JOURNAL OF
CONTEMPORARY MEDICINE

DOI: 10.16899/jcm.598318
J Contemp Med 2020;10(1):131-137

Review / Derleme

Fetal Sex Determination in Light of Interdisciplinary Current Studies: A Forensic Anthropological Approach

Disiplinlerarası Güncel Çalışmalar Işığında Fetal Cinsiyet Tahmini: Adli Antropolojik Bir Yaklaşım

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Abstract

Aim: In this study, it was aimed to highlight the situation and importance of fetal sex determination in the context of forensic anthropology. Suggestions have been made about the development of this subject with current approaches and interdisciplinary perspectives.

Material and Method: Fetal osteology is one of the topics that needs to be developed within forensic anthropology. New techniques are needed both due to lack of sample and to the limited methods that can be applied to this age group. In this respect, a literature review was conducted within the scope of studies on fetal sex determination. It has been revealed how the applications of different disciplines in this field can be adapted to forensic anthropology.

Results: The vast majority of current studies emphasize that ilium gives reliable results in fetal sex determination. Often evaluated in both clinical applications and forensic anthropological studies, this bone is subjected to morphological and morphometric analyses.

Conclusion: Perspectives of interdisciplinary approaches will enable the development of fetal sex determination studies within forensic anthropology and will play an important role in identification studies. The use of technological tools such as ultrasound for fetal sex determination is thought to be useful for the development of forensic anthropological techniques as in clinical applications. Testing new techniques on fetal sex prediction is expected to contribute to many areas such as growth development, genetics, embryology, neonatology and endocrinology.

Keywords: Forensic anthropology, fetal development, sex determination by skeleton, human identification, interdisciplinary studies

Öz

Amaç: Bu çalışmada, fetal cinsiyet tahmininin adli antropoloji bağlamındaki yeri ve öneminin vurgulanması amaçlanmıştır. Bu konunun güncel yaklaşımlarla ve disiplinlerarası bakış açılarıyla geliştirilmesine dair öneriler sunulmuştur.

Gereç ve Yöntem: Fetal osteoloji, adli antropoloji içerisinde geliştirilmesine ihtiyaç duyulan konulardan biridir. Gerek örneklem yetersizlikleri gerekse bu yaş grubuna uygulanabilecek yöntemlerin henüz kısıtlı olmasından dolayı yeni tekniklere ihtiyaç duyulmaktadır. Bu açıdan, fetal cinsiyet tahminine yönelik çalışmalar kapsamında bir literatür araştırması yapılmıştır. Farklı bilim dallarının bu konudaki uygulamalarının adli antropolojiye nasıl uyarlanabileceği ortaya konulmuştur.

Bulgular: Mevcut çalışmaların büyük bir çoğunluğu ilium'un fetal cinsiyet tahmininde güvenilir sonuçlar verdiğini vurgulamaktadır. Hem klinik uygulamalarda hem de adli antropolojik çalışmalarda sıklıkla değerlendirilen bu kemik, morfolojik ve morfometrik analizlere tabi tutulmaktadır.

Sonuç: Disiplinlerarası yaklaşımların bakış açıları, fetal cinsiyet tahmini çalışmalarının adli antropoloji içerisinde de gelişmesini sağlayacak ve kimliklendirme çalışmalarında önemli rol oynayacaktır. Fetal cinsiyet tahmini için ultrason gibi teknolojik araçların kullanılması klinik uygulamalarda olduğu gibi adli antropolojik tekniklerin geliştirilmesi için de yararlı olacağı düşünülmektedir. Fetal cinsiyet tahmini konusunda yeni tekniklerin test edilmesinin büyüme gelişme, genetik, embriyoloji, neonatoloji ve endokrinoloji gibi pek çok alana da katkıda bulunması beklenmektedir.

Anahtar Kelimeler: Adli antropoloji, fetal gelişim, iskelet ile cinsiyet belirlenmesi, insan kimliklendirme, disiplinlerarası çalışmalar

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Received *(Geliş Tarihi)*: 09.07.2019 **Accepted *(Kabul Tarihi)*:** 30.11.2019

INTRODUCTION

There are many reasons for determining fetal sex, such as medical or social motives. For example, sex determination may be needed in the prenatal period as some of the embryonic diseases develop sex-specific.[1] Fetal sex determination is not needed only in clinical researches. This issue has an important place in terms of forensic anthropology, also should be supported and developed with current approaches. Special expertise is needed in the analysis of bones and skeletons of unidentified persons for the criminal investigation and the administration of justice.[2] Forensic anthropology plays an effective role in such cases.

Forensic anthropology, as a branch of science that conducts biological profiling and identification studies through skeletal remains, encounters many obstacles when it comes to fetal skeletal remains. Studies on fetal skeletons are more limited than adult and child skeletons, making fetal age and sex estimation more difficult. The fact that skeletal remains have been mixed, especially in mass burials, is also a disadvantage in terms of interpretation of fetal skeletal remains.

In this review study, fetal sex determination will be approached from a forensic anthropological perspective. The aim of this study is to reveal the deficiencies in fetal sex determination methods and to emphasize the importance of interdisciplinary researches in this area.

Evaluation of Sex Determination From Forensic Anthropological Perspective

Numerous methods have been developed in terms of biological anthropology in estimating sex with skeletal remains. Therefore, these methods are also used in forensic anthropology. The use of sex determination methods based solely on the evaluation of bone size is often inadequate. Geometric morphometric analysis created by a combination of morphological and metric methods yields more reliable results.[3] Two methods are used as the basis for sex estimation through skeletal data in forensic anthropology: Morphological (descriptive) or metric (quantitative) methods.[3] Morphological methods are associated with genetic control and are based on the interaction between sex-related growth and development factors. In addition, it also considers environmental factors that may differ by sex.[4] However, morphological methods are evaluated by categorical scales based on the researchers' judgments.[5]

Metric methods, on the other hand, evaluate the growth and development processes in females and males morphologically as in morphometric methods. Also, this methods are far from subjective judicial evaluations. Morphometric methods are specific to populations. Therefore, these methods may not yield reliable results at the same rate in different populations.[6]

Emergence and Development of Fetal Sex Estimation Studies

Sexual dimorphism indicators for immature skeletal remains are evaluated based mostly on morphological differences in the pelvis and skull, just as in adults.[7] Modern morphometric techniques show that fetal ilia is reliable in revealing sex differences.[8] Because this section shows morphological differences between sexes in fetuses as in adults.[9] Early scientific studies involving sex determination of fetal skeletal remains have focused more to greater sciatic notch of the ilium. It is therefore important for forensic anthropologists to correctly identify ilium and to know pelvis development well (**Figure 1** and **Table 1**).

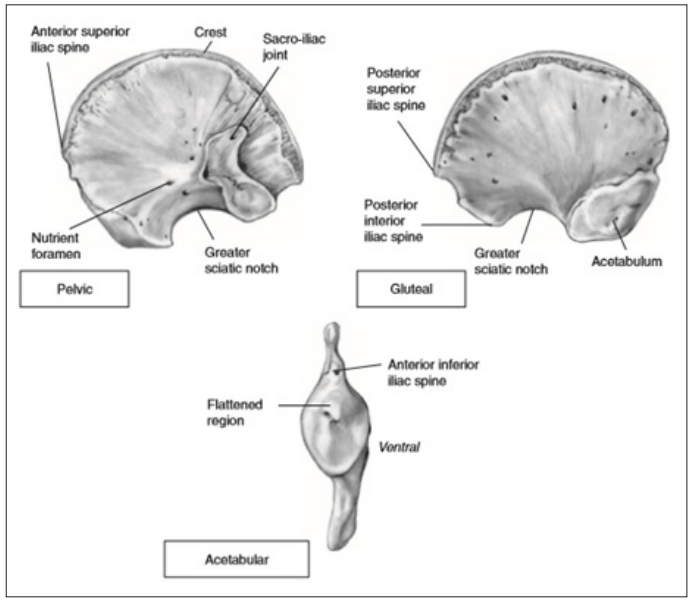

Figure 1. Right Perinatal Ilium [10]

Table 1. Development of Ilium in Fetal Period [11]

2-3 months	Ilium commences ossification
4-5 months	Ischium commences ossification and ilium is recognizable
5-6 months	Pubis commences ossification
6-8 months	Ischium is recognizable in isolation

The fetus bones are composed of more parts than children and adults. These bones haven't taken their characteristic shapes yet. Therefore, some bones are more difficult to identify during the fetal period. Hence, in the first stage of sex determination, it is necessary to consider in detail the developmental stages of each bone.

Research on the differences between the sexes of fetal ilia began in 1876 with Fehling's study stating that male and female pelvises could be distinguished as early as the 4th fetal month.[12] Thomson (1899) also noted that after studying a small sample of only eight pelvises, indicators of sex differences could be seen as early as the 4th fetal month.[13] Boucher (1955) conducted a blind study on sex differences in the sciatic notch of fetal ilia. In this study, ilia samples were taken from 20 stillborn babies whose sex was known during six months. According to the indice created, the differences between male and female values were significant. Indice values were higher in females than in males. Therefore, it has been stated that fetal ilium can be used in sex determination.[14] Boucher (1957) adapted the same study to the British whites group as she did on American black and white fetuses. However, no significant differences between the sexes were found for this sample (15). This study is a good example of how the indices created would not yield reliable results for each population.

Fazekas and Kósa's (1978) extensive research on fetal skeletons is one of the important precursors in this field. It is also noted in the study that ilium ossified early in the third month of the prenatal period, and from this stage the iliac crest became more convex.[12]

Schutkowski's study (1987) evaluated 43 females and 61 males in the lunar month from 4 to 10 of the fetal period. The raw data in the research of Fazekas and Kósa (1978) was used in this study. The dimensions of hip and thigh bones of the sample, whose sex is known before, were analyzed and discriminant functions were evaluated (**Figure 2**).

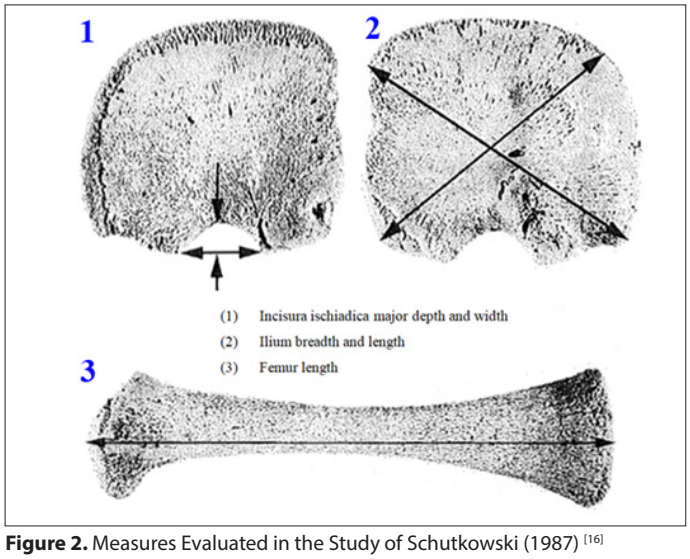

Figure 2. Measures Evaluated in the Study of Schutkowski (1987) [16]

As a result, more than 70% of the sample was grouped into fetal and neonatal periods. This study highlights that the detection of early ontological indicators of sex in paleodemographic studies is important in defining sex distribution in skeletal populations.[16]

In the study of Cera Holcomb (1992), photographs of the ventral side of 133 fetal ilia known from age and sex from Washington University Trotter Collection were digitized and trace coordinates were used in subsequent analyses. Overall shape differences in fetal iliac of males and females are statistically significant between the sexes according to the elliptic Fourier analysis.[8]

Dhooria ve arkadaşlarının yaptığı meta-analizde sensitivite, spesifite oranları sırasıyla ortalama olarak %80,3 (73,7–85,9), %100 (98,7–100) bulunmuştur.[10] Çalışmamızdaki değerler bu haliyle literatürdeki çalışmalar ile uyumlu olup EBUS-TBİA yönteminin tanı ve evrelemede son derece önemli bir yöntem olduğunu destekler özelliktedir. Komplikasyon oranının düşük olduğu dikkate alınacak olursa özellikle cerrahi işlem ve kemo-radyoterapi sonrası yeniden evrelemede ilk seçilecek işlem olma özelliğini korumaktadır.Holcomb and Konigsberg (1995) photographed the ventral surface of the ilium of 133 fetuses with known age and sex from the Trotter Collection of Washington University based on uncertain results from previous studies on sex determination by fetal ilium morphometry. Some trace coordinates have been identified in the digital versions of the photos. As a result of the analyses, significant sexual dimorphism was identified in the anteriorposterior region of the maximum depth of sciatic notch.[9]

Ridley's study (2002) aimed to make sex prediction of fetus and early infants using cranium and mandible bones. It was stated that cranial bones in this age group didn't show enough morphological differences to be used in sex estimation and therefore, these bones didn't provide useful indicators for sex estimation in forensic applications.[17] Morphognostic analyses were performed in fetus and infant mandibles in Schutkowski's study (1993). This study shows that there are significant morphological differences between the sexes. It is stated that this situation is caused by developmental biological differences between males and females.[18] Stull and Godde (2013) investigated the characteristics of sexual dimorphism in the humerus and femurs of infants between birth and 1 year of age. 85 femoral and 45 humeral radiographic images were examined and length and width measurements were recorded. As a result of discriminant analysis, it was stated that this application was a reliable method for sex determination.[19]

Devadas et al. (2017) examined 100 hip bones of 50 fetuses including 25 females and 25 males. Morphometric analyzes were performed on the greater sciatic notch. The index was calculated from the width and depth of the greater sciatic notch. As a result, statistically significant differences were found between the sexes. Greater sciatic notch was found to be wider in females than in males. In males this area is deeper than for females. This indicator is demonstrated to be a gauge of sexual dimorphism with 100% accuracy. It has been emphasized that this region is the best parameter for fetal sex estimation and can also be considered as a reliable indicator in forensic context.[20]

Luv et al. (2017) examined the pelvic bones of 34 fetuses, including 22 males and 12 females (**Image 1**). In this study, bilateral fetal pelvic bones were obtained from dead fetuses between 16 38 weeks. These bones were separated from soft tissues by taking horizontal sections. Fetal bone samples were examined in two groups as <22 weeks and >22 weeks. The age reference point of the sample was determined according to the beginning of ossification and stages of development. Morphologically and morphometrically evaluations were made in this study. As a result, in the >22 weeks group, fetal ilium was 100% reliable in the sex estimation. Therefore, it was concluded that fetal ilium can be used as a potential tool for forensic researches in the assessment of sex in severely fragmented and isolated bodies.[21]

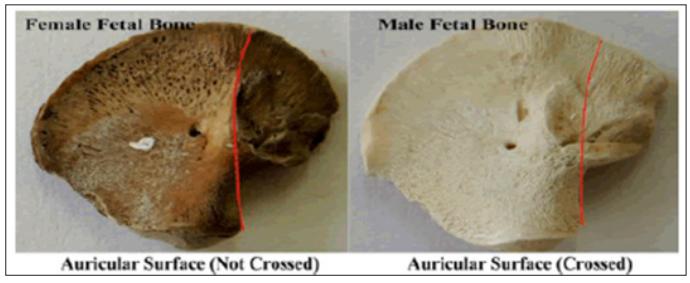

Image 1. Evaluation of Arches on the Auricular Surface in Sex Prediction [21]

Technological Approaches That Can Be Used In Verifying Skeletal Data

Observation of the endometrium with sonography allows reliable fetal sex determination through internal pelvic components. This technique can be considered reliable in cases where gestational age is known.[22]

Although bone is always thought of as a problematic tissue in terms of obtaining images due to the nature of ultrasound waves, it is possible to obtain quality images with optimum equipment adjustment. In addition to the study of bone development, such ultrasonographic applications are of great importance for epidemiological studies.[23]

Today, it is known that sex-specific maternal-placental-fetal interaction plays an important role in placental functions and pathologies. Therefore, invasive tests (amniocentesis and patchy sampling) can be performed as a result of clinical evaluations.[1] Sex determination of the fetus can be an important criterion in studies using placenta samples (24). Such studies would also be instrumental in the diversification of forensic anthropological research based on fetal sex prediction.

Case Study 1. Mahon et al. (2009) examined the feasibility of using 3-dimensional ultrasound (3DUS) to investigate the effects on fetal femoral development. In this context, fetal thigh measurements were taken between 19 34 weeks of gestation from 517 females. In addition to standard femoral length measurements, femoral cross-sectional areas and volume were obtained from volumetric data sets (**Figure 3**).

This study concluded that measurements of fetal femur size and volume were reproducible and could be used to study biological differences between samples. In addition, it was determined that femur volume was higher in male fetuses at 19th weeks of gestation.[23]

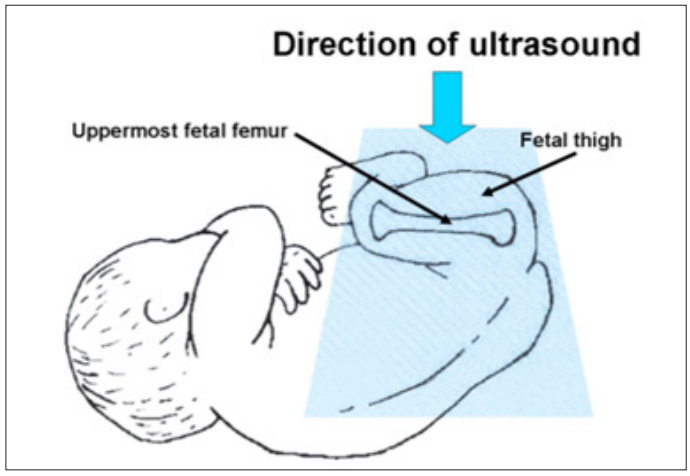

Figure 3. Fetal Femoral Measurements Through Ultrasound [23]

Case Study 2. With the development of image processing techniques, it was also possible to successfully observe clear genital appearance in ultrasound images. Sex and weight estimation can be made in fetuses using ultrasound images. Aditya et al. (2013) developed a method using thresholding and canny segmentation to increase the accuracy rate in estimating these variables. The method aims to calculate the percentage of white level in the processed image to determine the sex of the fetus (**Image 2**).

Image 2. Ultrasound image of female fetus shows "three lines " indicating labia and clitoris in area marked with red hoop [25]

Canny edge detection has been used for segmentation. Then parameters such as femur length (FL), biparietal diameter (BPD) and abdominal circumference (AC) were measured to estimate fetal weight. The fact that the results are equal to or greater than 46% indicates that the fetus will be considered a male. Images with a white level of less than 46% indicate that the fetus is female. In the context of increasing the accuracy of fetal sex determination of this method, it is stated that screening procedures can be further developed and applied in low cost two dimension ultrasound machine.[25]

Role of Genetic Studies in Fetal Sex Determination

When it comes to fetal sex determination, genetic applications may be needed for many reasons. For example, in cases with a high risk of serious genetic diseases affecting a particular sex, prenatal fetal sex determination applications can be made. Early prenatal diagnosis of fetal sex is necessary to optimize pregnancy management in families known to be at risk for certain genetic disorders.[26]

Genetic and chromosomal studies for the detection of sex-related genes offer the most reliable methods Chromosomal analyses began to be developed in 1956 and were used clinically soon after.[27] Chromosomal analysis requires divisible viable cells. The use of any organ in genetic sex determination methods applied with special techniques is an important advantage and reliable results are obtained in all stages of pregnancy. It is highly likely to obtain DNA from very ancient and nonviable tissues.[27]

Identification of cffDNA (cell-free fetal DNA) in maternal circulation has led to the development of olmayan noninvasive prenatal diagnostic tests veren that allow fetal sex determination without risk in pregnancy.[26,28] Thus, it was possible to identify Y chromosome sequences in maternal blood and determine fetal sex noninvasively in the first trimester. This process can significantly reduce the number of procedures to be performed in pregnancies at risk of sexrelated diseases, thereby optimizing the management of such pregnancies. According to the studies, it was concluded that fetal sex can be determined with high reliability in early pregnancy stages where cffDNA analysis doesn't result in ultrasound imaging.[26] In addition to its importance in the forensic context, it is recommended that Y-STR profile be used as an alternative technique for fetal sex determination in the third trimester of pregnancy.[29]

As in forensics or archaeology studies, DNA can be obtained from highly degraded tissues. The petrous bone was found to be suitable for STR (Short Tandem Repeat) with electrophoresis. This method compares DNA loci in samples.[27]

RESULTS

Both conventional morphometric measurements and molecular sex determination methods have limitations. Being aware of these limitations is very important for judicial evaluation of human remains. In this context, all available methods of sex estimation should be used for reliable results when creating biological profile.[30]

Various hormones, chemicals and teratogenic agents show sex-related effects not only after birth but also in utero.[27, 31] Understanding the effects of these hormones will be useful for fetal sex determination. The effects of sex hormones on skeletal development and indicators that directly reflect sex characteristics should be investigated.[3] In this respect, the impact of sex on growth and development processes should continue to be investigated.

Studies on fetal ilia have been carried out since the late 1800s. In most of these studies, pelvic components are reported to be reliable in the determination of sex for preadulthood as well as for adulthood. Since the late 1800s, no reliable technique has been developed for a different region.[32] Sex demography of pre-adulthood is an issue that needs to be emphasized both forensic anthropological and bioarchaeological aspects.[32]

CONCLUSION

Past studies have revealed that growth and development show differences between the sexes in the early stages. However, in most of the studies, age ranges have been determined with wide intervals. This is a disadvantage for forensic anthropological fetal sex determination. Focusing

only on morphological indicators in fetal osteology studies can also be misleading. Therefore, metric studies should be developed.

There are study examples in which different parts of the body are referenced to verify the data obtained. Fetal sex prediction researches can be expanded with combinations of genetic applications and various imaging techniques. However firstly, it should be determined whether the applied method is suitable for the sample examined and whether it gives reliable results. Different methods can be tested on populations. Using population-specific data in sex estimation researches is effective in achieving reliable results. Because not every technique has the same reliability ratio for each sample. When applying existing sex prediction techniques, the age group of the sample can be extended until postnatal period as there may be differences in growth and development between populations.

Interdisciplinary cooperation is important in fetal sex prediction studies. The studies will contribute not only to forensic anthropology but also clinically. These studies will provide significant improvements in the future of growth and development research and early detection of sex-based diseases. In this context, information on fetal mortality, health and socioeconomic status of the population can be obtained.

Forensic anthropology should work in conjunction with fields such as embryology, neonatology, developmental biology within the scope of fetal osteology. In particular, genetically based studies should be considered in the development of new techniques as they provide reliable results.

ETHICAL DECLARATIONS

Status of Peer-review: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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