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Estrogen effects on plasma volume, arterial blood pressure, interstitial space, plasma proteins, and blood viscosity in sheep

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In adult castrated ewes the infusion of 17β-estradiol for 3 weeks was associated with a 12% increase in body weight, a 20% increase in whole blood volume (mainly due to a 27% increase of plasma volume), a 13% decrease in mean arterial blood pressure, and a 40% increase in heart rate. The change in plasma volume correlated with the change in estradiol concentration ($r = 0.72$). Most of the fluid was retained in the interstitial space, as represented by a 6 kg weight gain, 10% of which was in the intravascular compartment. Whole blood and plasma viscosity increased 16% and 21%, respectively, thus reversing some of the blood volume effects toward a hyperdynamic cardiovascular state. We conclude that many of the cardiovascular and hematologic changes with estrogen administration are similar to the changes observed during pregnancy, with the proposed requirement of decrease in mean arterial blood pressure as a condition for blood volume expansion. (AM J OBSTET GYNECOL 1986;155:195-201.)

Key words: Estrogen, blood volume, blood pressure, pregnancy

Human pregnancy is characterized by a 35% to 45% increase in blood volume from the nonpregnant values, mainly secondary to a 45% to 60% increase in plasma volume and a 20% to 30% increase in red cell mass.[1] Among other cardiovascular changes, diastolic arterial pressure decreases about 10 to 15 mm Hg and heart rate increases 10 to 15 bpm.[2] Also, plasma protein concentrations alter to affect both the Starling forces, and thus fluid flux across the capillaries, and blood viscosity, which is a major determinant of vascular resistance.[3]

During human pregnancy the concentrations of several hormones that play a role in fluid regulation increase in maternal plasma, including progesterone, deoxycorticosterone, aldosterone, and prolactin. A large increase also occurs in the concentration of 17β-estradiol and other estrogens. Experimentally it has been shown that estrogen administration to postmenopausal women, men with prostate cancer, and laboratory animals results in blood volume increases secondary to plasma volume expansion.[4]

In an effort to test the hypothesis that some of the cardiovascular and hematologic changes of pregnancy, such as the increase in plasma and whole blood volumes, heart rate, and decrease in mean arterial blood pressure, are a function of the circulating estrogen concentration, we infused 17β-estradiol into castrated ewes for 3 weeks and measured whole blood, plasma, and red cell volumes, arterial pressure, heart rate, plasma protein concentrations, and plasma and whole blood viscosities. The studies were not meant to reproduce exactly the endocrinologic milieu of pregnancy.

Material and methods

Five adult ewes were used in the present study. Four to 7 days after they were received from a local breeder,

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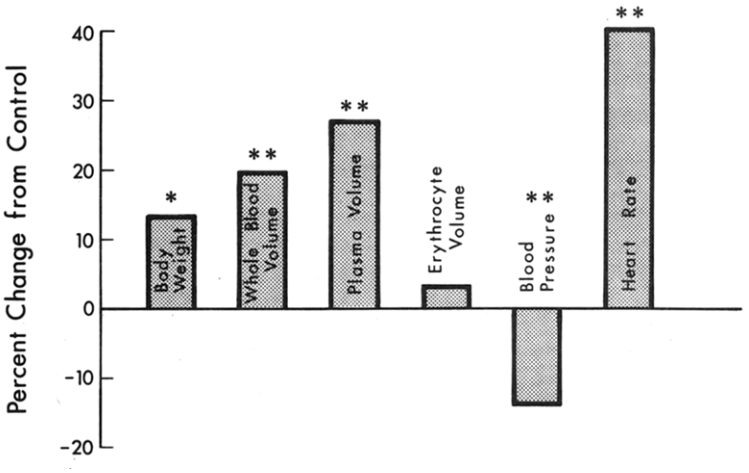

Fig. 1. Percent change from control values for body weight, whole blood, plasma, and erythrocyte volumes, mean arterial blood pressure, and heart rate. * = $p < 0.05$; ** = $p < 0.01$.

they were oophorectomized under spinal anesthesia. We placed Tygon catheters in a hindlimb artery and vein and exteriorized them to a flank pouch. Subsequently we flushed the catheters daily with 5 ml of heparinized saline solution (50 IU of heparin per milliliter) and allowed the animals to recover from the surgical stress for 3 weeks. Then we placed the ewe in a special metabolism cart and for 3 weeks monitored their body weight, arterial blood pressure, heart rate, and blood volumes. We then continuously infused 17β-estradiol (Sigma Chemical Co., St. Louis) at the rate of either 6.7 or 33.6 mg/kg/day for 3 weeks. Three ewes were infused in a random manner with both doses with a 4-week interval between infusions, during which time the various functions were measured. Of the other two ewes, one was infused with the lower dose, while the other received the higher dose. Thus four ewes were infused at each dose. In addition, three ewes were infused at the rate of 3.3 mg/kg/day, but no significant changes were observed in any of the parameters measured. In essence this constituted a control experiment for infusion of the vehicle. The 17β-estradiol was dissolved in ethanol and then diluted in normal saline solution. The rate of saline infusion was 4 ml/hr, for a total of 96 ml/day; the concentration of ethanol in saline solution was 0.1%. This minimized the effects of both saline volume and ethanol on the ewe's cardiovascular system. Then at weekly intervals we determined arterial blood pressure, heart rate, plasma, erythrocyte, and whole blood volumes, blood plasma protein concentrations, and plasma viscosity.

Blood volume measurements. We determined the total blood volume by dilution of chromium 51-labeled red cells, and calculated the plasma volume indirectly from the whole blood volume and the microhematocrit.[5] The day of the experiment we labeled 3 ml blood with ^{51}Cr using standard techniques.[5] We obtained a 0.5 ml blood sample for background radioactivity and then injected the tagged erythrocytes through the venous catheter, which was flushed three times the catheter volume with heparinized saline solution. Thereafter we withdrew 0.5 ml blood samples at 10, 20, and 30 minutes through the arterial catheter. To clear the catheter, we withdrew a volume equal to six times that of the catheter before each sample was obtained and then replaced that blood. We measured the sample radioactivity in a gamma counter (Packard Instrument Co., Downers Grove, Illinois) and corrected it for hematocrit and sample weight. Then by the use of a computer program, we extrapolated the decay curve back to zero time and used this value to calculate whole blood volume. Whole blood, plasma, and erythrocyte volumes were expressed both in absolute values and per weight of the ewe.

Arterial pressure and heart rate. Arterial blood pressure was recorded with a pressure transducer (Gould, P23Db, Cleveland) on a polygraph (Gould, Model 200), which was connected to an on-line computer (Texas Instrument, 990/10, Houston). Following a 1-hour period of adaptation, the mean arterial pressure and heart rate were obtained for an additional hour, averaged over 1-minute intervals, and stored for later analysis.

Plasma protein determinations. At weekly intervals we withdrew arterial blood into a sterile, nonheparinized plastic syringe and immediately transferred it into an appropriate glass tube. We determined fibrinogen concentration by a timed conversion of fibrinogen to fibrin by topical thrombin, using a fibrometer (Fibrosystem, Cockeysville, Maryland) to detect clot formation. Each time we constructed a standard curve, using dilutions of a commercial plasma of known concentration (Data-Fi Fibrinogen Calibration Reference, Dade Diagnostics Inc., Aguada, Puerto Rico).

Total plasma protein and albumin concentrations were determined by the biuret method[6] and a modified bromocresol green binding method,[7] respectively. The

Table I. Cardiovascular and hematologic values in ewes before and after 3 weeks infusion with 17β estradiol*

Variable	*Control*	*Change*	*% change*
Body weight (kg)	55.11 ± 2.14	6.44† ± 1.83	11.7†
Whole blood volume (ml)	3,355 ± 124.5	653.8‡ ± 125.9	19.5‡
Plasma volume (ml)	2,346 ± 94.2	627.5‡ ± 117.9	26.7‡
Erythrocyte volume (ml)	1,009 ± 62.2	17.9 ± 57.6	1.8
Whole blood volume (ml/kg)	61.1 ± 1.4	4.26† ± 1.03	7.0†
Plasma volume (ml/kg)	42.8 ± 1.7	5.58† ± 2.12	13.0†
Erythrocyte volume (ml/kg)	18.2 ± 0.5	−1.48 ± 0.66	−8.1
Hemoglobin (gm/dl)	12.0 ± 0.6	−0.22 ± 0.39	−1.8
Hematocrit (%)	30.0 ± 1.3	−0.97 ± 1.04	−3.2
Blood pressure (mm Hg)	90.9 ± 1.9	−12.0‡ ± 2.9	−13.2‡
Heart rate (bpm)	65.3 ± 5.0	26.3‡ ± 6.0	40.3‡
Estrone (pg/ml)	4.59 ± 1.20	155.8‡ ± 130.2	3,394‡
17β-Estradiol (pg/ml)	20.0 ± 8.8	6,242‡ ± 1,103	31,210‡

*Mean ± SEM, n = 8.
†$p < 0.05$.
‡$p < 0.01$.

plasma globulin was determined by the difference of these values.

Blood viscosity determinations. We measured the absolute viscosity of distilled water, whole blood, and plasma at 37° C in centipoise, using a Coulter capillary viscometer (Coulter Electronics Ltd., Hialeah, Florida). Viscosity was calculated relative to distilled water.

Estrogen assays. Unconjugated unbound and bound serum estrone and 17β-estradiol were measured by radioimmunoassay by means of a modification of the method of Abraham et al.[8] (Radioassay Systems, Carson, California). The intra-assay and interassay coefficients of variation were 7% and 13%, respectively, for both hormones.

Data analysis. From the control and experimental measurements as well as paired differences between experimental and control values, we computed mean, SD, and SE of the means for each variable under consideration. We tested differences between means by a paired *t* test at the 0.05 and 0.01 levels.

Results

Body weight. During the 3 weeks before estradiol infusion there was no significant change in body weight or other parameter. In contrast, during the 3 weeks of estradiol infusion the ewe's body weight increased about 12% (6.4 ± 1.8 kg, mean ± SEM) from 55.1 ± 2.1 kg ($p < 0.05$) at both 17β-estradiol doses (Fig. 1 and Table I).

Blood volume. Fig. 1 also shows the changes of whole blood volume, which gradually increased about 19% (654 ± 126 ml) at 3 weeks, from 3355 ± 125 ml at both 17β-estradiol doses ($p < 0.01$). When calculated per kilogram of body weight, whole blood volume increased about 7% from a control value of 61.1 ± 1.4 ml/kg ($p < 0.05$) (Table I).

Essentially all of the whole blood volume increase could be accounted for by the rise in plasma volume, which by the end of 3 weeks increased about 27% (628 ± 118 ml) from 2346 ± 94 ml ($p < 0.01$). With the lower dose the rate of increase was gradual over the entire 3 weeks, while at the higher dose the increase was relatively rapid, occurring in 1 to 2 weeks. Again, when normalized to body weight, plasma volume increased 13% (5.6 ± 2.1 ml/kg) from a control value of 42.8 ± 1.7 ml/kg ($p < 0.05$) (Table I). The whole blood and plasma volume changes returned to control values by two weeks after infusion. Erythrocyte volumes did not change significantly from the control values of 1009 ± 62 and 18.2 ± 0.5 ml/kg (Table I).

Estrogen concentration. The plasma concentrations of 17β-estradiol and estrone demonstrated very large increases (Table I). The mean plasma estradiol concentrations were 174 ± 17, 4616 ± 616, and 6991 ± 875 pg/ml at the infusion rates of 3.3, 6.7, and 33.6 mg/kg/day, respectively. For estrone these values were 6.5 ± 2, 179.6 ± 21.2, and 239.3 ± 87 pg/ml, respectively. As shown in Fig. 2, the change in plasma volume was highly correlated with the change in 17β-estradiol concentration ($r = 0.72$, $p < 0.001$).

Arterial blood pressure and heart rate. The mean arterial blood pressure decreased 13% (12 ± 2.9 mm Hg) from 90.9 ± 1.9 mm Hg ($p < 0.01$) following the 3-week 17β-estradiol infusion. In turn, the heart rate increased about 40% (26 ± 6 bpm) from a control value of 65 ± 5 bpm ($p < 0.01$) (Table I and Fig. 1).

Blood plasma proteins and viscosity. As shown in Fig. 3 and Table II, plasma fibrinogen concentration increased 86% (148 ± 54 mg/dl) from 173 ± 15 mg/dl. The plasma albumin concentration decreased 7% from 3.7 ± 0.8 gm/dl, while the globulin increased 24% from 3.9 ± 0.4 gm/dl. The albumin-to-globulin ratio decreased 31% from 0.99 ± 0.13 (Fig. 3).

Plasma viscosity increased 21% from 1.58 ± 0.17 centipoise ($p < 0.01$), while whole blood viscosity increased 16% from 3.05 ± 0.30 centipoise ($p < 0.01$)

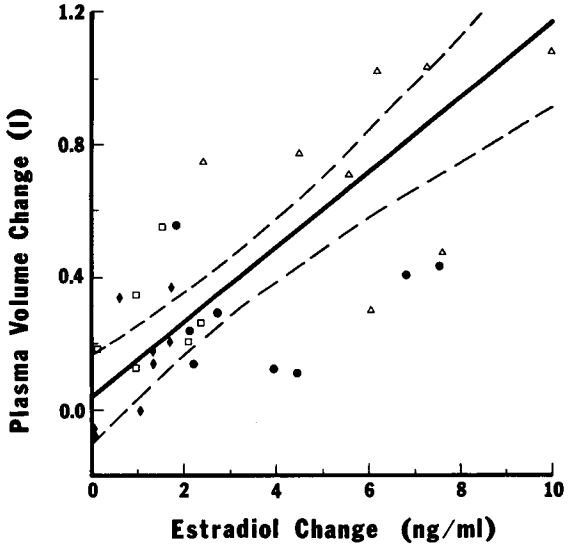

Fig. 2. Change in plasma volume (L) as a function of increase in circulating 17β-estradiol concentrations (ng/ml) following 2 and 3 weeks of estradiol infusion at 3.3 mg/kg/day (□), n = 6; 6.7 mg/kg/day (●), n = 8; and 33.6 mg/kg/day (△), n = 8. ♦ represents the recovery values 3 weeks following the infusion of the two higher doses (i.e., 6.7 and 33.6 mg/kg/day, n = 8).

(Table II and Fig. 3). As observed earlier, in three sheep infused with 17β-estradiol at 3.3 mg/kg/day, no significant changes occurred in any of the parameters measured.

Comment

Hemodynamics. The first aim of this study was to test the hypothesis that profound hematologic and cardiovascular changes, such as occur during pregnancy, can be secondary to elevated circulating estrogen concentrations. Human pregnancy is associated with some rather striking cardiovascular adaptations, such as 40% and 55% increases in whole blood and plasma volumes, respectively, several liters of fluid accumulation in the interstitial space, a 10% increase in heart rate, 30% increase in cardiac output, and decreases in both systemic vascular resistance and mean arterial blood pressure.[1, 9]

Basically two theories have been postulated to explain these pregnancy-associated changes. Burwell et al.[10, 11] in the late 1930s first postulated that the placental circulation acts as an arteriovenous shunt. In experimental animals the opening of a shunt produces an immediate fall in vascular resistance, which in turn results in a drop in arterial pressure and cardiac output. Within a few seconds vasoconstriction results in compensatory blood flow redistribution. Heart rate increases, as does cardiac output, while arterial pressure gradually returns toward normal values. The decrease of renal perfusion, acting probably through the renin-angiotensin-aldosterone system, results in fluid retention with blood volume expansion within a matter of minutes to hours.[12, 13]

Table II. Plasma protein and viscosity changes in ewes before and after 3 weeks' infusion of 17β-estradiol*

	Control	*Change*	*% change*
Fibrinogen (mg/dl)	173 ± 14.8	148 ± 54.0†	85.5†
Albumin (gm/dl)	3.67 ± 0.75	−0.25 ± 0.09‡	−6.8‡
Globulin (gm/dl)	3.85 ± 0.41	0.92 ± 0.02†	23.9†
Albumin/globulin ratio	0.99 ± 0.13	−0.31 ± 0.07†	−31.3†
Whole blood viscosity (centipoise)	3.05 ± 0.30	0.50 ± 0.17†	16.4†
Plasma viscosity (centipoise)	1.58 ± 0.17	0.33 ± 0.12†	20.9†

*Mean value ± SEM, n = 8.
†$p < 0.01$.
‡$p < 0.05$.

The second theory proposes that the hormonal changes during pregnancy, chiefly large increases in circulating estrogens, increase blood volume, cardiac output, and uteroplacental blood flow.[4] Again, the mechanism probably involves estrogenic mediated stimulation of the renin-angiotension system, producing an increase in aldosterone, with resultant sodium and fluid retention,[14] as well as direct action on the uteroplacental vessels.[15, 16] Estrogen administration also has been shown to decrease the number of angiotensin receptors[17] and to increase the amount of vasodilatory prostaglandins.[18] In pregnant women aldosterone secretion increases markedly, reaching a peak near the end of gestation.[19]

The infusion of either angiotensin II[20] or aldosterone[21] into experimental animals on a long-term basis has been shown to result in transistory fluid retention. However, the mean arterial pressure increase in turn results in increased glomular filtration rate, so that blood volume returned to a normal value. Saline infusion demonstrates a similar effect.[22] Only when renal arterial pressure is maintained within normal levels with a servomechanism as control is it possible to keep the fluid volume persistently elevated.[20, 21, 23] Thus it appears that a prerequisite to maintaining an expanded blood volume is to maintain a relative or absolute decrease in renal arterial pressure.

During the course of gestation in sheep, whole blood and plasma volumes normally increase only about 6% and 8%, respectively.[24] Whole blood and plasma volumes in sheep with twins and triplets increase 9% and 12%, respectively (Ueda et al., unpublished observations). Circulating estrogen concentrations are comparably low in this species, rising from 20 to 400 pg/ml only during the last few days of gestation.[25]

The present findings that in castrated sheep the

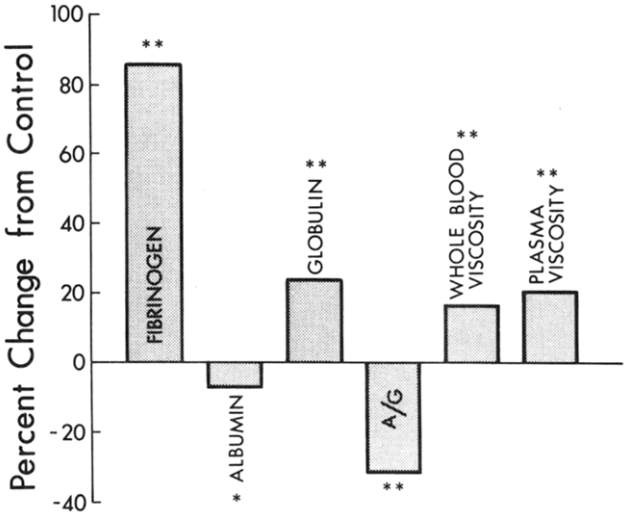

Fig. 3. Percent change from control values for fibrinogen, albumin, globulin, albumin-to-globulin ratio, and whole blood and plasma viscosities. * = $p < 0.05$; ** = $p < 0.01$.

chronic administration of 17β-estradiol resulted in decreased mean arterial pressure, increased heart rate, expansion of the interstitial space as reflected by the body weight, and expansion of the blood volume agree with the general mechanisms for volume expansion proposed previously.[4, 20, 21] Although the circulating doses of estrogen in the present study were greater than the physiologic, this should not detract from the value of the study because we were attempting to demonstrate an effect in a time length shorter than the normal duration of pregnancy.

Plasma proteins and blood viscosity. The second aim of the present study was to test the hypothesis that plasma protein concentrations and blood viscosity can reflect circulating estrogen concentrations. Human pregnancy is associated with marked changes in plasma protein concentrations. For instance, fibrinogen and globulin concentrations increase about 50% and 13%, respectively, while albumin decreases about 30%, so that the albumin-to-globulin ratio decreases about 36%.[13, 26] Also, in women using oral contraceptives the increase of fibrinogen and globulin concentrations has been alleged to result from the elevated circulating estrogen concentrations.[27, 28] In this study 17β-estradiol infusion resulted in plasma protein changes similar to those observed during the course of gestation and in women on oral contraceptives (Fig. 3 and Table II).

Because both fibrinogen and globulin are known to increase both plasma and whole blood viscosity,[29, 30] the substantial viscosity increases observed in the present study are not surprising. Additionally, the moderate decline in albumin would enhance viscosity.[29, 30] These results vary somewhat from the findings reported in pregnancy. During pregnancy the changes in whole blood viscosity are more complex than those in plasma, because the hematocrit decreases as pregnancy proceeds. These hematocrit changes are superimposed on any baseline changes in plasma viscosity. Because a declining hematocrit decreases whole blood viscosity, this effect counteracts an increase resulting from plasma protein changes. Such an interaction between a rising plasma viscosity and a falling hematocrit would account for the decline in whole blood viscosity that most investigators have noted as term approaches.[12, 31-34]

Not all authors are, however, in agreement on whether whole blood viscosity declines during pregnancy.[35] At least part of this disagreement can be attributed to differences in the shear rate at which the viscosity determinations were carried out. During measurements made at low shear rates the red cells interact in such a fashion that the apparent whole blood viscosity is increased. Increases in fibrinogen enhance this aggregation and thus increase the measured viscosity of whole blood in at least two ways: The first is by directly enhancing plasma viscosity. The second is by enhancing cell-cell interaction, particularly at low shear rates. Capillary viscometers such as the one employed in this study are inherently high shear devices and as such are quite insensitive to cell-cell interactions. Because of this characteristic they respond to the increase in plasma viscosity that a heightened fibrinogen will cause but are unaffected by any viscosity increase resulting from fibrinogen-enhanced red cell aggregation.

Measurements of plasma viscosity are more straightforward, and the results obtained are essentially independent of the shear rate applied, because cell-cell interactions are excluded. Furthermore, in a series of measurements made over time there are no variations in hematocrit to complicate the interpretation. It is thus surprising that during the course of pregnancy some authors report no change or a slight increase,[36] others report a decrease,[34] and still others claim to have ob-

served an increase during early pregnancy followed by a decline at term.[31]

From this confusion in the literature it is clear that no experimental model, even a complex one, will succeed in reproducing such discordant results. The present studies do, however, provide strong evidence that estrogen, in isolation, produces a marked rise in fibrinogen and globulin, a moderate decrease in albumin, and an unequivocal enhancement of plasma viscosity. As such, the findings may help to sort out the effects of the hormonal changes that occur in women taking oral contraceptive medication.[37] They may also, if due respect is accorded the more complex hormonal interplay surrounding pregnancy, provide some understanding of both the normal physiology and the pathophysiology of this state.[38-40]

In conclusion, estrogen administration reproduced several of the hemodynamic changes observed in human pregnancy. A perfect reproduction of these alterations cannot be expected because of differences in species and the overall hormonal milieu.

We propose that during pregnancy increasing estrogen concentrations decrease systemic vascular resistance and mean arterial pressure, with increased glomerular filtration rate. Estrogen may also affect the lymphatic vessels and interstitial space compliance, as evidenced by the fact that most of the fluid retained is in the interstitial space. These changes result in expanded plasma and interstitial fluid volumes. A possible mechanism for these changes would be as follows: estrogen results in increases in renin, angiotensin, and aldosterone with resultant fluid retention. Estrogen administration has been shown to decrease the number of angiotension receptors and to increase the amount of vasodilatory prostaglandins in the uterine vein of castrated rabbits treated with estrogen. A combination of both factors would result in decreased tone in blood and lymphatic vessels, with decreased arterial pressure and increased heart rate. Uteroplacental vascular shunts may also contribute to these changes.

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Pregnancy-induced hypertension: Development of a model in the pregnant sheep

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Selected hemodynamic, renal, and biochemical parameters were assessed in chronically instrumented third-trimester pregnant ewes and in the same ewes after induction of pregnancy toxemia. Ewes with induced pregnancy toxemia developed hypertension, proteinuria, ketonuria, decreased glomerular filtration rate, decreased cardiac output, and decreased left uterine artery blood flow. Histological and transmission electron microscopy revealed the development of renal morphologic changes consistent with those observed in human pregnancy-induced hypertension. These studies have elucidated that pregnancy-induced hypertension can be produced experimentally in the pregnant ewe. Furthermore, the pathophysiologic features of ovine pregnancy toxemia are similar to those of human preeclampsia, and therefore the sheep provides a suitable animal model to study the human condition, which still remains a major complication of pregnancy, jeopardizing both mother and fetus. (AM J OBSTET GYNECOL 1986;155:201-7.)

Key words: Pregnancy-induced hypertension, sheep, preeclampsia, renal

Toxemia states of pregnancy are known to occur in various animal species although none to date has been found to be a precise model for human preeclampsia. The human condition is a hypertensive disorder peculiar to pregnancy and has been associated with proteinuria,[1] a decrease in blood volume,[2] thrombocytopenia,[3] uteroplacental ischemia,[4] prostaglandin deficiency,[5, 6] increased thromboxane A_2 production,[7] increased activity of the sympathetic nervous system[8] and the renin-angiotensin system,[9] and renal morphologic changes.[10]

Several investigators have compared ovine pregnancy toxemia to human pregnancy-induced hypertension. In the ewe, the toxemic syndrome of pregnancy demonstrates a close resemblance to human preeclampsia with proteinuria and uteroplacental isch-

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