



Revisiting the effects of menopause on the skin: Functional changes, clinical studies, in vitro models and therapeutic alternatives

Thamile Luciane Reus*, Carla Abdo Brohem, Desiree Cigaran Schuck, Marcio Lorencini

Grupo Boticário, Research & Development, São José dos Pinhais, Brazil

ARTICLE INFO

Keywords:
Menopause
Skin
Physiology
Cosmetics

ABSTRACT

Menopause is a stage in a woman's life characterized by twelve months of amenorrhoea. This transition happens due to changes in ovarian follicular activity, leading to endocrine, biological and clinical modifications. The main hormones related to these changes and symptoms are oestradiol, LH, FSH, AMH, Inhibin B and GnRH. It is important to point out that the skin is very affected by all these hormone changes, leading to a decrease in collagen content, water content, elasticity, thickness and impacting on all skin layers quality. Aiming to help women go through this period of their lifetimes with a better quality of life, cosmetic and pharmaceutical industries have studied formulations to improve skin quality. In order to study the safety and efficacy of these products, *in vitro* methods have been developed in order to mimic menopause and aged skin. In addition to that, many clinical methodologies for skin features assessment have also been improved and applied to evaluate the efficacy of treatments or compounds for menopause. Studying and improving skin models and skin evaluation methodologies may help in the identification of therapeutic targets, treatments, drugs and cosmetics along with new insights for future research in the dermatology field.

1. What is menopause?

Menopause consists of a stage in a woman's life when reproductive capacity ceases (De Souza and Araújo, 2015). This happens due to a decline in ovarian follicular activity (Nair, 2014). Clinically, it can be defined as the period of time in a woman's life after twelve consecutive months of amenorrhoea with no other pathological or physiological aetiology. It is important to point out, however, that it is not a sudden process; in most women, menopause is preceded by a period (generally four years) during which many endocrine, biological and clinical changes are occurring (Burger, 2006).

Due to the life expectancy in most countries, many women are expected to live one-third of their lives in this time period (Burger, 2006). However, this landmark goes beyond the absence of menstruation and oestrogen decline (Parand avar et al., 2014); menopause brings with it many insecurities as it generally occurs during the period of life when women play important roles in society (Monteleone et al., 2018). Approximately one billion women have experienced menopause worldwide, and this process is associated with three types of crisis: social, biological and psychological (Parand avar et al., 2014). Consequently, women who have undergone this phase of life need to have support that meets their needs at this moment (Hoga et al., 2015). This is why

menopause matters. To date, there are 1050 clinical trials that have been performed or are happening worldwide regarding menopause and their symptoms (U.S. National Library of Medicine, 2019).

2. What is behind the scenes? The key players

What happens to a woman's body that leads to these symptoms? Many physiological changes happen in this period, and several hormones are key players in these alterations. Oestrogens are the most well-known of the hormones linked to menopausal symptoms. The decline in ovarian oestrogen production is certainly responsible for most of the consequences of menopause. However, many different protagonists contribute to this decline, such as hypothalamic and ovarian ageing, as well as environmental, lifestyle and genetic factors, leading to changes in many other hormones other than oestrogen (Davis et al., 2015).

The menopausal transition involves a time of hormonal instability, and the endocrine changes are primarily a consequence of the reduced number of ovarian follicles. Among all the hormones, FSH, anti-Mullerian hormone (AMH), inhibin B and oestradiol are the hormones that exhibit more subtle changes during this period of time (Burger et al., 2007; Su and Freeman, 2009). The changes during menopause

* Corresponding author at: Grupo Boticário, Research & Development, Rui Barbosa Avenue, 4110, São José dos Pinhais, Paraná, Zip Code 83055-320, Brazil.

E-mail addresses: thamile.reus@grupoboticario.com.br (T.L. Reus), carla@grupoboticario.com.br (C.A. Brohem), desirees@grupoboticario.com.br (D.C. Schuck), marciolo@grupoboticario.com.br (M. Lorencini).

<https://doi.org/10.1016/j.mad.2019.111193>

Received 12 July 2019; Received in revised form 22 November 2019; Accepted 2 December 2019

Available online 04 December 2019

0047-6374/ © 2019 Elsevier B.V. All rights reserved.

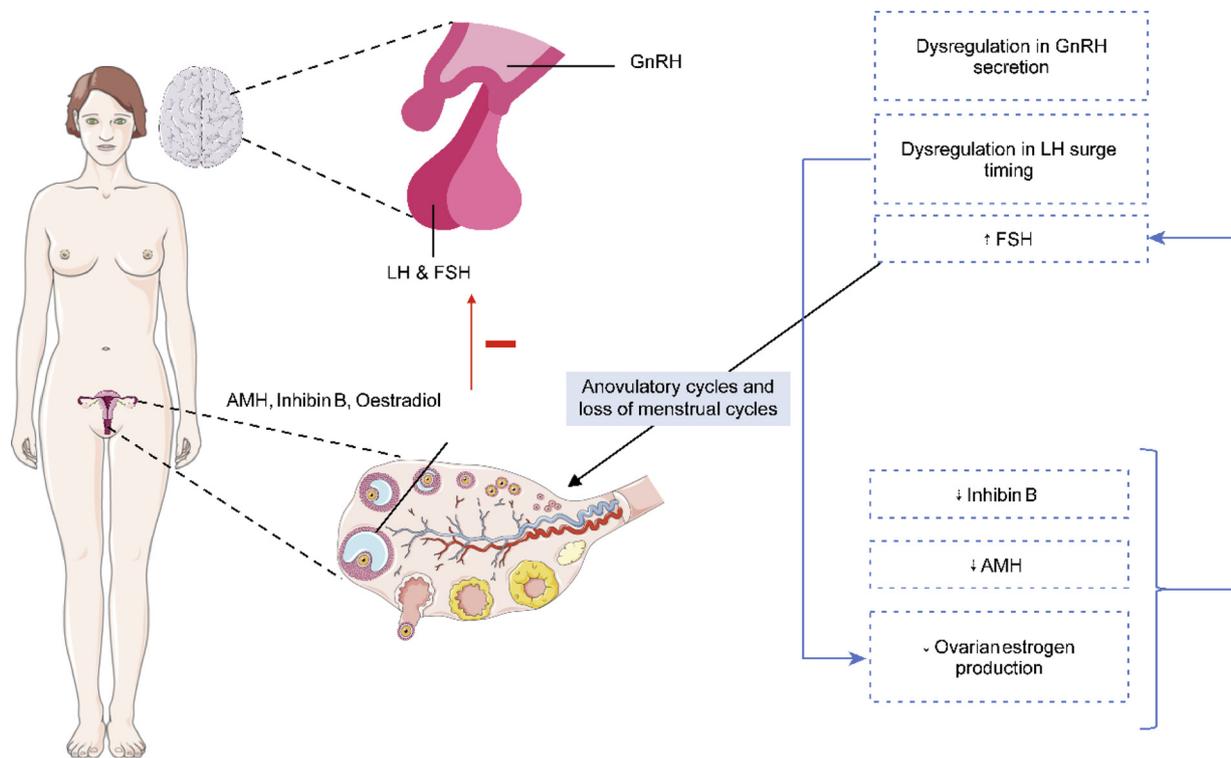


Fig. 1. Menopause takes part mainly in the hypothalamus and uterus, and many hormones are involved in the symptomatology. Menopause happens due to communication between the hypothalamus and the uterus. Changes in hormones lead to dysregulation of important processes in female endocrine physiology (SMART Imagebase; SMART, 2019).

are not limited to the ovaries; they happen in both the uterus and the brain. In the uterus, there is a decline in AMH, inhibin B and oestrogen, resulting in a drop in their negative feedback on FSH release and thus leading to FSH upregulation. This rise in FSH culminates in anovulatory cycles and loss of menstrual cycles. Simultaneously, in the brain, events are occurring in the hypothalamus. Hypothalamic ageing is associated with desynchronized production and release of hormones such as GnRH (gonadotropin-releasing hormone) and LH (luteinizing hormone) (Fig. 1). It is important to point out that these events are not isolated; they influence each other along the whole process with positive and negative feedback (Davis et al., 2015). In addition to the most commonly cited female sex hormones, we can also cite changes in hormones such as the androstenedione/SHBG (Sex Hormone-Binding Globulin) ratio, cortisol, norepinephrine and IGF-1 (Insulin-like growth factor 1), which are also related to the pathophysiology of menopause (Atapattu et al., 2015).

Since there is a significant change in hormones, researchers have attempted to define and determine kinetic correlations to establish biomarkers of menopause. Among many possible markers, the most emblematic ones are inhibin B and FSH (Overlie et al., 2005).

3. Skin and menopause

Changes in hormones may lead to many adverse effects and therefore impact many different areas of a woman's life, with resultant physical, psychological and sexual changes (Nair, 2014). Menopausal symptoms represent a very distressing stage in most women's lives. These symptoms involve changes in the central nervous system, skin, mucosa, hair, weight, metabolism, sexual function, and urogenital and musculoskeletal systems (Monteleone et al., 2018), resulting in an increased production of inflammatory mediators, vascular reactivity, endothelial proliferation, fat redistribution and increased visceral adiposity and a narrowed thermoneutral zone of the hypothalamus (Atapattu et al., 2015).

The skin is the largest organ in the human body and has many functions, such as temperature maintenance, immune surveillance, regulation of nutrients and fluids, etc (Farage et al., 2015). The skin is affected by physiological changes that happen with ageing process and is strongly affected by oestrogens. The hypo-oestrogenism that accompanies menopause intensifies the effects of intrinsic and environmental ageing since oestrogens play a key role in collagen increase, skin thickness and skin moisture (Verdier-Sévrain et al., 2006). Many studies have shown that there is a positive correlation between levels of circulating oestrogens and perceived age, attractiveness, skin health and facial coloration in women (Lephart, 2018).

Oestrogens are important regulators of female physiology and pathology and regulate target gene expression through 3 different receptors: ER α (Oestrogen Receptor α), Oestrogen Receptor β (ER β) and G protein-coupled oestrogen receptor 1 (GPER1). Oestrogen signalling is also linked with epigenetic mechanisms, such as posttranslational histone modifications, microRNAs (miRNAs) and DNA methylation (Vrtačnik et al., 2014). Menopausal physiological changes are very noticeable in the skin once it loses its structural architecture and therefore leads to a more deficient wound healing capacity (Wilkinson and Hardman, 2017).

Along with ageing and environmental factors, menopause and oestrogen loss have a very expressive impact on skin quality (Brincaat et al., 2005). The skin changes that happen during menopause are strongly related to the effects on skin components. Studies have shown that oestrogen receptors are found on the cell components of the skin. Therefore, cell metabolism in the dermis is affected by low concentrations of oestrogens, impacting collagen content, glycosaminoglycans and water content (Fig. 2). Water content is directly decreased due to a reduction in hydrophilic glycosaminoglycans (Raine-Fenning et al., 2003), and collagen content decreases due to other mechanisms.

It is known that oxidative stress in human fibroblasts leads to a decrease in procollagen I synthesis. Studies have shown that 17- β oestradiol plays a protective role in fibroblasts and keratinocytes against

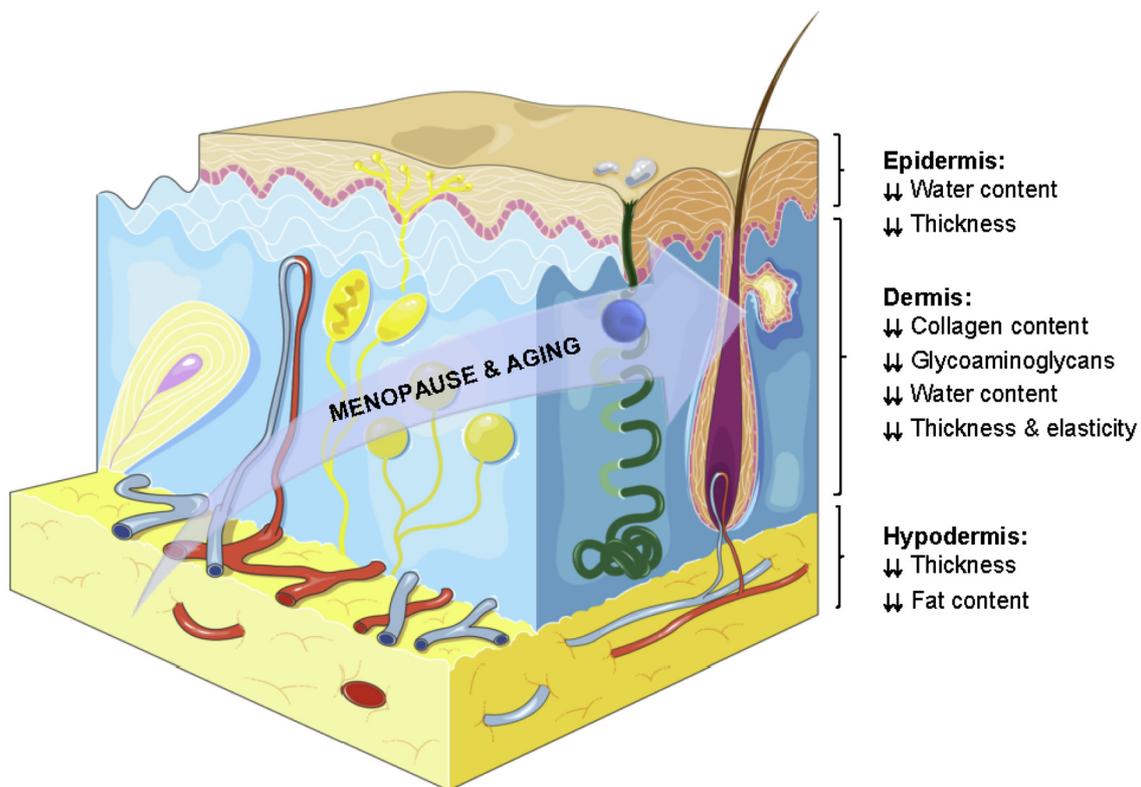


Fig. 2. Menopause and ageing lead to many changes in all skin layers (SMART Imagebase; SMART, 2019).

oxidative stress, therefore leading to an increase in pro-collagen I synthesis (Bottai et al., 2012). It has also been demonstrated that in postmenopausal women, there is a difference in collagen subtypes in the dermis. Affinito et al. (1999) evaluated 14 premenopausal and 18 postmenopausal women and observed that the postmenopausal women had a significant reduction in collagen types I and III, and this correlation was mainly related to oestrogen deficiency rather than chronological age. All these data indicate that the reduced collagen content in menopausal women is an oestrogen-related phenomenon (Affinito et al., 1999). Son et al. (2005) also demonstrated that the topical application of 17β -oestradiol led to an increase in pro-collagen I, tropoelastin and fibrillin-1 mRNA (messenger RNA) and protein expression and a reduction in MMP-1 (matrix metalloproteinase-1) protein levels. Topical 17β -oestradiol also increases the proliferation of keratinocytes and epidermal thickness (Son et al., 2005).

Furthermore, connective tissue ageing is accompanied by a progressive accumulation of AGEs (Advanced Glycation End-Products). Studies have suggested that AGEs are responsible for shortened, thinned and disorganized collagen fibrils and for limiting fibre-fibre and fibril-fibril sliding. This culminates in a decrease in tissue viscoelasticity, wound healing and scar formation (Gautieri et al., 2016; Van Putte et al., 2016). Additionally, in recent years, the correlation between ageing ovaries and AGEs has been investigated. Studies have suggested that ovulatory dysfunction is highly influenced by AGEs. The accumulation of AGE products in ovarian follicles might lead to early ovarian ageing or decreased glucose uptake by granulosa cells, culminating in alterations in follicular growth (Pertynska-Marczewska and Diamanti-Kandarakis, 2017).

Ageing and undesirable effects in menopause are strongly associated with collagen atrophy and, consequently, an increase in extensibility and a decrease in elasticity (Calleja-Agius and Brincat, 2012) (Fig. 2). Thus, oestrogen replacement in menopause leads to an increase in collagen content, dermal thickness and elasticity. This hormone also plays an important role in connective tissue turnover (Calleja-Agius and Brincat, 2012).

Preclinical and clinical studies have been performed to understand the relation between oestrogens and skin and to improve skin quality in women undergoing menopause. In 2017, Chen et al. evaluated hydration of the stratum corneum (SC), recovery of the permeability barrier function, integrity and cohesion of the SC and irritant dermatitis in ovariectomized mice (animal model of human menopause) with or without β -oestradiol replacement. They observed that a treatment using subcutaneous slow-releasing E2 (17β -estradiol) tablets restored all menopausal changes; in mice without hormone replacement, lower levels of desmoglein-1 and differentiation markers of epidermis were also detected. Therefore, this demonstrates that skin dryness is not only linked to low water content but also impairs structural and functional changes in neum (SC), recovery of the permeability barrier function, integrity and cohesion of the SC and irritant dermatitis in ovariectomized mice (animal model of human menopause) with or without β -oestradiol replacement. They observed that a treatment using subcutaneous slow-releasing E2 (17β -estradiol) tablets restored all menopausal changes; in mice without hormone replacement, lower levels of desmoglein-1 and differentiation markers of epidermis were also detected. Therefore, this demonstrates that skin dryness is not only linked to low water content but also impairs structural and functional changes in the SC and the skin (Chen et al., 2017) (Fig. 2).

Studies of oestrogen replacement in postmenopausal women have shown that hormone replacement therapy may increase collagen content, dermal thickness, elasticity and hydration (Brincat et al., 2005) (Fig. 2). However, even though hormone replacement therapy (HRT) has been largely used, many studies have shown some risks associated with this treatment, such as breast cancer. Therefore, it is very important to consider the risks and benefits of this type of treatment for skin ageing. To minimize the risks, many studies have been conducted to develop new drugs that are selective for oestrogen receptors (Selective ER modulators – SERMs) (Verdier-Sévrain et al., 2006). According to the U.S. National Library of Medicine, there are 81 clinical studies involving SERMs and menopause, mainly related to vasomotor symptoms (clinicaltrials.gov).

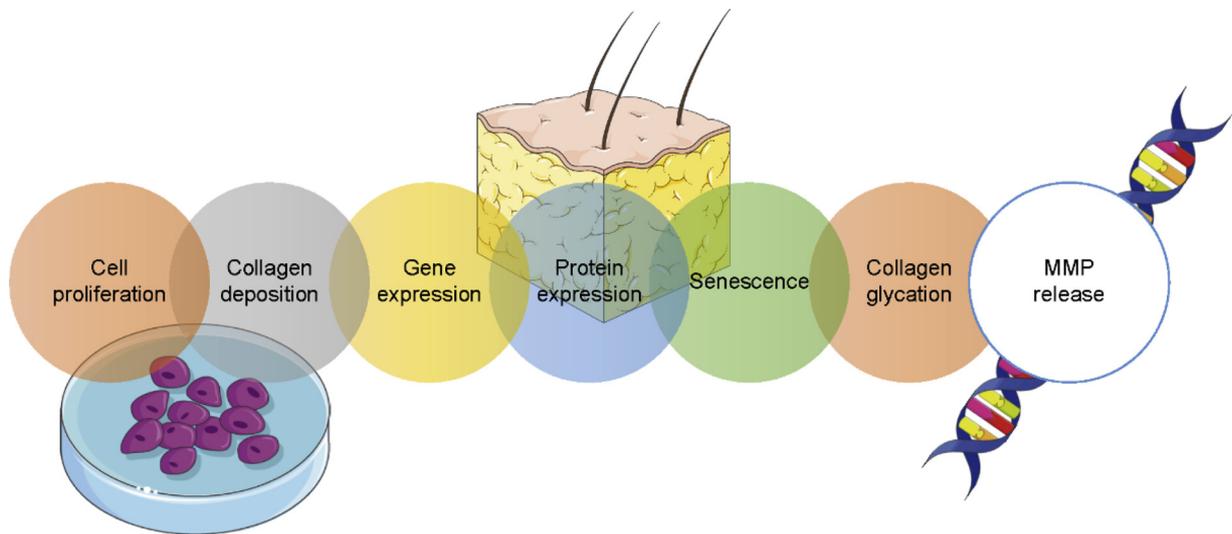


Fig. 3. *In vitro* methods may lead to some answers regarding menopause changes in skin (SMART Imagebase; SMART, 2019).

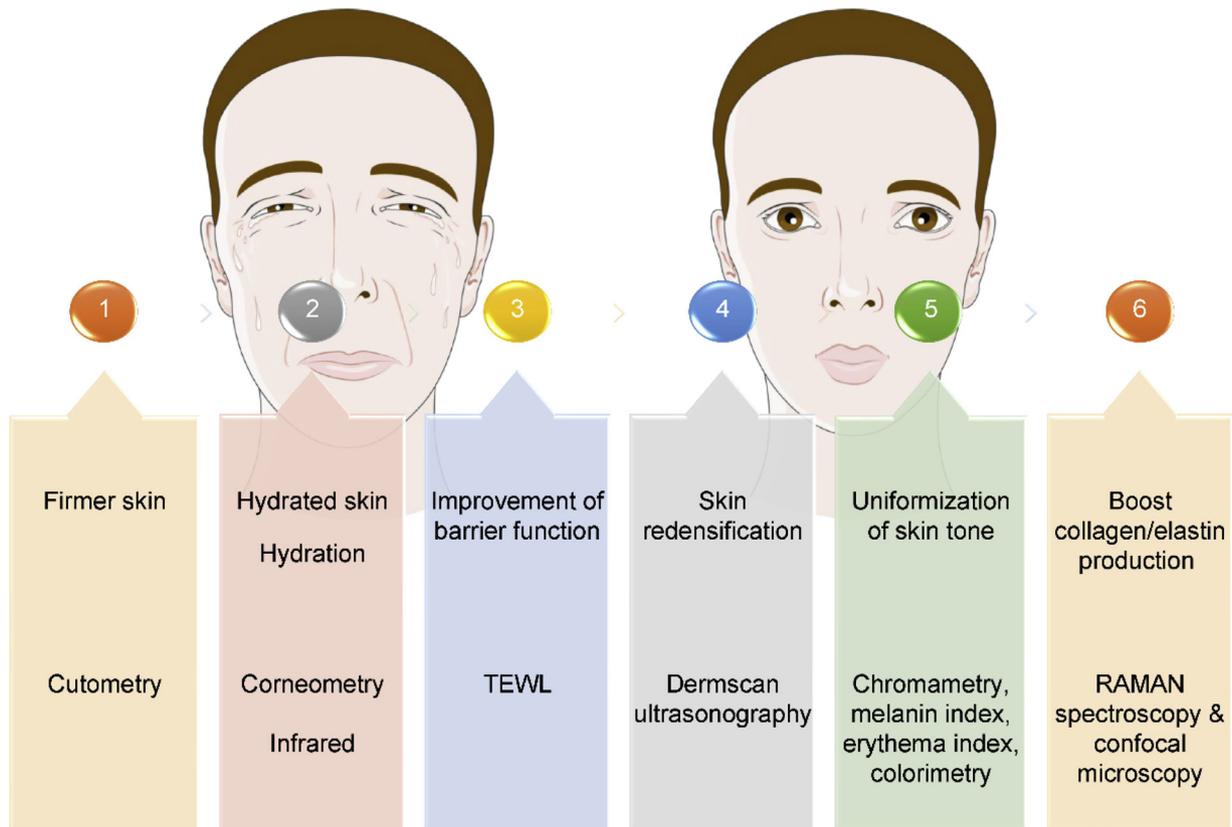


Fig. 4. Main claims for cosmetics in skin care during menopause and the clinical methodology that best supports them (SMART Imagebase; SMART, 2019).

In 2012, Sainthillier et al. performed a study with 150 women from 50 to 80 years and demonstrated three grades of skin maturity in menopausal women according to physiological and clinical patterns. They also found that the most important variables to differentiate these maturity grades were wrinkles on the cheeks, upper lip, elastosis, roughness and spots (Sainthillier et al., 2013).

Additionally, menopause and ageing bring along some changes in the hypodermis. Atrophy of the hypodermis is observed, leading to wrinkle formation (Levakov et al., 2012) (Fig. 3).

Other authors have also reviewed the impact of menopause and its changes on skin. In 2005, Hall and Phillips revisited the effects of low oestrogen levels, the effects on skin quality and also the benefits of

oestrogen replacement (Hall and Phillips, 2005). In 2012, Blume-Peytavi et al. reviewed the effects of menopause on both skin and hair. They listed the main skin and hair disorders that happen in menopause and the also main treatment options for those disorders. They also pointed out that it is very important that the treatment of these conditions should be specific to this population (Blume-Peytavi et al., 2012). In 2013, Herman et al. evaluated the main skin care noninvasive procedures and also home care procedures that are indicated for menopausal women in order to avoid the main skin disorders related to this life period. They pointed out that it is very important to moisturize and nourish the skin, stimulate fibroblasts and induce skin renovation (Herman et al., 2013).

4. Skin and menopause: *in vitro* methods that mimic this milieu

Many changes happen during menopause. The key players, the pathophysiology and the potential treatments have been studied and represent a coherent research theme among scientists, the cosmetic and pharmaceutical industries. Simultaneously, there is also increasing pressure to replace animal testing (McNamee et al., 2009; Hartung, 2011), especially in the cosmetic industry; therefore, *in vitro* methods have become essential to innovation in the cosmetic industry.

Since skin is one of the most affected organs during this life period, many *in vitro* methods that mimic menopause and aged skin have been developed (Fig. 3). Moving basic science into the clinic has been strongly supported. In this scenario, skin models represent an important tool for the discovery, research and development of drugs, cosmetics and therapies for pathologies related to skin. Screening potential substances *via* skin models dramatically increases the success rate in clinical trials (Yousuf et al., 2018). Because menopause is very complex, finding only one model to study and to rely on to evaluate its effects on skin is not possible. However, a combination of methodologies is possible and can bring some positive results towards understanding the main effects and possible treatments for menopausal skin. We describe some studies and the main methodologies used for understanding menopause below (Fig. 4).

1) Primary human dermal fibroblasts:

Human dermal fibroblasts can be used to mimic the menopause skin milieu. Remoué et al., 2013 established a model using fibroblasts from abdominal areas and younger donors (18–30 years). Cells from passages 4–6 were cultured in 4 steps as follows:

Step 1: confluence reaching;

Step 2: collagen production stimulation under non-menopausal conditions; First, cells were cultured in medium containing hormones in concentrations corresponding to the average serum levels in non-menopausal women: 17 β -oestradiol (750 pM), progesterone (60 nM), dehydroepiandrosterone (20 nM), growth hormone (5 ng/mL) and IGF-1 (200 ng/mL).

Step 3: FBS and growth supplement starvation; Cells were subcultured in the same conditions described above, although with FBS and growth supplement starvation.

Step 4: test phase with nonmenopausal vs. menopausal conditions. This phase is responsible for stimulating menopause, and cells are cultured in medium with hormone levels compatible with menopause: 17 β -oestradiol (60 pM), progesterone (6 nM), dehydroepiandrosterone (2 nM), growth hormone (1.5 ng/mL) and IGF-1 (100 ng/mL).

After cell treatment and menopause stimulation, several aspects related to menopause and skin were evaluated by using cellular and molecular approaches, such as cell proliferation, matrix metalloproteinase-1 and metalloproteinase-3 (MMPs) release, collagen deposition and procollagen gene expression.

The results showed that this model was responsible for generating a time-dependent decrease in cell proliferation, collagen deposition and the type III/type I collagen ratio; it also demonstrated an increase in MMP release. Altogether, these results reflect most of the effects that are observed *in vivo*.

1) *In vitro* photoaged normal human fibroblasts:

Aged skin and menopausal skin share many features with regard to collagen content, deposition, elasticity and turnover. Therefore, aged skin models may also help to mimic the menopause milieu.

Exposure to ultraviolet radiation leads to premature skin ageing (photoaging), bringing along wrinkles, altered pigmentation and skin tone loss, with profound alterations in the collagenous extracellular matrix (Fisher et al., 1997). Additionally, it is known that in photo-damaged skin, there is dramatic collagen fragmentation with a

posterior clumping of the damaged collagen (Fligiel et al., 2003).

Yoshimoto et al., 2018 proposed a method using normal human fibroblasts from foreskin. Cells were submitted to UVA (ultraviolet A) irradiation with 3.6 J/cm²/day (1 h per day for 10 days) by using a fluorescence lamp emitting a UVA spectrum (340–410 nm) with no filter. After irradiation, cells were evaluated regarding different characteristics related to ageing and senescence, such as the senescence marker β -galactosidase, reactive oxygen species (ROS) evaluation, and p16 expression.

The results demonstrated that repeated UVA radiation of cells led to typical senescence markers, such as increased SA- β -galactosidase staining, flattening and larger cells with a larger diameter ratio, higher levels of ROS, yellowish coloration (accumulation of oxidized proteins, carbonylated proteins and advanced glycation end products) and increased p16 expression.

Therefore, despite particularities, this model could represent an additional and complementary approach for evaluating menopause aged skin. However, it is important to point out that photoaged skin and menopause skin have some differences, especially with regard to collagen content (Rittié et al., 2008); for that reason, there should be some reservations before using this model exclusively to represent menopause.

1) Epidermis model with IGF-1 knockdown

Insulin-like growth factor 1 (IGF-1) is mandatory in the early stages of skin development and is known to be related to longevity in animals. Therefore, the knockdown of IGF-1 receptor (IGF-1R) may contribute to hormonal ageing.

In Mainzer et al., 2018 proposed an epidermis model using normal human keratinocytes (NHKs) from female donors. IGF-1R knockdown was accomplished by lentiviral transduction expressing a small hairpin RNA targeting IGF-1R mRNA, and then, positive cells were selected with puromycin. These cells were then cultured with fibroblasts (reconstructed human epidermis - RHE) to mimic the epidermis. The organotypic epidermis model was then evaluated regarding ageing features by evaluating cell proliferation, colony forming assay, adhesion assay, immunolabelling, quantification of epidermal thickness, gene expression analysis by real-time PCR and protein expression analysis by Western blotting.

The results showed that RHE with IGF-1R knockdown had a loss of function of the *Stratum basale*. Additionally, the keratinocytes with IGF-1R knockdown presented an extended cell cycle, a reduction in proliferation and adhesion potential and a larger sensitivity to oxidative stress.

This model is very interesting for mimicking the hormonal ageing milieu, however, this technique also brings along some undesirable effects due to the residual imprecision typical for viral gene transfer systems. Also, a very critical point is that it is very difficult to generate large vector stocks in the same production run (Schambach et al., 2013).

1) *In vitro* and *ex vivo* skin ageing models for antiglycation and anti-elastase potential

Glycation is a consequence of ageing and happens especially in dermal proteins such as type I collagen and elastin. In Bogdanowicz et al., 2016 performed a study to evaluate the antielastase and anti-glycation MMP-12 potential of glycylglycine oleamide (GGO). Therefore, they evaluated a combination of models for assessing these endpoints:

Noncellular

- 1) A noncellular *in vitro* study of collagen by collagen extraction from donor tissues; collagen glycation was then assessed by measuring the collagen-bound AGES (advanced glycation end products);

Table 1

Main endpoints evaluated for the cosmetic industry and the clinical methodology that best fits its substantiation.

Endpoint:	Methodology:
Firmness/elasticity	Cutometry (Jenkins et al., 2014)
Hydration	Corneometry/Infrared (Xu et al., 2016; Zhang et al., 2005)
Barrier function	Transepidermal water loss (TEWL) (Jenkins et al., 2014)
Skin colour	Chromametry (Jenkins et al., 2014), Melanin index, Erythema Index (Xu et al., 2018), Colorimetry (Castanedo-Cazares et al., 2018).
Collagen/elastin content	RAMAN spectroscopy (Caetano et al., 2017; Darvin et al., 2014)
Collagen subtypes/elastin content	Confocal microscopy (Moalli et al., 2004)
Redensification/ epidermis and dermis thickness	Dermscan ultrasonography (Sanz et al., 2016)

- 2 Noncellular *in vitro* study of the anti-elastase (MMP-12) potential by diffusion of elastase in agarose gel;
- 3 *Ex vivo* approach by immunostaining of human skin explants for fibrillin-1 detection;
- 4 *In vitro* approach by using fibroblasts from skin explants; cells were then used for collagen retraction assay;

Altogether, the results demonstrated that the substance that was under investigation (GGO) prevented glycation and maintained the elastic function of the skin. Even though this kind of study is far from an ideal model for menopause, it is indeed a viable approach for the screening of potential antiglycation and anti-elastase substances, which are *claims* that are becoming more common among skin care cosmetics.

5. Skin and menopause: clinical studies

As mentioned above, according to the U.S. National Library of Medicine, there are 1050 clinical trials related to menopause that have taken or are taking place in the world (Clinicaltrials.gov). Even though these studies are spread worldwide, most are being conducted in the United States (455) and Europe (257). They also cover a wide range of topics, including “vaginal atrophy”, “climacteric symptoms”, “bone mineral density”, “oestradiol”, “hot flashes”, “hormone replacement therapy”, and “menopausal osteoporosis” (U.S. National Library of Medicine, 2018).

Since menopause dramatically impacts skin quality (Brincat et al., 2005), evaluating skin features has become a promising endpoint for evaluating the efficacy of treatments or compounds for menopause. These features include wrinkles, collagen content, elasticity/firmness, water content, dermis thinning, and spots (Calleja-Agius and Brincat, 2012; Raine-Fenning et al., 2003; 3). The table below lists these promising endpoints and the clinical methodology that answers the question, and Fig. 4 shows some cosmetic claims for skin care and the clinical methodology suggested for its substantiation (Table 1).

Even though there are many clinical studies with menopause as a topic, few study the effect of substances on skin quality. According to the U.S. National Library of Medicine (2018), when the words “menopause” and “skin” are used, there is only one study that is listed. The study named “Effect of DHEA on Skin Aging in Postmenopausal Women” has been conducted by Quebec-Universite Laval with 150 volunteers since 2005, and no results regarding skin features have been published thus far.

In Marini et al., 2012 evaluated the effects of Pycnogenol® on skin elasticity and hydration in 20 healthy postmenopausal women. To assess that, cutometry and corneometry were performed.

In Sainthillier et al., 2013 performed a clinical study with 150 women aged between 50–80 years and conducted biometric measurements, such as corneometry and cutometry, to evaluate skin maturity.

Similarly, in 2014, Jenkins et al. conducted a double-blind randomized controlled human clinical trial to evaluate the effect of an oral supplement on facial wrinkles in postmenopausal females. To assess that, skin was evaluated by photography, cutometry, transepidermal water loss, chromametry and histological biopsy analyses (collagen and

elastin evaluation).

All of these studies yielded very good results, and they demonstrated that clinical studies are very valuable to answer questions regarding the efficacy of potential treatments and substances. Thus, clinical studies should accompany *in vitro* approaches.

6. Conclusion

The quality of the skin and the menopausal process are closely linked. Regardless of the time in one's life, women desire to feel good about their skins. To accomplish this, skin treatments, cosmetics and cosmeceuticals have been developed. A good quality treatment may lead to an increase in skin density, thickness, hydration and firmness and a decrease in wrinkles (Herman et al., 2013). Therefore, studying and improving skin models may enable a better representation of the challenges facing the clinics and may aid in the identification of therapeutic targets, treatments, drugs and cosmetics along with new insights for future research in the dermatology field.

References

- Affinito, P., Palomba, S., Sorrentino, C., Di Carlo, C., Bifulco, G., Arienzo, M.P., Nappi, C., 1999. Effects of postmenopausal hypoestrogenism on skin collagen. *Maturitas* 3, 239–247.
- Atapattu, P.M., Fernando, D., Wasalathanthri, S., de Silva, A., 2015. Menopause and exercise: linking paphthophysiology to effects. *iMedPub J Special Issue*.
- Blume-Peytavi, U., Atkin, S., Gieler, U., Grimalt, R., 2012. Skin Academy: hair, skin, hormones and menopause - current status/knowledge on the management of hair disorders in menopausal women. *Eur. J. Dermatol.* 22 (3), 310–318.
- Bogdanowicz, P., Haure, M.J., Ceruti, I., Bessou-Touya, S., Castex-Rizzi, N., 2016. Results from *in vitro* and *ex vivo* skin aging models assessing the antiglycation and anti-elastase MMP-12 potential of glycyglycine oleamide. *Clin. Cosmet. Investig. Dermatol.* 9, 143–150.
- Bottai, G., Mancina, R., Muratori, M., Di Gennaro, P., Lotti, T., 2012. 17 β -estradiol protects human skin fibroblasts and keratinocytes against oxidative damage. *J Eur Acad Dermatol Venereol.*
- Brincat, M.P., Baron, Y.M., Galea, R., 2005. Estrogens and the skin. *Climacteric* 8 (2), 110–123.
- Burger, H.G., 2006. Physiology and endocrinology of the menopause. *Med. J.* 34 (1), 27–30.
- Burger, H.G., Hale, G.E., Robertson, D.M., Dennerstein, L., 2007. A review of hormonal changes during the menopausal transition: focus on findings from the Melbourne Women's Midlife Health Project. *Hum. Reprod. Update* 13 (6), 559–565.
- Calleja-Agius, J., Brincat, M., 2012. The effect of menopause on the skin and other connective tissues. *Gynecol. Endocrinol.* 28 (4), 273–277.
- Caetano, L.V.N., Mendes, T.O., Bagatin, E., Miot, H.A., Soares, J.L.M., Enokihara, M.M.S.S., Martin, A.A., 2017. *in vivo* confocal raman spectroscopy for intrinsic aging and photoaging assessment. *J. Dermatol. Sci.* 88 (2), 199–206.
- Castanedo-Cazares, J.P., Hernández-Blanco, D., García-Cortés, J.D., 2018. Medina-aguiar, L., torres-élvarez, B. 'Análisis de la pigmentación cutánea em una muestra. *Gaceta Médica de México* 68–73.
- Chen, Y., Yokozeki, H., Katagiri, K., 2017. Physiological and functional changes in the stratum corneum restored by oestrogen in an ovariectomized mice model of climacterium. *Exp. Dermatol.* 26 (5), 394–401.
- Davis, S.R., Lambrinoudaki, I., Lumsden, M., Mishra, G.D., Pal, L., Rees, M., Santoro, N., Simoncini, T., 2015. Menopause. *Nat Rev Dis Primers* 15004.
- Darvin, M.E., Richter, H., Ahlberg, S., Haag, S.F., Meinke, M.C., Le Quintrec, D., Doucet, O., Lademann, J., 2014. Influence of sun exposure on the cutaneous collagen/elastin fibers and carotenoids: negative effects can be reduced by application of sunscreen. *J. Biophotonics* 9, 735–743.
- De Souza, N.L.S.A., Araújo, C.L.O., 2015. Marco female aging, Menopause: her experience in a literature review. *Rev. Kairós Gerontol.* 18 (2), 149–165.
- Farage, M.A., Miller, K.W., Maibach, H., 2015. Degenerative Changes in Aging Skin.

- Textbook of Aging Skin. Springer, pp. 1–18.
- Fisher, G.J., Wang, Z.Q., Datta, S.C., Varani, J., Kang, S., Voorhees, J.J., 1997. Pathophysiology of premature skin aging induced by ultraviolet light. *N. Engl. J. Med.* 337 (20), 1419–1428.
- Fligiel, S.E., Varani, J., Datta, S.C., Kang, S., Fisher, G.J., Voorhees, J.J., 2003. Collagen degradation in aged/photodamaged skin in vivo and after exposure to matrix metalloproteinase-1 in vitro. *J. Invest. Dermatol.* 120 (5), 842–848.
- Gautieri, A., Passini, F.S., Silván, U., Guizar-Sicarios, M., Carimati, G., Volpi, P., Moretti, M., Redaelli, A., Berli, M., Snedeker, J.G., 2016. Advanced glycation end-products: Mechanics of aged collagen from molecule to tissue. *Matrix Biol.* 59, 59–95.
- Hall, G., Phillips, T.J., 2005. Estrogen and skin: The effects of estrogen, menopause, and hormone replacement therapy on the skin. *J. Am. Acad. Dermatol.* 555–568.
- Hartung, T., 2011. From alternative methods to a new toxicology. *Eur. J. Pharm. Biopharm.* 77, 338–349.
- Herman, J., Rost-Roszkowska, M., Skotnicka-Graca, U., 2013. Skin care during the menopause period: noninvasive procedures of beauty studies. *Postepy Dermatol Alergol.* 30 (6), 388–395.
- Hoga, L., Rodolpho, J., Gonçalves, B., Quirino, B., 2015. Women's experience of menopause: a systematic review of qualitative evidence. *JBI Database System. Rev. Implement. Rep.* 13 (8), 250–337.
- Jenkins, G., Wainwright, L.J., Holland, R., Barrett, K.E., Casey, J., 2014. Wrinkle reduction in post-menopausal women consuming a novel oral supplement: a double-blind placebo-controlled randomized study. *Int. J. Cosmet. Sci.* 36 (1), 22–31.
- Lephart, E.D., 2018. A review of the role of estrogen in dermal aging and facial attractiveness in women. *J. Cosmet. Dermatol.* 17 (3), 282–288.
- Levakov, A., Vuckovic, N., Dolai, M., Mocko Kacanski, M., Bozanic, S., 2012. Age-related skin changes. *Med. Pregl.* (5–6), 191–195.
- Mainzer, C., Remoué, N., Molinari, J., Rousselle, P., Barricello, C., Lago, J.C., Sommer, P., Sigaudou-Roussel, D., Debret, R., 2018. In vitro epidermis model mimicking IGF-1-specific age-related decline. *Exp. Dermatol.* 27 (5), 537–543.
- Marini, A., Grether-Beck, S., Jaenicke, T., Weber, M., Burki, C., Formann, P., Brenden, H., Schönlauf, F., Krutmann, J., 2012. Pycnogenol® effects on skin elasticity and hydration coincide with increased gene expressions of collagen type I and hyaluronic acid synthase in women. *Skin Pharmacol. Physiol.* 25 (2), 86–92.
- McNamee, P., Hibatallah, J., Costabel-Farkas, M., Goebel, C., Araki, D., Dufour, E., Hewiit, N.J., Jones, P., Kirst, A., Le Varlet, B., Macfarlane, M., Marrec-Fairley, M., Rowland, J., Schellau, F., Scheel, J., 2009. A tiered approach to the use of alternatives to animal testing for the safety assessment of cosmetics: Eye irritation. *Regul. Toxicol. Pharmacol.* 54, 197–209.
- Moalli, P.A., Talarico, L.C., Sung, V.W., Klingensmith, W.L., Shand, S.H., Meyn, L.A., Watkins, S.C., 2004. Impact of menopause on collagen subtypes in the arcus tendineus fasciae pelvis. *Am J Obstet Gynecol.* 190 (3), 620–627.
- Monteleone, P., Mascagni, G., Giannini, A., Genazzani, A.R., Simoncini, T., 2018. Symptoms of menopause - global prevalence, physiology and implications. *Nat. Rev. Endocrinol.* 14 (4), 199–215.
- Nair, P.A., 2014. Dermatitis associated with menopause. *J. Midlife Health.* 5 (4), 168–175.
- Overlie, I., Mørkrid, L., Andersson, A.M., Skakkebaek, N.E., Moen, M.H., Holte, A., 2005. Inhibin A and B as markers of menopause: a five-year prospective longitudinal study of hormonal changes during the menopausal transition. *Acta Obstet. Gynecol. Scand.* 84 (3), 281–285.
- Parandavar, N., Mosalanejad, L., Ramezani, S., Ghavi, F., 2014. Menopause and crisis? Fake or real: comprehensive search to the depth of crisis experienced: a mixed-method study. *Glob. J. Health Sci.* 6 (2), 246–255.
- Pertynska-Marczewska, M., Diamanti-Kandarakis, E., 2017. Aging ovary and the role for advanced glycation end products. *Menopause.* 24 (3), 345–351.
- Raine-Fenning, N.J., Brinca, M.P., Muscat-Baron, Y., 2003. Skin aging and menopause: implications for treatment. *Am J Clin Dermatol.* 4 (6), 371–378.
- Remoué, N., Molinari, J., Andres, E., Lago, J.C., Barricello, C., Moreira, P.L., 2013. Development of an in vitro model of menopause using primary human dermal fibroblasts. *Int. J. Cosmet. Sci.* 35 (6), 546–554.
- Rittié, L., Kang, S., Voorhees, J.J., Fisher, G.J., 2008. Induction of collagen by estradiol / difference between Sun-Protected and Photodamaged Human Skin In Vivo. *Arch. Dermatol.* 144 (9), 1129–1140.
- Sainthillier, J.M., Mac-Mary, S., Monnier, D., Mermet, P., Tarrit, C., Mudry, C., Humbert, P., 2013. Exploratory study of the typology of various grades of mature skin. *Skin Res Technol.* 19 (1), e507–e514.
- Sanz, M.T., Campos, C., Milani, M., Foyaca, M., Lamy, A., Kurdian, K., Trullas, C., 2016. Biorevitalizing effect of a novel facial serum containing apple stem cell extract, procollagen lipopeptide, creatine, and urea on skin aging signs. *J. Cosmet. Dermatol.* 15 (1), 24–30.
- Schambach, A., Zychlinsk, D., Ehrnstroem, B., Baum, C., 2013. Biosafety features of lentiviral vectors. *Hum. Gene Ther. Clin. Dev.* 24, 132–142.
- SMARTSMART - Servier Medical ART - 3000 free medical iFree Medical Images Available at: <http://smart.servier.com/2019>.
- Son, E.D., Lee, J.Y., Lee, S., Kim, M.S., Lee, B.G., Chang, I.S., Chung, J.H., 2005. Topical Application of 17β-Estradiol Increases Extracellular Matrix Protein Synthesis by Stimulating TGF-β Signaling in Aged Human Skin In Vivo. *J. Invest. Dermatol.* 124, 1149–1161.
- Su, H.I., Freeman, E.W., 2009. Hormone changes associated with the menopausal transition. *Minerva Ginecol.* 61 (6), 483–489.
- U.S. National Library of Medicine, 2019. Clinical Trials: Menopause. available at: <https://clinicaltrials.gov/ct2/results?cond=&term=menopause&cntry=&state=&city=&dist>.
- Van Putte, L., De Schrijver, S., Moortgat, P., 2016. The effects of advanced glycation end products (AGEs) on dermal wound healing and scar formation: a systematic review. *Scars, Burns & Healing.* 1–14.
- Verdier-Sévrain, S., Bonté, F., Gilchrist, B., 2006. Biology of estrogens in skin: implications for skin aging. *Exp Dermatol.* 15 (2), 83–94.
- Vrtačnik, P., Ostanek, B., Mencej-Bedrač, S., Marc, J., 2014. The many faces of estrogen signaling. *Biochem. Med.* 24 (3), 329–342.
- Wilkinson, H.N., Hardman, M.J., 2017. The role of estrogen in cutaneous ageing and repair. *Maturitas.* 103, 60–64.
- Xu, Y., Guo, S., Wei, C., Li, H., Chen, L., Yin, C., Zhang, C., 2016. The comparison of adipose stem cell and placental stem cell in secretion characteristics and in facial antiaging. *Stem Cells Int.* 2016 pp. 7315830.
- Xu, Z., Xing, X., Zhang, C., Chen, L., Flora Xiang, L., 2018. A pilot study of oral tranexamic acid and Glycyrrhizin compound in the treatment of recalcitrant Riehl's melanosis. *J. Cosmet. Dermatol.*
- Yoshimoto, S., Yoshida, M., Ando, H., Ichihashi, M., 2018. Establishment of photoaging in vitro by repetitive UVA irradiation: induction of characteristic markers of senescence and its prevention by PAPANAL with potent catalase activity. *Photochem. Photobiol.* 94 (3), 438–444.
- Yousuf, Y., Amini-Nik, S., Jeschke, M.G., 2018. 2-overall perspective on the clinical importance of skin models. *Skin Tissue Models.* 39–54.
- Zhang, S.L., Meyers, C.L., Subramanyan, K., Hancewicz, T.M., 2005. Near infrared imaging for measuring and visualizing skin hydration. A comparison with visual assessment and electrical methods. *J. Biomed. Opt.* 10 (3) pp. 031107.