There is evidence that ligamentous laxity is influenced by female hormones, as joint hypermobility is more common in women than in men (8). Pregnancy also dramatically alters soft tissue structure and properties. During pregnancy, estrogen and relaxin levels are elevated, which affect fibroblast function. Estrogen reduces type I collagen synthesis and fibroblast proliferation (13). Relaxin decreases the synthesis and secretion of interstitial collagen, increases the expression of the matrix metalloproteinase procollagenase, and decreases the production of tissue inhibitor of metalloproteinase by human dermal and lung fibroblasts (26, 27). The pubic symphysis, which is directly associated with parturition, increases threefold in joint space during the 5 days before parturition in guinea pigs (28). There is also evidence that pregnancy increases joint laxity, as documented in a female patient after anterior cruciate ligament (ACL) reconstruction (7). Therefore, it is plausible that the effects of increased levels of estrogen and relaxin on soft tissues could have therapeutic benefits in the treatment of joint contracture.

In this study, we hypothesize that pregnancy could offer a protective effect against the development of joint contracture caused by immobilization because of increased levels of estrogen and relaxin. To study this hypothesis, a joint contracture model in a rat was used (29). In this model, the ACL was contracted, which leads to development of a flexion contracture after 2 wk. We examined whether pregnancy would 1) prevent the progression of the joint contracture caused by immobilization and 2) change the biomechanical properties of the knee ligaments and pubic symphysis. From this work, we hope to demonstrate the potential function that the hormones could produce in a ligament unrelated to the specific process of parturition.

**MATERIALS AND METHODS**

After receiving approval from our institution’s Institutional Animal Care and Use Committee, 20 Sprague-Dawley rats were used. All animals were caged individually in holding rooms at constant temperature (21°C) and humidity (40%) under a 12:12-h light-dark cycle. Rats were fed ad libitum and had free access to water. Pregnant rats were determined by examining the vaginal smears for estrous cycle day (22). After final examination, all pregnant rats were housed with nonpregnant rats in the same room. Nonpregnant rats were used as controls. Rats were housed in a balanced manner to assure similar average body weights among the pregnant and nonpregnant groups. Immobilization of the knee with the joint in full extension was performed in all animals. After immobilization, continuous passive motion was initiated in all rats to prevent knee contracture. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
female rats were obtained with an average preexperimental weight of 266 ± 19 g (SD). Ten rats were early pregnant (days 5–7) (average weight = 253 g), and ten were nonpregnant aged-matched controls (average weight = 279 g). For the pregnant group, we used rats in days 5–7 because it has been reported that relaxin levels rise on day 10 of pregnancy (20). The gestation time for a rat is 21–22 days, so, after an immobilization period of 14 days, pregnant rats were 1–2 days before delivery. Weights were not measured at the time of death; however, pregnant rats were much heavier as they had >10 fetuses.

Operative procedure. Intramuscular anesthesia with ketamin (90 mg/kg) and xylazine (9.0 mg/kg) was administered. The hindlimbs were shaved and washed with Betadine (povidone-iodine). Under aseptic conditions, the operation was performed as previously described by Wilson and Dahners (29). In brief, a lateral skin incision was made in the hindlimb, and the femur and tibia were exposed through an intermuscular approach. The knee was immobilized in 150° of flexion by using a 3-0 steel suture wire passed extraperiosteally around the midshaft of the femur and tibia (Fig. 1). The immobilized side was randomized for each animal, whereas the contralateral knee served as the control. After 14 days of unrestrained activity in their cages, the rats were killed with an overdose of pentobarbital. Both hind legs and the pubic symphysis were harvested within 30 min of death and stored at –20°C.

Mechanical testing procedure. On the day of testing, the hind legs were thawed to room temperature. Specimens were microscopically dissected, and the suture wire was removed. The anterior capsule of the knee was dissected, isolating just the posterior capsule, ACL, and the posterior cruciate ligament as the only structures limiting extension.

The femur was held with a three-pronged clamp, and a 0.005 N/mm extension moment was applied to the tibia (29). The degree of joint contracture was assessed by measuring the femorotibial angle on both hind legs with the use of a goniometer. Full extension was assigned 0°, so a larger femorotibial angle would be the result of a joint contracture. Measurements were made before and after the posterior capsule was cut.

The proximal femur and distal tibia were then potted in polymethylmethacrylate (PMMA) blocks. Material testing of the knee medial collateral ligament (MCL) and ACL was performed by using a servohydraulic testing machine (MTS Systems, Minneapolis, MN). Specimens were first preconditioned by applying 0.5 mm of displacement for 10 cycles at a rate of 0.2 Hz. At this elongation, the ligaments were just beyond the toe region of the loading curve. Then a distraction to failure was prescribed at a rate of 5 mm/min.

To test the MCL, the femur and tibia were aligned in the horizontal plane in ~60° flexion to relax the MCL. A hook was inserted around the MCL, and a force perpendicular to the ligament was applied until ligamentous failure (Fig. 2A). Testing the MCL in this nontraditional manner allowed us to take a measurement of properties for an extra-articular ligament without disrupting the intra-articular ACL. The applied force vs. the perpendicular displacement of the hook was used to characterize the structural properties of the MCL. Stiffness was calculated from the linear portion of the load-displacement curves and ultimate load obtained from the peak. After MCL failure, the medial and lateral menisci were excised at the anterior and posterior horn of the meniscus.

The tibia and femur were then oriented in the test machine with the joint in 60° of knee flexion aligning the anterolateral bundle of the ACL parallel with the direction of load application (Fig. 2B) (5). The tibia and femur were distracted until ACL failure. The applied force vs. linear distraction was used to characterize the ACL structural properties of stiffness and ultimate load.

The mechanical properties of the pubic symphysis were also measured. The pelvis was cut off above the acetabulum, and each side was potted in a PMMA block (Fig. 2C). The

Fig. 1. Radiograph (lateral view) of the flexed rat knee with 3-0 suture wire immobilizing the tibia to the femur.

Fig. 2. Testing configurations for the medial collateral ligament (A), anterior cruciate ligament (B), and pubic symphysis (C).
shape of the pubic joint was assumed to be an ellipse, and the major and minor diameters were measured with a caliper. The PMMA blocks were distracted until tensile failure of the pubic symphysis occurred. The alignment of loading to the pubic symphysis was perpendicular to the joint surface. The force vs. displacement curve was used to calculate stiffness and ultimate load.

Statistical analysis. A paired t-test was used to compare measurements between the control and immobilized knee. An unpaired t-test was used to compare results between the nonpregnant and pregnant groups. In all cases, a significance level of \( P < 0.05 \) was used.

RESULTS

Knee joint contractures, as measured by the femorotibial angle, were successfully created in both the nonpregnant and pregnant groups (Fig. 3). With the capsule intact, the joint angles in the control limbs were not significantly different for the two groups (34 and 35° for pregnant and nonpregnant groups, respectively). For the immobilized limb, the joint angle measured with the capsule intact was significantly greater than control for both the nonpregnant (53°; \( P < 0.05 \)) and pregnant groups (44°; \( P < 0.05 \)). Comparison of the joint contracture between the nonpregnant and pregnant groups showed a nearly significant decrease in contracture with pregnancy (\( P = 0.06 \)).

Cutting the capsule resulted in a small but statistically significant decrease in joint angle for both limbs in the pregnant group and the immobilized limb in the nonpregnant group (\( P < 0.05 \)). The nonpregnant control group was nearly significant in the decrease (\( P = 0.06 \)).

During failure testing, MCLs all failed at the insertion to the tibia. The ultimate load of the MCL (Fig. 4) significantly decreased with immobilization in the nonpregnant and pregnant rats (Table 1), although there was no statistically significant difference between pregnant (49% of control) and nonpregnant animals (62% of control) (\( P = 0.051 \)). The stiffness of the MCL (Fig. 4) decreased with immobilization, but the difference was statistically significant only in the pregnant rats (12.1 N/mm for control vs. 8.9 N/mm for immobilized rats; \( P < 0.001 \)). There was no statistically significant difference in MCL stiffness between nonpregnant (82% of control) and pregnant rats (75% of control) (\( P = 0.12 \)).

During tensile testing, the femur-ACL-tibia complexes all ruptured in the midsubstance of the ACL. The ultimate load of the ACL (Fig. 5) significantly decreased with immobilization in nonpregnant (39.6 N for immobilized vs. 47.8 N for control rats, \( P = 0.04 \)) and pregnant animals (34.6 N for immobilized vs. 49.3 N for control rats; \( P < 0.001 \)). The stiffness of the ACL (Fig. 5) did not significantly change with either immobilization or pregnancy (102% vs. 101% of control for pregnant and nonpregnant groups, respectively; \( P = 0.46 \) (Table 1).

The cross-sectional area of the pubic symphysis was measured, as it is a joint associated with parturition and therefore should have altered properties during pregnancy (27). The cross-sectional area of the pregnant rat pubic symphysis (7.3 mm²) was significantly larger than that of nonpregnant rats (6.2 mm²; \( P < 0.001 \); Table 2). The ultimate load for the nonpregnant and pregnant groups (19.8 and 21.2 N, respectively).

![Fig. 3. Joint contracture measured by the femorotibial angle in 10 nonpregnant rats compared with that of 10 pregnant rats in either immobilized or control knees, with the capsule intact (left) and cut (right). Values are means ± SD.

![Fig. 4. Ultimate load (N) and stiffness (N/mm) of the medial collateral ligament. Values are means ± SD.](http://jap.org)

Table 1. Structural properties of MCL and ACL after immobilization reported as a percentage of control

<table>
<thead>
<tr>
<th></th>
<th>MCL</th>
<th>ACL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultimate load, %</td>
<td>61.5 ± 17.0*</td>
<td>49.1 ± 13.2†</td>
</tr>
<tr>
<td>Stiffness, %</td>
<td>82.4 ± 15.7</td>
<td>74.6 ± 11.1†</td>
</tr>
</tbody>
</table>

Values are means ± SD. MCL, medial collateral ligament; ACL, anterior cruciate ligament. *\( P < 0.05 \); †\( P < 0.01 \) (immobilized knee vs. control).
and stiffness (19.1 N/mm and 22.2 N/mm, respectively) were not significantly different ($P > 0.05$; Table 2).

**DISCUSSION**

Although the effects of hormones on ligament are still not clear, the female hormones have the potential to alter the properties of soft tissues, including ligaments, thus providing a potential treatment for joint contracture. Many studies have shown that the fibroblasts in various organs respond to the hormonal treatment (14, 17, 26, 27). During pregnancy, ripening and dilation of the rat cervix at term involves a widespread reduction in the density and organization of collagen fibers (20). The interpubic ligament is lengthened during pregnancy (14, 16, 28).

Although the present study showed that pregnancy reduced the developing joint contracture caused by immobilization, there was not a significant difference compared with the nonpregnant control animals. A power analysis calculated that a sample size of 45 rats would have been necessary to detect a significant difference between the pregnant and nonpregnant groups. Nonetheless, these initial test results do suggest that something related with pregnancy, such as estrogen and relaxin, may play a role in preventing the development of joint contracture.

Clinically, relaxin is applied for the treatment of systemic sclerosis (19). It has also been reported that there is a relationship between pregnancy and increased transient laxity in the knee joint after ACL reconstruction (7). However, stretching of soft tissues during pregnancy stimulates fibroblast activity (23), causing the cell to be more easily affected by the hormones (28). Therefore, we cannot conclude from the present study whether increasing the levels of relaxin or estrogen will reduce joint contracture in a nonpregnant model. With further study, hormone therapy may eventually find an application for treating conditions related to soft tissue alterations.

Hormones need receptors to perform a function. Previous studies (13, 18) showed that the ACL has the receptors for estrogen, progesterone, and relaxin (9), as does the uterus. However, there has been no report that the MCL has these hormone receptors. Individual effects of each hormone on the structural properties of ligaments are still controversial. Slauterbech et al. (21) reported that the load to failure of the ACL declines significantly in estrogen-treated rabbits. However, Stickland et al. (22) reported that estrogen exerted no influence on the mechanical properties of sheep knee ligaments. These reported differences may be because of the different species being studied or other variables such as drug dosing.

In both the MCL and ACL, there was a decrease in ultimate load after immobilization in the pregnant and nonpregnant groups. Because failures in the MCL group occurred at insertion, the effect of immobilization might initiate a weakening at this location. The test method employed for failure of the MCL would subject these fibers to a high shearing load. Meanwhile, as failure in the ACL occurred at the ligament midsubstance, immobilization effects would be concentrated on the ligament substance. In addition, the stiffness also decreased in the MCL but did not change in the ACL. These findings seem contrary to what would be expected in the presence of a stiff joint. Previously, Woo et al. (30) documented that immobilization results in a reduction of ligament properties. Therefore, the surrounding tissue at the joint must be responsible for the contracture. In fact, the joint angle significantly decreased when the capsule was cut, suggesting that it is partially responsible for the contracture. A recent study by Trudel and Uhthoff (24) discriminated between myogenic and arthrogenic contributions to joint contracture. Their findings demonstrate that articular structures, including the capsule, are primarily responsible. Future work studying the biology of the joint capsule in pregnant specimens is needed to determine whether its composition is altered with contracture.

Although pregnancy alters the biomechanical properties of the pubic symphysis, the ultimate load and stiffness were not different between the pregnant and nonpregnant groups. This finding is likely because of the mode of testing. The test results (stiffness and ultimate load) are a composite measure of the bone outside the PMMA block and the ligament. A more sensitive test of just the ligament or perhaps measuring viscoelastic characteristics of the ligament would likely show differences between the two groups.

**Table 2. Structural properties of the pubic symphysis**

<table>
<thead>
<tr>
<th></th>
<th>Cross-Sectional Area, mm²</th>
<th>Ultimate Load, N</th>
<th>Stiffness, N/mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonpregnant</td>
<td>6.2 ± 0.5</td>
<td>19.8 ± 4.6</td>
<td>19.1 ± 4.7</td>
</tr>
<tr>
<td>Pregnant</td>
<td>7.3 ± 0.8*</td>
<td>21.2 ± 3.7</td>
<td>22.2 ± 8.4</td>
</tr>
</tbody>
</table>

Values are means ± SD. *$P < 0.01$ vs. nonpregnant rats.
EFFECT OF PREGNANCY ON JOINT CONTRACTURE

The present study has several limitations. One is that cross-sectional areas of ACL and MCL were very small and were not able to be measured. Therefore, we are only able to report the structural properties. A second limitation is that we did not directly measure the hormone levels in the animals. There is evidence that the concentrations of relaxin and estrogen maintain a high level before parturition (12, 20). Because the specimens in this study were evaluated just before parturition, hormone levels should be heightened. Finally, increased weight during pregnancy and decreased activity levels may contribute to changes in the ligaments as demands are altered. However, because the experimental knees were fixed in both the pregnant and nonpregnant specimens, these factors are likely less influential.

In summary, this study demonstrated that although immobilization reduces structural properties of the intraarticular and extraarticular ligaments in the knees of rats, it increases the magnitude of joint contracture. The trend toward reduced contracture formation in pregnant rats points toward a possible therapeutic role for female hormones in the prevention of postoperative and/or posttraumatic joint contracture. To avoid systemic effects, such therapy may need to be targeted to the specific joint.

The authors thank Dr. Chunfeng Zhao for assisting with rat surgeries. This study was supported with a research grant from the Mayo Foundation.

REFERENCES