

OESTRUS-LIKE SYMPTOMS IN PREGNANT BITCHES

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Abstract. Oestrogenization during pregnancy was described in a few animal species and in women. The authors of this paper observed oestrus-like symptoms during five pregnancies in four bitches (group S) and compared chosen parameters with those in pregnant bitches without such symptoms (group C). Studied bitches were examined by vaginoscopy, vaginal cytology and ultrasonography. Concentrations of progesterone, estrogens and testosterone were measured. Blood progesterone concentration during pregnancy was similar in groups S and C. There were statistically significant differences in concentration of estrogens from 21st to 35th day postmating (P from 0.01 to 0.001). Testosterone concentrations showed statistically significant differences between group S and C only on 42nd and 56th day postmating ($P \geq 0.01$). We did not observe signs of embryonic resorption or miscarriage in group S, and bitches delivered 6, 5, 7, 8 and 11 puppies. Higher estrogens concentration in pregnant bitches with oestrus-like symptoms suggests hormonal extra supplementation, but the source/es of estrogens remained unknown. Additional research would be necessary to discover the source of estrogens in pregnant bitches.

Key words: dog, pregnancy, oestrus, estrogens

INTRODUCTION

Physiological course of pregnancy is ensured by many complicated mechanisms of neurohormonal regulation. Key role in this regulation play: progesterone (P4), prolactin, relaxin and luteinizing hormone (LH) [Nett et al. 1976, Concannon

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and Verstegen 1998, Johnston et al. 2001]. The main hormone responsible for supporting pregnancy is progesterone. Plasma progesterone concentration increases from the end of pro-estrus to the peak approximately 20–30 days after the LH surge, and then slowly declines to basal concentration immediately before parturition. Rapid fall of progesterone concentration, approximately on the 64th day after LH peak, is caused by luteolytic action of PGF 2α . At that point, concentration of progesterone reaches $\leq 2 \text{ ng} \cdot \text{ml}^{-1}$. Pregnancy associated progesterone produced by canine corpus luteum (CL) is significantly higher than in nonpregnant females, but it is rapidly metabolized in the periphery and utilized by the placenta, resulting in similar plasma concentrations of native progesterone in both pregnant and nonpregnant bitches [Onclin and Verstegen 1997]. Diurnal changes in the concentration of progesterone were observed. It was found that in the morning (8.00 am) concentration of progesterone is two times higher than in the afternoon (3.00 pm). Such changes were not observed in the concentration of estrogens, relaxin nor prolactin [Marinellia et al. 2009]. Secretion of progesterone by corpora lutea is stimulated by luteotropic activity of PGE and LH, and in the second half of pregnancy, by prolactin [Concannon and Verstegen 1998, Johnston et al. 2001, Hoffmann et al. 2007b, Marinellia et al. 2009]. The role of estrogens (E) in the regulation of pregnancy in the bitch has not been clearly identified, and relevant opinions could be generally divided into three groups. Currently dominates the conception that the level of estrogens in pregnant bitches does not change during the pregnancy, or only minor oscillations appear within chosen breeds of dogs [Austad et al. 1976, Steinetz et al. 1990, Concannon and Verstegen 1998]. Another group of authors demonstrates an increase in estrogens concentrations directly before parturition [Onclin et al. 2002, Luz et al. 2006]. However, some researchers observed the increase in estradiol concentration between 20th and 25th day of pregnancy, and then decrease to reference values before parturition [Hadley 1975, Tibold and Thuroczy 2009].

In some species such as rats and rabbits [Thomas and Dobson 1989, Concannon et al. 1997] estradiol is secreted by the luteal cells and plays a role in luteal regulation. Given that placenta does not produce 17 β -estradiol, its most probable source is the corpus luteum. In conjunction with prolactin, 17 β -estradiol is necessary to support production of progesterone by the corpus luteum through its influence on uptake, synthesis and transformation of cholesterol [Hoffmann et al. 2007 a].

Androstenedione is the major circulating androgen during the follicular and luteal phases of ovarian cycle. Androstenedione levels during the luteal phase parallel those reported for progesterone in pregnant and nonpregnant bitches, including maintenance of elevated levels throughout gestation, and an abrupt decline at parturition [Concannon and Castracane 1985, Bielańska-Osuchowska 2001].

During ovarian steroidogenesis a fraction of the androstenedione is converted to testosterone, which in turn undergoes conversion to estradiol by an enzyme aromatase. Testosterone (T) levels reached peaks near the time of the preovulatory luteinizing hormone peak, and were reduced to near the limits of detection throughout the luteal phase [Concannon and Castracane 1985].

Occurrence of oestrus during pregnancy is described in few animal species. It happens frequently in cattle and rarely in mares [Tomasgard and Benjaminsen 1975, Nett et al. 1976, Concannon and Castracane 1985]. Specific oestrus behavior and characteristic changes in the cervical mucus are observed in cattle, but typical concentrations of hormones, characteristic for oestrus, does not occur [Nett et al. 1976, Concannon and Castracane 1985]. So far it has not been reported to occur in bitches. In this paper, authors describe oestrus-like symptoms in the pregnant bitches. The aim of the study was to monitor the course of pregnancy in affected bitches, and to compare their blood P4, E and T concentration to those in control bitches.

MATERIAL AND METHODS

Animals and diagnosis. The study was carried out on 9 pregnant bitches (five clinically healthy – control group (C), aged 2.87 ± 1.45 years, and four with oestrus-like symptoms – studied group (S), aged 1.99 ± 0.9 years of different breeds (one Shih-Tzu, two German Shepherd Dogs (GSD), and one Irish Setter), with a mean weight of 21.2 ± 8.72 kg. Shih-Tzu was observed in two consecutive pregnancies, other bitches one time – in total 5 pregnancies. Diagnosis was based on anamnesis, general clinical examination, and gynecological examination (abdominal palpation, vaginal cytology, and vaginoscopy).

USG. Ultrasound scanner (Honda 4000, Japan) with a 5/7.5 MHz probe was used to examine the reproductive system every 7 days, starting from day 21st to 23rd after mating. Before the scan, skin was shaved, disinfected and coated with USG gel. Echography was performed for the purpose of the recognition, and the monitoring of the course of pregnancy. Heart rate, fetal movements, and the condition of placentas and amniotic fluid were evaluated. Ovaries were tested in order to detect tumors or cysts as a possible source of estrogens.

Cytology. The examination was performed at the time of clinical oestrus-like signs in pregnant bitches. Cytological examination of vaginal smears was carried out every other day started from day 21st after mating to the end of clinical oestrus-like symptoms (33–35 days after mating). Samples were collected with a cotton swab from anterior vagina. The vulvar lips were gently separated with fingers, and the swab was passed into dorsal aspect of the vulva, avoiding clitoral fossa. Next, the swab was advanced in craniodorsal direction, towards vertebral

column, then rotated and withdrawn. Color and type of secretions were observed. The cotton tip of the swab was rotated gently on a degreased glass slide. Pappenheim-type stain was used for cell staining.

Vaginoscopy. The examination was executed once on day 21st–23rd after mating for the purpose of the estimation of changes in the vagina at the time of oestrus-like symptoms in pregnant bitches. The vaginoscopy discontinued because of the absence of owners' assent.

Blood samples and hormonal profiles. Blood samples were collected from supraradial vein every 7 days started from 21st day after mating. Measurements of progesterone and total estrogens concentrations based on immunofluorescence assay, using commercial immunoenzymatic tests for quantitative determination of P4 and E (Pointe Scientific Poland). Concentration of estrogens was measured after extraction by ethyl acetate. Measurement of fluorescence was taken with a Pointe 2000 apparatus. Every analysis was performed two times in each series. Efficiency of extraction oscillated between 92 and 99%. Assay sensitivity and intra-series errors were $0.05 \text{ ng} \cdot \text{ml}^{-1}$ ($0.8 \text{ nmol} \cdot \text{l}^{-1}$) and 8.0% for P4, and $10 \text{ pg} \cdot \text{ml}^{-1}$ ($37 \text{ pmol} \cdot \text{l}^{-1}$) and 9.6% and for E, respectively. Measurement of testosterone concentration was made by immunofluorescent assay with commercial immunoenzymatic tests for quantitative determination of testosterone (DPC, USA). Fluorescence was measured with the Immulite 2000 apparatus. Assay sensitivity and intra-series error were $0.15 \text{ ng} \cdot \text{ml}^{-1}$ ($0.5 \text{ nmol} \cdot \text{l}^{-1}$) and 8.2% respectively.

Statistical analysis. Results were presented as arithmetic means and standard deviations. The significance of differences was calculated using Student's *t*-test and the Mann-Whitney-Wilcoxon test, prepared with the program Statistica for Windows (Statistica 10.0, StatSoft, Cracow, Poland). P-value was set at 0.05.

RESULTS

During first pregnancy the Shih-Tzu owners registered a haemorrhagic vulvar discharge between the 23rd to 25th day after mating, and few days later the vulva enlargement was observed. In first 7 days of observation the color was red, and then it turned pink.

During examination the bitch lifted up her tail as in male acceptance period. Echography showed 6.16 ± 0.75 fetuses during observation period with fetal heart rate 176.66 ± 18.35 . No pathological signs in the course of pregnancy were observed. The studied bitch gave birth to six normally developed puppies on day 61 after mating (4 female and 2 male).

The second pregnancy in this bitch has been monitored since the day of mating. First signs of haemorrhagic vulvar discharge were noticed on day 21st after mating, and the vulva was mildly enlarged. Until the 26th day after mating, ha-

emorrhagic discharge increased and then became pale pink (35 days after mating). The small discharge was observed till the parturition. Echography showed 5 fetuses with heart rate 171.22 ± 17.22 . The bitch gave birth to five normal newborns on 62nd day after mating (3 female and 2 male). During both pregnancies there was no clinical signs characteristic for miscarriage or premature parturition. No tumors or cysts were diagnosed on the ovaries.

In two GSD bitches haemorrhagic vulvar discharge and vulva enlargement between the 21st to 23rd days after mating were observed. Discharge was red, and then became pink during the first nine days of observation. At the time of clinical examination the bitches lifted up their tails as in male acceptance period. In one bitch, the echography showed 7.5 ± 1.05 fetuses with heart rate 185.0 ± 23.45 , while 7.83 ± 0.75 fetuses with heart rate 177.67 ± 18.62 were shown in another. No pathological signs were observed at the course of pregnancies in these two bitches. They gave births to seven (4 female and 3 male) and eight normal puppies on 65th and 66th day after mating (4 female and 4 male).

In Irish Setter bitch the first bloody vaginal secretion and vulva enlargement appeared between the 21st to 23rd day post mating. Vulvar discharge red in color was observed for 5 days only. Sonographic examination revealed 10.2 ± 1.17 fetuses with heart rate 172.67 ± 18.34 and no pathological signs at the course of pregnancy. The bitch gave birth to eleven puppies on 66th day after mating (6 female and 5 male). In observed group we had together 37 puppies (21 female – 57.76% and 16 male – 42.24%). In control group bitches were born 42 puppies (22 female – 52.38% and 20 male – 41.62%). There were no statistically significant differences between observe and control groups.

In all studied bitches vaginal cytology showed a lot of erythrocytes and large intermediate cells (42 ± 6.7), superficial-intermediate (29 ± 7.8), superficial (cornified) cells (19 ± 9.7), neutrophils (8 ± 3.2) and solitary parabasal cells (2 ± 2.1). Cytological picture resembled late proestrus or early oestrus in all studied bitches.

In following days the gradual increase in the number of parabasal cells was observed, with simultaneous decrease in the number of superficial and intermediate cells, and on 35 the day after mating only parabasal cells and neutrophils were found.

Vaginoscopy showed plication, hyperemia and softening of vaginal mucosa, and a lot of thick mucus in the external cervical orifice.

Concentration of progesterone in blood during pregnancy was similar in groups S and C. There were no significant differences on the other days of the observation (Fig.1). Blood estrogens concentration during pregnancy was considerably higher in group S than in group C. There were statistically significant differences in concentration of estrogens at the days 21st, 28th and 35th postmating (P from

0.01 to 0.001). From the 49th day, concentration of estrogens slowly increased in the blood of both groups of bitches (Fig. 2).

Concentration of testosterone in blood during pregnancy was similar in both groups. There were statistically significant differences only on 42nd and 56th day of observation (P from 0.01 to 0.001) (Fig. 3).

DISCUSSION

This publication introduces cases of the occurrence of oestrus-like symptoms in bitches connected with the high concentration of estrogens in the blood. To the authors' knowledge such cases were not described hitherto in dogs. Oestrous symptoms had a transitory character and remained for 5 to 10 days. Similar conditions were described in other species. Mares in late pregnancy occasionally show signs of heat, such as elevation of the tail, frequent urination, and "winking" of the lips of the vulva. They stand stretched out, as if receptive to being bred. It is likely that these signs are the result of hormones produced by the developing gonads of the fetus that is being carried, or by the placenta [Tomasgard and Benjaminsen 1975, Bielańska-Osuchowska 2001, Verstegen-Onclin and Verstegen 2008]. In pregnant mares estrogens (equilin, equilenin, dihydroequilin-17 α and dihydroequilenin-17 α) have been identified in the urine, and are presumably synthesized by the placenta [Nett et al. 1976]. Mares are more likely to show this behavior when they are in groups. While these are behavioral signs of heat, there are no accompanying changes in the ovaries or relaxation of the cervix. So by a strict definition, they are not truly in oestrus [Tomasgard and Benjaminsen 1975].

It appeared that 3.08% to 10.8% of the pregnant cows showed oestrous behaviour during pregnancy (EBP) in such an intensity that they would have been considered in oestrus [Thomas and Dobson 1989, Dijkhuizen and Van Eerdenburg 1997]. EBP was observed at all stages of pregnancy, although more often between 121 and 240 days, occurred more than once per pregnancy, and was also seen in successive pregnancies [Thomas and Dobson 1989, Dijkhuizen and Van Eerdenburg 1997]. Changes typical for a true oestrus, like a metoestrous bleeding, hormonal changes and ovulation, were not observed in pregnant cows showing oestrus. Seven of nine pregnant cows at oestrus stood willingly to be mounted by a bull [Thomas and Dobson 1989, Dijkhuizen and Van Eerdenburg 1997].

In bitches normal concentration of estrogens at the point of ovulation amounts about 35 pg · ml⁻¹, over the next 9 days it falls to 15–20 pg · ml⁻¹ and from the 10th day it rises again to 25 pg · ml⁻¹. In nonpregnant bitches this situation is similar, but the values are lower – after ovulation the concentration falls to 10–15 pg · ml⁻¹, and then rises to 15–20 pg · ml⁻¹ [Concannon 1996, Concannon et al. 1997, Verstegen-Onclin and Verstegen 2008]. In our study the concentration of

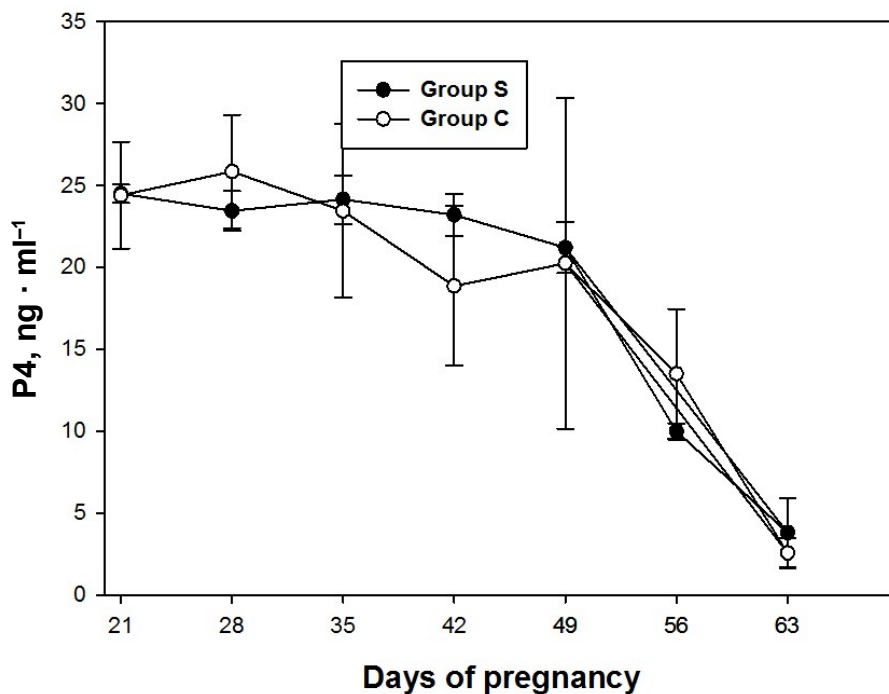


Fig. 1. Comparison of blood levels of progesterone (P4) in group S and C; no statistical differences between group C and S observed at any point

Rys. 1. Porównanie poziomów progesteronu (P4) we krwi w grupach S i C; brak różnic statystycznie istotnych pomiędzy grupami C i S w każdym z punktów

estrogens in bitches of studied group was essentially higher compared to this in control group, which is admitted as a typical range for dioestrus phase. We did not manage to determine source/es of estrogens. However, the lack of ovarian pathologies suggested an extra supplementation from corpora lutea and/or placenta.

The observation of pregnancy-specific increases in fecal concentrations of estradiol, testosterone and progesterone following implantation suggests that the CL of pregnant dogs synthesize and secrete more of these steroids than those of non-pregnant dogs. The pregnancy-specific source is presumed to be luteal because there is no evidence of placental steroid secretion in this species so far [Gudermuth et al. 1998]. However, regulation of CL in bitches is not fully recognized and is currently being investigated. The CL in a pregnant bitch produces and contains more testosterone ($54.7 \text{ pg} \cdot \text{ml}^{-1}$) and fewer estrogens ($45.4 \text{ pg} \cdot \text{ml}^{-1}$) than a corpus luteum in nonpregnant bitches in the same phase of cycle ($21.2 \text{ pg} \cdot \text{ml}^{-1}$ and

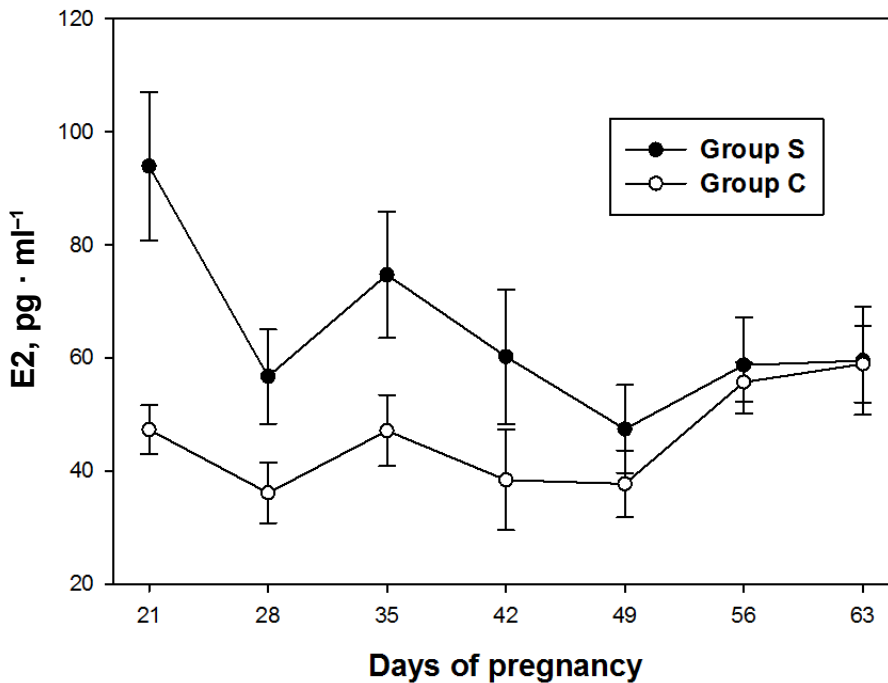


Fig. 2. Comparison of estrogens blood levels in group S and C. Statistical differences between groups were observed from 21st to 35th day (from $P \leq 0.01$ to $P \leq 0.05$). Statistical differences between groups were observed on days 21, 28 and 35

Rys. 2. Porównanie poziomów estrogenów we krwi w grupach S i C; różnice istotne statystycznie pomiędzy grupami od 21. do 35. dnia (od $P \leq 0,01$ do $P \leq 0,05$); różnice istotne statystycznie pomiędzy grupami w 21., 28. i 35. dniu

69.2 pg · ml⁻¹ respectively). Circulating blood volume during pregnancy is 40% bigger than in nonpregnant bitches, and hormonal secretion during pregnancy is more efficient. Therefore probably the blood concentration of estrogens is higher in pregnant bitches than in nonpregnant dioestrus bitches [Hadley 1975].

Prolactin is a required gonadotropin in dogs [Concannon 1996, Concannon and Verstegen 1998, Günzel-Apel et al. 2009]. The increase in prolactin concentration is reported to occur after implantation, beginning between 25th and 30th day [Günzel-Apel et al. 2009], when we observed increases in the level of estrogens and, at about the time when pregnancy-specific elevations in the fecal ovarian steroids occurred [Gudermuth et al. 1998]. Suppression of prolactin secretion by dopamine agonist administration after 8th day causes decline in progesterone con-

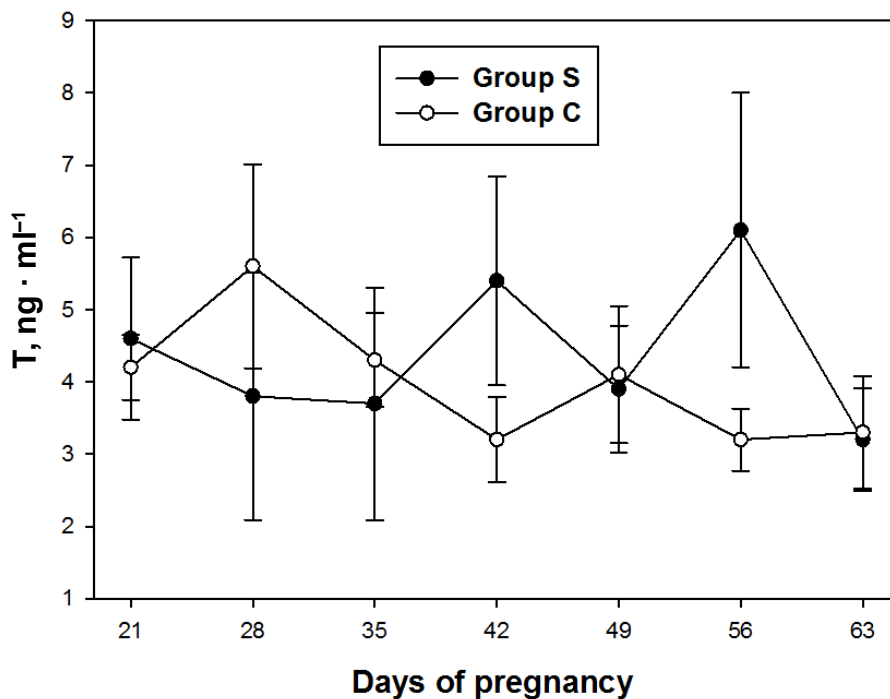


Fig. 3. Comparison of testosterone blood levels (T) in group S and C; no statistical differences (from $P \leq 0.01$ to $P \leq 0.05$) between group C and S observed at any point (except the 42nd and 56th day)

Rys. 3. Porównanie poziomów testosteronu (T) we krwi w grupach S i C; brak różnic istotnych statystycznie (od $P \leq 0,01$ do $P \leq 0,05$) pomiędzy grupami S i C w każdym z punktów (z wyjątkiem 42. i 56. dnia)

centration and/or permanent luteolysis in both pregnant and nonpregnant bitches [Günzel-Apel et al. 2009]. The prolactin may be a supportive agent, which maintains the luteal secretion of both P4 and E. The role of testosterone in pregnant bitches remains unknown. In the present study testosterone concentration was not elevated at the time of clinical symptoms of the heat, which leads to the conclusion that testosterone have not taken part in the development of these symptoms.

CONCLUSIONS

In the present publication we described very rare cases of oestrus-like symptoms in pregnant bitches. They were caused by extremely elevated estrogens con-

centration of unknown origin. Observed clinical symptoms did not have influence on the course of pregnancy and the number of puppies born, which was confirmed by clinical, and ultrasonic examinations. In studied bitches, concentration of P4 was sufficient to support progesterone block, simultaneously preventing opening of cervix uteri and miscarriage. However, high concentration of estrogens caused clinical symptoms of heat. Due to the limited quantity of cases described in the study it is not possible to state explicitly that oestrus-like symptoms do not have the influence on the course of the pregnancy in bitches. Therefore in every case of oestrus-like symptoms in pregnant bitch it is recommended to monitor the course of the pregnancy. Additional research would be necessary to establish the source of the hormone.

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OBJAWY RUJOPODOBNE U CIĘŻARNYCH SUK

Streszczenie. Występowanie estrogenizacji podczas ciąży opisano u kilku gatunków zwierząt oraz u kobiet. Autorzy niniejszej pracy obserwowali objawy rujopodobne u czterech suk w ich pięciu ciążach (grupa badana) i porównali wybrane parametry z sukami ciężarnymi bez objawów rujopodobnych (grupa kontrolna). Suki grupy badanej miały wykonane badanie waginoskopowe, cytologię pochwy i badanie USG. We krwi obwodowej zmierzono stężenie progesteronu, estrogenów i testosteronu. Stężenie progesteronu w obu grupach było podobne. Stwierdzono znacząco wyższe stężenie estrogenów w 21. i 35. dniu po pokryciu u suk grupy badanej. Stężenie testosteronu było statystycznie istotnie wyższe w grupie badanej w 42 i 56 dni po pokryciu. Nie obserwowano resorpcji ani poronienia płodów w grupie badanej suk, które urodziły odpowiednio 6, 5, 7, 8 i 11 szceniąt. Wysokie stężenie estrogenów u ciężarnych suk sugeruje dodatkowe ich wydzielanie, lecz nie zostało poznane ich źródło. Dodatkowe badania nad ustaleniem źródła estrogenów u ciężarnych suk wydają się być niezbędne.

Słowa kluczowe: pies, ciąża, ruj, estrogeny

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