RESEARCH ARTICLE

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Microscopic Evaluation of Ovarian Surface Epithelium Following Treatment with Conjugated Estrogens in a Mouse Model

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Abstract

Background: This study was designed to evaluate the effect of different concentrations of conjugated equine estrogens (CEE) on the ovarian epithelium of female CD1 mice. **Methods:** Twenty-four female mice at 7 months with irregular estrus cycles were randomly divided into four groups of 6 mice each. Group one was considered as a control group and received a daily dose of 0.5ml of propylene glycol, for three weeks, while those in the treatment groups received a daily dose of 14µg/kg, 28µg/kg and 56µg/kg conjugated equine estrogens, respectively. **Results:** The results from this study showed a strong correlation between elevated concentrations of CEE and histological changes in ovarian surface epithelium (OSE). They also showed that administration of high-dose estrogen created the conditions for excessive proliferation of OSE which may progress into the development of cysts in the ovaries. **Conclusion:** This study concluded that high concentrations of CEE may increase the chances of developing epithelial ovarian cancer.

Keywords: Ovary- Propylene glycol- histology- hormone- Mice

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Introduction

Ovarian cancer is one of the most common gynecological malignancies and is ranked third after cervical and uterine cancers worldwide Sung et al., 2020). In the Arab countries, ovarian cancer ranked fourth among top ten diagnosed cancer cases in women from different age groups (Oetashi and Al Kalbani, 2013). Ovarian cancer also has the worst prognosis and the highest mortality rate, and it is three times more lethal than breast cancer (Yoneda et al., 2013; Coburn et al., 2017). Ovarian cancer is a heterogeneous disease and the subtypes are very different from other gynecological cancers such as breast and endometrial cancers with respect to origins, behaviors, mutations, markers, and prognosis and responses to therapy (Mungenast and Theresia, 2014). Epithelial ovarian cancer (EOC) constitutes the majority of malignant ovarian tumors in adult women and is the commonest cause of gynecological cancer-associated death with the highest mortality rate of all cancers in the female reproductive system with a five-year survival of only 45% (Sung et al., 2020; Canadian Cancer Society, 2016). Ovarian neoplasms are classified into distinct morphological categories based on the epithelial appearance into tumors of serous, mucinous, clear cell, endometrioid, seromucinous, squamous, Brenner and undifferentiated (carcinoma) type (Jayson et al., 2014; Nagamine and Mikami, 2020). Approximately 90% of ovarian cancers originate from the ovarian surface epithelium (OSE) or the fallopian tube surface epithelium (Torpy, 2014; Dietl, 2014; Webb and Jordan, 2016; Reid et al., 2017; Shabir and Gill, 2020). However, the origin and pathogenesis of epithelial ovarian cancer remains poorly understood (Kurman and Shih, 2010; Farr et l., 2015).

The OSE is a monolayer of squamous to cuboidal cells surrounding the ovary, which are repeatedly exposed to estrogens and play an active role in ovulatory wound repair (Fathalla, 1971; Choi et al., 2001). During the reproductive life of a woman, OSE secret enzymes that digest walls of preovulatory follicles to facilitate the release of oocyte into the fallopian tube (Land, 1993;

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Murdoch, 1996). OSE at the site of rupture becomes exposed to stromal microenvironment (Wu et al., 1992, 1993) and fragments of OSE become invaginated in the ovarian cortical stroma giving rise to epithelial crypts and inclusion cysts (Womg and Leung, 2007; Auersperg, 2013). These structures regarded as preneoplastic and may promote malignant transformation of entrapped OSE by generating a microenvironment enriched with growth factors, cytokines and hormones (Berchuck, 1997; Auersperg, 2013). Higher frequency of inclusion cysts is observed in postmenopausal than in premenopausal women (Kronenberg et al., 2008).

Established risk factors for the development of EOC are age, infertility, and family history, whereas oral contraceptive use, increasing parity, hysterectomy or tubal ligation decreases the risk (Riman et al., 2002; Kazerouni et al., 2006; Hunn and Rodriguez, 2012; Rice et al., 2014; Zhang et al., 2019; Momenimovahed et al., 2019). In addition, well-established associations between reproductive physiognomic and EOC support an involvement of reproductive steroid hormones in the etiology of EOC (Riman et al., 2004; Schock et al., 2014). Furthermore, studies reported that steroid hormones, including estrogens, progesterone and androgens, are involved in ovarian carcinogenesis (Pearce et al., 2009; Koskela-Niska et al., 2013a, Blanco et al., 2017). Menopausal hormone replacement therapy (HRT) is widely used to treat postmenopausal symptoms and reduce bone loss. Findings from epidemiological studies investigating the effects of HRT on carcinogenesis are contradictory. The use of physical or oral contraceptives including HRT have been reported to increases the risk of ovarian cancer (Morch et al., 2009; Tsilidis et al., 2011; Urban et al., 2015; Simin et al., 2017). However, several studies found no relationship between them (Hunn and Rodriguez, 2012: Li et al., 2015; Rasmussen et al., 2017) and others studies found a positive association in individual histological subtype (Morch et al., 2012; Yang et al., 2012; Koskela-Niska et al., 2013b).

Conjugated equine estrogens (CEE) are commonly used in postmenopausal HRT, and administration of CEE in animal model reduces ovulation and promotes proliferation of the OSE (Murdoch and Van Kirk, 2002; Laviolette et al., 2014) as well as the emergence of ovarian cysts (Bai et al., 2000; Laviolette et al., 2010). However, the effects of these hormones on the OSE are not fully understood. Estrogens favor neoplastic transformation of the OSE, and their mechanisms of action on the tumorigenic process are complex, involving considerable genetic alterations (Hua et al., 2018). Several studies found a significant estrogen increase in EOC risk (Lacey et al., 2006; Beral, 2007; Rasmussen et al., 2017) but others reported no significant positive associations (Simin et al., 2017; Coughlin et al., 2000). Other studies have suggested there may be a small increased risk of EOC associated with longer-term use of estrogen (Ho, 2003; Wentzensen et al., 2016). Data about the association of HRT with ovarian cancer, therefore, is still controversial (D'Alonzo et al., 2019). The aim of the present study was to evaluate the carcinogenic effects of three different concentrations of CEE on the histological structural changes of ovarian surface epithelium of the adult female mice at cellular level following a twenty-one-day period.

Materials and Methods

Animals

Twenty-four female CD-1 mice of 7 months of age and weighing between 30 and 35g, were used in this study following approval by the Animal Ethics Committee at University of Nizwa. Mice were divided into four groups of six mice per cages and housed in a small animal facility at University of Nizwa on a 12-hour light cycle with access to ad libitum food and water with controlled room temperature (24-25oC) and humidity (30-35%). One week after adaptation, the first set represents control group and received a daily dose of 0.5ml of propylene glycol (vehicle). The second, third and fourth groups received a daily dose of 14µg/kg, 28µg/kg and 56µg/kg conjugated equine estrogens (CEE), respectively. The conjugated equine estrogens were diluted in 0.5 ml of propylene glycol and administered daily in the morning by gavage for 21 days. Gavage were performed with a standard metal tube supplied by the Stem Cells and Regenerative Medicine Department at the Natural and Medical Research Center, University of Nizwa.

Histology

A day after final treatment, mice were decapitated under deep anesthesia of 5% isoflurane for 5 minutes. Both ovaries were dissected using stereomicroscope and examined with the naked eye for gross abnormal changes then immersed in 10% formaldehyde solution for tissue processing. Tissue preparations were accomplished by using the Automated Tissue Processer instrument (ATP 140). In this process tissues were dehydrated by graded concentrations of ethanol 50%, 70%, 90%, 1h each and 3 changes of 100% ethanol for 1, 2 and 2½h, respectively. Tissues were then cleared with 3 changes of xylene for 1, 1, and 2h, respectively, then infiltrated in 3 changes of melted paraffin wax at 60oC for 1h each. Samples were then embedded in pure paraffin wax by using the Embedding Center Machine (AEC 380).

Paraffin blocks were sectioned by Rotary Microtome (AEM 450) to produce thin sections of 5 µm thickness, three slides being therefore prepared for each block. Sections were collected with glass slides and placed on hot plate. Sections were then placed in xylene for 2 periods of 5 minutes each to remove wax, then hydrated in 2 changes of pure ethanol of 5 minutes each, then in 95% ethanol and then in 70% ethanol for 2 minutes each, then in distilled water for 2 minutes. Each section stained with Hematoxylin and Eosin (H and E), dehydrated with 95% ethanol for 3 to 5 dips, 2 changes of 100% ethanol for 2 minutes each, and then cleared with xylene for 5 minutes each. Finally, slides were mounted with DPX and coverslip and screened under digital light microscope. Ten randomly selected non-overlapping fields were investigated by histologists under light microscopy for each slide at different magnifications.

Results

Light microscopic observation of ovary sections showed significant difference among groups 1, 2, 3 and 4. Control group (1) showed normal ovarian morphology with uniform surface epithelium, ovarian follicles, and connective tissue (stroma) as shown in Figure 1A-1C. Group 4 showed significant changes in the ovarian morphology with thickening of surface epithelium as shown in Figure 4A-4C. The mice in the control group sections showed normal ovarian tissue made up of cortex and medulla (Figure 1A-1C). The cortex consists of surface epithelium, tunica albuginea, ovarian follicles, and stroma. The medulla consists of blood vessels, lymphatic vessels, and nerve fibers in stroma (Figure 1A). The ovarian follicles are in different stages of follicular development consisting of primordial, primary, and secondary follicles (Figure 1B). The structure of primordial follicles includes monolayer of squamous epithelium surrounding the ooplasm and oocyte. Primary follicles are larger in size and made up of theca layer of endocrine cells, granulosa layer of cuboidal epithelium, zona pellucida of microvilli, ooplasm and oocyte (Figure1B). The secondary follicles are much larger in size and made up of theca layer, granulosa layer with an antrum fluid, zona pellucida, ooplasm and oocyte (Figure1B). The ovarian surface epithelium (OSE) made up of monolayer of squamous epithelium (Figure 1C).

In group 2, tissues from the ovaries exhibited no change in the morphology after three-week treatment with CEE (Figure 2A-2C). In group 3, the ovaries exhibited slight thickening in the OSE and increased cellular contents of the theca-interstitial tissue in stroma (Figure 3A-3C). The cortex and medulla showed normal morphological details (Figure 3A) and the structure of developing ovarian follicles made up of theca layer, granulosa layer, zona pellucida, ooplasm and oocyte ovarian follicles (Figure 3B). In stroma, however, an increased number of theca-interstitial cells was observed. The OSE showed abnormal morphology with slight thickening of OSE exhibiting multilayer epithelium (Figure 3C). In group 4, the ovaries exhibited marked alterations to the OSE morphology after three-week treatment with CEE (Figure 4A-4C). The ovarian follicles in the cortex and the medulla showed normal morphological details with the exception that the stroma exhibited an increased number of cells in the theca-interstitial tissue (Figure 4A, 4B and 4C). The OSE appeared thicker than those observed in group 3

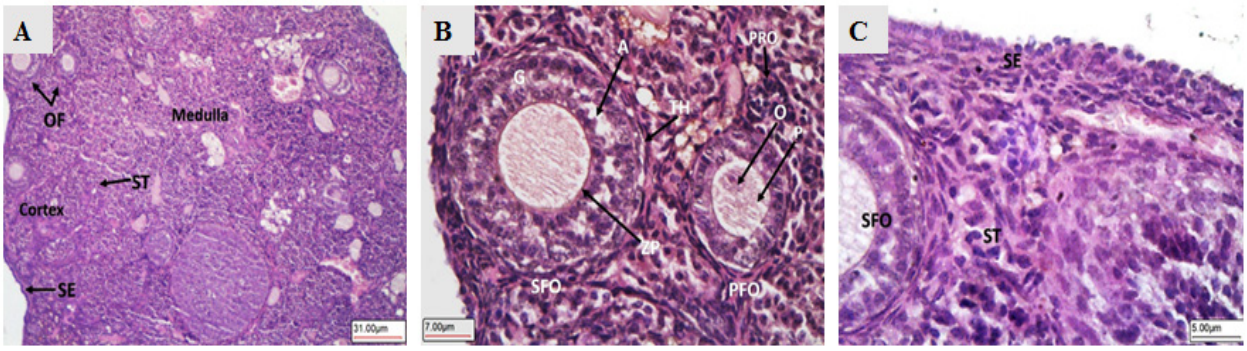

Figure 1. Light Microscopy Images of Hematoxylin and Eosin (H&E) Stained Samples of Ovary from the Control Group. Figure 1A, showing the ovarian cortex and medulla. The cortex is made up of OSE (SE), ovarian follicles (OF), and stroma (ST). Figure 1B, the cortex consists of primordial follicle (PRO), primary follicle (PFO), and secondary follicle (SFO). Both primary and secondary follicles structures include theca layer of endocrine cells (TH), granulosa layer of cuboidal epithelium (G), ooplasm (P) and oocyte (O). SFO with antrum fluid (A). Figure 1C, the OSE made up of monolayer of squamous epithelium (SE). Secondary follicle (SFO), stroma (ST). Mag. 100X, 400X and 600X, for A, B, and C, respectively.

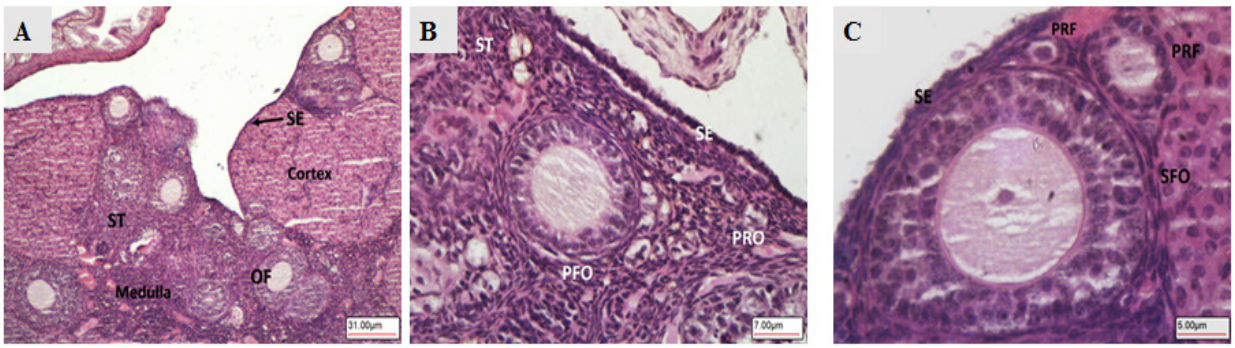

Figure 2. Histology Image of H&E-stained Samples of Ovary from Group Two Normal Ovarian Cortex and Medulla. Figure 2A, the cortex is made up of surface epithelium (SE), follicles (OF), and stroma (ST). Figure 2B, the cortex consists of primordial follicle (PRO), primary follicle (PFO), and stroma (ST). Figure 2C, the OSE made up of monolayer of squamous epithelium (SE). Primordial follicle (PRF), secondary follicle (SFO). Mag. 100X, 400X and 600X, for A, B and C, respectively.

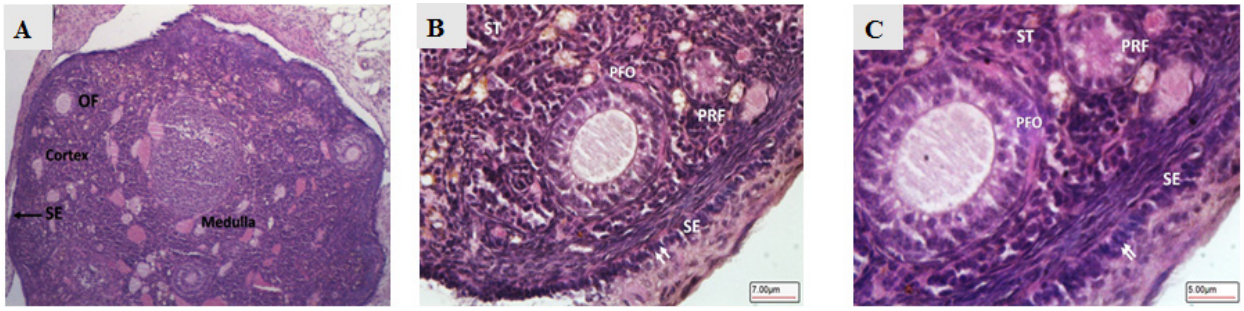

Figure 3. Histology Image of H&E Stained Samples of Ovary from CEE Treated Group Three Showing Ovary with Cortex and Medulla. Figure 3A, showing the cortex consists of normal ovarian follicles (OF) and abnormal OSE (SE). The cortex appeared denser than those observed in groups 1 and 2. Figure 3B, showing OSE (SE), primordial follicle (PRF) and primary follicle (PFO). Stroma (ST). Slight thickening observed in the OSE (double arrow) and the stroma (ST). Figure 3C, showing slight thickening of OSE (SE) made up of multilayered squamous epithelial cells (double arrow) and slight increase in the number of theca-interstitial cells in the stroma (ST). Primordial follicle (PRF) and primary follicle (PFO). Mag. 100X, 400X and 600, for A, B and C, respectively.

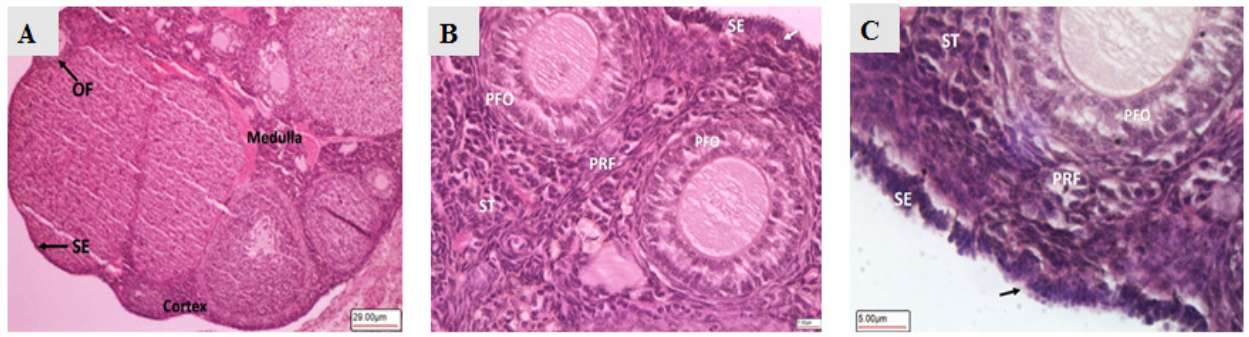

Figure 4. Histology Image of H&E Stained Samples of Ovary from CEE Treated Group Four Showing Ovary with Cortex and Medulla. Figure 4A showing the cortex consists of normal ovarian follicles (OF) and abnormal OSE (SE). The cortex appeared denser than those observed in other groups. 2Figure 4B, showing OSE (SE), primordial follicle (PRF) and primary follicle (PFO). Stroma (ST). Marked thickening (arrow) observed in the OSE. Figure 4C, showing marked thickening of OSE (SE) made up of increased multilayered squamous epithelial cells (arrow), and increased number of theca-interstitial cells in the stroma (ST). Primordial follicle (PRF) and primary follicle (PFO). Stroma (ST). Mag. 100X, 400 and 600X, for A, B and C, respectively.

with increased multilayered squamous epithelium clearly evident (Figures 4B and 4C).

Discussion

Estrogen replacement therapy was initially prescribed for the treatment of menopausal symptoms in the early 1940s. However, due to increased incidents of endometrial cancer in women in the 1970s, estrogen hormonal therapy declined and as a result progestin was introduced to HRT formulations which led to improved management of symptomatic menopause and prevention of chronic diseases (McNeil, 2017). Reports of increased risks of breast cancer and cardiac disease in the early 2000 led to closure of the estrogen and progestin therapy after 5 years of follow-up (Rossouw et al., 2002). Nevertheless, estrogen remain the most effective treatment prescribed to date for the management of menopausal symptoms following a thorough evaluation of the risks and benefits (Hill et al., 2016). Significant association between ovarian cancer and HRT remains controversial largely due to insufficient data. Observational studies reported association of estrogen use vary substantially by histologic subtype and appeared mainly associated with non-serous cancers (Trabert et al., 2016). However, a recent meta-analysis of studies by histologic subtypes reported menopausal HRT may increase the risk of serous and endometrioid tumors (Liu et al., 2019).

Estrogen is a steroid hormone produced primarily in the ovaries in females and to a lesser extent, in the testes in males and plays an important role in reproductive development, brain functions, cardiovascular remodeling and bone homeostasis. However, high concentrations of estrogen also promote ovarian, mammary and endometrial tumorigenesis (Hua et al., 2018). Estrogen and gonadotropins are known to control the ovary and are strong regulators of surface epithelium proliferation. Studies have reported that gonadotropins and estrogens stimulate surface epithelium cell proliferation (Bai et al., 2000; Hess et al., 1999; Song et al., 2020) through hormone receptors that are present on the surface of cells of the surface epithelium (Kuroda et al., 2001; Saddick, 2014). Evidence showed that estrogen receptors alpha and beta are present in 60–100% of ovarian tumors resected from patients (Lindgren et al., 2004; De Stefano et al., 2011). Other studies have found an epithelial ovarian cancer cell line, PE04, containing high levels of cytosolic estrogen receptor-like binding (Nash et al., 1989).

The monolayer of OSE is a heterogeneous population with multiple morphologies observable in primary cultures and animal models (Okamura and Katabuchi, 2003). Ovarian surface epithelial (OSE) cells exposed to elevated levels of estradiol develop a subpopulation that exhibit loss of cell polarity, contact inhibition, and forms multilayered foci of dysplastic cells with increased susceptibility to transformation (Vuong et al., 2018). Defects in cell polarity are often reported in human cancer (Fish and Molitoris, 1994; Bissell and Radisky, 2001) and loss of contact inhibition usually results in outgrowth of cells from the epithelial monolayer (mcclatchey and Yap, 2012). In this study, the three-week treatment with CEE administrated in adult female mice have demonstrated that estrogen induced OSE dysplasia as a direct result of prolonged exposure to estrogen. Tissue samples obtained from the ovaries of different mice in group three treated with CEE showed slight thickening of the ovarian surface epithelium (OSE) exhibiting multilayered squamous epithelium. Samples from group four treated with CEE, however, showed significant thickening of OSE in the ovaries. OSE exhibited an increase in the multilayers of squamous epithelium. Similar findings also reported in other studies (Perniconi et al., 2008; Vuong et al., 2017). Epithelial cells exhibit contact behavior for proper formation of the epithelial monolayer by inhibiting cell proliferation upon contact with neighboring cells. Studies have reported that loss of contact inhibition result in outgrowth of cells from the epithelial monolayer (Mcclatchey and Yap, 2012) similar to the multilayered OSE observed in our study.

The CEE treated groups in the present study also exhibited an increase in the number of theca-interstitial cells in the stroma of the ovaries of all samples. The theca layer of ovarian follicle is a connective tissue surrounding the granulosa cells and responsible for the production of androgens under the influence of gonadotropins stimulation (Fowler et al., 2011; Allen et al., 2016; Richards et al., 2017; Babu et al., 2000; Li et al., 2007). The theca-derived androgens are then converted into estradiol by granulosa cells (Fortune and Armstrong, 1978). which in turn may increase OSE proliferation. The entrapment of OSE cells in inclusion cysts increases the chance of OSE neoplastic transformation, as a consequence of stromal androgen-rich milieu (Rao and Slotman, 1991; Risch, 1998). Consistent with this androgen theory, women with polycystic ovary syndrome (PCOS) have a higher risk of developing ovarian cancer, as a result of anovulation and higher levels of circulating androgen. Recent studies reported that 70% of PCOS cases have elevated testosterone levels (Rosenfield and Ehrmann, 2016). Although the androgen theory may explain the effect of HRT on the OSE, it is conflicting and yet to be proven, and the derivation and roles of cells within the theca are less well defined (Richards et al., 2017; Mizushima and Miyamoto, 2019).

The role of hormonal therapy in the development of ovarian cancer remains unclear, and the mechanism underlying the association of menopausal HRT with ovarian cancer have not yet been fully established. A recent study of heterogeneity demonstrated possible mechanisms whereby activation of various cancer-associated pathways may increase OSE dysplasia as a result of prolonged exposure to estrogen (Vuong et al., 2018). This could be attributed to the fact that exogenously administered estrogens in postmenopausal women may be several-fold higher than endogenous estrogens (Trabert et al., 2016). Other studies have suggested that high levels of gonadotropins during menopause promote ovarian cancer (Stadel, 1975; Feng et al., 2017). These findings suggest that menopausal HRT may have a role in decreasing the risk of cancer by reducing gonadotropins level. However, these benefits might be overcome by estrogen-induced ovarian cell proliferation (Vuong et a., 2018; Gramer and Welch, 1983). Estrogen stimulates the proliferation of OSE cells whereas progesterone promotes apoptosis of ovarian cells. The weaker risk effect of HRT than estrogen alone may be because progesterone counteracts the proliferative effect of estrogen on ovarian cells (Lau et al., 1999; Syed et al., 2001; Mukerjee et al., 2005). Interactions between genetic, hormonal and environmental factors may impact ovarian cancer risk in an individual patient, but an understanding of the interactions and the order of risk factors is still incomplete.

In conclusion, hormonal replacement therapy may increase the risk of human ovarian cancer as exogenous estradiol accelerates the onset of ovarian cancer in mouse models. Administration of a high-dose of conjugated equine estrogen in our experimental model (CD1 mice) resulted in cellular proliferation and morphological dysplasia of ovarian superficial epithelium. This work supports earlier studies which have shown that estradiol increases epithelial cell susceptibility to tumor initiation. Further studies are needed to better understand the consequences of prolonged exposure to exogenous steroid hormones for disease prevention and to mitigate the risk associated with hormone therapy.

Author Contribution Statement

I.A, AS, WH, MM, FJ, and IK. conceived the experimental design. I.A. IK, AK, AH, SH and SV. performed the experiment, data observation and acquisition. I.A, SK, AS, WH, MM, FJ, SV, AA, SY, and KR, analyzed and interpreted the data. I.A, IK, AK, AS, WH, MM, FJ and AA. IA and IK wrote the paper. All authors reviewed the manuscript, read and approved the final manuscript.

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Mashmuli, I. Jamshidi-Adgani, and S. Vakilian performed the experiment, data observation and acquisition. I, Amri, I. Kadim S.Khalaf, A. Amri, S, Al-Yaqoobi and K. Al-Riyami analyzed and interpreted the data. A. Amri, I. Kadim A. Abdulziz wrote the paper. All authors reviewed the manuscript, read and approved the final manuscript. The authors declare that they have no conflict of interest.

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Ethical approval

The use of the animals was approved by the Animal Ethics Committee at University of Nizwa.

Competing Interests

The authors declare that they have no conflict of interest.

References

Allen JJ, Herrick SL, Fortune JE (2016). Regulation of steroidogenesis in fetal bovine ovaries: differential effects of LH and FSH. *J Mol Endocrinol*, **57**, 275–86.

Alonzo M, Bounous VE, Villa M, et al (2019). Current Evidence of the Oncological Benefit-Risk Profile of Hormone Replacement Therapy. *Medicina (Kaunas)*, **55**, 573.

Auersperg N (2013). Ovarian surface epithelium as a source of ovarian cancers: unwarranted speculation or evidence-based hypothesis?. *Gyn Onco*, **2013**, 1–6.

Babu PS, Krishnamurthy H, Chedrese PJ, et al (2000). Activation of extracellular-regulated kinase pathways in ovarian granulosa cells by the novel growth factor type 1 follicle-stimulating hormone receptor. Role in hormone signaling and cell proliferation. *J Biol Chem*, **275**, 27615–26.

Bai W, Oliveros-Saunders B, Wang Q, et al. (2000). Estrogen stimulation of ovarian surface epithelial cell proliferation. *In Vitro Cell Dev Biol Ani*, **36**, 657-666.

Beral V (2007). Million Women Study Collaborators. Ovarian cancer and hormone replacement therapy in the Million Women Study. *Lancet*, **369**, 1703–10.

Berchuck A, Carney M (1997). Human ovarian cancer of the surface epithelium. *Biochem Pharma*, **54**, 541-4.

Bissell MJ, Radisky D (2001). Putting tumours in context. *Nat Rev Cancer*, **1**, 46–54.

Blanco Jr, Luis Z, Elisabetta K, et al (2017). Steroid hormone synthesis by the ovarian stroma surrounding epithelial ovarian tumors: a potential mechanism in ovarian tumorigenesis. *Mod Path*, **30**, 563–576.

Canadian Cancer Society (2016). Canadian Cancer Statistics 1–142 (Canadian Cancer Society.

Choi KC, Kang SK, Tai CJ, et al (2001). Estradiol up-regulates antiapoptotic Bcl-2 messenger ribonucleic acid and protein in tumorigenic ovarian surface epithelium cells. *Endocrino*, **142**, 2351–60.

Coburn S, Bray F, Sherman M, et al (2017). International patterns and trends in ovarian cancer incidence, overall and by histologic subtype. *Int J Cancer*, **140**, 2451–60.

Coughlin SS, Giustozzi A, Smith SJ, et al (2000). A meta-analysis of estrogen replacement therapy and risk of epithelial ovarian cancer. *J Clin Epidemiol*, **53**, 367-75.

Cramer DW, Welch WR (1983). Determinants of ovarian cancer risk. II. Inferences regarding pathogenesis. *J Natl Cancer Inst*, **71**, 717–21.

De Stefano I, Zannoni GF, Prisco MG, et al (2011). Cytoplasmic expression of estrogen receptor b (ERb) predicts poor clinical outcome in advanced serous ovarian cancer. *Gyneco Onco*, **122**, 573–9.

Dietl (2014). Revisiting the pathogenesis of ovarian cancer: the central role of the fallopian tube. *Arch Gynecol Obstet*, **289**, 241-246.

Farr R, Nezhat RA, Camran N, et al (2015). Tanja Pejovic. New insights in the pathophysiology of ovarian cancer and implications for screening and prevention. A*mer J Obst Gyneco*, **213**, 262-7.

Fathalla MF (1971). Incessant ovulation-a factor in ovarian neoplasia?. *Lancet*, **2**, 163.

Feng D, Zhao T, Yan K, et al (2017). Gonadotropins promote human ovarian cancer cell migration and invasion via a cyclooxygenase 2-dependent pathway. *Oncol Rep*, **38**, 1091-8.

Fish EM, Molitoris BA (1994). Alterations in epithelial polarity and the pathogenesis of disease states. *N Engl J Med*, **330**, 1580–8.

Folsom AR, Anderson JP, Ross JA (2004). Estrogen replacement therapy and ovarian cancer. *Epidemiol*, **15**, 100-4.

Fortune JE, Armstrong DT (1978). Hormonal control of 17 beta-estradiol biosynthesis in proestrous rat follicles: estradiol production by isolated theca versus granulosa. *Endocrinol*, **102**, 227–35.

Fowler PA, Anderson RA, Saunders PT, et al (2011). Development of steroid signaling pathways during primordial follicle formation in the human fetal ovary. *J Clin Endocrinol Metab*, **96**, 1754–62.

Hess S, Gulati R, Peluso JJ (1999). Hepatocyte growth factor induces rat ovarian surface epithelial cell mitosis or apoptosis depending on the presence or absence of an extracellular matrix. *Endo*, **140**, 2908-16.

Hill DA, Crider M, Hill SR (2016). Hormone Therapy and Other Treatments for Symptoms of Menopause. *Am Fam Physician*, **94**, 884-9.

Ho SM (2003). Estrogen, progesterone and epithelial ovarian cancer. *Rep Biol Endocrinol*, **7**, 1-73.

Hua H, Hongying Z, Qingbin K, et al (2018). Mechanisms for estrogen receptor expression in human cancer. *Exp Hematol Oncol*, **7**, 1-11.

Hunn J, Rodriguez GC (2012). Ovarian cancer: etiology, risk factors, and epidemiology. *Clin Obstet Gynecol*, **55**, 3–23.

Jayson GC, Kohn EC, Kitchener HC, et al (2014). Ovarian cancer. *Lancet*, **11**, 1376-88.

Kazerouni N, Greene MH, Lacey Jr JV, et al (2006). Family history of breast cancer as a risk factor for ovarian cancer in a prospective study. *Cancer*, **107**, 1075–83.

Kronenberg HM, Memed S, Polonsky KS et al (2008) In Williams Textbook of Endocrinology. Chapter: The Physiology and Pathology of the Female Reproductive Axis, 11th edn. New York, NY, USA: Elsevier Health Sciences (ISBN: 9781416029113).

Koskela-Niska V, Lyytinen H, Riska A, et al (2013a). Ovarian cancer risk in postmenopausal women using estradiol progestin therapy - a nationwide study. *Climacteric*, **16**, 48–53.

Koskela-Niska V, Pukkala E, Lyytinen H, et al (2013b). Effect of various forms of postmenopausal hormone therapy on the risk of ovarian cancer–a population-based case control study from Finland. *Int J Cancer*, **133**, 1680–8.

Kurman RJ, Shih IM (2010). The Origin and pathogenesis of epithelial ovarian cancer-a proposed unifying theory. *Amer J Surg Patho*, **34**, 433.

Kuroda H, Mandai M, Konishi I, et al (2001). Human ovarian surface epithelial (OSE) cells express LH/hCG receptors,

and hCG inhibits apoptosis of OSE cells via up-regulation of insulin-like growth factor-1. *Int J Cancer*, **91**, 309-315.
Lacey JV, Louise A, Brinton, MF, et al (2006). Menopausal Hormone Therapy and Ovarian Cancer Risk in the National Institutes of Health–AARP Diet and Health Study Cohort. *JNCI J Nation Cancer Inst*, **98**, 1397–1405.
Land JA (1993). Ovulation, ovulation induction and ovarian carcinoma. *Bailliere's Clini Obst Gynae*, **7**, 455–72.
Lau KM, Mok SC, Ho SM (1999). Expression of human estrogen receptor-alpha and -beta, progesterone receptor, and androgen receptor mRNA in normal and malignant ovarian epithelial cells. *Proc Natl Acad Sci U S A*, **96**, 5722–7.
Laviolette LA, Hodgkinson KM, Minhas N, et al (2014). 17β-estradiol upregulates GREB1 and accelerates ovarian tumor progression in vivo. *Int J Cancer*, **135**, 1072-84.
Laviolette L, Kenneth GA, Elizabeth A, et al (2010). 17β-Estradiol Accelerates Tumor Onset and Decreases Survival in a Transgenic Mouse Model of Ovarian Cancer. *Endo*, **151**, 929–38.
Li Y, Ganta S, Cheng C, et al (2007). FSH stimulates ovarian cancer cell growth by action on growth factor variant receptor. *Mol Cell Endocrino*, **267**, 26–37.
Li K, Husing A, Fortner RT, et al (2015). An epidemiologic risk prediction model for ovarian cancer in Europe: the EPIC study. *Br J Cancer*, **112**, 1257–65.
Lindgren PR, Cajander S, Backstrom T, et al (2004). Estrogen and progesterone receptors in ovarian epithelial tumors. *Mole Cell Endo*, **221**, 97–104.
Liu Y, Ma L, Yang X, et al (2019). Menopausal Hormone Replacement Therapy and the Risk of Ovarian Cancer: A Meta-Analysis. *Front Endocrinol (Lausanne)*, **3**, 801.
Mcclatchey AI, Yap AS (2012). Contact inhibition (of proliferation) redux. *Curr Opin Cell Biol*, **24**, 685–94.
McNeil M (2017). Menopausal hormone therapy: understanding long-term risks and benefits. *JAMA*, **318**, 911-3.
Mizushima T, Miyamoto H (2019). The Role of Androgen Receptor Signaling in Ovarian Cancer. *Cells*, **8**, 176.
Momenimovahed Z, Azita T, Safoura T, et al (2019). Ovarian cancer in the world: epidemiology and risk factors. *Inter J Womens Heal*, **11**, 287–99.
Morch LS, Lokkegaard E, Andreasen AH, et al (2009). Hormone therapy and ovarian cancer. *JAMA*, **302**, 298–305.
Morch LS, Lokkegaard E, Andreasen AH, et al (2012). Hormone therapy and different ovarian cancers: a national cohort study. *Am J Epidemiol*, **175**, 1234–42.
Mungenast F, Theresia T (2014). Estrogen biosynthesis and action in ovarian cancer. *Front Endo Exp Endo*, **5**, 192, 1-12.
Mukherjee K, Syed V, Ho SM (2005). Estrogen-induced loss of progesterone receptor expression in normal and malignant ovarian surface epithelial cells. *Oncogen*, **24**, 4388–400.
Murdoch WJ (1996). Ovarian surface epithelium, ovulation and carcinogenesis. *Bio Rev Cambridge Philo Soc*, **71**, 529–43.
Murdoch WJ, Van Kirk EA (2002). Steroid hormonal regulation of proliferative, p53 tumor suppressor, and apoptotic responses of sheep ovarian surface epithelial cells. *Mol Cell Endocrinol*, **186**, 61-7.
Nagamine M, Mikami Y (2020). Ovarian Seromucinous Tumors: Pathogenesis, Morphologic Spectrum, and Clinical Issues. *Diagn (Basel)*, **31**, 77.
Nash JD, Ozols RF, Smyth JF, et al (1989). Estrogen and anti-estrogen effects on the growth of human epithelial ovarian cancer in vitro. *Obst Gyn*, **73**, 1009-1016.
Okamura H, Katabuchi H (2003). Cell biology of human ovarian surface epithelial cells and ovarian carcinogenesis. *Med Electron Microsco*, **36**, 74–86.
Ortashi O, Al Kalbani M (2013). Gynecological Cancer Services in Arab Countries: Present Scenario, Problems and Suggested Solutions. *Asian Pac J Can Prev*, **14**, 2147-50.
Pearce CL, Chung K, Pike MC, et al (2009). Increased ovarian cancer risk associated with menopausal estrogen therapy is reduced by adding a progestin. *Cancer*, **115**, 531–9.
Perniconi SE, Simões MJ, Simões RS, et al (2008). Proliferation of the superficial epithelium of ovaries in senile female rats following oral administration of conjugated equine estrogens. *Clinics (Sao Paulo)*, **63**, 381-8.
Rasmussen ELK, Hannibal CG, Dehlendorff C, et al (2017). Parity, infertility, oral contraceptives, and hormone replacement therapy and the risk of ovarian serous borderline tumors: a nationwide case-control study. *Gynecol Oncol*, **144**, 571–6.
Rao BR, Slotman BJ (1991). Endocrine factors in common epithelial ovarian cancer. *Endocrinol Rev*, **12**, 14-26.
Reid BM, Permuth JB, Sellers TA (2017). Epidemiology of ovarian cancer: a review. *Canc Biol Med*, **14**, 9–32.
Rice MS, Hankinson SE, Tworoger SS (2014). Tubal ligation, hysterectomy, unilateral oophorectomy, and risk of ovarian cancer in the Nurses' Health Studies. *Fertil Steril*, **102**, 192–8.
Richards JS, Ren YA, Candelaria N, et al. (2018). Ovarian Follicular Theca Cell Recruitment, Differentiation, and Impact on Fertility: 2017 Update. *Endocr Rev*, **39**, 1-20.
Riman T, Dickman PW, Nilsson S, et al (2002). Risk factors for invasive epithelial ovarian cancer: results from a Swedish case-control study. *Am J Epidemiol*, **156**, 363 – 73.
Riman T, Staffan N, Ingemar R (2004). Persson. Review of epidemiological evidence for reproductive and hormonal factors in relation to the risk of epithelial ovarian malignancies. *Acta Obstet Gynecol Scand*, **83**, 783–95.
Risch HA (1998). Hormonal etiology of epithelial ovarian cancer, with a hypothesis concerning the role of androgens and progesterone. *J Natl Cancer Inst*, **90**, 1774-86.
Rosenfield RL, Ehrmann DA (2016). The Pathogenesis of Polycystic Ovary Syndrome (PCOS): The Hypothesis of PCOS as Functional Ovarian Hyperandrogenism Revisited. *Endocrinol Rev*, **37**, 467-520.
Rossouw JE, Anderson GL, Prentice RL, et al (2002). Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the Women's Health Initiative randomized controlled trial. *JAMA*, **288**, 321-33.
Saddick SY (2014). Ovarian surface epithelium receptors during pregnancy and estrus cycle of rats with emphasis on steroids and gonadotropin fluctuation. *Saudi J Biological Sci*, **21**, 232–7.
Shabir S, Gill PK (2020). Global scenario on ovarian cancer – Its dynamics, relative survival, treatment, and epidemiology. *Adesh Uni J Medi Sci Res*, **2**, 17-25.
Schock H, Heljä-Marja SA, Zeleniuch-Jacquotte, K, et al (2014). Early pregnancy sex steroids and maternal risk of epithelial ovarian cancer. *Endocr Relat Cance*, **21**, 831–44.
Simin J, Tamimi R, Lagergren J, et al (2017). Menopausal hormone therapy and cancer risk: an overestimated risk?. *Eur J Cancer*, **84**, 60–8.
Song K, Dai L, Long X, et al (2020). Follicle-stimulating hormone promotes the proliferation of epithelial ovarian cancer cells by activating sphingosine kinase. *Sci Rep*, **10**, 13834.
Stadel BV (1975). Letter: The etiology and prevention of ovarian cancer. *Am J Obstet Gynecol*, **123**, 772–4.
Sung H, Jacques F, Rebecca L. et al (2021). Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *Ca Cancer J Clin*, **71**, 209–49.
Syed V, Ulinski G, Mok SC, et al (2001). Expression of gonadotropin receptor and growth responses to key

reproductive hormones in normal and malignant human ovarian surface epithelial cells. *Cancer Res*, **61**, 6768–76.
Trabert B, Brinton LA, Anderson GL, et al (2016). Circulating Estrogens and Postmenopausal Ovarian Cancer Risk in the Women's Health Initiative Observational Study. *Cancer Epidemiol Biomar Prev*, **25**, 648-56.
Torpy JM, Burke AE, Golub RM (2011). Ovarian cancer. *J Am Med Assoc*, **305**, 2484.
Tsilidis KK, Allen NE, Key TJ, et al (2011). Oral contraceptive use and reproductive factors and risk of ovarian cancer in the European Prospective Investigation into Cancer and Nutrition. *Br J Cancer*, **105**, 1436–42.
Urban N, Hawley S, Janes H, et al (2015). Identifying post-menopausal women at elevated risk for epithelial ovarian cancer. *Gynecol Oncol*, **139**, 253–60.
Vuong NH, Cook DP, Forrest LA, et al (2018). Single-cell RNA-sequencing reveals transcriptional dynamics of estrogen-induced dysplasia in the ovarian surface epithelium. *PLoS Genet*, **14**, e1007788.
Vuong NH, Salah OS, Vanderhyden BC (2017). 17β-Estradiol sensitizes ovarian surface epithelium to transformation by suppressing Disabled-2 expression. *Sci Rep*, **7**, 16702.
Webb PM, Jordan SJ (2016). Epidemiology of epithelial ovarian cancer. *Best Pract Res Clin Obstet Gynaecol Bpobgyn*, **8**, 6.
Wentzensen N, Poole EM, Trabert B, et al (2016). Ovarian Cancer Risk Factors by Histologic Subtype: An Analysis from the Ovarian Cancer Cohort Consortium. *J Clin Oncol*, **20**, 2888-98.
Wong AS, Leung PC (2007). Role of endocrine and growth factors on the ovarian surface epithelium. *J Obst Gyn Res*, **33**, 3-16.
Wu S, Rodabaugh K, Martinez-Maza O, et al (1992). Stimulation of ovarian tumor cell proliferation with monocyte products including interleukin-1, interleukin-6, and tumor necrosis factor-a. *Amer J Obst Gyn*, **166**, 997–1007.
Wu S, Boyer CM, Whitaker RS, et al (1993). Tumor necrosis factor a as an autocrine and paracrine growth factor for ovarian cancer: monokine induction of tumor cell proliferation and tumor necrosis factor-a expression. *Canc Res*, **53**, 1939–44.
Yang HP, Trabert B, Murphy MA, et al (2012). Ovarian cancer risk factors by histologic subtypes in the NIH-AARP Diet and Health Study. *Int J Cancer*, **131**, 938–48.
Yoneda A, Lendorf ME, et al (2012). Breast and ovarian cancers: a survey and possible roles for the cell surface heparan sulfate proteoglycans. *J Histochem Cytochem*, **60**, 9–21.
Zhang Y, Ganfeng L, Mengjie L, et al (2019). Global patterns and trends in ovarian cancer incidence: age, period and birth cohort analysis. *BMC Cancer*, **19**, 1-14.