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IMPLICATIONS FOR DETERMINING FETAL MATURITY: A PATTERN OF PRINCIPAL OSSIFICATION SITES IN THE HAND AND FOOT BONES OF FETUSES

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Abstract:
Fetal maturity is one of the most important key components in prenatal care giving evidence on fetal growth and well-being. The goal of this study is to establish a comprehensive pattern of ossification in the bones that constitute human fetal hands and feet, thereby constructing an easy-to-use reference guide to help assess gestational age.

The study included a total of 55 fetuses, with gestational ages ranging from eight weeks to nine months. Specimens were fixed in 70% alcohol before they were processed following a modified Staples and Schnell technique. All the ossification centers were identified through clearing and staining techniques followed by radiographic analysis from six to nine months of gestation.
The research showed how ossification commences, in the order of metacarpals at nine weeks, proximal phalanges at ten weeks, middle phalanges at fourteen weeks and terminal phalanges back to nine weeks. Ossification begins in the foot bones following a similar sequence. Female fetuses ossify first as compared to males while the right side bones develop first than left side ones. The carpal and tarsal bones have no ossification centers until six months of gestation.

The results highlight the importance of skeletal differentiation in assessing fetal maturity. A definite timetable for osteogenesis establishes an accurate basis for estimating post-conception age and identifying developmental abnormalities thereby enhancing prenatal care. It is important that future

studies be conducted to investigate genetic and environmental influences on bone development patterns as well as their long-term implications.
Keywords: Fetal maturity, Ossification patterns, Hand bones, Foot bones

Introduction:
The determination of fetal maturity is a crucial aspect of prenatal care, providing valuable insights into the development and well-being of the fetus. One of the methods to assess fetal maturity involves the study of ossification patterns in the hand and foot bones. [1-3] Ossification, the process of bone formation, occurs at specific sites and follows a distinct chronological pattern, which can be used as an indicator of fetal maturity. [4-6]

There are several reasons why it's important to study ossification sequence in fetal bones. 1st, the accurate assessment of gestational age is essential especially if conception date cannot be accurately established. Next, anomalies of development can be detected earlier hence providing reasons for timely medical intervention. In conclusion, knowledge on the timeline of ossification is helpful in understanding general aspects of fetal skeletal development as well as growth. [7-10]

This research will mainly focus on studying the major sites of ossification in hand and foot bones among fetuses within eight weeks old to nine months old. The objective is to determine a detailed pattern of ossification that would enable more precise estimation of fetal maturity. Thereby this research intends educating clinicians and scientists who are concerned with fetal evaluation and care by documenting appearing and maturing centers of ossification in these bones which provide an all-inclusive reference tool.

Material and Methods:
Ethical approval: The MNR Medical College, Sangareddy, India granted the institutional ethics committee permission for this study. All the parents of fetuses signed a written waiver of informed consent.The embryos and fetuses of both sexes, either aborted or following medical termination of pregnancy, with gestational ages ranging from 8 weeks to 9 months, were preserved in 70% alcohol.

They came from a number of South Indian hospitals. Every fetus was examined visually to ascertain its sex; however, because of the extremely small sample size, the sexes were combined for computation.
This research did not include fetuses that were decalcifying in Bouin's solution, dissected, wounded, dehydrated, or otherwise aberrant. Thus, fifty five fetuses were the final sample size. Recorded were the individuals' sex, crown heel length (CHL), and crown rump length (CRL). Forty seven specimens of CRL varying in size from 20 mm to 25 mm were subjected to defatting in acetone for seven days and dehydration in 70% alcohol for twenty-four hours. The bigger fetuses were washed in 1% KOH until transparent, 2% KOH for two days, and 5% KOH for four days. Embryos that were not fully formed were kept in 2% KOH for two days, and subsequently in 1% or 5% after that became translucent.

The concentration of the KOH solution is dependent upon the size of the specimen in order to avoid over maceration and specimen dissolution. Thus, careful examination is necessary. After being dyed with 1% alizarin red S in 1% KOH solution, the cleaned specimens' ossified areas took on a purple hue. For processing the specimens, we modified the procedure described by Staples and Schnell. [11]
Eight fetuses between six and nine months gestation had their hands and feet radiographed to look for signs of ossification centres in the tarsal and carpal bones. As previously indicated, these samples were then cleaned and dyed. Each specimen was stained, and then allowed to solidify in a benzyl alcohol and glycerine solution before being stored in pure glycerol. Photographs of the specimens were taken using the close-up lens. Because it may identify tiny regions of ossification at an early stage of growth, the clearing and staining approach was preferred for smaller fetuses. [12]

Results:
After being cleared and stained, fifty-seven specimens with CRLs ranging from 20 to 205 mm in both sexes were examined to determine whether ossification centres were present in the different hand and foot bones. On each side, there were two clavicle centres and one mandibular centre in a male embryo measuring 22 mm CRL (seventh week). The ossification centre was absent from the limb bones. The hand and foot bones in neither sex had an ossification centre, even at 28 mm CRL (eighth week).

Table 1: Size, number of seen specimens, sex, and gestational age of each specimen.

Specimen observed					
Gestational age in weeks	**Size of fetus in mm**		**sex and number of specimens**		**Total numbers**
	CRL	CHL	Males	Females	
7	22	-	1	-	1
8	28	30-40	3	5	8
9	37	48	-	1	1
10	41	52	-	2	2
10	47	74	2	-	2
12	71	92-107	1	1	2
13	77	100	3	-	3
13	81	125	2	-	2
14	92	127	-	1	1
14	96	136	2	1	3
15	99	138	-	1	1
15	105	150	-	1	1
16	108	161	-	3	3
16	110	155-180	1	2	3
17	110	190	1	-	1
18	140	208	1	-	1
18	145	212	2	2	4
20	172	202-216	4	5	9
22	205	310	1	-	1
24-36	206-310	310-450	3	6	9
Total			**27**	**30**	**57**

The distribution of ossification was noted in the hand bones from the radial to the ulnar side and from the tibial to the fibular side in the foot bones. Until one female fetus measuring 108 mm CRL (16th week) had the capitate, hamate, and triquetrum on both sides, as well as the trapezium and trapezoid on the left, the centres for the carpal and tarsal bones were absent until six months.
In the ninth week of pregnancy (37 mm CRL and 48 mm CHL), the ossification centre for the metacarpal emerged. The proximal phalanges appeared in the tenth week (41 mm CRL and 52 mm CHL), the middle phalanges arrived in the fourteenth week (92 mm CRL and 127 mm CHL), and the terminal phalanges appeared in the ninth week (37 mm CRL and 48 mm CHL). As a result, the terminal phalange ossification centre emerges before the proximal and intermediate phalange ossification centres.
In a 108 mm CRL fetus (16th week), centres of ossification were seen in the calcaneus and talus on both sides, as well as the navicular and cuboid on the right. The metatarsal ossification centre (71 mm CRL and 92 107 mm CHL), proximal phalanges (92 mm CRL and 127 mm CHL), intermediate phalanges (96 mm CRL and 136 mm CHL), and terminal phalanges (47 mm CRL and 75 mm CHL) all emerged in the 12th, 14th, and 10th week.
The centres were seen in female fetuses earlier than in male fetuses. Compared to foot bones, hand bones were ossified sooner. Compared to the left side bones, the right side bones experienced

ossification earlier. Carpal bone centres are absent from the hands and feet roentgenograms of eight fetuses between six and nine months of pregnancy. At nine months gestation, centres for the calcaneus, talus, and cuboid were seen in one male and two female fetuses.

Discussion:

The determination of fetal maturity through the study of ossification patterns in the hand and foot bones provides critical insights into fetal development and gestational age. This study has successfully documented the chronological emergence of ossification centers, offering a valuable reference for prenatal care.

Ossification Patterns and Gestational Age

In fact, this study has been successful in documenting the chronological emergence of ossification centers as a useful resource for prenatal care. Such results therefore indicate that ossification centers may appear as early as ninth week of pregnancy commencing with the metacarpal bones followed by proximal, middle and terminal phalanges respectively.[13-16] Further**,** the first appearance of ossification centers occur in the terminal phalanges before occurring in proximal and intermediate phalanges thus suggesting some developmental hierarchy of bone formation. N E Budorick et al found similar finding. This seems to be consistent with earlier findings showing that fetal ossification is predictable.

Gender and Laterality Differences

Female Fetuses have shown an advanced stage of ossification than Male Fetuses. Magdalena Grzonkowska (2023) found similar findings. These sex differences in bone maturity suggest genetic or hormonal influences that need further investigation.[17-18] The right side bones were found to have ossified faster than their left counterparts; probably due to some variations in vascular supply or mechanical factors during fetal growth period. Understanding these differences can enhance the precision of fetal maturity assessments. [19-23]

Clinical Implications

Identifying ossification centers is vital for estimating gestational age, especially when the conception date is uncertain. This estimation is crucial for monitoring fetal development, planning medical interventions, and managing high-risk pregnancies. Early detection of developmental anomalies through ossification centers allows for timely interventions, potentially improving prenatal care outcomes.

Future research should investigate the genetic and environmental factors influencing ossification patterns and examine the effects of maternal health and nutrition on fetal bone development. Longitudinal studies tracking fetal development from infancy into childhood could offer deeper insights into the long-term implications of early ossification patterns.

Conclusion:

This study underscores the importance of ossification patterns in determining fetal maturity. The documented chronological emergence of ossification centers in the hand and foot bones provides a reliable framework for assessing gestational age and identifying developmental anomalies. These findings contribute significantly to the field of prenatal care, offering a valuable reference for clinicians and researchers in their efforts to ensure optimal fetal development and health.

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