Androgen in the Uterus: A Compensator of Estrogen and Progesterone

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ABSTRACT : Pivotal roles of steroid hormones in uterine endometrial function are well established from the mouse models carrying the null mutation of their receptors. Literally androgen belongs to male but interestingly it also detected in female. The fluctuations of androgen levels are observed during reproductive cycle and pregnancy, and the functional androgen receptor is expressed in reproductive organs including uterus. Using high throughput methodology, the downstream genes of androgen have been isolated and revealed correlations between other steroid hormones. In androgen-deficient mice, uterine responses to exogenous gonadotropins are impaired and the number of pups per litter is reduced dramatically. As expected androgen has important role in decidual differentiation through AR. It regulates specific gene network during those cellular responses. Recently we examined the effects of steroid hormonal complex containing high level of androgen. Interestingly, on the contrary to the androgen-alone administration, the hormonal complex did not disturb the decidual reaction and the pubs did not show any morphological abnormality. It is suspected that the complexity of communication between other steroid hormone and their receptors are the reasons. In summary, androgen exists in female blood and it suggests the importance of androgen in female reproduction. However, the complex interactions with other hormones are not fully understood compared with estrogen and progesterone. The further studies to evaluate the possible role of androgen are needed and important to provide the *in vivo* rational for the prevention of associated pregnancy complications and help human's health.

Key words : Androgen, Androgen receptor, Uterus, Decidualization, Hormonal complexes.

The regulation of proliferation and differentiation in uterus is mainly accomplished by traditional sex steroid hormones. This hormonal regulation is achieved through the control of the levels of circulating hormones, the local ligand availability modulated by metabolizing enzymes expressed within endometrial tissue, and the amount of their specific receptors. Estrogen and progesterone are pivotal in uterine responsibility to embryo implantation and maintaining the pregnancy. Estrogen and its receptors (ERs) are involved in proliferation of epithelial cells and progesterone receptor (PR) expression regulation. In rodent, estrogen is a triggering signal of embryo implantation as seen in protocol for delayed implantation model (Brandon, 1993). On the other hand, progesterone and PRs are involved in differentiation. Progesterone is antiproliferative in the epithelium and involved in proliferation and differentiation of stromal cells (Cheon et al., 2002).

Estrogen surges appear just before ovulation and maintain low levels until just before delivery. In the case for progesterone, its level is continuously increased after ovulation and kept the increased levels during the pregnancy. Estrogen receptors and progesterone receptors are localized in luminal and glandular epithelial cells, stroma cells, and

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myometrium in the stage specific manners in uterus (Cheon et al., 2002). ER-alpha (ERα) is the predominant ER in the adult uterus and localized in epithelium, stroma and myometrium. ER-beta (ERβ) is expressed in both stroma and epithelial cells either alone or together with ERα (Weihua et al., 2002). PR is also localized in epithelium, stroma and myometrium as like ERs. Its intensity in these cells is dramatically changed by physiological status during pregnancy; PR localized both in epithelial cells and stroma cells on day 2, 3 and 4 of gestation, after then PR only localized in stroma cells (Cheon et al., 2002; Weihua et al., 2000).

In addition to estrogen and progesterone, some other factors including endocrine and paracrine factors are also known as regulating factors for uterine preparation during pregnancy. Interestingly, in female blood, high level of androgen is detected during estrus cycle and pregnancy. In addition, androgen receptors (ARs) are expressed in breast, ovary and uterus (Castracane & Asch, 1995; Ruizeveld de Winter et al., 1991). In 5α-reductase-deficient mice and in testicular feminized female (Tfm/Tfm) mice, the defects in parturition and the accelerated ovarian aging are appeared, respectively (Lyon & Glenister, 1980; Mahendroo et al., 1996). ARs were also observed in corpus luteum (CL) throughout gestation and it contributes to the maintenance of function of the CL with lactogenic hormones in pregnant rodent (Szoltys et al., 2007).

From the slight view of the functions of androgen in female, it is become clear that androgens are functional molecule in female reproductive tracts with estrogen and progesterone. However, its roles in female reproductive physiology still largely remain obscure but its role is important to understand the physiological phenomenon in uterus during pregnancy and reproductive cycle. One of the interesting focuses of research is the differentiation of uterine cells during embryo implantation in medical and basic sciences to understand the biological role and to overcome the related diseases. Therefore, in this review we focused on the androgen in uterus to help the understanding of possible role of androgen in female reproduction.

SYNTHESIS OF ANDROGEN IN FEMALE

Androgens in female mammals are synthesized in ovary and adrenal gland both before and after menopause (Burger, 2002; Rittmaster, 1995). Also the elongating blastocysts and placenta are source of androgens in some species of mammals (Spencer & Bazer, 2004). In female, androgens are detected in blood during reproductive cycle and its levels in target organs are controlled through counter-current transfer as like the other steroid hormone. High levels of androgens are detected in the uterine environment during early pregnancy, a seemingly common phenomenon among mammals, including primate, rat, mice, pig etc (Bonney et al., 1984; Stone & Seamark, 1985; Slomczynska et al., 2008; Stefańczyk-Krzymowska et al., 1998).

Testosterone is the major circulating androgen in female, exhibits cyclical blood serum levels during the menstrual cycle, peaking around ovulation (Dawood & Saxena, 1976; Stahl et al., 1976). Tissue androgen levels and conversion of androstenedione to testosterone are higher in secretory phase than proliferative endometrium (Bonney et al., 1984). Moreover, a rise in circulating androgen levels in the late luteal phase is associated with a conception cycle and levels continue to rise in early pregnancy (Castracane et al., 1998). During pregnancy, androstenedione significantly reduces with the decline in luteal weight (Castracane & Asch, 1995). On the other hand, testosterone levels are decreased after menopause about half of youth peaks (Bancroft & Cawood, 1996).

EXPRESSION OF ANDROGEN RECEPTORS IN UTERUS

Androgens are transcription factor in the target cells through its intracellular receptors, and the evaluation of the expression and expression regulation are important to understand the role of androgen. From the studies in

various species, it is revealed that ARs expression is time dependent and cell-specificity during reproductive cycle and pregnancy (Ruizeveld de Winter et al., 1991). Subcellular localization of AR is cytoplasm and karyoplasms (Duda et al., 2004). In the presence of androgen, AR is mainly localized in karyoplasms but androgen-AR complex shuttles between the nucleus and the cytoplasm. In the uteri of pregnant pig, AR is localized in the nucleus of the epithelial cells stromal cell and muscle cell. It is not detected in luminal epithelial cell except for very weak staining on day 10 (Slomczynska et al., 2008). During highest expression in the endometrium at peri-implantation localization of AR is over-lapped with that of ERα.

AR is localized in the epithelium, stroma and myometrium of uterus. During pregnancy, endometrial levels of AR mRNA and protein are greater at early than at mid- or late-pregnancy. In rat, AR is localized exclusively in the stroma and in the myometrium, where it colocalized with ERα but not with ERβ (Pelletier et al., 2000; Weihua et al., 2002). In canine, there is a basal expression of AR in the uterus throughout the estrus cycle. AR is observed in cells of the surface epithelium, glandular ducts, basal glands and stroma of the endometrium and in myometrial smooth muscle cells. In some case, AR is localized much strongly in the cytoplasm of epithelial cells from proestrus to early metestrus, when the cells were secretory active, in stromal cells during pregnancy and in the postpartum period. During the estrus cycle stroma cells are stained with higher intensities for AR than epithelial cells (Vermeirsch et al., 2002). In porcine, AR is detected during estrus cycle, early pregnancy and periimplantation period (Cardenas & Pope, 2003; Kowalski et al., 2004; Slomczynska et al., 2008). AR mRNA is detected in the endometrium up to day 18 p.c., but after then it is not detectable. On the other hand, AR is detected strongly in glandular epithelium and stromal cells at through day 90 of pregnancy. AR is also detected in the myometrium but on day 90 the immunostaining is present only in a limited number of cells. In glandular epithelium, AR is consistently higher than in the other cell types and the intensity of nuclear staining is stronger than in stroma and the myometrium, whereas the number of stained cells is dramatically decreased compared with the stroma and myometrium (Cardenas & Pope, 2003; Kowalski et al., 2004; Slomczynska et al., 2008). In human and primates, during the normal cycle AR is expressed strongly in stromal cells. As in the other species, in human, AR is localized in epithelium, stroma and myometrium, and AR mRNA expression fluctuates during menstrual cycle (Mertens et al., 1996, 2001). AR levels increase during the proliferative phase followed by a decline during the secretory phase with less protein detectable in the secretory epithelial cells (Mertens et al., 1996, 2001). During early pregnancy ARs are detectable in the deciduas (Milne et al., 2005). Progesterone specific antagonist treatment enhances stromal AR and greatly increased AR expression in the glands (Slayden et al., 2001). From those, although the preimplantation period and reproductive period are different from between species, we know that AR is expressed commonly in the endometrium and mainly temporally localized in stroma cells, and in decidualizing stromal cells commonly in mammals.

Expression regulation of AR during menstrual cycle in mammals including human is controlled by changes in systemic levels of estrogen and progesterone (Slayden et al., 2001). Basically the expression regulation of AR is related with the steroid hormones but paracrine regulation is also known (Cardenas & Pope, 2003; Mertens et al., 1996; van Loon et al., 1988). Estrogen and androgen up-regulate the AR expression level (Apparao et al, 2002; Cardenas & Pope, 2003). Progesterone and epidermal growth factor (EGF) suppress the AR expression in both epithelium and stroma (Slayden et al., 2001). However, interestingly AR mRNA levels are induced weakly or no by estrogen alone in ovariectomized model but it are dramatically induced by combined treatment of estrogen and testosterone (Slayden & Brenner, 2004). EGF can control the melanoma antigen gene protein-11 (MAGE-11), a coregulator of AR, activity in endometrium (Bai &

Wilson, 2008).

Transcriptional activity of AR depends on two critical domains, activation function 1 (AF1) in the N-terminal region and activation function 2 (AF2) in the ligand-binding domain (Heinlein & Chang, 2002). MAGE-11 functions as an AR coregulator by binding the AR N-terminal FXXLF motifs (Bai et al., 2008). MAGE-11 increases the AR transcriptional activity through increased AR stabilization and SRC/p160 coactivator (SRC1, TIF2 and AIB1 9SRC3) recruitment. MAGE-11 binds on the N-terminal transactivation domain and increases AR transcriptional activity during the establishment of uterine receptivity in the human female (Bai et al., 2008).

Interesingly, estrogen can inhibit the expression of MAGE-11. The importance of ERα-mediated down-regulation of genes is that it is observed at the period of establishing the timing of receptivity to implantation (Somkuti et al., 1997). The opposing actions of cAMP and estrogen on AR and MAGE-11 appear to provide a closely linked regulatory mechanism for controlling AR transcriptional activity in the cycling endometrium. The actions of these signaling molecules occur within a context of inhibitory progesterone effects on estrogen and androgen signaling and the influence of growth factors and hormones in a dynamic interrelationship between the stroma and epithelium (Lessey, 2003; Jabbour et al., 2006).

PHYSIOLOGICAL ROLE OF ANDROGEN IN UTERUS

From the profiling of AR expression and expression regulation, it has been expected that androgen has function in female reproduction. In actual fact, reduced fertility in AR null mouse is associated with prolonged estrus cycles and diminished endometrial growth (Hu et al., 2004). High plasma androgen levels are associated with adverse reproductive outcome, including recurrent miscarriage (Bussen et al., 1999). Those findings give confidence about the hypothesis that androgen and its receptor have important roles in female reproduction. In addition, the uterus is a very sensitive target organ to steroid hormones and it has been suggested that androgen may play an important role in morphological and functional changes of endometrium.

Hormonal regulation of function in target cell is generally regulated by counter-current transfer that locally increases concentrations of hormone and its regulatory substances, coregulators and the levels of its receptors. The biological roles of androgen are mediated through its receptor (AR). The primary function of AR is a ligand-activated transcription factor (Lee & Chang, 2003). Ligand-bound AR regulates gene expression by binding to hormone response elements in the transcriptional machinery. Testosterone diffuses into target cells and activates the receptor directly or can be converted to 5α-dihydrotestosterone (DHT). Androgen-AR complex forms homodimers and binds to the regulatory regions of target genes to either promote or inhibit transcription. In males, AR is expressed in reproductive organs such as prostate, in which AR activity in the secretory epithelial cells and surrounding stromal smooth muscle cells is required for its development, maintenance and function (Abate-Shen & Shen, 2000).

The known work of androgen is the modulation of both myometrial and endometrial growth in uterus. It promotes the growth and differentiation of the uterus in a manner similar to, yet distinct from, estrogen. In ovariectomized rodent, androgen administration induces the increase of the uterine weight to the intact rats. In epithelium, it modestly increases epithelial cell height and antagonizes the tropic effects of estrogen but unlike estrogens may take several weeks to reach full efficacy (Nantermet et al., 2005). Ligand-activated AR is able to interact with the ERα forming heterodimers and result in decrease the transactivational activity of ER (Armstrong et al., 1976; Panet-Raymond et al., 2000). In epithelium AR directly mediate the antiproliferative effects of androgens (Tuckerman et al., 2000) and antiandrogens inhibit the effect of estrogen on proliferation in rats (Weihua et al., 2002) and nonhuman primates (Slayden & Brenner, 2004). In stroma, androgens are similar to

estorgens when act positively on stroma-epithelail interaction. In myopetrium, androgens induce gene expression more extensively than in endometrial cells. Interestingly, the effects of AR on the myometrium are not blocked by estrogen, while mibolerone antagonized the full action of estrogen. Thus AR-selective agonists induce uterine weight increase in ovariectomized rats. Both AR and ER influence the expression of genes involved in the signal transduction cascade and exert many effects in this manner rather than by direct activation of transcription by steroid hormonal receptor (Nantermet et al., 2005). From them, it is clear that androgens regulate the tropic environment and architecture of the rodent uterus via a gene expression program in pregnant uterus (Nantermet et al., 2005).

Androgen-AR activation can modify the estrogenic gene expression profile which reconstitutes the tropic environment required for stroma and epithelial growth and differentiation (Nantermet et al., 2005). Estrogen produces profound changes in the gene expression profile of uterine tissue, with nearly all of the 503 regulated transcripts responding. And the majority of transcripts significantly regulated by DHT are included within this set of estrogen-responsive genes. Of the transcripts regulated by DHT, 85.9% are altered in qualitatively the same manner by estrogen (Nantermet et al., 2005). Therefore, although broadly similar in estrogen and androgens abilities to stimulate uterine growth, important differences between the two hormones are clearly exist.

During endometrial preparation for implantation, complicated interactions take place between the epithelium and stroma areas (Nantermet et al., 2005). The ligand-bound AR interacts with ERα and these are regulating the endometrial gene expression and uterine growth for embryo implantation (Duda et al., 2004; Kowalski et al., 2004). Estrogen in the presence of ERα can activate transcription from AP-1 (activator protein-1) sites, while tamoxifen can activate AP-1 site via ERβ (Paech et al., 1997). In uterine proliferation, ERβ is suspected that it does not involve in epithelial cell proliferation and ARs do not colocalized in uterine cells. It is usually assumed that the ER- and AR-dependent pathways are sequential stems in one pathway (Fujimoto et al., 1994, 1995; Weihua et al., 2002). For example, in estrogen-induced epithelial cell proliferation, ERα induces stromal AR and AR amplifies the ERα signal by induction of IFG-1.

On the other hand, paradoxically, androgen can inhibit the action of estrogen in the cellular levels in both *in vitro* and *in vivo*. Systemic treatment with dihydrotestosterone blocked the stimulatory effects of estrogen on ER and PR levels and endometrial mitosis. Androgen blocks estrogen action in the oviduct and uterus in ovariectomized macaques (Hirst et al., 1992). This antiestrogenic effect is also observed in human endometirum. Androstenedione inhibits human endometrial cell growth and secretory activity *in vitro* when the medium contained phenol red in amounts adequate to provide an estrogenic stimulus (Rose et al., 1988; Tuckerman et al., 2000). This antiestrogenic effect of androgen is mediated by AR (Blasberg et al., 1998; Nanterment et al., 2005). This can be illustrated by the examination of Cardenas and Pope (2003), down regulation of ERs, particularly ERα in the endometrial stroma and myometrium, is a mechanism in the antagonism of estrogenic effects of DHT in uterus. It is also suggested that one of the reasons for antiproliferative effects of androgen to any estrogen-dependent stroma-epithelial interactions involved in glandular mitosis is the high androgen-sensitivity of stroma (Brenner et al., 2002).

Androgen also has relation with progesterone in uterus. During the decidual differentiation in human androgen has important role in endometrial differentiation (Cloke et al., 2008; Talbi et al., 2006). Antiprogestins inhibit estradiol-stimulated endometrial growth in women and nonhuman primates through up-regulation of ARs expression. In decidualizing cells, small ubiqitin-like modifier (SUMO)-1 suppresses the posttranslational modification of AR and is accounted for increased responsiveness to androgen. ARs regulate the expression of distinct decidual gene networks from progesterone. In comparison to PR, AR governs the

expression of a limited decidual gene pool, responsible for cytoskeletal organization and inhibition of cell motility and proliferation (Cloke et al., 2008). These cell functions under AR control may be critical for coordinated trophoblast invasion and placental development. Using the AR-deficient mice model, the possible roles of AR in uterus are more cleared (Hu et al., 2004).

An AR coregulator, MAGE-11 is expressed in a temporal fashion in endometrium of normally cycling women. Highest levels of MAGE-11 mRNA and protein occur in the mid-secretory stage, coincident with the window of uterine receptive to embryo implantation. MAGE-11 binds on the N-terminal transactivation domain and increases AR transcriptional activity during the establishment of uterine receptivity in the human (Bai et al., 2008). From these it is revealed that androgen-dependent transcriptional event is important for developing and maintaining the window of receptivity to embryo implantation (Bai et al., 2008).

Delayed implantation can be initiated by testosterone propionate (TP). Dihydrotestosterone is also able to initiate implantation in the delayed-implantation model. A high dose of TP causes aberrant expression of prostaglandin endoperoxide synthase 2 and microsomal prostaglanding E synthase at implantation sites after delayed implantation. It is hypothesized that high doses of TP may disturb peri-implantation development or may be involved in early pregnancy loss by disturbing the uterine prostaglanding system (Diao et al., 2008). Androgen specific antagonist, hydroxyflutamide delay the initiation of implantation, fetal development, and parturition in pregnant rats and suppressed decidualization in pseudo pregnant rats (Chandrasekhar et al., 1990).

In addition (to) IGF-1, it is also explored that testosterone induce the expression of HOXA10 needed in embryo receptivity of the uterus. Testosterone also prevents the increased expression of HOXA10 which is induced by estradiol or progesterone (Cermik et al., 2003). In human endometrial stroma cell, testosterone significantly inhibits MMP-1 expression through AR in both cultured media and cell lysates in a dose-dependent manner (Ishikawa et al., 2007).

DEFECTS ASSOCIATED WITH THE ANDROGEN AND ANDROGEN RECEPTORS IN UTERUS

Interestingly, critical defects have been detected in androgen-related conditional model animals. In here, we described only a few phenomena concerned with pregnancy. Both lack and excess of circulating androgens in premature ovarian failure and polycystic ovary syndrome, respectively, are associated with increased risk of early fetal loss and late obstetric complication due to impaired placental function, such as preeclampsia (Abdalla et al., 1998; Castracane & Asch, 1995; de Vries et al., 1998). High levels of serum androgens combined with the AR in the endometrium have been associated with poor fertility. Elevated androgens in women with recurrent miscarriages may specifically antagonize estrogen action directly in the endometrium (Okon et al., 1998). Decreased levels of glycodelin secretion are associated with the hyperandrogenism during the secretory phase (Okon et al., 1998).

Exogenous androgen can have inhibitory effects on female reproductive systems, including induction of endometrial atrophy (Futterweit & Deligdisch, 1986; Miller et al., 1986). It has been suggested that during normal cycles any androgen effects on the endometrium would be mediated by the stroma and that, during progesterone antagonist, elevated AR could play some roles in the endometrial suppressive effects induced by progesterone antagonist.

Androgen replacement therapy has been proposed in postmenopausal women to improve several age related problems, such as reduced sexual function, cognitive decline, hot flashes, and depression, and osteophorosis (Braunstein, 2002; Burd & Bachmann, 2001; Greene & Dixon, 2002; Khosla & Bilezikian, 2003). It is also used to for the management of endometriosis (Redmond, 1998), hirsutism (Carmina, 2002), and in contraception (Schneider, 2003).

CONCLUDING REMARKS

Androgen replacement therapies have been applied to improve the physiological strength but the side effects also have been reported and showed negative effects. Recently we examine (d) the effects of hormonal complexes including androgen (testosterone and its other metabolites such as 19-nortestosterone) and estrogen. As depicted from above, the effects of androgen on uterine endometrium during pregnancy is complex because the expression of its downstream genes can be modulated by the hormonal resume. 19-nortestosterone alone disturbed the rhythmicity of estrus cycle when it was treated continuously. However, interestingly, exogenously given hormonal complexes did not show any developmental disturbance throughout pregnancy. In addition the uteri primed with hormonal extracts can respond normally to the artificial decidual stimulation (Cheon et al., unpublished data). It may be the results of complex communication between hormones by the hormonal regimes at the specific physiological condition.

Put together with our results, we can prospect the importance of androgen in female reproduction. For example, the blood level of androgen is one of criteria of polycystic ovary syndrome. PCO contain increased numbers of preantral follicles with a specific increase in primary follicles. Testosterone increases the number of primary follicles through suppression the atresia (Qureshi et al., 2008). Increased androgen level also induces increase of AMH production in granulose cell and inhibition of inhibin-B production (Andersen & Lossl, 2008). Using AR-deficient mice and the high throughput methodology, the downstream genes of androgen in uterus are identified and the possible roles are exposed recently.

The identification of human AR signature genes could be exploited to assess the decidual responses before pregnancy, especially in patients with relative androgen deficiency, including older women and patients with premature ovarian failure receiving fertility treatment with donor oocytes. Such translational studies may provide AR modulators for the prevention of associated pregnancy complications.

In summary, ARs are expressed predominantly in mammalian endometrial stroma throughout the reproductive cycle. The roles and expression of androgen and AR are under the control of themselves, estrogen, progesterone and other factors. It is now clear that the role of androgen and its receptors is also important factors in differentiation of uterine endometrium. It seems that AR-mediated effects would occur mainly through the stroma. AR-mediated effects of normal levels of endogenous androgens would be most prominent in the proliferative and periovulatory periods. Besides, androgen levels and AR in female also showed relationship with various abnormalities. But the functional role of androgen and androgen receptor still remain to be determined. Thus it remains an important challenge to determine the function of uterine AR to improve and dissolve the current problems in female reproduction and development.

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