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Uterine differentiation as a foundation for subsequent fertility

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Uterine differentiation in cattle and sheep begins prenatally, but is completed postnatally. Mechanisms regulating this process are not well defined. However, studies of urogenital tract development in murine systems, particularly those involving tissue recombination and targeted gene mutation, indicate that the ideal uterine organizational programme evolves epigenetically through dynamic cell–cell and cell–matrix interactions that define the microenvironmental context within which gene expression occurs and may ensure adult tissue stability. In the cow and ewe, transient postnatal exposure of the developing uterus to steroids can produce immutable changes in adult uterine tissues that may alter the embryotrophic potential of the uterine environment. Thus, success of steroid-sensitive postnatal events supporting uterine growth and development can dictate the functional potential of the adult uterus. Studies to determine effects of specific steroidal agents on patterns of uterine development during defined neonatal periods, as well as the functional consequences of targeted neonatal steroid exposure in the adult uterus, should enable identification of critical developmental mechanisms and determinants of uterine integrity and function. Extreme adult uterine phenotypes (lesion models) created in cattle and sheep by strategic postnatal steroid exposure hold promise as powerful tools for the study of factors affecting uterine function and the rapid identification of novel uterine genes.

Introduction

The uterus is an essential reproductive organ. Functions of the uterus in domestic ruminants include generation of the luteolytic signal required for ovarian cyclicity, transport and maturation of spermatozoa, recognition and reception of embryos, provision of an embryotrophic environment for conceptus development, and expulsion of the fetus and placenta at parturition (Bartol, 1999). These functions are borne by the uterine mucosa or endometrium and smooth muscle or myometrium. Developmental determinants of uterine function are not well defined. However, studies of laboratory animals (Mori and Nagasawa, 1988), humans (Mori and Nagasawa, 1988; Cooper and Kavlock, 1997), wildlife species (Cooper and Kavlock, 1997), and domestic ungulates, including sheep (Bartol *et al.*, 1988a, 1997), cattle (Hancock *et al.*, 1994; Bartol *et al.*, 1995; King *et al.*, 1995; Bartol *et al.*, 1996; Bartol and Floyd, 1996) and pigs (Bartol *et al.*, 1993), indicate that exposure of developing uterine tissues to agents that disrupt critical organizational events can have lasting effects on reproductive health. Thus, while genetic potential for uterine competence and reproductive success may be defined at conception, success of developmental events regulating uterine growth, morphogenesis and cytodifferentiation ultimately determines phenotypic potential of the uterus to support essential reproductive processes.

The fact that disruptive effects of steroids can have long-term consequences for uterine function, and that the nature of such effects reflects specific conditions of steroid exposure, suggests a strategy

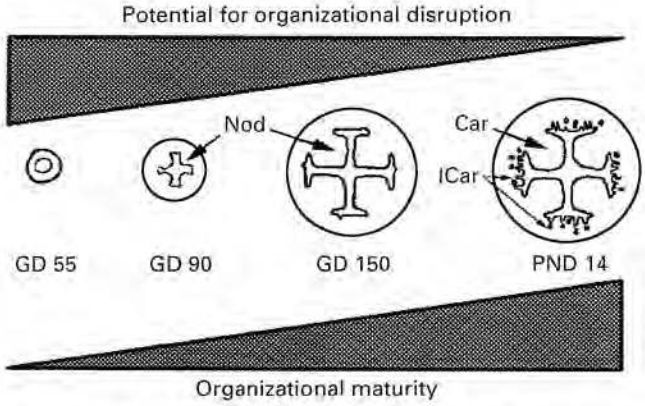

Fig. 1. Schematic representation of ovine uterine morphogenesis between gestational day (GD) 55 and postnatal day 14 (PND 14; birth = PND 0). Significant caruncular morphogenesis occurs prenatally (GD 150 = term), while genesis and proliferation of endometrial glands (adenogenesis) is a postnatal event in both sheep and cattle (Nod: precaruncular nodule; Car: caruncle; ICar: intercaruncular area). In general, the potential for organizational disruption of uterine development is inversely related to tissue maturity.

for identification of critical, steroid-sensitive developmental periods, and a scheme for the creation of extreme adult uterine phenotypes (lesion models) of use in studies of uterine function. The objectives of this review are to: (1) summarize primary developmental events that support uterine organization; (2) present evidence that the functional integrity of adult uterine tissues is determined, in part, by the success of steroid-sensitive postnatal uterine organizational events; (3) describe strategies for creation of extreme adult uterine phenotypes in domestic ruminants, based on the concept of endocrine disruption; and (4) present evidence of the utility of such models for study of uterine development and function. Where possible, emphasis will be placed on uterine development in sheep and cows.

Uterine Organogenesis

The uterus develops as a specialization of the paramesonephric ducts, which give rise to the infundibula, oviducts, uterus, cervix and anterior vagina (Bartol, 1999). Paramesonephric fusion occurs between gestational day (GD) 34 and 55 in sheep (Wiley *et al.*, 1987), and GD 55 and 60 in cattle (Marion and Gier, 1971). Fusion is partial in both species, producing a bicornuate uterus that supports intercornual migration of embryos.

Uterine histogenesis has been described to some extent for both cattle (see Marion and Gier, 1971; Atkinson *et al.*, 1984; and references therein) and sheep (Wiley *et al.*, 1987; Bartol *et al.*, 1988a,b). Differentiation of paramesonephric tissues into histologically discernible zones indicating endometrium and presumptive myometrium is evident in the ovine fetus by GD 55, and in the bovine fetus by GD 70. Definitive uterine tissue layers, including the adluminal zone of densely packed endometrial stroma or stratum compactum, the deeper more loosely arranged stromal cells of the stratum spongiosum, and both inner and outer layers of myometrial smooth muscle, are evident in sheep and cattle by GD 90–100. Caruncles, raised aglandular structures that are macroscopic features of the adult endometrium in sheep and cattle, emerge during fetal life as precaruncular nodules (Fig. 1). Extensive prenatal caruncular morphogenesis defines both the number and distribution of these structures along the uterine wall. Genesis of uterine glands

(adenogenesis) begins during the last month of gestation when short epithelial invaginations appear along the uterine mucosa surrounding the base of precaruncular nodules. Endometrial morphogenesis is completed postnatally with continued growth of caruncles, extensive proliferation of endometrial glands, and establishment of definitive aglandular caruncular and intensely glandular intercaruncular endometrial areas.

Organizational Mechanisms

Mechanisms regulating growth and differentiation of the paramesonephric duct axis in ruminants are not well defined. Presently, much is inferred about these mechanisms from studies of laboratory species. Jost (1953) established the paradigm that prenatal urogenital tract development in female mammals is an ovary-independent process. Uterine development is also unaffected for defined periods after ovariectomy at birth in the mouse (Bigsby and Cunha, 1985), rat (Branham and Sheehan, 1995), pig (Tarleton *et al.*, 1998) and sheep (Bartol *et al.*, 1988a,b). Thus, uterine organizational mechanisms are ovary-independent and may be steroid-independent for some period before and after birth.

Roles for ligand-dependent nuclear receptors

The extent to which members of the nuclear receptor superfamily of ligand-regulatable transcription factors are required to support uterine development during pre- and early postnatal life is unclear. However, female mice lacking functional oestrogen receptor-α (ER) or progesterone receptor (PR) genes were born with complete reproductive tracts, indicating that uterine organogenesis, at least in the mouse, does not require ER, PR or their cognate ligands (Korach *et al.*, 1996; Lydon *et al.*, 1996). Uterine hypoplasia in ER-null mice confirmed that an active ER system is required for uterine growth (Korach *et al.*, 1996). Uterine hypoplasia was also observed in weaned mice lacking receptors for 1α, 25-dihydroxy vitamin D3 (Yoshizawa *et al.*, 1997). In addition, the fact that retinyl palmitate administered to neonatal pigs perturbed early postnatal uterine development (Vallet *et al.*, 1995), and that retinoids can affect homeogene expression (see below, and Marshall *et al.*, 1996), indicates that retinoic acid receptors may mediate uterine organizational events.

Ontogeny of steroid receptor expression and function in developing ungulate uterine tissues are incompletely characterized. In the cow, PR mRNA was not detected consistently in dispersed fetal uterine cells from mid- to late gestation, whereas ER mRNA was detected on GD 100–110 and increased on GD 185–200 (Malayer and Woods, 1998). Oestrogen did not bind to or affect DNA synthesis in cultured bovine mesonephric cells obtained on GD 50–59 (Winters *et al.*, 1993). However, oestrogen responsiveness was reported for cultured uterine cells from GD 185–200 (Malayer and Woods, 1998). Thus, neither PR nor functional ER may be present in developing uterine tissues prenatally until after gross uterine morphology and basic histoarchitectural features of the uterine wall have emerged (see above). Since exposure of the immature urogenital tract to steroids can affect the integrity of adult tissues (see below), and steroids are present in the fetal circulation throughout gestation, ontogeny of steroid sensitivity in developing uterine tissues may reflect a natural strategy to ensure organizational success.

Expression of functional steroid receptors during the perinatal period may be necessary for normal uterine development (Bartol *et al.*, 1988b; Malayer and Woods, 1998; Tarleton *et al.*, 1998). Nuclear steroid receptors mediate classical ligand-dependent events and enhance target cell responsiveness to peptide growth factors by coupling with membrane receptor-mediated signal transduction pathways (Smith, 1998). Thus, expression of ER-positive (ER+) character in uterine cells later in development, as described for cows (Malayer and Woods, 1998), may define the point at which oestrogens begin to elicit trophic effects on the fetal uterus and(or) increase the sensitivity of ER+ uterine target cells to paracrine mediators of oestrogen action such as insulin-like growth factor I (IGF-I) or epidermal growth factor (EGF). Like the ER-null mouse (Korach *et al.*, 1996), both IGF-I-null and EGF receptor (EGFR)-null mice have hypoplastic uteri (Baker *et al.*, 1996; Hom *et al.*, 1998).

Uterotrophic effects of oestradiol were limited to epithelium and virtually absent from fibromuscular stroma when EGFR expression, also documented in the fetal bovine uterus (Malayer and Woods, 1998), was eliminated in mice (Hom *et al.*, 1998). Thus, compartment-specific cross-talk between growth factor and steroid receptor signalling pathways may affect uterine growth in the fetus and neonate.

Homeogenes as effectors of uterine organization and tissue stability

Homeogenes are primary effectors of tissue organization. Mammalian homeogenes encode transcription factors that determine regional tissue identities along anteroposterior body axes, and may maintain functional stability of adult tissues (Taylor *et al.*, 1997). Expression of homeogenes Hoxa-9, -10, -11, -13 and Msx1 is uniform along the murine paramesonephric duct prenatally, but becomes restricted spatially during the first two weeks of postnatal life such that oviducts express Hoxa-9, the uterus Hoxa-10, -11 and Msx1, the cervix Hoxa-11 and -13, and the anterior vagina Hoxa-13. This pattern persists in adult murine and human genital tracts and may be subject to steroid regulation (Pavlova *et al.*, 1994; Taylor *et al.*, 1997).

Disruption of homeogene expression is associated with homeotic tissue transformations. In adult ewes, prolonged exposure to oestrogen causes permanent infertility associated with destabilized cervical histoarchitecture and redifferentiation of the cervix toward a more anterior, uterine-like phenotype (Adams and Sanders, 1993; Adams, 1995). This partial homeotic transformation can be accompanied by development of uterine glandular cysts and adenomyotic lesions (Adams, 1995). Similarly, uteri of mice lacking functional Hoxa-10 (Benson *et al.*, 1996) or Hoxa-11 genes (Gendron *et al.*, 1997) displayed partial homeotic transformation toward a more anterior, oviduct-like phenotype. Impaired ability to form uterine glands was observed in Hoxa-11 mutants (Gendron *et al.*, 1997). Mice lacking functional Hoxa-10, Hoxa-11 or Hmx3 genes cannot support embryo development due to aberrant expression of critical uterine proteins (Gendron *et al.*, 1997; Wang *et al.*, 1998). Uterine expression of Wnt-5a was altered in Hmx3-null mice (Wang *et al.*, 1998). Stromal expression of Wnt-5a affects uterine epithelial expression of Msx1, which may be important for maintenance of epithelial receptivity to stromal and conceptus signals (Pavlova *et al.*, 1994). Thus, structural and functional stability of uterine tissues may evolve and be maintained through steroid-sensitive mechanisms operating to ensure spatially unique, tissue-specific patterns of homeotic gene expression yet to be defined in domestic ruminants.

Cell–cell and cell–matrix interactions and selective stabilization

Uterine development requires continuous reciprocal interactions between epithelium and underlying stroma (Bigsby, 1991). The communication network that develops through these interactions involves paracrine-acting factors and their receptors that may include homeogene products and Wnts (see above, and Moon *et al.* 1997,), hepatocyte growth factor (HGF; Sugawara *et al.*, 1997), EGF (Hom *et al.*, 1998), heparin-binding EGF (HB-EGF; Zhang *et al.*, 1998), transforming growth factors (TGF; Takahashi *et al.*, 1994; Godkin and Dore, 1998), IGF (Stevenson *et al.*, 1994), keratinocyte growth factor (KGF; Koji *et al.*, 1994), vascular endothelial growth factor (VEGF; Grant *et al.*, 1995; Torry and Torry, 1997), and others. Specific regulatory interactions that must evolve for successful uterine development reflect communication between epithelial, stromal and endothelial cells within the context of their extracellular matrix (ECM; Ettinger and Doljanski, 1992; Grant *et al.*, 1995). These relationships direct local patterns of gene expression and dictate cellular responsiveness to gene products (Ettinger and Doljanski, 1992). Therefore, the ideal uterine organizational programme may be determined epigenetically through selective stabilization of specific cell–cell and cell–ECM interactions. This organizational model predicts that each configuration of cells and ECM during the course of development increases the likelihood of the next, and decreases the likelihood of others that might be less desirable (Ettinger and Doljanski, 1992). Conversely, aberrations in initial conditions would be amplified through development with severe implications for end-organ integrity. If applicable to domestic ruminants, this model predicts

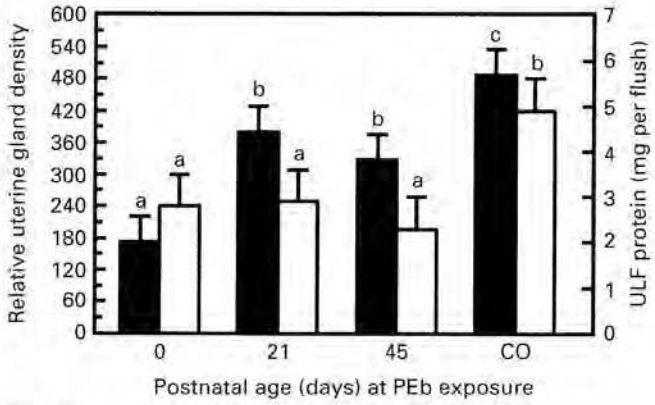

Fig. 2. Effects of chronic (about 200 days) administration of progesterone plus oestradiol benzoate (PEb), beginning on either postnatal day 0 (PND 0 = birth), 21 or 45, on relative endometrial gland density (dark bars) and uterine luminal fluid (ULF) protein content (open bars) in adult beef heifers during dioestrus. Treated heifers ($n = 5$ per group) received a single implant containing progesterone (100 mg) and oestradiol benzoate (10 mg). Control (CO) heifers were not exposed to progesterone and oestradiol benzoate. Uteri were obtained from heifers at approximately 15 months of age on day 12 of a $PGF_{2\alpha}$-induced oestrous cycle. For each response, bars with different letters are significantly different ($P < 0.01$). (Adapted from Bartol *et al.*, 1995.)

that disruption of critical primary conditions that define the programmatic context for uterine development should alter the capacity of uterine tissues to develop and function properly.

Postnatal Disruption of Uterine Development

The idea that disruption of development during specific 'critical' periods could have enduring effects on adult tissues is not new. Perinatal exposure of rodents to steroids, including oestrogens progestins and androgens, can disrupt uterine development, initiate uterine lesions and impair fertility (Sananes *et al.*, 1980; Mori and Nagasawa, 1988; Ohta, 1995). Tissue susceptibility to such organizational effects of steroids tends to be inversely related to age or tissue maturity and directly related to dosage and duration of exposure (Fig. 1).

Postnatal exposure to steroids can have lasting effects in domestic ungulates (Bartol *et al.*, 1993, 1995; Spencer *et al.*, 1993; King *et al.*, 1995). Moreover, the potential for inappropriate exposure of developing tissues in domestic animals to either natural hormones or xenobiotics is real. Exposure can occur: (1) physiologically, as a consequence of aberrant production of hormones during critical periods; (2) by diet, as a consequence of the consumption of bioactive agents such as phytoestrogens or mycotoxins (Adams, 1995); (3) pharmacologically, as a consequence of the intentional use of endocrinologically active agents to enhance performance traits (Hancock *et al.*, 1994; King *et al.*, 1995); and (4) unintentionally, as a consequence of the presence of endocrine-active industrial pollutants in the environment (Cooper and Kavlock, 1997). Compounds that disrupt development by altering critical endocrinological events are categorized as endocrine disruptors (EDs).

Postnatal exposure to steroidal endocrine disruptors in cows

Commercially, beef calves are often exposed to steroidal agents released from implants designed to enhance growth performance. In the United States, implants approved for female calves intended

for use as breeding replacements contain either the oestrogenic compound zeranol alone (36 mg) (Schering-Plough Animal Health Corp., Union, NJ), or a combination of progesterone (100 mg) and oestradiol benzoate (10 mg) (Fort Dodge Animal Health, KS; Vetlife, Norcross, GA).

Effects of postnatal exposure to zeranol on bovine reproductive performance were related to both period of exposure and dosage. In numerous trials, pregnancy rates decreased by an average of 35% in yearling heifers given single zeranol implants at birth, but were essentially unaffected by the same treatment initiated between one and 14 months of age, suggesting a critical period for oestrogen sensitivity during the first postnatal month. However, pregnancy rates were depressed by as much as 40% in heifers treated with two or more zeranol implants between one and 11 months of age (Hancock *et al.*, 1994; Bartol and Floyd, 1996). Chronic exposure to zeranol for 300 days from birth delayed puberty and reduced pre- and postpubertal uterine diameter in beef heifers (King *et al.*, 1995). Similarly, exposure of heifers to several compounds for one year from PND 84, including trenbolone acetate, oestradiol, zeranol, or a combination of TBA and oestradiol, had variable but consistently negative effects on adult uterine wet weights (Moran *et al.*, 1990). These anti-uterotrophic effects could reflect lesions of the central nervous system, altered gonadotrophin secretion and lack of uterotrophic support from the ovary. However, abortion frequency increased between GD 25 and 45 in heifers exposed to zeranol from birth to PND 300, indicating that zeranol-induced uterine lesions that affected attachment of the conceptus.

Treatment of beef heifers with an implant designed to release both progesterone and oestradiol benzoate for approximately 200 days beginning on either PND 0, 21 or 45 reduced adult uterocervical wet weight by 35%, myometrial area by 23%, and endometrial area by 27%, regardless of age at first exposure to progesterone and oestradiol benzoate (Bartol *et al.*, 1995). Effects were accompanied by a marked decrease in uterine glandularity that was most severe when exposure began at birth (Fig. 2). Uterine luminal fluid protein content was reduced by approximately 45% in heifers exposed to progesterone and oestradiol benzoate (Fig. 2). Thus, generalized uterine hypoplasia, endometrial aplasia and altered uterine protein content were observed in cyclic adult heifers 13.5 to 15 months after initiation of chronic (about 200 day) exposure to progesterone and oestradiol benzoate on or before PND 45, and a potentially critical period of uterine sensitivity to developmental disruption induced by progesterone and oestradiol benzoate was identified between birth and PND 21 (Bartol *et al.*, 1995).

Subsequently, crossbred beef heifers were assigned to one of five groups at birth (groups I–V; n = 5 or 6 heifers per group). Heifers in groups I–III received a single progesterone and oestradiol benzoate implant at birth, while those in groups IV and V served as unexposed controls (CO). All heifers were laparotomized on PND 21, when each uterus was measured and progesterone and oestradiol benzoate implants were removed from calves in group I. This created a group of uterine-intact adults exposed to progesterone and oestradiol benzoate for 21 days from birth. In addition, on PND 21, heifers in groups II (progesterone and oestradiol benzoate) and V (CO) were hemi-hysterectomized to permit evaluation of short-term treatment effects on uterine histoarchitecture. Jugular blood samples were taken from heifers at 16 months of age during dioestrus, before and after administration of oxytocin (100 iu), and plasma was assayed for 13,14-dihydro-15-keto$PGF_{2\alpha}$ (PGFM) as a reflection of oxytocin-inducible uterine prostaglandin generating ability (Wolfenson *et al.*, 1993). Uteri were obtained at slaughter during dioestrus at 26 months of age. Tissues were processed for histomorphometry, and endometrial samples were assayed for oxytocin receptor concentrations (Spencer *et al.*, 1995; F. F. Bartol and M. A. Mirando, unpublished).

Anti-uterotrophic effects of progesterone and oestradiol benzoate were evident by PND 21, when both uterine horn length (141.6 versus 78.2 ± 5.0 mm) and volume (5019 versus 2020 ± 59 mm^3) were reduced ($P < 0.05$) in treated heifers. Nascent uterine glands were present in both groups on PND 21, but were more frequently branched and appeared less stable structurally in heifers exposed to progesterone and oestradiol benzoate (Fig. 3). Chronic exposure to progesterone and oestradiol benzoate, exceeding 21 days from birth, was required to produce overt effects on adult bovine uterine size and endometrial histoarchitecture. Uterine weights (Fig. 4a) indicated that anti-uterotrophic effects of chronic exposure to progesterone and oestradiol benzoate alone (group III) were approximately equivalent to neonatal hhx (group V). Effects of hhx combined with chronic

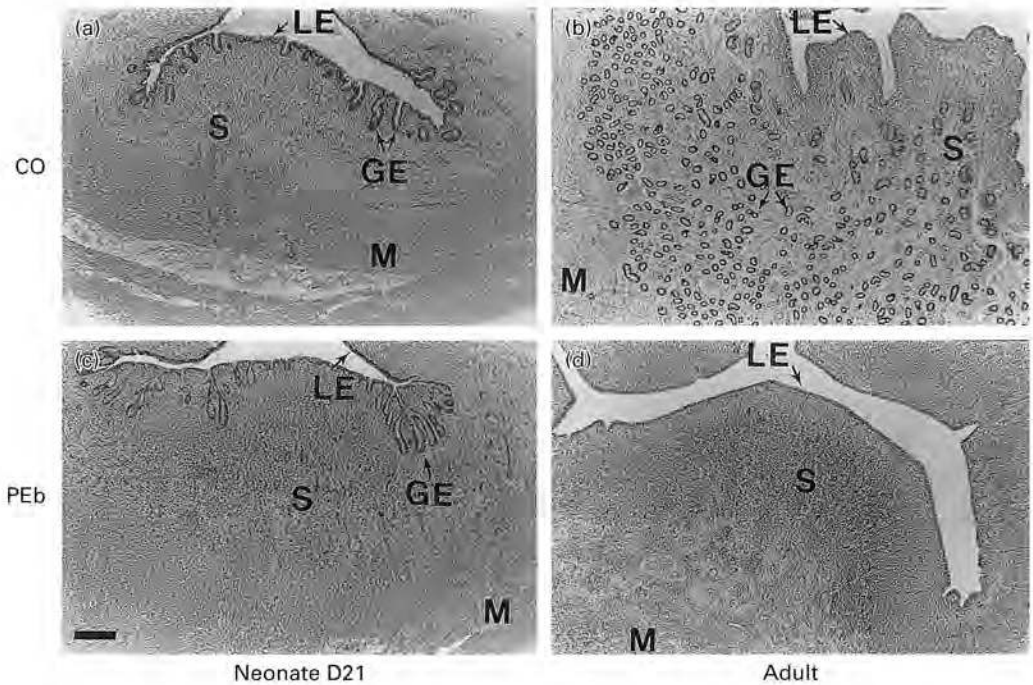

Fig. 3. Postnatal histogenesis of the bovine uterine wall and extreme effects of exposure to progesterone plus oestradiol benzoate (PEb) from birth on adult uterine histoarchitecture. Photomicrographs show histology of the uterine wall in individual animals hemihysterectomized on neonatal day 21 (a and c), and in the contralateral uterine horn of the same animals at 26 months of age during dioestrus (b and d). Micrographs (a) and (b) illustrate normal histogenesis in a representative control heifer (CO) not exposed to progesterone and oestradiol benzoate. Micrographs (c) and (d) illustrate an extreme consequence of chronic exposure to progesterone and oestradiol benzoate from birth in an adult heifer. Nascent endometrial glands (GE) were present in both CO and heifers exposed to progesterone plus oestradiol benzoate on PND 21 (a versus c). Chronic exposure to progesterone plus oestradiol benzoate reduced or eliminated endometrial glands in adults (b versus d). No endometrial glands were found in multiple serial sections of the adult uterus (d) that was exposed neonatally to progesterone plus oestradiol benzoate. However, this neonatally hemihysterectomized heifer displayed oestrous cycles of normal duration. LE: luminal epithelium, GE: glandular epithelium; S: endometrial stroma; M: myometrium. Haematoxylin and eosin staining. Scale bar represents 300 μm.

progesterone and oestradiol benzoate were additive, producing a 52% reduction in uterine mass in group II heifers (Fig. 4a). The reduction in adult endometrial glandularity, expected with chronic exposure to progesterone and oestradiol benzoate, was most severe for group II heifers, in which few or no endometrial glands were found (Fig. 3). Consistently, both peak uterine PGFM response and endometrial OTR concentrations were reduced ($P < 0.07$) in group II heifers (Fig. 4).

Treatment-induced loss of oxytocin-sensitive prostaglandin-generating uterine parenchyma may explain the reduced peak PGFM response observed in group II heifers (Fig. 4). However, all heifers displayed regular oestrous cycles of normal duration, including the group II heifer in which no endometrial glands were found (Fig. 3). Thus, uterine glandular epithelium may not be essential for normal cyclicity in cattle. Results also indicate that evidence of normal cyclicity is not necessarily evidence of normal endometrial integrity.

Results show that transient postnatal exposure to steroids can have specific, extreme and lasting effects on the adult bovine endometrium that could alter the embryotrophic potential of the uterine environment (Martal *et al.*, 1997). Effects reflect particular conditions of exposure and tend to be

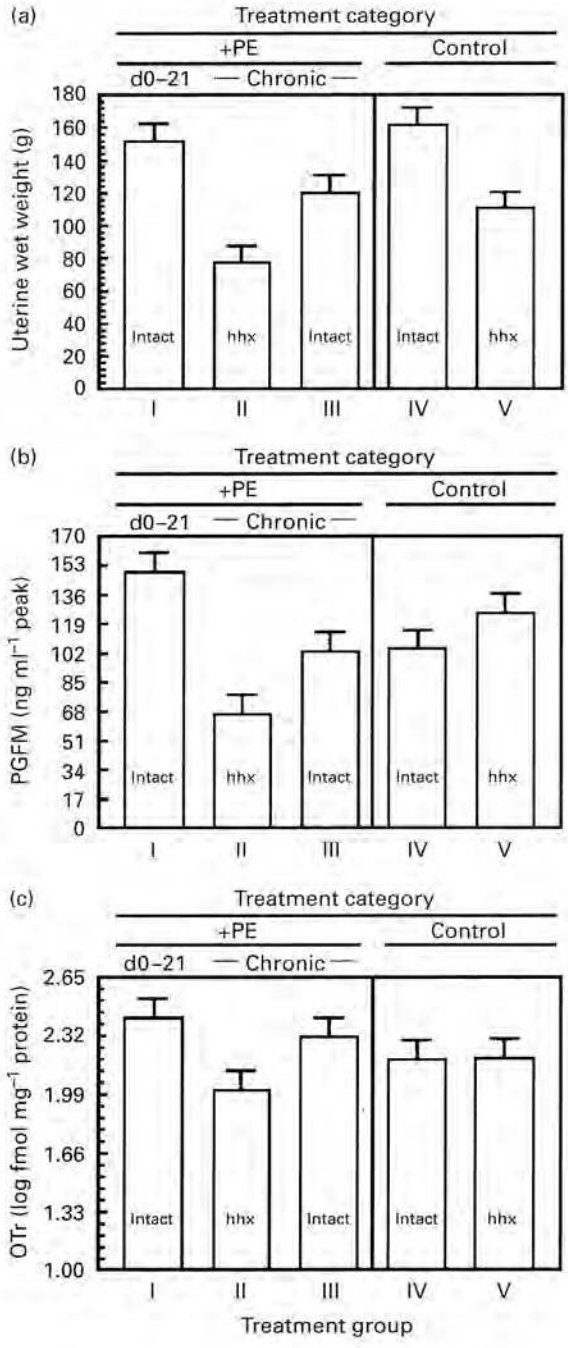

Fig. 4. Effects of neonatal exposure to progesterone plus oestradiol benzoate (PEb) from birth to neonatal day 21 (d0–21), or for approximately 200 days from birth (chronic), and hemihysterectomy (hhx) on neonatal day 21, on specific uterine responses in adult beef heifers (n = 5–6 per treatment group). Responses (least squares means + SEM) illustrated are: (a) uterine wet weight; (b) peak peripheral plasma concentrations of 13,14-dihydro-15-keto $PGF_{2\alpha}$ (PGFM) in response to an oxytocin (100 iu) challenge during dioestrus; and (c) endometrial oxytocin receptor density (OTr). Uterine weight (a) was reduced by neonatal progesterone plus oestradiol benzoate

more pronounced when initiated at birth. Studies also showed that postnatal exposure to steroidal endocrine disruptors of uterine development could be used to create unique adult uterine phenotypes.

Exposure to progestin from birth and uterine development in sheep

Endometrial glands proliferate rapidly between PND 0 and 26 in the ewe (Bartol *et al.*, 1988a,b). Exposure of lambs to a 19-nor-progestin (NOR; 17α-acetoxy-11β-methyl-19-norpreg-4-ene-3,20 dione) from PND 0 to 13 prevented gland development, as reflected by their absence in tissues from PND 13 (Bartol *et al.*, 1988a). Withdrawal of the progestin block permitted genesis of poorly organized uterine glands between PND 13 and 26 (Fig. 5A). Similarly, patterns of *in vitro* uterine protein synthesis characteristic of tissues obtained from NOR-exposed ewes on PND 13 and from NOR-withdrawn ewes on PND 26 were not identical to those observed during the normal morphogenetic transition between PND 0 and PND 13 (Fig. 5b). It was proposed that: (1) withdrawal of uterine tissues from a progestin-dominated prenatal environment at birth provides an endocrine cue for initiation of uterine adenogenesis; (2) this organizational programme could be disrupted by postnatal exposure to NOR; and that (3) prolonged exposure to NOR from birth should disintegrate critical organizational events sufficiently to produce a stable, extreme endometrial phenotype in adult ewes characterized by the absence of uterine glands, an organizationally induced uterine gland 'knock-out' (UGKO).

The 'UGKO' phenotype was created in adult ewes exposed to NOR for 32 weeks from birth (Bartol *et al.*, 1997). Uteri were obtained from NOR-exposed UGKO ewes during follicular ($n = 5$) and luteal ($n = 2$) phases of the ovarian cycle, and from one ewe with inactive ovaries. In striking contrast to intensely glandular endometrium obtained from control ewes during dioestrus (Fig. 6), endometrium from UGKO ewes was aglandular (7/8), or contained a few glandular cysts (1/8, not shown). This extreme phenotype may be induced by transient exposure to NOR for no more than 8 weeks from birth (T. E. Spencer, T. L. Ott, F. W. Bazer and F. F. Bartol, unpublished). Studies to determine whether UGKO ewes can cycle normally and conceive, and whether pregnancy can be established and maintained in an aglandular uterus are underway.

Distinct patterns of endometrial gene expression were identified between control and UGKO endometrium using mRNA differential display PCR. The majority (> 95%) of over 80 cDNAs cloned to date were amplified from control and absent from UGKO samples (Fig. 7). If structural differences between UGKO and control tissues are reflected at the transcriptional level, many differentially expressed mRNAs should be specific to the epithelium. Consistently, an antisense cRNA probe generated from endometrial cDNA DD54, identified as described above, hybridized specifically to uterine luminal and glandular epithelium from normal cyclic and pregnant ewes (Fig. 8a). Northern blot analysis revealed two major endometrial transcripts of approximately 2 kb and 6 kb, and DD54 expression increased during dioestrus, suggesting endocrine regulation of this epithelial gene product (Fig. 8b and unpublished results). The DD54 cDNA lacks sequence homology with known genes as determined using the BLAST algorithm (National Center for Biotechnology Information, NIH, Bethesda, MD). Results illustrate the immediate utility of the UGKO model for discovery of potentially novel genes encoding uterine proteins required for establishment of an embryotrophic uterine microenvironment in domestic ungulates (Martal *et al.*, 1997).

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exposure (+PE < Control, $P < 0.06$) and hhx ($P < 0.01$). Among groups exposed to progesterone plus oestradiol benzoate (I–III), uterine weight was lower in group II than in groups I and III ($P < 0.01$). Relative to intact controls (group IV), short-term exposure to progesterone plus oestradiol benzoate (d0–21, group I) did not affect uterine weight. Peak PGFM concentrations (b), defined as the maximum value detected for each heifer within 45 min after oxytocin, were not affected by progesterone plus oestradiol benzoate exposure or hhx alone. However, among progesterone plus oestradiol benzoate exposed heifers, peak PGFM values were lower ($P < 0.07$) in group II than in group III. Identical relationships were detected for endometrial OTR density (c).

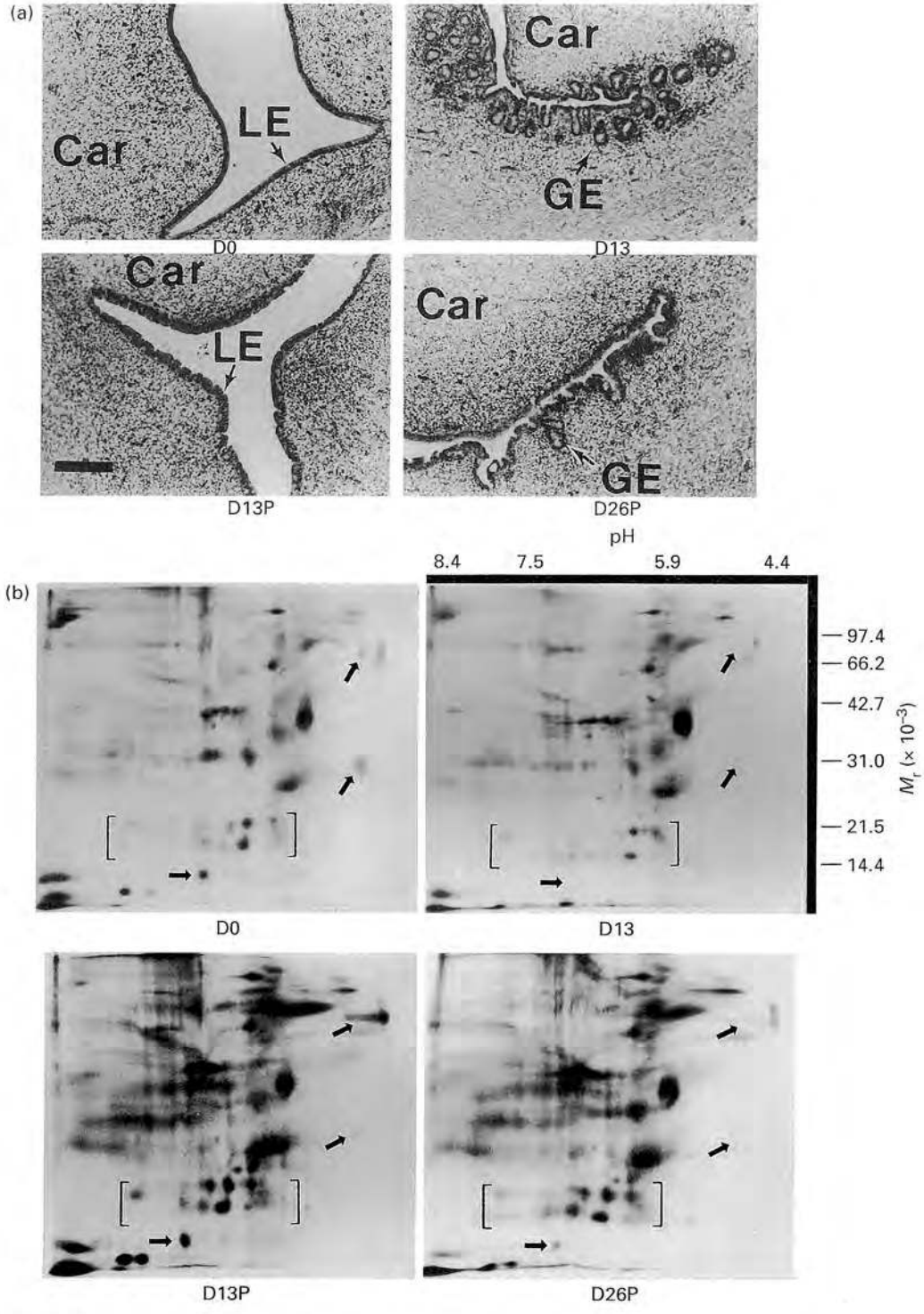

Fig. 5. For legend see facing page.

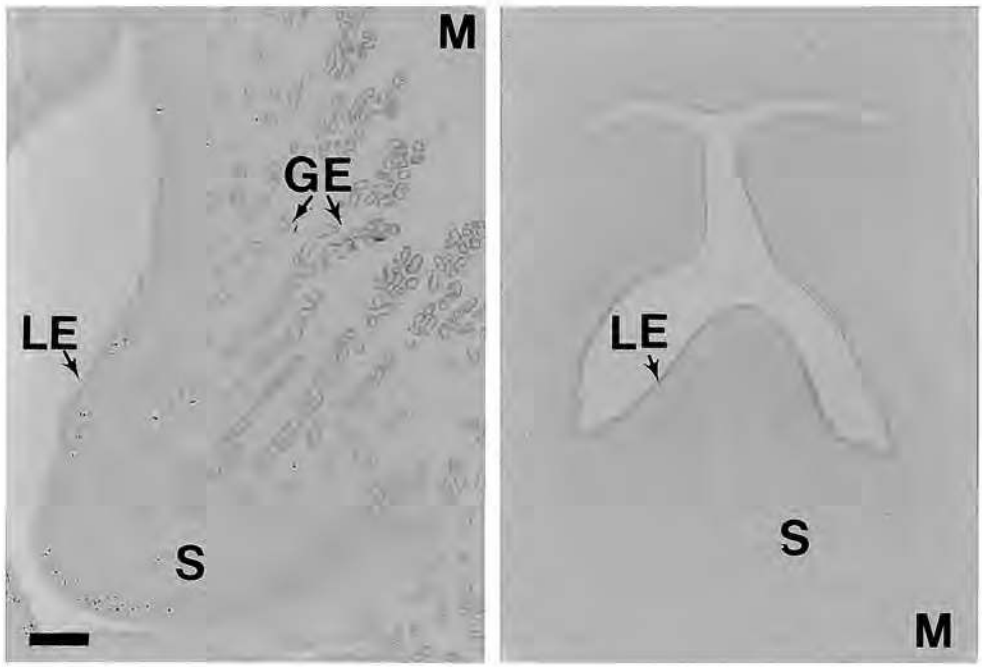

Fig. 6. Histological characterization of the uterine gland 'knock-out' (UGKO) phenotype in adult ewes. Photomicrographs depict normal adult endometrial histology for a typical control ewe, and the UGKO phenotype in an adult ewe that was exposed to a 19-norprogestin (19-norpreg-4-ene-3,20 dione) for 32 weeks from birth. Note the intense endometrial glandularity characteristic of control endometrium (left), compared with the glandless condition found in the UGKO endometrium (right). Tissues were obtained during dioestrus. LE: luminal epithelium; GE: glandular epithelium; S: endometrial stroma; M: myometrium. Scale bar represents 33 μm.

Fig. 5. Effects of chronic neonatal exposure of ewe lambs to 19-norprogestin (P; 19-norpreg-4-ene-3,20 dione) from birth on: (a) postnatal histogenesis of the endometrium (Car: caruncle; LE: luminal epithelium; GE: glandular epithelium); and (b) patterns of uterine protein synthesis *in vitro* in neonatal ewes ovariectomized at birth. (a) Endometrial glands are absent at birth (D0), but present throughout the intercaruncular endometrium by postnatal day (PND) 13 (D13). When ewe lambs are exposed to progestin from birth to PND 13, endometrial adenogenesis is inhibited and uterine glands are absent on PND 13 (D13P). Withdrawal of the progestin block on PND 13 permits some gland development as observed on PND 26 (D26P), although newly formed endometrial glands are structurally abnormal (D26P versus D13). (b) Uterine tissues of the type illustrated in (a) were explanted under defined conditions in the presence of L-4,5-[^{3}H]leucine and labelled proteins in explant medium were identified by fluorography of dried two-dimensional PAGE gels. Labelled uterine products were separated by isoelectric focussing in the first dimension (pH) and SDS-PAGE in the second ($M_r \times 10^{-3}$). Changes in patterns of uterine protein synthesis associated with uterine development between birth (D0) and PND 13 (D13) are illustrated by the top fluorographs. Some proteins produced by normally glandless uterine tissues from postnatal day 0 are no longer produced, or produced in the same relative abundance by tissues from postnatal day 13, in which endometrial glands are normally present (D0 versus D13, arrows). Chronic exposure of ewe lambs to progestin from birth inhibits gland genesis, restores production of some uterine proteins and induces production of others (D0 versus D13P, arrows and brackets). Tissues obtained on PND 26 after withdrawal of the progestin block to adenogenesis on PND 13 (D26P) displayed suppressed production of some proteins normally associated with uterine gland development (arrows), but relatively stable production of other proteins induced by progestin exposure (brackets). Scale bar represents 200 mm.

Fig. 7. Progestin-induced inhibition of uterine gland genesis alters transcriptional activity in adult ovine endometrium. An autoradiograph of [^{32}P-α]ATP-labelled cDNAs generated by mRNA differential display PCR (DD-PCR) and separated by electrophoresis on a 4.5% acrylamide–urea sequencing gel is shown. Typical results from duplicate endometrial total RNA samples obtained from normal control (left lanes) and uterine gland 'knock-out' (UGKO; right lanes) endometrium are shown (see Fig. 6). Evidence of differential gene expression is illustrated by the presence of bands in control lanes (arrows) and the absence of these bands in UGKO lanes.

Fig. 8. Expression of endometrial DD54 mRNA. (a) Representative dark-field photomicrographs of DD54 mRNA expression in endometrium obtained from cyclic and pregnant ewes detected by *in situ* hybridization analysis. The cDNA corresponding to DD54 was identified by mRNA DD-PCR as in Fig. 7. Cross-sections of uteri from cyclic and pregnant ewes were hybridized with [^{35}S]UTP-labelled sense and antisense DD54 cRNA probes and hybridization signals visualized by autoradiography. Tissues shown are from a cyclic ewe on day 1 after oestrus (D1C), and a pregnant ewe on day 19 (D19Px). Note intense hybridization signal in luminal epithelium (LE) and glandular epithelium (GE). No signal above background was detected with the labelled sense probe (D1C Sense). (b) Expression of endometrial DD54 mRNA detected by northern hybridization analysis. Two DD54 mRNA transcripts of approximately 2 kb and 6 kb (arrows) were identified in endometrial total RNA (20 mg) from cyclic (days 1, 5, 9 and 15 after oestrus) and pregnant ewes (Px; days 15 and 19) with a radiolabelled antisense cRNA probe generated from DD54 cDNA template. Expression of this epithelial gene product increased during dioestrus. Scale bar represents 40 μm.

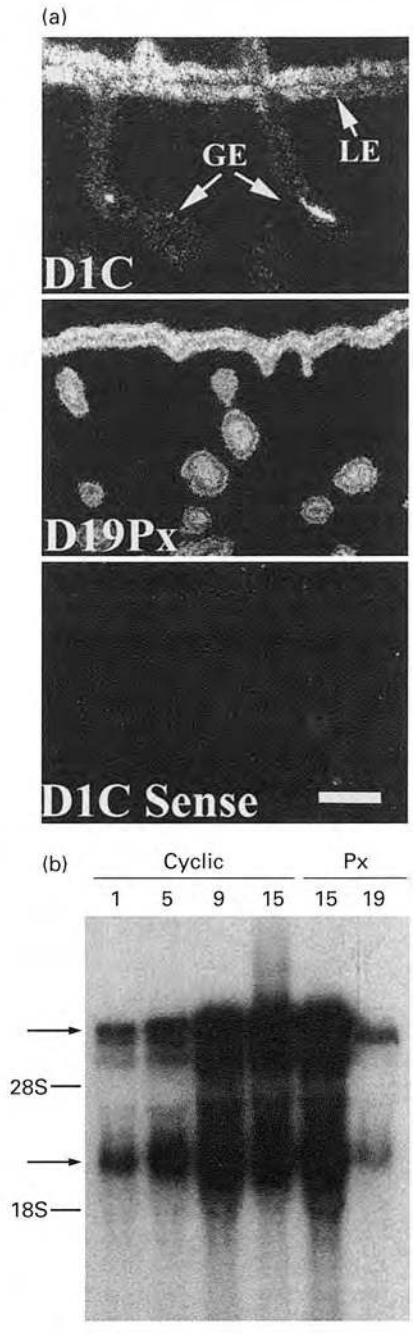

Fig. 8. For legend see facing page.

Conclusions

The extent to which embryo mortality (Martal *et al*, 1997) or uteroplacental dysfunctions associated with fetal growth retardation in domestic ruminants are attributable to uterine lesions induced by disruption of development is unknown. However, the fact that such lesions can be induced indicates that reproductive performance could be affected in this way. Since, in both cattle and sheep, postnatal uterine organizational events are steroid sensitive, mechanisms regulating these events are likely to involve steroid receptors and their co-activators and co-repressors (Hirotaka *et al.*, 1997). How the ideal uterine organizational programme evolves, and the extent to which disruption of organizationally critical cell–cell and cell–matrix interactions may affect the fate of uterine tissues and cells are topics that warrant investigation if factors affecting uterine capacity to support reproduction in domestic ruminants are to be defined.

Studies in cows and ewes indicate that normal and aberrant programmes of uterine organization, and consequences of organizational disruption, can be delineated by comparing patterns of uterine development and function in neonatally steroid exposed and unexposed animals. Physiological, biochemical and molecular comparisons of normal and lesioned adult uterine tissues should enable identification of factors affecting uterine function, developmental determinants of uterine integrity, biological markers of exposure to steroidal disruptors of uterine development, and rapid identification of novel uterine genes. Such studies will facilitate the design of environments and refinement of management guidelines to ensure that genetic potential for reproductive performance is realised, and will aid in efforts to evaluate the potential reproductive impact of environmental exposure to endocrine disruptors during development.

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