Objective: The aim of this study was to detect the incidence of estrogen receptors in human hip joint capsule and ligamentum teres.

Methods: The study included biopsies of the ligamentum capitis femoris (LCF) and hip joint capsule from 15 patients undergoing hip surgery for developmental dysplasia of the hip (DDH) and from the control hips of 15 cases of intrauterine fetal death. Mean age was 10.3 (range: 6 to 18) months at the time of surgery. Full-thickness 1x1 cm anterior capsule and LCF portions were taken as biopsy specimens. An immunohistochemical study using monoclonal antibody against estrogen receptors was performed to identify the rate of target estrogen cells in the hip joint capsule and LCF.

Results: Estrogen receptor (ER) staining rates were 1.6±0.2% for the LCF and 1.3±0.2% for the hip joint capsule in the control groups, and 2.5±0.3% for the LCF and 2.0±0.3% for the hip joint capsule in the DDH groups. Estrogen receptor staining rates in the LCF and hip joint capsule control groups were significantly lower than that in the DDH groups (p<0.001). In both groups, ER rates were significantly lower in the hip joint capsule than in the LCF (p<0.01).

Conclusion: The high rate of ERs in the LCF and hip joint capsule appears to support the effect of estrogen in the etiology of the DDH.

Key words: Developmental dysplasia of the hip; estrogen receptor; hip capsule; ligamentum capitis femoris.

Developmental dysplasia of the hip (DDH) is a neonatal condition with various causes. Within the outline provided by the genetic code, embryonic, fetal and childhood development of the hip continue to a variety of environmental and biomechanical factors.° Currently, only few laboratory studies have provided information on hormonal factors effective on the etiology of DDH.°

Undue laxity of the hip joint capsule and ligamentum teres at the time of birth is the most important structural deformity that permits the initial dislocation.

Estrogen has been shown to affect the composition and structure of a variety of tissues.° Estrogen receptor (ER) proteins in target cells are necessary for hormone action.° It is very likely that estrogen may influence the
structure and composition of the hip capsule and liga-
mentum teres as it does in other tissues. The exact
pathophysiology of the etiology of DDH is unclear.
Therefore, the aim of this study was to analyze the inci-
dence of ERs in the ligamentum capitis femoris (LCF)
and hip capsule in order to explore the hormonal effect
on the development of DDH.

Materials and methods
Approval was obtained from the local scientific depart-
ment of our hospital and consent to study participation
from the families prior to surgery. The study included
LCF and hip joint capsule biopsies from hips of 15
patients undergoing hip surgery for DDH (open
reduction through medial or anterolateral approaches,
Salter’s osteotomy) and from 15 cases of intrauterine
fetal death. All dislocated hips were unilateral. Fetal
hips were examined and found to be normal. Mean age
of the subjects was 10.3 (range: 6 to 18) months at the
time of surgery. The 60 specimens were divided into
DDH and control groups and further divided into
LCF and hip joint capsule groups. Full-thickness 1x1
cm anterior capsule and LCF portions were taken as
biopsy specimens. All babies undergoing surgery for
hip dysplasia were female.

Commercial monoclonal estrogen receptor anti-
body Estrogen Receptor Ab-11 (Clone 1D5) MW:
67kDa (Fisher Scientific, Loughborough, UK) was
obtained. A sample of breast carcinoma specimen was
used for both positive and negative controls. For
the negative controls, IgG1 immunoglobulin was substi-
tuted for the anti-estrogen receptor antibodies. One
positive and one negative control sample were includ-
ed in all assays.

Tissue sections were deparaffinized in xylene and
hydrated in a graded series of alcohol. Slides were then
placed in Tris-buffered saline for 15 minutes followed
by washing in 0.1% Tween/phosphate-buffered saline
(pH: 7.4) solution. Blocking of nonspecific antibody
binding was achieved with non-immune serum for 30
minutes at room temperature. The slides, including
positive and negative controls, were incubated with the
respective primary antibodies for 16 hours at room
temperature in a humidity chamber. Subsequently the
secondary link antibody (LabVision; Biotinylated Goat
Anti-polyvalent) followed by streptavidin-biotin per-
oxidase complex were separately applied in a humidity
chamber. The substrate diaminobenzidine (DAB)
tetrahydrochloride containing %0.024 hydrogen per-
oxidase in phosphate-buffered saline (pH: 7.4) was
applied. Sections were dehydrated in graded alcohol
and coverslips were applied with Entellan (Merck,
Darmstadt, Germany).

Estrogen receptor staining was quantified as the
rate of positive stained cells in 100 connective tissue
cells. Statistical analyses were performed by independ-
ent samples t-test and paired samples t-test. P values
of less than 0.05 were considered statistically significant.
Data were analyzed using the SPSS for Windows
v.11.5 computing program (SPSS Inc., Chicago, IL,
USA).

Results
Estrogen receptor positive staining was located in nuclei
of some connective tissue cells of all categorized tissue
samples (Figs. 1 and 2). Estrogen receptor positive stain-
ing values are shown in Table 1. Estrogen receptor posi-
tive staining for the LCF and hip capsule control
groups were significantly lower than those of the DDH
groups (p<0.001). Estrogen receptor positive staining
rate was 1.6±0.2 per 100 connective tissue cells for LCF
and 1.3±0.2 for hip capsule in the control group, where-
as it was 2.5±0.3 for the LCF and 2.0±0.3 for the hip
capsule DDH groups. In both groups, we found ER
positive staining significantly lower in the hip capsule
specimens when compared to LCF samples (p<0.01).

Discussion
The possible effect of maternal hormones on the hip
capsule leading to joint laxity has been previously
described in the literature.[3-6] Prior studies have
hypothesized that a newborn’s response to maternal
hormones may explain the higher incidence of DDH
in females. However, maternal hormones affect both
male and female fetuses. As detection of specific recep-
tors on tissue can prove hormonal activity, we decided
to explore estrogen receptors in the hip joint.

Hormone action at the cellular level begins with
the association of the hormone and its specific recep-
tor. Estrogen receptor is a nuclear protein synthesized
in cytoplasm and then rapidly transferred to the nucle-
us.[8] Estrogen and progestins bind their intracellular
receptors and cause conformational changes of the lat-
ter.

Wynne-Davies described hereditary ligamentous
laxity as one of two major mechanisms for the inheri-
tance of DDH.[1] Peripheral joint laxity produced in the
fetus is attributed to the maternal hormones entering
fetal circulation during the second and third trimesters.[1]

Collagen content is negatively influenced by estro-
gen. We believe that this might be the major cause
behind the relaxation of connective tissue. Studies have
shown the laxity producing effect of estrogen over con-
nective tissue.
Liu et al. reported that the laxity producing effect of estrogen on anterior cruciate ligament caused female athletic injury and reduced collagen synthesis with increasing amounts of 17beta-estradiol levels.\textsuperscript{[7]} In another study, Akeson et al.\textsuperscript{[6]} showed that administration of 17beta-estradiol administration decreased the content of soluble collagen fractions in periarticular tissue. They proposed that the therapeutic use of 17beta-estradiol in the short-term treatment of injuries or diseases results in enforced immobilization manifested as joint contractures.

Andren et al. analyzed the excretion of conjugated estriol, conjugated estrone and 17beta-estradiol of newborns with DDH and found higher levels in the DDH group than the controls.\textsuperscript{[10]} In another study, the association between 17beta-estradiol from umbilical cord blood and neonatal hip instability was evaluated and high levels of 17beta-estradiol tended to be associated with an increased risk of neonatal hip instability in girls.\textsuperscript{[11]}

Nakamura et al. studied TGF-beta1 and reported that TGF-beta1 induced a dose-dependent contraction of collagen gels containing bovine trabecular meshwork cells.\textsuperscript{[12]} Previous studies suggested that estrogens suppress TGF-beta-induced gene expression.\textsuperscript{[13]} We believe that a decrease in collagen contraction due to the effect of estrogen through TGF-beta on collagen may increase the laxity of tissues around the hip.

Shynlova et al. reported that the levels of collagen Type 1 and Type 3 in rat uterine tissue correlated with gestational changes in the plasma estrogen/progesterone ratio.\textsuperscript{[14]} Administration of estrogen causes a decrease in the total amount of collagen in the rat hip joint capsule, skin, aorta, and tail tendon.\textsuperscript{[15-18]}

Estrogen receptors were found in all samples. Estrogen receptors in the LCF and hip capsule specimens of the control group were significantly lower than in the DDH group. This result may be attributed to the receptor up-regulating effect of estrogen. As the positively stained ER concentration was higher in LCF samples than hip joint capsule samples, LCF may be affected more by estrogen than the hip joint capsule. This difference in receptor rates may not be limited only to the hip joint but may also be valid for other joints. Therefore, similar receptor studies should be performed for other tissues as well.

**Table 1.** Estrogen receptor positive staining for the LCF and hip capsule of the DDH group and the control group.

<table>
<thead>
<tr>
<th></th>
<th>DDH group</th>
<th>Control group</th>
<th>p value</th>
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<tbody>
<tr>
<td>LCF ER (%)</td>
<td>2.5±0.3</td>
<td>1.6±0.2</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Hip capsule ER (%)</td>
<td>2.0±0.3</td>
<td>1.3±0.2</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>p value</td>
<td>p&lt;0.01</td>
<td>p&lt;0.01</td>
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</table>
A disadvantage of our study was that the mean ages of the control and the DDH group were different, which may have affected the receptor rates.

In conclusion, the high rate of ERs in LCF and hip joint capsule appears to support the effect of estrogen in the etiology of DDH.

Conflicts of Interest: No conflicts declared.

References