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DEVELOPMENT OF THE HIP JOINT IN HUMAN EMBRYOS AND FETUSES

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The development of the hip joint was studied using a total of 11 externally normal Japanese embryos (Carnegie stage 19-22) and fetuses (crown-rump length (CRL) 29.8-168.0 mm) with light and scanning electron microscopy (SEM). The articular cavity was first identified at 29.8 mm, and developed as a single continuous space at 78.0 mm. From reconstruction analysis, the lateral and medial circumflex femoral arteries extended into the anterior and posterior parts of the femoral head, respectively, whereas the capitis femoris artery distributed the small area of the top region at 168.0 mm. These data on Japanese were essentially equivalent to those previously reported on Caucasoid. The present SEM study revealed that the surface of the condyle became less irregular and subsurface collagen fibers developed from a loose network to a dense layered structure from 58.0 mm to 168.0 mm.

Key words: hip joint/development/human/light microscopy/electron microscopy

The congenital dislocation of the hip joint is a relatively common anomaly in humans and causes significant motor dysfunction. The normal development of the hip joint has been therefore repeatedly examined to supply the basic informations for the pathogenesis of its anomalies including the congenital dislocation (1-12, the older references therein). However, most of these studies were on Caucasoid, and only one sytematic study on a large number of Japanense embryos and fetuses has not been fully documented (8). Although basic developmental events and their timing are essentially the same between the human races, there have been noted subtle differences in timing and a number of measurements among studies based on different human races, which may be related with different incidences of congenital anomalies and postnatal diseases (13,14). Further, most of the reports on the development of the hip joint were not published recently and mainly utilized macroscopic or light microscopic observation on the development of the femur (1) and the joint cavity (2,3,11), or the vascularization pattern in the femoral head (4,5,10,11). Electron microscopic observation of the surface morphology of the femoral head in humans has been described only for the postnatal period (6,7), but not for the prenatal period.

The present study, therefore, used externally normal Japanese embryos and fetuses, which are a part of a large number of sample collections and from which a series of studies on normal human prenatal development have been performed including the aforementioned previous only one systematic study on the normal development of the hip joint in Japanese (8,15-20). Firstly, we light microscopically observed these embryos and fetuses, described the basic developmental events of the hip joint, and compared these findings with those previously reported on Caucasoid. Secondly, using scanning electron microscopy, we observed and described the normal development of the surface and subsurface ultrastructures of the joint condyle.

MATERIALS AND METHODS

Specimens used in the present study were a total of 11 externally normal human embryos and fetuses (Tables 1 and 2) obtained as previously described (15-20).

For light microscopic observation (LM), specimens were fixed by immersion in either 10% formalin or Bouin's solution at room temperature for an appropriate period and decalcified by immersion in 5% trichloroacetic acid. Samples were embedded in parafin, serially sectioned at five micrometer thick, and stained with hematoxylin and eosin.

Table 1. List of human embryos studied

Carnegie stage	Specimen No.	Crown-rump length, (mm)	Estimated postovulation days
19	70252	15.9 ±0.30*	44**
21	70931	21.1 ±0.26*	48**
22	71039	22.8 ±0.53*	50**

* : Nishimura et al. (13).
** : Nishimura (14).

Table 2. List of human fetuses studied

Specimen No.	Crown-rump length, (mm)	Body weight (g)	Sex	Lunar month
70503	29.8		f	3**
F2802	41.0		m	3**
35234	58.0	13.5	f	3**
F2440	75.0	96.0	m	4**
F2838	120.0	108.0	m	4**
F3220	130.0	170.0	m	5**
F1976	158.0	300.0	f	5**
F2372	168.0	420.0	f	5**

m : male f : female
** : Nishimura (14).

Footnote : This study is dedicated to the late Professor Osamu Tanaka, Department of Anatomy, Shimane Medical University, who originally designed and conducted this study.

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The femoral heads of the two human fetuses of crown-rump length (CRL) 75.0 mm and 168.0 mm (Table 2) were reconstructed using transparent sheets to examine the three dimensional distribution pattern of arterial supply into the femoral head. The contour of the femoral head and the sites of the feeding arteries were traced on transparent sheets and the sheets were accumulated with a spacer between the two consecutive sheets. The reconstructed model was photographed and analyzed three dimensionally.

For electron microscopy, human fetuses fixed similarly to samples for LM were rinsed with water and refixed by immersion in 2.5% glutaraldehyde and 2% paraformaldehyde buffered with 0.1 M sodium phosphate (pH 7.4). The femoral heads were divided into two halves and the one half was prepared for scanning electron microscopy (SEM) in a routine method as described (15,16,19). The other half of the specimen was immresed in 2 N sodium hydroxide solution at room temperature for two to seven days to digest cartilagenous ground substance and to expose subsurface network of the collagen fibers. These specimens were further prepared for SEM observation as similarly to those of the other halves. The samples were observed with a Hitachi S-800 scanning electron microscope at 20 kV.

RESULTS

Light microscopic findings

The cartilagenous primordia of the femoral head and acetabulum were already formed in the embryos examined (Fig. 1a, and data not shown). The articular cavity of the hip joint was not observed in embryos till stage 22, although the mesenchymal condensation, which has been described as the interzone (2), developed at the future joint cavity region (Fig. 1a). In the fetus of CRL 29.8 mm, the joint cavity was first identified in this study (Fig. 1b). In the forming joint cavity, remnant mesenchymal cells and fibers still existed (Fig. 1b). There was no feeding artery localized in the forming femoral head (Fig. 1b). In the fetus of CRL 58.0 mm, the primordium of the capitis femoris ligament (ligamentum teres) was identified, and the cartilage canal through which the feeding vessels invaded was first observed at the transitory region from the femoral head to the neck (data not shown, see below). In the fetus of CRL 75.0 mm, the mesenchymal tissue was no more observed in the joint cavity, which was now a single continuous space except for the ligamentum teres entirely surrounding the spherical femoral head and bordered by the primordial joint capsule at the periphery (Fig. 1c). Feeding arteries were apparently observed in the deeper part of the femoral head than in the former fetus (Fig. 1c). In the fetus of CRL

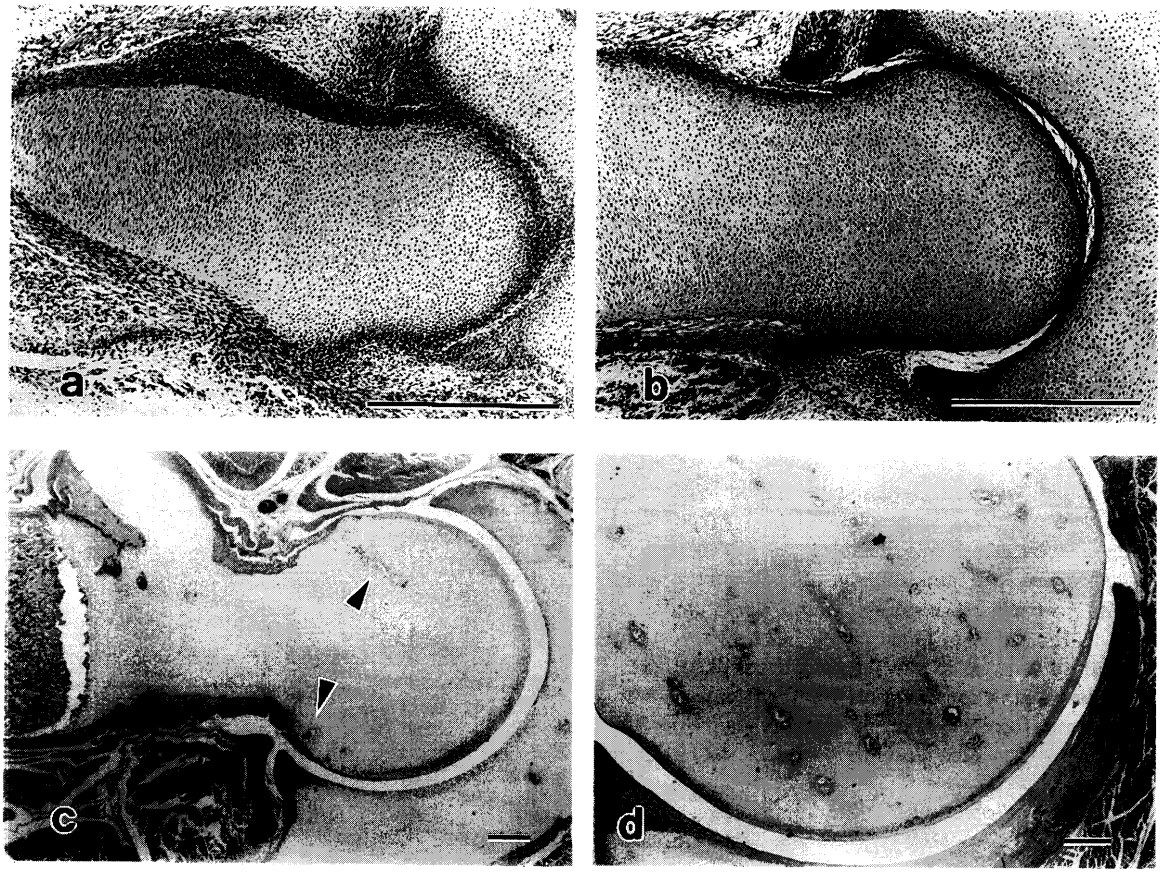

Fig. 1. Photomicrograph of the hip joint. a: The articular space of the hip joint is not observed in a Carnegie stage 22 human embryo. b: The articular space of the hip joint is first identified in a CRL 29.8 mm human fetus. c: Cartilage canals with vessels (arrowheads) are distributed in the lower half of the femoral head in a CRL 75.0 mm fetus. d: Cartilage canals are diffusely found in the whole head in a CRL 168.0 mm fetus. Bars = 500μm

168.0 mm, branches of feeding arteries were distributed dispersely in the femoral head (Fig. 1d). Other samples with intermediate CRLs showed basically the same findings with those described for the smallest one within that range (data not shown).

The pattern of arterial supply of the developing femoral head

From analysis of reconstructed models of the femoral head, the lateral circumflex femoral artery extended into the anterior lower part of the femoral head, whereas the medial circumflex femoral artery into the posterior lower part in the fetus of CRL 75.0 mm (Fig. 2). In the fetus of CRL 168.0 mm, the femoral head was fed by three arteries: the limited area of the top part was distributed by branches of the capitis femoris artery which was a branch from the acetabular branch of the obturator artery and ran through the ligamentum teres, whereas the lower major part of the head by extensive branches almost equally from the lateral and medial circumflex femoral arteries from the anterior and posterior aspects, respectively (Fig. 2).

Surface ultrastructures of the femoral head

SEM observation of the femoral head of the fetus of CRL 58.0 mm revealed its irregular surface with swellings which had generally round contours and flat tops and probably corresponded to the underlying cartilage

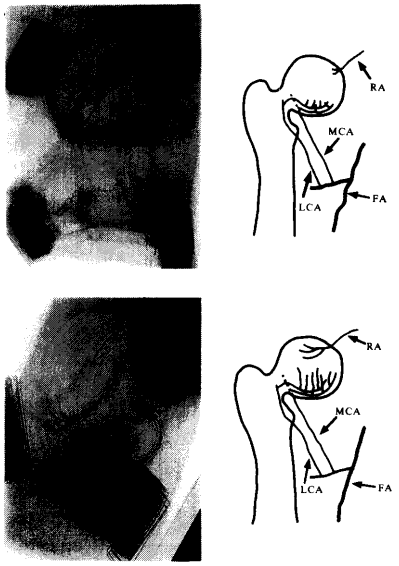

Fig. 2. In the reconstruction analysis, the femoral head has three feeding arteries, the lateral (LCA) and medial circumflex femoral arteries (MCA) from the femoral artery (FA), and the ramus acetabularis (RA) from the obturator artery. Left photos are reconstrcuted models and right figures are the illustrations based on the models. Upper: LCA and MCA invading into the head from the neck of the femoral head in a CRL 75.0 mm fetus. Lower: LCA and MCA invaded into further broader area and RA en tered into the femoral head from the top in a CRL 168.0 mm fetus.

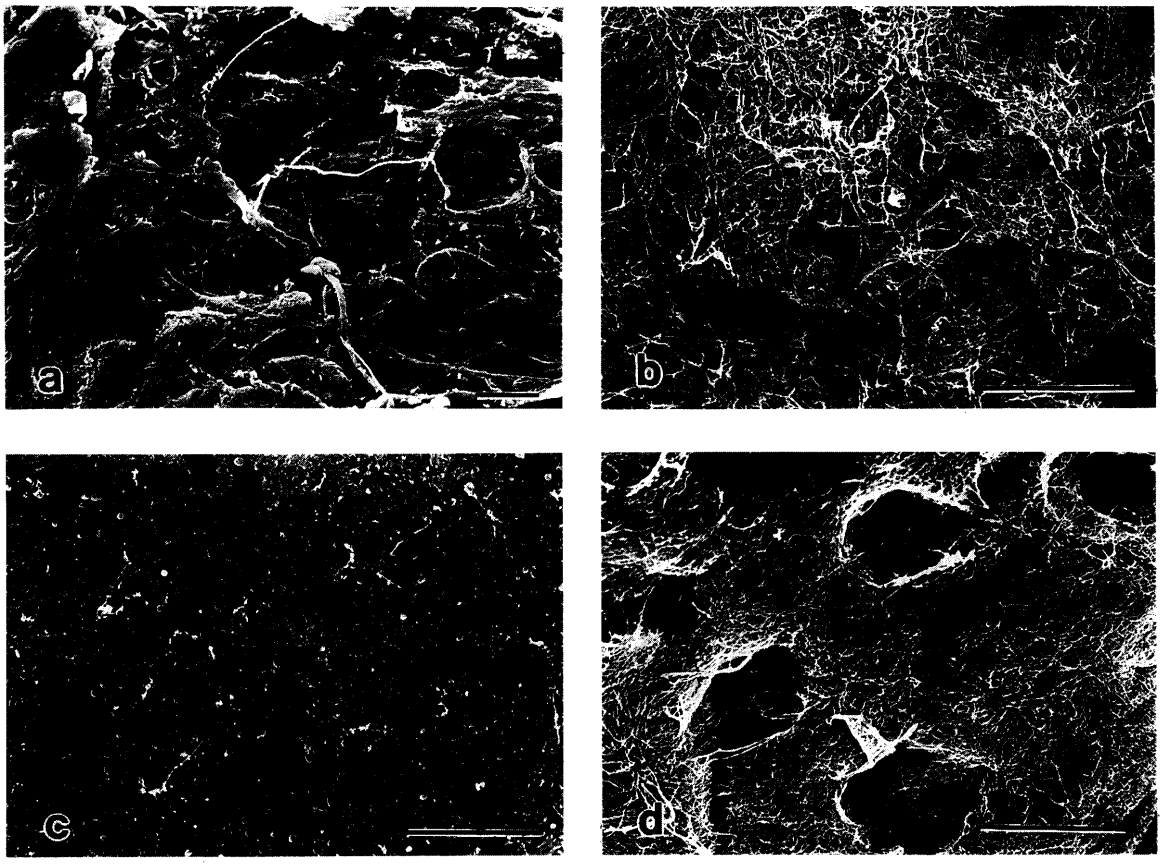

Fig. 3. SEM view of the surface of the femoral head. a: The surface is rough with fibrous structures and swellings in a CRL 58.0 mm fetus. b: After digestion of the specimen shown in a with 2 N NaOH solution. Rough meshwork is noted without layered packing. c: The surface of the femoral head in a CRL 168.0 mm fetus is smoother than that shown in a. d: The digested femoral head of the specimen in c exhibited dense and layered fine fibrils. Bars = 10μm

cells. Thick or fine fibrous structures were also observed that merged from the surface fibrous material and ran randomly forming a network over the surface (Fig. 3a). After digestion of cartilagenous ground substance of the specimen by sodium hydroxide treatment, collagen fibers ran rather loosely without forming any layered strucutre (Fig. 3b). In the fetus of CRL 168.0 mm, the articular surface was much less irregular than that of the former fetus (Fig. 3c). After digestion, hollowed depressions margined by bundles of collagen fibers, which probably corresponded to the places where the cartilage cells were located, were scattered in densely packed and layered fine collagen fibers (Fig. 3d).

DISCUSSION

The present study described the basic developmental events of the hip joint in Japanese embryos and fetuses, and demonstrated that they were essentially equivalent to those reported for Caucasoid (1-7,10-12). The joint cavity has been reported in previous studies on Caucasoid to first appear as a discontinuous space in the interzone, a mesenchymal condensation between the acetabulum and femoral head, at 30.0 mm (2) or 34.0 mm (3). In the present study, we first observed the joint cavity at 29.8 mm. A previous report on Japanese specimens mentioned that the joint cavity formation had completed at around 33 mm (8), which together with the present finding are almost the same with others on Caucasoid. Cartilage canals have been shown to appear at 49 to 50 mm (2), or by 57 mm (1), whereas we first noted it in the fetus of 58 mm CRL. Although we observed the first well-delineated fibrous ligamentum teres at 58 mm, some authors reported it to develop earlier (2,3). This may be due to a larger number of specimens around this size in these previous reports rather than a significant racial difference.

Regarding vascularization, we first recognized the invasion of the feeding vessels into the head at 58 mm, and, at 75 mm, the lateral and medial circumflex femoral arteries already fed the broad area of the femoral head. The vessels within the ligamentum teres with branches into the top part of the femoral head was first evidently observed in the fetus of 168 mm CRL. Previous studies on Caucasoid documented the first cartilage canal as mentioned above (1-3)and vascularization of the ligamentum teres first at 60 mm (2) or 61 mm (3), but the branches into the femoral head were noted only after further increase in the vascularity of the ligament by 195 to 198 mm (2). These findings are thus compatible with ours.

Vascularity patterns of the femoral head by the three feeding arteries, the lateral and medial circumflex femoral arteries and the artery within the ligamentum teres, have been described only from the last trimester of gestation and early postnatal life (4,5). In the present study, the lateral and medial circumflex femoral arteries supply the anterior and posterior region of the small lower part of the head, respectively, at 75 mm. At 168 mm, the artery through the ligamentum teres added a minor supply into the limited top part of the head, whereas the two circumflex arteries vascularized the larger area of the lower part of the head almost equally from anterior and posterior aspects. This pattern is compatible with those observed in fetuses of seven months of gestation (5), which are approximately one month older than the largest fetus in the present study. In these fetuses, the lateral circumflex (anterior) and medial circumflex (posterior) arteries approximately equally supply the proximal femoral chondroepiphysis and growth plate (5). Subsequent early postnatal vascular development is characterized by regression of the lateral circumflex system and concomitant development of the medial circumflex system (5). Although this transition of the vascular pattern from the lateral to medial circumflex arterial supply during early postnatal life may be related to the change in the position of the lower expremities, dramatic increase in the articular movement, or a purely genetic program, no experimental data to support any of these possibilitites have been so far available and the precise mechanisms remain unknown.

Whatever the mechanism for the change of the vascular pattern in the postnatal life, the present data revealed that both the lateral and medial circumflex arteries extend and supply the head almost equally. Therefore, there appear to be no differential mechanisms which operate for their development during the prenatal life.

The vessels within the ligamentum teres was observed with some branches into the small area of the condyle head at 168 mm in the present study. There has been argument on the role of the the vessels of the ligamentum teres to supply the femoral head during the prenatal and early postnatal life (4,5,10,11). Most of authors have agreed that the vascularity of the ligament itself increases in the latter half of the prenatal life, but only the articular cartilage or at most a minor area of the head is supplied by these vessels. The present finding also supported this notion.

Surface ultrastructures of the condyle of the hip joint have been observed only for postnatal life (6,7, and references therein) and in relation with pathological conditions (21, and references therein). In the adult, surface depressions with diameters from 15 to 30 μm were observed and interpreted as due to the collapse of the surface layer into the underlying lacunae in the collagen fiber network (6), although the existence of these depressions *in vivo* has not yet been established. In the present study on fetuses, we did not observe depressions with the corresponding sizes nor subsurface lacunae, but noted swellings with around 5 μm diameter. These swellings appeared to correspond to subsurface cartilage cells, since after digestion, we observed hollows in the dense meshwork of the collagen fibers suggesting the location of the cells. Although any specific orientation of the fibers in the network was noted, the fibers became apparently denser and more layered during the develoment from 58 mm to 168 mm. It has been argued whether the formation of collagen fiber network which is organized in different orientations depending on the depth from the articular surface is guided by the compression and tension forces to which they are subject, or purely by genetic programs (7). Interestingly, the

present findings on human fetuses of 58 mm and 168 mm roughly corresponded to those on rats of embryonic day 18 and postnatal day 6, respectively (24). This suggests that the development of the surface ultrastrucutres during this period is not affected by mechanical forces, since similar changes happen both prenataly in humans and postnataly in rats, although rats by postnatal day 6 still do not move very actively. However, human fetal movements have been shown to develop fairly early (22). Most of the movements described during the course of gestation have already emmerged before 16 weeks (22) and movements of the lower extremity are relatively active (23). Therefore, it remains still uncertain and intriguing to examine whether the development of the condyle including the surface structures is affected by articular movements. The results from our separate study using the expreimental inhibition of the fetal movements of the hip joint in rats suggested that development of the surface structures of the femoral head is significantly influenced by fetal movements (24).

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