



## Review article

## Sex hormones and stroke: Beyond estrogens

Farida Sohrabji\*, Andre Okoreeh, Aditya Panta

Women's Health in Neuroscience Program, Neuroscience and Experimental Therapeutics, Texas A&amp;M College of Medicine, Bryan, TX 77807, United States of America



## ARTICLE INFO

## Keywords:

Menopause  
Ischemic stroke  
FSH  
Testosterone

## ABSTRACT

Stroke risk and poor stroke outcomes in postmenopausal women have usually been attributed to decreased levels of estrogen. However, two lines of evidence suggest that this hormone may not be solely responsible for elevated stroke risk in this population. First, the increased risk for CVD and stroke occurs much earlier than menopause at a time when estrogen levels are not yet reduced. Second, estrogen therapy has not successfully reduced stroke risk in all studies. Other sex hormones may therefore also contribute to stroke risk. Prior to menopause, levels of the gonadotrophin Follicle Stimulating Hormone (FSH) are elevated while levels of the gonadal peptide inhibin are lowered, indicating an overall decrease in ovarian reserve. Similarly, reduced estrogen levels at menopause significantly increase the ratio of androgens to estrogens. In view of the evidence that androgens may be unfavorable for CVD and stroke, this elevated ratio of testosterone to estrogen may also contribute to the postmenopause-associated stroke risk. This review synthesizes evidence from different clinical populations including natural menopause, surgical menopause, women on chemotherapy, and preclinical stroke models to dissect the role of ovarian hormones and stroke risk and outcomes.

## 1. Menopause, estrogen deficiency and stroke outcomes

The importance of ovarian hormones as a risk factor for stroke is evident in comparisons of the incidence of female strokes before and after menopause. Premenopausal women have a much lower incidence of stroke as compared to young males, however at the menopause transition (ages 45–54), the incidence of stroke is double that of men (Reeves et al., 2008; Towfighi et al., 2007). In tandem with increased risk, stroke outcomes are also worse (reviewed in (Sohrabji, 2015)). Women account for 60% of stroke-related deaths (Lloyd-Jones et al., 2010), even after normalization for age. A Canadian stroke registry study reported that 10% of women stroke patients were discharged to long term care as compared to 5% men (Kapral et al., 2005), despite the fact that stroke size tends not to be different in males and females (Silva et al., 2010). Moreover, 5-yr stroke recurrence is disproportionately higher in females (20%) as compared to males (10%) in the 45–64 age range (Roger et al., 2011).

Increased stroke risk and severity among older women led to the hypothesis that the loss of ovarian hormones, principally estrogens, at menopause may be a contributory factor. However, analysis of hormone use and stroke incidence in pre and postmenopausal women does not support this conclusion entirely. For example, a multicenter case-controlled study showed that increased lifetime exposure to estrogen

was associated with a lower risk of stroke, supporting the idea that estrogens are beneficial (Alonso de Lecinana et al., 2007). In contrast, a case-control study in Northern California Kaiser Permanente facilities reported no benefit for stroke risk in postmenopausal women who took hormone therapy relative to those not taking hormones (Petitti et al., 1998). More ominously, the Women's Health Initiative (WHI) study, which had a significant impact on menopause medicine, concluded that hormone use actually increased stroke risk. This randomized, double blind, placebo-controlled multicenter trial compared the risk of myocardial infarction, stroke and dementia in women who consumed daily conjugated equine estrogens (CEE) (Hendrix et al., 2006), CEE + progestins (Wassertheil-Smoller et al., 2003) or placebo. Hormone therapy groups showed an increased risk for stroke; however, subgroup analyses indicated that most of this risk was seen in the older age groups. In the CEE trial, stroke risk was significantly elevated in the 60–69 year old group but not the 50–59 year old group (Hendrix et al., 2006). In an observational analysis of postmenopausal women in the Nurse's Health Study, estrogen and estrogen + progestin use increased the risk of stroke irrespective of the age of the user or time since menopause (Grodstein et al., 2008). However, the observational arm of the WHI study showed no increased risk for stroke in the CEE or CEE + progestin arm (Prentice et al., 2008; Prentice et al., 2006). A possible factor in the discrepancy between the WHI trial and the WHI observational study

\* Corresponding author at: Neuroscience and Experimental Therapeutics, MREB 4102 State Hwy 47, Texas A&M College of Medicine, Bryan, TX 77807, United States of America.

E-mail address: [Sohrabji@medicine.tamhsc.edu](mailto:Sohrabji@medicine.tamhsc.edu) (F. Sohrabji).

<https://doi.org/10.1016/j.yhbeh.2018.10.010>

Received 30 August 2018; Received in revised form 22 October 2018; Accepted 22 October 2018

Available online 20 November 2018

0018-506X/ © 2018 Elsevier Inc. All rights reserved.

was that the initiation of hormones was much earlier in the latter study. However other health characteristics among this group can also impact stroke risk in conjunction with hormone therapy (HT). In the observational trial (SHOW study) HT users were more likely to be normotensive and lean as compared to non-users in this study (Bushnell, 2009) which was not the case in the WHI study, where hypertension incidence was similar in CEE users and non-users (Hendrix et al., 2006). A similar interaction between HT and hypertension was seen in the Danish Nurses study, where normotensive women who used hormone therapy were not different from controls, while the risk for stroke was elevated among hypertensive women who used hormone therapy (Lokkegaard et al., 2003).

In addition to comorbid conditions, hormone treatment effects are also modified by the timing of treatment. Data from a prospective study of Swedish women showed that stroke risk was significantly decreased in women who initiated hormone treatment prior to menopause (Li et al., 2006). In a population-based nested case-control study of 50–69 year old women, HT did not significantly elevate ischemic stroke risk (Arana et al., 2006), further supporting the idea that HT at ages closer to the menopause may be harmless for stroke. Coronary artery calcification, a surrogate marker of cardiac disease, was reduced by estrogen in the youngest cohort of the WHI study (50–59 years) (Manson et al., 2007), also signifying that estrogen's effects can be modulated by the age of the user. Finally, a study of non-users of HT found that stroke-related mortality in women 65 and older was higher in women with higher levels of endogenous estrogen (Maggio et al., 2009), implying that elevated levels of hormones in late life, whether exogenous or endogenous, may exert a deleterious effect on stroke. The issue of timing of treatment was directly tested in the Kronos Early Estrogen Replacement study (KEEPS) (Miller et al., 2009) and the Early versus Late Intervention Trial with Estradiol (ELITE) study (Hodis et al., 2016). The KEEPS study was a prospective, randomized, controlled trial study where the primary outcome measure was cardiovascular risk measured by carotid intima thickness, coronary artery calcium, as well as other ancillary measures. Participants were women who were within 3 years of menopause and received either oral CEE or transdermal 17 $\beta$ -estradiol. (Miller et al., 2009) The study found that there was no difference in carotid intima thickness (CIMT) in the oral CEE, transdermal 17 $\beta$ -estradiol or placebo-treated groups. (Harman et al., 2014) Moreover, although hormone treatment did not affect cognitive function (Gleason et al., 2015), it improved sleep quality and vasomotor symptoms (Cintron et al., 2018). These findings suggest that hormone therapy for healthy, early postmenopausal women does not increase cardiac disease indicators. The ELITE trial tested the effect of oral 17 $\beta$ -estradiol treatment (with or without progesterone by vaginal gel) on early (< 6 years) and late (> 10 years) post-menopausal women. This study showed that progression of CIMT was influenced by the timing of estradiol treatment. Thus estradiol treatment to the early menopausal group had a lower rate of progression of CIMT as compared to placebo controls while CIMT measures in estradiol treated late post-menopausal group were no different from the placebo group (Hodis et al., 2016). The ELITE study therefore suggested a protective role for 17 $\beta$  estradiol for early menopausal females. Neither study examined stroke as an endpoint, but extrapolating from the CVD marker, these studies suggest that estrogens are not deleterious when given to women at the early stage of menopause. In summary, the evidence linking estrogen therapy and stroke risk, is modified by several intervening variables. The modification by 'age' (early or late menopause) suggest that other sex hormones may also influence this association.

## 2. If not estrogen, then what?

**The aging ovary:** The normal reproductive ovary secretes hormones under the regulation of two pituitary gonadotropins, follicle-stimulating hormones (FSH) and luteinizing hormone (LH) in a tightly regulated cycle (Hoffman et al., 2016) (Fig. 1). In this section, three

types of ovarian secretions, steroids, gonadal peptides, and growth factors, will be reviewed.

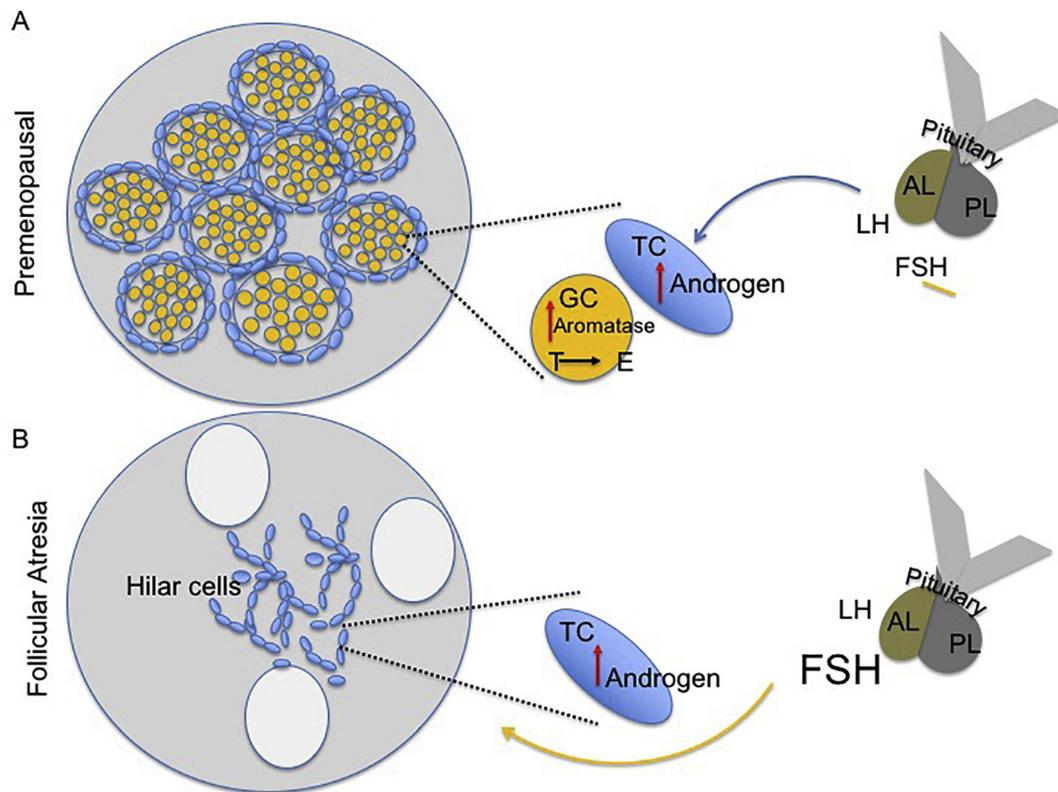
Ovarian steroids are essential for preparing the uterine endometrium for pregnancy (Alford & Nurudeen, 2013). Specifically, in response to LH stimulation, ovarian theca cells produce the androgens, DHEA, testosterone, and androstenedione, which diffuse into the granulosa cells (Jeppesen et al., 2012; Channing et al., 1980). The granulosa cells, in turn, upregulate aromatase in response to FSH to produce 17 $\beta$ -estradiol (E2) (Richards & Kersey, 1979) (Fig. 1). After ovulation, the corpus luteum forms, and its theca and granulosa cells increase its production of the other major sex steroid, progesterone (Niswender et al., 2000).

The ovarian peptide hormones regulate ovarian function and health. Inhibin, activin, and follistatin modulate pituitary gonadotropin release (Sharpe, 1982; Welt et al., 1999; Vale et al., 1990; Carroll et al., 1989). Insulin-like growth factors (IGF) -1 & -2 are produced by ovarian granulosa cells and are involved in follicle development (Hoffman et al., 2016; Hernandez et al., 1989; Mason et al., 1993; Oliver et al., 1989; Parikh et al., 2010). FSH also regulates IGF binding proteins and stimulates ovarian IGF-1 synthesis (Adachi et al., 1995). Anti-mullerian hormone (AMH) is produced by pre-antral and early antral follicles and reflect the approximate size of the primordial follicle pool and maybe the best biochemical marker of ovarian function (Peluso et al., 2014). AMH levels gradually decline with age and loss of the primordial follicle pool. At menopause, AMH is undetectable (La Marca & Volpe, 2006).

After menopause, the ovary becomes unresponsive to pituitary gonadotropins, and ovarian hormone production declines (Seifer et al., 1999). Estrone (E1), which is produced mostly by the aromatization of androstenedione in fat, replaces 17 $\beta$ -estradiol (E2) as the dominant circulating estrogen (Szymczak et al., 1998; Pasqualini et al., 1996; Batrinos, 2013). However, estrone levels are at lower quantities than premenopausal E2 (Grodin et al., 1973; Cauley et al., 1989). Ovarian testosterone production is preserved and regulated by the gonadotropin LH (Longcope, 2001; Ushiroyama & Sugimoto, 1995). The growth factors and gonadal peptides are not produced in appreciable amounts in postmenstrual women (Longcope, 2001; Rinaudo & Strauss, 2004). In non-human primates, the data is contradictory with studies showing ovarian hormone decline with age, and other showing that menstruation occurs in late life (Walker & Herndon, 2008). Rodents, like humans, also cease cyclic hormones expression during reproductive senescence. Rats and mice both have 4–5 day estrus cycles, and both species will display age-related changes in cycle length and pattern, although these changes may occur at different chronological ages depending on the species and strain. Thus, Fisher rats do not become acyclic until 18+ months of age (Zeynalov et al., 2014), while Sprague Dawley rats are acyclic at 10–12 months of age (LeFevre & McClintock, 1988; Jezierski & Sohrabji, 2001). Among mice strain differences have not been well studied, however, C57/B6 mice, one the most commonly used transgenic and wild type model, are acyclic at 13–16 months of age (Nelson et al., 1982). With age, rodents display an acyclic pattern of persistent estrus where estradiol levels are still measurable, and FSH levels are low (Koebele & Bimonte-Nelson, 2016). This is followed by persistent diestrus, where estradiol levels are undetectable and FSH levels are elevated (Sohrabji et al., 2013). In rodents, the role of estrogen has been thoroughly studied, however, the actions of other ovarian and pituitary hormones is poorly understood (see Table 1 for summary).

### 2.1. FSH as a risk factor for CVD and stroke

Follicle Stimulating Hormone (FSH) stimulates maturation of follicles and estrogen synthesis, and free estrogen, in a negative feedback loop, inhibits FSH secretion from the pituitary. However, serum FSH starts rising above the normal level before menopause, when the level of estrogen is still normal (Klein & Soules, 1998). Unlike estrogen, FSH



**Fig. 1.** Schematic representation of the pituitary-ovary axis at (A) pre-menopause and (B) menopause. (A) Pituitary gonadotrophin release acts on theca cells to stimulate androgen synthesis, while FSH increases aromatase in granulosa cells, which facilitates conversion of testosterone to estrogen. Estrogen subsequently suppresses FSH. Menopause: Age-related follicular atresia is associated with loss of granulosa cells and theca cells that accumulate as hilar cells. Pituitary secretions elevate androgens in theca cells, which is not converted to estrogens due to granulosa cell loss, thus increasing T:E ratio and FSH due to loss of negative feedback. LH: Luteinizing hormone, FSH: Follicle stimulating hormone, TC: theca cells, GC: granulosa cells, AL: anterior lobe of the pituitary, PL: posterior lobe of the pituitary.

has an unfavorable effect on lipid profiles. FSH promotes lipogenesis and fat storage, which is reduced in the FSH receptor knockout animals (Liu et al., 2015; Zareba et al., 2016). Pre-menopausal women with serum FSH > 7 IU/L have significant elevation of serum total cholesterol compared to pre-menopausal women with serum FSH < 7 IU/L (Chu et al., 2003). Adipose tissue, which is the main repository for cholesterol, secretes pro-inflammatory molecules such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and Interleukin-6 (IL-6), which are associated with development of atherosclerotic plaques and insulin

resistance (Choi et al., 2013; Crawford et al., 2017). Not surprisingly, in postmenopausal women, FSH levels also correlate well with vascular inflammation (Figuroa-Vega et al., 2015; Poljak et al., 2018). In the SWAN study, low FSH was associated with low intima media thickness (a measure of cardiac health), as compared to medium or high FSH levels (El Khoudary et al., 2016). Higher levels of FSH and Vascular Cell Adhesion Molecule (VCAM)1, an adhesion molecule associated with atherosclerotic lesions, are found in blood samples from postmenopausal women as compared to premenopausal women, with a

**Table 1**  
Sex differences in stroke outcomes: Experimental studies.

Sex differences in stroke outcomes	Potential mechanism
Young female rats have smaller infarcts than male rats and this sex difference is eradicated by bilateral ovariectomy (Alkayed et al., 1998; Park et al., 2006)	Estrogen deficiency worsens stroke outcomes
Estrogen treatment to ovariectomized young females is neuroprotective (Rusa et al., 1999; Selvamani & Sohrabji, 2010a; Simpkins et al., 1997b; Dubal et al., 1998b)	
Age differences in stroke outcomes	Potential mechanism
Worse stroke outcomes in middle-aged/aged females as compared to young females or to age-matched males	Toxicity due to low estrogen levels -Estrogen treatment to ovariectomized middle-aged or aged females is toxic (Leon et al., 2012; Selvamani & Sohrabji, 2010b) Toxicity due to elevated levels of FSH FSH receptor knockout in mice: Promote bone health (Yeh et al., 1996; Yeh et al., 1997; Sun et al., 2006) Estrogen + FSH treatment: No atheroprotection (Li et al., 2017) Toxicity due to increased T:E ratio Testosterone exacerbates stroke infarction Castrated males have better stroke outcomes than gonadally-intact males (Cheng et al., 2009; Yang et al., 2002; Hawk et al., 1998)
	Future direction Use estrogen levels that suppress FSH or improve T:E ratio, instead of matching pre-senescent levels of estrogen  Test stroke outcomes in animals with FSH over-abundance or FSH receptor k/o.  Decreasing free testosterone during postmenopausal transition could improve stroke outcome

positive correlation between these molecules (Li et al., 2017). In vitro, FSH is shown to elevate VCAM1 in endothelial cells and increase adhesion of human monocytes to endothelial cells (Li et al., 2017). Interestingly, in a 22-site population-based study of postmenopausal women in East China, high levels of FSH were associated with a low risk of atherosclerotic cardiovascular disease, and low obesity (Wang et al., 2017). In fact two recent studies report that FSH levels are better predictor of metabolic disease in postmenopausal women than CRP (C Reactive Protein), adiponectin and leptin (Stefanska et al., 2014; Stefanska et al., 2012). Thus, the effect of FSH effect is non-linear, and may affect risk factors for cardiovascular disease differently from vascular inflammation.

In addition to the cardiovascular health, FSH also negatively impacts bone health. Elevated level of circulating FSH in the premenopausal period (when estrogen levels are normal) is associated with post-menopausal bone turnover; especially bone resorption (Sowers et al., 2003). When estrogen level is low, such as cases of amenorrhea, a high level of FSH is strongly correlated with decreasing bone density (Devleta et al., 2004). Hormone therapy is generally shown to prevent bone loss in estrogen-deficient women, but a subgroup of postmenopausal women still lose bone mass despite HT. A retrospective study showed that serum FSH level is a good predictor of bone mineral density in patients with hormonal replacement therapy (Kawai et al., 2004). These findings are supported by animal studies which showed that mice with FSH receptors knock out or simple hypophysectomy can preserve bone density despite severe hypogonadism (Yeh et al., 1996; Yeh et al., 1997; Sun et al., 2006).

## 2.2. Androgens

The ratio of estrogen and androgen is also a critical factor for physiologic homeostasis. Estradiol, the most active estrogen, is primarily produced by ovary, whereas testosterone is produced by the ovary (25%), the adrenal gland (25%) and by peripheral conversion of androstenedione and dehydroepiandrosterone (produced by ovarian stroma and adrenal gland) (50%) (Burger, 2002). After menopause, the source of circulating testosterone is: 50% by ovaries, 10% by adrenal gland and 40% by peripheral conversion of androstenedione and dehydroepiandrosterone (Liu et al., 2001). During the postmenopause period, the ovary increases secretion of testosterone possibly via stimulation by elevated LH, and this secretion sometimes span up to 10 years after menopause (Ala-Fossi et al., 1998; Sluijmer et al., 1995; Fogle et al., 2007). As a consequence, estrogen levels decline steeply after menopause (Liu et al., 2001; Burger, 1996) whereas testosterone level remain more or less unchanged, leading to a state of relative androgen excess (Longcope et al., 1986; Rannevik et al., 1995; Torr ns et al., 2009). Like FSH, excess androgen also has unfavorable effects on lipid profiles. It decreases HDL level and increases triglyceride, LDL and total cholesterol (Sarrel, 1998; Reiner, 1996; Kaunitz, 1997). Testosterone is also positively correlated with insulin resistance and type-2 diabetes in elderly population (Oh et al., 2002). Thus one possibility is that estrogen therapy may be more effective if the imbalance between androgen and estrogen is normalized, especially during the transition during the pre- and post- menopause stage.

## 2.3. Sex hormone binding globulin (SHBG)

Studies have stressed that along with androgen and estrogen, sex hormone binding globulin (SHBG) be included in evaluating risk factors for cardiovascular diseases and mortality in postmenopausal women. SHBG levels tend to decrease across the menopause transition (Burger et al., 2000; Gambera et al., 2004). Low SHBG significantly correlates with the incidence of non-insulin-dependent diabetes mellitus (NIDDM), and stroke (Lindstedt et al., 1991; Lapidus et al., 1986; Laaksonen et al., 2004), and high level of SHBG in postmenopausal women is strongly associated with decreased risk of type-2 diabetes

(Ding et al., 2009). Similar to high level of FSH and androgen, low SHBG is associated with increased triglyceride (Masarei et al., 1980), decreased HDL-C (Haffner et al., 1993) and, in elderly men, it is associated with coronary heart disease (CHD) mortality (Kalme et al., 2005). A high level of free testosterone and low level of SHBG is also associated with elevated triglyceride and low level of HDL (Haffner & Valdez, 1995). FAI (Free Androgen Index: Free Testosterone level/SHBG) increase during the menopausal transition is also strong risk factor for metabolic syndromes occurring around the menopausal phase (Torr ns et al., 2009; Shelley et al., 1998). Thus, in addition to low estrogen levels SHBG may also modify cardiovascular pathologies.

## 3. Early menopause

Two populations can inform the debate on early menopause and stroke risk:

### 3.1. Chemotherapy for breast cancer

More than 250,000 women will be diagnosed with breast cancer every year, and women with ER+ tumors (Hammond et al., 2010) will be prescribed adjuvant therapy involving endocrine agents. Tamoxifen (TAM), a selective estrogen receptor modulator (SERM) is the most prescribed therapy for premenopausal breast cancer patients. Alternately, women may be prescribed aromatase inhibitors (AI) which block estrogen synthesis. The third-generation AIs such as anastrozole, exemestane and letrozole have largely replaced tamoxifen as the preferred treatment for HR+ breast cancer in postmenopausal women (Fabian, 2007) and are more effective in women with ER+ /PR- tumors. In premenopausal females, AIs are only used if TAM treatment fails (Forward et al., 2004), and then usually in conjunction with ovarian suppression drugs such as GnRH agonists (Forward et al., 2004). Despite their overall effectiveness, however, long term usage of these treatments reveal moderate to severe side effects.

Both drugs are shown to increase disease-free survival, and to reduce cancer-related mortality. At the same time, cardiovascular disease has emerged as the single greatest non-cancer cause of death, accounting for approximately 35% of non-breast cancer mortality for survivors 50 years of age and older. The accelerated menopause phenotype is well recognized in this population, including changes in bone, uterine and cardiovascular health. Neurologic changes due to breast cancer therapies are only recently recognized and only infrequently included in the overall assessment of breast cancer survivors. A recent paper (Fiske & Blaustein, 2017) clearly outlines this gap in our knowledge of estrogen signaling on neurologic disease such as AD. A similar case may be made for cerebrovascular stroke, since TAM and LTZ causes menopause-like phenotype and effectively places women into a stroke-prone demographic, where outcomes are very poor.

#### 3.1.1. Tamoxifen

Initially a failed contraceptive compound, tamoxifen (TAM) or ICI146,474 was successfully repurposed as adjuvant breast cancer therapy due to its antagonist actions on estrogen receptors (ER) in this tissue (Jordan, 1997). TAM is metabolized to 4-hydroxytamoxifen and can exert both antagonist and agonist actions at the estrogen receptor, depending on the cis/trans conformation of its metabolites (Robertson et al., 1982), and/or the type of steroid receptor coactivators present in the cell. (Gallo & Kaufman, 1997) Thus, while its action on breast tumor cells is antagonistic, TAM is an agonist on bone tissue and the uterus. Studies have repeatedly shown that TAM use increases the risk for endometrial cancer, (Chen et al., 2014; Davies et al., 2013; Fisher, 1999), pulmonary emboli (ATLAS study) (Davies et al., 2013), and increased mortality due to ischemic heart disease and stroke (NSABP B-14) although patient events were low in the latter (Fisher, 1999). Neural effects of these endocrine therapies are also noted, specifically in cognitive function. In a Dutch study, TAM treatment resulted in

significantly lower scores on cognitive performance, especially verbal, memory and executive functioning (Schilder et al., 2010). Patients treated for 5–6 months with either TAM or anastrozole (a related AI) showed significant cognitive decline from baseline scores (Collins et al., 2009), although most studies show that the two classes of drugs themselves do not differ in their cognitive effects (reviewed in (Phillips et al., 2011)). In addition to tissue-specific effects, TAM is also anti-angiogenic, preventing angiogenesis and endothelial tube formation in vitro (Johnson et al., 2017), reducing VEGF-induced angiogenesis in vivo in a matrigel preparation (McNamara et al., 2001) as well as in the uterus (Helgestam et al., 2012), indicating the potential for widespread inhibition of angiogenic signaling in multiple organs.

### 3.1.2. Letrozole

Letrozole, a 3rd generation aromatase inhibitor, effectively inhibits estrogen synthesis throughout the body, and is a highly effective adjuvant therapy for post-menopausal patients. LTZ is a potent non-steroidal aromatase inhibitor, both in vivo and in vitro (Bhatnagar, 2007). However, LTZ results in significant side effects such as arthralgia and higher grade cardiac events (Regan et al., 2011). Unlike, TAM which has tissue-specific effects, LTZ inhibits estrogen synthesis in all tissues. As a result, it has significant side effects that resemble menopausal symptoms including vaginal dryness, hot flashes, loss of libido, musculoskeletal pain and loss of bone mineral density. Joint pain or arthralgia which is associated with increased levels of inflammatory cytokines, occurs in 50% of women prescribed AI, leading to 20% non-compliance with an otherwise effective drug (Niravath, 2013). Women on LTZ have elevated levels of inflammatory mediators including CRP, eotaxin, MCP-1 (Monocyte Chemoattractant Protein-1) (Bauml et al., 2015) and in rodents, a three week course of LTZ causes ovarian cysts with elevation of CRP and oxidative stress (Kafali et al., 2004). Similar to TAM, LTZ exerts suppressive effects on angiogenesis in ovarian tissue (Hirakawa et al., 2014). By inhibiting estrogen synthesis and signaling, both LTZ and TAM have the potential to impair stroke recovery.

Among breast cancer survivors, cardiovascular disease (CVD) accounts for approximately 35% of non-breast cancer mortality for survivors 50 years of age and older, making this the single largest non-cancerous cause of death. Cancer is commonly seen in stroke patients and a recent study reported that the rates of hospitalization for stroke among cancer patients (urogenital, breast, prostate being the most frequent) were significantly higher than non-cancer patients in 1997, and remained high in 2006, while hospitalization rates actually fell among non-cancer patients for stroke during the same time frame (Sanossian et al., 2013). Meta-analyses of breast cancer treatment trials showed that tamoxifen is associated with an increased risk of stroke (Bushnell, 2011; Sohrabji et al., 2017; Braithwaite et al., 2003). Compared to AI, thromboembolic events and transient ischemic attacks (TIAs) are more common with TAM treatment (Coates et al., 2007).

Unlike TAM, LTZ is more likely to lead to elevated levels of FSH (Bajetta et al., 1999). Interestingly, this group is also more likely to suffer bone fractures compared to TAM (Rabaglio et al., 2009), although estrogens are equally reduced in both these treatments (Rossi et al., 2009). Indirectly, these data support the idea that elevated FSH levels, occurring as a result of estrogen depletion, is likely to be deleterious to traditional targets of estrogen. Bone loss is reported to occur 2–3 years prior to the final menstrual period (FMP) (Sowers et al., 2010), a time at which estrogens are not yet low but FSH levels are significantly elevated (Randolph et al., 2011).

Far less, however, is known about the effects of breast cancer hormone therapy on stroke recovery. A retrospective analysis of stroke among cancer patients (urogenital, breast and gastrointestinal, being the most frequent) and controls found that cancer patients had a poorer neurological condition at discharge and a trend towards a longer stay in the stroke unit (Brumm et al., 2016), both indicative of worse stroke outcomes. The Bergen NORSTROKE study found that ischemic stroke was more prevalent in cancer patients, and the median NIHSS score (an

indicator of stroke severity) was significantly higher in cancer patients than non-cancer patients (Selvik et al., 2014). Given the mixed cancer population in these study, and the lack of information on endocrine therapy use in this group, the recovery from stroke in patients with TAM or LTZ treatment remains an important gap in the health literature. A report from the Swedish National Hospital Discharge Registry combined with the Swedish Cause of Death Registry showed that stroke incidence was increased during the active phase of TAM treatment and reduced after the active period of treatment. Moreover, mortality from stroke also increased during the active drug period and fell during the post-treatment period (Rosell et al., 2011), indicating that both the risk and severity of stroke is affected by TAM treatment. Currently no studies are available on the effects of LTZ on stroke recovery.

Long term TAM or LTZ therapy is understudied in the context of stroke and is poorly studied in the preclinical literature. This group of patients show premature menopause, where both FSH levels are likely to be elevated (due to estrogen suppression) as well as an increased ratio of testosterone to estrogen.

### 3.2. Premature menopause resulting from Bilateral Salpingo-Oophorectomy (BSO)

Bilateral salpingo oophorectomy is a prophylactic surgery for women positive for BRCA1 and BRCA2 mutations to prevent ovarian or fallopian cancer, after their childbearing age. However the majority of cases involve benign disease. BSO is also usually performed along with hysterectomy in order to avoid ovarian pathologies or adnexal pain from postsurgical adhesions. In 1984, 1 out of 8 US women were reported to have undergone oophorectomy prior to natural menopause (Howe, 1984), however oophorectomy surgeries have decreased overall nationwide since that time. A recent study shows that the rate of elective bilateral salpingo-oophorectomy was 7.8 per 10,000 in 1998, which increased to 9.0 per 10,000 in 2001 and then fell to 7.4 per 10,000 in 2006 (Asante et al., 2010). Due to the increased prevalence of outpatient BSO surgeries, these numbers may be an underestimate since most databases reflect inpatient surgeries (Wright et al., 2013).

Oophorectomy reduces estrogens and testosterone levels and causes a corresponding rise in FSH levels (Cooper & Thorp Jr., 1999). In a community-dwellers study (Ranch Bernardo), testosterone levels were significantly reduced in women with BSO when compared to age-matched women with no surgery and women who undergo hysterectomy with preservation of ovaries (Laughlin et al., 2000). In contrast, FSH levels are significantly elevated in this population. Typically, FSH levels of 30 IU/L with one year without menses is indicative of menopause. Using a surgically intact referent group of women, women over 40 years of age with hysterectomy and unilateral oophorectomy were 2.49× more likely to exceed a 40 IU/L criteria and 19.17× more likely to exceed this criteria in women under 40 years of age (Moorman et al., 2011).

While BSO reduces ovarian cancer-related mortality in women inherited with BRCA1 and BRCA2 mutations (Domchek et al., 2006), it may do more harm than good for women (Parker et al., 2009a), including increased non-cancer mortality (Rocca et al., 2016; Evans et al., 2016). Among the earliest studies on this population were short term (3–6 months) assessments of cognition. Oophorectomy decreased scores on tests of cognition and recall and the MMSE, while estrogen therapy maintained scores at presurgical levels (Phillips & Sherwin, 1992; Sherwin, 1988; Farrag et al., 2002). Perhaps the most definitive studies of health risks for BSO come from longitudinal studies on a well characterized cohort in Olmstead County, MN, called the Mayo Clinic Cohort Study of Oophorectomy and Aging. Women in this cohort showed significant neurological and cardiovascular health risks (Rocca et al., 2016). In this cohort, bilateral oophorectomy performed at a younger age, in the absence of estrogen therapy, leads to higher risk for neurological diseases such as Parkinson (Hazard Ratio (HR): 1.8), Dementia (HR = 1.7), and depression (HR = 1.54) (Parker et al., 2009a; Rocca

et al., 2008a; Rocca et al., 2008b; Rocca et al., 2007a). Similarly, an increased risk of cognitive impairment was also found after unilateral or bilateral oophorectomy before menopause (Rocca et al., 2007b). Estrogen therapy to women with BSO after 49 years of age showed no increased risk for dementia (Parker et al., 2009a).

In addition to neurological disorders, bilateral oophorectomy also led to cardiovascular disorders in women. Compared to pre-menopausal women, oophorectomy in the absence of estrogen therapy in age-matched women increased the risk for MI by > 2-fold (Parker et al., 2009a; Garcia et al., 2016). Similarly, an observational study on 29,380 women participating in The Nurses' Health Study (NHS) with age range of 30–55 also found that compared to women with ovarian conservation, oophorectomy without estrogen therapy increased the risk for MI (Parker et al., 2009b). BSO after the age of 50 increases the risk for stroke, which is attenuated by estrogen therapy (Lai et al., 2018).

Increase in serum lipids, reduced carotid artery blood flow, and increased atherosclerosis due to the reduced circulating level of estrogen in the oophorectomized women could be the reason for these cardiovascular conditions. BSO before 45 years, with no estrogen therapy, increased all-cause mortality (Domchek et al., 2006; Evans et al., 2016; Parker et al., 2009b; Rocca et al., 2006). In many of these studies, HT reduced the risk for stroke. While this may indicate that estrogen is a mediator of stroke effects (Rocca et al., 2012), it does not preclude the involvement of FSH, since estrogen therapy is known to suppress FSH (Lambrinoudaki et al., 2011).

#### 4. Preclinical models

##### 4.1. Lessons learnt from preclinical studies

While it is not practical to study stroke risk in preclinical models, a significant literature is available on the role of sex hormones in stroke recovery. Ischemic stroke is usually caused by mechanical or biochemical occlusion of the lumen of a major brain vessel, typically the middle cerebral artery (MCA) which results in a corticostriatal infarct, or bilateral carotid artery occlusion that results in hippocampal cell death, among others (Macrae, 2011). Stroke-induced neural damage is usually assessed by a variety of measures such as infarct volume (extent of dead tissue), changes in vascularity (microvessel density, tortuosity, length), changes in blood brain barrier permeability, central and peripheral inflammation, and stroke impairment and recovery is measured by short term and long-term deficits in sensory motor function, cognition, depressive-like behaviors, among others.

In rodents, ischemic stroke results in a smaller infarct and better cerebral blood flow in young females compared to age-matched, normoglycemic (Alkayed et al., 1998) or diabetic (Toung et al., 2000) males. This sex difference is eradicated when females are bilaterally ovariectomized, supporting the idea that gonadal hormones may underlie these sex differences. This idea received further support from studies showing that the extent of ischemic damage was inversely related to circulating levels of estrogen (Liao et al., 2001). Over the last 20 years, studies have overwhelmingly shown that estrogen treatment to ovariectomized female rats or mice is neuroprotective, an umbrella term typically referring to reduced infarct volume, reduced inflammation and improved motor (behavioral) performance (Rusa et al., 1999; Dubal et al., 1998a; Simpkins et al., 1997a; Selvamani & Sohrabji, 2010a). Thus, replacement with 17 $\beta$ -estradiol or its inactive stereoisomer 17 $\alpha$  estradiol (Simpkins et al., 1997b) as well as the conjugate equine estrogen preparations (McCullough et al., 2001), all reduced infarct volume in female animals. Exogenous estrogen replacement was shown to be neuroprotective when given prior (Dubal et al., 1998b) or subsequent to the injury (Liu et al., 2005; Yang et al., 2003).

However, the effects of estrogens on stroke are not always neuroprotective. In some cases, this has been attributed to the age of the animal or the type of ischemic injury.

Type of injury: Most studies use a transient focal ischemia, where

blood flow to the middle cerebral artery (MCAo) is disrupted and then reinstated. In this model, estradiol is typically shown to improve stroke infarction. However, in permanent ischemia models, several studies have shown that estrogen increased infarct volume (Bingham et al., 2005; Carswell et al., 2004; Gordon et al., 2005).

Hormone effects are more complicated in the context of aging. In general, studies agree that older female rats/mice have worse outcomes as compared to young females. In fact, older females have larger infarct volumes than age matched males (Manwani et al., 2011), indicating a virtual reversal of the sex difference seen in young animals. This loss of 'female advantage' appears to be related to ovarian function, such that acyclic middle-aged female rats also display significantly larger infarct volumes as compared to young females (Selvamani & Sohrabji, 2010a). These studies used reproductive senescent rats, defined as animals with multiple previous successful pregnancies, current reproductive failure, and vaginal cytology indicative of constant diestrus (a low estrogen state). In this group, serum levels of estradiol are low and FSH levels are elevated, consistent with a 'menopausal' pattern (Sohrabji et al., 2013). Thus, the large infarcts seen in this group compared to young normally cycling females is consistent with the hypothesis that ovarian aging impairs stroke recovery. Moreover estradiol treatment for 2–4 weeks, which decreased infarct volumes (Selvamani & Sohrabji, 2010a; Glendenning et al., 2008) and reduced sensory motor impairment in young females, paradoxically, increased infarct volume in reproductive senescent females (Selvamani & Sohrabji, 2010a).

The anomalous effects of estrogens on stroke outcomes in animal models are reminiscent of the paradoxical effects of this hormone in clinical studies. We proposed (above) that some of these paradoxical effects of estrogen may be related to the altered endocrine environment in aging such as testosterone levels or other sex hormones (FSH). Preclinical studies could be informative on the role of these hormones, especially in the context of aging, however, they have not been exploited. Most studies use a 2-group approach, comparing ovariectomized (OVX) vehicle-treated animals with OVX + estrogen-treated animals, or a 3-group approach comparing gonadally intact, OVX and OVX + estradiol treated animals. Few studies have incorporated an estrogen receptor antagonist, to define the locus of estrogen action. One study that used the pan-estrogen receptor antagonist, ICI182,780, noted that this drug increased striatal (but not cortical) infarction (Sawada et al., 2000) presumably by blocking endogenous estrogen signaling. However, no differences were reported in infarct volume between the wild type and ER-alpha knock out (ERKO) mouse, suggesting that estrogen may act via receptor independent process. (Sampei et al., 2000). Conversely, exogenous estradiol is reported to improve infarct volume in the OVX wild type but not in the OVX ERKO mouse (Dubal et al., 2001) or in neuron-specific ER knock out mouse (Elzer et al., 2010). These studies did not report whether infarct volume in the untreated OVX mouse is similar to WT, thus it is not clear if the baseline in these models is altered. A third strategy involves the use of the aromatase knock-out or ARKO, where all endogenous estrogen synthesis is inhibited. In this model, MCAo results in a larger infarct volume in the ARKO mouse as compared WT or the ovariectomized WT mouse, and infarct volume is reduced by estradiol treatment (McCullough et al., 2003). Moreover, WT animals pretreated with the aromatase inhibitor fadrozole for 1 week also showed worse infarct volumes after MCAo (McCullough et al., 2003).

The OVX model, which shares similarities with the BSO population, may also result in elevated FSH. Thus, an alternate explanation for the effects of estrogen on stroke outcomes in the OVX model is that this treatment improves stroke outcomes by suppressing FSH. Few studies have tested if estrogen treatment to OVX rats (or mice) reduces FSH levels and whether co-treatment with FSH negates the effects of estradiol. In a study of aortic atherosclerotic lesions in the ApoE $^{-/-}$ , OVX mice showed increased lesions which were reduced by estradiol. However, co-treatment of estradiol and FSH abolished the protective effect of estradiol (Li et al., 2017), suggesting that the atheroprotective

effect of estradiol was driven by its negative regulation of FSH. No such studies have been performed for stroke studies.

In the case of ovary-intact populations, there is some concordance of the human and preclinical data, such that stroke risk is elevated in older postmenopausal women and stroke outcomes are worse in reproductive senescent rats. In these groups, toxicity of elevated FSH levels may be a concern, as also the increased testosterone to estrogen ratio may be a probable cause for worse stroke outcomes. In rodent models, testosterone has been shown to exacerbate infarction. Thus, castrate males display lower infarct volumes as compared to gonadal-intact males and testosterone replaced castrate males (Cheng et al., 2009; Yang et al., 2002; Hawk et al., 1998). Thus, larger infarct sizes in reproductive senescent females may also result from elevated testosterone levels. A corollary to this hypothesis is that estrogen treatment to this group is toxic or ineffective because it fails to suppress elevated FSH levels or fails to restore homeostatic levels of testosterone and estrogen. It also raises the intriguing idea that ovariectomy to this group may actually improve stroke outcomes as compared to ovary intact females. These alternate strategies have not been tested thus far.

## 5. Conclusions

Overall, the evidence linking estrogen loss to increased stroke risk is fairly strong, however, the data on estrogen therapy and stroke risk is more ambiguous. In reviewing other cohorts of early menopause (surgical or through chemotherapy) suggests that other sex hormones may also be modify this risk. In the preclinical literature, the variability in stroke outcomes based on age, dose, type of injury may be better explained by considering a global change in ovarian hormones and gonadotrophins.

## Acknowledgements

Support by grants from the National Institutes of Health AG042189 and NS074895 to FS.

## References

- Adachi, T., et al., 1995. Regulation of IGF binding proteins by FSH in human luteinizing granulosa cells. *J. Assist. Reprod. Genet.* 12 (9), 639–643.
- Ala-Fossi, S.L., et al., 1998. Ovarian testosterone secretion during perimenopause. *Maturitas* 29 (3), 239–245.
- Alford, C., Nurudeen, S., 2013. Chapter 4. Physiology of reproduction in women. In: DeCherney, A.H. (Ed.), *Current Diagnosis & Treatment: Obstetrics & Gynecology*. 11e The McGraw-Hill Companies, New York, NY.
- Alkayed, N.J., et al., 1998. Gender-linked brain injury in experimental stroke. *Stroke* 29 (1), 159–165 (discussion 166).
- Alonso de Lecinana, M., et al., 2007. Risk of ischemic stroke and lifetime estrogen exposure. *Neurology* 68 (1), 33–38.
- Arana, A., et al., 2006. Hormone therapy and cerebrovascular events: a population-based nested case-control study. *Menopause* 13 (5), 730–736.
- Asante, A., et al., 2010. Elective oophorectomy in the United States: trends and in-hospital complications, 1998–2006. *Obstet. Gynecol.* 116 (5), 1088–1095.
- Bajetta, E., et al., 1999. Double-blind, randomised, multicentre endocrine trial comparing two letrozole doses, in postmenopausal breast cancer patients. *Eur. J. Cancer* 35 (2), 208–213.
- Batrinou, M.L., 2013. Premenopause: the endocrinology of reproductive decline. *Hormones (Athens)* 12 (3), 334–349.
- Bauml, J., et al., 2015. Arthralgia among women taking aromatase inhibitors: is there a shared inflammatory mechanism with co-morbid fatigue and insomnia? *Breast Cancer Res.* 17 (1), 89.
- Bhatnagar, A.S., 2007. The discovery and mechanism of action of letrozole. *Breast Cancer Res. Treat.* 105 (Suppl. 1), 7–17.
- Bingham, D., Macrae, I.M., Carswell, H.V., 2005. Detrimental effects of 17 $\beta$ -Oestradiol after permanent middle cerebral artery occlusion. *J. Cereb. Blood Flow Metab.* 25 (3), 414–420.
- Braithwaite, R.S., et al., 2003. Meta-analysis of vascular and neoplastic events associated with tamoxifen. *J. Gen. Intern. Med.* 18 (11), 937–947.
- Brumm, A.J., et al., 2016. Astrocytes can adopt endothelial cell fates in a p53-dependent manner. *Mol. Neurobiol.* 54 (6), 4584–4596.
- Burger, H.G., 1996. The menopausal transition. *Baillieres Clin. Obstet. Gynaecol.* 10 (3), 347–359.
- Burger, H.G., 2002. Androgen production in women. *Fertil. Steril.* 77 (Suppl. 4), S3–S5.
- Burger, H.G., et al., 2000. A prospective longitudinal study of serum testosterone, dehydroepiandrosterone sulfate, and sex hormone-binding globulin levels through the menopause transition. *J. Clin. Endocrinol. Metab.* 85 (8), 2832–2838.
- Bushnell, C., 2009. Stroke Hormones and Outcomes in Women (SHOW) study: is the 'healthy-user effect' valid for women after stroke? *Women's Health (Lond. Engl.)* 5 (5), 485–496.
- Bushnell, C., 2011. Depression and the risk of stroke in women: an identification and treatment paradox. *Stroke* 42 (10), 2718–2719.
- Carroll, R.S., et al., 1989. Inhibin, activin, and follistatin: regulation of follicle-stimulating hormone messenger ribonucleic acid levels. *Mol. Endocrinol.* 3 (12), 1969–1976.
- Carswell, H.V., et al., 2004. Differential effects of 17 $\beta$ -estradiol upon stroke damage in stroke prone and normotensive rats. *J. Cereb. Blood Flow Metab.* 24 (3), 298–304.
- Cauley, J.A., et al., 1989. The epidemiology of serum sex hormones in postmenopausal women. *Am. J. Epidemiol.* 129 (6), 1120–1131.
- Channing, C.P., et al., 1980. Ovarian follicular and luteal physiology. *Int. Rev. Physiol.* 22, 117–201.
- Chen, J.-Y., et al., 2014. Endometrial cancer incidence in breast cancer patients correlating with age and duration of tamoxifen use: a population based study. *J. Cancer* 5 (2), 151–155.
- Cheng, J., et al., 2009. Age-dependent effects of testosterone in experimental stroke. *J. Cereb. Blood Flow Metab.* 29 (3), 486–494.
- Choi, S.H., Hong, E.S., Lim, S., 2013. Clinical implications of adipocytokines and newly emerging metabolic factors with relation to insulin resistance and cardiovascular health. *Front. Endocrinol.* 4, 97.
- Chu, M.C., et al., 2003. Elevated basal FSH in normal cycling women is associated with unfavourable lipid levels and increased cardiovascular risk. *Hum. Reprod.* 18 (8), 1570–1573.
- Cintron, D., et al., 2018. Effects of oral versus transdermal menopausal hormone treatments on self-reported sleep domains and their association with vasomotor symptoms in recently menopausal women enrolled in the Kronos Early Estrogen Prevention Study (KEEPS). *Menopause* 25 (2), 145–153.
- Coates, A.S., et al., 2007. Five years of letrozole compared with tamoxifen as initial adjuvant therapy for postmenopausal women with endocrine-responsive early breast cancer: update of study BIG 1-98. *J. Clin. Oncol.* 25 (5), 486–492.
- Collins, B., et al., 2009. Cognitive effects of hormonal therapy in early stage breast cancer patients: a prospective study. *Psychooncology* 18 (8), 811–821.
- Cooper, G.S., Thorp Jr., J.M., 1999. FSH levels in relation to hysterectomy and to unilateral oophorectomy. *Obstet. Gynecol.* 94 (6), 969–972.
- Crawford, E.D., et al., 2017. The potential role of follicle-stimulating hormone in the cardiovascular, metabolic, skeletal, and cognitive effects associated with androgen deprivation therapy. *Urol. Oncol.* 35 (5), 183–191.
- Davies, C., et al., 2013. Long-term effects of continuing adjuvant tamoxifen to 10 years versus stopping at 5 years after diagnosis of oestrogen receptor-positive breast cancer: ATLAS, a randomised trial. *Lancet* 381 (9869), 805–816.
- Devleta, B., Adem, B., Senada, S., 2004. Hypergonadotropic amenorrhea and bone density: new approach to an old problem. *J. Bone Miner. Metab.* 22 (4), 360–364.
- Ding, E.L., et al., 2009. Sex hormone-binding globulin and risk of type 2 diabetes in women and men. *N. Engl. J. Med.* 361 (12), 1152–1163.
- Domchek, S.M., et al., 2006. Mortality after bilateral salpingo-oophorectomy in BRCA1 and BRCA2 mutation carriers: a prospective cohort study. *Lancet Oncol.* 7 (3), 223–229.
- Dubal, D.B., et al., 1998a. Estradiol protects against ischemic injury. *J. Cereb. Blood Flow Metab.* 18 (11), 1253–1258.
- Dubal, D., et al., 1998b. Estradiol protects against ischemic injury. *J. Cereb. Blood Flow Metab.* 18, 1253–1258.
- Dubal, D.B., et al., 2001. Estrogen receptor alpha, not beta, is a critical link in estradiol-mediated protection against brain injury. *Proc. Natl. Acad. Sci. U. S. A.* 98 (4), 1952–1957.
- El Khoudary, S.R., et al., 2016. Trajectories of estradiol and follicle-stimulating hormone over the menopause transition and early markers of atherosclerosis after menopause. *Eur. J. Prev. Cardiol.* 23 (7), 694–703.
- Elzer, J.G., et al., 2010. Neuronal estrogen receptor-alpha mediates neuroprotection by 17beta-estradiol. *J. Cereb. Blood Flow Metab.* 30 (5), 935–942.
- Evans, E.C., et al., 2016. Salpingo-oophorectomy at the time of benign hysterectomy: a systematic review. *Obstet. Gynecol.* 128 (3), 476–485.
- Fabian, C.J., 2007. The what, why and how of aromatase inhibitors: hormonal agents for treatment and prevention of breast cancer. *Int. J. Clin. Pract.* 61 (12), 2051–2063.
- Farrag, A.K., et al., 2002. Effect of surgical menopause on cognitive functions. *Dement. Geriatr. Cogn. Disord.* 13 (3), 193–198.
- Figuerola-Vega, N., Moreno-Frias, C., Malacara, J.M., 2015. Alterations in adhesion molecules, pro-inflammatory cytokines and cell-derived microparticles contribute to intima-media thickness and symptoms in postmenopausal women. *PLoS One* 10 (5), e0120990.
- Fisher, B., 1999. Highlights from recent National Surgical Adjuvant Breast and Bowel Project studies in the treatment and prevention of breast cancer. *CA Cancer J. Clin.* 49 (3), 159–177.
- Fiske, S.T., Blaustein, J.D., 2017. Treatments for breast cancer that affect cognitive function in postmenopausal women. *Policy Insights Behav. Brain Sci.* 4 (2), 170–177.
- Fogle, R.H., et al., 2007. Ovarian androgen production in postmenopausal women. *J. Clin. Endocrinol. Metab.* 92 (8), 3040–3043.
- Forward, D.P., et al., 2004. Clinical and endocrine data for goserelin plus anastrozole as second-line endocrine therapy for premenopausal advanced breast cancer. *Br. J. Cancer* 90 (3), 590–594.
- Gallo, M.A., Kaufman, D., 1997. Antagonistic and agonistic effects of tamoxifen: significance in human cancer. *Semin. Oncol.* 24 (1 Suppl 1), S1 (-71-s1-80).
- Gambera, A., et al., 2004. Androgens, insulin-like growth factor-I (IGF-I), and carrier proteins (SHBG, IGFBP-3) in postmenopause. *Menopause* 11 (2), 159–166.

- Garcia, M., et al., 2016. Cardiovascular disease in women: clinical perspectives. *Circ. Res.* 118 (8), 1273–1293.
- Gleason, C.E., et al., 2015. Effects of hormone therapy on cognition and mood in recently postmenopausal women: findings from the randomized, controlled KEEPS-cognitive and affective study. *PLoS Med.* 12 (6), e1001833.
- Glendenning, M.L., Lovekamp-Swan, T., Schreihof, D.A., 2008. Protective effect of estrogen in endothelin-induced middle cerebral artery occlusion in female rats. *Neurosci. Lett.* 445 (2), 188–192.
- Gordon, K.B., Macrae, I.M., Carswell, H.V.O., 2005. Effects of 17 $\beta$ -oestradiol on cerebral ischaemic damage and lipid peroxidation. *Brain Res.* 1036 (1), 155–162.
- Grodin, J., Siiteri, P., MacDonald, P., 1973. Source of estrogen production in postmenopausal women. *J. Clin. Endocrinol. Metab.* 36 (2), 207–214.
- Grodstein, F., et al., 2008. Postmenopausal hormone therapy and stroke: role of time since menopause and age at initiation of hormone therapy. *Arch. Intern. Med.* 168 (8), 861–866.
- Haffner, S.M., Valdez, R.A., 1995. Endogenous sex hormones: impact on lipids, lipoproteins, and insulin. *Am. J. Med.* 98 (1A), 40S–47S.
- Haffner, S.M., et al., 1993. Decreased sex hormone-binding globulin predicts noninsulin-dependent diabetes mellitus in women but not in men. *J. Clin. Endocrinol. Metab.* 77 (1), 56–60.
- Hammond, M.E., et al., 2010. American society of clinical oncology/college of American pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. *J. Oncol. Pract.* 6 (4), 195–197.
- Harman, S.M., et al., 2014. Arterial imaging outcomes and cardiovascular risk factors in recently menopausal women: a randomized trial. *Ann. Intern. Med.* 161 (4), 249–260.
- Hawk, T., et al., 1998. Testosterone increases and estradiol decreases middle cerebral artery occlusion lesion size in male rats. *Brain Res.* 796 (1–2), 296–298.
- Helmestam, M., et al., 2012. Tamoxifen modulates cell migration and expression of angiogenesis-related genes in human endometrial endothelial cells. *Am. J. Pathol.* 180 (6), 2527–2535.
- Hendrix, S.L., et al., 2006. Effects of conjugated equine estrogen on stroke in the Women's Health Initiative. *Circulation* 113 (20), 2425–2434.
- Hernandez, E., et al., 1989. Rat ovarian insulin-like growth factor I (IGF-I) gene expression is granulosa cell-selective: 5'-untranslated mRNA variant representation and hormonal regulation. *Endocrinology* 125 (1), 572–574.
- Hirakawa, H., et al., 2014. Inhibitory effects of aromatase inhibitor on estrogen receptor- $\alpha$  positive ovarian cancer in mice. *J. Ovarian Res.* 7, 4.
- Hodis, H.N., et al., 2016. Vascular effects of early versus late postmenopausal treatment with estradiol. *N. Engl. J. Med.* 374 (13), 1221–1231.
- Hoffman, B.L., et al., 2016. Reproductive endocrinology. In: Williams Gynecology. 3e McGraw-Hill Education, New York, NY.
- Howe, H.L., 1984. Age-specific hysterectomy and oophorectomy prevalence rates and the risks for cancer of the reproductive system. *Am. J. Public Health* 74 (6), 560–563.
- Jeppesen, J.V., et al., 2012. LH-receptor gene expression in human granulosa and cumulus cells from antral and preovulatory follicles. *J. Clin. Endocrinol. Metab.* 97 (8), E1524–E1531.
- Jezierski, M.K., Sohrabji, F., 2001. Neurotrophin expression in the reproductively senescent forebrain is refractory to estrogen stimulation. *Neurobiol. Aging* 22 (2), 309–319.
- Johnson, K.E., et al., 2017. Tamoxifen directly inhibits platelet angiogenic potential and platelet-mediated metastasis. *Arterioscler. Thromb. Vasc. Biol.* 37 (4), 664–674.
- Jordan, V.C., 1997. Tamoxifen: the herald of a new era of preventive therapeutics. *J. Natl. Cancer Inst.* 89 (11), 747–749.
- Kafali, H., et al., 2004. Letrozole-induced polycystic ovaries in the rat: a new model for cystic ovarian disease. *Arch. Med. Res.* 35 (2), 103–108.
- Kalme, T., et al., 2005. Sex hormone-binding globulin and insulin-like growth factor-binding protein-1 as indicators of metabolic syndrome, cardiovascular risk, and mortality in elderly men. *J. Clin. Endocrinol. Metab.* 90 (3), 1550–1556.
- Kapral, M.K., et al., 2005. Sex differences in stroke care and outcomes: results from the Registry of the Canadian Stroke Network. *Stroke* 36 (4), 809–814.
- Kaunitz, A.M., 1997. The role of androgens in menopausal hormonal replacement. *Endocrinol. Metab. Clin. N. Am.* 26 (2), 391–397.
- Kawai, H., Furuhashi, M., Suganuma, N., 2004. Serum follicle-stimulating hormone level is a predictor of bone mineral density in patients with hormone replacement therapy. *Arch. Gynecol. Obstet.* 269 (3), 192–195.
- Klein, N.A., Soules, M.R., 1998. Endocrine changes of the perimenopause. *Clin. Obstet. Gynecol.* 41 (4), 912–920.
- Koebele, S.V., Bimonte-Nelson, H.A., 2016. Modeling menopause: the utility of rodents in translational behavioral endocrinology research. *Maturitas* 87, 5–17.
- La Marca, A., Volpe, A., 2006. Anti-Müllerian hormone (AMH) in female reproduction: is measurement of circulating AMH a useful tool? *Clin. Endocrinol.* 64 (6), 603–610.
- Laaksonen, D.E., et al., 2004. Testosterone and sex hormone-binding globulin predict the metabolic syndrome and diabetes in middle-aged men. *Diabetes Care* 27 (5), 1036–1041.
- Lai, J.C.-Y., et al., 2018. The risk of stroke after bilateral salpingo-oophorectomy at hysterectomy for benign diseases: a nationwide cohort study. *Maturitas* 114, 27–33.
- Lambrinoudaki, I., et al., 2011. Sex hormones in postmenopausal women receiving low-dose hormone therapy: the effect of BMI. *Obesity (Silver Spring)* 19 (5), 988–993.
- Lapidus, L., et al., 1986. Concentrations of sex-hormone binding globulin and corticosteroid binding globulin in serum in relation to cardiovascular risk factors and to 12-year incidence of cardiovascular disease and overall mortality in postmenopausal women. *Clin. Chem.* 32 (1), 146–152.
- Laughlin, G.A., et al., 2000. Hysterectomy, oophorectomy, and endogenous sex hormone levels in older women: the Rancho Bernardo Study 1. *J. Clin. Endocrinol. Metab.* 85 (2), 645–651.
- LeFevre, J., McClintock, M.K., 1988. Reproductive senescence in female rats: a longitudinal study of individual differences in estrous cycles and behavior. *Biol. Reprod.* 38 (4), 780–789.
- Leon, R.L., et al., 2012. Worsened outcome from middle cerebral artery occlusion in aged rats receiving 17 $\beta$ -estradiol. *Endocrinology* 153 (7), 3386–3393.
- Li, C., et al., 2006. Risk of stroke and hormone replacement therapy. A prospective cohort study. *Maturitas* 54 (1), 11–18.
- Li, X., et al., 2017. Follicular stimulating hormone accelerates atherogenesis by increasing endothelial VCAM-1 expression. *Theranostics* 7 (19), 4671–4688.
- Liao, S., et al., 2001. Association of serum estrogen level and ischemic neuroprotection in female rats. *Neurosci. Lett.* 297 (3), 159–162.
- Lindstedt, G., et al., 1991. Low sex-hormone-binding globulin concentration as independent risk factor for development of NIDDM: 12-yr follow-up of population study of women in Gothenburg, Sweden. *Diabetes* 40 (1), 123–128.
- Liu, Y., et al., 2001. Relative androgen excess and increased cardiovascular risk after menopause: a hypothesized relation. *Am. J. Epidemiol.* 154 (6), 489–494.
- Liu, R., et al., 2005. 17 $\beta$ -Estradiol attenuates blood-brain barrier disruption induced by cerebral ischemia-reperfusion injury in female rats. *Brain Res.* 1060 (1–2), 55–61.
- Liu, X.M., et al., 2015. FSH regulates fat accumulation and redistribution in aging through the G $\alpha$ /Ca<sup>2+</sup>/CREB pathway. *Aging Cell* 14 (3), 409–420.
- Lloyd-Jones, D., et al., 2010. Heart disease and stroke statistics—2010 update: a report from the American Heart Association. *Circulation* 121 (7), e46–e215.
- Lokkegaard, E., et al., 2003. Increased risk of stroke in hypertensive women using hormone therapy: analyses based on the Danish Nurse Study. *Arch. Neurol.* 60 (10), 1379–1384.
- Longcope, C., 2001. Endocrine function of the postmenopausal ovary. *J. Soc. Gynecol. Investig.* 8 (1, suppl), S67–S68.
- Longcope, C., et al., 1986. Steroid and gonadotropin levels in women during the perimenopausal years. *Maturitas* 8 (3), 189–196.
- Macrae, I.M., 2011. Preclinical stroke research—advantages and disadvantages of the most common rodent models of focal ischaemia. *Br. J. Pharmacol.* 164 (4), 1062–1078.
- Maggio, M., et al., 2009. Relationship between estradiol levels and 9-year mortality in older women: the Invecchiare in Chianti study. *J. Am. Geriatr. Soc.* 57 (10), 1810–1815.
- Manson, J.E., et al., 2007. Estrogen therapy and coronary-artery calcification. *N. Engl. J. Med.* 356 (25), 2591–2602.
- Manwani, B., et al., 2011. Functional recovery in aging mice after experimental stroke. *Brain Behav. Immun.* 25 (8), 1689–1700.
- Masarei, J.R., et al., 1980. HDL-cholesterol and sex-hormone status. *Lancet* 1 (8161), 208.
- Mason, H., et al., 1993. Insulin-like growth factor-I (IGF-I) inhibits production of IGF-binding protein-1 while stimulating estradiol secretion in granulosa cells from normal and polycystic human ovaries. *J. Clin. Endocrinol. Metab.* 76 (5), 1275–1279.
- McCullough, L.D., et al., 2001. Postischemic estrogen reduces hypoperfusion and secondary ischemia after experimental stroke. *Stroke* 32 (3), 796–802.
- McCullough, L.D., et al., 2003. Aromatase cytochrome P450 and extragonadal estrogen play a role in ischemic neuroprotection. *J. Neurosci.* 23 (25), 8701–8705.
- McNamara, D.A., et al., 2001. Tamoxifen inhibits endothelial cell proliferation and attenuates VEGF-mediated angiogenesis and migration in vivo. *Eur. J. Surg. Oncol.* 27 (718), 714–718.
- Miller, V.M., et al., 2009. Using basic science to design a clinical trial: baseline characteristics of women enrolled in the Kronos Early Estrogen Prevention Study (KEEPS). *J. Cardiovasc. Transl. Res.* 2 (3), 228–239.
- Moorman, P.G., et al., 2011. Effect of hysterectomy with ovarian preservation on ovarian function. *Obstet. Gynecol.* 118 (6), 1271–1279.
- Nelson, J.F., et al., 1982. A longitudinal study of estrous cyclicity in aging C57BL/6J mice: I. cycle frequency, length and vaginal cytology. *Biol. Reprod.* 27 (2), 327–339.
- Niravath, P., 2013. Aromatase inhibitor-induced arthralgia: a review. *Ann. Oncol.* 24 (6), 1443–1449.
- Niswender, G.D., et al., 2000. Mechanisms controlling the function and life span of the corpus luteum. *Physiol. Rev.* 80 (1), 1–29.
- Oh, J.Y., et al., 2002. Endogenous sex hormones and the development of type 2 diabetes in older men and women: the Rancho Bernardo study. *Diabetes Care* 25 (1), 55–60.
- Oliver, J.E., et al., 1989. Insulin-like growth factor I gene expression in the rat ovary is confined to the granulosa cells of developing follicles. *Endocrinology* 124 (6), 2671–2679.
- Parikh, G., et al., 2010. Vitamin D regulates steroidogenesis and insulin-like growth factor binding protein-1 (IGFBP-1) production in human ovarian cells. *Horm. Metab. Res.* 42 (10), 754–757.
- Park, E.M., et al., 2006. Inducible nitric oxide synthase contributes to gender differences in ischemic brain injury. *J. Cereb. Blood Flow Metab.* 26 (3), 392–401.
- Parker, W.H., et al., 2009a. Effect of bilateral oophorectomy on women's long-term health. *Women's Health (Lond. Engl.)* 5 (5), 565–576.
- Parker, W.H., et al., 2009b. Ovarian conservation at the time of hysterectomy and long-term health outcomes in the nurses' health study. *Obstet. Gynecol.* 113 (5), 1027.
- Pasqualini, J., et al., 1996. Concentrations of estrone, estradiol, and estrone sulfate and evaluation of sulfatase and aromatase activities in pre- and postmenopausal breast cancer patients. *J. Clin. Endocrinol. Metab.* 81 (4), 1460–1464.
- Peluso, C., et al., 2014. AMH: an ovarian reserve biomarker in assisted reproduction. *Clin. Chim. Acta* 437, 175–182.
- Petitti, D.B., et al., 1998. Ischemic stroke and use of estrogen and estrogen/progestogen as hormone replacement therapy. *Stroke* 29 (1), 23–28.
- Phillips, S.M., Sherwin, B.B., 1992. Effects of estrogen on memory function in surgically menopausal women. *Psychoneuroendocrinology* 17 (5), 485–495.
- Phillips, K.A., Ribí, K., Fisher, R., 2011. Do aromatase inhibitors have adverse effects on cognitive function? *Breast Cancer Res.* 13 (1) (p. 203-203).
- Poljak, Z., et al., 2018. Are GnRH and FSH potentially damaging factors in the

- cardiovascular system? *Pharmazie* 73 (4), 187–190.
- Prentice, R.L., et al., 2006. Combined analysis of Women's Health Initiative observational and clinical trial data on postmenopausal hormone treatment and cardiovascular disease. *Am. J. Epidemiol.* 163 (7), 589–599.
- Prentice, R.L., et al., 2008. Conjugated equine estrogens and breast cancer risk in the Women's Health Initiative clinical trial and observational study. *Am. J. Epidemiol.* 167 (12), 1407–1415.
- Rabaglio, M., et al., 2009. Bone fractures among postmenopausal patients with endocrine-responsive early breast cancer treated with 5 years of letrozole or tamoxifen in the BIG 1-98 trial. *Ann. Oncol.* 20 (9), 1489–1498.
- Randolph, J.J.F., et al., 2011. Change in follicle-stimulating hormone and estradiol across the menopausal transition: effect of age at the final menstrual period. *J. Clin. Endocrinol. Metab.* 96 (3), 746–754.
- Rannevik, G., et al., 1995. A longitudinal study of the perimenopausal transition: altered profiles of steroid and pituitary hormones, SHBG and bone mineral density. *Maturitas* 21 (2), 103–113.
- Reeves, M.J., et al., 2008. Sex differences in stroke: epidemiology, clinical presentation, medical care, and outcomes. *Lancet Neurol.* 7 (10), 915–926.
- Regan, M.M., et al., 2011. Assessment of letrozole and tamoxifen alone and in sequence for postmenopausal women with steroid hormone receptor-positive breast cancer: the BIG 1-98 randomised clinical trial at 8.1 years median follow-up. *Lancet Oncol.* 12 (12), 1101–1108.
- Reiner, Z., 1996. The effects of androgens and other sex hormones on serum lipoproteins. *Lijec. Vjesn.* 118 (Suppl. 1), 33–37.
- Richards, J.S., Kersey, K.A., 1979. Changes in theca and granulosa cell function in antral follicles developing during pregnancy in the rat: gonadotropin receptors, cyclic AMP and estradiol-17  $\beta$ . *Biol. Reprod.* 21 (5), 1185–1201.
- Rinaudo, P., Strauss, J.F., 2004. Endocrine function of the postmenopausal ovary. *Endocrinol. Metab. Clin.* 33 (4), 661–674.
- Robertson, D.W., et al., 1982. Tamoxifen antiestrogens. A comparison of the activity, pharmacokinetics, and metabolic activation of the cis and trans isomers of tamoxifen. *J. Steroid Biochem.* 16 (1), 1–13.
- Rocca, W.A., et al., 2006. Survival patterns after oophorectomy in premenopausal women: a population-based cohort study. *Lancet Oncol.* 7 (10), 821–828.
- Rocca, W.A., et al., 2007a. Increased risk of cognitive impairment or dementia in women who underwent oophorectomy before menopause. *Neurology* 69 (11), 1074–1083.
- Rocca, W., et al., 2007b. Increased risk of cognitive impairment or dementia in women who underwent oophorectomy before menopause. *Neurology* 69 (11), 1074–1083.
- Rocca, W., et al., 2008a. Increased risk of parkinsonism in women who underwent oophorectomy before menopause. *Neurology* 70 (3), 200–209.
- Rocca, W.A., et al., 2008b. Long-term risk of depressive and anxiety symptoms after early bilateral oophorectomy. *Menopause* 15 (6), 1050–1059.
- Rocca, W.A., et al., 2012. Premature menopause or early menopause and risk of ischemic stroke. *Menopause* 19 (3), 272–277.
- Rocca, W.A., et al., 2016. Accelerated accumulation of multimorbidity after bilateral oophorectomy: a population-based cohort study. *Mayo Clin. Proc.* 91 (11), 1577–1589.
- Roger, V.L., et al., 2011. Heart disease and stroke statistics—2011 update: a report from the American Heart Association. *Circulation* 123 (4), e18–e209.
- Rosell, J., et al., 2011. Time dependent effects of adjuvant tamoxifen therapy on cerebrovascular disease: results from a randomised trial. *Br. J. Cancer* 104 (6), 899–902.
- Rossi, E., et al., 2009. Endocrine effects of adjuvant Letrozole compared with tamoxifen in hormone-responsive postmenopausal patients with early breast cancer: the HOBIE trial. *J. Clin. Oncol.* 27 (19), 3192–3197.
- Rusa, R., et al., 1999. 17 $\beta$ -estradiol reduces stroke injury in estrogen-deficient female animals. *Stroke* 30 (8), 1665–1670.
- Sampei, K., et al., 2000. Stroke in estrogen receptor- $\alpha$ -deficient mice. *Stroke* 31 (3), 738–743 (discussion 744).
- Sanossian, N., et al., 2013. Trends in cancer diagnoses among inpatients hospitalized with stroke. *J. Stroke Cerebrovasc. Dis.* 22 (7), 1146–1150.
- Sarrel, P.M., 1998. Cardiovascular aspects of androgens in women. *Semin. Reprod. Endocrinol.* 16 (2), 121–128.
- Sawada, M., et al., 2000. Estrogen receptor antagonist ICI182,780 exacerbates ischemic injury in female mouse. *J. Cereb. Blood Flow Metab.* 20 (1), 112–118.
- Schilder, C.M., et al., 2010. Effects of tamoxifen and exemestane on cognitive functioning of postmenopausal patients with breast cancer: results from the neuropsychological side study of the tamoxifen and exemestane adjuvant multinational trial. *J. Clin. Oncol.* 28 (8), 1294–1300.
- Seifer, D.B., et al., 1999. Women with declining ovarian reserve may demonstrate a decrease in day 3 serum inhibin B before a rise in day 3 follicle-stimulating hormone. *Fertil. Steril.* 72 (1), 63–65.
- Selvamani, A., Sohrabji, F., 2010a. Reproductive age modulates the impact of focal ischemia on the forebrain as well as the effects of estrogen treatment in female rats. *Neurobiol. Aging* 31 (9), 1618–1628.
- Selvamani, A., Sohrabji, F., 2010b. The neurotoxic effects of estrogen on ischemic stroke in older female rats is associated with age-dependent loss of IGF-1. *J. Neurosci.* 30 (20), 6852–6861.
- Selvik, H.A., et al., 2014. Prior cancer in patients with ischemic stroke: the Bergen NORSTROKE study. *J. Stroke Cerebrovasc. Dis.* 23 (5), 919–925.
- Sharpe, R., 1982. Cellular aspects of the inhibitory actions of LH-RH on the ovary and testis. *J. Reprod. Fertil.* 64 (2), 517–527.
- Shelley, J.M., et al., 1998. Relationship of endogenous sex hormones to lipids and blood pressure in mid-aged women. *Ann. Epidemiol.* 8 (1), 39–45.
- Sherwin, B.B., 1988. Estrogen and/or androgen replacement therapy and cognitive functioning in surgically menopausal women. *Psychoneuroendocrinology* 13 (4), 345–357.
- Silva, G.S., et al., 2010. Gender differences in outcomes after ischemic stroke: role of ischemic lesion volume and intracranial large-artery occlusion. *Cerebrovasc. Dis.* 30 (5), 470–475.
- Simpkins, J.W., et al., 1997a. Estrogens may reduce mortality and ischemic damage caused by middle cerebral artery occlusion in the female rat. *J. Neurosurg.* 87 (5), 724–730.
- Simpkins, J., et al., 1997b. Estrogens may reduce mortality and ischemic damage caused by middle cerebral artery occlusion in the female rat. *J. Neurosurg.* 87, 724–730.
- Sluijmer, A.V., et al., 1995. Endocrine activity of the postmenopausal ovary: the effects of pituitary down-regulation and oophorectomy. *J. Clin. Endocrinol. Metab.* 80 (7), 2163–2167.
- Sohrabji, F., 2015. Chapter 9 - cerebrovascular stroke: sex differences and the impact of estrogens A2 - Duncan. In: Kelli, A. (Ed.), *Estrogen Effects on Traumatic Brain Injury*. Academic Press, San Diego, pp. 125–141.
- Sohrabji, F., Bake, S., Lewis, D.K., 2013. Age-related changes in brain support cells: implications for stroke severity. *Neurochem. Int.* 63 (4), 291–301.
- Sohrabji, F., Park, M.J., Mahnke, A.H., 2017. Sex differences in stroke therapies. *J. Neurosci. Res.* 95 (1–2), 681–691.
- Sowers, M.R., et al., 2003. Endogenous hormones and bone turnover markers in pre- and perimenopausal women: SWAN. *Osteoporos. Int.* 14 (3), 191–197.
- Sowers, M.R., et al., 2010. Amount of bone loss in relation to time around the final menstrual period and follicle-stimulating hormone staging of the transmenopause. *J. Clin. Endocrinol. Metab.* 95 (5), 2155–2162.
- Stefanska, A., et al., 2012. Association of follicle-stimulating hormone and sex hormone binding globulin with the metabolic syndrome in postmenopausal women. *Clin. Biochem.* 45 (9), 703–706.
- Stefanska, A., et al., 2014. Association of FSH with metabolic syndrome in postmenopausal women: a comparison with CRP, adiponectin and leptin. *Biomark. Med* 8 (7), 921–930.
- Sun, L., et al., 2006. FSH directly regulates bone mass. *Cell* 125 (2), 247–260.
- Szymczak, J., et al., 1998. Concentration of sex steroids in adipose tissue after menopause. *Steroids* 63 (5–6), 319–321.
- Torr ns, J.I., et al., 2009. Relative androgen excess during the menopausal transition predicts incident metabolic syndrome in mid-life women: SWAN. *Menopause* 16 (2), 257.
- Toung, T.K., et al., 2000. Estrogen decreases infarct size after temporary focal ischemia in a genetic model of type 1 diabetes mellitus. *Stroke* 31 (11), 2701–2706.
- Towfighi, A., et al., 2007. A midlife stroke surge among women in the United States. *Neurology* 69 (20), 1898–1904.
- Ushiroyama, T., Sugimoto, O., 1995. Endocrine function of the peri- and postmenopausal ovary. *Horm. Res. Paediatr.* 44 (2), 64–68.
- Vale, W., et al., 1990. The inhibin/activin family of hormones and growth factors. In: *Peptide Growth Factors and Their Receptors II*. Springer, pp. 211–248.
- Walker, M.L., Herndon, J.G., 2008. Menopause in nonhuman primates? *Biol. Reprod.* 79 (3), 398–406.
- Wang, N., et al., 2017. Follicle-stimulating hormone, its association with cardiometabolic risk factors, and 10-year risk of cardiovascular disease in postmenopausal women. *J. Am. Heart Assoc.* 6 (9), e005918.
- Wassertheil-Smoller, S., et al., 2003. Effect of estrogen plus progestin on stroke in postmenopausal women: the Women's Health Initiative: a randomized trial. *JAMA* 289 (20), 2673–2684.
- Welt, C.K., et al., 1999. Female reproductive aging is marked by decreased secretion of dimeric inhibin. *J. Clin. Endocrinol. Metab.* 84 (1), 105–111.
- Wright, J.D., et al., 2013. Nationwide trends in the performance of inpatient hysterectomy in the United States. *Obstet. Gynecol.* 122 (2 Pt 1), 233–241.
- Yang, S.H., et al., 2002. Testosterone increases neurotoxicity of glutamate in vitro and ischemia-reperfusion injury in an animal model. *J. Appl. Physiol.* 92 (1), 195–201.
- Yang, S.H., et al., 2003. The use of estrogens and related compounds in the treatment of damage from cerebral ischemia. *Ann. N. Y. Acad. Sci.* 1007, 101–107.
- Yeh, J., Chen, M.-M., Aloia, J., 1996. Ovariectomy-induced high turnover in cortical bone is dependent on pituitary hormone in rats. *Bone* 18 (5), 443–450.
- Yeh, J., Chen, M., Aloia, J., 1997. Effects of 17 $\beta$ -estradiol administration on cortical and cancellous bone of ovariectomized rats with and without hypophysectomy. *Bone* 20 (5), 413–420.
- Zareba, P., et al., 2016. Androgen deprivation therapy and cardiovascular disease: what is the linking mechanism? *Ther. Adv. Urol.* 8 (2), 118–129.
- Zeynalov, E., et al., 2014. Reproductive senescence blunts response of estrogen receptor- $\alpha$  expression to estrogen treatment in rat post-ischemic cerebral microvessels. *PLoS One* 9 (7), e102194.