

Formation and early development of the corpus luteum in pigs

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Numerous corpora lutea form from the multiple follicles that ovulate during the oestrous cycle of pigs. Vascular elements invade the follicle from the theca compartment, first centripetally, and subsequently by lateral branching of centripetal veins and arteries. The vessels are the vehicle for dispersion of steroidogenic theca cells throughout the corpus luteum. Mitosis occurs in both the theca and granulosa layers before ovulation, and in luteal cells well into the luteal phase. Luteal cell proliferation undergoes gradual restriction as the corpus luteum matures, but the mechanisms of exit from the cell cycle are unknown. The extracellular ligands that direct luteinization and maintain the corpus luteum include LH, prolactin, insulin and insulin-like growth factors (IGFs). These ligands induce qualitative and quantitative changes in steroid output, with progesterone as the principal product. These changes upregulate the cholesterol synthetic pathways to increase substrate availability. The intracellular regulation of luteinization is complex. A model is presented in which LH stimulates arachidonic and linoleic acid metabolism to produce ligands for the nuclear proteins of the peripheral peroxisome activator receptor family. These ligands have downstream effects on cell differentiation and exit from the cell cycle. Luteal function is maintained by interactions among ligands, cholesterol regulatory proteins and constitutively expressed and regulated transcription factors.

Introduction

The success of gestation in the pig is entwined inextricably with the establishment and maintenance of the corpus luteum. This complex transitory organ develops from follicles as a consequence of ovulation and secretes the progesterone necessary for creating a uterine environment hospitable for survival of embryos and fetuses. Pigs differ from ruminants because the corpus luteum is required for the entire 16 weeks of gestation. Its importance is underlined by the fact that its principal synthetic product, progesterone, can, by itself, maintain gestation in ovariectomized gilts. The focus of this review is the process of luteinization and its aim is to identify the principal unanswered questions about luteal differentiation in pigs. Where possible, answers to these questions are proposed.

Corner (1919) described both the theca and granulosa precursors of luteal cells and the

histomorphological changes that follow ovulation and result in redistribution of theca cells within the corpus luteum. Both the final destinations of theca cells and the process of luteal angiogenesis, their proposed vehicle of dispersion, are not understood completely. Luteinization is believed to represent terminal differentiation of the granulosa component of the follicle, but little is known about the mechanisms for exit of luteal cells from the cell cycle in any species, and its timing in pigs has not been addressed. Furthermore, the mechanisms effecting the logarithmic increase in steroid output that occurs within a few days after the commencement of luteinization are only beginning to be recognized.

Formation of the pig corpus luteum

Preovulatory follicle development in the pig has been investigated extensively (for review see Cox, 1997). A well-documented finding is heterogeneity of the developmental process (Grant *et al.*, 1989), resulting in asynchrony in ovulation and consequent corpus luteum formation (Pope *et al.*, 1990). Ovulation and luteinization have been studied less. The evolution of a pig follicle to a young corpus luteum is shown (Fig. 1). The period from the onset of the LH surge to first ovulation was estimated as a mean of 44 h (Soede *et al.*, 1994) and in equine chorionic gonadotrophin (eCG)-hCG-treated gilts, the mean interval ranges from 34 to 48 h (Hunter, 1972). During the period between the ovulatory stimulus and expulsion of the ovum, the follicle undergoes changes, including hyperaemia and theca cell hypertrophy. There is rearrangement of the compact layers of granulosa cells to looser associations, characterized by reduced intercellular contact (Fig. 2a,b). Vascular remodelling begins before ovulation and incipient invagination occurs at the sites of major vein and artery complexes (Fig. 1). In samples taken approximately 24 h after ovulation, vascular and associated thecal tissues have not yet breached the follicle wall, but the regions of invagination are still substantially deeper and wider than in preovulatory follicles (Corner, 1919). Soon thereafter, there is evidence of focal decomposition of the follicle wall (Corner, 1919) and invasion of vascular elements into the granulosa compartment. The vascular components spearhead the invasion at the apices of the invaginations and bear theca cells to the lumen of the follicle (Fig. 1). Lateral vascularization throughout the former granulosa compartment occurs, apparently concomitant with the centripetal growth of the arteries and veins.

Corner (1919) described the invasion of the follicle compartment by the vascular elements and theca. He reported the dispersion of thecal cells throughout the luteal parenchyma, where they become disseminated singly or in groups of two or three among the granulosa descendants. Furthermore, Corner (1919) noted that many thecal cells remain near the periphery of the follicle or associate with the vessels that have invaded. His identification was based on the morphological characteristics of these cells and their responses to fixatives. Although the contribution of theca cells to the corpus luteum is not disputed, their ultimate fate and distribution have not been confirmed. It is assumed, but has not been shown conclusively, that theca cells become the small luteal cells that can be separated from their larger counterparts, presumably granulosa cell descendants, on density gradients (Pitzel *et al.*, 1990). There are no definitive markers that can be recognized after the differentiation of the theca precursors into theca-luteal cells. Understanding the fate of theca-luteal cells will allow better understanding of the important processes in luteal formation, the persistence of cell division, angiogenesis and migration of theca cells.

Ligand induction and maintenance of luteinization

In pigs, luteinization and maintenance of the corpus luteum depends on members of at least three protein families: the gonadotrophins, the cytokine-prolactin family and the family of

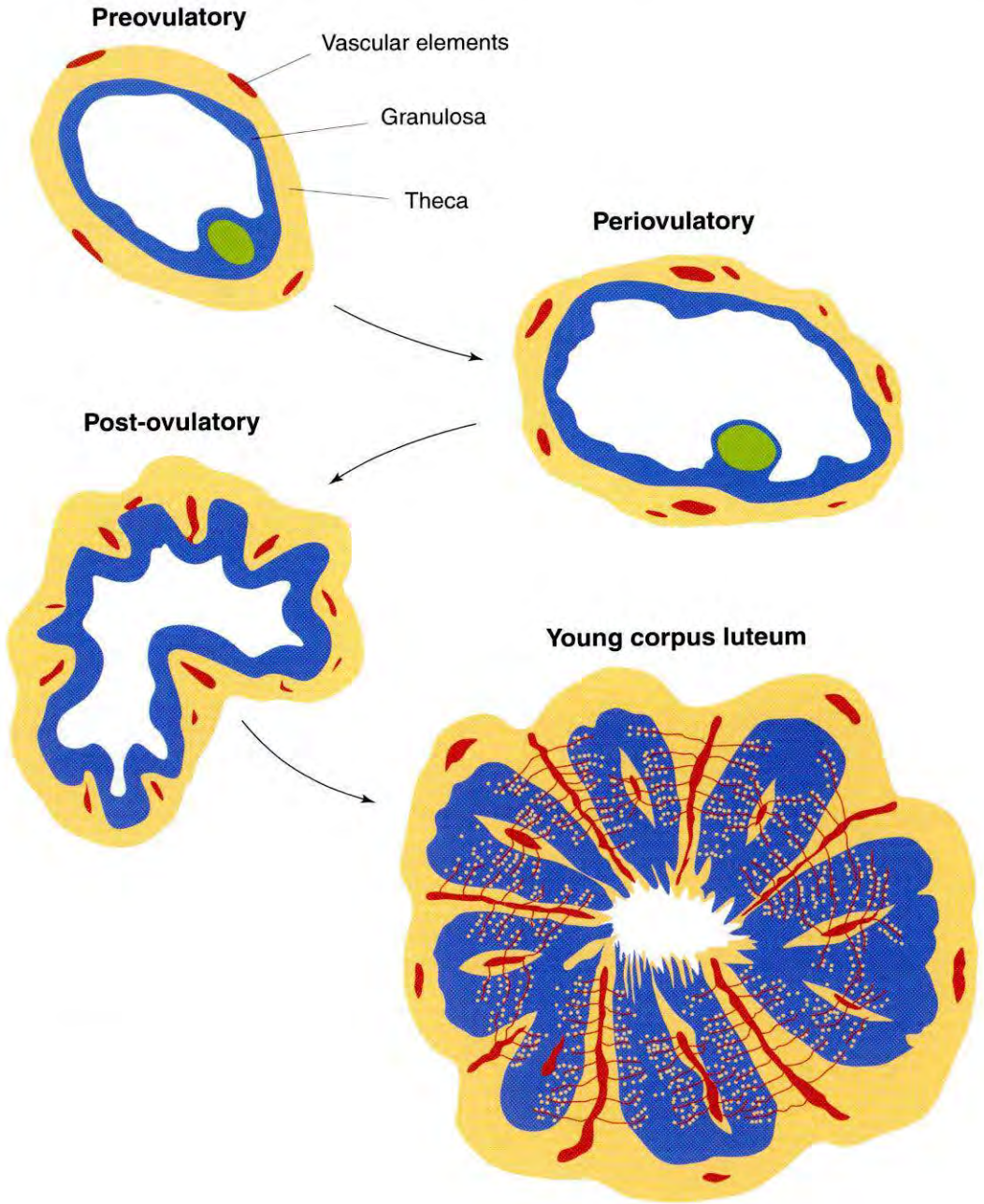


Fig. 1. Development of a pig corpus luteum from a preovulatory follicle. The preovulatory follicle undergoes hyperaemia and enlargement (periovulatory stage) before expulsion of the ovum (post-ovulatory stage) and formation of the young corpus luteum. The granulosa compartment is depicted in blue, the theca and its descendants in yellow, and the vascular elements in red. Incipient intrusion of the vascular elements occurs during the periovulatory period, but the theca-granulosa barrier is not breached until after ovulation. The vascular elements invade the corpus luteum in a centripetal direction, as well as laterally into the luteal parenchyma. Blood vessels are believed to carry theca cells into the luteal parenchyma and to disperse them throughout the corpus luteum.

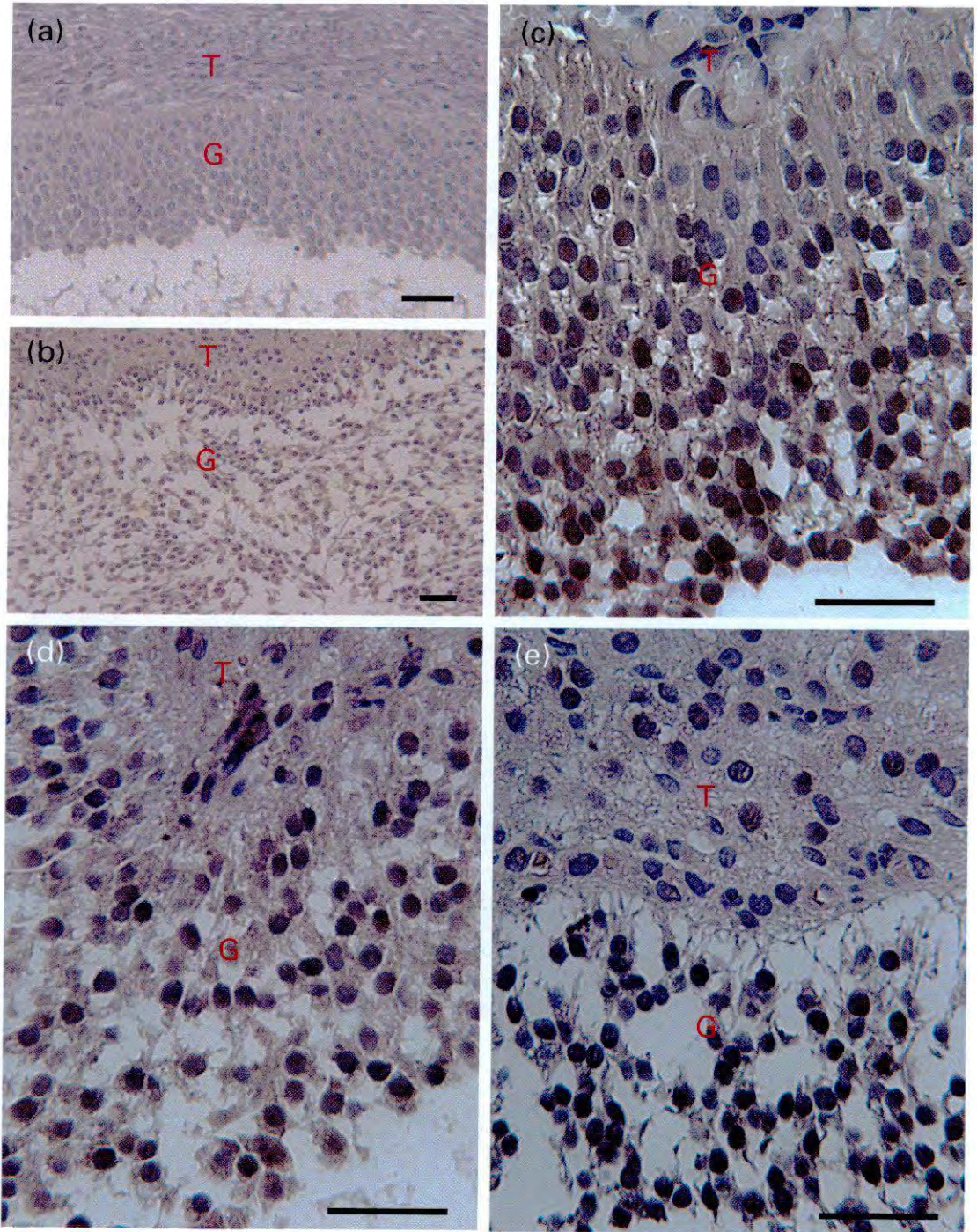


Fig. 2. Photomicrographs of the periovarian period in follicles from gilts treated with hCG to induce ovulation. Expression of proliferating cell nuclear antigen (PCNA), a marker for cell division, was identified by immunohistochemistry. In all panels cell nuclei with the characteristic brown diaminobenzidine stain are positive for PCNA. (a) Cross-section through a large antral (7 mm in diameter) pig follicle subjected to immunolocalization procedures but not exposed to the anti-PCNA antibody. (b) Theca-granulosa interface at

insulin and insulin-like growth factors (IGFs). The proximal stimulus for ovulation is the preovulatory release of LH from the pituitary, as in other mammals. In most of the species studied, including pigs (Coté *et al.*, 2001), gonadotrophins provoke expression of an inducible form of the cyclo-oxygenase enzyme (COX-2) by the granulosa cells that catalyses the first rate-limiting step in conversion of arachidonic acid to prostaglandins (Sirois and Richards, 1992). The sequence of COX-2 expression after ovulation has not been investigated in pigs, but it is clearly present and inducible in the corpus luteum at days 9 and 17 of the oestrous cycle (Diaz *et al.*, 2000). A second catalytic route for arachidonic acid is via the lipoxygenase enzymes, resulting in hydroxyeicosatetranoic acids (HETEs). Pharmacological interruption of this pathway reduces the rate of ovulation in pigs (Downey *et al.*, 1998).

The LH surge is the stimulus for initiation of other events in luteinization, particularly the qualitative and quantitative changes in steroidogenesis. Studies of hypophysectomy in pigs have shown that the pituitary is necessary for luteal support throughout gestation, that LH is essential in the early corpus luteum and that prolactin plays an important role later in pregnancy (Li *et al.*, 1989). Both LH and prolactin have luteotrophic effects on pig granulosa cells *in vitro* (Chedrese *et al.*, 1988).

Luteinization of pig granulosa cells *in vitro* occurs only when serum is present in the medium (Picton *et al.*, 1999). Serum-borne elements, particularly insulin and IGFs, contribute to luteinization and support of the corpus luteum during the luteal phase. Plasma membranes of pig luteal cells have receptors for IGF-I, and IGF-I binding proteins are expressed differentially in the pig corpus luteum throughout the luteal phase (Wandji *et al.*, 2000a). Insulin and IGFs are essential for the steroidogenic changes that characterize luteal formation *in vitro* (Pescador *et al.*, 1999; Sekar *et al.*, 2000). Most of the downstream genes that are modulated by these ligands in the corpus luteum have not yet been identified.

Angiogenesis in the pig corpus luteum

The source of the vasculature that perfuses and maintains the pig corpus luteum is the veins and arteries that invade after ovulation. Histological evidence indicates that the major vessels grow rapidly in a centripetal direction, arriving at the antrum of the former follicle (Fig. 1). Lateral development from the invading vascular components also occurs rapidly (Corner, 1919). The morphology and regulation of angiogenesis in the corpus luteum are the subject of at least five recent reviews (for example see Fraser and Lunn, 2001). Although none of these reviews considers pigs, the principles are expected to apply to this species. The rate of neovascularization in the corpus luteum appears to be higher than for any known tissue, including human tumours. A current view of regulation suggests that LH precipitates the angiogenic cascade (Fraser and Lunn, 2001). However, the principal stimulus inducing neovascularization is hypoxia, and a case has been made for hypoxia as an angiogenic stimulus in corpora lutea (Reynolds *et al.*, 2000). Results in other species implicate acidic and basic fibroblast growth factors (a and bFGFs), particularly bFGFs, and vascular endothelial growth factor (VEGF) as the key mediators of luteal angiogenesis (Reynolds *et al.*, 2000). The source of the FGFs is presumed to be the luteinized granulosa cells (Reynolds *et al.*, 2000) and this is consistent with the pattern of occurrence of FGF mRNA in pig follicles and corpora lutea (Guthridge *et al.*, 1992). VEGF is thought to stimulate proliferation of endothelial cells

Fig. 2. continued

38 h after hCG treatment, showing the dispersion of the granulosa layers at approximately 3 h before expected ovulation. (c) Cross-section of an 8 mm follicle taken from an untreated gilt and (d) 24 h after hCG and (e) 38 h after hCG administration. T: theca compartment. G: granulosa compartment. Scale bars represent 25 μm .

(Fraser and Lunn, 2001) and migration of endothelial cells into the granulosa parenchyma (Reynolds *et al.*, 2000). Its source may be theca-derived pericytes (Reynolds *et al.*, 2000) or granulosa–luteal cells (Fraser and Lunn, 2001). VEGF is found in pig follicular fluid and is produced by pig follicles during gonadotrophin-stimulated development (Barboni *et al.*, 2000). Both the theca and granulosa compartments contribute, but the latter predominates in overall VEGF synthesis (Barboni *et al.*, 2000). LH greatly reduces expression of VEGF by pig granulosa cells, both *in vivo* and *in vitro* (Barboni *et al.*, 2000). These findings contrast with the current view that LH drives angiogenesis via induction of VEGF and its downstream targets in granulosa cells (Fraser and Lunn, 2001). Whether this anomaly reflects differences in experimental design or fundamental differences among pig, ruminant and primate angiogenesis deserves further investigation. Connective tissue growth factor (CTGF) and a tumour inhibitor, MAC-25, have recently emerged as potential modulators of angiogenesis in pig corpora lutea (Wandji *et al.*, 2000b). Although there are some intriguing new findings, the nature of angiogenic stimuli and processes in formation of pig corpora lutea remain unknown.

Luteinization and exit from the cell cycle

Luteinization is the final phase in differentiation of theca and granulosa cells, a process that begins with formation of the primordial follicle before birth. There is much evidence that pig follicular growth results from hyperplasia of granulosa cells (Morbeck *et al.*, 1992). After ovulation, the prevailing view, derived from other species, is that luteinization results in exit of granulosa cells from the cell cycle. Reprogramming from the proliferative to the differentiated state in rat granulosa cells is complete within 5–7 h after the LH surge (Richards, 2001a). Although functional changes in mammalian theca cells have been described, little is known about their proliferation during luteinization *in vivo*. In pigs, both theca (Englehardt *et al.*, 1991) and granulosa cells (Pescador *et al.*, 1999; Picton *et al.*, 1999) proliferate and undergo luteinization when cultured with serum. This luteinization, defined as the loss of cytochrome P450aromatase expression and synthesis of large amounts of progesterone as the principal steroid product, can be avoided by culture in serum-free, insulin-supplemented medium (Picton *et al.*, 1999). Granulosa cells incubated under the latter conditions continue to proliferate, albeit somewhat less robustly than in the presence of serum.

Much of the growth of the pig corpus luteum is the result of cellular hypertrophy (Corner, 1919; Ricke *et al.*, 1999) and little information is available on the contribution of cell proliferation. Corner (1919) confirmed that cell division was occurring by observation of the mitotic spindle in theca cells of the periovulatory follicle and in follicles undergoing luteinization. We recently examined cell proliferation by immunohistochemistry using a mitotic marker, proliferating cell nuclear antigen (PCNA), in gilts treated with hCG to induce ovulation (B. D. Murphy, F. Coté, J. Sirois and B. R. Downey, unpublished). Late preovulatory follicles (6–8 mm in diameter) displayed the mitotic signal in many cells of the theca. The granulosa cell compartment consists of compact layers of cells, all of which express PCNA strongly (Fig. 2c). At 24 h after hCG administration, the theca cells are hypertrophied and display numerous PCNA positive cells (Fig. 2d). The granulosa layer is expanded; there is loss of the compactness of the cell layers and evidence of mitosis throughout (Fig. 2d). Ovaries removed at 30 and 34 h after hCG administration display greater dissociation of the granulosa cell layers, while retaining the same pattern of mitosis in the theca and the granulosa compartments (data not shown). At 38 h after hCG administration, a few hours before expected ovulation, the rate of mitosis has increased in the theca layers (Fig. 2e). The granulosa cells are nearly dissociated (Fig. 2b); however, the PCNA signal is present, particularly in the most interior layers of cells (Fig. 2e).

Ricke *et al.* (1999) demonstrated extensive mitosis in the corpus luteum during the first 4 days after ovulation. About 25–40% of the mitotic cells are derived from the theca or granulosa, whereas the other mitotic cells represent proliferation of vascular elements (Ricke *et al.*, 1999). Co-localization of mitotic and steroidogenic enzyme signals indicates that luteal steroidogenesis and cell division occur in proliferating cells, particularly early in luteal development. Over the lifespan of the corpus luteum, the labelling index decreases (Ricke *et al.*, 1999) and the mitotic signal is observed rarely in large luteal cells. Thus, it appears that terminal differentiation occurs in the pig corpus luteum. Nonetheless, it is equally apparent that the rat model of rapid and irreversible exit from the cell cycle soon after the ovulatory stimulus (Richards *et al.*, 1998) does not apply to pigs. A more appropriate model includes continued mitosis of the steroidogenic cells well into the luteal phase, with increasing restriction in the capacity to proliferate.

The mechanisms of terminal differentiation in the corpus luteum have begun to be studied, mostly in rodents (Richards *et al.*, 1998; Richards, 2001a,b). The initiation of mitotic division is under the control of members of the retinoblastoma protein family, retinoblastoma protein (pRb), p107 and p130 (Classen and Dyson, 2001). In the hyperphosphorylated state, these proteins permit cell division by liberating the transcription factors of the E2F family, which then upregulate the genes responsible for progression of the cell cycle. Hypophosphorylation of these proteins results in mitotic quiescence by the opposite mechanism, the sequestering of E2Fs (Morgan, 1995). In luteinization, a general pattern emerges in which pRb and p130 are dephosphorylated, thereby impairing the expression and activity of positive regulators of the cell cycle, cyclins D1, D2 and E (Green *et al.*, 2000; Hampl *et al.*, 2000). A second level of regulation is recognized, on the basis of a phenotype of luteal disruption in mice bearing the null mutation for p27^{Kip1} (Kikokawa *et al.*, 1996), a member of one of the two families of inhibitors of the cyclin-dependent kinases (Zhu and Skoultchi, 2001). In these mice, differentiation of luteal cells appears to occur, as indicated by cytochrome P450_{side chain cleavage} (P450_{scc}) expression, and cyclin D2 activity is repressed; however, active DNA synthesis persists (Tong *et al.*, 1998). Further evidence for a role for these repressors of cyclin-dependent kinases (CDKs) comes from a study which showed that expression of p27^{Kip1} and p21^{Cip1} is increased in granulosa cells of hypophysectomized rats treated with ovulatory doses of hCG (Robker and Richards, 1998). The expression of p27 proteins remains high throughout the lifespan of the corpus luteum in pregnant mice (Hampl *et al.*, 2000). CDK4, expressed in mouse granulosa cells and in the mouse corpus luteum of gestation, complexes with p27 and cyclin D3 (Hampl *et al.*, 2000). A different disruption in the luteal phenotype emerged after null mutation of CDK4, which enhanced expression of p27^{Kip1} (Tsutsui *et al.*, 1999). It took the form of entrapment of oocytes and aberrant luteal organisation. Cell cycle regulators can participate in both cell proliferation and differentiation, and CDKs regulate transcription of genes that result in differentiation in some tissues (Zhu and Skoultchi, 2001), which could explain the phenotype of the CDK4 knockout mice.

Although progress has been made, our knowledge remains rudimentary. There is no specific information on exit from the cell cycle for pigs, and there is no information on the cell cycle dynamics of theca cells in development of the corpus luteum.

Differentiation of theca and granulosa cells into their luteal counterparts

Granulosa and theca cells undergo functional differentiation during luteinization. In most of the species studied, the theca of the follicle responds to LH stimulation by synthesis of androgens. These diffuse across the basement membrane to the granulosa compartment where they are aromatized to oestrogens, under the influence of FSH. The latter portion of the

scheme occurs in pigs, as 3β -hydroxysteroid dehydrogenase (3β -HSD), which converts pregnenolone to progesterone, is found in the theca of the growing follicle only (Driancourt *et al.*, 1998). However, pig follicles deviate from the general pattern because the theca cells express aromatase and produce oestrogens that complement the production of granulosa cells (Tsang *et al.*, 1985). In many species, oestrogen concentrations are low or absent in the corpus luteum. In contrast, P450aromatase expression persists in pig corpora lutea (Meduri *et al.*, 1996) and large and small cells of pig corpora lutea maintain the capability to secrete oestrogen (Lemon and Loir, 1977). However, luteinization in pigs engenders alteration of the pattern of steroid synthesis in favour of progesterone, particularly the granulosa cells (Lavoie *et al.*, 1997), as indicated by the early acquisition of the expression of P450scc, which converts cholesterol to pregnenolone, and of 3β -HSD (Meduri *et al.*, 1996). This change is accompanied by a large increase in total steroid output, which requires an extensive increase in the supply of cholesterol, the substrate for steroid synthesis. In pigs, luteinization is characterized by coordinated upregulation of cholesterol synthesis, importation and intracellular trafficking genes, including the low density lipoprotein receptor and sterol carrier protein 2 (Lavoie *et al.*, 1997), steroidogenic acute regulatory protein (StAR), P450scc (Lavoie *et al.*, 1997; Pescador *et al.*, 1997, 1999) and Niemann-Pick C1 protein (Song *et al.*, 1998). Expression of StAR is a key event in granulosa cell remodelling (Pescador *et al.*, 1997, 1999); thus, factors that control this transition are believed to be essential regulators of luteinization.

The pattern of expression of gonadotrophin receptors changes as a pig follicle becomes a corpus luteum. The FSH receptors present on granulosa cells of the follicle are lost during luteinization (Liu *et al.*, 1998). Binding analysis of LH receptors indicated an initial downregulation, relative to granulosa cells from periovulatory follicles, followed by recovery in the number of receptors as the luteal phase progresses (Gebarowska *et al.*, 1997). Autoradiographic studies indicate that hCG binding in newly formed corpora lutea is mainly peripheral and is more widespread in mid-cycle corpora lutea (Gebarowska *et al.*, 1997). Immunohistochemical localization of the LH receptor supports this interpretation of the early stage: downregulation after ovulation and restriction of the signal to the periphery and to the presumed theca-derived component of the corpus luteum (Meduri *et al.*, 1996). However, the analyses diverge at this point, as immunolocalization using a monoclonal antibody indicates that, although the LH receptor signal increases at mid-cycle, it is present at the spokes of vascular invasion only, interpreted as restriction to the theca descendants (Meduri *et al.*, 1996). In contrast, *in situ* analysis indicates that both large and small cells throughout the pig corpus luteum contain LH receptor mRNA (Yuan and Lucy, 1996). Furthermore, LH receptor mRNA and protein were highly abundant, as demonstrated by RT-PCR and immunolocalization in large and small cell populations isolated from pig corpus luteum (Kaminski *et al.*, 2000). Data from the corpus luteum and the large number of studies demonstrating luteinized granulosa cell responses to LH (Murphy and Silavin, 1989) provide evidence for the persistence of the LH receptor on both theca and granulosa descendants in pig corpora lutea.

Transcriptional regulation of luteinization

The subject of transduction of signals and signalling pathways involved in luteinization has been reviewed recently (Murphy, 2000). Hence, in the present review, greater emphasis is placed on gene transcription and its role in the intricacies of corpus luteum formation. Transcription is not the sole regulator of gene expression, but it is clearly the most significant. It is a complex process that includes chromatin modification, coactivator recruitment, and synthesis and activation of transcription factors. Frequently, two or more transcription factors interact to produce differential

expression of a gene. As noted above, luteal formation involves changes in the expression of numerous known, and presumably many more unknown, gene products. It is not within the scope of this review to consider all permutations; thus, we will focus on factors known to act on genes related to steroidogenesis and to provision of steroid substrate. Where pertinent, factors involved in differentiation and exit from the cell cycle are also discussed.

The classic linear route for transduction of gonadotrophin signals is via cAMP activation of protein kinase A and subsequent phosphorylation of cAMP response binding protein (CREB; Richards, 2001b). CREB exists in three isoforms and is considered to be synthesized constitutively (Zelevnik and Somers, 1999). Phosphorylated CREB transactivates gonadotrophin-regulated steroidogenic genes including P450_{scc} (Watanabe *et al.*, 1994) and P450_{aromatase} (Michael *et al.*, 1997). StAR transcription, which is dependent on cAMP (Stocco, 2001), is modulated by CREB phosphorylation (D. M. Stocco, personal communication). Similarly, transcription of pig Niemann–Pick C protein 1 (NPC-1) is CREB-dependent (N. Gévry and B. D. Murphy, unpublished). Little is known about CREB expression in pigs, other than that its coding sequence is found in the pig genome (R. L. Mattari and J. A. Carroll; GenBank Accession U95009). Notwithstanding, in primates, CREB expression is abolished in granulosa cells during luteinization (Somers *et al.*, 1995). These observations are consistent with a model in which LH acts through cAMP to initiate luteinization of granulosa cells, which then become refractory to this messenger (Zelevnik and Somers, 1999). This view is not compatible with observations that LH and cAMP can stimulate progesterone from large and small pig luteal cells *in vitro* (Hunter, 1981) and that exogenous LH increases circulating progesterone concentrations from the pig corpus luteum (Watson and Maule Walker, 1978).

Among the transcription factors implicated clearly in luteinization is the CCAAT/enhancer binding protein β (C/EBP- β). C/EBP- β expression is induced rapidly in rat granulosa cells in response to an ovulatory dose of hCG (Sirois and Richards, 1993), and granulosa cells in the C/EBP- β knockout mice do not luteinize (Sterneck *et al.*, 1997). Furthermore, antisense inhibition of C/EBP- β expression interferes with ovulation and luteinization in rats, with no apparent reduction in COX-2 expression (Pall *et al.*, 1997). C/EBP- β is a transactivator of StAR transcription (Reinhart *et al.*, 1999). C/EBP- β is present in pigs and its expression changes during adipocyte differentiation *in vitro* (Yu and Hausman, 1998). Therefore, it is likely that C/EBP- β expression will prove to be a vital controlling factor in luteinization in pigs.

The six transcription factors (GATA 1–6) of the GATA family are proteins with two zinc fingers that bind to the consensus promoter elements (A/T)GATA(A/G) (Molkentin, 2000). The GATA-1, -2 and -3 subfamily regulates haematopoiesis and lymphopoiesis primarily, whereas the subfamily comprising GATA-4, -5 and -6 is expressed in endoderm-derived tissues, including the cardiovascular and digestive systems (Molkentin, 2000). Modulation of the abundance of GATA transcription factors is associated with luteinization in mice and expression of one isoform, GATA-4, is reduced in the follicle after ovulation, whereas GATA-6 is expressed highly in both developing and mature corpora lutea (Heikinheimo *et al.*, 1997). In contrast, GATA-4 is expressed constitutively in rat granulosa cells and interacts with acutely regulated C/EBP- β in induction of StAR transcription (Silverman *et al.*, 1999). GATA-4 induces transcription of both StAR and P450_{aromatase} in mouse ovaries (Tremblay and Viger, 2001). Deletion and mutational analysis of the mouse StAR promoter demonstrated that the GATA-4 element interacts with other enhancers, including steroid factor 1 (SF-1) and AP-1, in translating the cAMP stimulus (Wooten-Kee and Clark, 2000). Both the pig StAR and NPC-1 (N. Gévry and B. D. Murphy, unpublished) promoters have GATA response elements within the first 200 bp upstream of the ATG transcription start site. Given the importance of StAR and NPC-1 for luteinization in pigs, it is likely that GATA-directed transcription contributes to the synthesis of progesterone in this species.

Other transcription factors essential to luteal steroidogenesis are the sterol regulatory element binding proteins (SREBPs). There are three known isoforms of this basic-helix-loop-helix-leucine zipper protein: SREBP-1a and -1c (also known as ADD-1) are the products of alternative splicing, whereas SREBP-2 arises from a second gene (Osborne, 2000). The SREBPs are present in cells as integral membrane proteins embedded in the endoplasmic reticular or nuclear membranes (Brown and Goldstein, 1999). In the event of intracellular sterol depletion, a second element, sterol cleavage-activating protein, cleaves the N-terminal SREBP fragment, which then relocates to the nucleus and interacts with decanucleotide segments of the gene promoters (Brown and Goldstein, 1999). Transcription of genes associated with increased intracellular cholesterol is induced, including the cascade for *de novo* cholesterol synthesis and those related to low density lipoprotein (LDL) receptor-mediated cholesterol importation. Given the increases in cholesterol required to accomplish luteal synthesis of progesterone, it is not surprising that SREBP isoforms are important regulators of *de novo* synthesis and importation of cholesterol into the corpus luteum (Lopez and McLean, 1999; Shea-Eaton *et al.*, 2001). In addition, over-expression of transcriptionally active SREBP-1a, -1c and -2 increases transcription of StAR (Christenson *et al.*, 2001; Shea-Eaton *et al.*, 2001) and SREBP-1a synergizes with cAMP induction of StAR transcription (Z. T. Ruiz-Cortes and B. D. Murphy, unpublished). Nevertheless, basal StAR transcription in human granulosa lutein cells appears to be unaffected by sterol depletion or repletion and, thus, the physiological significance of SREBP in regulation of StAR is unclear (Christenson *et al.*, 2001). SREBPs appear to have the greatest effects on transcription in concert with other factors and may also integrate endocrine signals. Recent evidence indicates that cAMP may act via CREB to amplify SREBP-mediated transcription of HMG-CoA-reductase (Dooley *et al.*, 1999). Downstream signals in the mitogen-activated kinase pathway stimulate SREBP-induced transcription of the LDL promoter directly (Kotzka *et al.*, 2000). Induction of StAR transcription involves interaction of SREBP with Yin Yang 1 (YY1) (Christenson *et al.*, 2001) and the orphan nuclear receptor of the steroid-thyroid superfamily, SF-1 (Shea-Eaton *et al.*, 2001). Thus, the SREBPs are important in luteinization. They may act on cholesterol homeostasis genes in the corpus luteum and also on transcription of StAR, a key step in luteinization of granulosa cells.

SF-1 is implicated in luteinization and steroidogenesis (Wehrenberg *et al.*, 1997). It is essential for expression of StAR, key steroidogenic enzymes (Hanley *et al.*, 2000) and nonsteroidogenic proteins specific to bovine luteinization (Ivell *et al.*, 1999). In equine follicles, SF-1 expression decreases between the ovulatory stimulus and expulsion of the oocyte (Boerboom *et al.*, 2000). In rat granulosa cells, a reduction in SF-1 expression occurs concomitantly with the decrease in P450aromatase expression (Fitzpatrick *et al.*, 1997). SF-1 expression recovers during early development of the ovine corpus luteum and is expressed constitutively during the mid-luteal phase (Juengel *et al.*, 1998). Pig SF-1 has been identified and sequenced, and is present in the pig corpus luteum of pregnancy (Pilon *et al.*, 1998). Its pattern of expression during luteal formation has not been investigated.

The peroxisome proliferator-activated receptors (PPARs), another group of receptors of the steroid-thyroid superfamily, are potential effectors of luteinization. Three distinct isoforms, PPAR α , γ and δ (also known as PPAR β) are recognized. Each is encoded by a separate gene and has different patterns of tissue and developmental distribution (Chinetti *et al.*, 2000). PPARs require a ligand to be activated and after activation, they form heterodimers with the retinoic acid X receptor (RXR) and interact with specific PPAR response elements in the promoter regions of target genes (Chinetti *et al.*, 2000). Natural ligands include eicosanoides derived from arachidonic acid catalysed by COX-2 (for example 15-deoxy-D-12-14-prostaglandin J2 (PGJ2) and prostacyclin) and 15-hydroxyeicosatetranoic acid (15-HETE), derived from catalysis of lipo-oxygenase (Chinetti *et al.*, 2000). Linoleic acid metabolites,

including 9- and 12-hydroxydecadanoic acid (9- and 12-HODE), which are also catalysed by lipo-oxygenases, also serve as natural ligands (Chinetti *et al.*, 2000). The thiazolidinedione family of synthetic compounds, the members of which are effective in treatment of non-insulin dependent diabetes, are high affinity ligands for PPAR γ . Two splice variants of PPAR γ are recognized, and PPAR γ 2 is expressed highly in adipose tissue and involved in terminal differentiation of adipocytes (Hansen *et al.*, 1999). In adipose tissue, a PPAR γ -induced cell cycle inhibition occurs in tandem with an increase in intracellular concentrations of p27^{Kip1} (Motomura *et al.*, 2000; Wakino *et al.*, 2000), which, as noted above, is related to the mechanism of cell cycle exit during luteinization in mice and rats (Robker and Richards, 1998). PPAR δ has been implicated in adipocyte proliferation (Hansen *et al.*, 2000). All three PPARs are expressed in pigs (Grindflek *et al.*, 1998; Houseknecht *et al.*, 1998; Ding *et al.*, 2000). PPAR α transactivates pig HMG-CoA reductase (Ortiz *et al.*, 1999). PPAR γ mRNA is present in pig granulosa cells during luteinization *in vitro* (Z. T. Ruiz-Cortés and B. D. Murphy, unpublished). Furthermore, a role for PPAR γ in luteinization is apparent, on the basis of studies that demonstrate stage-specific expression in bovine luteal cells (Lohrke *et al.*, 1998; Viergutz *et al.*, 2000). A preliminary report indicates that PPAR γ is expressed in theca and granulosa cells of pig preovulatory follicles and appears to be related to luteal differentiation (Schoppee *et al.*, 2000). Mechanistic information comes from studies of bovine luteal cells (Lohrke *et al.*, 1998) and pig granulosa cells (Gasic *et al.*, 1998; Schoppee *et al.*, 2000) in which trogladizone (a thiazolidinedione) and PGJ2 cause dose-dependent increases in steroid synthesis. Trogladizone also interferes with 3 β -HSD expression in pig granulosa cells (Gasic *et al.*, 1998); it is not known whether this is mediated by PPAR γ . Trogladizone reduces P450aromatase expression and activity in human granulosa cells (Mu *et al.*, 2000) and PGJ2 interferes with the cell cycle in bovine luteal cells (Viergutz *et al.*, 2000), thereby further implicating this receptor in luteinization.

Expression of PPARs is induced by other transcription factors involved in luteinization. C/EBP α upregulates PPAR γ (Wu *et al.*, 1999), as do endogenous lipid ligands produced as a result of SREBP expression (Patel *et al.*, 2001). In similar experiments, over-expression of SREBP induced transcription of PPAR γ (Fajas *et al.*, 1999). PPARs may also influence corpus luteum formation and function by interactions with CREB (Mizukami and Taniguchi, 1997). Over-expression of SREBPs enhances transactivation of target genes by PPAR γ (Fajas *et al.*, 1999). Mice bearing the null mutation for PPAR α have disrupted expression of SREBP-regulated cholesterol and fatty acid metabolism genes (Patel *et al.*, 2001). Thus, the PPAR family of nuclear receptors is involved in the process of luteinization and understanding its impact awaits further experimentation.

Conclusion: models and mechanisms of formation of the pig corpus luteum

Several pieces in the 'puzzle of luteinization' are now in place, but not enough for the full picture to emerge. An attempt can be made, albeit rife with speculation, to model the changes and mechanisms of early luteal development from the theca and granulosa components of preovulatory pig follicles. The events that are known to occur in pig theca cells during luteinization *in vivo* after the LH surge, steroid synthesis, proliferation, hypertrophy and migration, are shown (Fig. 3a). LH and cAMP upregulate StAR and other steroidogenic enzymes in the theca (Zhang *et al.*, 2000), presumably working through the linear pathway via phosphorylation of CREB. StAR and steroidogenic enzymes require the orphan nuclear receptor SF-1 for their expression. Serum and growth factors are potent stimulators of theca cell mitosis (May *et al.*, 1992), acting via tyrosine kinases, protein kinase C and mitogen-activated kinase pathways. Theca cell hypertrophy is observed during luteal formation

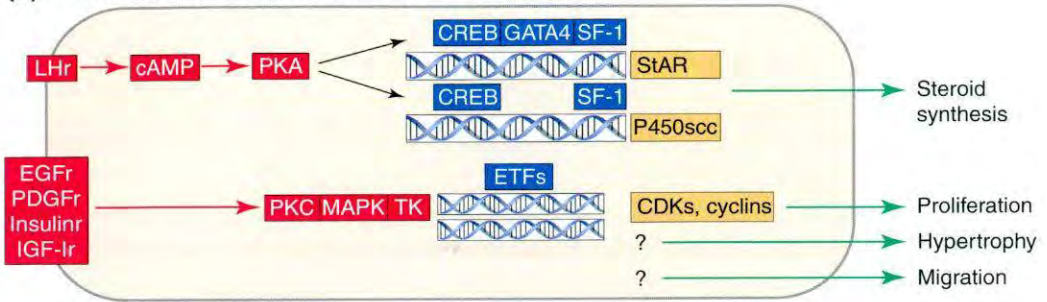
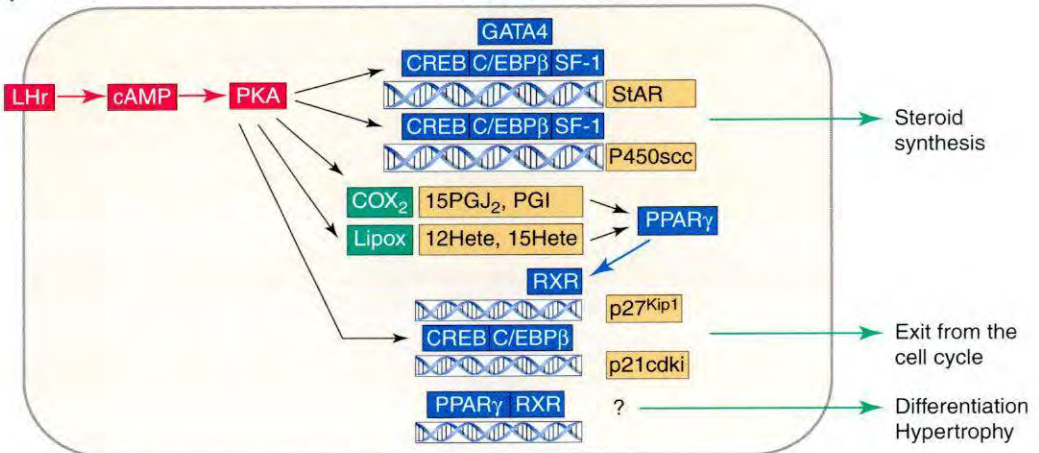
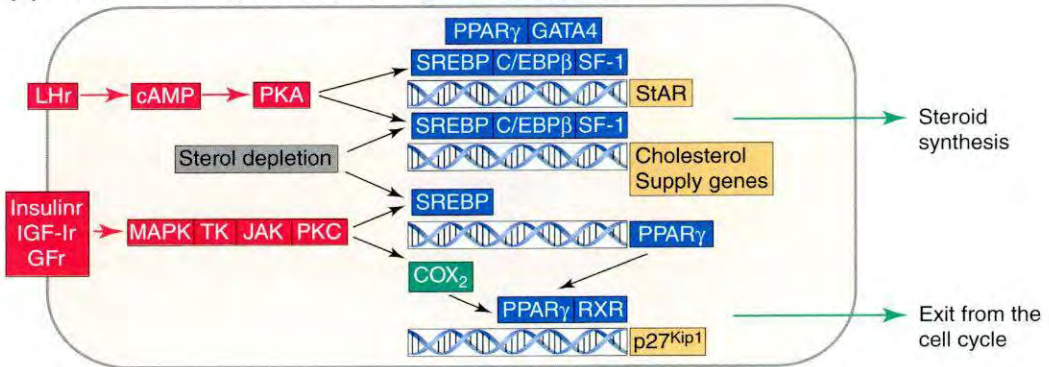
(a) Theca cells, 0–72 h after the ovulatory stimulus**(b)** Granulosa cells, 0–72 h after the ovulatory stimulus**(c)** Cells of the corpus luteum through the luteal phase

Fig. 3. Summary of the changes and regulation of luteinization in pigs. Elements in red are ligand pathways, blue are transcription factors and yellow are examples of regulated genes. (a) Theca cell between the preovulatory surge and approximately 24 h after ovulation. (b) Granulosa cell over the same period as in (a). (c) Generalized luteal cell during the early luteal phase. LHr: LH receptor; PKA: protein kinase A; CREB: cAMP response binding protein; GATA4: transcription factor GATA4; SF-1: steroid factor 1; StAR: steroidogenic acute regulatory protein; P450scc: cytochrome P450 side chain cleavage protein; EGFr: epidermal growth factor receptor; PDGFr: platelet-derived growth factor receptor; InsulInr: insulin receptor; IGF-Ir: insulin-like growth factor I receptor; PKC: protein kinase C; MAPK: mitogen-activated protein kinase; TK: tyrosine kinase; ETF: ETF transcription factor family; CDK: cyclin-dependent kinase; C/EBP β : CCAAT/enhancer binding protein β ; COX₂: cyclo-oxygenase enzyme; Lipox: lipo-oxygenase; PPAR γ : peroxisome proliferator-activated receptor γ ;

(Corner, 1919) and the migration of theca cells into the granulosa compartment is described in detail above. The factors controlling these profound and important changes are not currently known.

More is known about early events in granulosa cell differentiation, but a coherent synthesis eludes us. An attempt to reconcile available findings is shown (Fig. 3b). LH receptors appear at the antral follicle stage and respond to the ovulatory surge by expression of a number of genes, including COX-2. Given the role of lipoxygenase in ovulation (Downey *et al.*, 1998), its products may also be present during early luteinization. Both enzymes produce ligands for PPAR γ , which induces differentiation and exit from the cell cycle in adipocytes. Therefore, PPAR γ may serve multiple roles in granulosa cell luteinization: induction of differentiation, stimulation of steroidogenesis and initiation of exit from the cell cycle. Not represented in the scheme (Fig. 3b) are the roles of insulin and growth factors, which synergize with LH to convert granulosa cells to the luteal phenotype. In early luteinization in other species, CREB isoforms are present, and cAMP and CREB upregulate StAR expression and enzymes in the steroidogenic cascade. LH induces expression of C/EBP- β in preovulatory follicles, and this factor, together with GATA-4 and SF-1, enhances the transcription of StAR. The morphological evidence indicates that hypertrophy of granulosa cells is present as early as 24 h after ovulation or by 72 h after the ovulatory stimulus. The cellular events of hypertrophy and their mechanisms of control are not well understood.

The corpus luteum continues to evolve over its lifespan and, likewise, its regulation undergoes an evolution. Given the differential origin and variation in cell size of pig luteal cells, differing regulatory mechanisms must also exist. For simplicity, these have been generalized (Fig. 3c). The pituitary factors, LH and prolactin, are necessary for continued luteal steroidogenesis. If, as in other species, CREB is not expressed during luteinization, LH and cAMP must be acting through other mechanisms, perhaps independent of protein kinase A (Richards, 2001b). A role for SREBP is proposed via its classic effects on the genes that supply cholesterol, regulated by intracellular sterol concentrations. It may have a second role in transcriptional regulation of PPAR γ expression, and SREBP may be involved in expression of ligands that activate PPAR γ . As noted above, COX-2 expression is evident during the mid- and late luteal phases of the pig oestrous cycle (Diaz *et al.*, 2000) and may be a further source of ligands for PPAR γ . This receptor then has pleiotropic effects on the luteal cell, ensuring the completion of differentiation and exit from the cell cycle, and stimulating steroidogenesis directly. Growth factors and insulin participate in sterol homeostasis and in steroid synthesis via their effects on StAR and receptor-mediated importation of extracellular cholesterol.

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Fig. 3. continued

15PGI₂: 15-deoxy- Δ -12-14-prostaglandin I₂; PGI: prostaglandin I; 12HETE: 12-hydroxyeicosatetraenoic acid; 15HETE: 15-hydroxyeicosatetraenoic acid; RXR: retinoic acid X receptor; SREBP: sterol regulatory element binding protein; GFR: growth factor receptor; JAK: janus kinase.

References

- Barboni B, Turriani M, Galeati G, Spinaci M, Bacci ML, Forni M and Mattioli M (2000) Vascular endothelial growth factor production in growing pig antral follicles *Biology of Reproduction* **63** 858–864
- Boerboom D, Pilon N, Behdjani R, Silversides DW and Sirois J (2000) Expression and regulation of transcripts encoding two members of the NR5A nuclear receptor subfamily of orphan nuclear receptors, steroidogenic factor-1 and NR5A2, in equine ovarian cells during the ovulatory process *Endocrinology* **141** 4647–4656
- Brown MS and Goldstein JL (1999) A proteolytic pathway that controls the cholesterol content of membranes, cells, and blood *Proceedings National Academy of Sciences USA* **96** 11 041–11 048
- Chedrese PJ, Rajkumar K, Ly H and Murphy BD (1988) Dose response of luteinized porcine granulosa cells *in vitro* to prolactin: dependency on pre-exposure to human chorionic gonadotrophin *Canadian Journal of Physiology and Pharmacology* **66** 1337–1340
- Chinetti G, Fruchart JC and Staels B (2000) Peroxisome proliferator-activated receptors (PPARs): nuclear receptors at the crossroads between lipid metabolism and inflammation *Inflammation Research* **49** 497–505
- Christenson LK, Osborne TF, McAllister JM and Strauss JF, III (2001) Conditional response of human steroidogenic acute regulatory protein gene promoter of sterol regulatory element binding protein 1a *Endocrinology* **142** 28–36
- Classen M and Dyson N (2001) p107 and p130: versatile proteins with interesting pockets *Experimental Cell Research* **264** 135–147
- Corner GW (1919) On the origin of the corpus luteum of the sow from both granulosa and theca interna *American Journal of Anatomy* **27** 117–183
- Coté F, Sirois J, Doré M and Downey BR (2001) Induction of prostaglandin G/H synthase-2 in preovulatory follicles of equine chorionic gonadotropin/human chorionic gonadotropin treated prepubertal gilts *Biology of Reproduction* **63** (Supplement 1) 161 (Abstract)
- Cox NM (1997) Control of follicular development and ovulation rate in pigs *Journal of Reproduction and Fertility Supplement* **52** 31–46
- Diaz FJ, Crenshaw TD and Wiltbank MC (2000) Prostaglandin F2a induces distinct physiological responses in porcine corpora lutea after acquisition of luteolytic capacity *Biology of Reproduction* **63** 1504–1512
- Ding ST, Schinckel AP, Weber TE and Mersmann HJ (2000) Expression of porcine transcription factors and genes related to fatty acid metabolism in different tissues and genetic populations *Journal of Animal Science* **78** 2127–2134
- Dooley KA, Bennett MK and Osborne TF (1999) A critical role for cAMP response element-binding protein (CREB) as a co-activator in sterol-regulated transcription of 3-hydroxy-3-methylglutaryl coenzyme A synthase promoter *Journal of Biological Chemistry* **274** 5285–5291
- Downey BR, Mootoo JE and Doyle SE (1998) A role for lipoxygenase metabolites of arachidonic acid in porcine ovulation *Animal Reproduction Science* **49** 269–279
- Driancourt MA, Quesnel H, Meduri G, Prunier A and Hermier D (1998) Luteinization and proteolysis in ovarian follicles of Meishan and Large White gilts during the preovulatory period *Journal of Reproduction and Fertility* **114** 287–297
- du Mesnil du Buisson F and Dauzier L (1957) Influence d'ovariectomie chez la truie pendant la gestation *Compte Rendu Société de Biologie* **151** 311–313
- Ellicott AR and Dzuik PJ (1973) Minimum daily dose of progesterone and plasma concentration for maintenance of pregnancy in ovariectomized gilts *Biology of Reproduction* **9** 300–304
- Englehardt H, Gore-Langton RE and Armstrong DT (1991) Luteinization of porcine theca cells *in vitro*. *Molecular and Cellular Endocrinology* **75** 237–245
- Fajas L, Schoonjans K, Gelman L et al. (1999) Regulation of peroxisome proliferator-activated receptor gamma expression by adipocyte differentiation and determination factor 1/sterol regulatory element binding protein 1: implications for adipocyte differentiation and metabolism *Molecular and Cellular Biology* **19** 5495–5503
- Fitzpatrick SL, Carlone DL, Robker RL and Richards JS (1997) Expression of aromatase in the ovary: down-regulation of mRNA by the ovulatory luteinizing hormone surge *Steroids* **62** 197–206
- Fraser HM and Lunn SF (2001) Regulation and manipulation of angiogenesis in the primate corpus luteum *Reproduction* **121** 355–362
- Gasic S, Bodenbun Y, Nagamani M, Green A and Urban RJ (1998) Troglitazone inhibits progesterone production in porcine granulosa cells *Endocrinology* **139** 4962–4966
- Gebarowska D, Ziecik AJ and Gregoraszczyk EL (1997) Luteinizing hormone receptors on granulosa cells from preovulatory follicles and luteal cells throughout the oestrous cycle of pigs *Animal Reproduction Science* **49** 191–205
- Grant SA, Hunter MG and Foxcroft GR (1989) Morphological and biochemical characteristics during ovarian follicular development in the pig *Journal of Reproduction and Fertility* **86** 171–183
- Green C, Chatterjee R, McGarrigle HHG, Ahmed F and Thomas NSB (2000) p107 is active in the nucleolus of non-dividing human granulosa lutein cells *Journal of Molecular Endocrinology* **25** 275–286
- Grindflek E, Sundvold H, Klungland H and Lien S (1998) Characterisation of porcine peroxisome proliferator-activated receptors gamma 1 and gamma 2: detection of breed and age differences in gene expression *Biochemical and Biophysical Research Communications* **249** 713–718
- Guthridge M, Schmitt J, Bertolini J, Cowling J, Runting A, Katsahambas S, Drummond AE and Hearn MT (1992) Studies on basic fibroblast growth factor (FGF-beta) gene expression in the rat and pig ovary using *in situ* hybridization and quantitative reverse transcriptase-polymerase chain reaction techniques *EXS (Basel)* **61** 219–229
- HAMPL A, Pachernik J and Dvorak P (2000) Levels and

- interactions of p27, cyclin D3 and CDK4 during the formation and maintenance of the corpus luteum in mice *Biology of Reproduction* 62 1393–1401
- Hanley NA, Ikeda Y, Luo X and Parker KL (2000) Steroidogenic factor 1 (SF-1) is essential for ovarian development and function *Cellular and Molecular Endocrinology* 163 27–32
- Hansen JB, Petersen RK, Larsen BM, Bartkova J, Alsner J and Kristiansen K (1999) Activation of peroxisome proliferator-activated receptor gamma bypasses the function of the retinoblastoma protein in adipocyte differentiation *Journal of Biological Chemistry* 274 2386–2393
- Hansen JB, Zhang H, Rasmussen TH, Petersen RK, Flindt EN and Kristiansen K (2000) Peroxisome proliferator activator receptor delta (PPARdelta)-mediated regulation of preadipocyte proliferation and gene expression is dependent on cAMP signaling *Journal of Biological Chemistry* 276 3175–3182
- Heikinheimo M, Ermolaeva M, Bielinska M, Rahman NA, Narita N, Huhtaniemi IT, Tapanainen JS and Wilson DB (1997) Expression and hormonal regulation of transcription factors GATA-4 and GATA-6 in the mouse ovary *Endocrinology* 138 3505–3514
- Houseknecht KL, Bidwell CA, Portocarrero CP and Spurlock ME (1998) Expression and cDNA cloning of porcine peroxisome proliferator-activated receptor gamma (PPARGamma) *Gene* 225 89–96
- Hunter MG (1981) Responsiveness *in vitro* of porcine luteal tissue recovered at two stages of the luteal phase *Journal of Reproduction and Fertility* 63 471–476
- Hunter RHF (1972) Ovulation in the pig: timing in response to injection of human chorionic gonadotrophin *Research in Veterinary Science* 13 356–361
- Ivell R, Bathgate R and Walther N (1999) Luteal peptides and their genes as important markers of ovarian differentiation *Journal of Reproduction and Fertility Supplement* 54 207–216
- Juengel JL, Larrick TL, Meberg BM and Niswender GD (1998) Luteal expression of steroidogenic factor-1 mRNA during the estrous cycle and in response to luteotropic and luteolytic stimuli in ewes *Endocrine* 9 227–232
- Kaminski T, Gawronska B, Derecka K, Okrasa S and Przala J (2000) Gene expression and peptide localization for LH/hCG receptor in porcine small and large luteal cells: possible regulation by opioid peptides *Journal of Physiology and Pharmacology* 51 359–368
- Kikokawa H, Kineman RD, Manova-Todorova KO *et al.* (1996) Enhanced growth of mice lacking cyclin-dependent kinase inhibitor function of p27(Kip1) *Cell* 87 721–732
- Kotzka J, Muller-Wieland D, Roth C, Kremer L, Munck M, Schurmann S, Knebel B and Krone W (2000) Sterol regulatory element binding proteins (SREBP)-1a and SREBP-2 are linked to the MAP-kinase cascade *Journal of Lipid Research* 41 99–108
- Lavoie HA, Benoit AM, Garmey JC, Dailey RL, Wright DJ and Veldhuis JD (1997) Coordinate developmental expression of genes regulating sterol economy and cholesterol side chain cleavage in the porcine ovary *Biology of Reproduction* 57 402–407
- Lemon M and Loir L (1977) Steroid release *in vitro* by two luteal cell types in the corpus luteum of the pregnant sow *Journal of Endocrinology* 72 351–359
- Li Y, Molina JR, Klindt J, Bolt DJ and Anderson LL (1989) Prolactin maintains relaxin and progesterone secretion by aging corpora lutea after hypophysial stalk transection or hypophysectomy in the pig *Endocrinology* 124 1294–1304
- Liu J, Aronow BJ, Witte DP, Pope WF and La Barbera AR (1998) Cyclic and maturation-dependent regulation of follicle-stimulating hormone receptor and luteinizing hormone receptor messenger ribonucleic acid expression in the porcine ovary *Biology of Reproduction* 58 648–658
- Lohrke B, Viergutz T, Shahi SK, Pohland R, Wollenhaupt K, Goldammer T, Walzel H and Kanitz W (1998) Detection and functional characterisation of the transcription factor peroxisome proliferator-activated receptor gamma in lutein cells *Journal of Endocrinology* 159 429–439
- Lopez D and McLean MP (1999) Sterol regulatory element-binding protein-1a binds to cis elements in the promoter of the rat high density lipoprotein receptor SR-BI gene *Endocrinology* 140 5669–5681
- May JV, Bridge AJ, Gotcher ED and Gangrade BK (1992) The regulation of porcine theca cell proliferation *in vitro*: synergistic actions of epidermal growth factor and platelet-derived growth factor *Endocrinology* 131 689–697
- Meduri C, Vu Hai MT, Jolivet A, Takemori S, Kominami S, Driancourt MA and Milgrom E (1996) Comparison of cellular distribution of LH receptors and steroidogenic enzymes in the porcine ovary *Journal of Endocrinology* 148 435–446
- Michael MD, Michael LF and Simpson ER (1997) A CRE-like sequence that binds CREB and contributes to cAMP-dependent regulation of the proximal promoter of the human aromatase P450 (CYP19) gene *Molecular and Cellular Endocrinology* 134 147–156
- Mizukami J and Taniguchi T (1997) The antidiabetic agent thiazolidinedione stimulates the interaction between PPAR gamma and CBP *Biochemical and Biophysical Research Communications* 240 61–64
- Molkentin JD (2000) The zinc-finger containing transcription factors GATA-4, -5 and -6 *Journal of Biological Chemistry* 275 38 949–38 952
- Morbeck DE, Esbenshade KL, Flowers WL and Britt JH (1992) Kinetics of follicle growth in the prepubertal gilt *Biology of Reproduction* 47 485–491
- Morgan DO (1995) Principles of CDK regulation *Nature* 374 131–134
- Motomura W, Okumura T, Takahashi N, Obara T and Kohgo Y (2000) Activation of peroxisome proliferator-activated receptor gamma by troglitazone inhibits cell growth through the increase of p27KIP1 in human pancreatic carcinoma cells *Cancer Research* 60 5558–5564
- Mu YM, Yanase T, Nishi Y, Waseda N, Oda T, Tanaka A, Takayanagi R and Nawata H (2000) Insulin sensitizer, troglitazone, directly inhibits aromatase activity in human ovarian granulosa cells *Biochemical and Biophysical Research Communications* 271 710–713
- Murphy BD (2000) Models of luteinization *Biology of Reproduction* 63 2–11

- Murphy BD and Silavin SL (1989) Luteotropic agents and steroid substrate utilization *Oxford Reviews of Reproductive Biology* **11** 180–223
- Ortiz JA, Mallolas J, Nicot C, Bofarull J, Rodriguez JC, Hegardt FG, Haro D and Marrero PF (1999) Isolation of pig mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase gene promoter: characterization of a peroxisome proliferator-responsive element *Biochemical Journal* **337** 329–335
- Osborne TF (2000) Sterol regulatory element binding proteins (SREBPs): key regulators of nutritional homeostasis and insulin action *Journal of Biological Chemistry* **275** 32 379–32 382
- Pall M, Hellberg P, Brannstrom M, Mikuni M, Peterson CM, Sundfeldt K, Norden B, Hedin L and Enerback S (1997) The transcription factor C/EBP- β and its role in ovarian function; evidence for direct involvement in the ovulatory process *EMBO Journal* **16** 5273–5279
- Patel DD, Knight BL, Wiggins D, Humphreys SM and Gibbons GF (2001) Disturbances in the normal regulation of SREBP-sensitive genes in PPAR α -deficient mice *Journal of Lipid Research* **42** 328–337
- Pescador N, Stocco DM and Murphy BD (1997) Follicle-stimulating hormone and intracellular second messengers regulate steroidogenic acute regulatory protein (StAR) mRNA in luteinized granulosa cells *Biology of Reproduction* **57** 660–668
- Pescador N, Stocco DM and Murphy BD (1999) Growth factor modulation of steroidogenic acute regulatory protein and luteinization in the pig ovary *Biology of Reproduction* **60** 1453–1461
- Picton HM, Campbell BK and Hunter MG (1999) Maintenance of oestradiol production and expression of cytochrome P450 aromatase enzyme mRNA in long-term serum-free cultures of pig granulosa cells *Journal of Reproduction and Fertility* **115** 67–77
- Pilon N, Behdjani R, Daneau I, Lussier JC and Silversides DW (1998) Porcine steroidogenic factor-1 gene (pSF-1) expression and analysis of embryonic pig gonads during sexual differentiation *Endocrinology* **139** 3803–3812
- Pitzel L, Hubertus J and Wuttke W (1990) Effects of oxytocin on *in vitro* steroid release of midstage small and large porcine luteal cells *Endocrinology* **126** 2343–2349
- Pope WF, Xie S, Broermann DM and Nephew KP (1990) Causes and consequences of early embryonic diversity in pigs *Journal of Reproduction and Fertility Supplement* **40** 251–260
- Reinhart AJ, Williams SC, Clark BJ and Stocco DM (1999) SF-1 steroidogenic factor-1 and C/EBP- β CCAAT/Enhancer binding protein- β cooperate to regulate the murine StAR (steroidogenic acute regulatory protein) promoter *Molecular Endocrinology* **13** 729–741
- Reynolds LP, Grazul-Bilska AT and Redmer DA (2000) Angiogenesis in the corpus luteum *Endocrine* **12** 1–9
- Richards JS (2001a) Graafian follicle function and luteinization in nonprimates *Journal of the Society for Gynecologic Investigation* **8** (Supplement 1) S21–S23
- Richards JS (2001b) New signaling pathways for hormones and cyclic adenosine 3'5'-monophosphate action in endocrine cells *Journal of Molecular Endocrinology* **15** 209–218
- Richards JS, Russell DL, Robker RL, Dajee M and Alliston TN (1998) Molecular mechanisms of ovulation and luteinization *Molecular and Cellular Endocrinology* **145** 47–54
- Ricke WA, Redmer DA and Reynolds LP (1999) Growth and cellular proliferation of pig corpora lutea throughout the estrous cycle *Journal of Reproduction and Fertility* **117** 369–377
- Robker RL and Richards JS (1998) Hormone-induced proliferation and differentiation of granulosa cells: a coordinated balance between cell cycle regulators D2 and p27^{Kip1} *Molecular Endocrinology* **12** 924–940
- Schoppee PD, Guthrie HD and Veldhuis JD (2000) Expression of peroxisome proliferator activated receptor γ (PPAR γ) is associated with specific stages of follicular cell differentiation in porcine ovaries *Biology of Reproduction* **62** (Supplement 1) 195–196
- Sekar N, Garmey JC and Veldhuis JD (2000) Mechanisms underlying the steroidogenic synergy of insulin and luteinizing hormone in porcine granulosa cells: joint amplification of pivotal sterol-regulatory genes encoding the low-density lipoprotein (LDL) receptor, steroidogenic acute regulatory (stAR) protein and cytochrome P450 side-chain cleavage (P450_{sc}) enzyme *Molecular and Cellular Endocrinology* **159** 25–35
- Shea-Eaton WK, Trinidad MJ, Lopez D, Nackley A and McLean MP (2001) Sterol regulatory element binding protein-1 α regulation of the steroidogenic acute regulatory protein gene *Endocrinology* **142** 1525–1533
- Silverman E, Eimerl S and Orly J (1999) CCAAT enhancer-binding protein β and GATA-4 binding regions within the promoter of the steroidogenic acute regulatory protein (StAR) gene are required for transcription in ovarian cells *Journal of Biological Chemistry* **274** 17 987–17 996
- Sirois J and Richards JS (1992) Purification and characterization of a novel, distinct isoform of prostaglandin endoperoxide synthase induced by human chorionic gonadotropin in granulosa cells of rat preovulatory follicles *Journal of Biological Chemistry* **267** 6382–6388
- Sirois J and Richards JS (1993) Transcriptional regulation of the rat prostaglandin endoperoxidase synthase 2 gene in granulosa cells *Journal of Biological Chemistry* **268** 21 931–21 938
- Soede NM, Helmond A and Kemp B (1994) Perioovulatory profiles of oestradiol, LH and progesterone in relation to oestrus and embryonic mortality in multiparous sows using transrectal ultrasonography to detect ovulation *Journal of Reproduction and Fertility* **101** 633–641
- Somers JP, Benyo DF, Little-Ihrig LL and Zeleznik AJ (1995) Luteinization in primates is accompanied by a loss of a 43-kilodalton adenosine 3'5'-monophosphate response element binding protein isoform *Endocrinology* **136** 4262–4268
- Song J-H, Dobias M, Pescador N and Murphy BD (1998) Luteinization and gonadotrophins upregulate the Niemann-Pick gene in the porcine ovary *Biology of Reproduction* **58** (Supplement 1) 188 (Abstract)
- Sterneck E, Tessarollo L and Johnson PF (1997) An essential role for C/EBP β in female reproduction *Genes and Development* **11** 2153–2162

- Stocco DM (2001) STAR protein and the regulation of steroid hormone biosynthesis *Annual Reviews of Physiology* **63** 193–213
- Tong W, Kiyokawa H, Soos TJ, Park MS, Soares VC, Manova K, Pollard JW and Koff A (1998) The absence of P27Kip1, an inhibitor of G1 cyclin-dependent kinases, uncouples differentiation and growth arrest during the granulosa-luteal transition *Cell Growth and Differentiation* **9** 787–794
- Tremblay JJ and Viger RS (2001) GATA factors differentially activate multiple gonadal promoters through conserved GATA regulatory elements *Endocrinology* **142** 977–986
- Tsang BK, Ainsworth L, Downey BR and Marcus GJ (1985) Differential production of steroids by dispersed granulosa and theca interna cells from developing preovulatory follicle in pigs *Journal of Reproduction and Fertility* **74** 459–471
- Tsutsui T, Hesabi B, Moons DS, Pandolfi PP, Hansel KS, Koff A and Kiyokawa H (1999) Targeted disruption of CDK4 delays cell cycle entry with enhanced p27^{Kip1} activity *Molecular and Cellular Biology* **19** 7011–7019
- Viergutz T, Loehrke B, Poehland R, Becker F and Kanitz W (2000) Relationship between different stages of the corpus luteum and the expression of the peroxisome proliferator-activated receptor gamma protein in bovine large lutein cells *Journal of Reproduction and Fertility* **118** 153–161
- Wakino S, Kintscher U, Kim S, Yin F, Hsueh WA and Law RE (2000) Peroxisome proliferator-activated receptor gamma ligands inhibit retinoblastoma phosphorylation and G1 → S transition in vascular smooth muscle cells *Journal of Biological Chemistry* **275** 22 435–22 441
- Wandji SA, Gadsby JE, Simmen FA, Barber JA and Hammond JM (2000a) Porcine ovarian cells express messenger ribonucleic acids for the acid-labile subunit and insulin-like growth factor binding protein-3 during follicular and luteal phases of the estrous cycle *Endocrinology* **141** 2638–2647
- Wandji SA, Gadsby JE, Barber JA and Hammond JM (2000b) Messenger ribonucleic acids for MAC25 and connective tissue growth factor (CTGF) are inversely regulated during folliculogenesis and early luteogenesis *Endocrinology* **141** 2648–2657
- Watanabe N, Inoue H and Fujii-Kuriyama Y (1994) Regulatory mechanisms of the cAMP-dependent and cell specific expression of human steroidogenic P450_{sc} (CYP11) gene *European Journal of Biochemistry* **222** 825–834
- Watson J and Maule Walker FM (1978) Progesterone secretion by the corpus luteum of the early pregnant pig during superfusion *in vitro* with PGF-2 α , LH and oestradiol *Journal of Reproduction and Fertility* **52** 209–212
- Wehrenberg U, Wulff C, Husen B, Ken-ichirou M and Rune GM (1997) The expression of SF-1/Ad4BP is related to the process of luteinization in the marmoset (*Callithrix jacchus*) ovary *Histochemistry and Cell Biology* **107** 345–350
- Wooten-Kee CR and Clark BJ (2000) Steroidogenic factor-1 influences protein-deoxyribonucleic acid interactions within the cyclic adenosine 3'5'-monophosphate-responsive regions of the murine steroidogenic acute regulatory protein gene *Endocrinology* **141** 1345–1355
- Wu Z, Rosen ED, Brun R, Hauser S, Adelman G, Troy AE, McKeon C, Darlington GJ and Spiegelman BM (1999) Cross-regulation of C/EBP alpha and PPAR gamma controls the transcriptional pathway of adipogenesis and insulin sensitivity *Molecular Cell* **3** 151–158
- Yu ZK and Hausman GJ (1998) Expression of CCAAT/enhancer binding proteins during porcine preadipocyte differentiation *Experimental Cell Research* **15** 343–349
- Yuan W and Lucy MC (1996) Messenger ribonucleic acid expression for growth hormone receptor, luteinizing hormone receptor, and steroidogenic enzymes during the estrous cycle and pregnancy in porcine and bovine corpora lutea *Domestic Animal Endocrinology* **13** 431–444
- Zelevnik AJ and Somers JP (1999) Regulation of the primate corpus luteum: cellular and molecular perspectives *Trends in Endocrinology and Metabolism* **10** 189–193
- Zhang G, Garmey JC and Veldhuis JD (2000) Interactive stimulation by luteinizing hormone and insulin of the steroidogenic acute regulatory (STAR) protein and 17 α -hydroxylase/17,20-lyase (CYP17) genes in porcine theca cells *Endocrinology* **141** 2735–2742
- Zhu L and Skoultschi AI (2001) Coordinating cell proliferation and differentiation *Current Opinion in Genetics and Development* **10** 91–97