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OOPHORECTOMY DURING DIFFERENT STAGES OF PREGNANCY IN THE COW*

By

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LINDELL, J.-O., L.-E. EDQVIST and B. GUSTAFSSON: *Oophorectomy during different stages of pregnancy in the cow*. Acta vet. scand. 1981, 22, 553—565. — Thirteen cows of the Swedish Red and White Breed (SRB) were subjected to bilateral oophorectomy in the period 105—251 days of pregnancy. Eleven of the animals delivered their fetuses before the expected day of parturition, while 2 which were oophorectomized on days 205 and 212 calved at the expected time. Eleven of the cows had retained fetal membranes following abortion/parturition. Following surgery the peripheral plasma levels of progesterone dropped to levels about 10 % of the pretreatment levels. Animals oophorectomized between days 105 and 157 of pregnancy had a lower postsurgical progesterone concentration than cows operated on at days 205—251 of pregnancy. No increase of the peripheral plasma levels of estrone in connection with abortion was seen in cows operated on in early stages of pregnancy (< 200 days) while those operated on in later stages and maintaining the pregnancy for at least 17 days post oophorectomy showed an increase of estrone before parturition. The 2 cows that calved at the expected time had an increase of parturition estrone similar to that seen at spontaneous calving.

progesterone; estrone; pregnancy; abortion; cow.

An active corpus luteum (CL) is considered to be required for the maintenance of pregnancy in the bovine during the first two thirds of gestation (McDonald *et al.* 1953, Erb *et al.* 1968). Removal of the CL or oophorectomy after about day 200 has in some cases been shown to be compatible with the maintenance of pregnancy for up to 70 days (McDonald *et al.*, Estergreen *et al.* 1967, Erb *et al.*, Wenndorf & First 1977, Chew *et al.* 1979).

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However, parturition will in most cases occur prematurely. Dystocia and retained fetal membranes are common findings at such premature deliveries. These complications are suggested to be related to an insufficient progesterone production prior to parturition (*McDonald et al., Estergreen et al., Chew et al.*). Blood plasma levels of progesterone post oophorectomy in pregnant cows show that circulating progesterone declined within one day following oophorectomy (*Chew et al.*). However, in some cases the plasma progesterone concentration increased slightly again. These animals maintained pregnancy longer than those without increase in progesterone concentration after oophorectomy. The post oophorectomy progesterone concentration indicates an extra ovarian source, which most probably is the maternal adrenals (*Balfour et al. 1957, Wenndorf & First*).

The present study was designed to investigate blood plasma levels of progesterone and estrone, maintenance of pregnancy and course of parturition after oophorectomies at different stages of bovine pregnancy.

MATERIALS AND METHODS

Fourteen pregnant cows of the Swedish Red and White Breed (SRB) varying in age from 4 to 9 years were used. Thirteen of the cows were submitted to bilateral oophorectomy at pregnancy stages from 105 to 251 days. Surgery was performed on the standing animal after premedication with mebumal sodium (Mebumal, ACO, Sweden) and paravertebral anesthesia (1 % Xylocain, Astra, Sweden). Both ovaries were removed through an incision in the right flank region. In one cow sham operations were conducted on days 215 and 243 of pregnancy. On both these occasions the animal was subjected to the same surgical procedure as used in the other experimental animals, with the exception that the ovaries were left intact.

All animals were examined clinically at regular intervals before and after surgery. These examinations comprised daily inspections of the genital organs, the udder and the pelvic ligaments. In addition, vaginal inspection using a glass speculum and rectal examinations were carried out twice weekly. More frequent examinations were made when the animals showed imminent signs of parturition and after occurrence of abortion or parturition.

Blood samples were drawn daily from the jugular vein, starting 2 weeks before surgery and continuing through abortion or parturition and also through the immediate post partum period. Blood was collected into heparinized tubes and blood plasma was removed after centrifugation within 1 h after collection. The blood plasma samples were stored at below -18°C until the estrone and progesterone concentrations were analysed. Blood plasma levels of estrone were determined by a previously described radioimmunoassay procedure (Edqvist & Johansson 1972). This technique utilizes an antiserum to estradiol-17 β -17-succinyl-bovine-serum albumin. The cross-reaction percentage for the antiserum with estrone was 65 % (cross-reaction with estradiol-17 β , 100 %). Peripheral plasma levels of progesterone exceeding 1 ng/ml were determined by a competitive protein binding technique (Edqvist *et al.* 1970), while all levels below this figure were determined by radioimmunoassay (Kindahl *et al.* 1976) utilizing an antiserum to 11 α -hydroxyprogesterone-11-hemisuccinate-bovine-serum albumin. All hormonal measurements were made in duplicate.

For conversion of the figures to the S.I. system, the following factors should be used:

1 ng/ml of progesterone	3.23 nmol/l
1 ng/ml of estrone	3.71 nmol/l

RESULTS

Clinical outcome

Dependent on the stage of pregnancy at surgery and the interval between removal of the ovaries and delivery the animals have arbitrarily been divided into 3 groups (Table 1).

The 5 cows operated on between days 105 and 157 of their pregnancies all expelled their fetuses within 2 to 4 days of surgery (Table 1, Group I). These animals showed no visible signs of approaching delivery and, furthermore, no external signs of labour could be seen during the expulsion stage. All fetuses were dead at delivery. All the cows in Group I had retained fetal membranes (RFM) defined as membranes not expelled spontaneously within 24 h after delivery. During the immediate post partum period 2 of the animals in this group showed clinical signs of acute endometritis. They were subsequently treated with antibiotics and recovered within 3 days.

Table 1. Some clinical data on the animals used.

	Animal No.	Stage of pregnancy at surgery (days)	Stage of pregnancy at delivery (days)	Time interval from surgery to delivery (days)
Group I	1	105	107	2 ^a
	2	141	144	3 ^a
	3	151	155	4 ^a
	4	153	156	3 ^a
	5	157	161	4 ^a
Group II	6	210	213	3 ^a
	7	245	251	6 ^a
	8	249	254	5 ^a
	9	251	257	6 ^a
Group III	10	205	272	67 ^b
	11	212	286	74 ^b
	12	224	249	25 ^a
	13	242	259	17 ^a

^a fetal membranes retained for more than 24 h.

^b no retention of fetal membranes.

Of the remaining 8 animals which were operated on between days 205 and 251 of their pregnancies the interval between surgery and delivery varied between 3 and 74 days. Four of these animals aborted or delivered prematurely within 6 days after operation (Table 1, Group II), while the remaining 4 animals maintained their pregnancies for 17 to 74 days after surgery (Table 1, Group III). All deliveries in Groups II and III were preceded by signs of approaching parturition, these signs being more pronounced with advancing stage of pregnancy at delivery. The course of parturition was normal except in two cows (Nos. 7 and 11) which both had a prolonged stage of expulsion. In 1 of them (No. 7), which delivered twins, manual tractation was necessary. The 4 cows in Group II and 2 of those in Group III had RFM. With the exception of cow No. 6 all animals in Groups II and III gave birth to living calves. The calf of cow No. 12 died 8 h after parturition. All other calves survived and developed normally.

The cow that had sham operations on days 215 and 243 of pregnancy calved normally after a gestation period of 272 days, but had RFM.

Hormonal measurements

During the 14 days preceding the surgery, progesterone levels varied from 1.5 to 9.2 ng/ml. Relatively pronounced elevations of the progesterone concentration occurred in some animals in conjunction with surgery regardless of the stage of pregnancy. The mean plasma progesterone concentrations 36–60 h before and after operation, as well as 12–36 h before and after delivery, for the different groups of animals are given in Table 2.

Table 2. Peripheral plasma progesterone concentrations before and after surgery, and before and after delivery.

	Progesterone concentrations ng/ml			
	48±12 h before surgery	48±12 h after surgery	24±12 h before delivery	24±12 h after delivery
<i>Group I</i>				
mean±s.e.m.	4.1±0.6	0.4±0.1	0.3±0.1	0.1±0.1
range	2.9–5.0	0.3–0.6	0.1–0.5	0.1–0.2
<i>Group II</i>				
mean±s.e.m.	5.4±0.6	1.5±0.3	1.0±0.3	0.4±0.1
range	3.9–6.6	1.0–2.2	0.5–1.8	0.3–0.5
<i>Group III</i>				
mean±s.e.m.	5.0±0.9	1.1±0.2	0.8±0.1	0.6±0.2
range	2.9–7.0	0.8–1.6	0.6–1.1	0.3–1.1
Differences between means (Student's t-test)				
I:II	n.s.	***	*	**
I:III	n.s.	**	**	*
II:III	n.s.	n.s.	n.s.	n.s.

n.s. (not significant) = $P > 0.05$;

* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$.

Removal of the ovaries resulted in a pronounced decrease of the progesterone levels in all cows. The progesterone level measured 36–60 h after surgery in cows of Group I was significantly lower than in Groups II and III (Table 2). From 36–60 h after surgery and onward no dramatic change of the progesterone level occurred until after delivery, when a further reduction of the mean levels was found in all 3 groups of cows.

In Figs. 1–3 are presented individual data for cows Nos. 2, 8 and 11 representing Groups I, II and III respectively. Cows

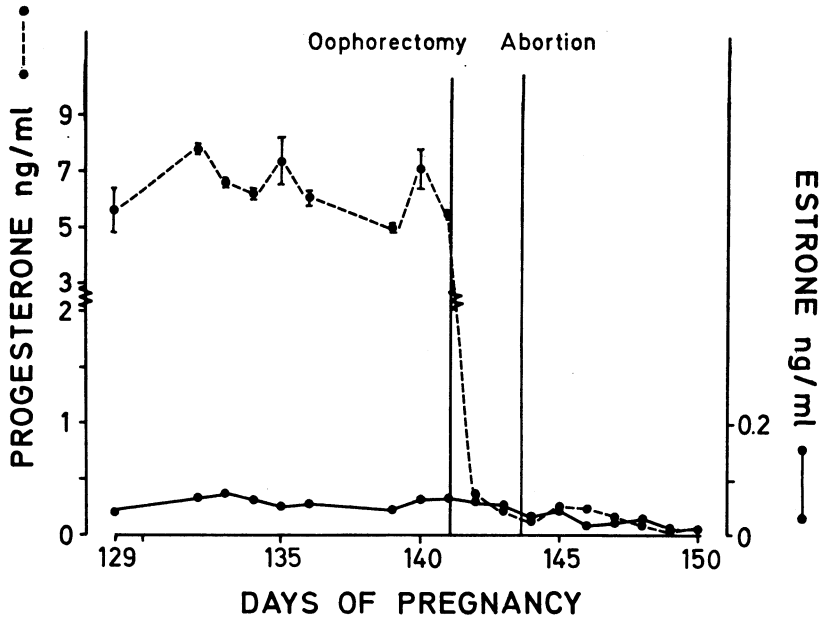


Figure 1. Peripheral blood plasma levels of progesterone (●—●) and estrone (●—●) in cow No. 2 (representing Group I).

Nos. 8 and 11 had elevated progesterone levels in conjunction with surgery (Figs. 2 and 3). The post oophorectomy decrease of progesterone was most pronounced in cow No. 2 (Group I), in which the level fell to below 0.5 ng/ml 12–36 h after surgery. In cows Nos. 8 and 11 (Groups II and III, respectively) the progesterone levels also decreased but reached a concentration of 1–1.5 ng/ml in the interval 12–36 h after surgery. In cow No. 8 this level was maintained for 3 days, after which a further reduction of the concentration occurred in conjunction with a premature delivery. Cow No. 11 maintained a peripheral blood plasma level of progesterone of about 1 ng/ml for 74 days, after which it calved at the expected time. A pronounced prepartum drop in the progesterone concentration could not be demonstrated in this cow.

Peripheral plasma levels of estrone at different intervals before and after surgery and delivery are presented in Table III. Cows in Group I had mean values below 100 pg/ml at all intervals studied. Presurgical estrone levels in animals of Group II were significantly higher than in Group I and III. 36–60 h after surgery there is a tendency to an increased mean value of estrone

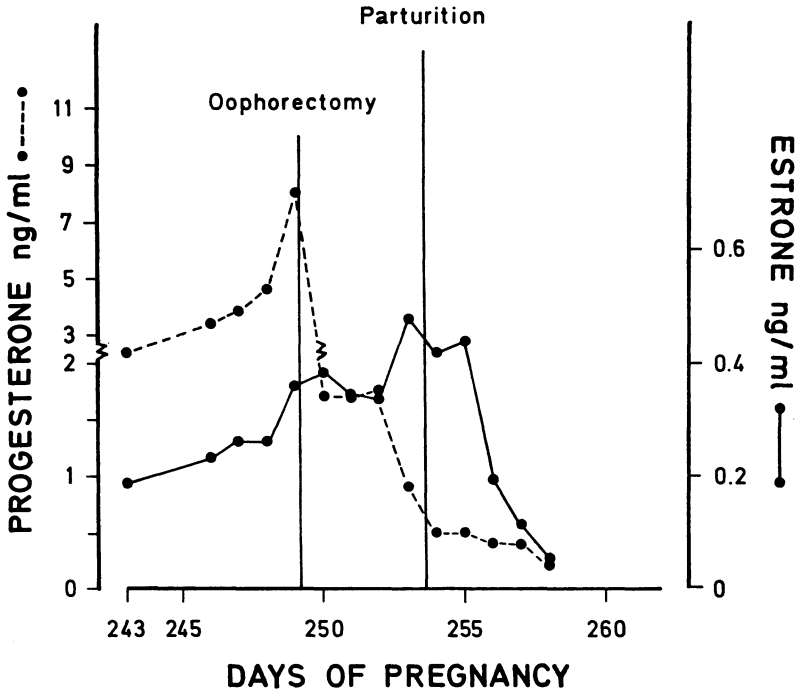


Figure 2. Peripheral blood plasma levels of progesterone (●---●) and estrone (●—●) in cow No. 8 (representing Group II).

for cows in Group II as compared to the presurgical level, while for cows in Groups I and II no such increase of the mean estrone level was recorded. Cows in Group III had highly elevated estrone concentrations prior to parturition, while no such marked prepartum increase was noted for the other 2 groups of animals. After delivery the estrone level diminished, although quite high levels were found in individual cows of Groups II and III in the interval 36—60 h after delivery.

Cow No. 2 showed no marked change of the estrone concentration in conjunction with surgery and abortion. There is, however, a tendency for the estrone level to decrease after abortion in the Group I animals. In cow No. 8 the estrone concentration increased in conjunction with surgery and continued to increase until parturition, after which it gradually decreased and reached a concentration below 0.05 ng/ml 5 days later. The estrone concentration in cow No. 11 showed no increase in conjunction with surgery and was maintained on a level of about 0.1—0.2 ng/ml

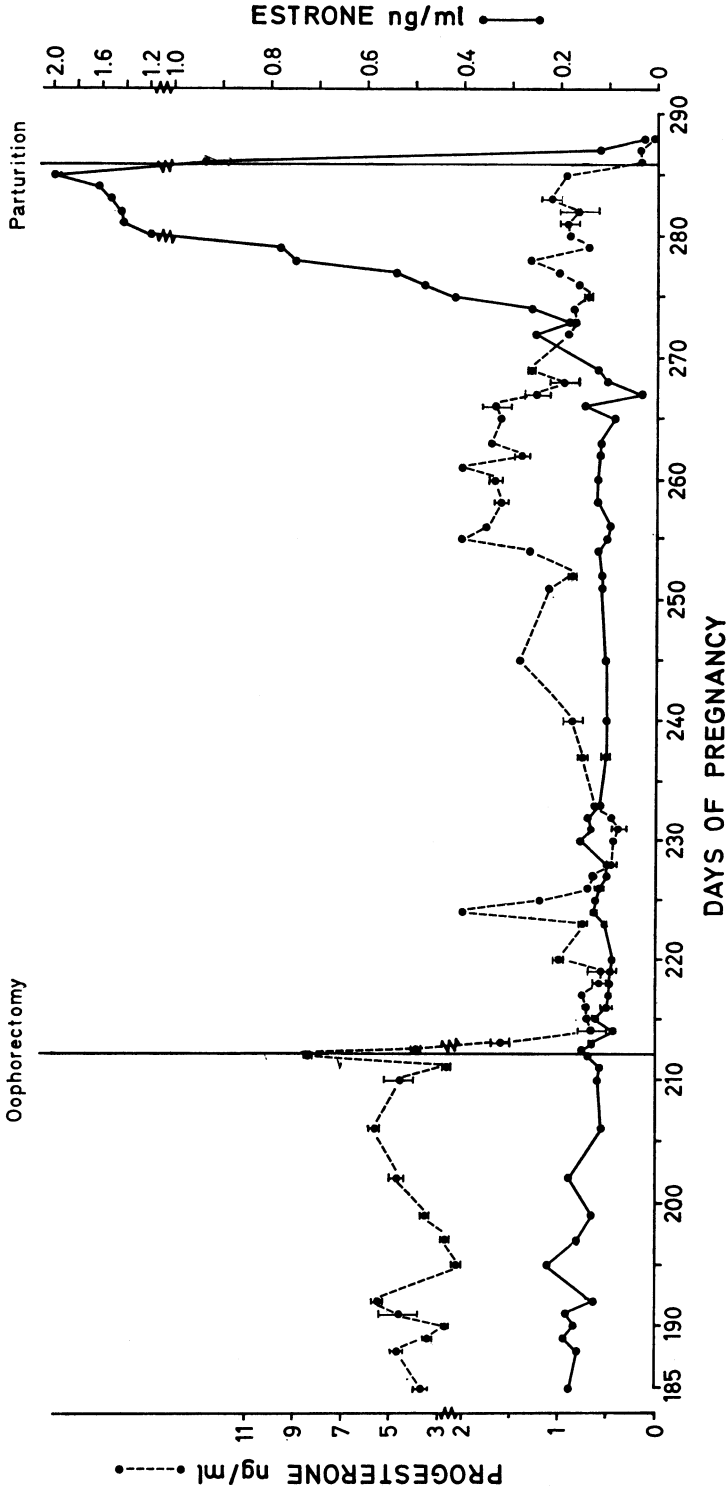


Figure 3. Peripheral blood plasma levels of progesterone (●---●) and estrone (●—●) in cow No. 11 (representing Group III).

Table 3. Peripheral plasma estrone concentrations before and after surgery, and before and after delivery.

	Estrone concentration pg/ml			
	48±12 h before surgery	48±12 h after surgery	24±12 h before delivery	24±12 h after delivery
Group I				
mean±s.e.m.	66±27.7	67±31.5	84±30.9	31±9.8
range	33—176	15—188	15—200	10—60
Group II				
mean±s.e.m.	274±32.1	391±115.8	365±20.5	307±123.5
range	212—364	176—720	328—420	80—588
Group III				
mean±s.e.m.	111±35.7	90±36.1	745±35.2	371±137.4
range	25—200	30—192	324—1570	125—600
Differences between means (Student's t-test)				
I:II	**	**	**	**
I:III	n.s.	n.s.	**	**
II:III	*	*	n.s.	n.s.

n.s. (not significant) = $P > 0.05$;

* = $P < 0.05$; ** = $P < 0.01$.

until around day 270 of pregnancy, after which it gradually increased and reached concentrations of about 2 ng/ml immediately prepartum. After delivery the concentration decreased rapidly and levels below 0.05 ng/ml were reached 3 days later.

DISCUSSION

The present study revealed that oophorectomy resulted in a marked drop in the plasma progesterone levels at all stages of pregnancy investigated. A similar sharp decrease in the progesterone concentration following oophorectomies has been reported earlier (*Chew et al.* 1979, *Hoffman et al.* 1979). Several studies have demonstrated that the CL of pregnancy in the cow is the main source of progesterone secretion, as evaluated by analyses of the progesterone content in CL tissue obtained at different stages of pregnancy (*Stormshak & Erb* 1961, *Axelsson et al.* 1975) and the determination of progesterone in ovarian venous plasma (*Fairclough et al.* 1975, *Evans & Wagner* 1976, *Hoffman et al.*). The post oophorectomy levels in late pregnant

cows indicate the presence of an extra-ovarian progesterone source in these animals as opposed to the early pregnant cows.

Several studies have indicated the maternal adrenal cortex to be a possible source of progesterone secretion in the pregnant cow (Short 1956, Stormshak & Erb, Comline et al. 1974). That this gland might be extra-ovarian source is further supported by an experiment in which cows were oophorectomized on day 215 of pregnancy (Wenndorf & First 1977). The majority of the animals continued their pregnancies but it was terminated within 4 days in cows additionally adrenalectomized. The results of the present study do not provide information on the nature of the extra-ovarian progesterone source but they confirm its existence.

The blood concentrations of progesterone and estrogen as related to the clinical outcome of the experiments indicate that the abortions in the early stages of pregnancy most likely were due to the very low post-surgical progesterone levels being insufficient for maintenance of pregnancy. The low estrogen concentrations in these animals throughout the investigation and the absence of preabortion increase in estrogen might be explained by an immature placenta.

In the other 2 groups it is obvious that the progesterone concentration per se was not the sole determining factor for termination of pregnancy. Animals in Group II aborted or calved within 6 days of surgery despite progesterone levels that were equal to those which maintained pregnancies for up to 74 days in Group III. The relatively rapid onset of labour after surgery in Group II animals might be related to the higher pre- and post-surgical estrogen concentrations than in Group III. These higher estrogen levels were probably an indicator that the fetal-placental unit had reached a stage where it had the ability to respond to surgical stress with endocrine events normally preceding parturition, e.g. with an increase of prostaglandin synthesis and release. Artificial induction of parturition in late pregnant cows with glucocorticoids involved a rise in estrogen concentration (Edqvist et al. 1972, Evans & Wagner 1976, Lindell et al. 1977) and a subsequent rise in $\text{PGF}_{2\alpha}$ (Lindell et al.). It is now known precisely at which stage of pregnancy corticosteroid administration can elicit estrogen rise and subsequent PG increase. It is obvious, however, that the possibility exists at about 240 days of pregnancy (Edqvist et al. 1972, Lindell et al.). Only 1 cow in

Group III had reached that stage of pregnancy at surgery. This might imply that the fetal placental unit was not yet ready to respond to surgical stress with an estrogen production large enough to stimulate PG synthesis and release. The results of the present study agree with those of *Wenndorf & First* and *Chew et al.*, who found the longest interval between surgery and calving in cows oophorectomized on day 215 of pregnancy.

The progesterone concentrations were in many cases elevated on the day of surgery. The blood samples from these animals were drawn after the cows had been transported to the operation room and it is reasonable to assume that they were submitted to a certain stress by the transport. Surgical stress has been shown to increase the plasma progesterone concentration in the pregnant goat (*Heap & Linzell* 1966, *Thorburn et al.* 1972).

Low progesterone levels before calving have been suggested to predispose the animal for retention of the fetal membranes (*McDonald et al.* 1954, *Chew et al.*), but also higher than normal estrogen levels (*Agthe & Kolm* 1975).

The present material is obviously too small to allow any conclusion on the hormonal background to RFM. However, comparing Group II and III it does not seem likely that low progesterone per se would be a significant factor. The 2 cows in Group III that expelled the placenta spontaneously showed a greater increase in estrogen before parturition than the other 2 cows and than cows in Group II. This might point to an insufficient prepartum concentration of estrogen as a crucial factor. It should, however, be emphasized that the 2 cows with spontaneous expulsion of the FM also had the longest gestation periods (272 and 286 days) which implies that other time related maturation processes in the placentomes might have been involved (*Grunert et al.* 1976).

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SAMMANFATTNING

Oophorektomi av kor under olika stadier av dräktigheten.

I försöket ingår 13 kor på vilka äggstockarna bortopererats vid dräktighetsstadier varierande från 105 till 251 dagar. Två kor vilka opererats å respektive dag 205 och 212 bar sina foster dräktighetstiden ut medan de övriga kalvade tidigare än beräknat. Hos två av de 13 djuren avgick efterbörden spontant inom 24 timmar efter aborten/förlossningen. Efter operationen sjönk progesteronvärdena till låga nivåer.

De kor vilka opererats under tidiga dräktighetsstadier (105—157 dagar) hade påtagligt lägre progesteronvärden efter operationen jämfört med de kor vilka opererats senare under dräktigheten (> 200 dagar). Detta kan sannolikt förklaras med en progesteronsekretion från de materna binjurarna under den sista tredjedelen av dräktigheten.

Efter oophorektomi vid tidiga dräktighetstider förändrades den materna blodöstronkoncentrationen ej påtagligt. Hos djur som opererats under sena dräktighetsstadier och som vidmakthöll dräktigheten i minst 17 dagar efter operation var östronnivån påtagligt förhöjd i samband med förlossningen. De två djur som opererades under sent dräktighetsstadium och som kalvade vid beräknad tidpunkt hade en prepartal östronökning som var snarlik den som ses före normalförlossning.

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