Endometritis and pyometra in bitches: a review

B. Kempisty[1,2], D. Bukowska[3], M. Wozna[3], H. Piotrowska[4], M. Jackowska[3], A. Zuraw[3], S. Ciesiolka[1], P. Antosik[3], H. Maryniak[5], E. Ociepa[1], Sz. Porowski[1], K.P. Brussow[6], J.M. Jaskowski[3], M. Nowicki[1]

[1]Department of Histology and Embryology, Poznan University of Medical Sciences, Poznan, Poland
[2]Department of Anatomy, Poznan University of Medical Sciences, Poznan, Poland
[3]Department of Veterinary Science, Poznan University of Life Sciences, Poznan, Poland
[4]Department of Toxicology, Poznan University of Medical Sciences, Poznan, Poland
[5]Institute of Zoology, Department of Animal Anatomy, Poznan University of Life Sciences, Poznan, Poland
[6]Department of Reproductive Biology, Leibniz Institute for Farm Animal Biology, Dummerstorf, Germany

Abstract: Endometritis-pyometra is the most frequent and complex pathology in domestic bitches. This process involves several immunological changes as well as molecular mechanisms responsible for inflammation in the female uterus. The various clinical stages of pyometra are associated with various symptoms. In this review, several aspects are described, including physiological and pathological mechanisms as well as molecular changes which take place during induction of endometritis-pyometra. The authors also highlight the important role of growth factors and their receptors in this process. It is well known that pyometra is a compound process which mainly involves immunological changes during inflammation. However, this review presents a new overview of this process, which includes changes at the molecular level, e.g., the altered expression of genes crucial for the development of this disease. Although pyometra is the most frequent disease of the reproductive tract in bitches, the molecular basis of this process is still not entirely understood.

Keywords: uterine diseases; cystic endometrial hyperplasia-pyometra complex; endometritis-pyometra complex

Contents

1. Physiological aspects of the oestrous cycle in bitches

The dog is the most important pet, a very important patient and an increasingly more important laboratory animal. However, the physiology of molecular regulatory mechanisms in reproduction remains unexplored. Bitches have a single oestrous cycle during a breeding season so they are monoestrous and have spontaneous ovulation. The

first oestrus appears at 6–14 months of age and its timing depends on the breed and size of a bitch. In bitches, the oestrous cycle lasts 5–12 months. It consists of 4 consecutive phases: anoestrus (depending on the breed lasting 80–240 days), pro-oestrus (3–16 days), oestrus (4–12 days) and a relatively long di-oestrus (60–90 days) (Concannon 2009). In domestic dogs, there is no relationship between the reproductive season and season of the year (the length of daylight). Furthermore, the duration of the period between heat (di-oestrus and an-oestrus) depends on the breed of a bitch. In German Sheppards the period between heats is relatively short; this breed enters the oestrus phase every 4–5 months (Grundy et al. 2002). It is said that the reproductive cycle of the bitch is one of the most primitive in all mammals. Anoestrus is the time of sexual inactivity, it ranges from variable to regular within bitches and is terminated by poorly understood interactions of environmental and endogenous factors. Anoestrus is followed by pro-oestrus; the time of vulvar swelling and bleeding, vaginal epithelial proliferation and cornification and vaginal secretion of pheromones. In vaginal smears parabasal cells predominate, followed by small and large intermediate squamous and cornified cells, and the cornification reaches the maximum at 1–6 days before the LH peak. In examination per vaginam the mucosa is oedematous, white, and lined with serosanguinous fluid. The serum estradiol concentration increases from 5–15 pg/ml to 40–120 pg/ml, and the pro-oestrus terminates with the preovulatory LH peak. Pro-oestrus behaviour is characterised by aggressive to ambivalent response to males. Clinical oestrus lasts until vaginal cytology manifests maximum cornification. Vaginoscopically, the mucosa appears wrinkled and crenulated. Oestrus is the time when bitch accepts a male and shows the receptive reflex, flagging and allowing intromission and copulation. Ovulation occurs spontaneously, usually at the beginning of this phase. The fertile cycle with oestrus behaviour can appear as early as two days before the LH surge or as late as 6 days after the surge. Serum progesterone increases from 1–3 ng/ml during the LH surge and rises rapidly. Oestrus is followed by meta-oestrus (di-oestrus) (Concannon et al. 1989). Behaviourally, it starts when oestrus activity finishes. Serum progesterone increases to 15–80 ng/ml in cycle days 20 to 35 and then slowly declines. Meta-oestrus is followed by anoestrus, which lasts on average for 140 days. Clinically, endometrium is repaired about 120 days after cycle without pregnancy and about 150 days after pregnancy. Vaginal cytology reveals a small number of parabasal cells and neutrophils. Serum oestradiol levels are low, 5–10 pg/ml, and serum progesterone remains at levels below 1 ng/ml (Concannon 2011).

Many of the regulatory mechanisms of canine reproduction differ distinctly from those in other species. The *corpus luteum* is the only source of progesterone in pregnant and non-pregnant bitches (Concannon 2011). In non–pregnant animals luteal regression and a decreasing level of progesterone are noted during the 30–90 days of di-oestrus. The luteal phase in a dog can be divided into two parts. The first is gonadotropin-independent and lasts for the first 40 days (when prostaglandins are probably the most important luteotropic component) and the second, gonadotropin-dependent one. Besides prostaglandins and progesterone, many factors regulate CL function, including CD4, CD8 lymphocytes, interleukins and trophic factors (Hoffmann et al. 2004; Engel et al. 2005). Then, cycle-dependent 3β-hydroxysteroid dehydrogenase and steroidogenic acute regulatory protein exert a pronounced influence (Kowalewski and Hoffmann 2008). Non-pregnant bitches experience a spontaneously prolonged luteal phase, often longer than in pregnancy (which is caused by the lack of maternal mechanisms for pregnancy recognition), and luteal function is independent of PGF2α – there is no uterine luteolytic mechanism.

It is difficult to detect changes in the cycle on the basis of external signs. Monitoring of serum progesterone concentration determines the expected time of ovulation, accurately indicating the time for insemination, it allows prediction of parturition and reveals whether the cycle is normal or not. Many changes in the oestrus cycle, e.g., anovulation, short inter-oestrus interval, persistent oestrus slow rise, "split heat", are important for recognising ovarian dysfunction.

Anovulation has been reported as uncommon (Arbeiter 1993), and is characterised by the failure of serum progesterone concentration to exceed 2 ng/ml during cytologic oestrus. An anovulatory cycle may be the result of an oestrogen signal which is insufficient to cause a LH surge, deficiency of the hypothalamus to secrete GnRH, or the pituitary to secrete LH or failure of the ovary and of attaining the LH surge (Meyers-Wallen 2007). It is recommended in such cases to apply no treatment because the ovulatory dysfunction is often temporary and the bitch may return to a normal cycle.

A "slow rise" is the most common unusual cycle (Meyers-Wallen 2007) and is defined as a plateau in serum progesterone at the level of 1–2 ng/ml for three and more days in oestrus, followed by a quick rise. The LH peak may occur at any time, and the causes of the "slow rise" may be similar to those of anovulation.

"Split heat" is described as an abnormally short duration of pro-oestrus or oestrus, serum progesterone concentrations remaining at the level typical for anovulation and in 1–2 months it is followed by a subsequent oestrus cycle, which is often a normal cycle, so the progesterone monitoring should be restarted.

An insufficient luteal phase is defined as a decrease in serum progesterone concentrations below 2 ng/ml before the end of di-oestrus. This may happen after ovulation in pregnant or non-pregnant animals and is not detected in everyday practice. It is recommended to conduct progesterone sampling during sonography exams in non-pregnant bitches at about the 30th day and later. A decrease in serum progesterone below 2 ng/ml is typical for the loss of pregnancy (Concannon 2011). It is suggested that progesterone be administered only when a pregnancy over 50 days is at risk (earlier it can induce pyometra and masculinisation of female foetuses): 2 mg/kg is administered *i.m.* every other day up to the 61th day and in this way parturition and prolactin secretion are induced but lactation may be delayed.

Persistent oestrus is described as an oestrus that lasts longer than 21 days, manifested as consequences in vaginal cytology in the form of more than 90% cornification, as an indicator of serum estrogen concentrations, while serum progesterone concentrations are in the preovulatory range, under 2 ng/ml (Concannon 2011). Persistent oestrus appears spontaneously (endogenous oestrogen production) or can be initiated by treatment with exogenous oestrogens or gonadotropins. A spontaneous persistent oestrus is usually a result of abnormal ovarian function and can be induced by a granulosa cell tumour. It can lead to unresponsiveness to gonadotropins, anovulation or a slow rise in progesterone. Bitches which manifest persistent oestrus have poor chances for fertility and oestrus lasting for more than one day leads to bone marrow hypoplasia and pyometra. In some cases, administration of hCG 22 IU/kg once daily for three days is recommended to induce di-oestrus or anoestrus.

In such cases treatment is problematic as it is often followed by pyometra and, therefore, ovariohysterectomy is frequently necessary as is histological examination of ovaries to identify granulosa cell tumours or follicular anomalies, when they are suspected.

2. The pathogenesis of endometritis-pyometra: clinical features

Uterine diseases directly connected with the oestrous cycle are an important cause of infertility in carnivorous females. Hormonal disorders resulting from the long proliferation phase, persistence of ovarian follicles or ovarian cysts and bacterial infections have a pronounced influence on the uterus, leading to its degeneration.

The cystic endometrial hyperplasia-pyometra complex (CEH/P), earlier called the endometritis-pyometra complex (EPC), is one of the most serious and most common uterine diseases in bitches (Kida et al. 2006). It is defined as the acute or chronic, polysystemic, di-oestrual disorder of the adult bitch, characterised by hyperplasia of the endometrium and infiltration of inflammatory cells and it may occur in all layers of the uterus. In the mid-1980s a disease entity was described, called the endometritis-pyometra complex, which consisted of three clinical forms: (a) pyometra, (b) chronic endometritis, and (c) cystic glandular endometrial hyperplasia (Zdunczyk et al. 2006). The etiopathogenesis has not been fully explained so far, but it is known that hormonal disorders and bacterial infections are the underlying causes of this complex and that the main pathological processes occur in the uterus. The ovarian abnormality and hormonal disorders resulting from the prolonged influence of estrogens (prolonged heat, ovarian cysts, active ovarian neoplasia) are thought to be the main cause of endometritis-pyometra complex (Kida et al. 2006). Changes in the concentration of ovarian steroid hormones in the blood and in their receptors in uteri with pyometra were investigated (de Bosschere and Ducatelle 2002). The estrogens used to prevent nidation also influence the development of pyometra. These hormones promote overproliferation of endometrium and lengthen the period in which the uterine cervix remains open. Progesterone also contributes to the development of pyometra. The use of progestagens for oestrus and ovulation synchronisation in bitches can also be a cause of pyometra. Nolte et al. (1990) reported that all bitches under two

years of age affected by pyometra had earlier been treated with hormones. The action of progesterone on canine uterus consists in decreasing its local immune reactivity, promotion of secretion in endometrial glands, a decrease in its motility and closing of the uterine cervix. Intrauterine introduction of *E. coli* cultures, ascending from the vagina in the luteal phase, causes inflammation within this organ (Johnston et al. 1985). It has been proven that progesterone induces the development of endometrium receptors which allow for the adhesion of *E. coli* colonies. Also, too late administration of progestagens in pro-oestrus in order to break the heat influences the inflammatory processes within the canine uterus. It is assumed that over 20% of the EPC cases are provoked by the use of hormones. CEH/P complex may be induced as well by uterine biopsies, scarification and uterine irritants such as suture material (Noakes et al. 2001).

Hormonal imbalance is thought to be the first cause of CEH/P complex, and the secondary factor of this disease is bacterial infection. From surgically removed uteri in most cases (70–80%) *E. coli* was isolated, in some cases staphylococci, streptococci and *Pseudomonas aeruginosa*. Considering β-haemolysis as an indirect indicator of pathogenicity, β-haemolytic *E. coli* strains were isolated more often from diseased than from healthy specimens (Arora et al. 2006).

Anamnesis is an important element of clinical investigation because CEH/P complex occurs in di-oestrus and the symptoms are often unusual and the behaviour of the animal is affected first. Typical pyometra develops after heat, in the luteal phase, 20–70 days after the end of oestrus (Bigliardi and Pamigiani 2004), and was described to occur in 93% patients within 12 weeks after heat (Borresen 1979). Changes within the endometrium, which are secondary to glandular hyperplasia and cystic degeneration, lead to pyometra. It is thought that the mentioned proliferative and secretive changes are the effect of endometrial overreaction to oestrogens and progesterone (hyperoestrogenisation). Also, dysfunction of the ovaries in bitches often contributes to degenerative changes in the uterus (because of the corpora luteal development in the ovaries the process develops into pyometra).

Hyperplasia of the endometrium is accompanied by a light red or brownish secretion from the reproductive tract of the bitch. The presence of this secretion is a characteristic sign of endometritis, but it depends on the extent of opening in the uterine cervix, and it may not appear in every case. In pyometra the secretion is thick, purulent, and yellow to reddish brown in colour, of a fetid odour or without any particular smell. This discharge from the reproductive tract can be observed in 80% of cases. The disease often causes general signs/symptoms, such as anorexia, weakness, polydipsia/polyuria, vomiting, increased body temperature, tachycardia and tachypnoe (Bedrica and Sacar 2004; Fransson et al. 1997). Ultrasound and X-ray examinations can reveal a fluid-filled uterus in a "closed" form of pyometra (Bigliardi and Pamigiani 2004).

Clinically a "closed" and "open" form of pyometra can be distinguished. The "closed" form does not give any general signs at the beginning; these appear only after intoxication with pathogens. When severe intoxication occurs, the body temperature rises, the abdomen can be distended, and the integument becomes tense. In bitches with pyometra the leukocyte concentration in blood is elevated to 15 000–60 000/mm^3 (Bigliardi and Pamigiani 2004). Upon histological examination uterine horns and the uterus body present large cystic endometrial glands and growing endometrial thickness, an inflow of inflammatory cells into the glandular and uterine luminal areas and bacterial colonies. A significant increase in the number of endometrial neutrophils and macrophages is observed during pyometra. The pathologic uterine condition is characterised by a prevalence of degenerated endometrial cells with cytoplasmic large vacuoles (foamy aspect). A decrease in nuclear size and degenerative effects on nuclear morphology in endometrial cells are more marked during pyometra in comparison with the physiological condition (Groppetti et al. 2010). There are two approaches to CEH/P complex treatment, pharmacological or surgical treatment. The pharmacological (hormonal) treatment is possible in the bitch if she is not older than six years, if in the clinical examination no advanced morphological lesions can be noted in the uterus and if the bitch is in a good general condition. The aim of the therapy is to decrease the concentration of serum progesterone by oestrogen, androgen or oxytocin, cabergoline and prostaglandin. Verstegen et al. (2008) suggested transcervical endoscopic catheterisation (TECT). Because of the risk of complications after pharmacological treatment, the most popular method of CEH/P treatment is the surgical removal of the uterus and ovaries or the uterus alone. The main advantage of the surgical method is the quick resolution of clinical symptoms, such

as anorexia, polydipsia, proteinuria, and toxaemia, which enables recovery of affected bitches. If the owner does not address the condition and no therapy is undertaken, very often the process is the cause of animal death (Blendinger et al. 1997).

Many authors claim that the breed of animal has no influence on the appearance of pyometra (Niskanen and Thrusfield 1998). It is estimated that CEH/P complex is the most common gynaecological disorder in dogs and it can affect 25% of the bitch population before 10 years of age (Egenvall et al. 2001). The average age of a bitch with CEH/P complex varies from six to 10 years (Niskanen and Thrusfield 1998) and 5.5 years in bitches treated with oestrogens. Cases of CEH/P have been reported after the first heat. Blendinger and Bostedt (1991) reported that among bitches affected by EPC 2% were younger than two years, 9.3% were at the age of 2–4 years, 28.5% were 5–7 years, 42% were 8–10 years, 15.5% were 11–13 years, and 2.3% were over 13 years of age.

3. The role of growth factors in the pathogenesis of endometritis-pyometra

Factors which may play a significant role in the appearance and development of various genital pathologies in bitches, manifesting autocrine and paracrine activity, include also growth factors and their receptors (growth factors receptors, GFs), (Gama et al. 2009). These may stimulate cell growth and proliferation and may inhibit apoptotic processes and stimulate neoangiogenesis, in this way also being potentially involved in pathogenesis of mammary tumours.

3.1. Insulin-like growth factors (IGFs)

Within the broad range of substances and factors affecting the organs, tissues and cells of living bodies insulin-like growth factors (IGFs) and the entire group of insulin-like growth factor-binding proteins (IGFBP) play a significant role in the genital system of the bitch. Their effects include, first of all, an increased synthesis of oestrogens and progesterone. They control the growth of ovarian follicles and development of the corpus luteum (Chamberlain and Spicer 2001). In the uterus, their function involves stimulation of mitosis both in endometrium and in embryo cells. This leads to hyperplasia of the endometrium and development of placenta, normal implantation and subsequent development of the embryo (Hull and Harvey 2000; Fabian et al. 2005). The family of insulin-like growth factors includes two proteins: IGF-I (somatomedin C) and IGF-II, which affect cells by acting through their respective receptors. Their accessibility in tissues is controlled by the proteins binding to insulin-like growth factors (IGFBP). The pathway of IGF signal transduction includes Ras-MAPK protein kinases, Shc proteins and/or PI3K kinases. Activation of IGF-IR leads mainly to mobilisation of intracellular pathways of mitogenic signal transmission. This is followed by increased expression of transcription factors and a stimulation of cell proliferation (Dolka et al. 2010).

In the uteri of bitches, levels of IGF depend on the phase of ovarian cycle and, thus, on the level of hormones, mainly of oestradiol and progesterone (Hull and Harvey 2000; Krakowski et al. 2008). The location of receptors for IGF growth factors is linked to the function played by them in the uterus, i.e., first of all, to stimulation of secretory function of the endometrium and mitogenic effects on cells of the mother and the foetus (Hull and Harvey 2000). IGF-I, manifesting high mitogenic activity within the uterus is thought to represent one of the principal factors which mediates the development of endometrial lesions, including cystic endometrial hyperplasia (CEH) (De Cock et al. 2002). Disturbances in the expression profile of genes coding for insulin-growth factors under the effect of alterations in the hormonal system during oestrus and di-oestrus phases may lead to the development of pathologic conditions involving endometrium and are classified as EPC syndrome.

3.2. Epidermal growth factors (EGFs)

The family of epidermal growth factors, which play a significant role in the shaping, proliferation, migration and differentiation of cells, includes EGF, TGF-α, HBEGF, amphiregulins. These act through EGF receptors (EGF-R). The epidermal growth factors are also significant for the maturation of epithelial cells, development of organ epithelium and for wound healing. Moreover, their physiological role is significant for the manifestation of pathological conditions, such as neoplasia (Gerstenberg et al. 1999; Tamada et al. 2002; Kida et al. 2010). Analysis of the expression of epidermal growth factors and

their receptors in the uterus was performed in a few species, and revealed respective inter-species differences. The level of their expression depends on sex hormones such as oestrogen and progesterone (Gerstenberg et al. 1999; Komatsu et al. 2003; Kida et al. 2010). Growth factors belonging to the EGF family are engaged in the process of hormonal induction. They also stimulate growth of the uterus. A significant effect was demonstrated on the proliferation of endometrial cells in the mouse uterus (Komatsu et al. 2003; Kida et al. 2010). In experiments on endometrium originating from bitches levels of TGFα and EGF-R transcription were found to depend on the phase of the sexual cycle (Tamada et al. 2005; Kida et al. 2010). Studies suggested that the factors are engaged in processes of growth, differentiation and regression of epithelial cells in the endometrium of bitches. Such a scenario highlights the potential of using the factors as molecular indices, serving for the identification of endometrial diseases in bitches.

3.3. Transforming growth factors superfamily (TGFs)

The superfamily of transforming growth factors beta (TGF-β) represents another group of multifunctional factors manifesting a variable effect on processes of cell growth, differentiation and migration, on formation and decomposition of extracellular matrix components and on the processes of chemotaxia and apoptosis (Stepien-Wyrobiec et al. 2008). In several diseases a decreased expression of TGF-β was demonstrated. The family encompasses three isoforms of TGF-β 1, 2, 3 synthesised in mammals, anti-Müllerian Hormone (AMH), two inhibins (A and B), three activins (A, B and AB), 20 bone morphogenetic proteins (BMP1-20) and nine growth differentiating factors (GDF1-9) (Knight and Glister 2006). The factors undergo expression in the ovarian follicle, i.e., in theca cells, granulosa cells and in the oocyte itself. They are responsible for the differentiation of follicles, proliferation and atresia of granulosa and theca cells, steroidogenesis, oocyte maturation and luteinisation of the follicle. In several diseases a disturbed expression of TGF-β was detected, including autoimmune diseases (TGF-β1) and ovarian tumours (inhibin). This fact may be of a pronounced significance for the selection of molecular markers aimed at detecting developmental abnormalities of several structures both in somatic cells and in cells of the germinal cell line (Flanders and Burmester 2003). Disturbances in the synthesis of TGF-β and in the mechanism of signal transduction linked to this protein family may lead to cell apoptosis. Several cell types synthesise TGF-β, and most of them carry specific receptors for the proteins. Activation of the TGF-β 1, 2 and 3 pathway proceeds through the same system of receptor stimulation (Bukowska et al. 2011). Caron et al. (2009) demonstrated that all isoforms of TGF-β undergo expression during regression of the basic decidua layer of uterine mucosa. Moreover, investigators have confirmed the apoptotic activity of TGF-β 1 on basal cells although confirmation of such a function in the remaining isoforms requires further experiments.

Studies on the expression profile of the described growth factors in normal endometrium and endometrium affected by endometritis-pyometra syndrome have not yet been the subject of detailed analyses. The listed examples may point to the usefulness of selecting growth factors as potential markers demonstrating an altered expression profile of gene expression in endometrium affected by individual disease units of EPC complex and in oocytes.

4. Immunological overlook on endometritis-pyometra

Pyometra is generally thought to develop due to an interaction between potentially pathogenic bacteria and variable levels of progesterone. As prevention against pathogen invasion the female genital system developed appropriate immune mechanisms. Innate immunity represents the most universal and the most rapid protective mechanism upon contact with pathogens (Silva et al. 2010).

Innate mechanisms are based on the activity of Toll-like receptors (TLR), which recognise and cooperate with pathogen-associated molecular patterns (PAMP) synthesised by microbes, initiating a reaction cascade including the early inflammatory response (Horne et al. 2008). TLR receptors reside on the surface of epithelial cells in, among other locations, the intestines, respiratory tract, and on macrophages. Their presence was detected also on both types of endometrial cells (epithelial and sublayer cells) in humans and in cattle (Silva et al. 2010). Out of thirteen membrane TLR receptors identified until now in mammals, TLR2 and TLR4

receptors have been most completely characterised as beingrelated to innate immunity to bacteria.

TLR4 is a receptor of signal transduction utilised by lipopolysaccharide (LPS) and heat shock proteins of gram-negative bacteria (Pioli et al. 2004). However, LPS alone is unable to cooperate with TLR4. The circulating LPS forms a receptor complex of CD14/MD-2/TLR4 by anchorage to LPS-binding protein (LBP) (Gioannini et al. 2004). It initiates a subsequent signalling cascade, which results in the production of pro-inflammatory cytokines and chemokines (Horne et al. 2008). The TLR2 receptor recognises structural elements of gram-positive bacteria (lipoteichoic acid, lipopeptide, peptidoglycan) (Zahringer et al. 2008).

One of cellular products yielded by TLR signalling involves cyclooxygenase-2 (COX2). Following the action of pro-inflammatory stimuli, such as LPS, interleukin-1β (IL-1β) and/or tumour necrosis factor alpha (TNFα), expression of prostaglandin E2 (PGES) genes is stimulated and coupled to expression of COX2, which delays synthesis of PGE2. PGE2 participates also in extinguishing inflammation by feedback inhibition of COX2 isoenzyme expression. (Mosca et al. 2007). Recent reports have shown that the transcription of COX2, PGES and PGFS genes is higher in the endometrium of bitches affected by pyometra induced by, e.g., *E. coli* (Silva et al. 2010).

Through the TLR-toll like receptor cells of non-specific immunity recognize PAMP. This recognition is followed by the entire chain of reactions, leading to the production of several inflammation-inducing agents responsible for the development of a full immune response. Abnormal function of these processes (including both their absence as well as their over-activity) provides causes for numerous disturbances. Thus, it seems sensible to select Toll-like receptors and their products as markers for identifying lesions in the endometrium in bitches suspected of endometritis-pyometra complex.

5. Conclusions

In this review physiological and pathological aspects involving molecular mechanisms of endometritis-pyometra were discussed. Moreover, the important role of growth factors in induction of this process in domestic bitches was highlighted. Although the pathological and clinical features of this process are well recognised, still little is known regarding the molecular changes that occur during pyometra development. In the future, results of experiments must include molecular assays which will clearly demonstrate the genetic grounds of this inflammatory disease. Also, the knowledge coming from molecular analyses may provide a new target in the clinical treatment of this most frequent disease in domestic bitches.

6. Acknowledgements

This study was made possible by grant number N N401 527940 from the Polish Ministry of Scientific Research and Higher Education.

M. W. was supported by "Scholarship support for Ph.D. students specialising in majors strategic for Wielkopolska's development", Sub-measure 8.2.2 Human Capital Operational Programme, co-financed by the European Union under the European Social Fund (No. POKL 8.2.2/30-165-11)

7. REFERENCES

Arbeiter K (1993): Anovulatory ovarian cycles in dogs. Journal of Reproduction and Fertility 47, 453–456.

Arora A, Sandford J, Browning GF, Sandy JR, Wright PJ (2006): A model for cystic endometrial hyperplasia/pyometra complex in the bitch. Theriogenology 66, 1530–1536.

Bedrica L, Sacar D (2004): A case of atypical hyperplasia-pyometra-complex in a female dog (in German). Tierarztliche Umschau 59, 433–439.

Bigliardi E, Pamigiani E (2004): Ultrasonography and cystic hyperplasia-pyometra complex in the bitch. Reproduction in Domestic Animals 39, 136–240.

Blendinger K, Bostedt H (1991): The age and stage of estrus in bitches with pyometra. Statistical inquiry and interpretive study of the understanding of variability (in German). Tierarztliche Praxis 19, 307–310.

Blendinger K, Bostedt H, Hoffmann B (1997): Hormonal state and effects on the use of an antiprogestin in bitches with pyometra. Journal of Reproduction and Fertility 51, 317–325.

Borresen B (1979): Pyometra in the dog. I. A pathophysiological investigation. II. Anamnestic, clinical and reproductive aspects. Nordisk Veterinaermedicin 31, 251–257.

Bukowska D, Kempisty B, Jackowska M, Wozna M, Antosik P, Piotrowska H, Jaskowski JM (2011): Differential expression of epidermal growth factor and

transforming growth factor beta isoforms in dog endometrium during different periods of the estrous cycle. Polish Journal of Veterinary Sciences 14, 259–264.

Caron PL, Frechette-Frigon G, Shooner C, Leblanc V, Asselin E (2009): Transforming growth factor beta isoforms regulation of Akt activity and XIAP levels in rat endometrium during estrous cycle, in a model of pseudopregnancy and in cultured decidual cells. Reproductive Biology and Endocrinology 7, 80.

Chamberlain CS, Spicer LJ (2001): Hormonal control of ovarian cell production of insulin-like growth factor binding proteins. Molecular and Cellular Endocrinology 182, 69–81.

Concannon PW (2009): Endocrinologic control of normal canine ovarian function. Reproduction in Domestic Animals 44, 3–15.

Concannon PW (2011): Reproductive cycles of the domestic bitch. Animal Reproduction Science 124, 200–210.

Concannon PW, McCann JP, Temple M (1989): Biology and endocrinology of ovulation, pregnancy and parturition in the dog. Journal of Reproduction and Fertility 39, 3–25.

De Bosschere H, Ducatelle R (2002): Estrogen alpha and progesterone receptor expression in cystic endometrial hyperplasia and pyometra in the bitch. Animal Reproduction Science 70, 251–259.

De Cock H, Ducatelle R, Tilmant K, De Schepper J (2002): Possible role for insulin-like growth factor-I in the pathogenesis of cystic endometrial hyperplasia pyometra complex in the bitch. Theriogenology 57, 2271–2287.

Dolka I, Malicka E, Motyl T, Sapierzynski R (2010): IGF and other growth factors in canine and human mammary tumors – their role in carcinogenesis and prognostic significance. Medycyna Weterynaryjna 66, 745–750.

Egenvall A, Hagman R, Bonnet B, Hedhammar A, Olsson P, Lagerstedt A (2001): Breed risk of pyometra in insured dogs in Sweden. Journal of Veterinary Internal Medicine 15, 530–538.

Engel E, Klein R, Baumgartner W, Hoffmann B (2005): Investigations on the expression of cytokines in the canine corpus luteum in relation to dioestrus. Animal Reproduction Science 87, 163–176.

Fabian D, Koppel J, Maddox-Hyttel P (2005): Apoptotic processes during mammalian preimplantation development. Theriogenology 64, 221–231.

Flanders KC, Burmester JK (2003): Medical applications of TGF-β. Clinical Medicine and Research 1, 13–20.

Fransson B, Lagerstedt AS, Hellmen E, Jonsson P (1997): Bacteriological findings, blood chemistry profile and plasma endotoxin levels in bitches with pyometra or other uterine diseases. Journal of Veterinary Medicine. Series A 44, 417–426.

Gama A, Gartner F, Alves A, Schmitt F (2009): Immunohistochemical expression of Epidermal Growth Factor Receptor (EGFR) in canine mammary tissues. Research in Veterinary Science 87, 432–437.

Gerstenberg C, Allen WR , Stewart F (1999): Factors controlling epidermal growth factor (EGF) gene expression in the endometrium of the mare. Molecular Reproduction and Development 53, 255–265.

Gioannini TL, Teghanemt A, Zhang D, Coussens NP, Dockstader W, Ramaswamy S, Weiss JP (2004): Isolation of an endotoxin-MD2 complex that produces Toll-like receptor 4-dependent cell activation at picomolar concentrations. Proceedings of the National Academy of Sciences U.S.A. 101, 4186–4191.

Groppetti D, Pecile A, Arrighi S, Giancamillo D, Cremonesi F (2010): Endometrial cytology and computerized morphometric analysis of epithelial nuclei: A useful tool for reproductive diagnosis in the bitch. Theriogenology 73, 927–941.

Grundy SA, Feldman E, Davidson A (2002): Evaluation of infertility in the bitch. Clinical Techniques in Small Animal Practice 17, 108–115.

Hoffmann B, Busges F, Engel E, Kowalewski MP, Papa P (2004): Regulation of corpus luteum-function in the bitch. Reproduction in Domestic Animals 39, 232–240.

Horne AW, Stock SJ, King AE (2008): Innate immunity and disorders of the female reproductive tract. Reproduction 135, 739–749.

Hull KL, Harvey S (2000): Growth hormone: roles in male reproduction. Endocrine 13, 243–250.

Johnston SD, Kiang DT, Seguin BE, Hegstad RL (1985): Cytoplasmatic estrogen and progesterone receptors in canine endometrium during the estrous cycle. American Journal of Veterinary Research 46, 1635–1638.

Kida K, Baba E, Torii R, Kawate N, Hatoya S, Wijewardana V, Sugiura K, Sawada T, Tamada H, Inaba T (2006): Lactoferrin expression in the canine uterus during the estrous cycle and with pyometra. Theriogenology 66, 1325–1333.

Kida K, Maezono Y, Kawate N, Inaba T, Hatoya S, Tamada H (2010): Epidermal growth factor, transforming growth factor-alpha, and epidermal growth factor receptor expression and localization in the canine endometrium during the estrous cycle and in bitches with pyometra. Theriogenology 73, 36–47.

Knight PG, Glister C (2006): TGF-beta superfamily members and ovarian follicle development. Reproduction 132, 191–206.

Komatsu N, Maekawa T, Takeuchi S, Takahashi S (2003): Epidermal growth factor and transforming growth

factor-α stimulate the proliferation of mouse uterine stromal cells. Zoological Science 20, 639–645.

Kowalewski MP, Hoffmann B (2008): Molecular cloning and expression of StAR protein in the canine corpus luteum during dioestrus. Experimental and Clinical Endocrinology and Diabetes 116, 158–161.

Krakowski L, Obara J, Wrona Z (2008): The role of immune-like growth factors in the reproductive function of females. Medycyna Weterynaryjna 68, 1091–1094.

Meyers-Wallen VN (2007): Unusual and abnormal canine oestrus cycles. Theriogenology 68, 1205–1210.

Mosca M, Polentarutti N, Mangano G, Apicella C, Doni A, Mancini F, De Bortoli M, Coletta I, Polenzani L, Santoni G, Sironi M, Vecchi A, Mantovani A (2007): Regulation of the microsomal prostaglandin E synthase-1 in polarized mononuclear phagocytes and its constitutive expression in neutrophils. Journal of Leukocyte Biology 82, 320–326.

Niskanen M, Thrusfield MV (1998): Associations between age, parity, hormonal therapy and breed, and pyometra in Finnish dog. Veterinary Record 143, 493–498.

Noakes DE, Dhaliwal GK, England GC (2001): Cystic endometrial hyperplasia/pyometra in dogs: a review of the causes and pathogenesis. Journal of Reproduction and Fertility 57, 395–406.

Nolte I, Volpert A, Brunckhorst D (1990): Formation, diagnostic, therapy and complications of the endometritis-pyometritis-complex in the bitch. Kleintierpraxis 11, 589–602.

Pioli PA, Amiel E, Schaefer TM, Connolly JE, Wira CR, Guyre PM (2004): Differential expression of Toll-like receptors 2 and 4 in tissues of the human female reproductive tract. Infection and Immunity 72, 5799–5806.

Silva E, Leitao S, Henriques S, Kowalewski MP, Hoffmann B, Ferreira-Dias G, da Costa LL, Mateus L (2010): Gene transcription of TLR2, TLR4, LPS ligands and prostaglandin synthesis enzymes are up-regulated in canine uteri with cystic endometrial hyperplasia-pyometra complex. Journal of Reproductive Immunology 84, 66–74.

Stepien-Wyrobiec O, Hrycek A, Wyrobiec G (2008): Transforming growth factor beta (TGF-beta): its structure, function, and role in the pathogenesis of systemic lupus erythematosus (in Polish). Postepy Higieny i Medycyny Doswiadczalnej 62, 688–693.

Tamada H, Tsubutani D, Kawate N, Inaba T, Matsuyama S, Imakawa K, Sakai S, Christenson RK, Sawada T (2002): Detection of transforming growth factor-α and epidermal growth factor receptor mRNA and immunohistochemical localization of their proteins in the ovine uterus during the early implantation period. Histochemical Journal 34, 383–390.

Tamada H, Tominaga M, Kida K, Kawate N, Inaba T, Matsuyama S, Sawada T (2005): Detection of transforming growth factor-α and epidermal growth factor mRNA and immunohistochemical localization of the canine uterus during the estrous cycle. Histology and Histopathology 20, 817–824.

Verstegen J, Dhaliwal G, Verstegen-Onclin K (2008): Mucometra, cystic endometrial hyperplasia, and pyometra in the bitch: advances in treatment and assessment of future reproductive success. Theriogenology 70, 364–374.

Zahringer U, Lindner B, Inamura S, Heine H, Alexander C (2008): TLR2-promiscuous or specific? A critical re-evaluation of a receptor expressing apparent broad specificity. Immunobiology 213, 205–224.

Zdunczyk S, Janowski T, Borkowska I (2006): Vaginal and uterine bacterial flora in bitches: physiological and inflammatory conditions. Medycyna Weterynaryjna 62, 1116–1119.

Received: 2012–09–25
Accepted after corrections: 2013–06–25

Corresponding Author:

Bartosz Kempisty, Poznan University of Medical Sciences, Department of Histology and Embryology,
6 Swiecickiego St., 60-781 Poznan, Poland
Tel. + 48 61 8546515, Fax + 48 61 8546510, E-mail: etok@op.pl