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# Effect of Estrogen on Fibrinogen Clotting Time in Rabbits

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**Abstract:** The aim of this study was to examine the effect of estrogen on fibrinogen clotting time in rabbit model experimentally. The study was performed on 14 white New Zealand race female prepubertary rabbits at two months old weighing meanly 1.4±0.3 kg. Blood samples was taken from ear veins of the rabbits and first fibrinogen clotting time levels were measured to obtain control values. Next day, female sex hormone preparate estradiol valerinate at a dose of 0.3 mg/kg were injected to the rabbits IM. In their examinations made on $10^{th}$ day of injection, they were evaluated as in oestrus and under the maximal effects of estrogen, and their blood samples were taken again to measure their fibrinogen clotting time levels. Fibrinogen clotting time levels were determined from blood samples taken before and after female sex hormone administration. Samples taken from ear veins of the rabbits were used for measurement of fibrinogen clotting time. In the study, fibrinogen clotting time values were found meanly 18.13±1.00 sec. from blood samples taken before estrogen injection versus 18.87±0.61 sec. from blood samples after 10 days of estrogen injection. In statistical assessment performed, there were not statistically significant differences (t=0.94, p>0.05) between fibrinogen clotting time values before and after estrogen administration. Our data revealed that estrogen administration caused a silghtly but insignificantly increase in fibrinogen clotting time values in the rabbits.

**Key Words:** Estrogen, fibrinogen clotting time, rabbit.

## Tavşanlarda Östrojenin Fibrinojen Pıhtılaşma Zamanına Etkisi

**Özet:** Bu çalışmanın amacı, deneysel olarak tavşan modelinde östrojenin fibrinojen pıhtılaşma zamanına (FPZ) etkisini araştırmaktı. Çalışma, ortalama olarak 1.4±0.3 kg ağırlığına sahip prepuberter dönemdeki 2 aylık beyaz Yeni Zellanda ırkı 14 tavşan üzerinde gerçekleştirildi. Tavşanların kulak venlerinden kan örnekleri alındı ve kontrol değerlerini elde etmek için ilk FPZ düzeyleri ölçüldü. Ertesi gün, tavşanlara dişi seks hormon preparatı östradiol valerinat 0.3 mg/kg dozda kas içine injekte edildi. Tavşanların injeksiyonunun 10. gününde yapılan muayenesinde, tavşanlar östrusta ve östrojenin maksimal etkisi altında olarak değerlendirildi ve FPZ düzeylerini ölçmek üzere tekrar kanları alındı. FPZ düzeyleri dişi seks hormon preparatı verilmeden önce ve sonra alınan kan örneklerinden tespit edildi. Çalışma, FPZ düzeyleri östrojen injeksiyonundan önceki kan örneklerinde ortalama olarak 18.13±1.0 sn olarak bulunurken, östrojen injeksiyonunun 10. gününde alınan kan örneklerinde 18.87±0.61 sn olarak bulundu. Yapılan istatistiksel değerlendirmede, östrojen verilmesi öncesi ve sonnrası FPZ düzeyleri arasında anlamlı farklılık (t=0.94, p>0.05) yoktu. Bulgularımız tavşanlara östrojen verilmesinin FPZ değerlerinde hafifçe fakat anlamlı olmayan bir artışa neden olduğunu gösterdi.

**Anahtar Sözcükler:** Östrojen, fibrinojen pıhtılaşma zamanı, tavşan.

## Introduction

Human fibrinogen is a glucoprotein weighing 340.000 daltons. It is composed of three pairs of peptide chains connected by disulfide bridges to form a molecule composed of symmetrical halves. Fibrinogen as a blood protein takes place in a variety of physiological and pathophysiological events. It is among the most important factors determining especially clotting time of the blood (1).

For the measurement of plasma fibrinogen level (PFL), the commonest laboratory method is to measure fibrinogen clotting time (FCT) by coagulome-

ter. According to our knowledge today, obtained FCT values by coagulometer are converted to PFL values by use of transformation tables. According to these tables increasing PFL values cause decreasing FCT values. In the later literature, the FCT values are preferred instead of PFL values (2). For this reason, in this study, the FCT values were preferred instead of PFL.

According to our knowledge today, it is known to be some differences between male and female PFLs. However, it is understood from basic books and literature research that the factors determining normal PFL or FCT are not known clearly. In addition, we know of no experimental study that have examined relationship between male and female sex hormones and plasma fibrinojen levels in animals. In this purpose, we investigated the effect of female sex hormones on FCT and consequently on PFL in rabbit model.

## Material and Method

### Trial animals and trial procedure

In this study, 14 white New Zealand race female prepubertary rabbits at two months old weighing meanly 1.4±0.3 kg were used. The rabbits were caged in laboratory animals section of Yüzüncü Yıl University (YYU) Medical School, providing medium temperature of 10±4ºC during experiment. The rabbits were fed with rabbit fodder prepared by Animal feeding section of YYU Veterinay Faculty, in addition to other foods such as cabbage, lettuce and water adlibutum during trial.

After the rabbits were bought and brought to our section, they were waited for adaptations to their new place for one week period, then blood samples were taken and their clotting time levels were measured so that they were taken as control. Next day, female hormone preparate estradiol valerinate at a dose of 0.3 mg/kg were injected to the rabbits IM. The rabbits were observed after the injection, In their examinations made on $10^{th}$ day of injection, they were evaluated as in oestrus and under the maximal effects of estrogen, then their blood samples were taken again so as to measure their fibrinogen clotting time values.

### Measurements of Fibrinogen clotting time levels

FCT levels were determined from blood samples taken before and after female sex hormone administration. Samples taken from ear veins of the rabbits were used for measurement of fibrinogen clotting time. Blood samples were taken into tubes containing EDTA within. FCTs were measured by coagulamater (Coulter IL MCL2) using ready kits (diagnostica-fibri prest automate kit). The results were expressed as seconds (3).

### Statistical assessments

Statistical analysis was done by "paired, two tailed student t" test using the Microsoft excel 7.0 computer program. The values of $p<0.05$ were accepted as statistically significant. The values obtained were shown as "mean ±SD".

## Results

In the study, FCT values were found meanly 18.13±1.00 sec. from blood samples taken before estrogen injection versus 18.87±0.61 sec. from blood samples after 10 days of estrogen injection. FCT values before and after 10 days of estrogen are shown in Table and Figure. In statistical assessment performed, there were no statistically significant diffrences ($t=0.94$ $p>0.05$) between FCT values before and after estrogen administration.

## Discussion

Fibrinogen is essentially a glycoprotein and exists within the plasma and platelets playing a role in blood coagulation as a basic physiologic reaction. Fibronogen is synthesized in liver parenchymal cells. Fibrinogen is an acute phase reactant and plasma level may increase as much as eight fold in response to inflammatory

Table. Fibrinogen clotting time values (sec) before and after 10 days of estrogen administration (n=14).

| | Before estrogen administration | Ten days after estrogen administration |
|---|---|---|
| | 18,1 | 19,6 |
| | 17,4 | 19,8 |
| | 16,9 | 18,7 |
| | 18,5 | 17,9 |
| | 18,9 | 18,8 |
| | 15,8 | 19,6 |
| | 18,4 | 18,6 |
| | 17,6 | 18,8 |
| | 17,4 | 19,3 |
| | 19,2 | 17,7 |
| | 19,4 | 19,1 |
| | 19,1 | 18,5 |
| | 18,5 | 19,2 |
| | 18,7 | 18,7 |
| Mean | 18,13 | 18,87 |
| SD | 1,009 | 0,61 |

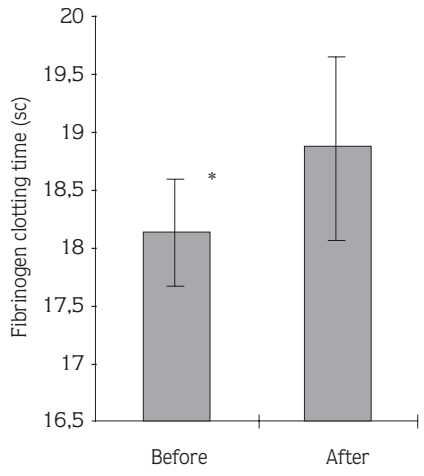


Figure. Fibrinogen clotting time values before and after 10 days of estrogen administration (n=14), (* t= 0.94, p>0.05)

challenge. The most important agents stimulating its synhtesis are glucocorticoids and IL-6 released from monocytes named as hepatocyte-stimulating factor (4,5).

Fibrinogen degradation products and some endotoxins activate fibrinogen synthesis. Although the role of hormones are not known clearly, it has been known that insulin has negative effect on fibrinogen production. Chronic alcoholism and smoking increase the fibrinogen synthesis (6,7,8).

It has been shown that fibrinogen is an important risk factor in cardiovascular diseases. In ischemic stroke patients, FCT is decreased and PFL is increased (9).

In pregnancy, thyroid binding globulin and cortisol binding globulin both increase, as do complement proteins and fibrinogen, the later resulting in a normally high ESR, in which estrogen and progesterone rise dramatically (10).

We know of no experimental study performed on laboratory animals that have examined the relationship between estrogen and FCT or PFL. But there is a number of studies in this subject performed on patients of different situation.

In a study performed to detect coagulation during the normal menstrual cycle, antithrombin III and and factor IV did not change; fibrinogen, however, showed a significant increase in the luteal phase compared to the follicular phase and midcycle. Fibrinogen showed a positive correlation with progestoren effect (11).

In a study to investigate coagulation system activation following estrogen treatment in postmenopausal women, significant increases in mean plasma PFL and fibrinopeptid A levels were seen (12). In another study to detect the effects of estrogen therapy and orchidectomy on coagulation in patients with prostatic cancer, during long-term estrogen treatment were found to have increased levels of factor VII, factor VIII-C and fibrinogen (13).

A clinical investigation was undertaken of the hemorrheologic effects of short-term administration of sex hormones. Estrogens and progesterone singly or in combination, were found to cause a rise in blood viscosity. Estrogens did so by raising hematocrit and plasma fibrinogen, parameters are similiarly raised in other conditions such as pregnancy and surgery (14).

In studies to detect hemorrheological effect of triphasic oral contraceptives, fibrinogen was increased either significantly or slighty (15,16,17).

As there are studies showing no significant difference in coagulation with estrogens in associated with cyproteron acetate at twelve months (18), there are investigations showing decreases in PFLs by estrogen and/or progesterone. In a study to detect the possible effect on coagulation of 17-ß-oestradiol-dhydrogesterone therapy in postmenapausal women for twelve months, fibrinogen was decreased slightly, but significantly below the inital value (19). In another study to investigate the effects of estrogen or estrogen/progestin regimens on heart disease risk factor in postmenapausal women showed that estrogen alone or in combination with a progestin improved lipoproteins and lowered PFLs without detectable effects on postchallenge insulin or blood pressure (3).

In the present study, FCT values were found meanly 18.13±1.00 sec. from blood samples taken before estrogen injection versus 18.87±0.621 sec. from blood samples after 10 days of estrogen injection. There were no statistically significant differences (t=0.94 p>0.05) between FCT values before and after estrogen administration. As a result, our data revealed that estrogen administration caused a slightly but insignificantly increase in fibrinogen clotting time values (or decrease plasma fibrinogen values) in the rabbits.

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