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Membrane progesterone receptor expression in mammalian tissues; a review of regulation and physiological implications

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Abstract

The recent discovery of a novel, membrane localized progestin receptor (mPR) unrelated to the classical progesterone receptor (PR) in fishes and its subsequent identification in mammals suggests a potential mediator of non-traditional progestin actions, particularly in tissues where PR is absent. While early studies on mPR focused on final oocyte maturation in fishes, more current studies have examined mPRs in multiple mammalian systems in both reproductive and non-reproductive tissues as well as in diseased tissues. Here we review the current data on mPR in mammalian systems including male and female reproductive tracts, liver, neuroendocrine tissues, the immune system and breast and ovarian cancer. We also provide new data demonstrating mPR expression in the RAW 264.7 immune cell line and bone marrow-derived macrophages as well as mPR expression and downstream gene regulation in ovarian cancer cells.

Keywords

membrane progesterone receptor; non-genomic; macrophage; breast cancer; ovarian cancer

Introduction

Non-nuclear actions of progestins have been studied for several years and are often attributable to extra-nuclear or membrane-associated activities of the classical progesterone receptor (PR), a ligand-activated transcription factor in the steroid hormone receptor super-family that has the ability to directly interact with and activate cytoplasmic protein kinases [1]. However, several studies also demonstrate actions of progestins in cells and tissues that are clearly devoid of PR. Extra-nuclear activity of progestins induces oocyte maturation in multiple non-mammalian species [2–4] and the receptor mediating this hormone-dependent action was recently identified as an alternative, membrane localized progestin receptor (mPR) which is unrelated to classical PR [2].

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Three mPR gene isoforms (mPR α (PAQR VII), mPR β (PAQR VIII), and mPR γ (PAQR V)), first identified in teleost fishes [2] and subsequently in humans [5], each encode a 7-transmembrane protein with high affinity and limited capacity progesterin binding [6]. mPRs are members of the recently defined progesterin and adiponectin Q receptor family (PAQR) [7,8] and, while not classical G protein coupled receptors (GPCRs), mPRs, when bound to progesterin, rapidly activate G proteins and their downstream signaling pathways.

While the majority of studies on mPRs focus on teleost oocyte maturation, mammalian homologues of mPRs have been identified in multiple species (sheep [9,10], pig [11], mouse [12–14], rat [15,16] and human [5,6,17,18]) and a growing number of studies have examined the regulation and activity of mPRs in mammalian biology (Table 1). Here we review previous reports and present new evidence of mPR signaling and function in mammalian reproduction, neuro-endocrine signaling, immune function and disease.

Reproductive Tissues

As mPR is critical for fish and amphibian gamete function [19], it is logical to consider the role of mPR activity in mammalian reproductive tissues. Similar to mPR induced hypermotility in teleost sperm [19,20], mPR α has been identified in mouse testis [21] and implicated in human sperm motility [19]. Progestins initiate both the acrosome reaction and hypermotility in mammalian sperm [22,23]. While the acrosome reaction is attributable to membrane localized PR [24], the identity of the hormone receptor mediating progesterin-induced human sperm hypermotility is less clear. A recent review by Thomas et al. [19] shows functional mPR α located on the midpiece and flagella of human sperm. The midpiece and flagella structures work in concert to drive sperm motility. Thus, the localization of mPR to these structures is suggestive of a connection between mPR and hypermotility. Additional evidence correlates sperm mPR expression to motility as low-motility human sperm expressed lower levels of mPR protein than did highly motile sperm. These preliminary data suggest that mPR may be an attractive target for therapeutic treatments to enhance male fertility and/or increase fertilization success during *in vitro* fertilization procedures.

While studies on mPR involvement in male reproductive function are limited, multiple groups have examined mPRs in female reproductive function. It is not known if mPR acts directly on the mammalian oocyte as it does in teleosts, but there is mounting evidence from multiple female reproductive tissues that mPRs are expressed and are hormonally regulated in the ovarian follicle and corpus luteum as well as the fallopian tube, endometrium, myometrium and fetal tissues. In the ovary, porcine ovarian cumulus cells express increasing levels of mPR β as the ovarian follicle matures; expression peaks near the time of germinal vesicle breakdown [11]. Cell surface mPR β expression is correlated with cumulus cell expansion as blockade of mPR β activation by incubation with specific anti-sera impairs cumulus expansion. The mechanism of mPR β involvement in cumulus expansion remains unknown but may relate to the ability of mPR β to traffic between the plasma membrane and endoplasmic reticulum; additionally, mPR β bound to anti-sera in the culture media retained mPR β in the plasma membrane and blocked trafficking back to the endoplasmic reticulum [11].

mPRs have been identified in the corpus luteum (CL) of sheep [9,10] and rat [15]. While the physiological actions and intracellular signaling of mPR in luteal cells remain unclear, the expression patterns of mPRs are regulated throughout estrous and pregnancy. In the sheep, mPR α expression increases throughout estrous before declining at the end of the estrous cycle [10]. The levels of mPR in the sheep CL correlate to serum progesterone levels but it remains unclear if this is a causal relationship. In the rat, the expression of each mPR

isoform is regulated throughout pregnancy. mPR α mRNA peaks midway through pregnancy and is down regulated at the end of pregnancy. Similarly, mPR β expression is high throughout pregnancy but declines during the last two days of pregnancy. Alternatively, mPR γ expression increases throughout pregnancy. Regulation of mPRs in rat CL during pregnancy appears to be under the control of prolactin wherein this hormone positively regulates mPR α and mPR β but negatively regulates mPR γ [15]. While progesterin and gonadotropins regulate mPR expression in fishes [2] this is the first report of mPR regulation by prolactin [15]; it is unclear if this pathway is unique to mammals.

mPRs are also expressed and activate G protein signaling in multiple tissues of the reproductive tract including fallopian tubes, endometrium, myometrium and in fetal tissues. mPR β and γ are both expressed in the ciliated cells lining the fallopian tubes of mice and humans [12,13]. Interestingly, while mPR β is present in the plasma membrane of the cilia proper, mPR γ is localized to the base of the cilia [13]. As the ciliated cells of the fallopian tubes help transport mature, released oocytes from the ovary to the uterus it is hypothesized that mPR β and γ work together to control cilia movement and gamete transport at times when serum progesterone levels are high (i.e. during post gamete release).

Two studies suggest that mPRs are regulated in uterine tissues throughout estrous and may be involved in term and preterm labor. mPR α , β and γ are regulated in human endometrium throughout estrous [17]. mPR α and β levels increase midway through the cycle while mPR γ levels decrease. However, the physiological relevance of mPR in the endometrium throughout estrous and pregnancy remain unknown. Extensive studies using human myometrial cells suggest that mPR activates G protein signaling pathways during pregnancy and labor [18]. In myometrial cells, mPR acts during the early stages of pregnancy to upregulate the transcriptional activity of classical PR co-expressed in this tissue. During labor activated mPR inhibits SRC2 expression and functionally down regulates PR transcriptional activity as measured by luciferase reporter gene assays while activating myosin light chain, thereby switching the myometrium to a contractile state. During labor, myometrial mPR levels change. During both term and preterm labor, mPR α levels decrease while mPR β levels decrease only during term labor; mPR γ levels appear unregulated [17]. These two studies suggest a regulatory role for mPRs in term labor. Alternative regulation of mPRs during pre-term labor may provide a useful molecular marker for identification of women at risk for this complication.

It is clear from multiple reports that mPRs are expressed in a variety of both male and female reproductive tissues. In males, mPRs appear linked to the regulation of sperm motility and these receptors may prove useful targets for male infertility treatments [19]. More focus has been given to mPRs role in mammalian female reproductive function. Multiple mPR isoforms are important throughout the female reproductive cycle including oocyte growth [11] transport of the oocyte from the ovary to the uterus [12,13], preparation of the uterus for implantation [9–11], gestation and fetal development [17] and parturition [18]. While the expression of both PR and mPRs in some tissues may provide functional redundancy to ensure reproductive success via progesterone driven actions, there are many instances where the two classes of progesterone receptors clearly act independently to initiate a physiological function. Given that the binding affinities of mammalian mPRs for synthetic progestins (both agonists and antagonists) differ greatly from those of classical PR that is co-expressed with these receptors [6], mPRs may present novel targets for reproductive therapies unique to mPR actions, while preserving the actions of classical PRs.

Immune Cells and Tissues

Progesterone has functional effects on a wide variety of immune cell subtypes, including monocytes, macrophages, dendritic cells, and lymphocytes. In murine monocytes and macrophages activated *in vitro* by lipopolysaccharide, progesterone decreased nitric oxide production and expression of nitric oxide synthase, IL-6 and IL-12 proteins [25–27]. Progesterone induced apoptosis in LPS-activated human monocytes and increased expression of the proinflammatory cytokine TNF α [28,29]. Reduced expression of cytokines has also been observed in activated dendritic cells from rats, mice and humans in response to progesterone treatment [30,31].

Additionally, progesterone has varying effects on mammalian lymphocytes. During pregnancy, the production of progesterone-induced blocking factor inhibits natural killer cell activity and increases production of asymmetric antibodies by B cells; these molecules block the binding of functional antibodies to their antigens [32,33]. In addition, progesterone impacts the polarization of T helper cells. T helper cells differentiate into Th1 or Th2 cells, each subpopulation characterized by differential cytokine expression. Th1 cells promote cell-mediated immunity while Th2 cells promote antibody-mediated immunity. Progesterone inhibits *in vitro* generation of murine and human Th1 cells [34,35] while enhancing Th2 generation [34]. In addition, progesterone directly alters cytokine production to induce Th2 cytokines by established human Th1 clones [35]. This promotion of Th2 responses may occur indirectly, since progesterone promotes dendritic cell production of IL-10, a Th2 promoting secreted cytokine [36]. The ability of progesterone to induce the immune system towards a Th2 like state is suggested to play a role in the maintenance of pregnancy [37–39].

While progesterone clearly modifies immune cell functions, the mechanisms of progesterone action (i.e. target proteins) on the mammalian immune system are poorly defined. Data describing the expression of the classical or nuclear PR in cells of the immune system has been inconsistent. While some studies suggest the presence of PR in mammalian immune cells [30,40,41], others found no evidence of PR expression [42–44]. As PR is often undetectable in immune cells, other mediators of progesterone action in immune cells are under investigation. Several reports demonstrate non-genomic or rapid effects of progesterone on human T cells [45–49]. Progesterone has been shown to inhibit sustained calcium signals that occurs after the initial events of T cell activation, and inhibit gene expression driven by nuclear factor of activated T Cells (NF-AT) [46]. In addition, progesterone, but not other steroid hormones, directly inhibited both voltage gated and calcium-gated potassium channels [45]. Chien, et al. demonstrated a progesterone-induced increase in intracellular calcium in human peripheral blood T cells, coupled to a concomitant decrease in intracellular pH due to progesterone acting on the Na⁺/H⁺ exchange I transporter [47,49]. In addition, concentrations of progesterone greater than or equal to 50 nM inhibited activation of T cells by a mitogenic lectin. These effects of progesterone most likely occur at the plasma membrane because they are fully recapitulated by treatment of cells with membrane impermeable progesterone-BSA conjugates [47]. While these researchers did demonstrate the expression of all mPR isoforms in the human T cells studied, no direct connection between mPR expression and the effects of progesterone on ion movement were established [48]. Interestingly, the anti-progestin, RU486 was antagonistic to both the Ca²⁺ increase and pH decrease induced by progesterone in T cells [48].

The above studies suggest that progesterone acts upon immune cells via a nongenomic mechanism, presumably at or near the plasma membrane, although direct evidence for a membrane-localized receptor is lacking. Recently however, human peripheral blood cells and isolated T cells from female donors, as well as the commonly studied human male Jurkat T cell line were shown to express mRNA transcripts for mPR α and mPR β , but not

mPR γ or PR [50]. Chien, et al., found slightly different results, observing mRNA transcripts for mPR α , mPR β , and mPR γ in peripheral blood T cells from male donors [48]. Dosiou, et al. further showed that Western blotting detected mPR α and mPR β proteins in plasma membranes from human T lymphocytes isolated from blood and from Jurkat cells. Additionally, radiolabeled progesterone bound to the cell membranes of T lymphocytes and Jurkat cells with an affinity appropriate for steroid receptor binding. Further, progesterone treatment of Jurkat cells resulted in the activation of an inhibitory G protein. While published data on mPR expression in the immune system thus far has focused on T cells, we have recently found mPR α mRNA and protein expression and mPR β mRNA expression in the murine RAW 264.7 macrophage cell line as well as in mouse bone marrow derived macrophages (BMDM) (Figure 1). In addition, BMDM, but not RAW 264.7 express mPR γ mRNA. Both the RAW 264.7 and BMDM cell lines were negative for PR by RT-PCR and Western blot (data not shown).

Progesterone elicits a diverse variety of functional effects on immune cell types representing multiple hematopoietic lineages including alteration of cytokine gene transcription, change in ion transport, and inhibition of nitric oxide production [51]. Many of these effects occur in cells that seemingly lack PR, potentially implicating mPRs as the signal initiators. To date, expression of mPRs on specific subsets of immune cells has been demonstrated by mRNA and protein in only T cells [48,50] and macrophages (Figure 1). Likewise, limited research suggests that progesterone binding to mPRs elicits functional effects in immune cells akin to those observed in Jurkat T cells, where progesterone stimulates the activity of inhibitory G proteins [50]. Other functional effects of progesterone on T cells, including alterations in intracellular calcium or pH and inhibition of T cell proliferation, have yet to be directly linked to mPR activity. Likewise, progesterone-mediated effects on other types of immune cells with respect to mPR-dependent signaling events still need to be investigated.

Neuroendocrine Tissues

Progesterone has multiple functions in the nervous system, including the regulation of mood and cognition, neuroprotection and neuroregeneration [52]. While these effects can occur through classical PR, a role for other progesterone-binding proteins or receptors in the nervous system has long been postulated. Progesterone and its derivatives bind to cell membranes of various rat brain tissues, suggesting non-classical mechanisms of action [53–55]. Non-genomic and rapid effects of progesterone within the central nervous system have been observed, including: effects on ion transport [56,57], neurotransmitter release [58], protein kinase activity [59,60] and nuclear translocation [57].

Early speculation regarding the identity of the receptor mediating the non-genomic effects of progesterone in the brain led to the discovery of a steroid binding site on the GABA $_A$ /benzodiazepine receptor chloride channel complex in rat brains [61]. This binding site has affinity for multiple steroid hormone metabolites, including the progesterone derivative 3 α -hydroxy-dihydroprogesterone found in blood and brain tissue and following stress [62]. Binding of steroids to this receptor leads to enhancement of GABA induced chloride flux [56]. The recent discovery of mPRs offers an alternative mediator of non-genomic progesterone effects in the nervous system. Analysis of mPR expression in various human tissues shows variable distribution of mPR α , mPR β , and mPR γ [5]. Using human tissue arrays and Northern blot analysis, Zhu, et al., showed broad expression of mPR β , but not mPR α or mPR γ , within the nervous system, including expression in the spinal cord, cerebral cortex, cerebellum, thalamus, pituitary gland, and caudate nucleus. Using a more sensitive assay (RT-PCR) Tang et al. found mRNA of all three mPRs (α , β , and γ) in human brain [8]. An mPR with 91% amino acid homology to human mPR α has been cloned from sheep and is expressed in both hypothalamus and pituitary tissues [9]. In rodents, low levels of mPR β

protein are present in mouse brain [13] and expression of mPR mRNA has been more specifically localized to the mediobasal hypothalamus of both the rat [63] and mouse [64]. All three mPR isoforms are expressed in the mediobasal area of the rat hypothalamus, in addition to classical PR-A and PR-B nuclear receptors [63]. Notably, as in the reproductive tract (discussed above), mPR α and mPR β expression in the brain varied with hormonal changes during estrous, with highest mPR expression during proestrous, suggesting receptor upregulation by estrogen and downregulation by progesterone. A similar pattern was seen for PR-B. mPR α and mPR β were also expressed in the mouse mediobasal hypothalamus as well as in the murine-derived GT1-7 neuroblastoma cell line [64]. mPR α and mPR β are known to activate inhibitory G proteins and treatment of GT1-7 cells with progesterone suppressed cAMP accumulation, leading to the suppression of gonadotropin release. The loss of cAMP production was abrogated by pretreatment with pertussis toxin, implicating inhibitory G protein activity.

It is clear that progesterone action is complex within the central nervous system, and progesterone may simultaneously engage multiple receptor types including membrane-associated or nongenomic PR activities (Table 1). While functional correlates to mPR expression are lacking, abundant expression of this new class of transmembrane progesterone receptors in the hypothalamus underlies the potential importance of these receptors in neuroendocrine function. Similarly, co-regulation of mPR α and mPR β mRNA expression along with PR-B during hormonal fluxuations during estrous, suggests mPR involvement in regulation of reproductive processes [63] and cross-talk between these two classes of progestin receptors [18].

Cancer

mPRs may also contribute to cancer development, proliferation, and metastasis. mPRs were first identified in human breast cancer biopsies and epithelial derived breast cancer cell lines by Dressing and co-workers [65]. mPR α message and protein are present in MCF-7 and SKBR3 breast cancer cell lines and mPR is associated with membrane localized progesterone binding. Of particular interest, mPR α mRNA is upregulated in breast tumor biopsies compared to normal tissue, suggesting a role for mPRs in breast tumor biology. Subsequent investigations confirmed the presence of mPR in breast cancer cell lines [66]. A recent study demonstrated mPR α mRNA and protein expression and signaling in classical PR null, basal type breast cancer cells (MDA-MB-468) and in human breast cancer biopsies [67]. In MDA-MB-468 cells, mPR α mediates epithelial to mesenchymal transition (EMT) through activation of the PI3K/Akt pathway [67]. mPR induced EMT likely occurs via transactivation of EGFR and indeed, both molecules are co-localized to caveolin rafts in the plasma membrane. While previous work suggests that mPR transactivates PR [18], this is the first study to suggest that mPR transactivates membrane localized tyrosine kinase receptors and provides new insight into mPR signaling networks and a role for mPRs in PR-null basal like tumors.

In addition to breast cancer, mPRs may also impact gynecologic malignancies of the female reproductive tract. mPR α is present in the HeLa cervical cancer cell line [66] and all three mPR isoforms have been observed in human ovarian cancer tissue specimens comprising each of the major histologic subtypes [68]. We have recently identified mPR expression in ovarian cancer cell lines of epithelial origin (Figure 2 A) [69]. However, in contrast to observations made in breast cancer cells, progesterone-stimulated mPRs increase intracellular cAMP levels in ovarian cancer cells in the presence of β 1,2-adrenergic receptor agonists [69]. mPR-mediated signaling also caused increased gene expression of the pro-apoptotic marker BAX, and occurred in a JNK1/2-dependent manner (Figure 2 B). While it remains unclear whether mPRs influence ovarian tumorigenesis and/or progression

positively or negatively, mPR-mediated control of key signaling intermediates (i.e. cAMP, p38, JNK, BAX) known to regulate proliferation, survival, and invasion implicates them in ovarian cancer pathology (Figure 3). Further studies are required to define the functional significance of mPR action in PR-positive and –negative gynecologic malignancies, as these molecules may provide unique targets for future cancer therapies.

Conclusions

While mPRs were first identified as critical to teleost oocyte maturation and reproductive success, they are abundantly expressed and hormonally regulated in a diverse array of normal mammalian tissues, and implicated in human breast and ovarian malignancies. mPRs may primarily influence both male and female reproduction; however, further study is needed to fully understand the contribution of these novel receptor signaling molecules to all facets of mammalian cell biology, including their functions within the reproductive and neuroendocrine axis, the CNS, and the immune system. A novel mPR specific agonist is now available [70] thus, studies of mPR in relation to cancer biology are timely and exciting, particularly, in tumors of the female reproductive system where significant findings could lead to alternative endocrine-based cancer treatments.

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Figure 1.

Expression of mPRs in murine macrophages. A. PCR products amplified from the RAW 264.7 cell line (R) or bone marrow-derived macrophages (B) with the indicated mPR primers with or without reverse transcriptase. B. Western blot analysis of mPR α protein expression in RAW 264.7 or bone marrow-derived macrophages. Anti-mPR α antibody kindly provided by Dr. Peter Thomas (University of Texas Austin).

Figure 2.
A. Quantitative-PCR confirmed that the SKOV-3 and ES-2 ovarian cancer cell lines positively express mRNA of each membrane-progesterone receptor (mPR) isoform ($-\alpha$, $-\beta$, $-\gamma$) (mean $\pm$ SD, n = 4). Relative to control MCF-7 breast cancer cells, SKOV-3 and ES-2 cells express minimal amounts of nuclear progesterone receptor (PR) mRNA. B. Targeted screening of 84 cancer pathway related genes (Human Cancer PathwayFinder™, RT² Profiler™ PCR Array, SABiosciences™) in SKOV-3 cells treated with progesterone (50 nM) for 24 hours revealed a greater than 1.5-fold increase in the expression of 23 distinct gene targets. (note: no genes demonstrated a greater than 0.5-fold decrease in expression.)

Figure 3.

The membrane-progesterone receptor (mPR) independently activates two distinct signaling pathways in the SKOV-3 and ES-2 ovarian cancer cell lines. A. Progesterone augmented cAMP levels increase cyclic AMP response element (CRE) transcriptional activity. B. Progesterone-stimulated mPRs activate the JNK1/2 and p38 MAPKs and increase *BAX* gene expression [69]. The mPR may activate JNK1/2 and p38 by directly stimulating ASK1 via the MAPK-GPCR scaffolding protein β -arrestin. Additionally, mPR-mediated JNK1/2 activity may stimulate *BAX* gene expression via the p53 tumor suppressor protein.

Table 1

mPR isoform tissue distribution, regulation and signaling in mammals

Species	Isoform	Tissues	mRNA/Protein	Regulation	Direction	Intracellular Signaling	Reference
Human	α , β , γ	Amnion, Chorion	mRNA, Protein				[17]
Human	α , β , γ	Endometrium	mRNA, Protein	Estrous, Parturition			[17]
Human	α , β , γ	Myometrium	mRNA, Protein	Estrous, Parturition		G protein signaling cascade	[17,18]
Human	α	Sperm	mRNA, Protein			G protein activation	[19]
Human	α	Testis	mRNA				[19]
Human	α , β , γ	T lymphocytes from male donors	mRNA			Calcium release, pH decrease	[48]
Human	α , β	Peripheral Blood	mRNA				[50]
Human	α , β	T lymphocytes from female donors	mRNA, protein				[50]
Human	α , β	Jurkat T cell line	mRNA, protein			G protein signaling cascade	[50]
Human	β	Brain (spinal cord, cerebral cortex, cerebellum, pituitary, thalamus, caudate nucleus)	mRNA				[5]
Mouse	β	Bone	Protein				[13]
Mouse	β	Brain	Protein				[13]
Mouse	β	Heart	Protein				[13]
Mouse	γ	Liver	Protein				[12]
Mouse	β	Lung	Protein				[13]
Mouse	β	ovary	Protein				[12,13]
Mouse	β	Prostate	Protein				[13]
Mouse	β	Spleen	Protein				[13]
Mouse	β , γ	Testis	Protein				[12,13]
Mouse	α	Testis	Protein				[19,21]
Mouse	β	Uterus	Protein				[13]
Mouse	α , β	Hypothalamus	mRNA				[64]
Mouse	α , β	GT1-7 Neuroblastoma cell line	mRNA			G protein signaling cascade	[64]
Mouse, human	β , γ	Fallopian Tube	mRNA, Protein	E2, P4 post eCG priming	↓		[12,13]
Pig	β	Granulosa Cell	mRNA, Protein	Estrous			[11]
Rat	β	Brain	mRNA				[15]

Species	Isoform	Tissues	mRNA/Protein	Regulation	Direction	Intracellular Signaling	Reference
Rat	α, β, γ	Corpus Luteum	mRNA, Protein	Prolactin, Pregnancy	$\alpha, \beta \uparrow; \gamma \downarrow$		[15]
Rat	γ	Kidney	mRNA				[15]
Rat	α	Liver	mRNA, Protein	Dioxin	$\uparrow$ Male Only		[16]
Rat	α, β, γ	Hypothalamus	mRNA	Estrous (α, β only)			[63]
Sheep	α	Corpus Luteum	mRNA	Estrous		Calcium release	[9,10]
Sheep	α	Hypothalamus, Pituitary	mRNA				[9,10]
Sheep	α	Ovary	mRNA				[10]
Sheep	α	Uterus	mRNA	Estrous			[9,10]