Original Article
What is the relationship between the breech presentation and hip dysplasia? An experimental study on a rat model

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Abstract: The relationship between breech presentation and Developmental Dysplasia of the Hip (DDH) had been demonstrated through epidemiological methods, but the mechanism of such correlation and the pathological process of breech-related hip dysplasia remain unclarified. The purposes of this study were: (1) to establish an animal model to best simulate breech presentation; (2) to investigate how breech presentation influenced the severity of DDH and (3) to analyze the pathological development of the acetabulum and the femoral head. Newborn rats were swaddled to keep the hip flexed and knees extended to simulate human breech presentation. At 0, 2, 4, 6, 8 day after birth the specimen of the rats was stained and postero-anterior pelvic picture of the rat skeletons were taken to observe the relationship of the acetabulum and the femoral head. Sections of the hip were stained with Safranin O-Fast Green to assess the histopathologic changes of the acetabulum and the femoral head. In the pelvic pactures, cartilage acetabular index (CAI), center-edge angle (CEA) and acetabular diameter/depth ratio (D/D) were measured. The incidence of DDH increased and the severity aggravated with the swaddling time. CAI in the experiment group was significantly larger than that of the control group since day 2. CEA decreased with time in the experiment group, while D/D increased obviously. Pathological changes of the hip joint emerged in the early stage of breech presentation, and aggravated with growth. Lateral bending of the ischium and thinner articular cartilage were observed in the dysplastic hips. In conclusion, the more time in breech position, the more incidence and severity of DDH. Breech-related DDH was a chronic process that proceeded from mild dysplasia to subluxation and then to frank dislocation.

Keywords: Developmental dysplasia of the hip, breech presentation, animal model, etiology, pathology

Introduction
Developmental dysplasia of the hip (DDH) is a spectrum of anatomical abnormalities of the hip. The incidence of DDH in infants is diverse in different area, which might be due to different genetic susceptibility and diagnostic methods [1]. Risk factors of DDH include female gender, breech presentation, first-born children and family history, among which breech presentation is the most frequently addressed [2-4]. Previous studies demonstrate the relationship between breech presentation and DDH through epidemiological methods [5, 6]. Few reports had looked into the mechanism of such correlation.

Since human studies are inevitably scarce because of ethical concerns, animal models became the first choice. Up till now there has been no exact method to induce intrauterine breech presentation, so studies exclusively utilized young animals to establish the model. Wilkinson had demonstrated that breech malpositioning might increase the likelihood of a hip disorder in generalized joint laxity [7], and proposed that this malpositioning might have a genetic origin [8]. Since then, there have been no studies concerning the pathological process of breech-related hip dysplasia.

The aims of this study were: (1) to establish an animal model to best simulate breech presentation; (2) to investigate how breech presentation influenced the severity of DDH and (3) to analyze the pathological development of the acetabulum and the femoral head.
Materials and methods

Animal model

The study utilized 270 newborn Wistar rats from 23 litters. 155 rats from 14 litters were swaddled as below. 115 rats from 9 litters were left untreated as controls. All the rats fed from their mothers, and care was taken to guarantee their normal intake. Rats were swaddled with medical tape (3 MD urapore, St.Paul, Minnesota) to keep the hips flexed and knees extended and the hindlimb lateral rotated so as to simulate human frank-breech presentation with lateral rotation.

Whole skeletal staining with alcian blue and alizarin red

185 newborn Wistar rats (110 from the experimental group and 75 from the control group) were euthanized by 5% chloral hydrate at 0, 2, 4, 6, 8 days after birth respectively. The 0 day subgroup in the experimental group were swaddled and euthanized right afterward. Remove the skin and internal organs carefully and fix the skeleton in 75% ethanol for more than 3 days. Cartilage tissue of the specimen was stained with 0.2% Alcian Blue 8GX (Sigma, St Louis, MO, USA) (dissolved in ethanol; glacial acetic acid = 7:3) and mineralized bone was stained with 0.008% Alizarin Red S (Sigma, St Louis, MO, USA) (dissolved in 0.5% potassium hydroxide). The specimen was then treated with a graded series of 0.5% potassium hydroxide/glycerol, and stored in glycerol with a crystal of thymol.

Gross observation

Postero-anterior pelvic picture of each rat skeleton was taken to observe the relationship of the acetabulum and the femoral head. Cartilage acetabular index (CAI), center-edge angle (CEA) and acetabular diameter/depth ratio (D/D) were measured at the same time (Figure 2). The measurement was performed by two experienced doctors (X.R. and C.S.) respectively to assess the inter-observer variation. One of the observers measured the data again one month afterward to assess the intra-observer variation. The average of the three results was used as the final data. Hips were categorized as dislocated, subluxated, mildly dysplastic and normal. Dislocation was defined as spatial change of the femoral head beyond the lateral edge of the acetabulum. Subluxation was defined as spatial change of the femoral head while still remaining under partial coverage of the acetabulum. The hip was identified as mild dysplasia when the acetabulum and the femoral head were generally in congruence but: (1) CAI or D/D was above 120% of the maximal value in the control group, or (2) CEA was below 80% of the minimal value in the control group.

Histological assessment

Altogether 85 rats (45 from the experimental group and 40 from the control group) were euthanized at 0, 2, 4, 6, 8 days after birth. The hips were dissected en bloc, fixed in 10% neutral buffered formalin, and then transferred to 10% EDTA solution for decalcification and par-
affin embedding. The paraffin blocks were sectioned coronally at a thickness of 4-μm. Sections with the deepest position of the acetabulum were deparaffinized, rehydrated, and stained with 0.5% Safranin O (Sigma, St Louis, MO, USA) (dissolved in EtOH: H₂O = 1:1) and Fast Green (Sigma, St Louis, MO, USA) (dissolved in EtOH: H₂O = 19:1) for 10 min respectively at room temperature.

### Statistical Analysis

Statistical analysis was performed with statistical package for social science (SPSS, version 17.0; Chicago, Illinois). Independent-samples t-test was used to analyze differences between groups. Chi-square test was used to compare the incidence of acetabular dysplasia in both genders. Bivariate correlation was used to explore the relationship of swaddling duration and incidence of hip dysplasia. The intra-class correlation coefficient (ICC) was adopted to investigate the intra- and inter-observer reliability. An ICC>0.75 was regarded as excellent, ICC 0.40 to 0.75 was fair to good, and ICC<0.40 was poor. P<0.05 was regarded as statistically significant.

### Results

In the study of the whole skeletal staining, 9 rats in the experimental group and 2 in the control group died during the experiment. The remaining rats in the experimental group developed as normally as the control group at each time point. 202 hips of 101 rats in the experimental group and 146 hips of 73 rats in the control group were finally included in the analysis. The intra- and inter-observer reliability was shown in Table 1. The incidence of DDH increased with the swaddling time (Table 2; Figure 3). It was 0% (0/34) at the baseline, 27.5% (11/40) at the 2nd day, 61.36% (27/44) at the 4th day, 70.45% (31/44) at the 6th day and 82.5% (33/40) at the 8th day. DDH aggravated with time, as reflected by increasing percentage of frank dislocation. The correlation between swaddling duration and incidence of DDH fit a positive rectilinear model ($r = 0.970$, $P = 0.006$). But the difference of the incidence between dislocated and subluxated was not significant in any group according to

### Table 1. The observer variation of data measured from the pictures

<table>
<thead>
<tr>
<th>Data</th>
<th>Intra-observer variation</th>
<th>Interobserver variation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intra-class correlation coefficient</td>
<td>95% confidence interval</td>
</tr>
<tr>
<td>CAI</td>
<td>0.847 (0.815, 0.874)</td>
<td>0.818 (0.779, 0.850)</td>
</tr>
<tr>
<td>CEA</td>
<td>0.942 (0.929, 0.953)</td>
<td>0.948 (0.936, 0.957)</td>
</tr>
<tr>
<td>D/D</td>
<td>0.925 (0.908, 0.939)</td>
<td>0.841 (0.807, 0.869)</td>
</tr>
</tbody>
</table>
Breech presentation and hip dysplasia

chi-square tests. CAI in the experiment group was significantly larger than that of the control group since day 2. CAI of the control group decreased with age, while in the experiment group it showed an upward tendency. Meanwhile CEA decreased with time in the experiment group, while D/D increased obviously. These two parameters in the control group remained constant in all ages (Table 3 and Figures 4-6).

Although female rats had higher DDH incidence in each time point, this gender difference was statistically significant only at the 2nd day (P = 0.031, Fisher probabilities in 2×2 table) (Figure 2). Then we compared the parameters of the male and female rats in the experiment group on

Table 2. The incidence of abnormality in the experiment group of different gender at each time point. (Mild dysplasia/subluxation/dislocation/total)

<table>
<thead>
<tr>
<th>The time points (day)</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>0/0/0/16</td>
<td>2/0/0/20</td>
<td>4/6/2/22</td>
<td>6/4/5/22</td>
<td>5/2/9/20</td>
<td>17/12/16/100</td>
</tr>
<tr>
<td>Female</td>
<td>0/0/0/18</td>
<td>9/0/0/20</td>
<td>6/7/2/22</td>
<td>5/5/6/22</td>
<td>4/2/11/20</td>
<td>24/14/19/102</td>
</tr>
<tr>
<td>Total</td>
<td>0/0/0/34</td>
<td>11/0/0/40</td>
<td>10/13/4/44</td>
<td>11/9/11/44</td>
<td>9/4/20/40</td>
<td>41/26/35/202</td>
</tr>
</tbody>
</table>

Figure 3. The rate of various pathoanatomical categorizations of the affected hips in experimental group (M: male; F: female).

Table 3. The Comparison of CAI, CEA and D/D Between the Experimental and Control Groups

<table>
<thead>
<tr>
<th></th>
<th>CAI</th>
<th>p value</th>
<th>CEA</th>
<th>p value</th>
<th>D/D</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 day in Exp</td>
<td>27.12±2.06</td>
<td>0.357</td>
<td>28.18±1.93</td>
<td>0.623</td>
<td>1.96±0.13</td>
<td>0.708</td>
</tr>
<tr>
<td>0 day in Col</td>
<td>27.61±2.08</td>
<td></td>
<td>28.43±2.08</td>
<td></td>
<td>1.97±0.12</td>
<td></td>
</tr>
<tr>
<td>2 day in Exp</td>
<td>34.85±2.45</td>
<td>0.000*</td>
<td>17.63±3.92</td>
<td>0.000*</td>
<td>2.86±0.18</td>
<td>0.000*</td>
</tr>
<tr>
<td>2 day in Col</td>
<td>27.54±2.82</td>
<td></td>
<td>27.82±2.84</td>
<td></td>
<td>1.90±0.12</td>
<td></td>
</tr>
<tr>
<td>4 day in Exp</td>
<td>39.16±2.73</td>
<td>0.000*</td>
<td>10.30±14.42</td>
<td>0.000*</td>
<td>3.10±0.44</td>
<td>0.000*</td>
</tr>
<tr>
<td>4 day in Col</td>
<td>20.80±2.04</td>
<td></td>
<td>31.10±3.04</td>
<td></td>
<td>1.86±0.11</td>
<td></td>
</tr>
<tr>
<td>6 day in Exp</td>
<td>40.34±3.39</td>
<td>0.000*</td>
<td>-1.14±35.33</td>
<td>0.000*</td>
<td>4.19±0.60</td>
<td>0.000*</td>
</tr>
<tr>
<td>6 day in Col</td>
<td>17.25±1.32</td>
<td></td>
<td>32.84±2.53</td>
<td></td>
<td>1.95±0.19</td>
<td></td>
</tr>
<tr>
<td>8 day in Exp</td>
<td>46.75±7.24</td>
<td>0.000*</td>
<td>-25.58±43.93</td>
<td>0.000*</td>
<td>5.18±0.53</td>
<td>0.000*</td>
</tr>
<tr>
<td>8 day in Col</td>
<td>17.07±1.68</td>
<td></td>
<td>32.43±2.73</td>
<td></td>
<td>1.91±0.16</td>
<td></td>
</tr>
</tbody>
</table>

All data were presented as mean ± standard deviation. *Indicates statistical significance compared to the controls at P<0.05. Exp Indicates experimental group; Col Indicates control group.
every time point and found almost no significant difference (Table 4).

Besides, we observed some changes in the gross morphology of dysplastic hips and affected pelvis, including lateral bending of the ischium and thinner articular cartilage (Figures 4-6).

Pathological changes in the acetabulum and femoral head

3 rats in the experimental group and 2 rats in the control group died before harvest. The remaining hips were included in the study. Safranin O-Fast Green staining presented red color on the cartilage of 0, 2, 4 day after birth. The red staining was lighter than day 6 and 8, especially that of day 0. In 0 day and control group of every day, the acetabulum was a deep-set cavity that almost totally enclosed the femoral head. The inner wall of the cavity was smooth. As growth proceeded, the absolute depth of the cavity increased and the femoral head increased in size and became more globular (Figure 7).

In the experimental group, the cavity was shallow and filled with hypertrophic soft tissue. The inner wall was rough and the femoral head became flat. At the 4th day, the coverage of the acetabulum to the femoral head was reduced. The femoral head pressed against the upper labrum while the upper labrum everted, leading to hip subluxation. At the 6th and 8th day, the hip became dislocated. The femoral head moved beyond the lateral edge of the acetabulum and a false acetabulum formed. Both the upper and the lower labrum became inverted. These pathological changes were more serious at day 8 (Figure 7A2, 7B2, 7C2).
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Discussion

Breech presentation had been reported to be a risk factor of DDH, while their relationship had been mostly approached through epidemiological methods [5, 6, 9]. The incidence of DDH in single breech presentation was 20% compared to 0.7% in normal cephalic presentation [10]. Breech presentation with vaginal birth could bring about a 17 fold increased risk of DDH [2]. Wilkinson proposed that delayed leg-folding, a prenatal phenomenon directly linked to breech presentation, had a percentage of as high as 20% in developing DDH in the future [8]. Although the association between breech presentation and DDH had been well-established, there had been no experimental study on its pathological process. Therefore, our study aimed at disclosing the pathogenesis of breech-related DDH based on a newborn rat model.

There are several limitations in this study. First of all, we used newborn rats to simulate breech presentation rather than establishing a real intrauterine model. At the beginning of this study we endeavored to establish such a model by connecting the skin of the hindlimb with the chest wall through intrauterine operation, but none of the operated pups survived the delivery. Second, a continuous observation of the hip joint in one animal would have been more conclusive, but radiological methods such as X-ray and MRI could not function well in such tiny and cartilaginous hips in newborn rats. Also, there are many forms of breech presentation, with the lower limbs stretched, flexed or one flexed and the other extended, but our study only investigated the most common form (Frank breech). We also keep the hindlimb laterally rotated because it was easy to perform in this model with medical tape. However, the different position of the hindlimb might have some influence on the incidence and severity of breech-related DDH.

Animal models had been frequently used to study the pathology of DDH. Hindlimb restric-
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Figure 6. The staining of rat skeleton (15×): (A, B) Were the rats of the 6 and 8 day in experimental group and (C, D) were the rats in control group. In (A) and (B), the femoral head was severely deformed and beyond the lateral edge of the acetabulum with lateral bending of the ischium and thinner articular cartilage compared with the hips in (C) and (D).

Table 4. The CAI, CEA and D/D of different genders in the experiment group

<table>
<thead>
<tr>
<th></th>
<th>CAI</th>
<th>p value</th>
<th>CEA</th>
<th>p value</th>
<th>D/D</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 day male</td>
<td>27.00±2.34</td>
<td>0.758</td>
<td>27.94±1.88</td>
<td>0.505</td>
<td>1.95±0.13</td>
<td>0.660</td>
</tr>
<tr>
<td>0 day female</td>
<td>27.22±1.83</td>
<td>28.39±2.00</td>
<td>1.97±0.13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 day male</td>
<td>34.30±2.56</td>
<td>0.157</td>
<td>16.55±3.79</td>
<td>0.083</td>
<td>2.81±0.20</td>
<td>0.080</td>
</tr>
<tr>
<td>2 day female</td>
<td>33.40±2.26</td>
<td>18.70±3.84</td>
<td>2.91±0.14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 day male</td>
<td>40.14±2.95</td>
<td>0.016*</td>
<td>9.45±13.70</td>
<td>0.704</td>
<td>3.14±0.51</td>
<td>0.526</td>
</tr>
<tr>
<td>4 day female</td>
<td>38.18±2.13</td>
<td>11.14±15.38</td>
<td>3.05±0.37</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 day male</td>
<td>41.00±3.34</td>
<td>0.201</td>
<td>-1.23±38.94</td>
<td>0.987</td>
<td>4.19±0.61</td>
<td>0.980</td>
</tr>
<tr>
<td>6 day female</td>
<td>39.68±3.39</td>
<td>-1.05±32.23</td>
<td>4.18±0.59</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 day male</td>
<td>46.90±8.50</td>
<td>0.898</td>
<td>-23.80±46.01</td>
<td>0.802</td>
<td>5.26±0.63</td>
<td>0.345</td>
</tr>
<tr>
<td>8 day female</td>
<td>46.60±5.95</td>
<td>-27.35±42.87</td>
<td>5.10±0.40</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All data were presented as mean ± standard deviation. *Indicates statistical significance between genders at P<0.05.

We fixed the rats in hip flexion and knee extension with medical tape to simulate the intrauterine breech posture that was most associated with DDH [10]. Our previous study used the same tape to mimic traditional straight-leg swaddle [14]. Compared to rigid fixations, the elasticity of medical tape could allow for minor movement which was closer to the natural circumstance of intrauterine breech position. Breech presentation was usually fixed by the time of 32 weeks of gestation when the legs were confined to different planes of rotation [8], so we started swaddling right...
after birth in order that the morphology and developmental potential of the hip was the closest to intrauterine status.

We examined some rats right after swaddling and found dislocation or dysplasia in none of the rats. This guaranteed that dislocation/subluxation/dysplasia was not directly caused by the manipulations of swaddling. After two days some of the hips showed dysplastic features but there were no dislocation/subluxation. On the fourth day on dislocation/subluxation app-
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...eared in more than half of the swaddled rats. Since then the percentage of frank dislocation increased while that of subluxated cases decreased, with the total number of affected hips rising dramatically. The longer the swaddling lasted, the higher the incidence and severity of DDH. This indicated that the pathogenesis of breech-related DDH was a chronic process, in which the affected hip developed gradually from mild dysplasia to subluxation and eventually to frank dislocation. Breech presentation may place mechanical restraint on the femoral head towards the acetabulum and thus lead to malpositioning of the hindlimb [2, 4]. This would further disturb the growth and molding of the 'socket'-like surface of the acetabulum. The acetabulum became so shallow that the femoral head would slip away from the acetabulum easily.

Pelvic deformities had been noticed in DDH patients as well as animal models. Delgado-Baeza reported anterior bending of the ilium, lateral tilt of the innominate bone and contralateral pelvic rotation in a DDH model of rats and attributed this change to disturbance of the normal growth of triradiate cartilage [15]. Our previous study found that in DDH rats, the ischium rotated up and forwardly around the posterior and vertical limbs of the triradiate cartilage complex respectively just as a lifted piece of Pizza [16]. In this study we observed lateral bending of the ischium and thinner articular cartilage. Our future study would focus on the developmental mechanism of such abnormalities.

Female gender had been well recognized as a risk factor of DDH. There was a female: male ratio of approximately 4:1 [6, 17-19]. In our study, however, we found no difference in the incidence of DDH between the two genders except in the 2 day group. Female gender was prone to hip dysplasia, as demonstrated by more hip dysplasia after swaddling for two days. Prolonged swaddling, however, overwhelmed the influence of gender and resulted in similar incidence of DDH. This overwhelming effect of swaddling was also observed in our previous study [14].

In the Safranin O-Fast Green staining, basophilic cartilage combined with safranin and showed red, while eosinophilic bone bound with fast green and showed green or blue. In this way the cartilage and the bonytissue were differentiat-ed. The mechanism was that safranin combined with chondroitin sulfate (CS) and keratan sulfate (KS) in the proteoglycan. In our study, safranin staining was light in 0, 2 and 4 days, suggesting that the expression of CS and KS was low in the early developmental stage of the hip. After two days of swaddling, the acetabular cavity was shallow and occupied by hypertrophic soft tissue. The inner acetabular wall was rough and the femoral head became flat. These phenomena indicated that pathological changes had existed ever since the early stage of DDH. These pathological changes would aggravate with age, so that treatment would be more challenging and the outcome would be less satisfactory.

In summary, this study investigated the pathological process of breech-related DDH. Breech presentation had adverse influence on the normal development of the hip. There was a positive linear correlation between swaddling time and the incidence of DDH. Breech-related DDH was a chronic process that proceeded from mild dysplasia to subluxation and then to frank dislocation. Lateral bending of the ischium and thinner articular cartilage was observed. Prolonged swaddling seemed to overwhelm the susceptibility of female gender. Early diagnosis would reduce the pathological changes and yield better outcome.

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Disclosure of conflict of interest
None.

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References
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