Morpho-physiological features associated with menopause: recent knowledge and areas for future work

IPYANA H. MWAMPAGATWA1*, MASUMBUKO Y. MWASHAMBWA2 and AINORY P. GESASE3

1Department of Maternal Health, School of Medicine, University of Dodoma, Dodoma, Tanzania
2Department of Surgery, School of Medicine, University of Dodoma, Dodoma, Tanzania
3Department of Biomedical Sciences, School of Medicine, University of Dodoma, Dodoma, Tanzania

Abstract: Menopause is the permanent cessation of menstruation resulting from loss of ovarian follicular activity which happens as a result of depletion of primary follicles which is basically an aging effect. Depletion of ovarian follicles is reflected as declined production of oestradiol which is currently known to be central to the morphologic and physiological changes that happen during the climacteric, menopause and post menopause periods. The cessation of oestradiol production is much more pronounced in tissues with oestrogen receptors such as bones, brain, blood vessels, central nervous system and the skin. But generally little is known on the subject and in particular the bioactive substances involved in the process such that there are some symptoms that menopause women experience which not only defy clinicians but also challenge the management of the condition. This article is presented to shade light to what is currently known, what is not known and stimulate future research which may reveal more understanding and advance our knowledge on management of women throughout the climacteric and menopausal periods.

Keywords: menopause, menstruation, ovarian follicles, oestriadiol, inhibin, mast cells

Introduction

Menopause is defined as the point in time when menstrual cycles permanently cease due to the natural depletion of ovarian oocytes from aging. The diagnosis is typically made retrospectively after the woman has missed menses for 12 consecutive months. It marks the permanent end of fertility and the average age of menopause is 51 years (WHO, 1996; Butler & Santoro, 2011; Santoro & Randolph, 2011). Research into why menopause occurs is centred mainly on ovaries because of abnormal follicular development and depletion of ovarian follicles (Judd & Furnet, 1994). The current view is that the follicles fail to develop because the ovaries become insensitive to follicle stimulation (FSH) hormone. However, recent findings have suggested the role of inhibin B and possibly other local paracrine factors in the recruitment and regulation of follicular development (Findlay et al., 2001).

Although the major symptoms of menopause is cessation of menstrual flow, irregularities in hormonal serum levels during perimenopausal period is thought to bring silent morphological and physiological changes in the body before the time of onset menopause. Systems that are commonly affected include the bones, blood vessels, heart, brain, urinary tract, gastrointestinal tract, skin and reproductive system (Nicks et al., 2010). The morphological changes that occur are commonly associated with complaints that in some cases need medical attention ((Burger, 1999; Nicks et al., 2010). Knowledge into such changes in various tissues is important not only for the understanding of the biology of menopause but also may have treatment implications and may be useful in counselling the woman on the expected body changes. This article reviews the cell biology of menopause and the associated morphological features of reproductive organs after menopause. The information is aimed at stimulating research on menopause that will add knowledge on the pathophysiology of menopause.

* Correspondence: Ipyana H. Mwampagatwa; E-mail: mwampagatwa@yahoo.com
Ovaries and the biology of menopause: the role for inhibin in the journey to menopause

Ovaries are paired organs situated on either side of the uterus and their primary functions are to release an oocyte during ovulation and secondly they secrete the female sex hormones. On cross section each ovary consists of the superficial ovarian cortex, the inner medulla and the hilus (Leeson et al., 1988). The ovarian cortex is made up of two components, namely the stroma and follicles. Presence of follicles makes the cortex to be larger than the medulla. The stroma of the cortex is basically the same as that of the medulla. It has amount of loose connective tissue consisting of nerve fibres, lymph vessels, blood vessels and numerous stromal cells also known as interstitial or fibroblast-like cells (Leeson et al., 1988). Stromal cells secrete small amounts of androgens and may differentiate into a variety of other cell types such as follicular theca internal cells, luteinizing stromal cells, enzymatically active stromal cells, smooth muscle cell, decidual cell, endometrial stromal type cells, adipocytes and stromal Leydig cells (Moore, 1988). Embedded within the cortical stroma are ovarian follicles; at birth there are nearly 2 million follicles in the cortex, however, they gradually undergo atresia throughout the life of a woman and at puberty there are about 400,000 follicles in the cortex for reproductive life (Moore, 1988).

Following puberty the ovaries begin to produce a single mature oocyte each month up to menopause. During each menstrual cycle 2-20 follicles are stimulated to grow, however, only one of these follicles attains full maturity and ovulate, the rest will degenerate by the process of atresia (Gosden & Faddy, 1994). The long held view is that development of ovarian follicle is controlled by the gonadotropins (Follicle Stimulating Hormone, FSH and Lutenizing Hormone, LH) secreted by the adenohypophysis. However, recent studies have suggested the presence of intrinsic ovarian paracrine system that trigger early development of follicles; and that the gonadotropins comes in late during the final maturation stages of follicles and ovulation (Buffet et al., 1998; Findlay et al., 2001). Studies that have utilized gene manipulation technique showed that early follicular development (from primordial to antral follicles) proceeded normally in animals that lack FSH and/or FSH receptors (Buffet et al., 1998). Follicular development is characterized by the growth of an oocyte and proliferation of the surrounding cells to form glandular tissue that secrete oestrogen and progesterone. These hormones maintain the morphological integrity of many organs in the body and they also prepare reproductive system for copulation, fertilization, implantation and initial growth of the embryo (Moore, 1988).

Under normal circumstances the process of follicular development occurs cyclically every month during the reproductive life, but as menopause approaches it becomes irregular up to the time when it stops completely (Richardson, 1993; Judd & Fiurnet, 1994; Prior, 2002). Abnormal fluctuations of sex hormones lead to gradual morphologic and physiological changes to most organs in the body. Events that precede menopause and pathways that lead to failure in follicular development are not clearly understood. It has been suggested that as the woman age advances the follicles are depleted from the cortex due to repeated ovulation and atretic change, and that ovarian sensitivity to FSH also decreases (Richardson, 1993; Gosden & Faddy, 1994). Similarly, it is widely believed that gradual depletion of the ovarian follicles impairs hormonal secretion, which further accelerate menopause (Richardson, 1993). However, the number of follicles that must remain in the ovaries for the maintenance of normal development to full maturity is not known. Whether the development of one ovarian follicle is dependent on the presence of a specific number of established other follicle has not been discussed.

Attainment of menopause is a gradual process and the journey is characterized by menstrual irregularities, ovulatory and anovulatory cycles up to the time when menstrual flow stops completely (Prior, 2002). This indicates that molecular pathways that control the menstrual cycles are gradually depleted from the ovarian cells. Studies on the hormonal status in women have indicated that
Menopause is associated with low serum levels of oestrogen, inhibin, insulin-like growth factors-I (IGF-I), anti-Mullerian hormone (AMH) and elevated serum levels of FSH (Klein et al., 2000; Sowers et al., 2008). AMH has been suggested to be an endocrine marker of follicle depletion or ovarian aging (Overlie et al., 2005; Sowers et al., 2008). Inhibin is a heterodimer containing α and β subunit (inhibin α and β) and it belongs to the TGF-β superfamily of proteins (Overlie et al., 2005). Inhibin B is secreted by the granulosa cells during early follicular development and in recent years it has attracted much attention because of its role in the regulation of early follicular development and secretion of FSH by the adenohypophysis (Messinis, 2000; Findlay et al., 2001; Jaatinen et al., 2002). Studies done in girls from birth to adulthood indicated that serum levels of inhibin are low below the age of 6 and reaches a peak between 12-18 years when the girls attain menarche (Bergada et al., 2001; Crofton et al., 2002).

Observations made in the elderly women showed that inhibin serum levels were lower in the women aged 46-52 years than in women aged 39-45 years (Seifer et al., 1999). The study also revealed that these women were still menstruating and the serum levels of FSH were normal, there was no difference between the old and younger woman. Putting together the findings indicate that abnormalities in inhibin secretion (i.e. low serum levels) occur much earlier when serum FSH levels are still normal (Richardson, 1993; Burger, 1999; Seifer et al., 1999). An indication that low inhibin levels remove the inhibitory effects to the pituitary gland, leading to elevated FSH serum levels (Ala-fossi et al., 1998, Buffer et al., 1998).

During reproductive life secretion of FSH is cyclical, with serum levels varying from low to high; this character maintains the sensitivity of follicular cells to FSH. Chronic elevation of FSH and prolonged exposure of ovarian cell to FSH may cause down regulation of the receptors, making the follicular cells insensitive to FSH leading to failure of the final maturation of ovarian follicles. This may explain anovulatory cycles that characterize perimenopausal period (Vagenakis, 1989). Chronic exposure of receptors to ligands has been shown to have a negative effect on the receptors expression (Alberts et al., 1994). Why inhibin levels decrease in women approaching menopause is not clear, it is possible that there may be local factors secreted by granulosa cells or the oocyte that may regulate inhibin secretion. Factors that control the secretion inhibition during reproductive life are still debatable; a few researchers have concentrated on the possible role of FSH (Messinis, 2000; Welt & Schneyer, 2001). However the relationship that exists between FSH and inhibin during perimenopausal period and at menopause does not strongly support the role of FSH in the regulation of inhibin secretion. At menopause low serum levels of inhibin occurs when the FSH levels are normal and they remain so even after menopause when there high serum level of FSH, an indication that factors other than FSH are responsible for the regulation of inhibin secretion.

Past studies have indicated the presence of local factors such as Insulin-like growth factor-I (IGF-I) in the control of inhibin secretion (Klein et al., 2000; Welt & Schneyer, 2001). During reproductive life IGF-I is expressed in the endometrial cells and in the ovarian tissue and studies have showed that in the ovaries it enhances proliferation of granulosa cells and secretion of steroid hormones (Messinis, 2000; Conover et al., 2001). Other observations have showed that as women approach menopause the levels of IGF-I decreases from ovarian tissues, and this is followed by a gradual decline in serum levels of inhibin B (Klein et al., 2000; Welt & Schneyer, 2001). Low IGF-levels have also been reported in the endometrium of postmenopausal women (Leone et al., 1993; Liverro et al., 1999). It is therefore suggested that IGF-I may act as a paracrine factor that enhances the secretion of inhibin B during reproductive period and that declining levels of IGF-I in ovarian tissues may lead to failure of inhibin B secretion by granulosa cells. It is known that the oocyte plays an important role in controlling its own fate by influencing follicular cell function.

Studies done in mice have showed that mice lacking oocyte secreted differentiation growth factor-9 (GDF-9) become infertile due to failure of follicular cells development and oocyte maturation.
GDF-9 is a protein that in human is encoded by the GDF9 gene (McGrath et al., 1995), it plays an important role in the development of primary follicles in the ovary. It has critical role in granulose cell and theca cell growth, as well as in differentiation and maturation of oocytes (Su et al., 2004). GDF-9 has been associated in premature cessation of ovary function, therefore has a significant role in fertility (McNatty et al., 2007).

**The uterus and menopause: the role of mast cells**

The uterus is the largest reproductive organ that is capable of undergoing the hormonal induced growth in order to accommodate the developing foetus. Its wall is made up of three layers; the outer perimetrium, the middle muscular layer (myometrium) and the inner mucosal layer (endometrium). Oestrogen and progesterone are known to influence development and growth of the uterus during menstrual cycle and during pregnancy; in the absence of sex hormones it becomes atrophic. The endometrium is a complex mucus membrane that undergoes cyclical changes in preparation for implantation and in the absence of pregnancy the functional part is shed off as menstrual flow. Ultrastructurally the endometrium is made up of the surface epithelium and a wide endometrial stroma that is continuous with the underlying layer of smooth muscles. Surface cells are columnar in shape and form a single layer of cells; some are ciliated while others are non-ciliated (Baraggino et al., 1980). Ciliated cells are numerous and beating of the cillum facilitates mobilization and distribution of endometrial fluid in the uterine cavity. None-ciliated cells secrete mucus and their luminal surface is densely covered with long microvilli, this increases the surface area of the endometrium. The luminal surface may show apical protrusions suggesting the occurrence of apocrine secretion.

Secretory cells also extend deep into the stroma where they line the endometrial glands (Leeson et al., 1988). The endometrial stoma contains the endometrial glands, extensive capillary network and fibroblast-like cells that are embedded in connective tissue network of collagen fibres. It is the most dynamic portion of the endometrium; it can grow up to 7mm thick during menstrual cycle. For the purpose of description it is divided into the basal and the superficial functional zone. The stroma of the basal zone is cellular and fibrous than the functional zone and it does not undergo cyclic changes during menstrual cycles. It serves principally as a source of tissue for the cyclic regeneration of the functional layer. The functional zone is superficial to the basal zone and it is subjected to cyclic changes that are brought about by the oestrogen and progesterone secreted by ovarian follicles (Amos et al., 1994). Endometrial tissue shows cyclical expression of oestrogen and progesterone receptors during menstrual cycle, this enables the sex hormones to induce growth of the endometrium and secretory activities (Amos et al., 1994).

Menopause brings atrophic changes to the endometrium; these are gradual and become more marked as time after menopause advances (Delgdisch et al., 1978). The surface cells become cuboidal in shape and they gradually lose cilia from the apical membrane (Delgdisch et al., 1978). Non-ciliated cells become flattened, microvilli become short and blunt, and as the length of post-menopausal period increases they disappear from the cells. Menopausal endometrium does not show features of active proliferation or secretion. The cytoplasm of glandular and stromal cells becomes clear with a few organelles and immunoreactivity for oestrogen and progesterone receptors decreases. There is fibrosis of the endometrial stroma, which is caused by increased amount of collagen fibres (Delgdisch et al., 1978). This makes the menopausal endometrium less cellular and as a result the stromal volume decreases steadily to approximately less than 0.3mm (Zalud et al., 1993). There is a decrease in number of endometrial glands, which become oriented parallel rather than perpendicular to the surface cells. It has also been shown that menopause is associated with a decrease in vascular distribution and resistances to endometrial blood flow (Zalud...
et al., 1993; Bonilla-Musoles et al., 1995). Although oestrogen and progesterone are the main hormones that causes endometrial cyclical change during reproductive life, the endometrium has been shown to releases some active molecules such as decidual associated protein-200, insulin growth factor (IGF), transforming growth factor (TGF), Interleukin-8 (IL-8) and telomerase enzyme, which have been shown to enhance endometrial growth and maintenance (Leone et al., 1993; Liverro et al., 1999; Arici, 2002). However, how these growth factors are linked to sex hormones has not been clearly described. The interesting phenomenon about the endometrium is that whereas the ovaries cease to operate permanently after menopause, the endometrium retains the capacity to undergo proliferation and growth upon stimulation (Sauer et al., 1993; Liverro et al., 1999). This is a unique feature that is present in human species. In other mammals it is the uterus that fails to support nidation; production of oocytes continues well into old age (Finn, 2001). Postmenopausal endometrium has been shown to support implantation and growth of pregnancy to term (Sauer, 1993; Finn, 2001).

Receptors for some powerful growth factors have been labelled in the endometrium cell and some of them remain so even after menopause. Studies done in the endometrium of postmenopausal women revealed the expression of TGF-beta 1, telomerase activity and insulin growth factor-1 receptors in the endometrium cells (Leone et al., 1993). It is not clear as to why such molecules continue to be expressed in the endometrium after menopause (Kyo et al., 1997; Liverro et al., 1999). They may play a role of maintaining the quiescent differentiated state of atrophic postmenopausal endometrium (Liverro et al., 1999).

The myometrium contains mainly the smooth muscle fibres and it forms the bulk of the uterus, measuring about 1.5cm at the thickest point. The bundles of muscle fibres are separated by connective tissue and individual smooth muscle cells are long measuring about 60µm. However, during pregnancy they increase greatly in size and may attain a length of approximately 600µm. The muscle fibres are arranged into two layers; the stratum subvasculare, which lies below the endometrium and stratum supravasculare (Leeson et al., 1988; Noe et al., 1999). It is generally known that thinning of the myometrium occurs after menopause. However, the exact ultrastructural changes that occur in the smooth muscle and to the elements of the interstitial space and the pathway that lead to such changes are not well understood. Both the strata vasculare and supravasculare have oestrogen and progesterone receptors. Nonetheless, during reproductive life the stratum vasculare is much more dynamic than stratum supravasculare (Noe et al., 1999). The smooth muscle cell exhibits a cyclic pattern of sex hormones receptor expression that is comparable to those in the endometrium (Koshiyama et al., 1996; Noe et al., 1999).

Oestrogen is known to support and maintain the uterine smooth muscle cells and also to increase the amount of contractile proteins, irritability and spontaneous muscular activities. Most researchers have associated the low serum levels of sex hormones during menopause with atrophic changes in the myometrium (Zalud et al., 1993; Koshiyama et al., 1996). Judging from the morphological features of the myometrium it is unlikely that the declining serum levels of sex hormones alone can explain atrophic changes seen after menopause. Other factors may be present in the aging myometrium that may work in conjunction with sex hormones to bring about menopausal changes.

Generally white blood cells are seen in the walls of reproductive organs, and they include lymphocytes, neutrophils, macrophages and mast cells (Choroszewska et al., 1989; Crow et al., 1991; White et al., 1997; Utrearas et al., 2000). Mast cells are in particular abundantly distributed in the myometrium and they lie in close association with smooth muscle cells (Crow et al., 1991; Milne et al., 2001). Stem cell factor (SCF) and its receptors, which regulate mast cell proliferation and differentiation, have also been localized in the myometrium (Mori et al., 1997). Mast cells, like most other leukocytes are known to release growth factors that may enhance tissue proliferation and
maintenance (Skeper et al., 2001; Artuc et al., 2002). These include the nerve growth factor (NGF), fibroblast growth factor (FGF), epidermal growth factor (EGF), vascular growth factor (VGF) and serotonin (Azmitia, 2001; Ribatti et al., 2001; Skaper et al., 2001; Artuc et al., 2002). FGF is known to regulate the proliferation and function of many classes of cell and in particular it enhances proliferation and maintenance of smooth muscle cells (Alberts et al., 1994). EGF is a powerful regulator of growth, differentiation and matrix synthesis by connective tissue cells and it has also been shown to stimulate mitosis in many classes of cells. NGF is known to influence growth of neuritis in tissues; in this way it helps to sustain cell survival (Alberts et al., 1994). VGF has been shown to stimulate angiogenesis, an important process during tissue growth and repair (Alberts et al., 1994). All these morphological phenomena are relevant to the uterus during the reproductive period.

Observations made in the postmenopausal uterus indicated that mast cell population decreased significantly in the myometrium, and that the remaining few cells did not show secretory activities (Crow et al., 1991). It is possible that mast cells may play an important role in the maintenance and support of the myometrium during reproductive life (Mori et al., 1997). Decreasing mast cell population and absence of their secretory activities during menopause may deprive the uterus with important active molecules needed for growth and maintenance. However, to what extent the decreased mast cell population affect the postmenopausal changes in the uterus awaits future studies. Similarly, there is no data on the expression profiles of some these trophic factor after menopause. Answers to these questions may provide valuable information towards the understanding the menopausal changes in the myometrium.

**Fallopian tubes and the cervix after menopause**

The cervix and fallopian tubes allows the uterine cavity to communicate with the vagina and ovaries, respectively. The mucosa of the fallopian tube shows extensive folding that increase towards the ovarian side and is lined by ciliated and non-ciliated secretory cells (Donnez et al., 1985). Other cells that have been demonstrated include the antigen presenting Langerhans cell and the myofibroblasts (Hagiwara et al., 1998). Ciliated cells are columnar in shape and accounts for roughly 20-30% of mucosal cell; the cilia beat in synchronized waves in the direction of the uterus (Donnez et al., 1985). Secretory cells account for 55-65% of mucosal cells and their luminal surface contains numerous microvilli. Both ciliated and non-ciliated cells express oestrogen and progesterone receptors and the concentration of these receptors varies with the phase of menstrual cycle (Nielsen et al., 2001). Studies done in monkey indicated that the presence of cilia on the mucosal cells depends on the presence of oestrogen and that castration caused atrophy and gradual loss of cilia (Nielsen et al., 2001). The main function of mucosal cells is to provide favourable environment for the transport of gametes and zygote and to nourish the embryo, by releasing embryotrophic factors before it reaches the uterine cavity (Valasquez et al., 2001).

The molecular pathways that regulate postmenopausal changes in the fallopian tubes are not clearly understood because there is limited research information. A few studies have reported the presence of trophic factors such as Interleukin-8 (IL-8), epidermal growth factor (EGF) and transforming growth factor (TGF) in the fallopian tube (Palter et al., 2001). Experiment done in human fallopian tube indicated that IL-8 receptor showed regional variation, they were more expressed in the distal portion of the fallopian tube (Palter et al., 2001). IL-8 is mainly produced by the fibroblasts, epithelium, hepatocyte and macrophages; in human fallopian tubes, the receptors were predominantly seen in the epithelium (Palter, 2001). IL-8 belongs to the class of interleukin that are known to mediate local interaction between the white blood cells. It is a potent angiogenic factor and is also known to stimulate cell proliferation and survival directly and/or by inducing
secretion of potent growth factors such as fibroblast growth factor-2 (FGF-2) and Insulin growth factor-1 (IGF-1) (Arici, 2002). Presence of IL-8 receptors in the fallopian tube suggests a physiological role. However, how it interacts with sex hormones and its role in menopause is open for future work. Another phenomenon that needs to be clarified is whether postmenopausal fallopian tube can support the establishment of pregnancy upon stimulation. Such knowledge is important not only for the general understanding on the biology of menopause, but also it will shed light on fertility problem caused by failure of the fallopian tubes.

The exposed part of the cervix (ectocervix) is lined by a protective stratified squamous epithelium, which show three zones: the basal (germinal) cell zone that provide new cell to the overlying layers, the midzone and the largest outer superficial zone that contain cells that are constantly shed off in a process called desquamation. The half-life for these cells is between 4-5 days, and oestrogen has been shown to accelerate proliferation, maturation and desquamation of superficial cells. Studies have showed that after menopause the squamous cells become atrophic and maturation does not occur (Pandit & Ouslander, 1997). Thinning of the epithelial lining result into inadequate protection of the sub-epithelial tissue against trauma, this may cause frequent bleeding and inflammation commonly seen after menopause (Pandit & Ouslander, 1997). The mucosa of the endocervix is highly folded, forming complex deep furrows known as plicae palmatae. It is lined by a single layer of columnar cells consisting of mucus and a few non-mucus secreting cells. Studies have showed that secretory cells are influenced by sex hormones; and that cervical mucus is subject to profound cyclic changes (Pandit & Ouslander, 1997). Oestrogen induces profuse, watery and alkaline secretion via exocytosis and apocrine secretory mechanisms, which facilitate sperm transport across the cervix.

After menopause the mucosa wall of the cervix shrinks and the folded nature gradually disappear to become smooth. The columnar cells become flat, the cilia disappear and secretory features decrease causing dryness of the vagina, which may cause dyspareunia (Pandit & Ouslander, 1997). Other cells that have been demonstrated in the cervical wall include the macrophages, dendritic cells, lymphocytes and neuroendocrine cells (Fetissof et al., 1985). The neuroendocrine cells have been shown to express serotonin immunoreactivity, but their roles and the immunohistochemical profile after menopause are not understood (Fetissof et al., 1985).

Observations made in tissues other than the genital organs have showed that serotonin may regulate the secretion of mucus, contraction of smooth muscles, cell proliferation and cell migration and maturation (Fujita et al., 1988). Studies done in the urethra indicated that the neuroendocrine cell extend to reach the luminal surface and have synaptic contact with afferent nerves (Dixon et al., 1973). Because of this morphological relationship it has been suggested that the neuroendocrine cells may represent sensory devices for detecting information about the luminal contents, and via afferent nerves associated with them, lead to local reflexes, such as muscular contraction (Dixon et al., 1973; Fujita et al., 1988). Solitary cells with endocrine functions are widely distributed in the body and have been shown to release a number of substances such as gamma amino-butyric acid, serotonin, gastrin, secretin and cholecystokinin (Davanger et al., 1994). In the gut some of these molecules have been shown to play important role during development and also to enhance growth and maintenance of gastric mucosa and age related decline in endocrine cell population have also been documented (Fiorica et al., 2000 & Larsson, 2000). To what extent the neuroendocrine cells are involved in the physiology of the cervix during the reproductive life and after menopause remains to be an interesting subject for future research.

Conclusion
Though our current knowledge on the morphological and physiological changes of menopause has centred on the ovary and cessation of production of oestradiol by the ovarian follicles, there is mounting scientific evidence that there are other bio-active substances that may be responsible for the process and explain the changes and clinical presentation observed throughout the climacteric and post menopausal periods. Further studies on the subject in future may reveal what is currently not known and therefore have an impact on management of postmenopausal symptoms.

References


