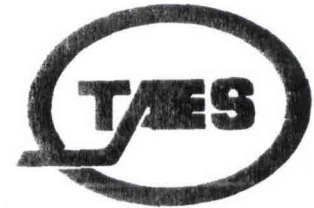# PUBLICATIONS

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CHAPTER 2

EFFECTS OF DIETARY MONENSIN AND SEX OF CALF ON PROFILES
OF SERUM PROGESTERONE AND ESTROGEN IN LATE PREGNANCY
OF FIRST-CROSS BRAHMAN-HEREFORD COWS

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SUMMARY

Two groups of eight first-cross Brahman-Hereford cows of equal
parity within groups were fed monensin and no monensin, respectively,
from day 246 ± .4 of gestation. The cows were group fed Coastal
bermudagrass hay _ad_ _libitum_ and either 0 (group NMON) or 200 mg (group
MON) of monensin incorporated in 1.8 kg of standard range cube (daily per
cow). The experimental purpose was to study concentration profiles of
sex steroids prepartum. Blood was sampled via tail vein or artery on
alternate days through day 270 of gestation and at least daily thereafter
until 3 days postpartum as well as within 1 hr after calving (day 0).
Progesterone (P_4), estrone (E_1) and estradiol-17β (Eβ) and estradiol -17α
(Eα) were measured in blood serum by radioimmunoassay. The data were
analyzed by least-squares and split-plot analysis of variance and by
stepwise-addition multiple regression. Diet did not affect body weight
and length, wither height, heart girth or condition score of cows,
calving traits or birthweight and vigor of calves. Periparturiently,
concentration profiles of the serum steroids were characteristic of other
published data. Concentrations of the serum steroids were not different
(P>.25) between groups initially, but serum P_4 increased (P<.01) about 3
ng/ml in cows of group NMON during the first 22 days of the feeding trial
and serum P_4 was higher (P<.01) on day 22 than in cows of group MON. In
contrast, concentrations of serum estrogens did not differ significantly
between diet groups until days 2 to 0 prepartum when concentrations of
serum E_1 and Eβ were higher (P<.05) in cows of group NMON than in cows

of group MON. Concentrations of serum $E\alpha$ started to decrease on either day -4 (group MON) or day -1 (group NMON). Cows carrying a female calf had higher concentrations of serum P_4 (P<.05) and lower concentrations of serum estrogens [E_1, P>.10; $E\beta$ and $E\alpha$, P<.10] prepartum than cows carrying a male calf. Differences in concentrations of the serum estrogens associated with calf sex were most evident during the last 18 days before calving.

INTRODUCTION

Dietary monensin (MON) when fed to cattle has been associated with increased feed efficiency in growing cattle (Potter et al., 1976; Raun et al., 1976) and brood cows (Randel and Rouquette, 1976; Turner et al., 1977). Moreover, dietary MON has been associated with a decreased interval from calving to first estrus in beef cows (Turner et al., 1977) and decreased time to onset of puberty in beef heifers (Moseley et al., 1977). Effects of dietary MON on reproductive traits were of special interest because of inference that MON may in some way also have a favorable effect on synchrony of endogenous hormones to control reproductive cycles.

Our purpose is to report effects of MON on profiles of serum progesterone (P_4), estrone (E_1), estradiol-17β ($E\beta$), estradiol-17α ($E\alpha$) and total estrogen during late pregnancy of first-cross Brahman-Hereford cows. During summarization of data it was found that sex of calf also was a source of variation. Therefore, data on effect of calf sex on concentrations of sex steroids in serum have been included.

EXPERIMENTAL PROCEDURE

The 16 cows in this experiment were among two groups of 35 first-cross Brahman-Hereford cows, respectively, that had been allotted

at random within age group to two diet treatments a minimum of 30 days prior to calving. One group was fed 200 mg of MON daily per cow. The MON was incorporated in 1.8 kg of a standard range cube (21% all plant protein) and group fed. The other group was group fed 1.8 kg of range cube without MON (NMON). In addition, the two groups were managed in separate dry, fall pastures and fed Coastal bermudagrass [Cynodon dactylon (L.) Pers] hay ad libitum. All cows had been bred artificially with semen from one Simmental bull. Cows were weighed, measured (wither height, body length from point of shoulder to pin bones and heart girth) and scored for body condition when placed on experimental diets and again prior to calving and within 24 hr after calving. Cows were checked for retained fetal membranes after calving and signs of dystocia during calving. Calves were weighed within 12 hr after birth.

Eight cows assigned to each diet were used to compare hormonal profiles during late pregnancy and parturition. There were two age groups, four 3- to 4-year-old cows (age code 1) and four 7-to 8-year-old cows (age code 2), in groups NMON and MON, respectively. These cows were placed on experimental diets between 243 and 249 days of pregnancy ($\bar{x} = 246 \pm .4$ days)

Blood was collected at 0800 hr by venipuncture of the tail vein or artery when cows were allotted to experimental diets and then on alternate days to day 270 of gestation, at least daily thereafter through day 3 postpartum and also within 1 hr after calving (day 0). After collection, blood was chilled immediately in ice water and then stored at 4 C for 12 hr before centrifugation at 1,300 x g for 20 minutes. Two aliquots of supernatant serum were stored at -23C until concentrations of P_4, E_1, and $E\beta$ and $E\alpha$ were measured by radioimmunoassay at Purdue

University using procedures as published (Chew et al., 1977; Erb et al., 1976b; Monk et al., 1976). Animal phases of the experiment were conducted at Texas A&M University Agricultural Research and Extension Center, Overton.

Preliminary analysis of data were done by least-squares analyses of variance (Harvey, 1960) to survey linear effects of certain covariants on concentrations of steroids by days prepartum. There were 23 serum samples from each of the 16 cows (n = 368). Diet and age codes of cows and sex of calf along with the interactions were included as discrete variables. Age code of cow and the associate interactions were not significant (P>.25). Therefore, age code of cow was deleted as a discrete variable. Subsequent analyses included sex of calf (SC), diet of cow (DC) and the SC x DC interaction. Thus, there were 12 df among cows within SC and DC to derive mean squares to test effects of SC and DC on the serum steroids. Body weight and length, heart girth, wither height, condition score and gestation length of cows, birth weight of calves, and linear, quadratic and cubic effects of days prepartum were included as covariants (10 df). There were 354 df in the residual mean squares to test significance of covariants. Other analyses (Harvey, 1960) additionally included quadratic, and quadratic and cubic effects of body weight of cows and birth weight of calves. Also, the data were analyzed to test for differences due to SC, DC and linear effect of days and their interactions with cows nested (Harvey, 1960). Prediction equations were calculated separately for each diet group of cows and for each sex of calf group of cows by stepwise-addition multiple regression (Nie et al., 1975) to illustrate changes in serum steroids from days -38 to -.5 prepartum. Computational limits were .001 for tolerance, .01 F

for inclusion and .005 F for deletion of independent variables from the equations. Each analysis contained one serum steroid [P_4, E_1, $E\beta$, $E\alpha$ or total estrogen ($E_1 + E\beta + E\alpha$)] as a dependent variable. Independent variables were (1) sex of calf, (2) diet treatments, (3) linear, quadratic and cubic effects of weight of cow and calf, and (4) linear to octic effects of days −38 to −.5 prepartum. Day −38 was day code 1 and day −.5 was day code 38.5 in the regression analyses. Unadjusted averages were used to illustrate changes in serum steroids for days 0 to 3 postpartum. Differences in concentrations of serum steroids among groups of cows were analyzed by split-plot analyses of variance. Between cow within group mean squares were used to test significance of differences among groups.

RESULTS AND DISCUSSION

General. Diet of cow and sex of calf were unrelated to body condition score or to size measurement of cows. Diet of cow had no effect on weight and vigor of calves. Average birth weights of nine male and seven female calves were not different significantly (P>.10). All cows delivered single births without signs of dystocia and none retained fetal membranes more than 12 hours. Average length of gestation was not different among groups NMON and MON or among male and female calves (overall mean was 29 ± 2 days). There were five male and three female calves from cows of group NMON and four calves of each sex from cows in group MON.

Results of least-squares analyses of variance (Harvey, 1960) revealed that body weight and length, wither height and heart girth of the cows were correlated significantly (P<.01; r = .62 to .85) with each other. Among measurements of cow size, only body weight was included in

later analysis. However, any one of the measurements of cow size would
have reflected nearly similar independent effects on concentrations of
any serum steroid.

Effect of Diet. Due to variation among individuals in length of
gestation, aligning data sequentially from days -38 to -.5 prior to
calving increased the variation among individuals for days on diet
treatments for each period relative to calving. However, cows in both
groups had been on diet treatments 7 ± 2 days (average ± standard error
here and elsewhere in the text) on code day -38 in the multiple
regression analyses (Nie et al., 1975). Moreover, concentrations of the
steroids in blood serum were not different (P>.25) among cows of groups
NMON and MON during the first 2 days on feeding trial (6.41 ± .6 vs 6.47
± .6 ng/ml of P_4, 297 ± 64 vs 291 ± 33 pg/ml of E_1, 50 ± 7 vs 63 ± 14
pg/ml of Eβ, 160 ± 20 vs 145 ± 14 pg/ml of Eα, and 507 ± 70 vs 499 ± 42
pg/ml of total estrogen, respectively).

Concentrations of serum P_4 in group NMON started to increase between
4 and 6 days after start of diet treatment, were increased (P<.01) about
3 ng/ml on day 22, and higher (P<.01) than cows in group MON on day 22.
In contrast to serum P_4, the serum estrogens were not different (P>.25)
between groups of cows from 0 to 22 days of diet treatment.
Concentrations of P_4 have been increased by underfeeding pregnant ewes
and heifers (Donaldson et al., 1970; Cumming et al., 1971), but it is
unknown if the pattern of increase was the same as in cows of group NMON.

Prepartum and postpartum changes in the serum steroids associated
with diet treatment are illustrated in figures 1 to 4. Regression
equations for the prepartum phase (days -38 to -.5) are shown in table 1.
Concentrations of the serum steroids were similar (P>.25) between diet

groups of cows postpartum. However, regression coefficients comparing diet groups of cows were significantly (P<.01) different from zero prepartum for serum P_4, E_1, Eβ and total estrogen, but not for serum Eα (table 2).

Differences in concentrations of serum P_4 between cows in groups NMON and MON were largest prior to day -20 and then decreased gradually each day until concentrations of serum P_4 were nearly identical on day -2 (figure 1). Across days -38 to -.5, serum P_4 approached significance (P<.10) and the day x diet interaction was significant (P<.01) as tested by least-squares analysis of variance. Both groups of cows showed typical decreases in serum P_4 the last 2 days before calving (Henricks et al., 1972; Hoffmann et al., 1973; Smith et al., 1973; Dobson and Dean, 1974; Chew et al., 1977) as reflected by significant (P<.01) D^8 regression coefficients (table 1).

Even though differences in the serum estrogens across days and the day x diet interaction were not significant (P>.10) between diet groups when tested by least-squares analyses of variance, other criteria indicated differences in concentration profiles. Average concentrations of serum E_1 (figure 2) and serum Eβ (figure 3) in cows of groups NMON and MON increased (P>.01) four- to fivefold between days -18 and -2 or -1, respectively. Concentration profiles of serum E_1 and Eβ (figures 2 and 3) show that both steroids increased more between days -4 and -2 in cows of group NMON than in cows of group MON. The magnitude of differences in serum E_1 between cows of groups NMON and MON is reflected in the larger negative regression coefficient for D^8 in group MON (table 1). Serum Eβ increased at a slower rate after day -6 in cows of group MON than in cows of group NMON (figure 3) as shown by the negative regression coefficient

for D^8 (P<.05) in group MON and the positive (P<.01) highest order function of day effects (D^4) in group NMON (table 1). Also, unadjusted differences between cows in groups NMON and MON were significant (P<.05) for serum E_1 and Eβ on day -1 and approached significance (P<.10) for serum E_1 on day 0.

Concentration profiles of serum Eα appeared to be different among days between cows in groups MON and NMON (figure 3). Cows in group MON compared to cows in group NMON had lower (P>.10) concentrations of serum Eα prior to day -17, increased at a faster rate until about day -8 and then started to decrease earlier prepartum (day -4 vs day -.5). The daily rate of decrease in serum Eα was significant (D^8; P<.01) in cows of group NMON (table 1).

Concentration profiles of serum total estrogen between groups NMON and MON (figure 4) were not distinguishable from profiles of serum E_1 (figure 2) because total estrogen averaged 75% E_1 and only 5% Eβ and 20% Eα prepartum. The regression coefficient reflecting rate of decrease in serum total estrogen the last few days prepartum was about threefold higher in cows of group MON than in cows of group NMON (D^8; table 1; both P<.01).

The tendency for cows in group MON to decrease in serum E_1, Eα and total estrogen the last 2 to 3 days prepartum (figures 2 to 4) is in general agreement with results reported by Robinson et al. (1971) and Smith et al. (1973). The tendency for cows in group NMON to increase in serum steroids until day -.5 is in general agreement with results reported by Henricks et al. (1972), Hoffman et al. (1973), Robertson (1974), Garverick et al. (1974) and Chew et al. (1977). The differences in late prepartum profiles of the serum estrogens between groups MON and

NMON indicate that differences due to diet of cows may be one variable causing discrepancies in results of the studies cited above.

Effects of Calf Sex. Average concentrations of serum steroids from days -38 prepartum to 3 days postpartum within groups of cows carrying male (group MC) and female calves (group FC) are illustrated in figures 5 to 8. Regression equations used to plot the daily changes in serum steroids prepartum are shown in table 3. The unadjusted averages illustrating changes postpartum were not significantly ($P>.10$) different for any serum steroid among days or between groups MC and FC (figures 5 to 8).

Independent regression coefficients attributable to sex of calf within diet treatments were significantly ($P<.01$) greater than zero for each serum steroid (table 2). Day x calf sex and calf sex x diet interactions were not significant for any serum steroid prepartum. However, comparison of effect of calf sex across days by two least-squares techniques resulted in different estimates of probability. Differences due to calf sex were not significant when only linear effects of diet, calf sex and days were included in the statistical model. Calf sex differences were significant for serum P_4 ($P<.05$) and approached significance ($P<.10$) for Eβ, Eα and total estrogen when diet and calf sex were used as discrete variables along with the covariates listed in the procedure section. The latter analysis should give the most valid estimate of differences due to calf sex because each covariate was correlated ($r = .12$ to $.22$) significantly ($P<.05$ to $P<.01$) with one or more of the serum steroids.

Concentrations of serum P_4 were higher consistently in group FC than in group MC until concentrations of serum P_4 began to equalize between

groups after day -7 (figure 5). The higher constant in the equation for group FC reflected the differences between groups FC and MC prior to day -7 since regression coefficients describing day effects were rather similar for each group (table 3).

In contrast to higher serum P_4 in group FC than in group MC, the serum estrogens averaged higher in group MC (table 2). Concentration profiles of serum E_1, Eβ and total estrogen for cows in groups FC and MC were essentially parallel prepartum (figures 6 to 8). In comparison, serum Eα averaged lower in cows of group MC prior to day -18 and increased more rapidly thereafter than cows in group FC (table 3; figure 7). Average concentrations of serum Eβ were about the same between cows in groups FC and MC prior to day -8 when cows in group MC showed more rapid daily increases through day -1 (figure 7) as reflected by a larger positive D^4 effect (table 3).

<u>Ratios of Progesterone to Total Estrogen</u>. Ratios of P_4 to total estrogen in serum were nearly identical among cows in groups NMON and MON for days -7 and 0. The ratios decreased linearly from 3.4 on day -7 to 1.3 on day -.5. The ratio was only .5 on day 0.

The effects of sex of calf on ratios of P_4 to total estrogen were independent of diet treatment. Cows in group FC consistently had a higher ratio of P_4 to total estrogen in serum prepartum. On day -20 the ratio was 14.7 in cows of group FC and 7.5 in cows of group MC (not shown). Ratios were decreased by day -7 in both groups of cows but those in group FC remained higher through day -.5 (figure 9). Each group of cows averaged only .5 on day 0 and showed parallel increases through day 1.5 postpartum. Increases in ratios occurred postpartum because serum P_4 was decreased to low concentration by day 0 in both groups, whereas total

estrogen in serum was still decreasing rapidly.

General Discussion

It is known that chronic dietary deficiencies decrease gross measures of reproductive efficiency (Leathem, 1961, 1970; Wiltbank et al., 1962; Lamond, 1969) as well as concentrations of sex steroids in systemic blood of animals (Setchell et al., 1965; Donaldson et al., 1970; Hill et al., 1965; Grewal et al. 1971; Gombe and Hansel, 1973; Howland, 1975). Underfeeding pregnant heifers and ewes caused increased concentrations of P_4 (Donaldson et al., 1970; Cumming et al., 1971), whereas underfeeding nonpregnant heifers caused decreased concentrations of P_4 within 1 week (Donaldson et al., 1970; Hill et al., 1970; Gombe and Hansel, 1973).

Moreover, concentrations of testosterone were decreased in systemic blood of male rats by underfeeding (Grewal et al., 1971; Howland, 1975) and rebounded above concentrations in controls within 2 days after return to fullfeeding (Howland, 1975). Thus, concentrations of sex steroids in systemic blood appear to be affected by even short-term variations in dietary energy.

The foregoing observations cannot explain differences in concentrations of serum steroids among cows in groups NMON and MON since there were no differences in weight gain of cows, vigor and weight of calves at birth, length of gestation or calving traits. Only the characteristic shift in rumen volatile fatty acids from acetate and butyrate to propionate (Raun et al., 1976; Potter et al., 1976; Turner et al., 1977) was observed in cows of group MON as compared to cows in group NMON (Randel et al., 1977). Proof that dietary MON increased feed efficiency and decreased age of puberty and interval from calving to

first estrus, whether or not daily gains were increased (Raun et al., 1976; Potter et al., 1976; Turner et al., 1977; Moseley et al., 1977), infers improved energy metabolism. Also, effects of MON on reproductive traits infer that synchrony of reproductive hormones associated with onset of puberty and return to estrus after calving occurred earlier in animals fed MON. Our results suggest that sex steroid metabolism also may be altered detectably by dietary MON and thus explain differences in concentrations of serum P_4 and the serum estrogens among groups NMON and MON (figures 1 to 4).

If we assume that energy metabolism was more nearly optimal in cows of group MON, then decreased concentrations of serum P_4 (figure 1) would be consistent with reports that underfeeding balanced rations to pregnant heifers and ewes increased P_4 (Donaldson et al., 1970; Cumming et al., 1971). Data are not available to make a similar analogy to explain lower concentrations of the serum estrogens in cows of group MON, especially during the last 3 to 7 days of gestation. It is possible that liver metabolism of sex steroids and their excretion is more efficient when energy metabolism is closer to optimum. If this should be true, then rates of synthesis and secretion of P_4 and the estrogens could have been similar in cows of both groups with generally lower serum concentrations in cows of group MON because of more efficient metabolism of the endogenous steroids.

It is difficult to explain the substantial differences in concentrations of serum steroids between groups MC and FC. Male calves generally average heavier at birth and generally have longer gestations than female calves. However, neither trait was different (P>.10) in this study. Moreover, effects of calf sex as illustrated in figures 5 to 8

were independent of cow weight and diet, and birth weight of calves. Therefore, any positive relationship between weight of placental tissue and birth weight of calves should have been accounted for in the statistical model. The placenta is the only major source of estrogen during pregnancy of cows (Mellin and Erb, 1965) and E_1 Eβ and E_2α have been identified and quantified in bovine fetal cotyledons (Gorski and Erb, 1959; Veenhuizen et al., 1960). The fetal calf of either sex seems an unlikely source of P_4, E_1 or Eβ because concentrations of E_1 and Eβ in fetal plasma remain unchanged during the terminal stages of pregnancy (Peterson et al., 1975; Hunter et al., 1977).

Hunter et al., (1977) postulated that placental synthesis and secretion of estrogen may be stimulated by physical activity of the fetus. Male calves appear stronger at birth than female calves and perhaps are more active in-utero. This possibility could explain differences in the serum estrogens because their concentrations increased more rapidly in cows of group MC than in cows of group FC during the last 4 (figure 6) to 18 (figure 7) days of pregnancy. However, the postulate of Hunter et al. (1977) gives no clue as to why concentrations of serum P_4 were lower (figure 5) in cows of group MC unless increased estrogen inhibited luteal function thereby causing decreased serum P_4. Moreover, Erb et al., (1976a) reported that concentrations of P_4 were lower and concentrations of total estrogen were higher in blood plasma of Holstein heifers and cows carrying a single female calf as compared to those carrying a single male calf. However, the latter comparisons were made with animals at earlier stages of pregnancy (days 69 to 249). Also, concentration profiles for serum E_1 (figure 6) were similar on day -38 and those for serum Eα (figure 7) were lower between days -38 and -21 in

cows of group MC compared to cows of group FC. Therefore, relationships between sex of fetus and concentrations of steroids may have changed prior to day -38 in our study.

TABLE 1. COEFFICIENTS OF MULTIPLE REGRESSION EQUATIONS FOR ESTIMATING PREPARTUM CONCENTRATIONS OF SERUM STEROIDS FOR COWS FED AND NOT FED MONENSIN.

	Group equations			
Steroid	Monensin		No monensin	
Progesterone(ng/ml)	K^a	6.15	K_4	9.50 *
	D_{7b}	.3707E-9 **	D_7	-.8746E-5 *
	D_8	-.1020E-10 **	D_8	.7907E-9 **
			D	-.1773E-10
Estrone (pg/ml)	K_2	446.64	K_3	525.87
	D_6	-.6358 **	D_5	-.0295 **
	D_8	.4229E-5 **	D_8	.1012E-3 **
	D	-.2357E-8	D	-.1032E-8
Estradiol-17β (pg/ml)	K_3	32.59	K	31.70
	D_5	-.1876E-2 *	D_4	-.2420 **
	D_8	.4954E-5 *	D	.7278E-4
	D	-.4833E-10		
Estradiol-17α (pg/ml)	K	164.18	K_2	268.58
	D_6	.5544 **	D_5	-.2036 *
	D_8	.6929E-6 **	D_8	.1341E-4
	D	-.4216E-9	D	-.1132E-9
Total estrogen (pg/ml)	K	380.60	K_3	812.80
	D_2	55.2582	D_5	-.0379 **
	D_6	-2.8581 **	D_8	.1187E-3 **
	D_8	.6341E-5 **	D	-.1143E-8
	D	-.3417E-8		

[a]Constant adjusted to group average of other covariates in the equation.

[b]Day effect. Day code 38.5 equals day -.5 in figures 1 to 4.

* P<.05; ** P<.01.

TABLE 2. REGRESSION COEFFICIENTS DESCRIBING AVERAGE DIFFERENCES IN SERUM
STEROIDS IN COWS DUE TO DIET TREATMENT AND CALF SEX.

Steroid	Group no monensin minus group monensin		Group male calf minus group female calf	
Progesterone (ng/ml)	1.71** ±	.28[a]	-1.63** ±	.28
Estrone (pg/ml)	273** ±	79	441** ±	88
Estradiol-17β (pg/ml)	23 ±	5	20** ±	5
Estradiol-17α (pg/ml)	44** ±	24	94** ±	26
Total estrogen (pg/ml)	345 ±	96	576 ±	108

[a]Regression coefficient ± standard error.

**Significantly different from zero (P<.01).

TABLE 3.   COEFFICIENTS OF MULTIPLE REGRESSION EQUATIONS FOR ESTIMATING PREPARTUM CONCENTRATIONS OF SERUM STEROIDS FOR COWS CARRYING SINGLE MALE AND SINGLE FEMALE CALVES.

Steroid	Group equations			
	Male calf		Female calf	
Progesterone (ng/ml)	k^a_{4b}	7.15 *	K_4	8.79
	D_7	-.5485E-5 **	D_7	-.4459E-5
	D_8	.6511E-9 **	D_8	.5713-9
	D	-.1518E-10	D	-.1399E-10
Estrone (pg/ml)	K	350.80	K_2	390.90
	D_2	43.5893	D_6	-.6939 **
	D_5	-2.1588	D_8	.4293E-5 **
	D_8	.1083E-3 **	D	-.2351E0-8
	D	-.1178E-8		
Estradiol-17β (pg/ml)	K_3	35.60	K_4	27.50
	D_4	-.5482E-2	D_8	.5721E-4 **
	D_8	.2671E-3 *	D	-.4825E-11
	D	-.2986E-10		
Estradiol-17α (pg/ml)	K_2	197.10	K_3	266.20
	D_4	-.1744	D_4	-.0319 **
	D_8	.6001E-3 *	D_8	.1232E-2 **
	D	-.1459E-9 *	D	-.1531E-9 **
Total estrogen (pg/ml)	K	594.40	K	183.10
	D_2	39.2550	D_2	116.2823
	D_5	-1.9959	D_5	-6.4023
	D_8	.1228E-3 **	D_8	.2165E-3 **
	D	-.1346E-8	D	-.22663-8

[a]Constant adjusted to group average of other covariates in the equation.

[b]Day effect.   Day code 38.5 equals day-.5 in figures 5 to 8.
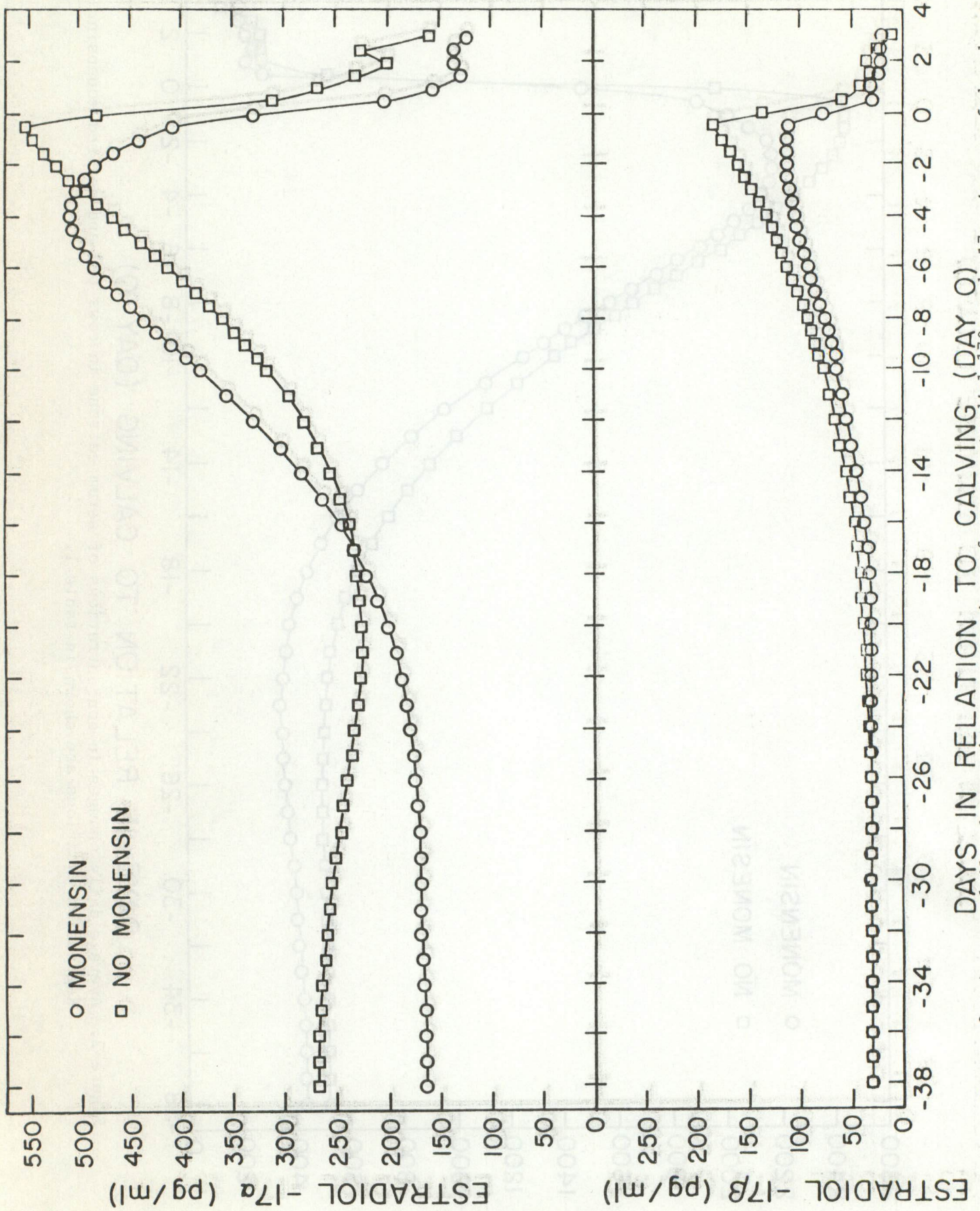
* P<.05; ** P<.01.
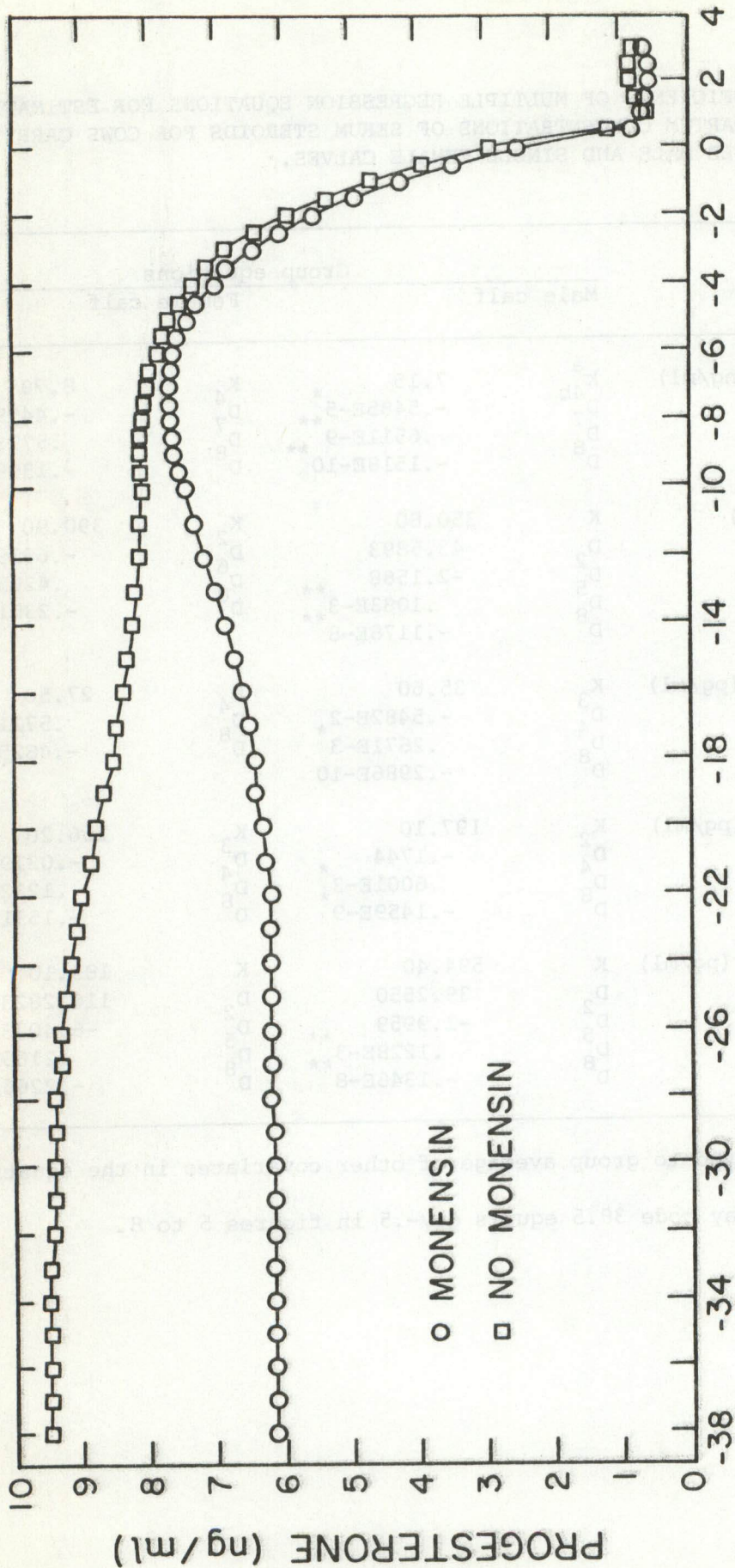
Figure 1. Average daily changes of serum progesterone in cows fed and not fed monensin. Regression equations are shown in table 1.
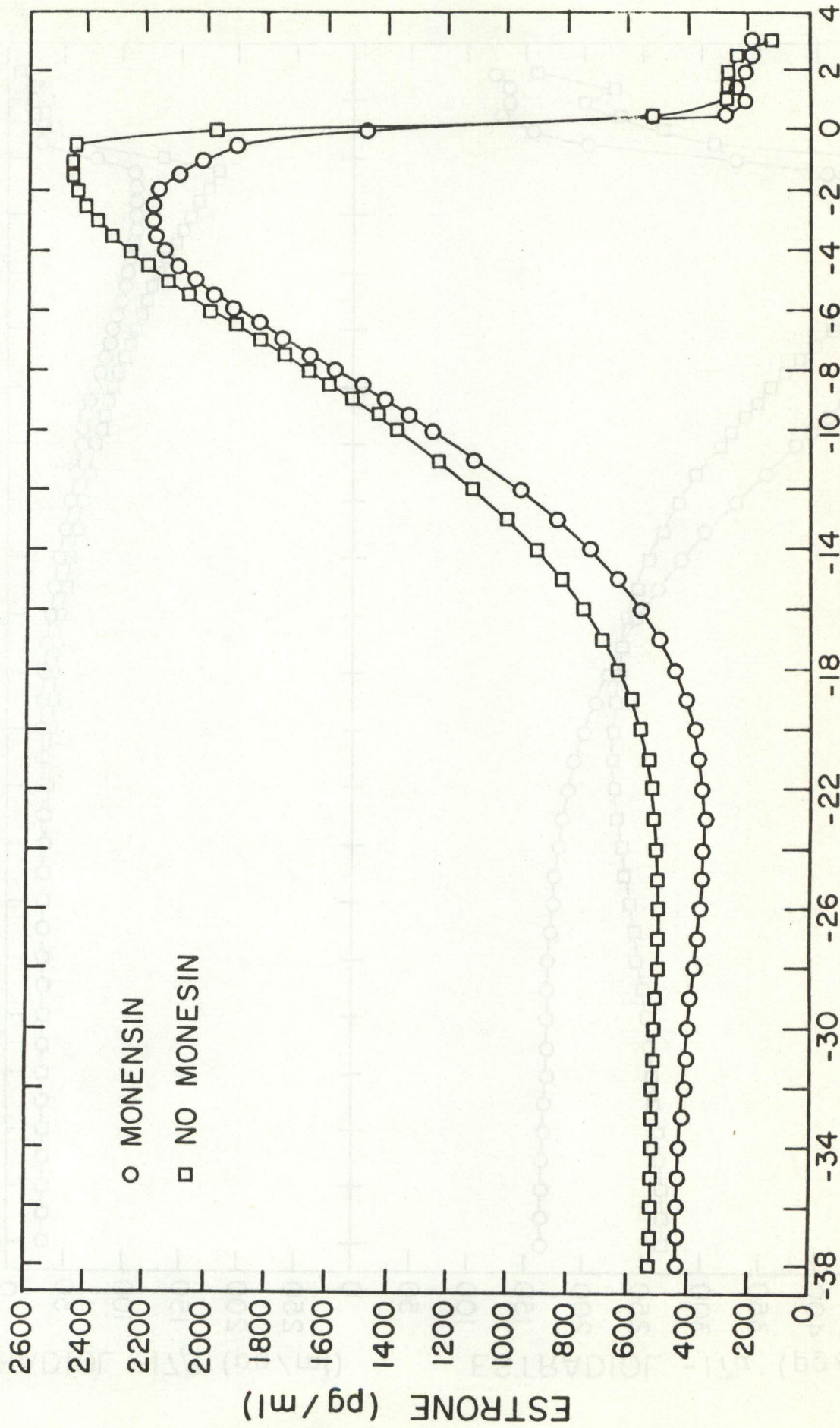
Figure 2. Average daily changes in concentrations of serum estrone in cows fed and not fed monensin. Regression equations are shown in table 1.
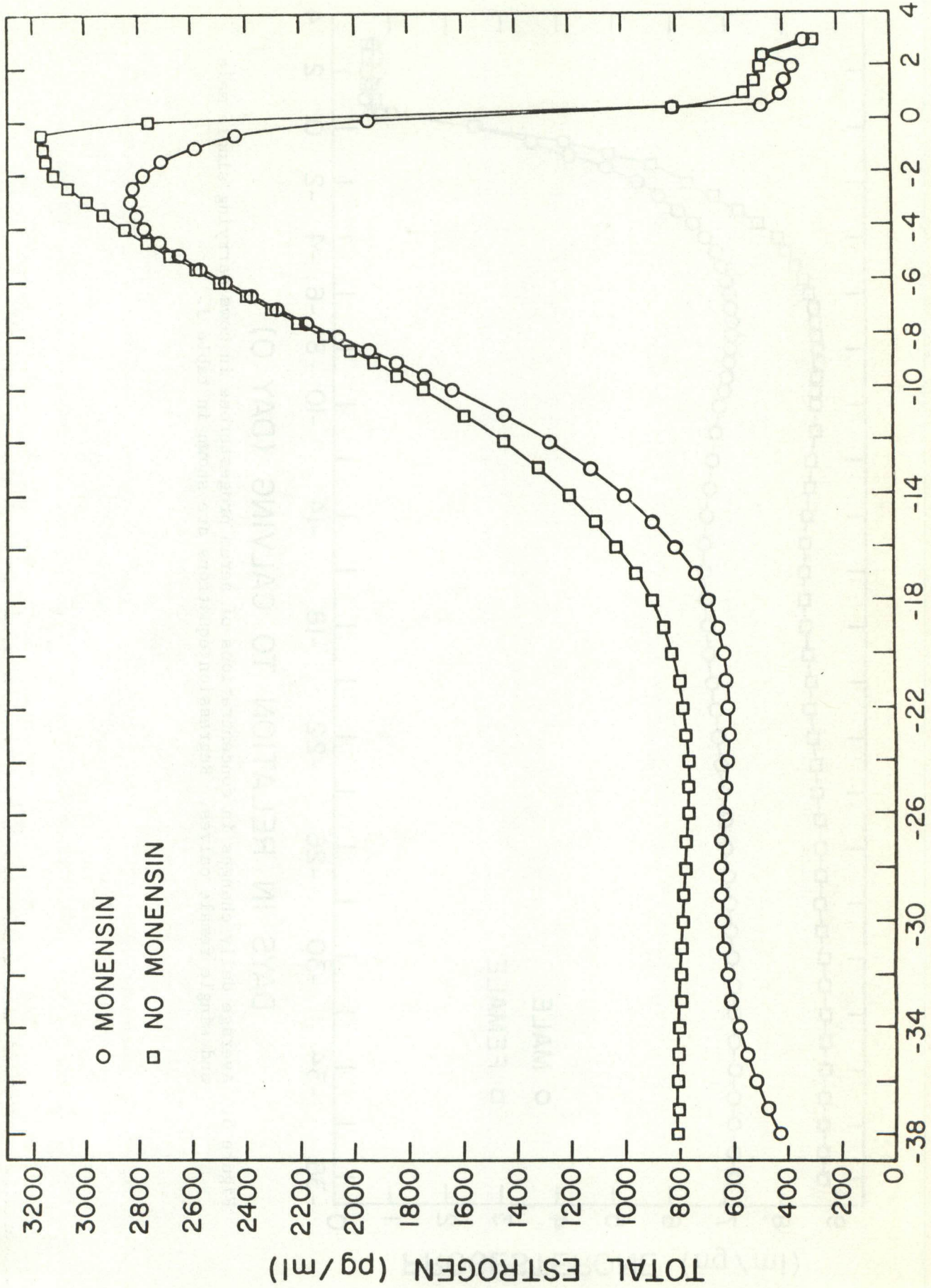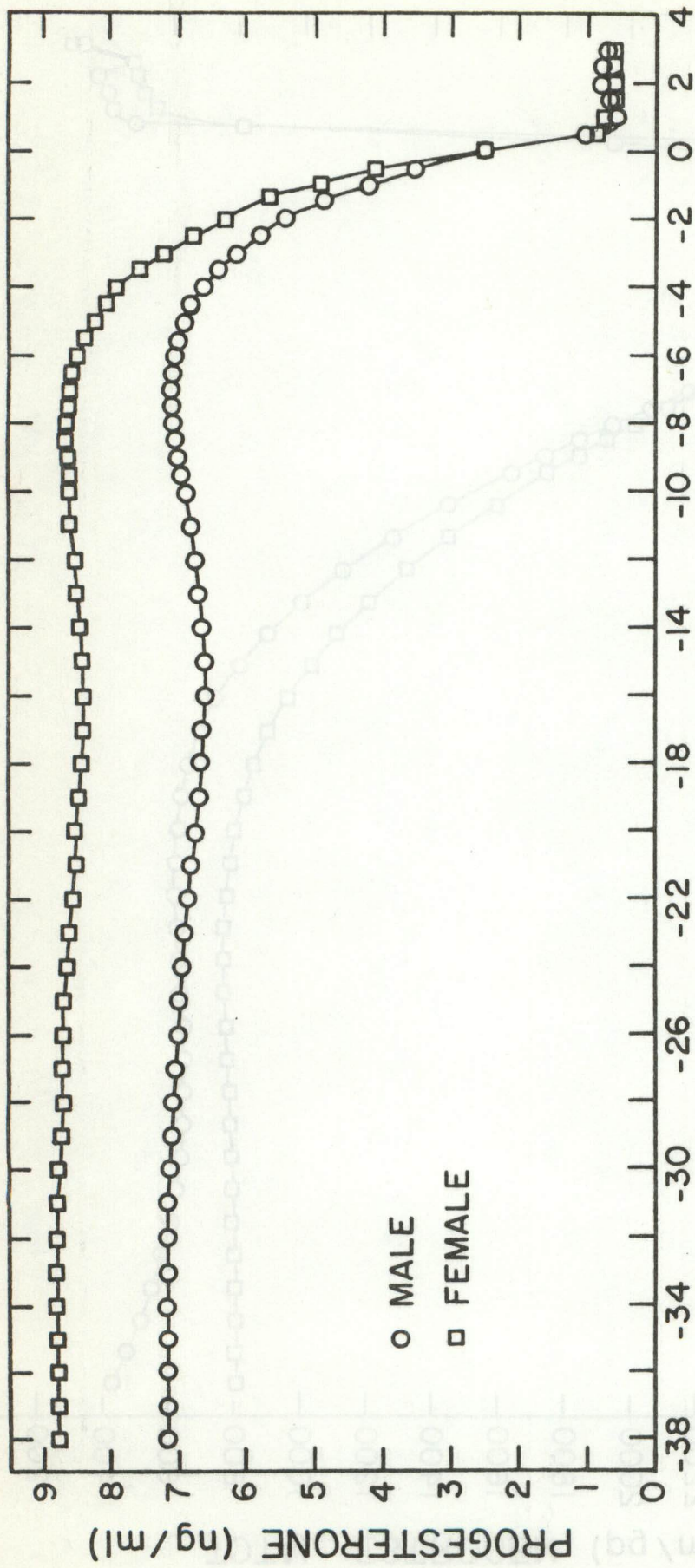
Figure 3. Average daily changes in concentrations of serum estradiol-17β and -17α in cows fed and not fed monensin. Regression equations are shown in table 1.

Figure 4. Average daily changes in concentrations of serum total estrogen in cows fed and not fed monensin. Regression equations are shown in table 1.

Figure 5. Average daily changes in concentrations of serum progesterone in cows carrying single male and single female calves. Regression equations are shown in table 3.
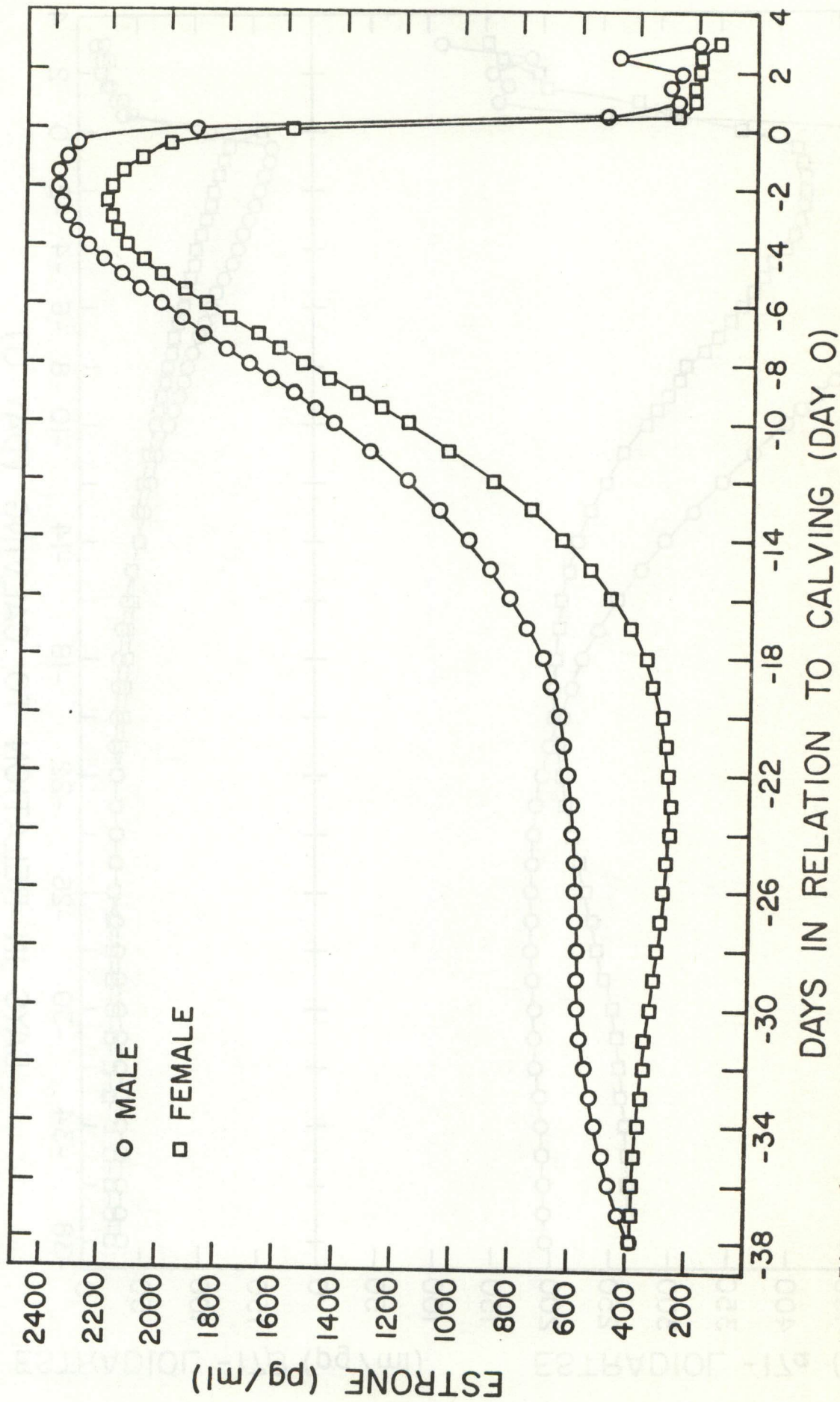
Figure 6. Average daily changes in concentrations of serum estrone in cows carrying single male and single female calves. Regression equations are shown in table 3.
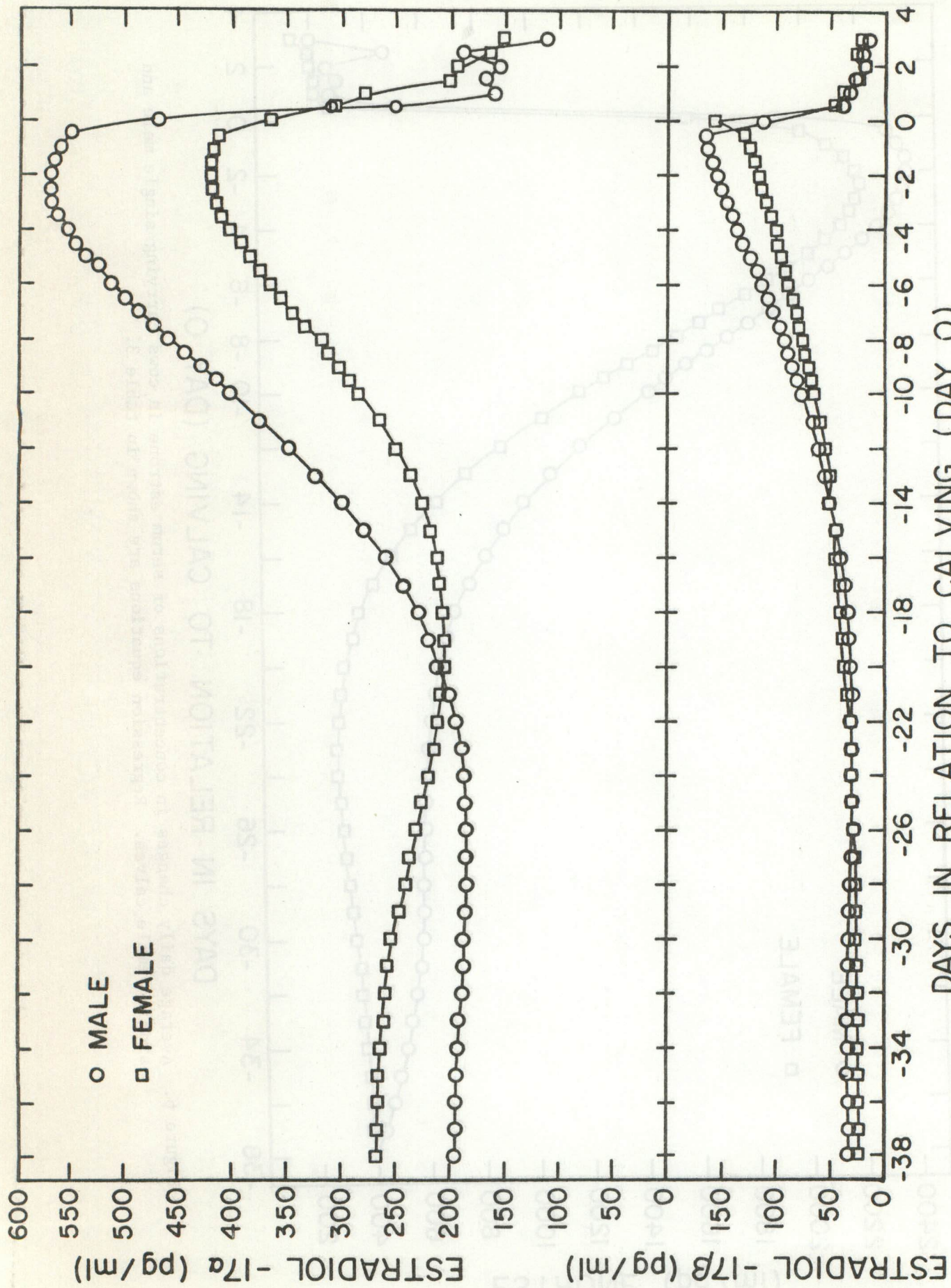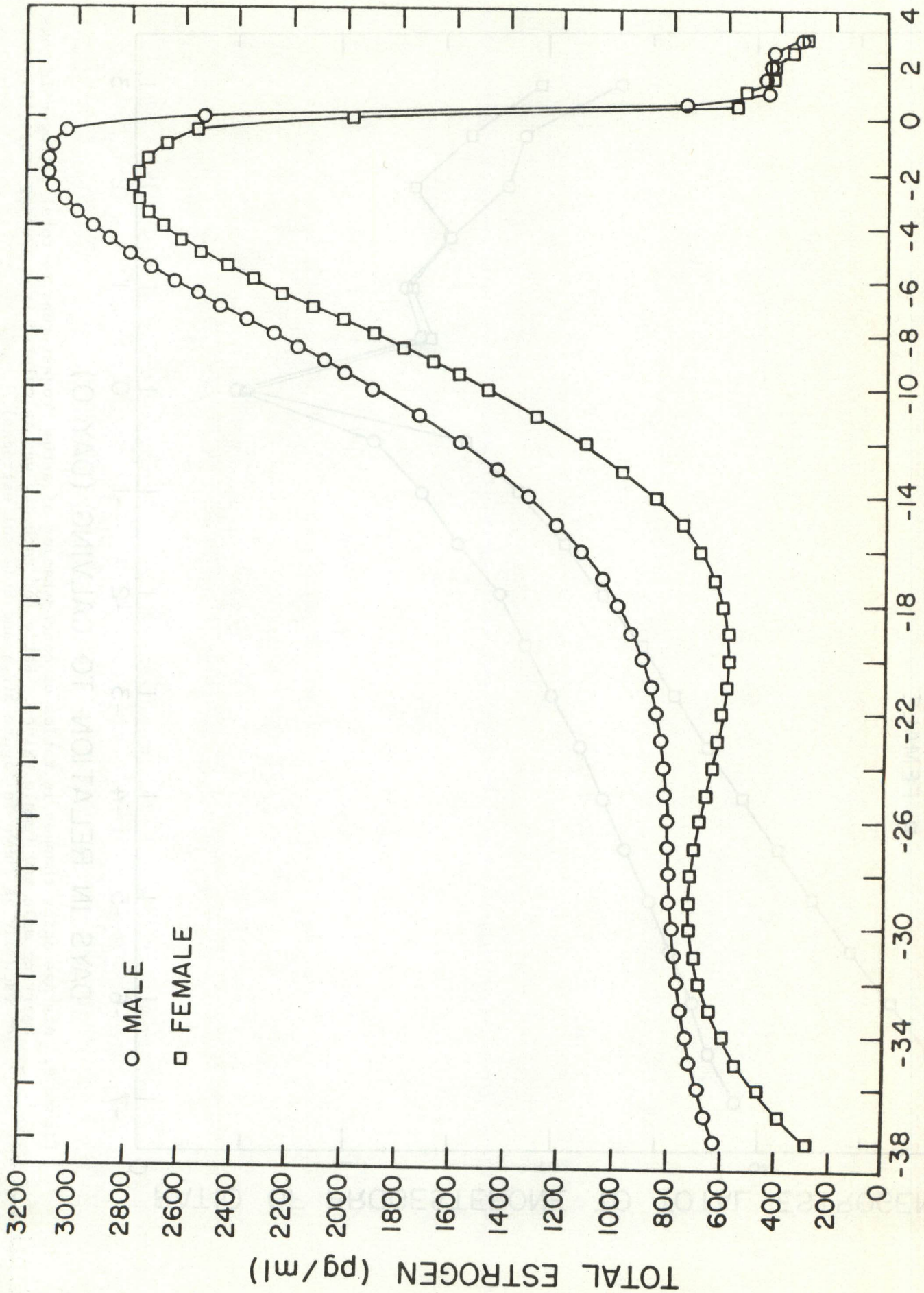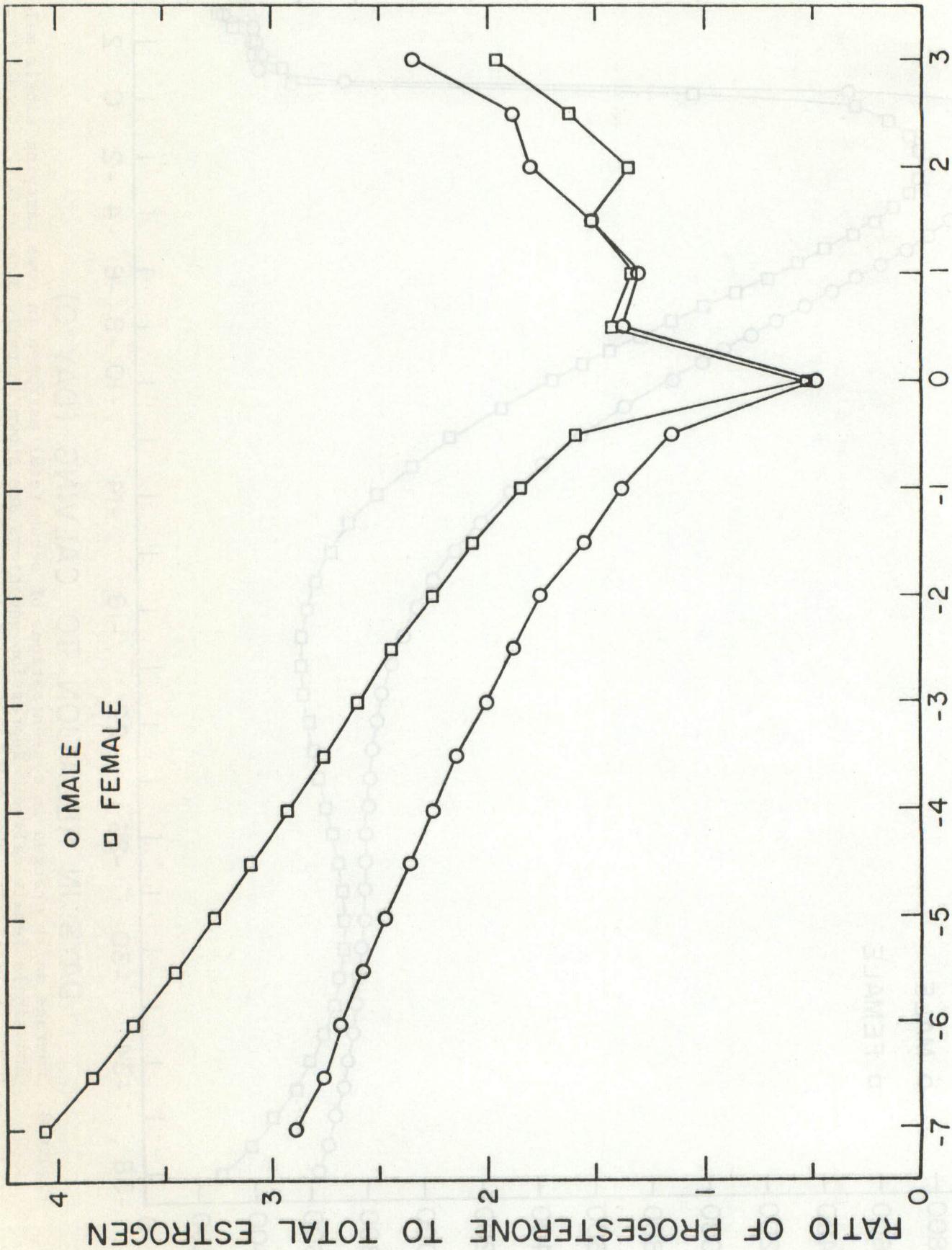
Figure 7. Average daily changes in concentrations of serum estradiol-17β and -17α in cows carrying single male and single female calves. Regression equations are shown in table 3.

Figure 8. Average daily changes in concentrations of serum total estrogen in cows carrying single male and single female calves. Regression equations are shown in table 3.

Figure 9. Average daily changes in ratios of concentration of serum progesterone to total estrogen in cows carrying male and female calves. Ratio for each day was calculated from average concentrations on each day as shown in figure 5(P_4) and 8 (total estrogen).

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