ABSTRACTS

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INFLUENCE OF PROGESTERONE WITHDRAWAL ON PREGNANCY RELATED PARAMETERS DURING POST-IMPLANTATION EARLY PREGNANCY LOSS

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Introduction
The dog is a multiparous species, thus normal canine pregnancy is characterized by a number of conceptuses. Integrity of pregnancy is maintained by sensitive interaction of hormones from the hypothalamo-pituitary-gonad axis on one hand and placental factors on the other hand. Insufficient secretion of progesterone by the developing corpora lutea may cause embryonic death prior to implantation occurring clinically unperceived. Death of implanted embryos is followed by so called “resorption” of the complete gestational sac, which may also not be detected by external inspection of the bitch but can be identified by ultrasound. Fetuses dying in progressed pregnancy are usually expelled from the uterus at abortion. Pregnancy can be terminated by withdrawal of progesterone leading to resorption or abortion depending on the stage of gestation at start of treatment. Induction of luteolysis by prostaglandin F2 or analogues alone or in combination with cabergoline during early post implantation pregnancy have shown to induce embryonic death asynchronously in the different conceptuses [9]. In the vast majority of studies on pregnancy termination by blockade of progesterone receptors an antigestagene (aglepristone) has been administered in mid pregnancy or later [6,7]. Nevertheless, clinical use of aglepristone for early post-implantation pregnancy termination has revealed asynchronous embryonic death. Single embryos may even survive and further develop to term if treatment efficiency is not controlled and treatment is not repeated. From these observations it is considered that early post-implantation pregnancy is a specifically stable condition. The aim of this paper is to compile the present knowledge of pregnancy related processes added with some results of a recent study considering both clinical and scientific aspects of early pregnancy loss in the bitch.

Definition of early pregnancy and early pregnancy loss
Considering pregnancy as a stable condition, its beginning may be correctly dated at the time of implantation, when blastocysts become attached to the endometrium by placental trophectoderm. By this implantation, occurring 22 to 23 after the LH-surge [3] or about 19 to 21 days after ovulation, is the prerequisite for early pregnancy detection by analysis of serum concentrations of relaxin deriving from the syncytiotrophoblast. Pre-implantation embryos may be detectable by high resolution ultrasound imaging the earliest day 17 to 19 after ovulation, which is very close to implantation. Therefore and because under clinical conditions early sonographic pregnancy diagnosis is usually performed not earlier than day 23 to 24 after ovulation, loss of pre-implantation embryos may not be realized. In case of mismating treatment before implantation efficiency of the substance used for prevention of implantation remains unclear, as presence of pre-implantation embryos cannot be proven under clinical conditions. Considering this, early pregnancy as well as early pregnancy loss should be related to the post-implantation period of gestation between day 20 and 28 (to 30) after ovulation (ov.), the latter coinciding with the completion of organogenesis of conceptuses. Pregnancy failure occurring during this stage of gestation is characterized by embryonic death followed by resorption, which may not be perceived unless ultrasound examinations are performed.

Embryonic death and resorption after progesterone withdrawal
Induction of embryonic death in early post-implantation pregnancy has been demonstrated to be a long lasting process. Subcutaneous injection of 20 µg kg⁻¹ PGF₂α (Dinoprost) three times daily from day 21 to 27 after ov. resulted in asynchronous death of embryos over 7 to 18 days in 5 individual beagle bitches [8]. Thus almost completely resorbed placental sites were observed at the same time beside live fetuses in different uterine locations. Resorptions were accompanied by local oedema of the placenta and endometrium, demonstrated by ultrasonography as thickened irregular shaped part of the placenta and by placental inflammation verified by histo-pathological examination of vaginal discharge. Furthermore haematological examinations during the treatment period revealed a significant increase of the number of leukocytes from on average 6.9 x 10³ µl⁻¹ before to 15.2 x 10³ µl⁻¹ at day 7 after the end of treatment, which is supposed to be related to the inflammatory intrauterine processes. No comparable changes in blood picture were evident in bitches that had been treated before implantation. Asynchrony of fetal death has also been described after one or multiple injections of the PGF₂α analogue cloprostenol (2.5 µg kg⁻¹) in progressed pregnancy [mean 35.5 (29 – 55) days after mating] of 67 bitches [5]. Abortion of all fetuses was found in about 80% of the bitches 8 days after treatment while repeated cloprostenol injection was followed by abortion in further nine bitches. Even in this late stage of gestation further injections did not result in complete abortion in 7.5 % of the bitches, obviously related to insufficient progesterone decrease. The antigestagen aglepristone has been successfully used for termination of mid pregnancy [6,7] with fetal expulsion occurring after 60 to 132 h. As part of a study on clinical, sonographic and blood coagulation changes as well as on morphological and histological features of placental sites showing embryonic resorption we administered aglepristone (AGLE, Alizine™, 10 mg kg⁻¹ sc on days 24 and 25 after ov.) or the PGF₂α-analogue cloprostenol (CLO, Cloprostenol™, 1.0 µg kg⁻¹ sc day 24 and 27 after ov.) in combination with the dopamine agonist cabergoline (CAB, Galastop™, 5 µg kg⁻¹ po daily from day 24 to 30 after ov.) in two consecutive pregnancies of three respectively two beagle bitches. Two additional bitches remained untreated and served as controls. In the second pregnancy ovariohysterectomy was performed in all dogs 30 to 34 days after ovulation. Daily sonographic examination revealed spontaneous resorptions (res) in both pregnancies of control bitch 1 (1st pregnancy 8 conceptuses: res 1 day 23, res 2 day 33 after ov. out of; 2nd pregnancy 10 conceptuses: res 1day 21, res 2 day 2, res 3 day 28 after ov.) as well as in the 1st pregnancy of bitch AGLE 1 (8 conceptuses: res. day 24 prior to start of treatment) and the 2nd pregnancy of bitch AGLE 3 (8 conceptuses: res. day 21 after ov.). In bitches AGLE 1 (1st pregnancy 8 conceptuses) and AGLE 2 (1st pregnancy 6 conceptuses) post-treatment embryonic death was first detected day 26 and day 28 and further resorptions followed one by one until day 36 after ov. In bitch AGLE 3 (1st pregnancy 10 conceptuses) a single resorption found on day 28 was followed by seven resorptions the next day and one further on day 31 after ov. After repeated treatment of all three bitches on days 36 and 37 after ov. the last live embryos died on day 39 (AGLE 2 and 3) and 41 after ov. (AGLE 1). Similar processes were observed in the CLO/CAB group. In both bitches seven of eight embryos (1 to 3 a day) died until day 36 over periods of eight days (bitch CLO/CAB 1) and four days (bitch CLO/CAB 2). Repeated treatment (day 36 and 37) led immediately to complete pregnancy termination. A brownish vaginal discharge was first observed on day 31, 28 and 50 after ov. in bitches AGLE 1, 2 and 3 respectively and on day 35 after ov. in both CLO/CAB treated bitches. Similar resorption patterns were seen in the second pregnancy. The ratio resorbed conceptuses/total number of conceptuses on the day of ovariohysterectomy was for control 1: 3/11, control 2: 0/3, AGLE 1: 4/6, AGLE 2: 6/6, AGLE 3: 3/9, CLO/CAB 1: 3/5 and CLO/CAB 2: 2/4. Non of the bitches showed vaginal discharge before ovariohysterectomy.

Changes in pregnancy related parameters due to progesterone deficiency

Hormones
Progesterone is known to be absolutely essential for establishment and maintenance of pregnancy. After an autonomous phase of progesterone synthesis from ovulation until about day 25 after ovulation, maintenance of luteal function is essentially supported by prolactin originating from the anterior pituitary gland. Prolactin is the main luteotropic factor both in the luteal phase of non pregnant and pregnant bitches. Due to its placental origin relaxin can be used for verification of canine pregnancy. Subcutaneous injections of low PGF$_2$ doses (Dinoprost, 20 µg kg$^{-1}$) three times daily from day 21 to 27 after ov. led to a sharp drop of progesterone concentration to <2 ng ml$^{-1}$ in all five bitches, but complete functional arrest of corpora lutea was obtained in only two dogs, while in the other three a re-increase of progesterone concentrations to >5 ng ml$^{-1}$ took place immediately after the end of treatment. This level was further obtained over a 12 day period in two bitches and for 32 days (until day 60 after ov.) in one bitch, indicating distinct luteal stability and regenerative power in this early stage of gestation. [8]. Administration of aglepristone (10 mg kg$^{-1}$ sc twice at 24 h interval) for mid-pregnancy termination resulted in premature cessation of luteal function and shortening of the interestrous interval indicating direct effect on the hypothalamic-pituitary level [6,7]. This is supported by an increase in prolactin release detected within 12 h after the second injection, which is supposed to result from blockade of pituitary progesterone receptors thus simulating insufficient ovarian progesterone secretion. The fact that this effect could not be demonstrated in bitches treated before implantation (12.8±3.8 days after ovulation) [6] may indicate interaction with presence of intrauterine conceptuses. Moreover, as the initial increase in prolactin has been shown to occur in coincidence with that of placental relaxin [3], it may be bound to early post-implantation pregnancy. The same relationship has to be taken into account when plasma PGFM concentrations are assessed regarding pre-implantation prevention or post-implantation termination of pregnancy. As long as pregnancy is not established, free floating blastocysts are probably degenerating due to deficient progesterone supply. The lack of increasing PGFM concentrations in the bitches treated with aglepristone prior to implantation [6] may indicate the absence of placental tissue, which is known to produce PGF$_2$ at the time of normal parturition and probably also due to placental inflammation [8] and to profound degenerative changes during the process of so called embryonic resorption [10]. In addition to these data, we could show in comparison to the untreated control bitches that aglepristone application in early pregnancy led to forced luteal regression after day 35 after ov. Under luteolytic medication (CLO/CAB) a sharp drop in progesterone concentrations was observed immediately after start of treatment and was followed by a continuous further decrease until day 60. However complete luteolysis had to be obtained by repeated treatment on day 36/37. Peripheral relaxin concentrations in control bitches increased gradually from early pregnancy (day 20-24 after ovulation) reaching highest values in late pregnancy (day 41-60). No direct changes in relaxin values were observed after aglepristone or cloprostenol/cabergoline administration, but relaxin concentrations dropped markedly after day 40 coinciding with death of the last fetuses.

**Blood coagulation**

In physiological pregnancy changes in blood coagulation dynamics have been demonstrated to be related to implantation and relaxin secretion, respectively [1,2]. Fibrinogen concentrations and the activities of plasminogen and plasin inhibitor are significantly increased during post-implantation pregnancy when compared with nonpregnant bitches, indicating activation of the coagulation system caused by epithelial and endothelial alteration during placentation. As this aspect has not been considered in disorders of canine pregnancy due to progesterone withdrawal so far, it was included in our study. In the two control bitches, patterns of fibrinogen concentrations and of plasminogen and plasin inhibitor activity were in accordance to the previously reported findings [1] and the mean courses were similar in both the aglepristone and the cloprostenol/cabergoline treated dogs. Nevertheless a re-increase in fibrinogen concentrations was evident in bitch AGLE 3 on days 45, 50 and 55 after ov.,
which is supposed to be related to intrauterine resorption processes after synchronous death of 7 embryos on day 29 and extrusion of remnants of gestational sacs as late as day 50 after ov. Activities of plasminogen and plasmin inhibitor remained completely unaffected.

**Perfusion of uteroplacental vessels and embryonic/fetal heart rate**

In addition to B-mode ultrasound embryonic/fetal heart rate was measured by M-Mode technique. Blood flow patterns of uteroplacental vessels were monitored every second day according to the indications found in literature [4] for signs of impending embryonic death. Before start of medications the average embryonic heart rate was 192 BPM in all three groups. In the sonographically intact embryos/fetuses heart rates increased moderately until mid gestation and remained constant (mean 235 BPM) as long as pregnancy was maintained. A clear decrease in heart rate prior to embryonic death could be realized in only one conceptus of three bitches each (bitch AGLE 2, day 24 after ov.: 127 BPM, spontaneous embryonic death; bitch CLO/CAB 1, day 31 after ov.: 135 BPM, induced embryonic death; bitch CLO/CAB 2, day 33 after ov.: 120 BPM, induced embryonic death). These three embryos died within 30 to 120 minutes after examination. Blood flow velocity patterns in small utero placental vessels of intact placental sites of control bitches were characterized by a gradual increase throughout pregnancy. Compared with the values found in both pregnancies of the control dogs bitches treated with aglepristone showed a significantly lower time averaged maximum velocity (TAMAX) on days 25/27 after ov. In the CLO/CAB treated dogs all blood flow velocities were significantly reduced on days 29/31 after ov. (5 to 7 days after start of treatment) indicating reduced perfusion probably due to increased contraction of myometrial and/or vascular muscles. Perfusion of the arteries in the placental sites at the stage of embryonic death and initial resorption showed large variation between individual locations without specific features concerning treatment. Blood flow could be easily detected in uteroplacental vessels within two days after embryonic death. Progressing resorption was accompanied by local reduction of uterine perfusion rendering differentiation of vessel more and more difficult.

**Morphological changes in intact placental sites and at various stages of resorption**

Macromorphological appearance of the excised uteri (day 30 to 34 after ov.) was characterized by a wide variation in size of gestational sacs and resorption sites. No relation was found between size and “age” of resorption sites. Inside of intact gestational sacs the typical structures including the zonary placenta and the marginal haematomas could be clearly distinguished. The latter could not or only partly be identified in resorptions. In intact gestational sacs allantoic fluid was clear and of watery consistency but reddish to brownish and mucous in resorption sites. In many of the latter embryonic/fetal remnants were identified. The placental labyrinth showed oedema to varying degree. The luminal (central) compartment of the placental labyrinth was partly or completely detached from the deeper compartment and showed progressed integrity loss. As previously described [10] the main event in resorption of gestational sacs after both spontaneous and induced embryonic death was hypoxic degeneration of the syncytiotrophoblast including appearance of haemorrhage and fibrin structures but no inflammatory cells between trophoblast populations. No signs of phagocytosis and no increased infiltration of maternal stroma by macrophages occurred compared to normal placental sites at the same stage of gestation. Coagulation necrosis observed in two of the placentation sites shortly after fetal death was morphologically consistent with infarcts, but no thromboses were visible neither in fetal nor in maternal blood vessels.

**Conclusions** - Despite the small number of bitches used in our study the clinical observations are confirmed regarding asynchronous and incomplete occurrence of embryonic death and resorption after administration of antigestagenic and luteolytic drugs in early post-
implantation pregnancy. The morphological features detected in intact gestational sacs and in different stages of resorption are in accordance with the Doppler sonographic findings regarding visualization of blood flow in uteroplacental vessels. The missing of clear changes in blood coagulation dynamics is supported by histological exclusion of inflammatory processes in the placentas and of thromboses in fetal or maternal blood vessels. It could be demonstrated that embryonic/fetal death is obviously a consequence of local progesterone deficiency followed by placental disintegration. The latter was accompanied by decreasing relaxin concentrations after death of the last fetus. For elucidation of the causal background of asynchrony in occurrence of embryonic death and progress of placental disintegration (i.e. local differences in hormone efficiency or hormone receptor distribution) further input in placental research is needed.

References