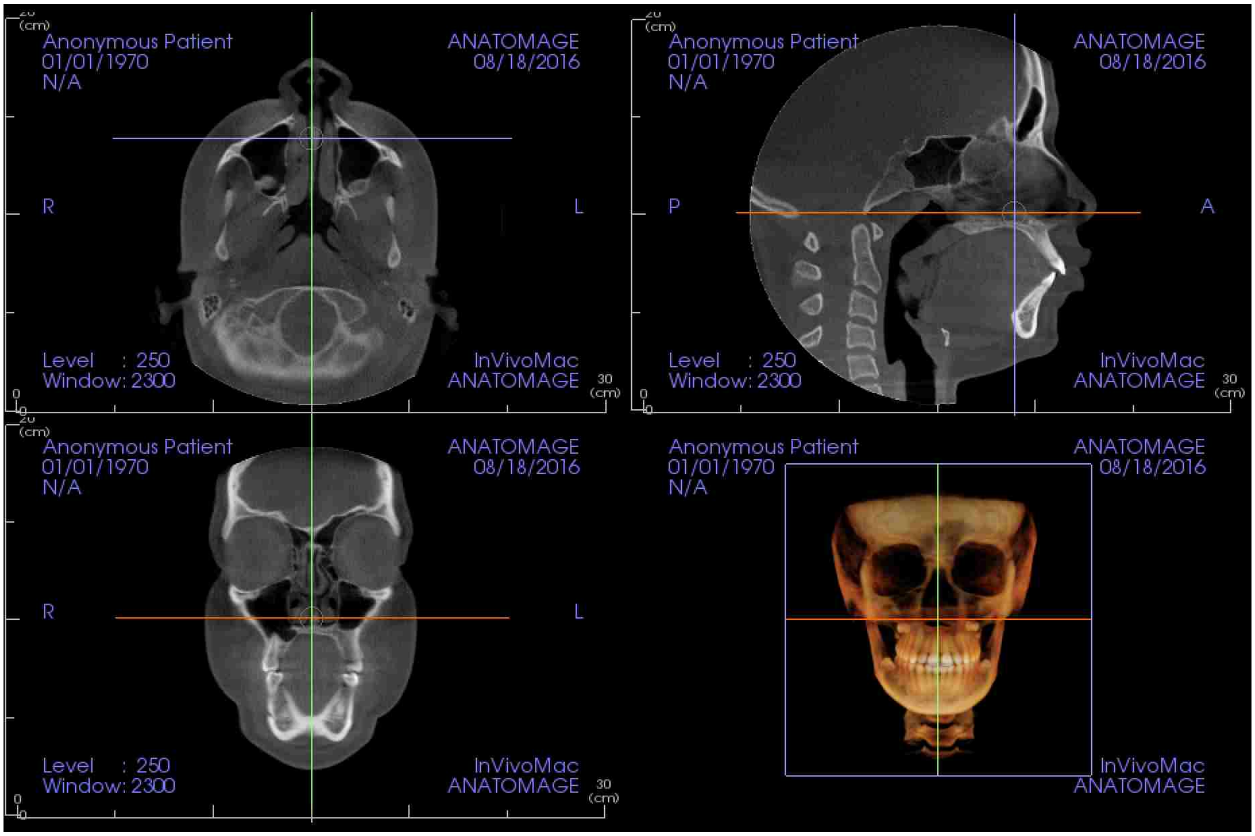


Figure 3.1 *Orientation of CBCT Images for Analysis in Midsagittal Plane*

Spheno-occipital synchondrosis fusion stage assignment was performed by a single observer (M.B.) for the entire sample. A second observer (H.B.) assigned fusion stage for ten percent of the sample. The length of the SOS was measured from the endocranial to the ectocranial border using the linear measuring tool in InVivoDental^{TM}. The best fit line was created with segmental measurements when one straight line failed to characterize the morphology of the SOS (Figure 3.2). Fusion length was measured with the same technique when necessary.

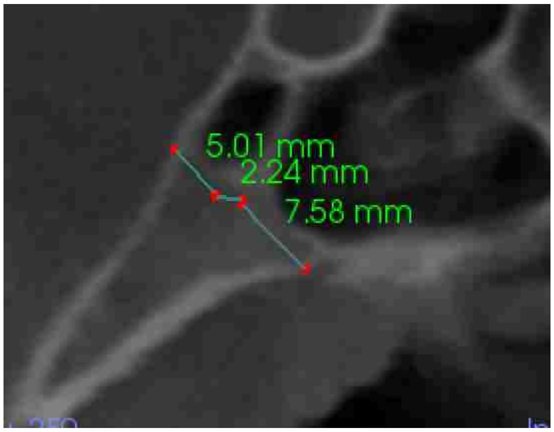


Figure 3.2 *Incremental Measurements*

The millimeter lengths of the SOS and fusion degree were recorded for each anonymized patient number in a Microsoft Excel spreadsheet. Percentage of fusion was calculated as length fused divided by length of synchondrosis:

$$\frac{\text{Length Fused (L Fu)}}{\text{Length of Synchondrosis (L Sy)}} = \% \text{ of } \textit{Fusion } (\% \textit{ Fu}).$$

Results were scored by the four-stage scoring system adopted by Franklin and Flavel (Table 3.1 and Figure 3.3) (Franklin & Flavel, 2014).

Table 3.1
Definition of the Scoring System used for Assigning Fusion Stage to the SOS

Stage		**Description**
0	Unfused	Completely open with no evidence of fusion between the basilar portion of the occipital and the sphenoid, no bone present in the gap.
1	Fusing endocranially	No more than half the length of the synchondrosis is fused proceeding endo- to ectocranially.
2	Fusing ectocranially	Greater than half the length of the synchondrosis is fused, the ectocranial border remains unfused.
3	Complete fusion	Completely fused with the appearance of normal bone throughout, a fusion scar may be present

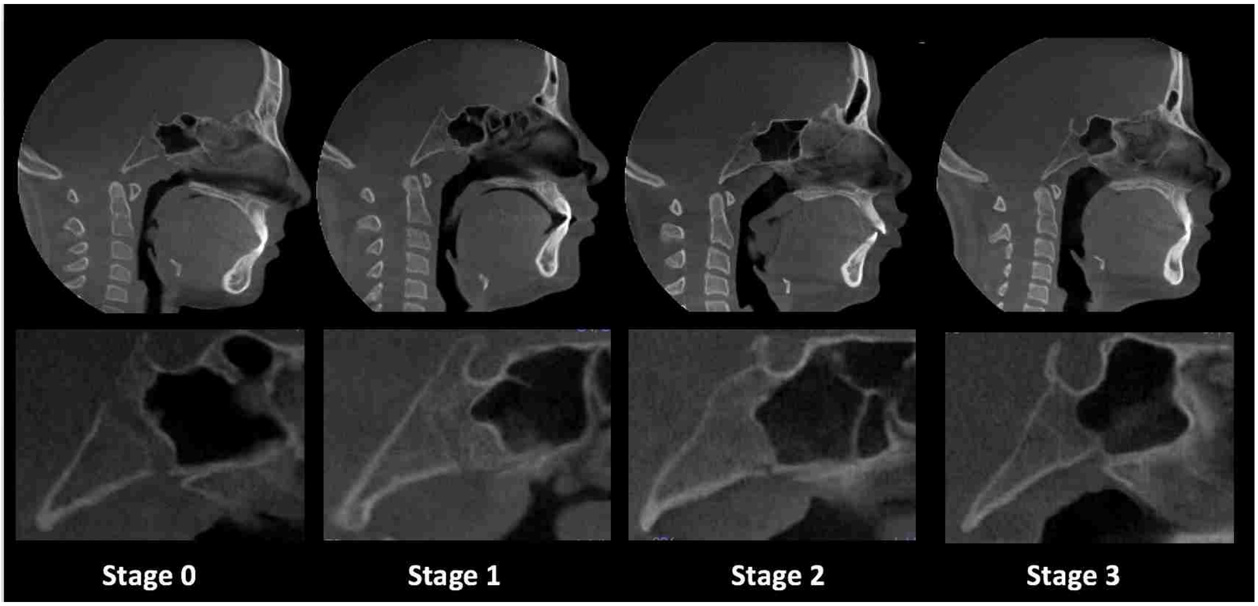


Figure 3.3 *Midsagittal CBCT Images Illustrating SOS Fusion Stage*

Statistics

Statistical analysis was performed using the data from the Microsoft Excel spreadsheet which was transferred into Statistical Package for the Social Sciences (SPSS) for Windows version 24.0. Inter- and intraobserver error were calculated with Cohen's Kappa coefficient.

Interobserver error calculation was based on the fusion scores assigned by observer H.B. to ten percent of the entire sample. Intraobserver error calculation was based on repeat assessment of 60 randomly selected scans, viewed under the same settings and viewing conditions, with a two-month interval between the first and second scoring sessions. All scoring assignments were made without observer knowledge of true chronological age and sex of the individuals.

Descriptive statistics were calculated to evaluate the mean and median ages for each fusion stage in addition to the ninety-five percent confidence interval for the mean age. Spearman correlation was used to assess the correlation between age and fusion stage. Linear regression parameters were used taking age as the dependent variable and degree of SOS fusion as the independent variable. Analysis of variance (ANOVA) was used to analyze the differences in group means of the sample. For comparison of mean age between fusion stages, the 1-way ANOVA test for multiple comparisons with the post hoc Bonferroni adjustment was used. All P values less than 0.05 were defined as significant. Linear regression and ANOVA analyses were performed separately for males and females in order to explore sexual dimorphism.

Chapter 4: Results

Age and Sex Distribution

The age distribution of the 374 individuals evaluated for this study ranged from 8-20 years with 166 males and 208 females. Figure 4.1 shows the distribution of age by sex.

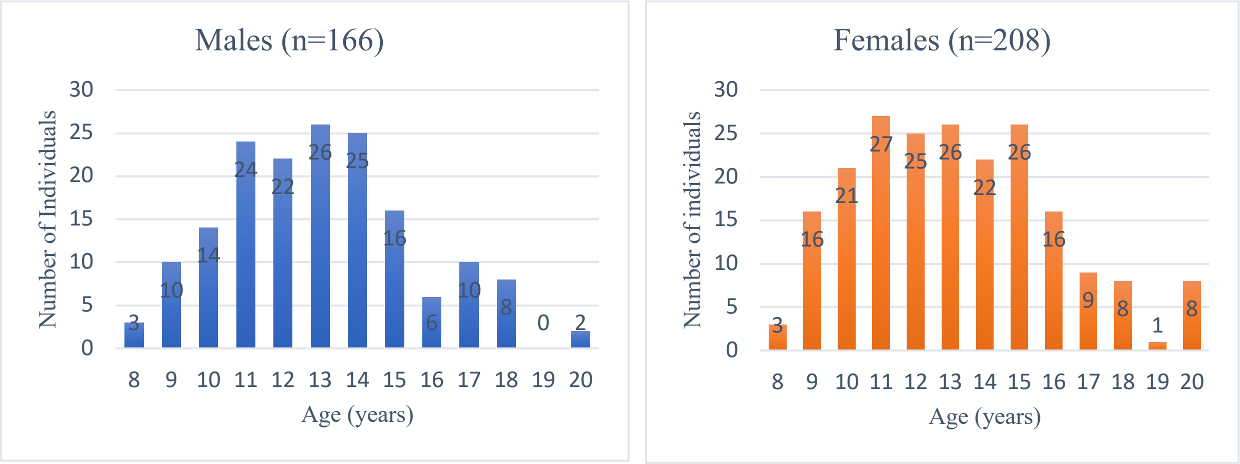


Figure 4.1 *Sample Distribution of Age by Sex*

Intraobserver and Interobserver Error Rate

Intra- and interobserver error testing were performed in order to evaluate the degree of reliability for methods employed in this study. Intraobserver error testing was performed on 60 (24 males, 36 females) randomly selected CBCT scans two months after initial scoring was completed. Interobserver error testing was performed on 37 (13 males, 24 females) of the randomly selected 60 CBCT scans subjected to intraobserver analysis. Cohen's Kappa coefficient was calculated for intra- and interobserver error rates.

A Kappa measurement of agreement score of 1 indicates a perfect correlation, whereas a score of 0 indicates no correlation. The Kappa measure of agreement between the first and second scoring sessions for the single observer (M.B.) for 60 images was 0.977 (p <0.001) (Table 4.1). The Kappa measure of agreement between the two observers (M.B. and H.B.) was 0.890 (p <0.001) (Table 4.1). There were no situations where fusion stage assignment by a second observer or repeated assessment by a single observer differed by more than one stage.

Table 4.1
Intra- and Interobserver Reliability with Cohen's Kappa Coefficient

	Intraobserver	Interobserver
	M.B.	H.B.
Number of cases	60	37
Kappa measurement of agreement	0.977	0.890
Significance	p <0.001	p <0.001

Research Question 1

Does the degree of fusion of the spheno-occipital synchondrosis, as visualized on midsagittal CBCT images, correlate with chronological age in a male/female subadult Hispanic population of Southern Nevada?

Hypothesis: The degree of spheno-occipital synchondrosis fusion correlates with chronological age and is statistically significant ($p < 0.05$).

Null Hypothesis: There is no statistically significant correlation between degree of spheno-occipital synchondrosis fusion and chronological age for this population. *The null hypothesis was rejected.* Spearman correlation was performed and statistically significant values ($p < 0.001$) for the correlation between age and fusion stage were observed. Table 4.2 presents the strong positive correlation between age and fusion stage for the male and female subadult Hispanic population of Southern Nevada. It is apparent that the progressive closure is correlated with chronological age for male and female subadult Hispanics of Southern Nevada.

Table 4.2
Correlation Between Age and Fusion Stage using Spearman Correlation

Spearman's rho	N	Correlation coefficient	Significance (2-tailed)
	374	0.719	$p < 0.001$

Research Question 2

Does mean age for each SOS fusion stage, as visualized on midsagittal CBCT images, demonstrate sexual dimorphism in a male/female subadult Hispanic population of Southern Nevada?

Hypothesis: Mean age for each SOS fusion stage is sexually dimorphic with females demonstrating earlier mean age for each fusion stage.

Null Hypothesis: Mean age for each SOS fusion stage is not sexually dimorphic for this population. *The null hypothesis was rejected.* Table 4.3 presents the descriptive statistics to determine mean age of for each fusion stage by sex. Females preceded males in median and mean age at each closure stage by an average of 1.78 years. Figures 4.2 and 4.3 graphically demonstrate the median and quartile ranges for the distribution of the study population by sex.

Table 4.3
Descriptive Characteristics for SOS Closure in Study Sample

Stages	Sex	n	Mean age (years)	SD	Median age	95% CI mean age	Min age-Max age
Stage 0	Male	50	10.90	1.36	11.03	10.51-11.28	8.49-14.31
	Female	16	9.86	0.90	9.65	9.38-10.34	8.48-11.99
Stage 1	Male	38	12.66	1.04	12.88	12.31-13.00	10.57-14.68
	Female	20	10.54	0.93	10.52	10.11-10.97	8.98-12.33
Stage 2	Male	26	13.87	0.90	13.86	13.51-14.24	12.41-15.58
	Female	23	11.42	1.09	11.47	10.95-11.89	9.80-13.53
Stage 3	Male	52	16.35	1.70	16.01	15.87-16.82	13.11-20.68
	Female	149	14.84	2.40	14.65	14.45-15.23	10.22-20.93

SD: standard deviation; CI: confidence interval

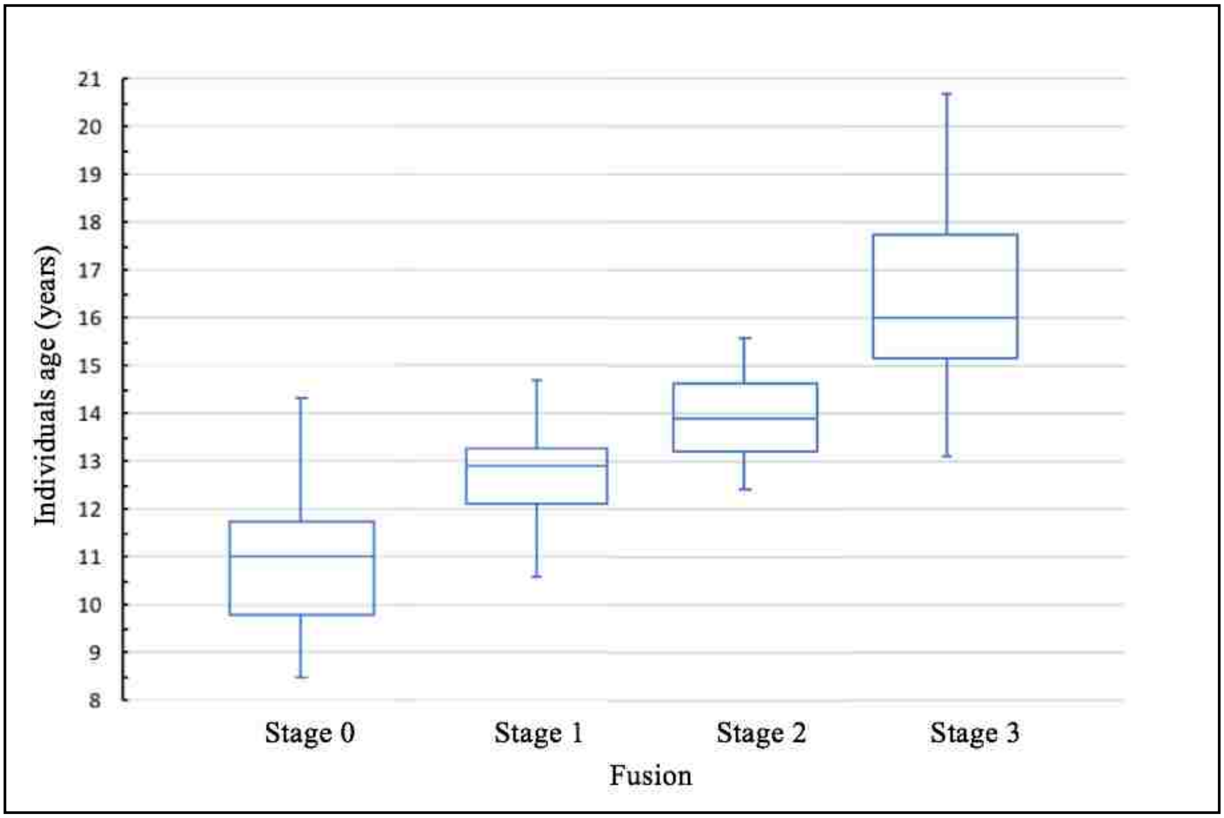


Figure 4.2 *Distribution of Males According to Age and SOS Fusion Stage (median and quartile range)*

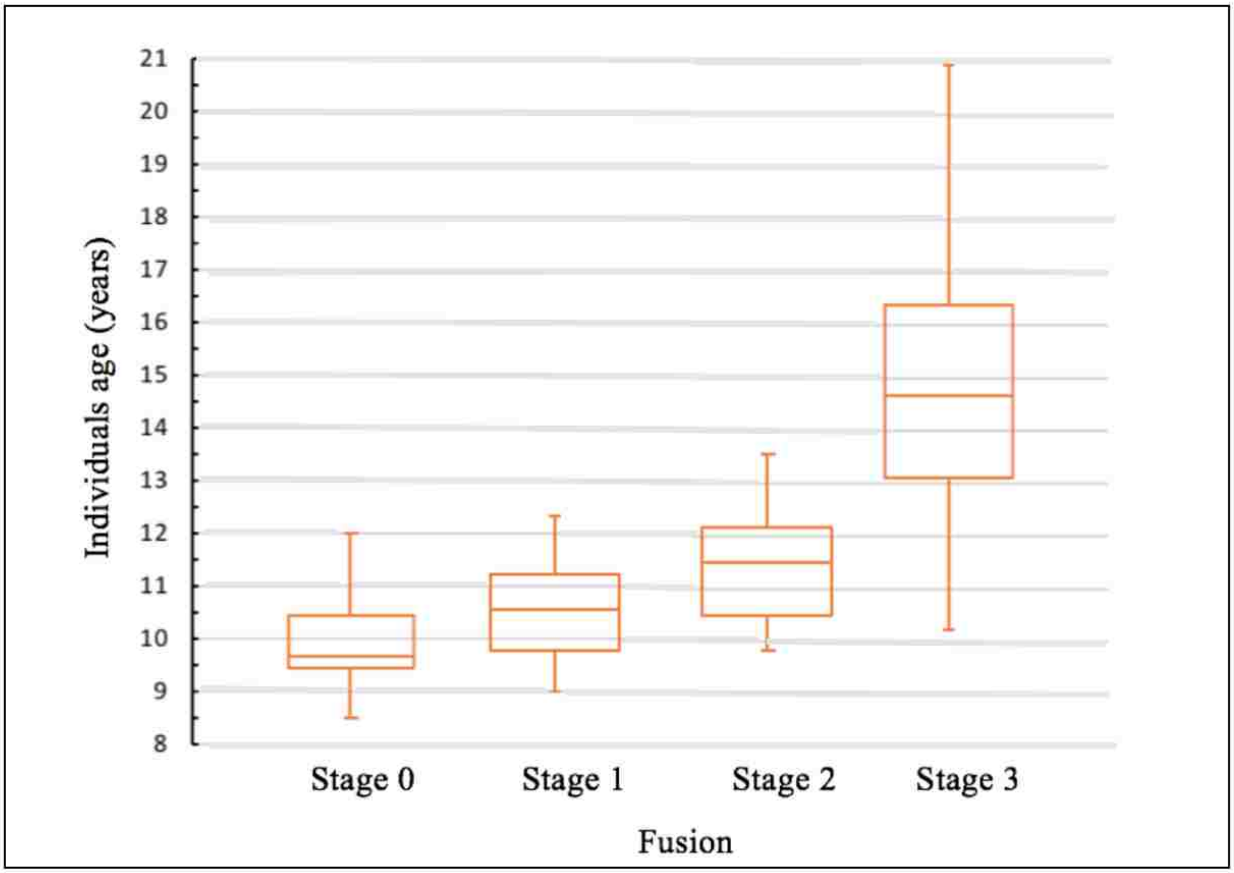


Figure 4.3 *Distribution of Females According to Age and SOS Fusion Stage (median and quartile range)*

Research Question 3

Do subadult Hispanic males of Southern Nevada demonstrate a statistically significant ($p < 0.05$) relationship between mean age and fusion stage? If so, between which stages do statistically significant differences in mean ages exist?

Hypothesis: Males have a positive correlation between age and fusion stage and demonstrate statistically significant differences ($p < 0.05$) in mean ages between all fusion stages.

Null Hypothesis: Males demonstrate no statistically significant ($p < 0.05$) correlation between age and fusion stage. *The null hypothesis was rejected.* Linear regression analysis was performed and statistically significant ($p < 0.001$) correlation values were observed. Males demonstrate a strong positive correlation ($r=0.847$) between age and fusion stage (Table 4.4). Figure 4.4 highlights the results from the linear regression analysis for males. Analysis of variance (ANOVA) was performed to analyze the differences between the mean ages per fusion stage (Table 4.5). One-way ANOVA with post hoc Bonferroni adjustment was used to determine where the significant differences occur in mean ages between fusion stages (Table 4.6). Together these analyses show subadult Hispanic males of Southern Nevada demonstrate significant difference ($p < 0.001$) between mean age and fusion stage and the differences are significant between all groups.

Table 4.4

Regression Output for SOS Fusion in Males

	Males
N (sample size)	166
F	417.64 (p <0.001)
Adjusted R^2	0.716
Standard Error of the Estimate	1.368
Pearson (*r*)	0.847

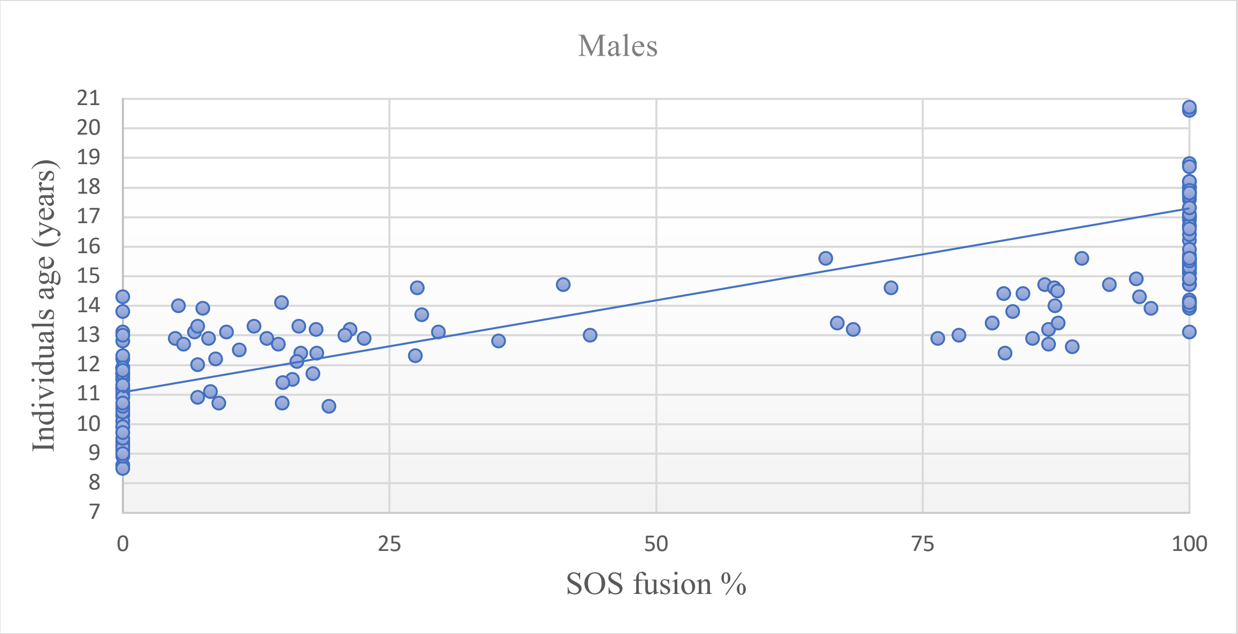


Figure 4.4 *Linear Regression Model for Males*

Table 4.5

ANOVA for Males for Difference in Mean Ages Between Fusion Stages

	Sum of Squares	df	Mean Square	F	Significance
Between groups	790.25	3	263.42	143.19	p <0.001
Within groups	298.03	162	1.84		

Table 4.6

One-way ANOVA with Post Hoc Bonferroni Adjustment for Males

Fusion Stage		Mean Difference	Std. Error	Sig. $p < 0.05$	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Fusion stage 0	Fusion stage 1	1.759	0.292	0.000	0.98	2.54
	Fusion stage 2	2.976	0.328	0.000	2.10	3.85
	Fusion stage 3	5.448	0.269	0.000	4.73	6.17
Fusion stage 1	Fusion stage 0	1.759	0.292	0.000	0.98	2.54
	Fusion stage 2	1.218	0.345	0.003	0.30	2.14
	Fusion stage 3	3.689	0.289	0.000	2.92	4.46
Fusion stage 2	Fusion stage 0	2.976	0.328	0.000	2.10	3.85
	Fusion stage 1	1.218	0.345	0.003	0.30	2.14
	Fusion stage 3	2.472	0.326	0.000	1.60	3.34
Fusion stage 3	Fusion stage 0	5.448	0.269	0.000	4.73	6.17
	Fusion stage 1	3.689	0.289	0.000	2.92	4.46
	Fusion stage 2	2.472	0.326	0.000	1.60	3.34

Research Question 4

Do subadult Hispanic females of Southern Nevada demonstrate a statistically significant ($p < 0.05$) relationship between mean age and fusion stage? If so, between which stages do statistically significant differences in mean ages exist?

Hypothesis: Females have a positive correlation between age and fusion stage and demonstrate statistically significant differences ($p < 0.05$) in mean ages between all fusion stages.

Null Hypothesis: Females demonstrate no statistically significant ($p < 0.05$) correlation between age and fusion stage. *The null hypothesis was rejected for differences in mean ages for fusion stages 0 & 3, 1 & 3, and 2 & 3. The null hypothesis was accepted for differences in mean ages for fusion stages 0 & 1, 0 & 2, and 1 & 2.* Linear regression analysis was performed and statistically significant ($p < 0.001$) values were observed. Females demonstrate a moderate positive correlation ($r=0.643$) between age and fusion stage (Table 4.7). Figure 4.5 illustrates the results from the linear regression analysis for females. ANOVA was performed to analyze the differences between the mean ages per fusion stage (Table 4.8). One-way ANOVA with post hoc Bonferroni adjustment was used to determine where the significant differences occur in mean ages between fusion stages (Table 4.9). Together these analyses show subadult Hispanic females of Southern Nevada demonstrate significant correlation ($p < 0.001$) between age and fusion stage, but the significant differences are only between the mean ages for fusion stages 0 & 3, 1 & 3, and 2 & 3.

Table 4.7

Regression Output for SOS Fusion in Females

	Females
N (sample size)	208
F	145.53 (p <0.001)
Adjusted R^2	0.411
Standard Error of the Estimate	2.171
Pearson (*r*)	0.643

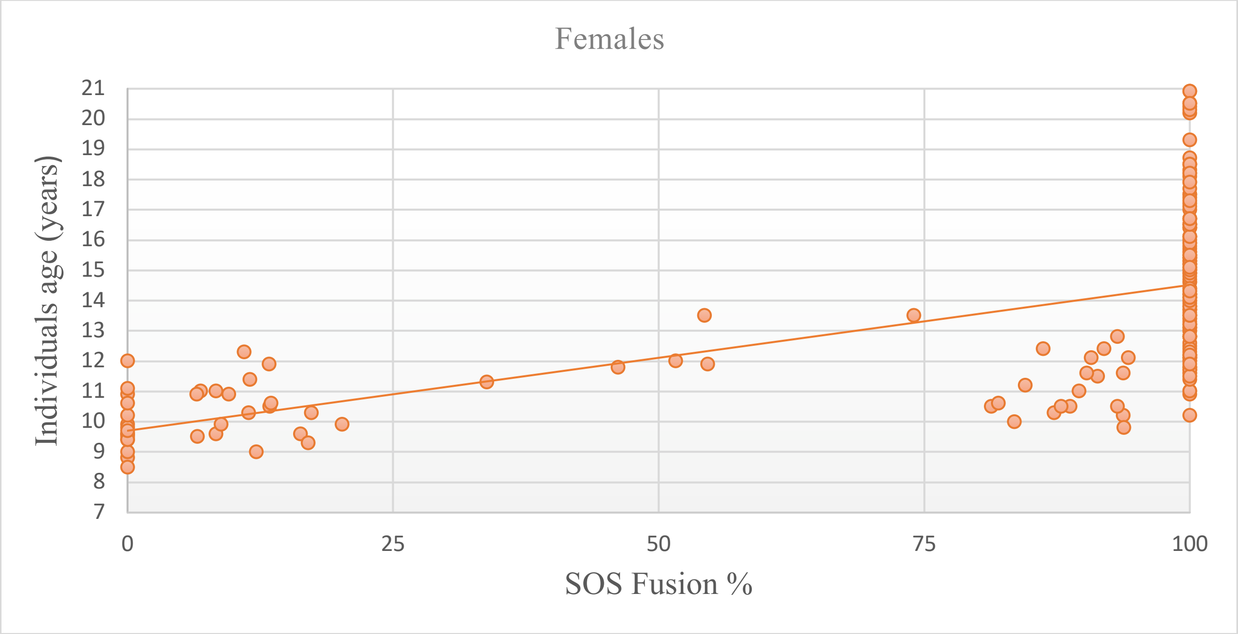


Figure 4.5 *Linear Regression Model for Females*

Table 4.8

ANOVA for Females for Difference in Mean Ages Between Fusion Stages

	Sum of Squares	df	Mean Square	F	Significance
Between groups	746.73	3	248.91	55.80	p <0.001
Within groups	909.91	204	4.46		

Table 4.9

One-way ANOVA with Post Hoc Bonferroni Adjustment for Females

Fusion Stage		Mean Difference	Std. Error	Sig. p <0.05	95% Confidence Interval	
					Lower Bound	Upper Bound
Fusion stage 0	Fusion stage 1	0.676	0.708	1.000	1.21	2.56
	Fusion stage 2	1.557	0.688	0.147	0.27	3.39
	Fusion stage 3	4.972	0.556	0.000	3.49	6.45
Fusion stage 1	Fusion stage 0	0.676	0.708	1.000	1.21	2.56
	Fusion stage 2	0.881	0.646	1.000	0.84	2.60
	Fusion stage 3	4.296	0.503	0.000	2.96	5.64
Fusion stage 2	Fusion stage 0	1.557	0.688	0.147	0.27	3.39
	Fusion stage 1	0.881	0.646	1.000	0.84	2.60
	Fusion stage 3	3.415	0.473	0.000	2.15	4.68
Fusion stage 3	Fusion stage 0	4.972	0.556	0.000	3.49	6.45
	Fusion stage 1	4.296	0.503	0.000	2.96	5.64
	Fusion stage 2	3.415	0.473	0.000	2.15	4.68

Research Question 5

Does a male/female subadult Hispanic population of Southern Nevada differ from other global populations in age when SOS is completely fused compared to the results from investigations using techniques other than CBCT analysis?

Hypothesis: Male/Female subadult Hispanics of Southern Nevada observationally demonstrate variable age at complete SOS fusion compared to the results of:

- Macroscopic studies by Coqueugniot and Weaver, 2007, Shirley and Jantz, 2011, Mahon et al., 2017,
- Conventional radiographic study by Powell and Brodie, 1963,
- Conventional CT studies by Madeline and Elster, 1995, Okamoto et al., 1996, El-Sheikh and Ramadan, 2006, Bassed et al., 2010, Franklin and Flavel, 2014, Can et al., 2014, Lottering et al., 2015.

All of which utilize techniques other than CBCT analysis for their study of various global populations.

Null Hypothesis: There is no observational difference between a male/female subadult Hispanic population of Southern Nevada and the results of the following studies utilizing techniques other than CBCT analysis in age when the SOS is completely fused:

- Macroscopic studies by Coqueugniot and Weaver, 2007, Shirley and Jantz, 2011, Mahon et al., 2017,
- Conventional radiographic study by Powell and Brodie, 1963,
- Conventional CT studies by Madeline and Elster, 1995, Okamoto et al., 1996, El-Sheikh and Ramadan, 2006, Bassed et al., 2010, Franklin and Flavel, 2014, Can et al., 2014, Lottering et al., 2015.

The null hypothesis was rejected. Table 4.10 outlines the aforementioned studies with population, sample size, age range, scoring scheme, and details for description of ages at complete SOS fusion. It is evident there is variability in results obtained among these reports.

Table 4.10

Summary of Age at Complete SOS Fusion Data for Relevant Studies Utilizing Visualization Techniques other than CBCT Analysis

Assessment	Author	Year	Population	Sex	Sample size	Age range	Scoring scheme	Age at complete fusion (years)
Macroscopic	Coqueugniot & Weaver	2007	Portuguese	Male	68	7-29	3-stage	19[a]
				Female	69			14[a]
	Shirley & Jantz	2011	American	Male	62	5-25	3-stage	17.4[b]
				Female	100			13.7[b]
	Mahon et al.	2017	South African Black	Male	74	12-30	3-stage	20[c]
				Female	73			20[c]
Conventional Radiography	Powell & Brodie	1963	American	Male	205	8-21	6-stage	13-16
				Female	193	6-18		11-14
Conventional CT	Madeline & Elster	1995	American	Male	115	0-18	5-stage	18[d]
				Female	74			16[d]
	Okamoto et al.	1996	Japanese	Male	253[e]	1-77	N/A	13
				Female				12
	El-Sheikh & Ramadan	2006	Arabian Gulf	Male	207[e]	9-23	2-stage	18[f]
				Female				16[f]
	Bassed et al.	2010	Australian (Victoria)	Male	458	15-25	5-stage	17[f]
				Female	208			17[f]
	Franklin & Flavel	2014	Australian (Western)	Male	169	5-25	4-stage	19.83[g]
				Female	143			18.62[g]
	Can et al.	2014	Turkish	Male	399	10-25	5-stage	20.34[g]
				Female	139			18.17[g]
	Lottering et al.	2015	Australian (Queensland)	Male	448	0-20	6-stage	15.6[b]
				Female	416			13.1[b]

[a] Youngest age at which complete SOS fusion was observed.

[b] Age when individual is most likely to transition from closing to closed SOS using maximum likelihood estimates.

[c] Complete fusion observed in all individuals above this age.

[d] Age of 95th percentile for complete fusion.

[e] Distribution of male and female not specified.

[f] Age at which SOS is completely fused in *all* individuals in the study.

[g] Mean age at complete fusion.

Research Question 6

Do results in mean age for each SOS fusion stage differ between this CBCT analysis of a male/female subadult Hispanic population of Southern Nevada compared to results obtained from the CBCT analysis of a modern American population reported in the literature by Alhazmi et al., 2017?

Hypothesis: Using CBCT technology, male/female subadult Hispanics of Southern Nevada observationally demonstrate different mean ages for each SOS fusion stage compared to the male/female modern American population studied by Alhazmi et al., 2017.

Null Hypothesis: Using CBCT technology, there is no observational difference between a male/female subadult Hispanic population of Southern Nevada and the CBCT study of a male/female modern American population studied by Alhazmi et al., 2017 in mean age of each SOS fusion stage. *The null hypothesis was accepted.* Table 4.11 summarizes the details of the present study and that performed by Alhazmi et al., 2017. It is apparent that there is observational similarity between the results in mean ages for all SOS fusion stages between the two American populations. The only exception is mean ages for fusion stages 1 and 2 for females in that they differ by more than one full year.

Table 4.11
Summary of Results from CBCT studies on American Populations

					Sample size	Age range	Scoring scheme	Mean age for fusion stage			
Assessment	Author	Year	Population	Sex				0	1	2	3
CBCT	Alhazmi et al.	2017	American	Male	361	6-20	4-stage	11.07	12.95	14.44	16.41
				Female	380			9.75	11.67	13.25	15.25
	Present study	2018	Hispanic American	Male	166	8-20	4-stage	10.90	12.66	13.87	16.35
				Female	208			9.86	10.54	11.42	14.84

Chapter 5: Discussion

The objective of this study was to evaluate the timing of spheno-occipital synchondrosis fusion and its correlation with chronological age in a contemporary male/female subadult Hispanic population of Southern Nevada. It was performed to explore and cultivate population specific age estimation standards. The results demonstrate that SOS fusion is positively correlated with chronological age and that complete fusion occurs during adolescence. Specifically, complete fusion occurred by a mean age of 16.35 (±1.70) years in males and 14.84 (±2.40) years in females. It was also evident in this study that SOS fusion and its progression occurs earlier in females by an average of 1.78 years. This finding is consistent with previous research indicating that a definite sex difference in the timing of skeletal maturation exists (Powell & Brodie, 1963; Konie, 1964; Scheuer & Black, 2000). Historically, complete SOS fusion in both sexes has be shown to occur between 17-25 years of age (Ford, 1958). Current research efforts reinforce the idea that complete fusion of the SOS is a defining mark of adolescence, and not an indicator of transition into adulthood as maintained by Enlow (Enlow & Hans, 1996).

Intraobserver and Interobserver Error Rate

Intraobserver error rate was calculated in order to evaluate whether fusion stage assignment derived from CBCT images could be reliably reproduced by a single examiner. Sixty randomly selected CBCT images were examined two months after initial data collection and the results were subjected to Cohen's Kappa correlation analysis. The test revealed a significant correlation (Kappa value =0.977, p <0.001) between initial and repeat fusion stage assignment. The strength of agreement is excellent which establishes reliable repeatability of SOS fusion scoring with a single examiner.

Interobserver error rate was calculated in order to evaluate the reliability of fusion stage assignment by a second examiner. Thirty-seven images were examined by a second examiner (H.B.) and the results were subjected to Cohen's Kappa correlation analysis. The test revealed a significant correlation (Kappa value=0.890, $p < 0.001$) between fusion stage assignment by the two examiners (M.B. and H.B.). The strength of agreement establishes reliable SOS fusion scoring between two examiners.

Visualization with CBCT Technology

Age estimation utilizing SOS fusion stage is variable across research protocols, and may be attributed to different methodological design, application of different scoring systems, and/or a reflection of true population variation. Macroscopic visualization, conventional radiographic imaging, and three-dimensional imaging modalities have all been employed to investigate the relationship between chronological age and SOS fusion (Coqueugniot & Weaver, 2007; Shirley & Jantz, 2011; Mahon et al., 2017; Powell & Brodie, 1963; Madeline & Elster, 1995; Okamoto et al., 1996; El-Sheikh & Ramadan, 2006; Bassed et al., 2010; Franklin & Flavel, 2014; Can et al., 2014; Lottering et al., 2015). Use of different visualization techniques limits the ability to directly compare results of age estimation studies. Technological advancements in the high-resolution capacity of CBCT imaging permits discrete characterization of the morphological fusion pattern of the SOS; thereby providing a foundation for future research especially with CBCT imaging.

One of the multiple factors that affects the resolution of images acquired from CBCT scans includes the sensor system associated with the various CBCT image acquisition devices, e.g. AFP NuTome, Imaging Sciences I Cat, Hitachi CBMercuRay, Sirona Galileos (Baba et al., 2002; Miles & Danforth, 2007). Contemporary CBCT scanners use one of two types of image detectors:

- Flat panel detector
- Image intensifying screen.

The latter detector introduces measurement inaccuracies of the acquired, reconstructed images by comparatively creating increased distortion at their peripheries (Abramovitch & Rice, 2014). The Hitachi CBMercuRay CBCT scanner employed at the UNLV SDM orthodontic residency clinic uses an intensifying screen for image acquisition.

Although the highest degree of spatial resolution could have been achieved analyzing images reconstructed from a flat panel detector, all images in this study were generated using the Hitachi CBMercuRay scanner (Baba et al., 2002). However, considering that the SOS is centrally located in a CBCT image, it is unlikely that the intensifying screen played a significant role in fusion stage scoring accuracy.

Despite the limitations between the two CBCT image detector systems, the use of either high resolution CBCT or CT imaging modalities remains advantageous in detecting fusion status to the various macroscopic and two-dimensional radiographic methods (Coqueugniot & Weaver, 2007; Shirley & Jantz, 2011; Mahon et al., 2017; Powell & Brodie, 1963). It is for this reason that standards derived from high resolution imaging studies should not be applied when analyzing macroscopic samples and vice versa.

Morphological Scoring of SOS Fusion

Fusion of the SOS has been scored according to four-stage (Franklin & Flavel, 2014) or five-stage methods (Madeline & Elster, 1995; Bassed et al., 2010) in the midsagittal plane, or a six-stage method when both the midsagittal and axial planes are analyzed (Lottering et al., 2015).

Some of the five and six-stage scoring methods include the presence of a fusion scar. Macroscopic evaluation performed by Shirley and Jantz suggested, however, that a fusion scar may remain at the site of the synchondrosis for decades after fusion is complete (Shirley & Jantz, 2011). Therefore, fusion scars should not be considered a recent sign of fusion and consequently not included in age estimation efforts (Shirley & Jantz, 2011).

Lottering et al. introduced a six-stage scoring scheme in an effort to more reliably correlate skeletal morphology to known chronological ages and decrease overlap between consecutive stages (Lottering et al., 2015). Unique to this CT project, Lottering et al. studied a population in age range 0-20 years. Stages 1 and 2 were characterized by age distributions of 0-4 years and 2-10 years, respectively. Therefore, accurate application of this six-stage scoring tier would inherently be limited to sample populations that included neonates. Radiographic imaging data would necessarily be limited for this age group. The current retrospective study did not include images from this age group because all CBCT scans were obtained from an orthodontic patient population with a minimum age of eight years old.

Furthermore, Scheuer and Black support methods that simplify fusion stages in order to reduce error rate between observers and therefore between populations studied (Scheuer & Black, 2000). A four-stage scoring system was selected for the current project. This decision was made because the sample population did not evaluate individuals 0-7 years old. Assessment of this age group would have been necessary for application of Lottering et al.'s six-stage system (Lottering et al., 2015). Additionally, five-stage scoring methods where not employed in the current study because incorporation of a fusion scar in scoring was excluded and subjectivity in fusion stage assignment was decreased. Consequently, the four-stage scoring method was chosen for this study because it was most appropriate for evaluation of the population age range.

Additionally, this model would theoretically increase inter- and intraobserver reproducibility and permit improved inter- and intrapopulation comparison.

Statistical Analysis

Based on a review of SOS fusion literature, Krishan and Kanchan noted that some studies evaluated SOS fusion in specific ethnic populations: whereas some other studies did not consider removing ethnicity as a variable (Krishan & Kanchan, 2013). Therefore, they stated, "It is a well-established fact that standards may vary in different populations and ethnic groups and thus the standards drawn for a particular population/ethnic group should not be employed on a different group" (Krishan & Kanchan, 2013).

Consequently, these forensic anthropologists recommended that future research regarding SOS closure in modern populations should use standard methodologies and techniques so ethnic differences can be more precisely compared and more effective standards developed (Krishan & Kanchan, 2013). Based on this recommendation, the current study performed statistical analysis with Spearman correlation and linear regression parameters. This approach was used in an attempt to promote consistency and comparability between this project and another recent CBCT SOS fusion investigation of an American population that was not limited to a specific ethnic group (Alhazmi et al., 2017).

By maintaining the same methodologies and statistical analyses as previous radiographic SOS fusion research, this project attempted to gain deeper insight into variation in a Southern Nevada subadult Hispanic population of similar societal modernization and socioeconomic status. It has been suggested that populations similar in socioeconomic status, general nutritional health, and societal modernization might demonstrate similar growth and development patterns

(Lottering et al., 2015). Furthermore, these characteristics may play a more critical role in the development of the SOS compared to ethnic differences alone.

Ethnic Intrapopulation Comparison to a Multiethnic American Population

Intrapopulation variation has been defined by Lottering et al. as the "biological variation observed within a single population, which is relatively homogenous for environmental and social influences" (Lottering et al., 2015). Although the study performed by Alhazmi et al. did not evaluate a sample population according to the previous definition of an intrapopulation variable, comparison of the results of the current SOS fusion study with the results from Alhazmi et al. demonstrates similarities across mean ages for each fusion stage (Table 4.11) (Alhazmi et al., 2017).

The largest differences in mean ages for SOS fusion observed between the current investigation and the Alhazmi et al. study existed between stages 1 and 2 for females. The present experiment found mean ages of 10.54 (±0.93) years for stage 1 and 11.42 (±1.09) years for stage 2. Alhazmi et al. reported a mean age of 11.67 (±0.93) years for stage 1 and 13.25 (±1.19) years for stage 2 (Alhazmi et al., 2017).

Alhazmi et al. did not consider the ethnic specificity that was the focus of the present study. They report the study sample as a "modern American population," without distinction of ethnic representation (Alhazmi et al., 2017). Comparing results of both projects could imply that Southern Nevada subadult Hispanic females demonstrate a faster fusion rate than a generalized multiethnic American population.

Southern Nevada Subadult Hispanic Females Lack Significant Differences in Mean Ages for Initial and Intermediate Fusion Stages (Stages 0 and 1; Stages 0 and 2; Stages 1 and 2)

Results of this study indicate non-statistically significant differences in mean ages for SOS fusion stages 0 & 1, 0 & 2, and 1 & 2 for females. Although the current study did not evaluate longitudinal data of SOS fusion, it could be contended that fusion of the synchondrosis might occur at a faster rate and in a shorter time frame for Southern Nevada subadult Hispanic females compared to males.

Alhazmi et al. evaluated longitudinal data in their research and reported that females had a higher rate of closure than males at the age of 10 years and that SOS closure takes longer in males (Alhazmi et al., 2017). A similar finding was reported by Konie indicating that progression from initial ossification of the synchondrosis to complete obliteration required more time in males than females (Konie, 1964). In another early CT study of SOS closure, Madeline and Elster noted that sutural closure staging can be difficult to assign (Madeline & Elster, 1995). They determined that fusion does not occur simultaneously along the entire length of the synchondrosis and such variability is especially true in females (Madeline & Elster, 1995).

Inferred from the findings of these studies, non-statistically significant differences in mean age between initial and intermediate fusion stages is also supported for multiethnic subadult American female samples (Alhazmi et al., 2017; Konie, 1964; Madeline & Elster, 1995). Thus, faster synchondrosis fusion rates for both Southern Nevada subadult Hispanic and multiethnic subadult American females is logically explained based on the finding statistically significant differences were calculated only for the most extreme stages of unfused and completely fused SOS.

Limitations

The presence of statistically significant differences between complete fusion and all other fusion stages in females can be explained by the fact that mean age at complete fusion for females in this sample may be overestimated. This could be due to the upper age limit of 20 years in this study far exceeding the age in which complete fusion in females is generally observed. Thus, inflating the mean age at complete female SOS fusion. The best fit line in the regression model demonstrates elevation, particularly at the scattered data points between 75% and 100% female fusion (Figure 4.5). This is supported by the descriptive data which indicated that 149 of the 208 sampled females manifested complete SOS fusion (Table 4.3). In this study, complete fusion was observed in 100% of females 14 years of age and older.

Genetic and geographic origin, as well as socioeconomic status, must be considered when reference studies are used for age estimation efforts (Schmeling et al., 2007). This is especially true when studying SOS fusion for age estimation in living individuals. It is acknowledged that the predominant limitation of the present study is the lack of an actual genetic component within the data for the Southern Nevada subadult Hispanic population evaluated. All patients self-reported Hispanic ethnicity and no genetic analyses were performed to identify specific Spanish speaking subpopulations in this retrospective study. Therefore, the associated limitation reflects that the population represents a homogenous Hispanic population. Realistically, however, the sample represents variable genotypes within a Spanish speaking group of individuals.

Every effort was made to obtain the largest sample possible in each age group for Southern Nevada subadult Hispanic male and female subjects. Despite these efforts, however, the greatest number of individuals clustered between the tenth and fifteenth years of age for both male and female populations analyzed. This is reflective of the ages at which most orthodontic treatment is initiated and completed. Future studies should include a more equal distribution of all ages examined. Larger sample sizes would also be beneficial to ensure statistical robustness of the results.

The current study employed an age estimation method utilizing CBCT imaging of SOS closure. Selected statistical analyses of a four-stage fusion scheme demonstrated reliability and reproducibility. However, this technique requires calibration and methodological considerations to appropriately assign correct chronological ages for the Southern Nevada subadult male/female Hispanic populations studied.

As the review of the SOS fusion scientific literature demonstrates, there is a vast range of techniques and staging methods that have been used to estimate chronological age at which the SOS fuses. As in the current project, some of the reviewed SOS fusion studies used CBCT techniques. However, they included a different ethnic population than this study or a multiethnic population which did not indicate an ethnic subpopulation as a variable (Sinanoglu et al., 2016; Alhazmi et al., 2017). Accurate comparison of various ethnic populations worldwide would necessarily require the use of coincident classification systems using similar visualization techniques.

Direction of Future Research

Although Schmeling et al. did not specifically study SOS fusion/chronological age estimation comparison, they proposed that the most relevant age estimation research should consider the following variables, "…various ethnic groups of similar socioeconomic status living in the same geographic region" (Schmeling et al., 2007). The current project followed the variable selection protocol advocated by these researchers. Based on the results of this study, future research regarding SOS fusion/chronological age estimation should also select various ethnic populations (e.g.: Asian, Black, Native Americans, etc.) with comparable socioeconomic status from similar geographic locations to test validity of ethnic specific age estimation standards.

Future research involving the presently analyzed Hispanic subadult male/female population from Southern Nevada should limit the upper age range to 14 + 2 years for the female sample. This should represent the minimum age at which complete SOS fusion is observed for 100% of the individuals examined. Thus, the suspected limitation noted in the current study regarding inflation of the mean age for the terminal SOS fusion stage for females should be avoided. Based on the current study, it would be advisable to limit the upper age range for selected ethnic population studied, with the understanding that it may differ from the upper age range in this project.

An understanding of spheno-occipital synchondrosis development and fusion has forensic anthropological relevance regarding age estimation. Additionally, knowledge of its development and fusion has applicability in the practice of forensic odontology, orthodontics, and dental implantology. In terms of its orthodontic significance, longitudinal evaluation of SOS closure would be practical to explore regarding its relationship to mandibular growth. Understanding this relationship could clarify whether the degree of SOS fusion could be utilized as an alternative parameter to evaluate skeletal maturation and individual growth potential. With the increasing popularity of CBCT imaging in the practice of orthodontics, determination of SOS fusion may also prove to be an easy to evaluate, more readily discernible indicator of growth potential than cervical vertebrae maturational status or hand-wrist ossification. Further population-specific data is necessary to validate consistent application of this method to clinical practice and could prove particularly advantageous for orthodontic and dental implant treatment planning.

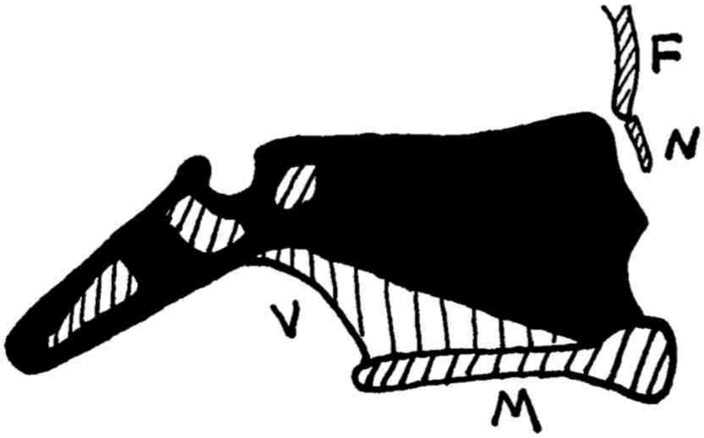
Conclusions

Consistent with previous reports in the scientific literature, this study found that the spheno-occipital synchondrosis fusion begins in adolescence and is completely fused by adulthood (Bassed et al., 2010; Shirley & Jantz, 2011; Franklin & Flavel, 2014). Additionally, this project supported the fact that females preceded male counterparts in SOS fusion progression and completion also observed by others (Bassed et al., 2010; Shirley & Jantz, 2011; Franklin & Flavel, 2014). The fusion stage of the spheno-occipital synchondrosis was found to be correlated enough with chronological age to permit reasonable age estimation in subadult Southern Nevada Hispanic males but remains questionable for subadult Hispanic females from the same location.

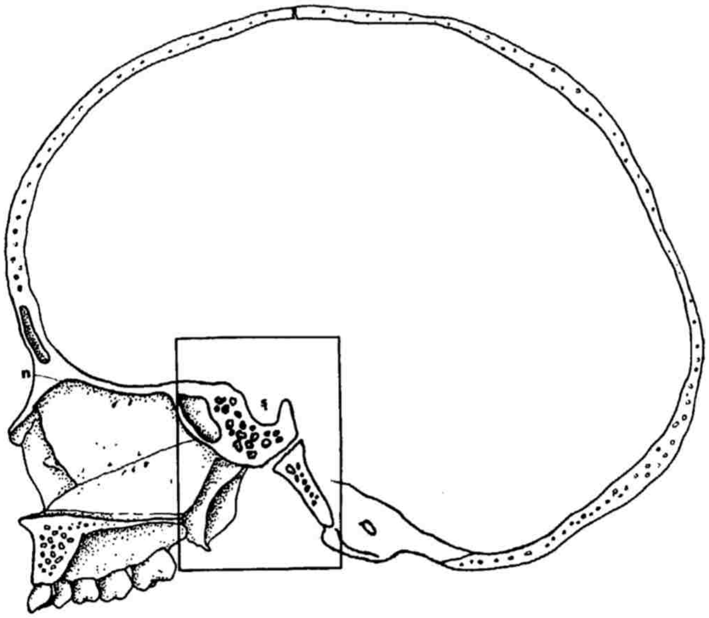
Individual skeletal markers of chronological age should not be used alone to make a determination of age estimation. Therefore, utilization of multiple defined skeletal and dental indices should be employed to achieve the most beneficial results to ensure accurate age estimation. This should increase diagnostic accuracy and improve age estimation in living individuals and human decedents requiring identification.

As evidenced in this study and in the scientific literature, inferences for age estimation for ethnic subpopulations from similar socioeconomic backgrounds and geographic locations is still unresolved. Thus, future research is strongly recommended in this regard to derive independent population age estimation standards. Together with previous age estimation research, this study supports the practicality and usefulness of appraising spheno-occipital synchondrosis fusion as an indicator of chronological age in both living and deceased individuals, particularly in the presence of other skeletal or dental indices.

Appendix A: Original, unmodified version of Figure 2.1, *Midsagittal view of fetal cartilaginous skull* (Scott, 1958)



Appendix B: Original, unmodified version of Figure 2.2, *Midsagittal view of the cranial base synchondroses* (Melsen, 1969)



Appendix C: UNLV Institutional Review Board Exemption Decision

UNLV Biomedical IRB - Exempt Review
Exempt Notice

DATE:	September 5, 2017
TO:	Edward Herschaft, DDS, MA
FROM:	Office of Research Integrity - Human Subjects
PROTOCOL TITLE:	[1104233-1] Analysis of spheno-occipital synchondrosis (SOS) fusion in a contemporary Southern Nevada subadult Hispanic population using archival cone-beam computerized tomography (CBCT) images.
ACTION:	DETERMINATION OF EXEMPT STATUS
EXEMPT DATE:	August 20, 2017
REVIEW CATEGORY:	Exemption category # 4

Thank you for your submission of New Project materials for this protocol. This memorandum is notification that the protocol referenced above has been reviewed as indicated in Federal regulatory statutes 45CFR46.101(b) and deemed exempt.

We will retain a copy of this correspondence with our records.

PLEASE NOTE:
Upon final determination of exempt status, the research team is responsible for conducting the research as stated in the exempt application reviewed by the ORI - HS and/or the IRB which shall include using the most recently submitted Informed Consent/Assent Forms (Information Sheet) and recruitment materials.

If your project involves paying research participants, it is recommended to contact Carisa Shaffer, ORI Program Coordinator at (702) 895-2794 to ensure compliance with the Policy for Incentives for Human Research Subjects.

Any changes to the application may cause this protocol to require a different level of IRB review. Should any changes need to be made, please submit a **Modification Form**. When the above-referenced protocol has been completed, please submit a **Continuing Review/Progress Completion report** to notify ORI - HS of its closure.

If you have questions, please contact the Office of Research Integrity - Human Subjects at IRB@unlv.edu or call 702-895-2794. Please include your protocol title and IRBNet ID in all correspondence.

Office of Research Integrity - Human Subjects
4505 Maryland Parkway . Box 451047 . Las Vegas, Nevada 89154-1047
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