Non-genomic progesterone receptors in the mammalian ovary: some unresolved issues

Tony Bramley

The University of Edinburgh, Division of Reproductive and Developmental Sciences, Simpson Centre for Reproductive Health, Academic Centre, New Royal Infirmary, 49 Little France Crescent, Old Dalkeith Road, Edinburgh EH16 4SB, Scotland, UK

In addition to their well-documented genomic effects, steroid hormones may also exert actions that are: (i) rapid, (ii) insensitive to inhibitors of transcription, (iii) mimicked by steroids coupled to cell membrane-impermeant molecules, and (iv) demonstrable in cells that do not express the classic genomic progesterone receptor (gPR). Such 'non-genomic' effects have been described for all the major classes of steroids (progesterone, oestrogens, androgens and corticoids), as well as for thyroid hormones, retinoids and vitamin D₃. Rapid, membrane-mediated effects of progesterone have been studied most intensively in human spermatozoa and in the *Xenopus* oocyte. However, similar non-genomic actions of progesterone and other steroids have now been described in a wide variety of different tissues in many species. The first putative membrane steroid receptor to be cloned was that for the pig membrane progesterone receptor (mPR). Subsequently, similar genes were cloned from rats and cattle, and two related mPRs have been described in humans. Despite accumulating evidence for cell-surface membrane actions of steroids, a number of uncertainties remain as to the properties and identity of such 'receptors' and their cellular actions. Furthermore, some rapid steroid effects may be mediated through membrane-associated 'classical' steroid receptors, and steroid receptors may be capable of activating other signalling pathways non-classically. This review focuses on some of these unresolved issues, taking as its model the actions of progesterone in the mammalian ovary.

Some of the earliest studies into steroid hormone action clearly showed that steroids could bind to specific, high-affinity binding sites (or receptors) localized on surface-membrane fractions of their target cells (Pietras and Szego, 1977). However, other laboratories demonstrated the presence of specific steroid receptors in the nuclear-cytosolic fractions of target cells and showed that such receptors could act as ligand-dependent transcription factors capable of modulating the activity of specific genes that were known to be regulated by steroid hormones. These findings led, understandably, to the concentration of effort on elaborating the classical genomic steroid hormone actions. However, there has been a recent resurgence of interest in the actions of steroids at the cell surface membrane, and a growing realization that some effects of steroid hormones clearly cannot be explained by a change in transcription (Table 1). A number of excellent reviews have appeared in the last few years that discuss in detail non-genomic effects of progesterone (Sutter-Dub, 2002) and other

steroids (Wehling, 1997; Revelli *et al.*, 1998; Falkenstein *et al.*, 2000a; Schmidt *et al.*, 2000; Kelly and Levine, 2001).

Progesterone is an important intermediate in the synthesis of androgens and oestrogens. It plays an important role in ovulation, atresia and luteinization *in vivo* (Telleria and Deis, 1994; Chaffin and Stouffer, 2000), and is essential for the continuation of early pregnancy in all mammalian species. However, the importance of genomic and non-genomic responses to progesterone in the mammalian ovary is still being debated. Some of the issues addressed in this review are:

- Which responses to progesterone are mediated through classical genomic effects, non-classical genomic or non-genomic actions?
- Which regions of the receptor protein or gene are detected by different antibodies or primers?
- What isoforms or variants of the genomic or membrane receptor(s) are expressed in progesterone target cells and in what proportions?
- How do cross-talk with other hormone and growth factor signalling processes, post-translational receptor

Email: tbramley@staffmail.ed.ac.uk

Table 1. Comparison of some characteristics of genomic and non-genomic effects of steroid hormones

	Genomic effect	Non-genomic effect
Latency of action?	Slow (normally several hours)	Rapid (seconds to minutes)
Inhibitors of transcription?	Effect blocked	Effect persists
Cells lacking a nucleus?	No effect demonstrable	Effect persists
Cells lack genomic receptor?	No effect demonstrable	Effect persists
Isolated cell membranes?	No effect demonstrable	Effects demonstrable
Cell-impermeable steroid?	No effect demonstrable	Effect persists
Inhibitors of 'classic' genomic steroid receptor?	Effect blocked	Effect persists

modification, and type of cell influence the cellular response to progesterone?

Classical genomic progesterone receptors

The genomic progesterone receptor is a member of the ligand-inducible DNA-binding superfamily of nuclear transcription factors that includes the receptors for the other steroid hormones, thyroid hormones, vitamin D, retinoic acid and a variety of 'orphan' receptors. The cDNA for the B-form of the (human) genomic progesterone receptor (gPR-B) consists of eight different exons separated by seven short introns, and codes for a 933 amino acid (116 kDa) protein containing a number of different functional domains (Fig. 1). Exon 1 codes for the N-terminal region of the receptor and for one of three activation function domains (AF-3). Exons 2 and 3 code for AF-1 and the DNA-binding domain (DBD) with its two zinc fingers. Exons 4–8 code for the ligand-binding domain (LBD) and AF-2. This region also codes for the hinge region, a nuclear localization signal (NLS), and regions important for receptor dimerization and binding of heat-shock proteins and immunophilins. Two other isoforms of the human gPR arise from the use of alternate promoters within the same gene. PR-A is N-terminally truncated (94 kDa), lacking 164 amino acids containing the AF-3 domain (Giangrande and McDonnell, 1999), whereas PR-C (60 kDa) lacks exon 1 and most of exon 2 (Wei and Miner, 1994).

Several other variants of gPR have been described (Fig. 1). One variant codes for a protein that lacks the NLS, hinge region and part of the LBD; another variant has a frameshift that gives rise to a truncated protein lacking much of the LBD (Hodges *et al.*, 1999). In addition, other PR variants have been described that have deletions of one or more exons (Misao *et al.*, 1998a; 2000) or that have previously unidentified 5' sequences spliced to novel exons, yet still retain those exons (exons 4–8) that code for the LBD (Hirata *et al.*, 2000, 2002). Variants lacking the LBD or DBD can modify the effects of full-length wild-type gPR-B (Vegeto *et al.*, 1993; Wei *et al.*, 1997; Hodges *et al.*, 1999; Misao *et al.*, 2000).

Effects of progesterone and anti-gestagens on ovarian function: classical genomic, non-classical genomic or non-genomic effects?

Some of the known responses of ovarian tissues to progesterone *in vitro* are listed for domestic animals (Table 2), humans and primates (Table 3) and rats (Table 4). Both the dose–response characteristics and sensitivity to anti-progestins of rapid and longer-term responses are distinct. Stimulation of intracellular Ca^{2+} and inositol trisphosphate (IP_3) (and protein kinase C $\beta 1$ (PKC- $\beta 1$)) by progesterone is detectable within seconds at picomolar levels and is not blocked by anti-gestagens. In general, longer-term actions (effects on steroidogenesis, apoptosis and proliferation; activation of protein or mRNA synthesis) are detectable only at much higher (nanomolar to micromolar) concentrations of progesterone, and are reversed by anti-gestagens (Tables 2–4), implying action via classical genomic progesterone receptors. To date, rapid Ca^{2+} , IP_3 , phospholipase C- β (PLC- β) responses have been studied only in pig granulosa cells, although similar rapid and specific responses to androgen and oestrogen have also been observed in pig granulosa cells (Lieberherr *et al.*, 1999) and to other steroids in cells lacking classical steroid receptors (Le Mellay *et al.*, 1999). Whether rapid response to very low progesterone concentrations is a general response in the ovarian cells of other mammalian species is still unclear.

The actions of progesterone and synthetic progestagens may differ (for review, see Swan *et al.*, 2002). Indeed, although [^3H]progesterone tracer bound readily to plasma-membrane binding sites in the bovine ovary (Rae *et al.*, 1998), 3β -glucuronide-11- ^{125}I -labelled tyramine–progesterone and progesterone coupled to radiolabelled BSA were poor ligands (Bramley *et al.*, 2002). Moreover, a number of synthetic progestagen agonists (levonorgestrel, medroxyprogesterone acetate, ORG2058, R5020) and antagonists (mifepristone, onapristone) with high activity for classical gPRs failed to compete for ovarian [^3H]progesterone binding sites (Rae *et al.*, 1998), indicating that progestagenic agonists, derivatives and antagonist analogues may not behave in the same manner as progesterone itself.

Fig. 1. Genomic structure and functional domains of the human progesterone receptor and its variants. DBD: DNA-binding domain; LBD: ligand-binding domain; PR: progesterone receptor. Arrows indicate transcription initiation sites.

Ovarian genomic progesterone receptors

The presence and cellular localization of gPR proteins and mRNA in ovarian tissues has been demonstrated by immunocytochemistry and *in situ* hybridization (Tables 5 and 6). Most of these studies have used antibodies and primers designed to detect either the ligand binding domain (LBD) of the gPR, or the N-terminus (to differentiate PR-A and PR-B isoforms). PR-A and PR-B have been localized simultaneously in the same types of cell (Mote *et al.*, 1999). It will be of interest to examine the distribution of PR-A and PR-B isoforms in types of ovarian cell at different stages of the ovarian cycle, and to study their responses to luteotrophic and luteolytic stimuli.

The co-expression of PR-A or PR-C in the same type of cell as PR-B modulates its activity (Vegeto *et al.*, 1993; Wei *et al.*, 1997). Moreover, genomic actions of progesterone receptor isoforms are affected differentially by their association with chaperones (Smith, 2000) and nuclear coactivators (Rowan and O'Malley, 2000), and are modulated by receptor phosphorylation (Orti *et al.*, 1992) and by cross-talk with other signalling pathways (mitogen-activated protein kinase (MAPK), Src, tyrosine kinases, phosphatidylinositol 3-kinase (PI3-K)) that are activated by growth factors and cytokines (Richer *et al.*, 1998; Boonyaratanakornkit *et al.*, 2001; Bagowski *et al.*, 2001). Furthermore, gPRs can exist in different functional states (Smith *et al.*, 2000), and can exhibit direct non-genomic actions in their unliganded (Weigel and Zhang, 1998) and undimerized states (Cohen-Solal *et al.*, 1993). Finally, PR-A and PR-B isoforms can activate different constellations of genes even within the same cell (Richer *et al.*, 2002), highlighting the enormous complexity of the intracellular processes involved in progesterone activation of a target cell.

Non-genomic membrane receptors for progesterone

Steroids can affect membrane-mediated events non-genomically in a number of ways (for example, by effects on membrane fluidity, or by direct effects on other G-protein-coupled receptors; for review, see Falkenstein *et al.*, 2000b). However, studies of rapid, steroid-specific responses to progesterone, mediated by the cell membrane, culminated in the isolation and cloning of a non-genomic (pig) progesterone receptor of 194 amino acids, with a single membrane-spanning domain (Falkenstein *et al.*, 1996; Meyer *et al.*, 1996). Homologous proteins were subsequently cloned in rats (Selmin *et al.*, 1996; Krebs *et al.*, 2000; Nolte *et al.*, 2000) and cattle (Cenedella *et al.*, 1999; Zhu *et al.*, 2001), and two putative mPR homologues (hpr6.6 and Dg6) were identified in humans (Gerdes *et al.*, 1998). The human mPR gene consists of three exons separated by two introns. The 5'-region lacks a typical TATA box, but has high homology to a transcription initiator consensus sequence. The proximal region is GC-rich, and a CpG island spans the putative transcription start site (Bernauer *et al.*, 2001). Several upstream regulatory DNA motifs were identified, including AP2, NF-AT, C/EBP and Ahr/Arnt (Bernauer *et al.*, 2001).

Although the rapid effects of progesterone are mediated at the cell surface membrane of a variety of types of cell in pig hepatocytes (Falkenstein *et al.*, 1998) and in Chinese hamster ovary cells transfected with mPR (Falkenstein *et al.*, 1999), progesterone binding is associated with the endo-membrane rather than the cell surface-membrane fraction. Nevertheless, the protein was exposed at the cell surface of human spermatozoa, as an antibody to the protein prevented induction of the acrosome reaction by progesterone, but not by other

Table 2. Effects *in vitro* of progesterone on the ovary of domestic species

Species	Type of cell	Effective dose of		Effect	Reference
		Progestagen	Antiprogestagen		
Pig	Granulosa	1 pmol P l ⁻¹ 1 pmol P l ⁻¹	1 µmol RU486 l ⁻¹ 1 µmol RU486 l ⁻¹ 30 nmol RU486 l ⁻¹ 30 nmol ONA l ⁻¹	Increased [Ca ²⁺], IP ₃ Increased [Ca ²⁺], IP ₃ , PKC-β1 Upregulated gPR and inhibited E ₂ /P accumulation	Machelon <i>et al.</i> , 1996* [†] Lieberherr <i>et al.</i> , 1999* [†] Wu <i>et al.</i> , 1999
	Stable granulosa cell line (JC-410)	3–10 µmol LNG l ⁻¹ 10 µmol P l ⁻¹ 0.1 µmol RWJ l ⁻¹	1–10 µmol RU486 l ⁻¹	LNG and RU stimulated P accumulation. P, RU and LNG increased P450scc transcription. RWJ inhibited P450scc expression.	Swan <i>et al.</i> , 2002 [‡]
	Theca		30 nmol RU486 l ⁻¹ 30 nmol ONA l ⁻¹	Inhibited P secretion	Wu <i>et al.</i> , 1999
Sheep	Ovarian surface epithelium cells	3 nmol P l ⁻¹		Upregulated p53 and inhibited E ₂ -stimulated proliferation	Murdoch and Van Kirk, 2002 [†]
		30 nmol P l ⁻¹	60 nmol RU486 l ⁻¹	Increased PARP activity	Murdoch, 1998 [†] , Murdoch <i>et al.</i> , 2001 [†]
Cow	Granulosa (+ forskolin/insulin)	1 µmol MPA l ⁻¹	0.1 µmol RU486 l ⁻¹ 1 µmol ONA l ⁻¹	Inhibited upregulation of oxytocin gene	Lioutas <i>et al.</i> , 1997 [§]
	Cumulus–oocyte complex	10 µmol P l ⁻¹		Inhibited E ₂ secretion	Mingoti <i>et al.</i> , 2002
	Corpus luteum	50 nmol P l ⁻¹	50 nmol RU486 l ⁻¹ 5 µmol ONA l ⁻¹	P inhibited and RU and ONA stimulated apoptosis induced by AG	Rueda <i>et al.</i> , 2000 [†]
		5 µmol P l ⁻¹		Decreased PGF _{2α} and prostacyclin secretion	Pate, 1988

*Demonstration of cell surface receptor action.

[†]Progestin effect reversed by gPR antagonist.[‡]Progestin effect not reversed by gPR antagonist.[§]Antiprogestin effect reversed by progestin.AG: aminoglutethimide; E₂: oestradiol; gPR: genomic progesterone receptor; IP₃: inositol trisphosphate; LNG: levonorgestrel; MPA: medroxyprogesterone acetate; ONA: onapristone (ZK98299); P: progesterone; P450scc: cytochrome P450 side-chain cleavage; PARP: pituitary adenyl cyclase activated regulatory protein; PKC-β1: protein kinase C β1; RU486: mifepristone; RWJ: RWJ26819.

Table 3. Effects *in vitro* of progesterone on the human ovary

Type of cell	Effective dose range		Effect	Reference
	Progestagen	Antiprogestagen		
Granulosa (luteinizing IVF cells)	1 nmol–10 $\mu\text{mol P l}^{-1}$	50 $\mu\text{mol RU486 l}^{-1}$	Induced apoptosis and stimulated caspase-3	Svensson <i>et al.</i> , 2001 [§]
	10 nmol P l^{-1}	10 $\mu\text{mol ORG}$		
		1 $\mu\text{mol RU486 l}^{-1}$	P reduced apoptosis and had protective effect on survival	Makrigiannakis <i>et al.</i> , 2000 [†]
		50 $\mu\text{mol RU486 l}^{-1}$	Reduced secretion of (E_2) P and relaxin	VanderVoort <i>et al.</i> , 2000
		50 $\mu\text{mol HRP l}^{-1}$		
		5–10 nmol RU486 l^{-1}	Suppressed P secretion	DiMattina <i>et al.</i> , 1986
		50–500 $\mu\text{mol RU486 l}^{-1}$	Inhibited 17-hydroxylase. No effect on E_2 secretion	DiMattina <i>et al.</i> , 1987
		10–100 nmol RU486 l^{-1}	No effect on basal, hCG- or PGE_2 -stimulated P	Greenberg <i>et al.</i> , 1990
Ovarian surface epithelium cells	640 nmol P l^{-1}	64 nmol RU486 l^{-1}	P inhibited proliferation and differentiation	Chaffkin <i>et al.</i> , 1993 [†]
	160 nmol P l^{-1}	100 nmol ORG l^{-1}	Suppressed proliferation. Enhanced proliferation	Ivarsson <i>et al.</i> , 2001 [†]
		10 nmol RU486 l^{-1}	Inhibited hCG-stimulated (but not basal) P secretion	Ottander <i>et al.</i> , 2000
Corpus luteum				
SKOV-3 ovarian cancer	30–300 nmol P l^{-1}	3 $\mu\text{mol RU486 l}^{-1}$	Inhibited uPA secretion and cell invasiveness	McDonnel and Murdoch, 2001 ^{*‡}
gPR-positive ovarian carcinoma cell lines		30–120 nmol RU486 l^{-1}	Inhibited proliferation (blocked G_0/G_1 phase of cell cycle) and downregulated gPR	Rose and Barnea, 1996

*Demonstration of cell surface receptor action.

[†]Progestin effect reversed by gPR antagonist.

[‡]Progestin effect not reversed by gPR antagonist.

[§]Antiprogestin effect not reversed by progestin.

E_2 : oestradiol; gPR: genomic progesterone receptor; HRP: horseradish peroxidase; ORG: ORG-5128; P: progesterone; PGE_2 : prostaglandin E_2 ; RU486: mifepristone; SKOV-3: human ovarian cancer cell line; uPA: urokinase plasminogen activator.

Table 4. Effects *in vitro* of progesterone on the rat ovary

Type of cell	Effective dose range		Effect	Reference
	Progestagen	Antiprogestagen		
Granulosa cells	1 $\mu\text{mol P l}^{-1}$		Enhanced LH- and FSH-stimulated steroidogenesis	Fanjul <i>et al.</i> , 1983
	1 $\mu\text{mol R5020 l}^{-1}$			
		300 nmol RU486 l^{-1}	Inhibited aromatase. Inhibited P secretion	Fortune and Vincent, 1983
		30 $\mu\text{mol RU486 l}^{-1}$		
	200 $\mu\text{mol P l}^{-1}$	64 $\mu\text{mol RU486 l}^{-1}$	P reduced and antagonist enhanced apoptosis and cell adhesion	Peluso and Pappalardo, 1994 [†]
		25 $\mu\text{mol RU486 l}^{-1}$	Antagonized hCG-induced apoptosis and suppressed expression of gPR	Svensson <i>et al.</i> , 2000
Large granulosa cells	640 nmol P l^{-1}		Inhibition of hCG- and forskolin-induced PACAP mRNA expression	Ko <i>et al.</i> , 1999
	64 nmol P l^{-1}	64 $\mu\text{mol RU486 l}^{-1}$	P inhibited apoptosis of large GC	Luciano <i>et al.</i> , 1994 [†]
			Stimulated bFGF secretion and inhibited apoptosis in large luteal cells	Peluso and Pappalardo, 1999*
Small granulosa cells	160 nmol P l^{-1}	64 $\mu\text{mol RU486 l}^{-1}$	P inhibited insulin-stimulated mitosis	Luciano and Peluso, 1995 [†]
Granulosa/SIGC	640 nmol P l^{-1}	640 nmol RU486 l^{-1} 640 nmol ONA l^{-1}	Inhibited apoptosis and MAPKK	Peluso <i>et al.</i> , 2001* [‡]
Luteinizing granulosa cells		10 $\mu\text{mol ONA l}^{-1}$	Inhibited hCG and forskolin on PAC-1 receptor expression	Ko and Park-Sarge, 2000
	150 nmol P l^{-1}	2 $\mu\text{mol ONA l}^{-1}$ 2 $\mu\text{mol RU486 l}^{-1}$	Induced GRE expression; RU486 and ONA antagonized P effect, but did not block LH-induction of gPR	Natraj and Richards, 1993 [†]
CL cells	10 $\mu\text{mol LNG l}^{-1}$		Inhibited LH and cAMP stimulated P secretion	Telleria and Deis, 1994
CL of pregnancy	0.1 $\mu\text{mol P l}^{-1}$		Suppressed IL-6 expression	Telleria <i>et al.</i> , 1998
	1–100 $\mu\text{mol R5020 l}^{-1}$		Stimulated P secretion	Telleria <i>et al.</i> , 1999
CL and SV40 sensitive cell line	10 nmol P l^{-1}	1 $\mu\text{mol RU486 l}^{-1}$	Inhibited 20 α -HSD expression	Sugino <i>et al.</i> , 1997 ^{†§}
CL-derived phagocytes	300 nmol P l^{-1}	100 nmol RU486 l^{-1}	Inhibited superoxide production	Sugino <i>et al.</i> , 1996 [†]

*Demonstration of cell surface receptor action.

[†]Progestin effect reversed by gPR antagonist.[‡]Progestin effect not reversed by gPR antagonist.[§]Effect may be explained by action at the glucocorticoid receptor.bFGF: basic fibroblast growth factor; CL: corpus luteum; GC: granulosa cell; gPR: genomic progesterone receptor; GRE: glucocorticoid response element; 20 α -HSD: hydroxysteroid dehydrogenase; IL-6: interleukin 6; LNG: levonorgestrel; MAPKK: mitogen-activated protein kinase kinase; ONA: onapristone (ZK98299); ORG: ORG-5128; P: progesterone; PACAP: pituitary adenylate cyclase activating polypeptide; PAC-1: PACAP-specific receptor-1; RU486: mifepristone.

Fig. 2. Genomic and non-genomic activation pathways of progesterone. DAG: diacylglycerol; GABA: gamma aminobutyric acid; MAPK: mitogen-activated protein kinase; MEK: mitogen-activated (extracellular) protein kinase; P: progesterone; PI3-K: phosphatidylinositol 3-kinase; PKA: protein kinase A; PKC: protein kinase C; PLC: phospholipase C; PTK: protein tyrosine kinase.

agonists (Falkenstein *et al.*, 1999; Buddhikot *et al.*, 1999).

What is the nature of the membrane receptor for progesterone?

There is conflicting data on the nature of the membrane progesterone receptor, and the signalling pathways used in different types of cell (Fig. 2). Studies of rapid, membrane-mediated events in several types of cell have identified a wide range of changes, including Ca^{2+} mobilization (influx or mobilization from intracellular stores), opening of Na^+ and Cl^- channels, and the activation of phospholipase C (leading to increased intracellular IP_3 and diacylglycerol generation), Pertussis toxin-insensitive G-protein-coupled receptors, PKC, tyrosine kinase and MAPK pathways (for review, see Wehling, 1997; Falkenstein *et al.*, 2000a). However, it is

unclear which of these changes are primary, and which are activated by other downstream events or by cross-talk with other signalling pathways. Although the rat ovarian mPR has some similarity to gamma amino butyric acid A receptors (GABA_A) (Peluso and Pappalardo, 1998), GABA agonists and antagonists failed to affect progesterone binding to bovine luteal membranes (Menzies *et al.*, 1999).

The two most widely used models for studies of the non-genomic progesterone receptor are human spermatozoa (which had been thought to lack the genomic PR; Castilla *et al.*, 1995) and the *Xenopus* oocyte (in which responses in enucleated cells, and membrane-impermeable derivatives of progesterone can mimic all the rapid changes induced by the steroid). However, doubts about these models have been raised as (a) spermatozoa appear to contain small amounts of gPR mRNA (Sachdeva *et al.*, 2000; Contreras and Llanos,

Table 5. Studies demonstrating expression of the gene for genomic progesterone receptor in ovarian tissues

Species	Primers (nucleotides)	Method	Type of cell	Reference
Human	2113–2343	RT–PCR (PR-B)	CL	Ottander <i>et al.</i> , 2000
	144–367	RT–PCR (PR-A/B)		
		Southern blots/ISH		
	1341–1360	RT–PCR/Southern blot	CL	Misao <i>et al.</i> , 1998a
Monkey	PR-A and PR-B	Quantitative RT–PCR	CL	Misao <i>et al.</i> , 1998b
	1817–36 and 2330–49	Southern blot	HOSE cells	Lau <i>et al.</i> , 1999
	LBD	RT–PCR, sequencing, restriction enzyme cleavage	Germinal epithelium, CL, luteinizing GC	Chandrasekher <i>et al.</i> , 1994
	LBD	RPase	CL, luteinized GC	Duffy <i>et al.</i> , 1996
Baboon	Full length hPR	Northern blot	CL	Duffy and Stouffer, 1995
Bovine		RPase/northern blot	CL	Hild-Petito and Fazleabas, 1997
		RT–PCR, sequencing	GC	Lioutas <i>et al.</i> , 1997
	Hinge/LBD	Northern blot	CL	Rueda <i>et al.</i> , 2000
			Periovulatory follicle	Cassar <i>et al.</i> , 2002
Rat	LBD	RT–PCR, ISH	GC of preovulatory follicles	Park-Sarge <i>et al.</i> , 1995
	LBD	RT–PCR	Transient expression in GC of luteinizing primed ovary	Park and Mayo, 1991

CL: corpus luteum; GC: granulosa cell; HOSE: human ovarian surface epithelium cells; hPR: human progesterone receptor; ISH: *in situ* hybridization; LBD: ligand-binding domain; PR-A/B; progesterone receptor-A or -B; RPase: ribonuclease protection assay.

Table 6. Studies demonstrating genomic progesterone receptor protein expression in ovarian tissues

Species	Antibody	Protein detected	Type of cell	Subcellular localization	Reference
Human	Range of mAbs	116 and 97 kDa	Ovarian tumours, CL, stroma, HOSE GC of dominant follicles	Nuclear	Press and Green, 1988 Iwai <i>et al.</i> , 1990
	mAb C262	120 and 90 kDa	CL	Nuclear?	Misao <i>et al.</i> , 1998a
	mAb C262	116 and 90 kDa	CL		Misao <i>et al.</i> , 1998b
	Neomarkers hPRa3	PR-B and PR-A	Ovarian cancers	Nuclear	Akahira <i>et al.</i> , 2000
	Neomarkers hPRa7	PR-A			
	Neomarkers hPRa2	PR-B			
	Novocastra mAb	120 and 90 kDa	Theca and small and large luteal cells	Nuclear	Suzuki <i>et al.</i> , 1994
	JZB39 mAb		60–80% of luteinizing GC	Nuclear	Greenberg <i>et al.</i> , 1990
	Abbott K68		Germinal epithelium, CL, cancer	Nuclear	Zeimet <i>et al.</i> , 1994
	Abbott		CL (theca; GC of some follicles)		Revelli <i>et al.</i> , 1996
Monkey	Abbott		CL	Nuclear	Ottander <i>et al.</i> , 2000
	JZB39 mAb		Germinal epithelium, stroma, theca, CL, GC of preovulatory follicles	Nuclear	Hild-Petito <i>et al.</i> , 1988
Baboon	JZB39 mAb		Luteinized GC	Nuclear	Duffy <i>et al.</i> , 1996
	JZB39 mAb		CL	Nuclear	Hild-Petito and Fazleabas, 1997
Rat	mAb C262	60 kDa	GC/SIGC	Cell membrane	Peluso and Pappalardo, 1998
	mAb C262		Small GC	Cell membrane	Peluso <i>et al.</i> , 2001
	mAb 928	115 and 85 kDa	Luteinizing GC in culture	Nuclear	Natraj and Richards, 1993
	mAb 928	97 and 66 kDa			
	mAb H928		Not present in CL of pregnancy Transient expression in GC of luteinizing ovary		Park-Sarge <i>et al.</i> , 1995 Park and Mayo, 1991
Pregnant rat	mAb LET81		GC and theca but not CL	Nuclear?	Telleria <i>et al.</i> , 1999
Pig	Novocastra mAb	120 and 80 kDa	CL, theca, GC of preovulatory follicles	Nuclear	Slomczynska <i>et al.</i> , 2000
Bovine	JZB39 mAb		Large and small luteal cells	Nuclear	Rueda <i>et al.</i> , 2000
	mAb C262	60 and 55 kDa	CL	Cell membrane	Bramley <i>et al.</i> , 2002

CL: corpus luteum; GC: granulosa cells; HOSE: human ovarian surface epithelium cells; mAb: monoclonal antibody; PR-A: progesterone receptor A; PR-B: progesterone receptor B; SIGC: spontaneously induced granulosa cells.

Epitopes: mAb C262: raised to C-terminal 14 amino acids of gPR; mAb 928: raised to hinge region of gPR; conformation-dependent; mAb JZB39: recognizes occupied and unoccupied human gPR.

2001), and (b) cloning of the receptor responsible for inducing oocyte maturation (xPR) has revealed it to be similar to the mammalian genomic progesterone receptor (Maller, 2001). xPR associates with p42 MAPK and activates oocyte maturation non-classically by signalling through the PI3-K pathway (Bagowski *et al.*, 2001).

Studies designed to identify and localize progesterone receptors in ovarian tissues have often used antibodies to the LBD of the gPR (Tables 5 and 6). However, antibodies raised to other regions of the gPR often give little or no signal in these same tissues (for review, see Press and Greene, 1988; El-Hefnawy *et al.*, 2000; Luconi *et al.*, 1998 – but see Luconi *et al.*, 2002). Furthermore, a commonly used monoclonal antibody raised to the C-terminal end of the gPR (mAb C262) also detects immunoactivity in isolated rat granulosa cell membranes (Peluso and Pappalardo, 1998, 1999; Peluso *et al.*, 2001) and in bovine luteal membranes and detergent extracts (Bramley *et al.*, 2002), indicating that the membrane steroid receptor may possess a domain with homology to the LBD of the gPR. Indeed, different methods of detecting mPR can identify proteins of markedly different sizes even in the same tissues and species (for review, see Bramley *et al.*, 2002). Finally, it will be of interest to establish whether gPR variants with deleted exons or novel 5'-regions (*vide infra*) are present in tissues with high amounts of membrane progesterone binding sites and, if so, to study their subcellular distribution at different stages of ovarian development and functional activity.

Conclusion

Progesterone can induce rapid and specific changes in a variety of tissues by acting via membrane-associated receptors. However, the nature of these receptors is presently unclear. Although membrane-associated, progesterone-specific receptors have been isolated and cloned from a range of tissues in a number of species, the protein isolated appears to be only one subunit of the (possibly dimeric) membrane receptor, and it has been difficult to demonstrate binding to the expressed protein. However, other studies indicate a membrane protein (or an associated protein) with homology to the ligand-binding domain of the genomic progesterone receptor. Further studies of these receptors in the ovary, and the ways in which they interact with other autocrine or paracrine factors, may lead to exciting advances in the understanding of the progesterone-dependent processes of ovulation, luteinization, follicular atresia and maternal recognition of pregnancy, and so permit the development of new methods of manipulating ovarian activity and fertility in humans and other mammalian species.

The author is most grateful to M. Rae and G. Scobie for their helpful discussions and comments, and to G. Menzies, for his excellent technical support.

References

Key references are identified by asterisks.

- Akahira J, Inoue T, Suzuki T *et al.* (2000) Progesterone receptor isoforms A and B in human epithelial ovarian carcinoma: immunohistochemical and RT-PCR studies *British Journal of Cancer* **83** 1488–1494
- Bagowski C, Myers JW and Ferrell JE, Jr (2001) The classical progesterone receptor associates with p42 MAPK and is involved in phosphatidylinositol 3-kinase signaling in *Xenopus* oocytes *Journal of Biological Chemistry* **276** 37 708–37 714
- Bernauer S, Wehling M, Gerdes D and Falkenstein E (2001) The human membrane progesterone receptor gene: genomic structure and promoter analysis *DNA Sequence* **12** 13–25
- Boonyaratnakornkit V, Scott MP, Ribon V, Sherman L, Anderson SM, Maller JL, Miller WT and Edwards DP (2001) Progesterone receptor contains a proline-rich motif that directly interacts with SH3 domains and activates c-Src family tyrosine kinases *Molecular Cell* **8** 269–280
- Bramley TA, Menzies GS, Rae MT and Scobie G (2002) Non-genomic steroid receptors in the bovine ovary *Domestic Animal Endocrinology* **23** 1–10
- Buddhikot M, Falkenstein E, Wehling M and Meizel S (1999) Recognition of a human sperm surface protein involved in the progesterone-initiated acrosome reaction by antisera against an endomembrane progesterone binding protein from porcine liver *Molecular and Cellular Endocrinology* **158** 187–193
- Cassar CA, Dow MPD, Pursley JR and Smith GW (2002) Effect of the preovulatory LH surge on bovine follicular progesterone receptor mRNA expression *Domestic Animal Endocrinology* **22** 179–187
- Castilla JA, Gil T, Molina J, Hortas ML, Rodriguez F, Torres-Munoz J, Vergara F and Herruzo AJ (1995) Undetectable expression of genomic progesterone receptor in human spermatozoa *Human Reproduction* **10** 1757–1760
- Cenedella RJ, Sexton PS and Zhu XL (1999) Lens epithelia contain a high-affinity, membrane steroid hormone-binding protein *Investigative Ophthalmology and Visual Science* **40** 1452–1459
- Chaffin CL and Stouffer RL (2000) Role of gonadotrophins and progesterone in the regulation of morphological remodelling and atresia in the monkey peri-ovulatory follicle *Human Reproduction* **15** 2489–2495
- Chaffkin LM, Luciano AA and Peluso JJ (1993) The role of progesterone in regulating human granulosa cell proliferation and differentiation *in vitro*. *Journal of Clinical Endocrinology and Metabolism* **76** 696–700
- Chandrasekhar YA, Melner MH, Nagalla SR and Stouffer RL (1994) Progesterone receptor, but not estradiol receptor, messenger ribonucleic acid is expressed in luteinizing granulosa cells and the corpus luteum in rhesus monkeys *Endocrinology* **135** 307–314
- Cohen-Solal K, Bailly A, Rauch C, Quesne M and Milgrom E (1993) Specific binding of progesterone receptor to progesterone-responsive elements does not require prior dimerization *European Journal of Biochemistry* **214** 189–195
- Contreras HR and Llanos MN (2001) Detection of progesterone receptors in human spermatozoa and their correlation with morphological and functional properties *International Journal of Andrology* **24** 246–252
- Di Mattina M, Albertson B, Seyler DE, Loriaux DL and Falk R (1986) Effect of the antiprogesterin RU486 on progesterone production by cultured human granulosa cells: inhibition of the ovarian 3 β -hydroxysteroid dehydrogenase *Contraception* **34** 199–206
- Di Mattina M, Albertson BD, Tyson V, Loriaux DL and Falk RJ (1987) Effect of the antiprogesterin RU486 on human ovarian steroidogenesis *Fertility and Sterility* **48** 229–233
- Duffy DM and Stouffer RL (1995) Progesterone receptor messenger ribonucleic acid in the primate corpus luteum during the menstrual cycle: possible regulation by progesterone *Endocrinology* **136** 1869–1876
- Duffy DM, Molskness TA and Stouffer RL (1996) Progesterone receptor messenger ribonucleic acid and protein in luteinized granulosa cells of Rhesus monkeys are regulated *in vitro* by gonadotropins and steroids *Biology of Reproduction* **54** 888–895
- El-Hefnawy T, Manna PR, Luconi M, Baldi E, Slotte JP and Huhtaniemi I (2000) Progesterone action in a murine Leydig tumor cell line

- (mLTC-1), possibly through a nonclassical receptor type *Endocrinology* **141** 247–255
- Falkenstein E, Meyer C, Eisen C, Scriba PC and Wehling M (1996) Full-length cDNA sequence of a progesterone membrane-binding protein from porcine vascular smooth muscle cells *Biochemical and Biophysical Research Communications* **229** 86–89
- Falkenstein E, Schmieding K, Lange A, Meyer C, Gerdes D, Welsch U and Wehling M (1998) Localization of a putative progesterone membrane binding protein in porcine hepatocytes *Cellular and Molecular Biology* **44** 571–578
- Falkenstein E, Heck M, Gerdes D, Grube D, Christ M, Weigel M, Buddhikot M, Meizel S and Wehling M (1999) Specific progesterone binding to a membrane protein and related nongenomic effects on Ca^{2+} -fluxes in sperm *Endocrinology* **140** 5999–6002
- Falkenstein E, Tillmann H-C, Christ M, Feuring M and Wehling M (2000a) Multiple actions of steroid hormones – a focus on rapid, nongenomic effects *Pharmacological Reviews* **52** 513–556
- *Falkenstein E, Norman AW and Wehling M (2000b) Mannheim classification of nongenomically initiated (rapid) steroid action(s) *Journal of Clinical Endocrinology and Metabolism* **85** 2072–2075
- Fanjul LF, Ruiz de Galarreta CM and Hsueh AJ (1983) Progesterin augmentation of gonadotropin-stimulated progesterone production by cultured rat granulosa cells *Endocrinology* **112** 405–407
- Fortune JE and Vincent SE (1983) Progesterone inhibits the induction of aromatase activity in rat granulosa cells *in vitro*. *Biology of Reproduction* **28** 1078–1089
- Gerdes D, Wehling M, Leube B and Falkenstein E (1998) Cloning and tissue expression of two putative steroid membrane receptors *Biological Chemistry* **379** 907–911
- Giangrande PH and McDonnell DP (1999) The A and B isoforms of the human progesterone receptor: two functionally different transcription factors encoded by a single gene *Recent Progress in Hormone Research* **54** 291–314
- Greenberg LH, Stouffer RL, Brenner RM, Molskness TA, Hild-Petito SA and Yu Q (1990) Are human luteinizing granulosa cells a site of action for progesterone and relaxin? *Fertility and Sterility* **53** 446–453
- Hild-Petito S and Fazleabas AT (1997) Expression of steroid receptors and steroidogenic enzymes in the baboon (*Papio anubis*) corpus luteum during the menstrual cycle and early pregnancy *Journal of Clinical Endocrinology and Metabolism* **82** 955–962
- Hild-Petito S, Stouffer RL and Brenner RM (1988) Immunocytochemical localization of estradiol and progesterone receptors in the monkey ovary throughout the menstrual cycle *Endocrinology* **123** 2896–2905
- Hirata S, Shoda T, Kato J and Hoshi K (2000) The novel form of the progesterone receptor cDNA in the human testis and detection of its mRNA in the human uterine endometrium *Oncology* **59** Supplement 1 39–44
- Hirata S, Shoda T, Kato J and Hoshi K (2002) The novel exon, exon T, of the human progesterone receptor gene and the genomic organization of the gene *Journal of Steroid Biochemistry and Molecular Biology* **80** 299–305
- Hodges YK, Richer JK, Horwitz HB and Horwitz LD (1999) Variant estrogen and progesterone receptor messages in human vascular smooth muscle *Circulation* **99** 2688–2693
- Ivarsson K, Sundfeldt K, Brannstrom M and Janson PO (2001) Production of steroids by human ovarian surface epithelial cells in culture: possible role of progesterone as growth inhibitor *Gynecologic Oncology* **82** 116–121
- Iwai T, Nanbu Y, Iwai M, Taii S, Fijii S and Mori T (1990) Immunocytochemical localization of oestrogen receptors and progesterone receptors in the human ovary throughout the menstrual cycle *Virchows Archives A Pathology, Anatomy and Histopathology* **417** 369–375
- Kelly MJ and Levine ER (2001) Rapid actions of plasma membrane estrogen receptors *Trends in Endocrinology and Metabolism* **12** 152–156
- Ko C and Park-Sarge O-K (2000) Progesterone receptor activation mediates LH-induced type-1 pituitary adenylate cyclase activating polypeptide receptor (PAC1) gene expression in rat granulosa cells *Biochemical and Biophysical Research Communications* **277** 270–279
- Ko C, In Y-H and Park-Sarge O-K (1999) Role of progesterone receptor activation in pituitary adenylate cyclase activating polypeptide gene expression in rat ovary *Endocrinology* **140** 5185–5194
- Krebs CJ, Jarvis ED, Chan J, Lydon JP, Ogawa S and Pfaff DW (2000) A membrane-associated progesterone-binding protein, 25-Dx, is regulated by progesterone in brain regions involved in female reproductive behaviors *Proceedings National Academy of Sciences USA* **97** 12 816–12 821
- Lau K-M, Mok SC and Ho S-M (1999) Expression of human estrogen receptor- α and - β , progesterone receptor, and androgen receptor mRNA in normal and malignant ovarian epithelial cells *Proceedings National Academy of Sciences USA* **96** 5722–5727
- Le Mellay V, Lasmoles F and Lieberherr M (1999) $\text{G}\alpha_{q/11}$ and $\text{G}\beta\gamma$ proteins and membrane signaling of calcitriol and estradiol *Journal of Cellular Biochemistry* **75** 138–146
- *Lieberherr M, Grosse B and Machelon V (1999) Phospholipase C- β and ovarian sex steroids in pig granulosa cells *Journal of Cellular Biochemistry* **74** 50–60
- Lioutas C, Einspanier A, Kascheike B, Walther N and Ivell R (1997) An autocrine progesterone positive feedback loop mediates oxytocin up-regulation in bovine granulosa cells during luteinization *Endocrinology* **138** 5059–5062
- Luciano AM and Peluso JJ (1995) Effect of *in vivo* gonadotropin treatment on the ability of progesterone, estrogen, and cyclic adenosine 5'-monophosphate to inhibit insulin-dependent granulosa cell mitosis *in vitro*. *Biology of Reproduction* **53** 664–669
- Luciano AM, Pappalardo A, Ray C and Peluso JJ (1994) Epidermal growth factor inhibits large granulosa cell apoptosis by stimulating progesterone synthesis and regulating the distribution of intracellular free calcium *Biology of Reproduction* **51** 646–654
- Luconi M, Bonaccorsi L, Maggi M, Pecchioli P, Krausz C, Forti G and Baldi E (1998) Identification and characterization of functional nongenomic progesterone receptors on human sperm membrane *Journal of Clinical Endocrinology and Metabolism* **83** 877–885
- Luconi M, Bonaccorsi L, Bini L, Liberatori S, Pallini V, Forti G and Baldi E (2002) Characterization of membrane nongenomic receptors for progesterone in human spermatozoa *Steroids* **67** 505–509
- McDonnell AC and Murdoch WJ (2001) High-dose progesterone inhibition of urokinase secretion and invasive activity by SKOV-3 ovarian carcinoma cells: evidence for a receptor-independent nongenomic effect on the plasma membrane *Journal of Steroid Biochemistry and Molecular Biology* **78** 185–191
- Machelon V, Nome F, Grosse B and Lieberherr M (1996) Progesterone triggers rapid transmembrane calcium influx and/or calcium mobilization from endoplasmic reticulum, via a Pertussis-insensitive G-protein in granulosa cells in relation to luteinization process *Journal of Cell Biochemistry* **61** 619–628
- Makrigiannakis A, Coukos G, Christofidou-Solomidou M, Montas S and Coutifaris C (2000) Progesterone is an autocrine/paracrine regulator of human granulosa cell survival *in vitro*. *Annals of the New York Academy of Science* **900** 16–25
- Maller JL (2001) The elusive progesterone receptor in *Xenopus* oocytes *Proceedings National Academy of Sciences USA* **98** 8–10
- Menzies GS, Howland K, Rae MT and Bramley TA (1999) Stimulation of binding of [^3H]-progesterone to bovine luteal cell-surface membranes: specificity of digitonin *Molecular and Cellular Endocrinology* **153** 57–69
- Meyer C, Schmid R, Scriba PC and Wehling M (1996) Purification and partial sequencing of high-affinity progesterone binding site(s) from porcine liver membranes *European Journal of Biochemistry* **239** 726–731
- Mingoti GZ, Garcia JM and Rosa-e-Silva AAM (2002) Steroidogenesis in cumulus cells of bovine cumulus-oocyte complexes matured *in vitro* with BSA and different concentrations of steroids *Animal Reproduction Science* **69** 175–186
- Misao R, Sun WS, Iwagaki S, Fujimoto J and Tamaya T (1998a) Identification of various exon-deleted progesterone receptor mRNAs in human endometrium and ovarian endometriosis *Biochemical and Biophysical Research Communications* **252** 302–306

- Misao R, Nakanishi Y, Fujimoto J and Tamaya T (1998b) Steroid receptor mRNA levels in human corpus luteum *Hormone Research* **50** 155–159
- Misao R, Nakanishi Y, Iwagaki S, Fujimoto J and Tamaya T (1998c) Expression of progesterone receptor isoforms in corpora lutea of human subjects: correlation with serum estrogen and progesterone concentrations *Molecular Human Reproduction* **4** 1045–1052
- Misao R, Nakanishi Y, Sun W-S, Iwagaki S, Fujimoto J and Tamaya T (2000) Identification of exon-deleted progesterone receptor mRNAs in human uterine endometrial cancers *Oncology* **58** 60–65
- Mote PA, Balleine RL, McGowan EM and Clarke CL (1999) Colocalization of progesterone receptors A and B by dual immunofluorescent histochemistry in human endometrium during the menstrual cycle *Journal of Clinical Endocrinology and Metabolism* **84** 2963–2971
- Murdoch WJ (1998) Perturbation of sheep ovarian surface epithelial cells by ovulation: evidence for roles of progesterone and poly(ADP-ribose) polymerase in the restoration of DNA integrity *Journal of Endocrinology* **156** 503–508
- Murdoch WJ and Van Kirk EA (2002) Steroid hormonal regulation of proliferative, p53 tumor suppressor, and apoptotic responses of sheep ovarian surface epithelial cells *Molecular and Cellular Endocrinology* **186** 61–67
- Murdoch WJ, Townsend RS and McDonnell AC (2001) Ovulation-induced DNA damage in ovarian surface epithelial cells of ewes: prospective regulatory mechanisms of repair/survival and apoptosis *Biology of Reproduction* **65** 1417–1424
- Natraj U and Richards JS (1993) Hormonal regulation, localization, and functional activity of the progesterone receptor in granulosa cells of rat preovulatory follicles *Endocrinology* **133** 761–769
- Nolte I, Jeckel D, Wieland FT and Sohn K (2000) Localization and topology of ratp28, a member of a novel family of putative steroid-binding proteins *Biochimica et Biophysica Acta* **1543** 123–130
- Orti E, Bodwell JE and Munck A (1992) Phosphorylation of steroid hormone receptors *Endocrine Reviews* **13** 105–128
- Ottander U, Hosokawa K, Liu K, Bergh A, Ny T and Olofsson JI (2000) A putative stimulatory role of progesterone acting via progesterone receptors in the steroidogenic cells of the human corpus luteum *Biology of Reproduction* **62** 655–663
- Park O-K and Mayo KE (1991) Transient expression of progesterone receptor messenger RNA in ovarian granulosa cells after the preovulatory luteinizing hormone surge *Molecular Endocrinology* **5** 967–978
- Park-Sarge O-K, Parmer TG, Gu Y and Gibori G (1995) Does the rat corpus luteum express the progesterone receptor? *Endocrinology* **136** 1537–1543
- Pate JL (1988) Regulation of prostaglandin synthesis by progesterone in the bovine corpus luteum *Prostaglandins* **36** 303–315
- Peluso JJ and Pappalardo A (1994) Progesterone and cell–cell adhesion interact to regulate rat granulosa cell apoptosis *Biochemical Cell Biology* **72** 547–551
- Peluso JJ and Pappalardo A (1998) Progesterone mediates its anti-mitogenic and anti-apoptotic actions in rat granulosa cells through a progesterone-binding protein with gamma aminobutyric acid A receptor-like features *Biology of Reproduction* **58** 1131–1137
- Peluso JJ and Pappalardo A (1999) Progesterone maintains large rat granulosa cell viability indirectly by stimulating small granulosa cells to synthesize basic fibroblast growth factor *Biology of Reproduction* **60** 290–296
- *Peluso JJ, Fernandez G, Pappalardo A and White BA (2001) Characterization of a putative membrane receptor for progesterone in rat granulosa cells *Biology of Reproduction* **65** 94–101
- Pietras RJ and Szego CM (1977) Specific binding sites for estrogen at the outer surfaces of isolated endometrial cells *Nature (London)* **265** 69–72
- Press MF and Greene GL (1988) Localization of progesterone receptor with monoclonal antibodies to the human progestin receptor *Endocrinology* **122** 1165–1175
- Rae MT, Menzies GS, McNeilly AS, Woad K, Webb R and Bramley TA (1998) Specific non-genomic, membrane-localized binding sites for progesterone in the bovine corpus luteum *Biology of Reproduction* **58** 1394–1406
- Revelli A, Pacchioni D, Cassoni P, Bussolati G and Massobrio M (1996) *In situ* hybridization study of messenger RNA for estrogen receptor and immunohistochemical detection of estrogen and progesterone receptors in the human ovary *Gynecological Endocrinology* **10** 177–186
- Revelli A, Massobrio M and Tesarik J (1998) Nongenomic actions of steroid hormones in reproductive tissues *Endocrine Reviews* **19** 3–17
- *Richer JK, Lange CA, Manning NG, Owen G, Powell R and Horwitz KB (1998) Convergence of progesterone with growth factor and cytokine signaling in breast cancer: progesterone receptors regulate signal transducers and activators of transcription expression and activity *Journal of Biological Chemistry* **273** 31 317–31 326
- Richer JK, Jacobsen BM, Manning NG, Abel MG, Wolf DM and Horwitz KB (2002) Differential gene regulation by the two progesterone receptor isoforms in human breast cancer cells *Journal of Biological Chemistry* **277** 5209–5218
- Rose FV and Barnea ER (1996) Response of human ovarian carcinoma cell lines to antiprogesterin mifepristone *Oncogene* **12** 999–1003
- Rowan BG and O'Malley BW (2000) Progesterone receptor coactivators *Steroids* **65** 545–549
- Rueda BR, Hendry IR, Hendry WJ III, Stormshak F, Slayden OD and Davis JS (2000) Decreased progesterone levels and progesterone receptor antagonists promote apoptotic cell death in bovine luteal cells *Biology of Reproduction* **62** 269–276
- Sachdeva G, Shah CA, Kholkute SD and Puri CP (2000) Detection of progesterone receptor transcript in human spermatozoa *Biology of Reproduction* **62** 1610–1614
- Schmidt BMW, Gerdes D, Feuring M, Falkenstein E, Christ M and Wehling M (2000) Rapid, nongenomic steroid actions: a new age? *Frontiers in Neuroendocrinology* **21** 57–94
- Selmin O, Lucier GW, Clark GC, Tritscher AM, Vanden Heuvel JP, Gastel JA, Walker NJ, Sutter TR and Bell DA (1996) Isolation and characterisation of a novel gene induced by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in rat liver *Carcinogenesis* **17** 2609–2615
- Slomczynska M, Krok M and Pierscinski A (2000) Localization of the progesterone receptor in the porcine ovary *Acta Histochemica* **102** 183–191
- Smith CL, Wolford RG, O'Neill TB and Hager GL (2000) Characterization of transiently and constitutively expressed progesterone receptors: evidence for two functional states *Molecular Endocrinology* **14** 956–971
- Smith DF (2000) Chaperones in progesterone receptor complexes *Seminars in Cell and Developmental Biology* **11** 45–52
- Sugino N, Shimamura K, Tamura H, Ono M, Nakamura Y, Ogino K and Kato H (1996) Progesterone inhibits superoxide radical production by mononuclear phagocytes in pseudopregnant rats *Endocrinology* **137** 749–754
- Sugino N, Telleria CM and Gibori G (1997) Progesterone inhibits 20 α -hydroxysteroid dehydrogenase expression in the rat corpus luteum through the glucocorticoid receptor *Endocrinology* **138** 4497–4500
- Sutter-Dub M-T (2002) Rapid non-genomic and genomic responses to progestogens, estrogens, and glucocorticoids in the endocrine pancreatic B cell, the adipocyte and other cell types *Steroids* **67** 77–93
- Suzuki T, Sasano H, Kimura N, Tamura M, Fukaya T, Yajima A and Nagura H (1994) Immunohistochemical distribution of progesterone, androgen and oestrogen receptors in the human ovary during the menstrual cycle: relationship to expression of steroidogenic enzymes *Human Reproduction* **9** 1589–1595
- Svensson ECh, Markstrom E, Andersson M and Billig H (2000) Progesterone receptor-mediated inhibition of apoptosis in granulosa cells isolated from rats treated with human chorionic gonadotropin *Biology of Reproduction* **63** 1457–1464
- Svensson ECh, Markstrom E, Shao R, Andersson M and Billig H (2001) Progesterone receptor antagonists Org 31710 and RU 486 increase apoptosis in human periovulatory granulosa cells *Fertility and Sterility* **76** 1225–1231
- Swan CL, Agostini MC, Bartlewski PM, Feyles V, Urban RJ and Chedrese PJ (2002) Effects of progestins on progesterone synthesis in a stable

- porcine granulosa cell line: control of transcriptional activity of the cytochrome P450 side-chain cleavage gene *Biology of Reproduction* **66** 959–965
- Telleria CM and Deis RP** (1994) Effect of RU486 on ovarian progesterone production at pro-oestrus and during pregnancy: a possible dual regulation of the biosynthesis of progesterone *Journal of Reproduction and Fertility* **102** 379–384
- Telleria CM, Ou J, Sugino N, Ferguson S and Gibori G** (1998) The expression of interleukin-6 in the pregnant rat corpus luteum and its regulation by progesterone and glucocorticoid *Endocrinology* **139** 3597–3605
- Telleria CM, Stocco CO, Stati AO and Deis RP** (1999) Progesterone receptor is not required for progesterone action in the rat corpus luteum of pregnancy *Steroids* **64** 760–766
- VandeVoort CA, Overstreet JW, Lasley BL and Stewart DR** (2000) Effects of progesterone receptor blockers on human granulosa–luteal cell culture secretion of progesterone, estradiol, and relaxin *Biology of Reproduction* **62** 200–205
- Vegeto E, Shabbaz M, Wen DX, Goldman M, O'Malley BW and McDonnell DP** (1993) Human progesterone receptor A form is a cell- and promoter-specific repressor of human progesterone receptor B function *Molecular Endocrinology* **7** 1244–1255
- Wehling M** (1997) Specific, nongenomic actions of steroid hormones *Annual Reviews of Physiology* **59** 365–393
- Wei LL and Miner R** (1994) Evidence for the existence of a third progesterone receptor protein in human breast cancer cell line T47D *Cancer Research* **54** 340–343
- Wei LL, Norris BM and Baker CJ** (1997) An N-terminally truncated third progesterone receptor protein, PR(C), forms heterodimers with PR(B) but interferes in PR(B)-DNA binding *Journal of Steroid Biochemistry and Molecular Biology* **62** 287–297
- Weigel NL and Zhang Y** (1998) Ligand-independent activation of steroid hormone receptors *Journal of Molecular Medicine* **76** 469–479
- Wu ER, Wang JZ, Zhao JL and Xiao BL** (1999) Flow cytometry of porcine ovarian cells: antiprogestins play an important role in progesterone receptor upregulation *Gynecological Endocrinology* **13** 333–339
- Zeimet AG, Muller-Holzner E, Marth C and Daxenbichler G** (1994) Immunocytochemical versus biochemical receptor determination in normal and tumorous tissues of the female reproductive tract and breast *Journal of Steroid Biochemistry and Molecular Biology* **49** 365–372
- *Zhu XL, Sexton PS and Cenedella RJ** (2001) Characterization of membrane steroid binding protein mRNA and protein in lens epithelial cells *Experimental Eye Research* **73** 213–219