THE ROLE OF IMMUNE CELLS IN THE SOW ENDOMETRIUM DURING DIFFERENT STAGES OF THE OESTROUS CYCLE AND EARLY PREGNANCY

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Abstract

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Approximately 30%(range 13-49%) of sows are culled due to fertility disturbances. Reproductive failure on farms are due to anoestrus, repeat breeding (regular and irregular return to oestrus), negative pregnancy diagnosis, abortion or failure to farrow. Reproductive problems are often difficult to diagnose. As a diagnostic tool, post-mortem examination (i.e. macroscopically and histologically) of the reproductive organs is useful when sows with reproductive failure are culled. The present review provides information about the distribution of immune cells, both in non-inseminated and inseminated pregnant sows endometrium, in order to be able to differentiate a physiological distribution of immune cells from pathological conditions. In non-inseminated sows, the infiltration and distribution of immune cells in the endometrium varied throughout the oestrous cycle, which suggests direct or indirect hormonal control. In both non-inseminated and inseminated pregnant sows, the distribution of T lymphocyte subpopulations indicate that the helper and cytotoxic functions of the immune system have primary locations in different tissue layers. In pre-ovulatory, inseminated and pregnant sows, the immunomodulation illustrated by the distribution of leukocytes, $CD2^+$, $CD4^+$, $CD8^+$ and MHC class II expressing cells, at the attachment sites on day 19, i.e. the low numbers on the surface epithelium and high numbers in the subepithelial layer, shows that the porcine trophoblast may influence the endometrium in developing the conditions required for embryonic attachment and survival

Keywords : immune cells, sow endometrium, artificial insemination, pregnancy

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***ผู้รับผิดชอบบทความ**

บทคัดย่อ

กัมพล แก้วเกษ

บทบาทของอิมมูนเซลล์ ที่อยู่ในเนื้อเยื่อบุโพรงมดลูกของสุกรนาง ในระยะต่างๆ ของวงรอบการเป็นสัด และของการตั้งท้องในระยะแรก

โดยทั่วไปประมาณร้อยละ 30(พิสัยร้อยละ 13-49) ของอัตราการคัดทิ้งแม่สุกรนั้นเกิดจากปัญหาทางการสืบพันธุ์ ปัญหาทางการสืบพันธุ์ในฟาร์มสุกรจะแสดงให้เห็นในลักษณะของการไม่แสดงอาการเป็นสัด ปัญหาการกลับสัด (กลับสัดตรงรอบและกลับสัดไม่ตรงรอบ) ทำการตรวจท้องแล้วพบว่าไม่ท้อง การแท้งหรือล้มเหลวในการเข้าคลอด โดยทั่วไปปัญหาที่เกิดจากความผิดปกติทางการสืบพันธุ์นั้น ยากที่จะวินิจฉัยด้วยการสังเกตจากอาการทางคลินิก ดังนั้นการตรวจวินิจฉัยอวัยวะของระบบสืบพันธุ์ของสุกร ทั้งทางมหกายวิภาค และจุลกายวิภาคจะมีประโยชน์อย่างยิ่ง เมื่อแม่สุกรถูกคัดทิ้งด้วยปัญหาทางการสืบพันธุ์ การศึกษาเชิงทบทวนบทความนี้มีวัตถุประสงค์ เพื่อนำเสนอข้อมูลที่เกี่ยวข้องกับอิมมูนเซลล์ ทั้งจำนวนและการกระจายตัว ตลอดจนบทบาทหน้าที่ของเซลล์เหล่านี้ในเนื้อเยื่อบุโพรงมดลูกของสุกรนาง ที่ไม่ได้รับการผสม และได้รับการผสม รวมทั้งสุกรที่ตั้งท้อง เพื่อใช้เป็นข้อมูลประกอบในการวินิจฉัยแยกแยะระหว่างสภาพปกติ และการเกิดพยาธิสภาพในเนื้อเยื่อบุโพรงมดลูก โดยทั่วไปจำนวนและการกระจายตัวของอิมมูนเซลล์ในเนื้อเยื่อบุโพรงมดลูกของสุกรนาง ที่ไม่ได้รับการผสม มีการเปลี่ยนแปลงไปตามระยะต่างๆ ของวงรอบการเป็นสัด ทั้งนี้อาจได้รับอิทธิผลจากฮอร์โมนไม่ทางตรงก็ทางอ้อม สำหรับจำนวนและการกระจายตัวของ T lymphocytes ในเนื้อเยื่อบุโพรงมดลูกของสุกรนาง ทั้งที่ได้รับการผสม และไม่ได้รับการผสมนั้น จะเห็นว่า T helper และ T cytotoxic มีการกระจายตัวต่างกันไปขึ้นอยู่กับชั้นต่างๆ ของเนื้อเยื่อบุโพรงมดลูก ในสุกรที่ได้รับการผสม และตั้งท้องจะมีการเปลี่ยนแปลงของจำนวน และการกระจายตัวของอิมมูนเซลล์อย่างเห็นได้ชัดบริเวณของเนื้อเยื่อบุโพรงมดลูกที่ตัวอ่อนของสุกรมาฝังตัว กล่าวคือมีการลดจำนวน และการกระจายตัวของอิมมูนเซลล์ชนิดต่างๆ ที่เยื่อบุผิว (surface epithelium) ของเนื้อเยื่อบุโพรงมดลูก เช่น leukocytes, $CD2^+$, $CD4^+$, $CD8^+$ และ MHC class II expressing cells แต่ขณะเดียวกันมีการเพิ่มจำนวน และการกระจายตัวของอิมมูนเซลล์เหล่านี้ที่ชั้นใต้เยื่อบุผิว (subepithelial layer) ของเนื้อเยื่อบุโพรงมดลูก แสดงให้เห็นว่า ตัวอ่อนและเนื้อเยื่อที่อยู่รอบๆ ตัวอ่อน (porcine trophoblast) ของสุกรมีส่วนในการทำให้เกิดการเปลี่ยนแปลงนี้ขึ้น ซึ่งอาจจะเป็นการกระตุ้นเนื้อเยื่อบุโพรงมดลูกให้เตรียมสภาพต่างๆ ให้พร้อมสำหรับการฝังตัวของตัวอ่อน และเพื่อการมีชีวิตอยู่ได้ของตัวอ่อนในโพรงมดลูกของสุกร

คำสำคัญ : อิมมูนเซลล์ เนื้อเยื่อบุโพรงมดลูกของสุกรนาง การผสมเทียม การตั้งท้อง

Introduction

During the oestrous cycle, the porcine uterus and especially its endometrium undergoes proliferation and differentiation in response to changes in sex steroid hormone levels. Treatment with high levels of oestradiol causes an increase in the uterine blood flow (Ford and Christenson, 1979) and in endometrial vascular permeability (Keys and King, 1988). In addition, oestradiol treatment results in the uterus being heavier and the endometrium thicker (Spencer et al., 1993; Tarleton et al., 1999). Furthermore, the progesterone phase is characterized by high secretory activity of the endometrium (Basha et al., 1979).

During early pregnancy, the endometrium will create an appropriate environment for the embryo, e.g. provide uterine secretions during early development (Dantzer, 1985; Roberts and Bazer, 1988). Up to days 11-12, the morphological changes of the sow endometrium, as observed by light microscopy, are similar for pregnant and non-pregnant sows (Sidler et al., 1986; Kaeoket

et al., 2003[a]). After fertilisation, pig embryos reach the uterus on days 2-3 and migrate through both uterine horns between days 6-12 (Dziuk, 1985), followed by attachment and placentation (Dantzer, 1985; review King, 1993). The establishment of pregnancy and the maintenance of an embryotrophic uterine environment both depend upon endometrial responses to maternal and conceptus signals (Dantzer, 1985; Pope et al., 1990). Thus, the ability of the uterus to recognise and integrate specific systemic and local signals, determines if pregnancy can become established and defines the environment in which embryonic and foetal growth occurs (review Bartol et al., 1993).

If we consider the immune functions, a normal uterine response at oestrus and after mating (natural mating or AI), causes an influx of neutrophils into the endometrium and uterine lumen (Lovell and Getty, 1968; Bischof et al., 1995; Rozeboom et al., 1998; Kaeoket et al., 2003[a]). This inflammatory response to spermatozoa seems to be a normal process in order to remove excess spermatozoa and bacteria, thereby preparing an optimal uterine environment for early embryos (Rozeboom et al., 1998, 1999). Seminal plasma itself can also induce a transient inflammatory response in the uterus (Bischof et al., 1994[a]; Rozeboom et al., 1999; Armstrong et al., 2000). In experimental models on uterine infection in gilts, it has been shown that oestrous cycle stages and progesterone levels are related to the uterine inflammatory response and the development of endometritis (De Winter et al., 1992, 1996). Furthermore, in the gilt, during early and mid pregnancy, leukocyte infiltration of the endometrium has been described (Bischof et al., 1995) and a variation in the infiltration of intraepithelial lymphocytes during days 10-20 of pregnancy has been reported (King, 1988). However, successful pregnancy in pigs is associated with intrauterine immunosuppression, i.e. suppression of major histocompatibility complex molecules and T cell responses which allow the embryos (semiallografts) to develop inside the uterus without being rejected (Croy et al., 1987; review Segerson and Beetham, 2000). The aims of this present review are to provide an overview of the immune system, with special emphasis on immune cells that are likely to be present in the sow endometrium and which suggest that immune cells in the sow endometrium, do indeed respond to changes in steroid hormone levels both during the oestrous cycle and the challenges of mating and embryo development.

The pig uterus

The uterus develops in the embryo from the mesodermal paramesonephric (Müllerian) ducts. It consists of a short uterine body and two long tubular uterine horns (60-200 cm) attached to broad ligaments, called mesometrium. Histologically, the uterus is composed of an inner mucosa, the endometrium, and a myometrium, with both inner circular and outer longitudinal smooth muscle layers. The outermost connective tissue layer is the perimetrium.

Morphological aspects of the endometrium

The endometrium is the mucosa that lines the uterine lumen. The surface epithelium varies from simple cuboidal and simple columnar to pseudostratified columnar (Fig. 1) and consists of secretory and ciliated cells. Also openings of numerous simple glands are found in the surface epithelium. The lamina propria/tunica submucosa contains a thin, highly vascularised subepithelial layer of loose connective tissue and a glandular layer, with glands surrounded by connective tissue, close to the myometrium (Fig. 1). The surface epithelial and the glandular cells change morphologically during the oestrous cycle and during pregnancy (Perry and Crombie, 1982; Stroband et al., 1986; Kaeoket et al., 2001[a], 2003[a]). Morphological changes, observed by light microscopy, are similar in the endometrium of non-pregnant and pregnant sows up to days 11-12 of pregnancy (Sidler et al., 1986; Kaeoket et al., 2001[a], 2003[a]).

The pregnant endometrium undergoes morphological changes during gestation, for instance, an increase in density and size of the capillaries underneath the

surface epithelium (Keys and King, 1990; Bischof et al., 1995; Kaeoket et al., 2003[a]) and mucosal foldings (anchoring protrusions of the surface epithelium towards the uterine lumen, Fig. 2) (Dantzer and Leiser, 1994; Kaeoket et al., 2003[a]), but its general structure is maintained, as placentation in the pig is a continuous, non-invasive process (Dantzer, 1985; review King, 1993). The trophoblast of the conceptus successively attaches to and remains in direct contact with the maternal uterine epithelium, starting on the mesometrial side of the uterus and establishing an epitheliochorial placenta (Dantzer, 1985; Stroband and Van der Lende, 1990; review King, 1993).

Infiltration of leukocytes in the pig endometrium

The defence mechanisms of the uterus against different agents or micro-organisms, consists of both anatomical barriers and local, cellular, immune functions. The anatomical barriers between the contaminated environment and the relatively sterile environment of the uterus include the vulva, the vestibule and the cervix. The myometrium provides physical propulsions in order to get rid of foreign material in the uterine lumen. The myometrial activity shows different characteristics depending on the stage of the oestrous cycle (Claus et al., 1989; Langendijk et al., 2002) and early pregnancy (Scheerboom et al., 1987).

Changes in the distribution of leukocytes in the porcine endometrium of non-pregnant sows and gilts during the oestrous cycle, have been reported (Corner, 1921; Sidler et al., 1986). Bischof et al. (1994a) described changes in the distribution of leukocytes, especially lymphocytes, in the endometrium of gilts. In addition, Hussein et al. (1983) reported that in sows there is a cyclic variation in the distribution of immunoglobulin-producing cells.

Inflammatory cells, especially neutrophils, have been seen to rapidly influx into the pigs endometrium and into the uterine lumen, in response to mating (Lovell and Getty, 1968; Rozeboom et al., 1998; Kaeoket et al., 2003[a]). This inflammatory response to the semen seems to be a normal process to remove excess spermatozoa and bacteria, thereby preparing an optimal uterine environment for the early embryos (Rozeboom et al., 1998, 1999). Seminal plasma itself can also induce a transient inflammatory response in the uterus (Bischof et al., 1994[b]; Rozeboom et al., 1999; Armstrong et al., 2000). The distribution of inflammatory cells in the endometrium during early pregnancy was described in gilts by Bischof et al. (1995). In addition, King (1988) found that intraepithelial lymphocytes, within the surface epithelium, decreased in number during early pregnancy (day 19, the first day of standing = day 0), compared with the corresponding period of the oestrous cycle, indicating that the developing pig conceptuses at this stage may initiate some processes to suppress the immune response at the placental interface, in order not to be lysed but to undergo differentiation and further development.

General aspects of the leukocytes

Immune responses can be divided into two types: *innate (non-adaptive), or non-specific or natural* immune responses, or *adaptive, or specific, or acquired* immune responses (Roitt et al., 1998). The differences between these types is that an *adaptive* immune response is highly specific for a particular pathogen. The innate response is generally effective, without prior exposure to a pathogen and does not change after repeated exposure to an infectious agent. However, the adaptive response improves with each successive encounter with the same pathogen: in effect the adaptive immune system "remembers" the infectious agent and can prevent it from causing disease at a later stage. Thus, the two properties of the adaptive immune response are specificity and memory (Roitt et al., 1998). The cells of the immune system arise from stem cells in the bone marrow, through two main lines of differentiation. The lymphoid lineage produces lymphocytes and the myeloid lineage produces phagocytes (monocytes/macrophages, neutrophils, eosinophils and basophils).

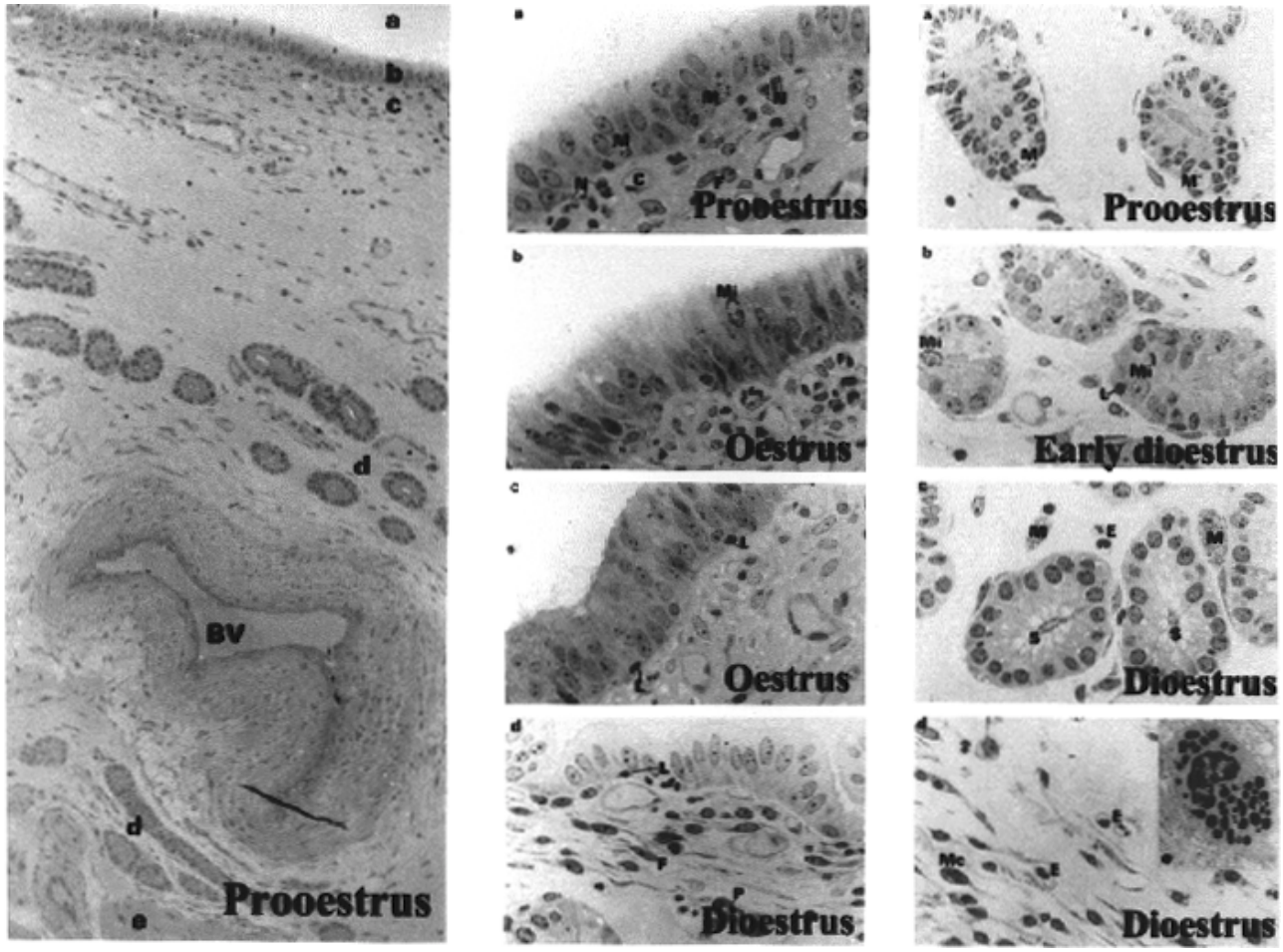

Fig. 1 Section of the porcine endometrium by light microscopy, *Left panel* (at prooestrus): (a) Uterine lumen, (b) surface epithelium, (c) subepithelial layer, (d) glandular layer and (e) myometrium. BV: blood vessel. *Middle panel*: (a) At prooestrus: F (fibroblast), N (neutrophils), M (macrophages within the surface epithelium), C (capillaries). (b) At oestrus: Mi (mitosis). (c) At oestrus: L (intraepithelial lymphocytes within pseudostratified epithelium). (d) At dioestrus: F (fibroblast), L (intraepithelial lymphocytes), and P (plasma cell. *Right panel*: (a) At pro-oestrus: M (macrophages within the glandular epithelium). (b) At early dioestrus: L (lymphocyte within the glandular epithelium), Mi (mitosis). (c) At dioestrus: E (eosinophils), M (macrophages in the connective tissue), S (secretory vesicles at supranuclear level). (d) At dioestrus: E (eosinophils), e (electron micrograph of eosinophil), Mc (mast cell). (Kaeoket et al., 2001[a])

Lymphocytes (B, T and NK cells) are typically small- to medium-sized cells, with large nuclei, surrounded by a thin rim of cytoplasm. B lymphocytes are genetically programmed to encode a surface receptor specific for a particular antigen (Roitt et al., 1998). After an antigen has been recognised, the B cells multiply and differentiate into plasma cells, which produce antibodies in the tissues. The major class of lymphocytes is the T cells, which can be divided into subpopulations of helper (T_H) cells and cytotoxic (T_C) cells (Roitt et al., 1998). In response to antigenic stimulation, T_H cells release cytokines which promote the proliferation and/or differentiation of other T cells, B cells, NK cells and monocytes/macrophages. T_C cells lyse cells that express non-self antigens, such as cells infected by viruses. Another class of lymphocytes is LGLs (large granulated lymphocytes) with numerous cytoplasmic granules that are capable of spontaneous lysis of a variety of tumour cells, as well as virally infected

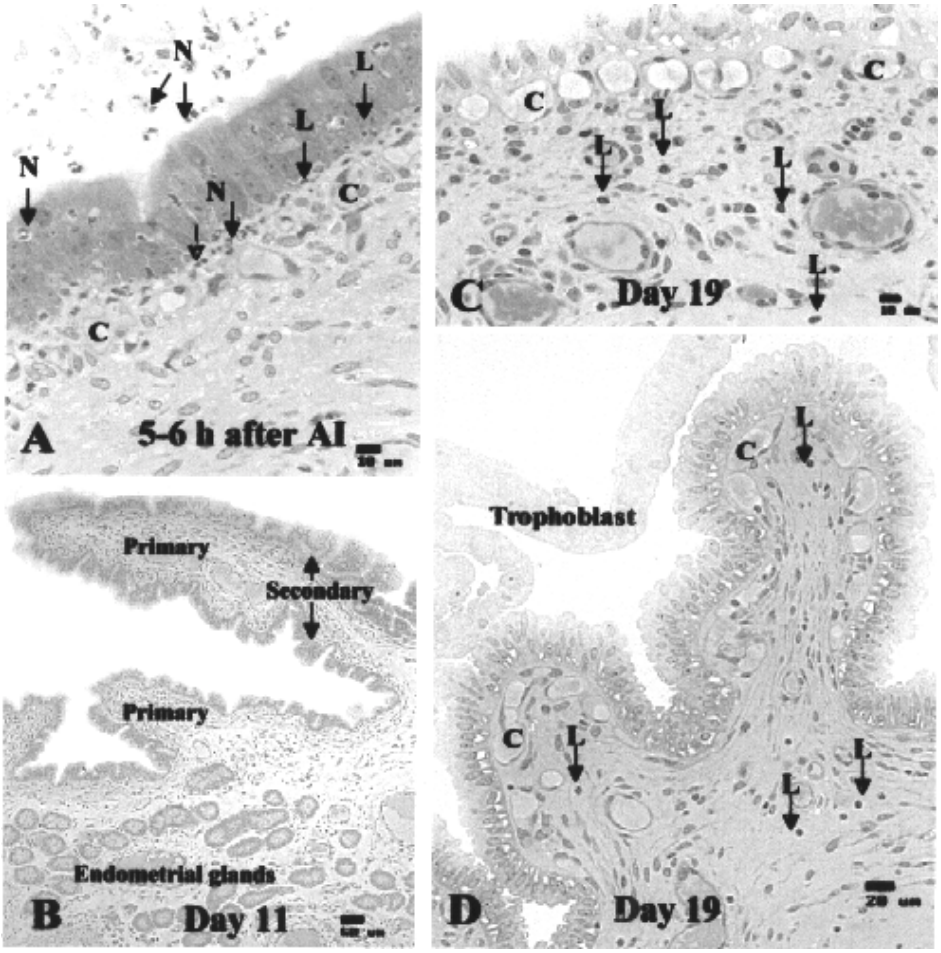

Fig. 2 Morphology of the porcine endometrium by light microscopy. (A) 5-6 h after AI: C, capillaries; N, neutrophils in the uterine lumen, neutrophils within the surface epithelium, neutrophils in the connective tissue; L, intraepithelial lymphocytes within the surface epithelium. (B) On day 11: F, primary and secondary folds of the surface epithelium. (C) On day 19: C, capillaries; L, lymphocytes. (D) On day 19: Anchoring protrusions of the surface epithelium; C, capillaries; L, lymphocytes in the connective tissue; T, trophoblast. (Kaeoket et al., 2003[a])

cells. This lymphocyte population is also known as NK cells. According to studies in humans (King et al., 1996) and rodents (review Croy et al., 1996) as well as pigs (review Engelhardt and King, 1996), these cells may play an important role in the endometrium during pregnancy.

Plasma cells are developed from B lymphocytes, inresponse to antigen. B cells are found in the lymph nodes and the spleen. The plasma cell progenitors migrate into the tissues and respond to antigen stimulation by division and differentiation into tissue plasma cells (Tizard, 1996). Plasma cells are terminally differentiated in tissue and they can no longer divide. Their lifespan varies from 3 days to 4 weeks (Tizard, 1996). Plasma cells are ovoid cells and have a round, eccentrically placed nucleus with unevenly distributed chromatin or a clumped heterochromatin pattern (Dellmann and Carithers, 1996), resulting in the nucleus resembling a clock face or cartwheel. The plasma cells also have an extensive cytoplasm, that is rich in rough endoplasmic reticulum, indicating a protein producing function. It has long been known that plasma cells are immunoglobulin-producing cells. For instance, it has been shown in humans (review Brandtzaeg, 1997) and pigs (Hussein et al., 1983) that these cells in the endometrium can produce IgA, IgG and IgM, which is important for the mucosal immune response.

Neutrophils consist of the majority of the poly-

morphonuclear granulocytes. They migrate from the bone marrow into the bloodstream and are then recruited to the tissues. Their life span in the pigs bloodstream is approximately 8 hours (Roth, 1999) and in tissue they live for a few days (Tizard, 1996). Neutrophils are round cells with a fine granular cytoplasm and a segmented nucleus. Electron microscopy shows that there are two types of granules in the cytoplasm. Primary (azurophilic) granules are lysosomes containing bactericidal enzymes such as hydrolases, myeloperoxidase, lysozymes, etc. Secondary (specific) granules contain enzymes such as lysozyme, collagenase and the protein lactoferrin (Tizard, 1996; Roth, 1999). Neutrophils are triggered to leave the blood vessels as a result of the endothelial cells expressing adhesive proteins that bind the neutrophils to the walls of small blood vessels (Tizard, 1996). In tissue, the neutrophils migrate along the chemotactic factor gradient toward the source of the factor and thus arrive at the site of infection (Roth, 1999). The neutrophils are thought to be the first line of defence, since they move rapidly towards foreign material and their function is to capture and destroy foreign material through phagocytosis. The process can be divided into four parts: chemotaxis, adherence, ingestion and digestion (Tizard, 1996). Neutrophils die after a short time at the site of infection. Hydrolytic enzymes and antibacterial peptides are then released and contribute to the inflammatory response and tissue destruction (Roth, 1999).

Eosinophils are the second major polymorphonuclear granulocytes. These cells leave the bone marrow in a relatively immature state and move directly to the spleen, where they reach maturity. Their half-life in blood circulation is only about 30 minutes, whereas in the tissues, they have a half-life of about two weeks (Tizard, 1996; review Costa et al., 1997). Eosinophils have a bilobed nucleus and contain different types of granules: *small, primary* granules that contain arylsulfatase, eosinophil peroxidase and acid phosphatase and crystalloid granules that contain four major proteins: major basic protein (MBP), eosinophilic cationic protein (ECP), eosinophil peroxidase (EPO) and eosinophil-derived neurotoxin (EDN) (Tizard, 1996). The primary function of eosinophils is to destroy foreign material. They degranulate in response to stimulation of their surface membranes and their activities are enhanced by cytokines, such as granulocyte-macrophages, colony stimulating factor (GM-CSF), IL-3 and IL-5 (review Costa et al., 1997). In addition, eosinophil-derived mediators such as major basic proteins and eosinophil peroxidase are known to have profound effects on tissue and vascular remodelling, associated with endometrial physiology (Jeziorska et al., 1995).

Mast cells originate in the bone marrow and are carried by the blood to the tissue location, where they mature into recognisable mast cells, under the influence of local cytokines (Church and Caulfield, 1993). Their life span is from weeks to months. Mast cells are round or elongated cells, with non-segmented or, occasionally, a bilobed or multilobed nucleus, with moderate condensation of the nuclear chromatin (review Costa et al., 1997). In the cytoplasm, there are numerous granules that are usually small and vary in appearance. The granules contain potent mediators and some of these products are stored in the cytoplasmic granules (e.g. histamine, heparin and cytokines). Other mediators are synthesised (e.g. prostaglandin) on activation of the cell by other stimuli. Histamine produces oedema of the surrounding tissue and increases diapedesis (i.e. the migration of blood cells through the walls of capillaries and venules into the tissue) (Dellmann and Carithers, 1996). Heparin is an anticoagulant and it also stimulates endothelial cell motility, promoting angiogenesis (Dellmann and Carithers, 1996). The mast cells in human and mice endometrium are thought to play a pivotal role in a variety of tissue changes including angiogenesis, inflammation and tissue remodelling (Jeziorska et al., 1995).

Macrophages originate in the bone marrow. Immature macrophages, found in blood, are called monocytes. Monocytes enter the bloodstream and circulate for a few days before migrating into the tissues

and developing into mature macrophages. They are relatively long-lived cells. Macrophages are round cells with abundant cytoplasm and a single nucleus, that may be round, bean-shaped, or indented. The cytoplasm contains mitochondria, rough endoplasmic reticulum, a Golgi apparatus and, on activation, many lysosomes and cytoplasmic vacuoles and granules. Phagocytosis by macrophages is similar to the process seen in neutrophils. When they become actively phagocytic, they increase in size, extend pseudopodia and become more irregular in shape (Dellmann and Carithers, 1996). Macrophages are attracted not only to bacterial products but also to molecules released from damaged cells and tissues, e.g. elastase and collagenase. At phagocytosis, neutrophils (the first line of defence) reach and attack foreign material first, and in the process of dying, they attract macrophages to the site of invasion. Macrophages are able to kill certain types of bacteria that are resistant to neutrophils. Thus, the important function of macrophages, is not only to remove dead and dying cells (Tizard, 1996), but also in the processing and the presentation of antigen to T lymphocytes, which in turn initiate the cell-mediated response by the B lymphocytes (Roth, 1999). For that reason, macrophages are said to be the second line of defence (Roitt et al., 1998). On the other hand, the migration of macrophages into the endometrium, both during the oestrous cycle and during pregnancy, may be one example of migration under a physiological condition, which changes and is dependant on hormonal (oestradiol and progesterone) variations (De and Wood, 1990).

Characteristics of lymphocyte subpopulations and the MHC class II molecule in the pig

Leukocytes express different molecules on their surfaces, which can be used to distinguish leukocyte subpopulations. Cell markers can be identified by specific monoclonal antibodies. A systematic nomenclature has been developed in which the term CD (cluster of differentiation) refers to groups (clusters) of monoclonal antibodies, each cluster binding specifically to a particular molecule (Roitt, 1998). At least 19 CD molecules have been identified on the surface of porcine leukocytes (Saalmüller et al., 1996). Porcine T lymphocytes have remarkable characteristics compared with other species (Lunney and Pescovitz, 1987). For example, they have a high proportion of T cells positive for both CD4 and CD8 (Pescovitz et al., 1994; Zuckermann and Husmann, 1996). It has been suggested that many of these dual expressing T cells are memory cells; however, the functional importance of having both CD4 and CD8 on the same cells is not known (Pescovitz et al., 1994; Zuckermann and Husmann, 1996). In addition, the ratio of $CD4^+$ to $CD8^+$ T cells in blood is normally about 0.6 in pigs (Lunney and Pescovitz, 1987), which is the reverse of the ratio found in humans (1.5-2.0). The significance of these differences, between porcine T lymphocytes and those of other species, is not completely understood.

Lymphocyte surface markers, such as markers for T cells and NK cells (CD2), T helper cells (CD4) and T cytotoxic cells and NK cells (CD8), have been applied to clarify functional aspects of lymphocyte subpopulations in the pig uterus (review Engelhardt et al., 1997). In the pig, lymphocyte subpopulations in peripheral blood and lymphoid tissues can be divided into four subsets, depending on the expression of the CD2, CD4, and CD8 molecules (Saalmüller et al., 1989; Hirt et al., 1990). Bischof et al. (1994[a], 1995) reported different phenotypes of lymphocyte subpopulations such as $CD2^+$, $CD4^+$ and $CD8^+$ T cells in the endometrium of gilts, during the oestrous cycle and pregnancy. The $CD2^+$ lymphocytes were predominant both in the surface epithelium and in the connective tissue, particularly during the early stage of the oestrous cycle and also in the connective tissue during days 18-21 of pregnancy. In addition, a subpopulation of $CD2^+CD4^-CD8^-$ T lymphocytes has been observed both in non-pregnant and the pregnant gilt endometrium (Bischof et al., 1994[a], 1995).

The MHC (major histocompatibility complex) class II molecules are expressed on different cell types, such as antigen-presenting cells (i.e. macrophages and

dendritic cells), lymphocytes (T and B cells) and endothelial cells, which have been seen in different species, such as pigs (Lunney and Pescovitz, 1987). MHC class II expressing cells have been found in the endometrium of non-pregnant and pregnant gilts (Bischof et al., 1994[a,b,] 1995), cows (Cobb and Watson, 1995) and mares (Watson and Dixon, 1993; Frayne and Stroke, 1994).

Discussion

Morphology of the endometrium

In a study by Kaeoket et al. (2001[a]), the morphology of the sow endometrium was studied on plastic sections using light microscopy. The data presented on mitotic activity, the height of the surface epithelium, pseudostratification of the surface epithelium, the height of the glandular epithelium and secretory vesicles in the glandular epithelium (Fig. 1) are similar to results by Leiser et al. (1988). Kaeoket et al. (2001[a]) reported that uterine oedema was found during late dioestrus, pro-oestrus and oestrus. It has been shown that an increased level of oestradiol-17β resulted in increased permeability of blood capillaries (Keys and King, 1988) which may be related to the development of uterine oedema. However, despite the plasma level of oestradiol-17β being low, oedema was also found at late dioestrus. As the tissue response to plasma levels of oestrogen depends on functional intracellular receptors (Stanchev et al., 1990; Sukjumlong et al., 2003), a reduction in the inhibitory effect of progesterone on the oestrogen receptors may explain the findings at late dioestrus. Another explanation could be that, at late dioestrus, the E_2 level might already have increased locally. Rojanasthien et al. (1988) showed, that during the oestrous period, the E_2 level increased in the utero-ovarian vein, 40-48 h earlier, compared to the levels in the jugular vein. Further studies are, however, needed in order to clarify the development of uterine oedema in the pig endometrium.

The results in pregnant sows (Kaeoket et al., 2003[a]) are in agreement with Sidler et al. (1986), who reported that in early pregnant sows, up to days 11-12, the morphology of the endometrium observed by light microscopy did not differ from the endometrium of non-pregnant sows. Furthermore, the increase in density and size of the capillaries, underneath the surface epithelium, observed on day 19 of pregnancy (Kaeoket et al., 2003[a], Fig. 2), is in agreement with the studies of Keys and King (1990), Bischof et al. (1995) and Dantzer and Leiser (1994). These changes reflect a locally induced interaction between the embryos and the endometrium that stimulates angiogenesis and vascular changes, leading to a switch from histotrophic to haemotrophic embryonic nutrition, that occurs during placentation in the pig (Keys and King, 1990; Dantzer and Leiser, 1994). It has been shown that oestradiol-17β is secreted by porcine blastocysts from day 11 of pregnancy (Fischer et al., 1985; King and Ackerley, 1985). This oestrogen release is concomitant with the induction of morphologically apparent changes, especially mucosal foldings and the development of a subepithelial vascular bed in the endometrium (Fig. 2) (Geisert et al., 1990; review Stroband and Van der Lende, 1990; Dantzer and Leiser, 1994; Kaeoket et al., 2003[a]).

Distribution of leukocytes in the different tissue compartments of the sow endometrium

In the following, different tissue compartments are discussed separately.

Surface and glandular epithelia

In non-mated sows (Kaeoket et al., 2001[a]), at oestrus, neutrophils were only occasionally found within the surface epithelium. Following insemination, the high infiltration of neutrophils within the surface epithelium 5-6 h after AI and 20-25 h after ovulation as reported by Kaeoket et al. (2003[a]; Fig. 2 and 3), is in agreement with earlier studies in gilts (Lovell and Getty, 1968; Bischof et al., 1995). In addition (Kaeoket et al., 2003[a]), the cytological findings after flushing of the uterine horns 5-6 h after AI and 20-25 h after ovulation (36-43 h after AI), are in agreement with Lovell and Getty (1968) and

Rozeboom et al. (1998), who reported an influx of neutrophils into the uterine lumen, following artificial insemination. Therefore, a transient inflammatory reaction in the form of the rapid migration of inflammatory cells, especially neutrophils, into the uterine lumen, is to be expected following mating during oestrus (Rozeboom et al., 1998). It has been suggested that spermatozoa trigger an influx of PMNs into the uterine lumen either via the activation of complement (Rozeboom et al., 1998, 1999) or of natural antibodies (Matthijs et al., 2000). In addition to spermatozoa, seminal plasma itself, has also been shown to induce a transient inflammatory response in the uterus (Bischof et al., 1994[b]; Armstrong et al., 2000).

During the oestrous cycle (Kaeoket et al., 2001[a]), intraepithelial lymphocytes (IELs) were found both within the surface and the glandular epithelia (Fig. 1). In this study, the nuclear morphology of IELs, within the surface epithelium, varied from round to small dot and irregular, which is in accordance with a study by King (1988). However, whether a small dot or irregular form of the nucleus represents a regressive stage of the lymphocyte, has yet to be determined. After pre-ovulatory insemination (Kaeoket et al., 2003[a]), the surface epithelium displayed a larger number of IELs which were observed 20-25 h and 70 h after ovulation, compared with other stages. However, the number did not differ from the number found in non-inseminated sows (Kaeoket et al., 2001[a]). On day 19 of pregnancy (Kaeoket et al., 2003[a]), the results showed that there was a significant reduction in the number of IELs in the surface epithelium (Fig. 3), compared with the earlier stages studied, which is in accordance with King (1988). As suggested by King (1988), the reduction in the number of IELs in the surface epithelium indicates that the porcine embryos at this stage may initiate some processes to suppress the immune response in order not to be lysed but to undergo differentiation and further development. Others have proposed that pregnancy in pigs is associated with a suppression of MHC molecules and T cell responses (Croy et al., 1987; review Segerson and Beetham, 2000).

In agreement with Leiser et al. (1988), *macrophages* were also observed intermingled with the epithelial cells of the surface epithelium and the endometrial glands at pro-oestrus and oestrus (Kaeoket et al., 2001[a]). The macrophages found within the epithelia were filled with cellular debris and nuclear fragments, indicating their phagocytic activities. In addition, Kaeoket et al. (2001[a]) showed that the number of macrophages within the surface and glandular epithelia were positively correlated with plasma levels of oestradiol-17β and negatively correlated with levels of P_4. In pre-ovulatory, inseminated sows (Kaeoket et al., 2003[a]), macrophages were observed within the surface of the glandular epithelia 5-6 h after AI and 20-25 h after ovulation (36-43 h after AI). The number of macrophages seemed to be only slightly higher than the number observed in the endometrium of non-mated sows (Kaeoket et al., 2001[a]). These results suggest epithelial cell death is the main reason for the attraction of macrophages at this stage.

Connective tissue of the subepithelial layer

During pro-oestrus and oestrus, in non-pregnant sows, with high levels of oestradiol-17β and a low level of progesterone (Kaeoket et al., 2001[a]), the infiltration of *neutrophils* was very high in the subepithelial layer, (Fig. 4) close to the surface epithelium. Bischof et al. (1994[a]) observed neutrophils on days 18-21 of the oestrous cycle, i.e. the gilts were probably at pro-oestrus/oestrus. It has been shown that in the endometrium, a high plasma level of oestradiol results in an increase in the permeability of blood capillaries (Keys and King, 1988). This may be a possible mechanism for high neutrophil infiltration of the subepithelial layer. Another possible explanation may be the influence of cytokines and chemotactic factors on inflammatory cell migration (Dunon et al., 1996). The neutrophil infiltration in non-mated animals shows that the endometrium and, especially the large numbers of neutrophils in the subepithelial layer, has a potential to respond quickly, if exposed to agents such as spermatozoa, at mating. In an acute inflammatory

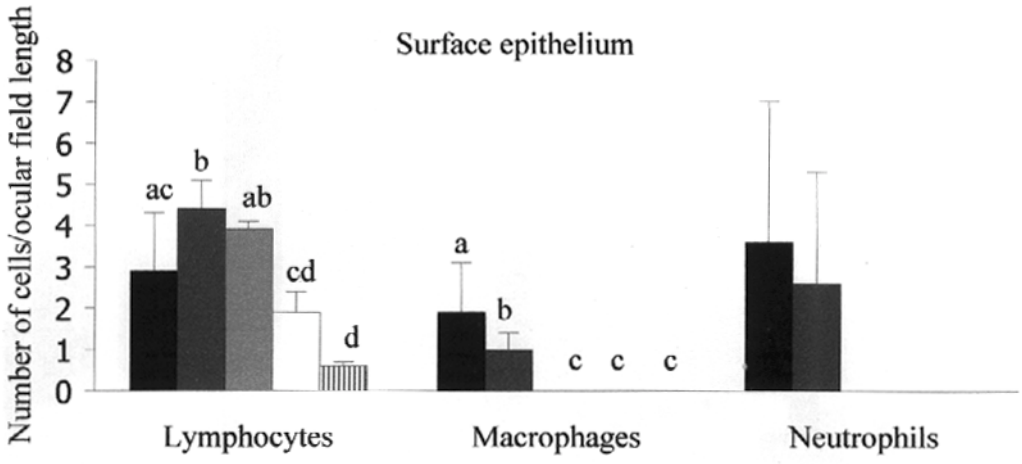

Fig. 3 Distribution of lymphocytes, macrophages and neutrophils within the surface epithelium of the porcine endometrium at 5-6 h (■) after AI, 20-25 h (■) and 70 h (■) after ovulation and on days 11 (□) and 19 (▥), after standing oestrus, presented as bars (mean + S.D.). Bar within the same type of cell marked by different letters, are significantly different ($p \leq 0.05$). (Kaeoket et al., 2003[a])

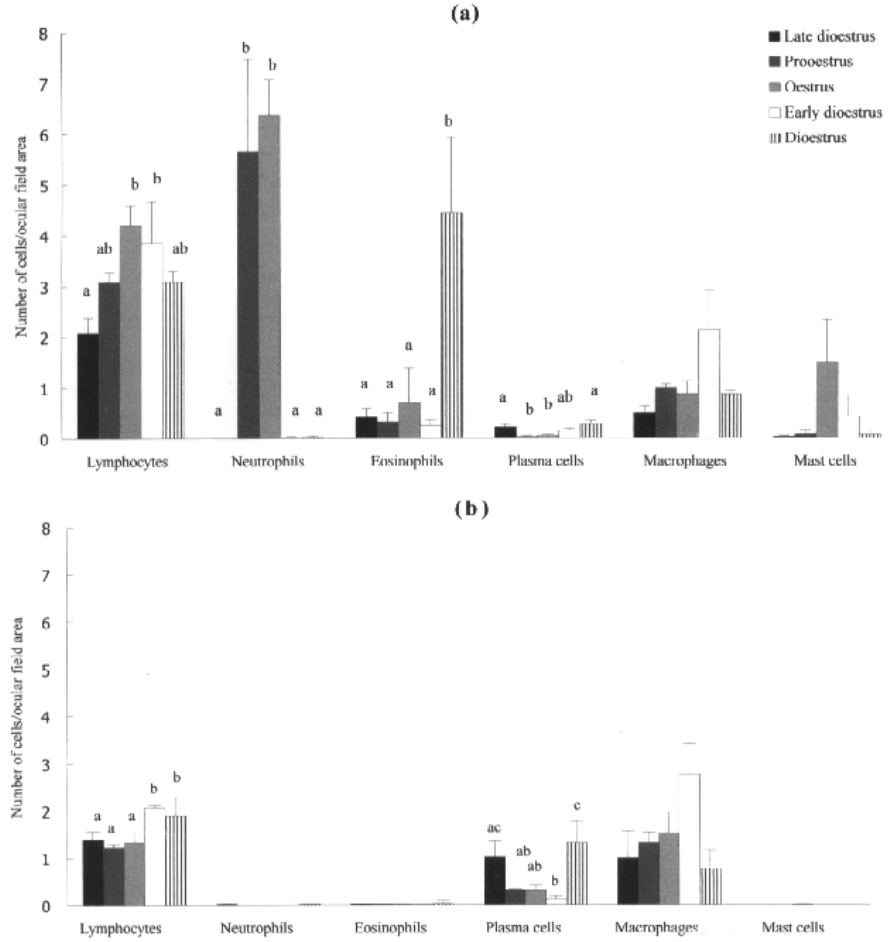

Fig. 4 Distribution of cells of the immune system in the connective tissue of the subepithelial (a) and glandular (b) layers of the sow endometrium, presented as bars (mean + S.D.). Bars within the same type of cell and marked by different letters, are significantly different ($p \leq 0.05$). (Kaeoket et al., 2001[a])

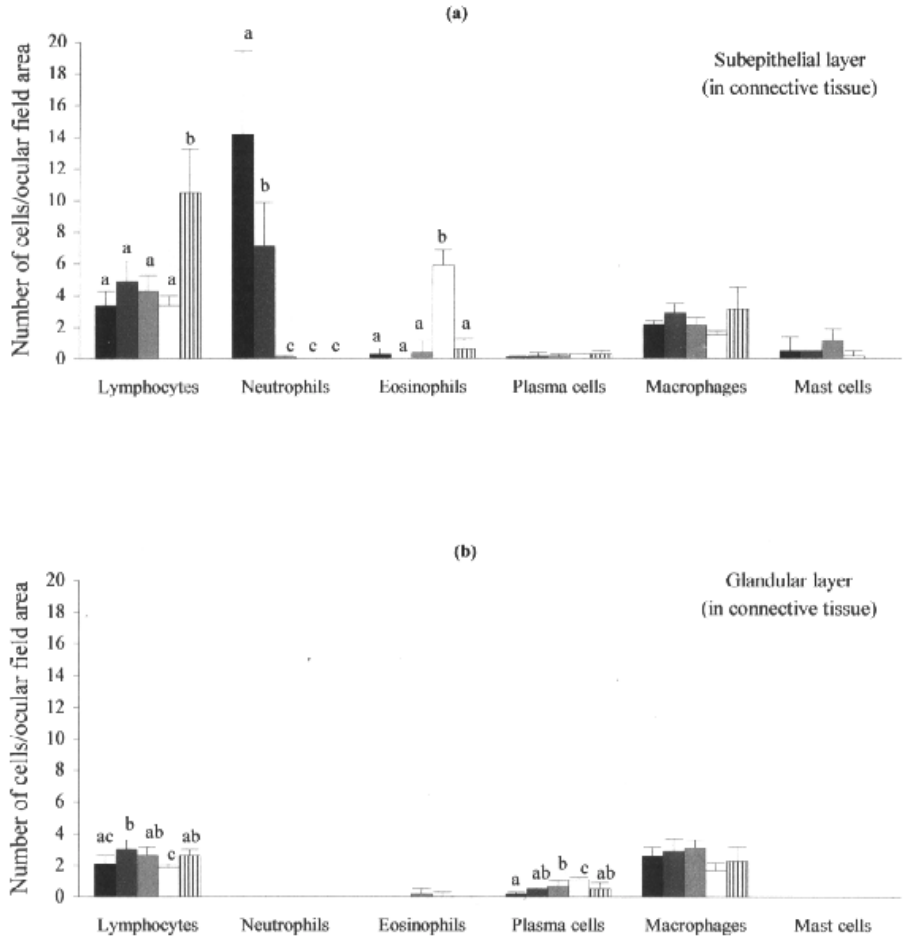

Fig. 5 Distribution of cells of the immune system in the connective tissue of the subepithelial (a) and glandular (b) layers of the sow endometrium 5-6 h (■) after AI, 20-25 h (▩) and 70 h (▨) after ovulation and on days 11 (□) and 19 (▥) after standing oestrus presented as bars (mean + S.D.). Bars within the same type of cell and marked by different letters, are significantly different ($p \leq 0.005$). (Kaeoket et al., 2003[a])

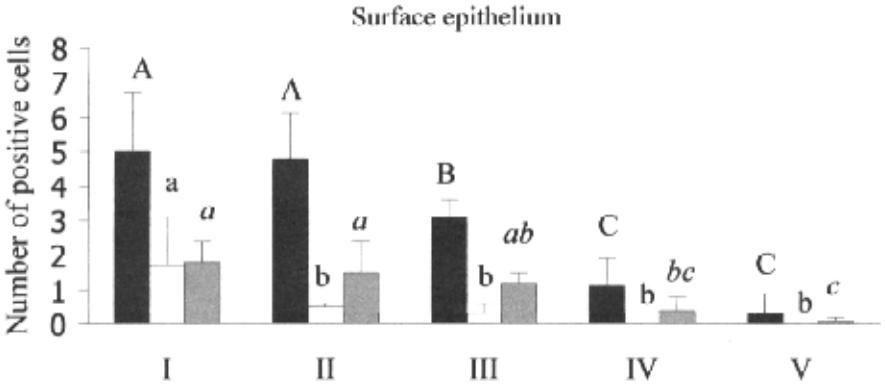

Fig. 6 Distribution of the CD2 (■), CD4 (▩) and CD8 (▨) positive cells, presented as bars (mean + S.D.) within the surface epithelium (cells/ocular field length) of the sow endometrium 5-6 h (gr. I) after AI, 20-25 h (gr. III) after ovulation and on day 11 (gr. IV) and 19 (gr. V), after the start of standing oestrus. Bars within the same type of positive cell are marked by different letters and are significantly different ($p \leq 0.005$). (Kaeoket et al., 2003[b])

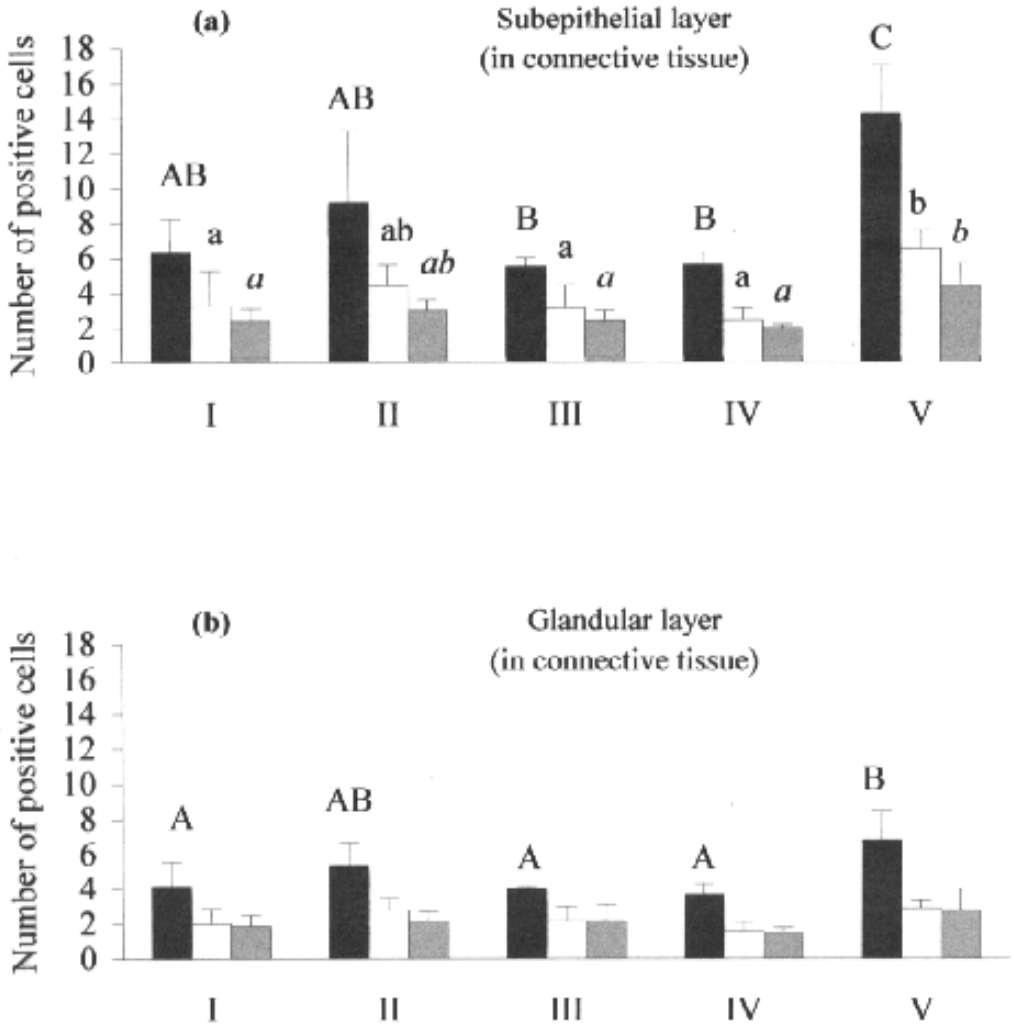

Fig. 7 Distribution of the CD2 (■), CD4 (■) and CD8 (□) positive cells, presented as bars (mean + S.D.) in the connective tissue of subepithelial (a) and glandular (b) layers (cells/ocular field area) of the sow endometrium 5-6 h (gr. I) after AI, 20-25 h (gr. II) and 70 h (gr. III) after ovulation and on day 11 (gr. IV) and 19 (gr. V) after the start of standing oestrus. Bars within the same type of positive cell are marked by different letters and are significantly different ($p \leq 0.005$). (Kaeoket et al., 2003[b])

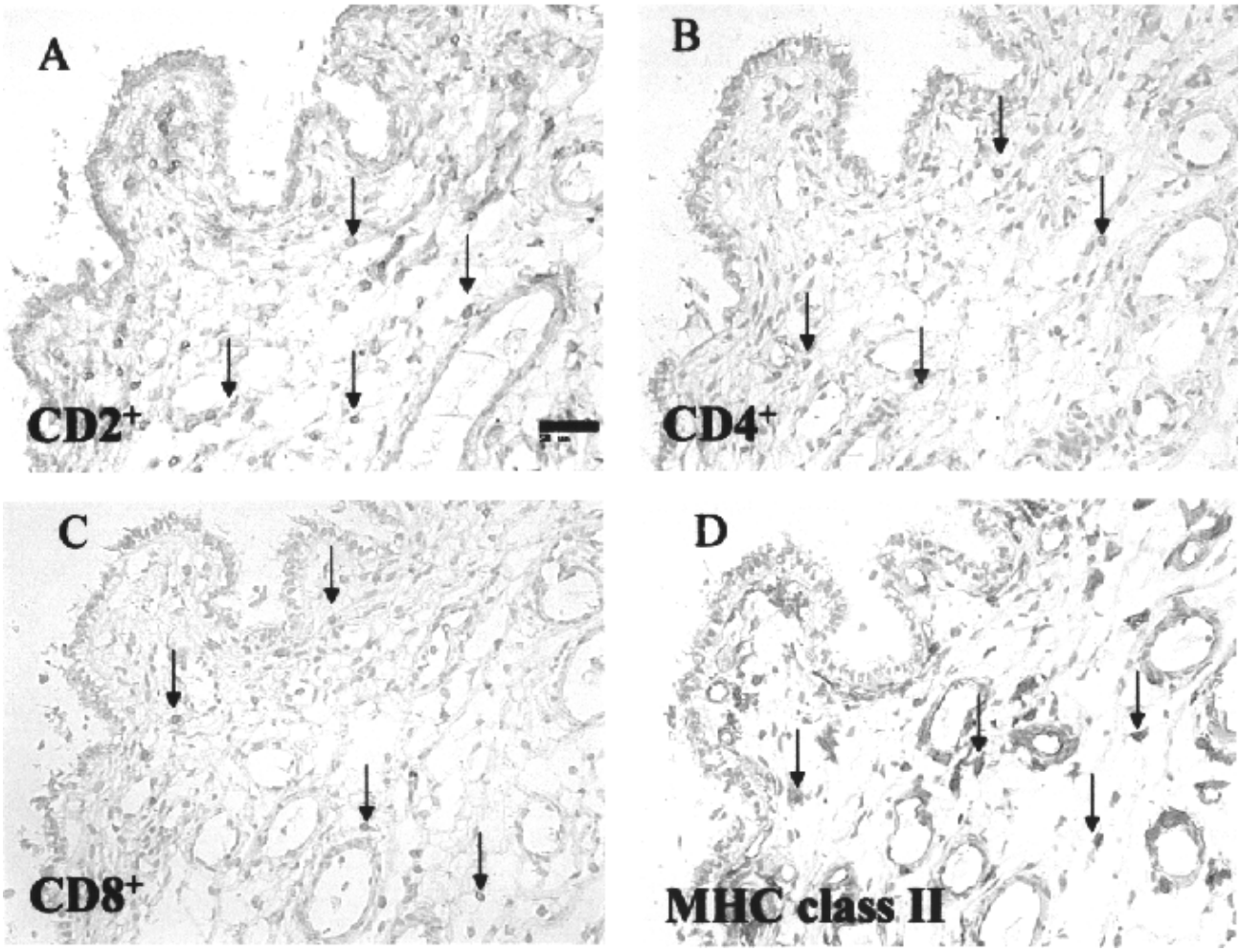

Fig. 8 Avidin-biotin peroxidase labeling with monoclonal antibodies to CD2 (A), CD4 (B), CD8 (C) and MHC class II (D) molecules, showing labeled cells within the surface epithelium and the connective tissue of the subepithelial layer of the sow endometrium. (A)-(D) on day 19, bar = 50 μm. (Kaeoket et al., 2003[b])

response to infection or mating, neutrophils are the first line of defence in the affected area. In mated sows (Kaeoket et al., 2003[a]), a high infiltration of neutrophils (Fig. 5) was observed in the subepithelial layer, 5-6 h after AI and 20-25 h after ovulation (36-43 h after AI), which is in agreement with earlier studies in newly mated gilts (Lovell and Getty, 1968; Bischof et al., 1995). The same reasons that causes for the large number of neutrophils in the surface epithelium in inseminated sows, may explain the high infiltration of neutrophils in the subepithelial layer.

The results in non-pregnant (Kaeoket et al., 2001[a]) and pregnant (Kaeoket et al., 2003[a]) sows, showed that lymphocytes were the dominating leukocyte cell type, taking into account all stages and both the connective tissue of the subepithelial and glandular layers together. This is consistent with earlier reports (Bischof et al., 1994[a], 1995; review Engelhardt et al., 1997). Furthermore, in pregnant sows (Kaeoket et al., 2003[a]), on day 19, in contrast to the surface epithelium having a lower lymphocyte infiltration, there was a high infiltration of lymphocytes in the subepithelial layer (Fig. 2 and 5), compared with the earlier stages. This is in accordance with studies by Bischof et al. (1995) who also showed a high infiltration of lymphocytes in the subepithelial layer of gilt endometrium on days 18-21. It has been suggested (Cross and Roberts, 1989; Lefèvre et al., 1990; La Bonnardière et al., 1991) that during early pregnancy (days 12-20), interferons (i.e. IFN-γ and -δ), secreted from the porcine trophectoderm, could play an active role in immune interactions with the mother, as their function is known to modulate leukocyte function. Particularly, IFN-γ is considered to be a potent activator of NK cells (review Reiter, 1993). Therefore, it is likely that these interferons may be involved in the attraction of lymphocytes to attachment sites, particularly to the underlying connective tissue of the subepithelial layer (review Engelhardt and King, 1996; Engelhardt et al., 2002[a,b]). It has also been suggested that conceptus-derived oestrogens (review Geisert et al., 1990) may play a direct role in the recruitment of lymphocytes to attachment sites (review Engelhardt et al., 1997). Furthermore, it has been shown that oestrogen receptors (ERβ) can be co-localised with leukocyte markers in the human cervix (Stygar et al., 2001). As described, the immune cells' activity in the surface epithelium (low) and subepithelial layer (high), at pregnancy, is quite different. The surface epithelium acts as a barrier between the maternal immune cell reaction and the developing embryos. In addition, it has been shown in earlier studies, that transplantation of porcine trophoblasts to different ectopic sites (i.e. the ovarian stroma, the outer wall of the uterus, beneath the spleen or kidney capsules) results in an invasion of trophoblast cells into the surrounding tissues (Samuel, 1971; Samuel and Perry, 1972). This also suggests that the surface epithelium of the pigs endometrium is crucial, as a barrier. The mechanism for this is not known.

In non-mated sows (Kaeoket et., 2001[a]), the largest number of macrophages in the connective tissue of the subepithelial layer was found during early dioestrus (day 4, the first day of standing oestrus = day 1), indicating that these cells may have migrated into the endometrium after oestrus, in order to phagocyte and remove dead and dying cells, as well as foreign materials, in the endometrium. In pre-ovulatory inseminated sows (Kaeoket et al., 2003[a]), macrophages were also observed in the subepithelial layer at all stages. The number in pregnant sows (Kaeoket et al., 2003[a]), up to day 11, was only slightly higher than found in non-mated sows (Kaeoket et al., 2001[a]). However, at day 19, the number was three times higher (subepithelial layer) than in non-pregnant sows (at pro-oestrus, Kaeoket et al., 2001[a]). It has been reported that progesterone may directly or indirectly, via other factors (e.g. GM-CSF, CSF-1), stimulate the monocyte/macrophage migration (review Miller and Hunt, 1996). Uterine macrophages not only exhibit immunological functions against invading agents but they may also be involved in endometrial growth, cell differentiation, tissue remodelling and embryonic development, via their cytokine and growth factor products (review Hunt and Pollard,

1992). It has been suggested in human, mouse and rats that macrophages produce immunosuppressive factors, i.e. TGF-β, TNF-α and PGE (review Miller and Hunt, 1996). It is suggested that these factors might inhibit the migration and proliferation of cytotoxic lymphocytes, thereby reducing the local immune response and potential immunological rejection of the embryos (review Pollard, 1991). However, in pre-ovulatory inseminated sows (on day 19, Kaeoket et al., 2003[b]), the number of T cytotoxic lymphocytes ($CD8^+$ cells), was not reduced in the subepithelial layer but in the surface epithelium. This shows that the immune functions are complex during pregnancy. Eosinophils are known to be elaborate inflammatory mediators, which are important factors in the hosts defence against microorganisms (review Costa et al., 1997). However, eosinophils have a variety of potent substances in their granules, which may have a function in tissue and vascular remodeling, associated with endometrial physiology, as shown in humans by Jeziorska et al. (1995). In non-pregnant (Kaeoket et al., 2001[a]) and pregnant (Kaeoket et al., 2003[a]) sows, the largest number of eosinophils was found in the connective tissue of the subepithelial layer at dioestrus (day 11, Fig. 2 and 3). Bischof et al. (1995) also observed a large number of eosinophils in the subepithelial layer during days 10-14 of pregnancy in gilts. Studies of eosinophil functions during angiogenesis in ewes (Murdoch and Van Kirk, 2000) and humans (Horiuchi and Weller, 1997; review Sherer and Abulafia, 2001) have shown that the vascular endothelial growth factor (VEGF), can be secreted by eosinophils and may contribute to vascular development. In addition, it has been suggested that VEGF plays an important roles in endometrial vascularisation and early embryonic development during early pregnancy, in pigs (Winther et al., 1999). Taking the results from non-pregnant and pregnant sows together, the eosinophil infiltration appears to be dependent on a high progesterone level, at a certain stage and is not related to insemination or pregnancy. Irrespective of embryos being present or absent, the eosinophils in the endometrium of sows under progesterone dominance, may be associated with the dynamic changes in structure and function of the endometrium, in preparation for a potential attachment of embryos. In addition, folds of the surface epithelium of the endometrium around day 11 (Fig. 2) are observed in both non-pregnant (Kaeoket et al., 2001[a] and Stroband et al., 1986) and pregnant (Kaeoket et al., 2003[a] and Stroband et al., 1986) animals. As early as 1921, Corner related the occurrence of primary and secondary folds of the surface epithelium, to the presence of eosinophils in the endometrium, during days 7-10 of non-pregnant and pregnant sows.

Connective tissue of the glandular layer

In non-pregnant (Kaeoket et al., 2001a), pre-ovulatory inseminated and pregnant (Kaeoket et al., 2003[a]) sows, taking all stages together, lymphocytes, macrophages and plasma cells were the most apparent leukocyte cell types in the glandular layer (Fig. 4 and 5). Neutrophils, eosinophils and mast cells were rarely found in this layer. This indicates that cells of the immune system may have primary locations in different tissue layers.

In both non-pregnant and pregnant sows, plasma cells were found to be more pronounced in the connective tissue associated with the uterine glands than in the subepithelial layer. However, the numbers of plasma cells were low. It has long been known that plasma cells are a part of the adaptive immune cell response, being IgG-, IgM- and IgA-producing cells (review Brandtzaeg, 1997). Hussein et al. (1983) described IgG-containing cells in the sow endometrium, with higher levels in the glandular layer than in the other layers, which corresponded with the results (Kaeoket et al., 2001[a], 2003[a]). In addition, in pathological cases, such as subacute endometritis in sows, plasma cells are commonly seen in the subepithelial layer (McEntee, 1990; Dalin, personal communication), i.e. an adaptive response.

Distribution of T lymphocyte subpopulations and MHC class II expressing cells in the sow endometrium

In the pig, lymphocyte subpopulations in peripheral blood and lymphoid tissues can be divided into four subsets, depending on the expression of the CD2, CD4 and CD8 molecules, i.e. $CD2^+CD4^+CD8^+$, $CD2^+CD4^+CD8^-$, $CD2^+CD4^-CD8^+$, $CD2^+CD4^-CD8^-$ T lymphocyte subsets (Hirt et al., 1990; Saalmüller et al., 1989).

The results of the study on lymphocyte subpopulations of the sow endometrium (Kaeoket et al., 2001[b], 2003[b]) showed that CD2 positivity (T lymphocytes and NK cells) was the most common T lymphocyte subpopulation observed at different stages of the oestrous cycle and during early pregnancy, which is in accordance with Bischof et al. (1994[a], 1995), studying non-pregnant and pregnant gilt endometrium. In the studies of sow endometrium (Kaeoket et al., 2001[b], 2003[b]), the total number of $CD2^+$ cells was always found to be larger than the summation of $CD4^+$ (T helper) and $CD8^+$ (T cytotoxic) cells, suggesting the presence of $CD2^+$ $CD4^-$ $CD8^-$ cells in the sow endometrium, which is in agreement with Bischof et al. (1994[a]). However, the functional aspect of these different subpopulations in the endometrium is not fully understood. In addition, the results from Kaeoket et al. (2003[b]), on the proportion of $(CD4^+ + CD8^+)/CD2^+$ cells, imply that there was a larger number of $CD2^+$ $CD4^-$ $CD8^-$ cells in the epithelia (Table 1), compared with the number in connective tissue layers. Considering all groups and tissue layers together, the largest number of $CD2^+$ $CD4^-$ $CD8^-$ cells was observed on day 19 of pregnancy, indicating the effect of pregnancy. The presence of embryos at this stage may initiate some processes that influence the T lymphocyte subpopulation.

Kaeoket et al. (2002[b], 2003[b]) showed that the $CD8^+$ cells were more common in the surface and the glandular epithelia, than $CD4^+$ cells. In the connective tissue (subepithelial and glandular layers) the situation was the opposite. This observation has also been made in studies on endometrium of other species such as bovines (Cobb and Watson, 1995) and non-pregnant women (Pace et al., 1991). Cells carrying the CD8 marker are predominantly cytotoxic (T_C) (review Engelhardt et al., 1997). Furthermore, Pescovitz et al. (1988) found that in peripheral porcine blood, some of the $CD8^+$ cells might be NK cells. The larger number of $CD8^+$ compared with the $CD4^+$ cells within the surface and glandular epithelia, suggests that cytotoxic functions of the immune system dominate in the epithelial tissue. The fact that $CD4^+$ cells are more common than $CD8^+$ cells in the connective tissue (subepithelial and glandular layers) suggests that, on the other hand, the T helper (T_H) activity (review Engelhardt et al., 1997) is primarily located in these layers.

In pre-ovulatory inseminated sows (Kaeoket et al., 2003[b]), the numbers of $CD2^+$, $CD4^+$ and $CD8^+$ cells in the endometrium of newly mated/pregnant sows, from oestrus up to day 11, were only slightly higher than the numbers observed in the endometrium of non-mated sows, at comparable stages of the oestrous cycle (Kaeoket et al., 2001[b]). In contrast, Bischof et al. (1995) showed a significantly larger number of $CD2^+$, $CD4^+$ and $CD8^+$ cells, both in the uterine epithelium and subepithelial stroma of non-mated gilts, 2-4 days post-oestrus when compared with mated gilts. The difference in results may be due to the use of sows in our studies. Another possible explanation may be that natural mating was used in the study on gilt endometrium by Bischof et al. (1995).

In the non-mated sows (Kaeoket et al., 2001[b]), no $CD8^+$ cells were found within the glandular epithelium at early dioestrus (approximately 70 h after ovulation) nor on day 11. However, in pregnant sows (Kaeoket et al., 2003[b]), $CD8^+$ cells were observed. This may be due to a subacute reaction following an acute response after insemination.

The pig placenta is of an epitheliochorial type (i.e. diffuse attachment between uterine epithelium and chorioallantoic membrane via interlocking microvilli) which means that there is no invasion of trophoblasts into the maternal endometrium (no decidualisation) (Dantzer et al., 1985; review King, 1993). As shown by Kaeoket et al. (2003[b]), the effects of pregnancy on

Table 1 The proportion of ($CD4^+$ + $CD8^+$)/$CD2^+$ T cells within the surface and glandular epithelia and in the connective tissue of the subepithelial and glandular layers of the porcine endometrium following pre-ovulatory insemination and at different stages during early pregnancy

Groups	Surface epithelium	Glandular epithelium	Subepithelial layer	Glandular layer
5-6 h after AI	0.68 ± 0.25^{a}	0.69 ± 0.18^{a}	0.87 ± 0.18	0.93 ± 0.07
20-25 h after ovulation	0.40 ± 0.11^{b}	0.43 ± 0.19^{bc}	0.87 ± 0.24	0.94 ± 0.11
70 h after ovulation	0.52 ± 0.16^{ab}	0.69 ± 0.13^{a}	0.99 ± 0.21	1.09 ± 0.26
Day 11	0.37 ± 0.10^{bc}	0.44 ± 0.05^{bc}	0.79 ± 0.13	0.85 ± 0.22
Day 19	0.09 ± 0.16^{c}	0.19 ± 0.09^{c}	0.76 ± 0.08	0.84 ± 0.04
Overal significance	$p \leq 0.01$	$p \leq 0.01$	NS	NS

NS = not significant

Means (± S.D.) within the same column followed by the different superscript letters are significantly different ($p \leq 0.05$). (Kaeoket et al., 2003[b])

lymphocyte distribution were observed on day 19 (Fig. 6). In addition, the results reported by Kaeoket et al. (2003[b]) on T lymphocyte subpopulations showed that there was a significant reduction in the numbers of $CD2^+$ (T cells and NK cells) and $CD8^+$ (T cytotoxic cells and NK cells) cells in the surface epithelium and no $CD4^+$ (T helper cells) cells were observed. Also the number of MHC class II expressing cells was low. As mentioned earlier, it is suggested that porcine embryos at this stage may initiate some processes to suppress the immune response in the surface epithelium (King, 1988).

The number of lymphocytes in the subepithelial layer on day 19, as shown Kaeoket et al. (2003[a]), was increased. This is in agreement with the results reported by Kaeoket et al. (2003[b]), which show that the T lymphocyte subpopulations ($CD2^+$, $CD4^+$ and $CD8^+$ cells; Fig. 7 and 8) were increased. Bischof et al. (1995) also found more $CD2^+$, $CD4^+$and $CD8^+$ cells in the gilt endometrium on days 18-21 of pregnancy, compared with days 2-4 and 10-14, respectively. Furthermore, in pre-ovulatory inseminated sows (Kaeoket et al., 2003[b]), the number of MHC class II expressing cells were also significantly higher on day 19 (Fig. 8). The presence of NK cells, T cells and MHC class II expressing cells in the subepithelial layer of the endometrium, may be supporting the surface epithelium, in limiting the trophoblast invasion. MHC class II molecules are found in a variety of immunologically important cell types (i.e. macrophages, monocytes, endothelial and epithelial cells) (review Farrar and Schreiber, 1993; review Cencič and La Bonnardière, 2002). It has been suggested that if trophoblast-derived IFN-γ crosses the uterine epithelium and reaches the connective tissue of the subepithelial layer, it may result in an induction of MHC class II antigen in this layer (review Cencič and La Bonnardière, 2002). Interferons secreted from porcine trophectoderm as shown by Lefèvre et al. (1990), La Bonnardière et al. (1991), D'Andréa and La Bonnardière (1998) and Cencič et al. (2002) may therefore be involved in the attraction (as seen on day 19, Kaeoket et al., 2003[b]) of both CD positive cells (T and NK cells) and MHC class II expressing cells, to the subepithelial layer underneath the site where the trophoblast is attached, to the surface epithelium.

Furthermore, it has been reported that IFN-γ can also be secreted by activated NK and T cells, as shown in humans (Mincheva-Nilsson et al., 1994; Kurago et al.,

1998) and pigs (Domeika et al., 2002). T and NK cells, via their IFN-γ production, may be involved in the endometrial vascularisation at the implantation/ placentation sites, which has been observed in humans (review King et al., 1997; review Redline et al., 2000) and mice (Ashkar et al., 2000) and suggested in the pig (review Cencič and La Bonnardière, 2002; Engelhardt et al., 2002[a,b]). In pregnant sows (Kaeoket et al., 2003[a]), an increase in endometrial vascularisation underneath the attachment sites on day 19 of pregnancy was observed.

The results reported by Kaeoket et al. (2001[b], 2003[b]) showed that MHC class II was consistently expressed in the endothelium and in other individual cells in all tissue layers. In non-mated sows, the distribution of MHC class II expressing cells (endothelial cells excluded) in the connective tissue of subepithelial and glandular layers (Kaeoket et al., 2001[b]) had a pattern similar to the distribution of macrophages (Kaeoket et al., 2001[a]). It has been reported that the administration of oestradiol, induced a significant increase in the number of MHC class II expressing cells in the rat (Zheng et al., 1988) and the equine (Frayne and Stokes, 1994) endometrium. In mated sows (Kaeoket et al., 2003[b]), larger numbers of MHC class II expressing cells were observed in the surface epithelium and subepithelial layer, up to 70 h after ovulation (approx. 83-93 h after insemination), compared with non-mated sows, at comparable stages. This means that MHC class II molecules were presented also on other cells than macrophages, because the number of macrophages in pregnant animals (Kaeoket et al., 2003[a]) was not higher than in non-pregnant animals (Kaeoket et al., 2001[a]). This indicates an increased immune response in terms of antigen presentation in response to insemination.

Conclusion

To conclude, in non-inseminated sows there is a variation in the distribution of leukocytes during different oestrous cycle stages, which suggests direct or indirect hormonal control. The pattern of immune cells in the endometrium represents a physiological variation. In pre-ovulatory, inseminated and pregnant sows, immune cells in the sow endometrium do respond to the challenges of both mating and embryo growth. The immunomodulation is illustrated by the distribution of leukocytes, $CD2^+$, $CD4^+$, $CD8^+$ and MHC class II expressing cells, at the attachment sites, on day 19, i.e. the low numbers in the surface epithelium and the high numbers in the subepithelial layer, shows that the porcine trophoblast may influence the endometrium to develop the conditions required for embryonic attachment and survival in pigs.

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