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Menopause: new frontiers in the treatment of urogenital atrophy

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Abstract. – OBJECTIVE: Urogenital atrophy is a degenerative process that may occur during menopause causing debilitating disorders and painful symptomatology. Estrogen therapy slows the onset of atrophy, but it requires ongoing therapy to maintain its effectiveness. To mitigate the degenerative evolutions associated with menopause, patients may benefit from new therapeutic approaches, such as the use of mesenchymal stem cells. Among the many sources, the adipose tissue is considered one of the smartest, due to its abundance and easy access. This study investigated the feasibility and potential benefits of using an autologous adipose tissue to treat the symptoms of urogenital atrophy.

PATIENTS AND METHODS: In 2014, the first three women affected by post-menopausal urogenital atrophy were treated with injections of autologous and micro-fragmented adipose tissue (Lipogems®). Clinical outcomes were determined at 3, 6, 9, 12, 18, 24, and 36 months by evaluating vaginal dryness, burning, itching, stranguria, sensitivity, and dyspareunia. Pre- and 36 months post-op biopsies and vaginal discharge were also collected.

RESULTS: The three women reported a significant improvement of the symptoms at 6 months with complete resolution at 9 months. This benefit, subjectively reported and confirmed by clinical evaluation, remained constant without recurrence at least until 36 months. Immunohistochemical analysis revealed a total recovery of vaginal vitality with production of glycogen, vasculature hyperplasia and regeneration of the epithelium and subcutaneous tissue at 36 months. The analysis of vaginal discharge showed a restoration of an acid pH with the colonization of lactobacilli. No postoperative complications nor adverse events were recorded.

CONCLUSIONS: The results of these first three cases pointed to autologous and micro-fragmented adipose tissue as a safe, feasible and effective therapeutic approach for the treatment of post-menopausal urogenital atrophy.

Key Words:
Menopause, Urogenital atrophy, MSCs, ASCs, Micro-fragmented adipose tissue, Regenerative medicine, Lipogems®.

Introduction

Menopause is one of the most significant events in a woman's life and brings to a number of physiological changes that permanently affect the life of a woman. It is associated with a marked reduction in endogenous oestrogen production, which induces various deleterious effects on the female urogenital system[1]. While vasomotor symptoms diminish over time until disappearance, genitourinary tract disorders, susceptible to hormone deficiency, progress and worsen over the years. The vaginal epithelium becomes atrophied and dry, with the consequence of vaginal discomfort, itching, dyspareunia[2] and reduced colonization of lactobacilli with pH rise due to less lactic acid production[3]. This induces an imbalance of the saprophytic flora[4], with a consequent growth of coliform bacteria causing inflammation, vaginal, urethral, and bladder infections, and bad smell[5]. An inflamed epithelium can also contribute to urinary symptoms, including frequency, urgency, and incontinence[6]. Diminished estrogen production affects also collagen turnover, playing a major role in the onset of vaginal prolapse[7,8]. Finally, also the bladder-urethral segment has a progressive evolution towards atrophy. Due to menopausal hypoestrogenism, the urethra is subjected to a decrease in the intermediate and squamous superficial cells[9] and the musculature of the distal, proximal, and genitourinary tract undergoes the same degenerative process. The reduction in the submucosal vascular plexus and the decreased tension of connective tissue decrease intramural pressure, favouring urine leakage[10].

Because estrogen deficiency is the primary cause of atrophic urogenital changes, postmenopausal estrogen therapy is the most logical choice of treatment. Systemic estrogen therapy is the gold standard to slow-down or block the onset of urogenital atrophy, but it requires ongoing therapy to

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maintain its effectiveness[11,12]. Unfortunately, only 25% of women in menopause undergoes hormone replacement therapy (HRT) and/or topical estrogenic therapy, while the remaining 75% does not care or uses gels, lubricants, and moisturizers that provide short-term relief but cannot resolve the hormonal deficiency with the consequence of a constant and continuous tissue degeneration. Vaginal dryness, dyspareunia, pain, burns, cystitis and urine leakage increase over time affecting mental and sexual wellbeing[13].

To mitigate the degenerative processes associated with menopause and vulvovaginal tissue aging[14], patients may benefit from regenerative medicine. Recently, the regenerative capabilities of fat with mesenchymal properties (adipose-derived mesenchymal stem cells – ASCs) have been widely explored[15-17]. Through trophic, mitogenic, anti-scarring, anti-apoptotic, immunomodulatory, and anti-microbial actions, produced by a large amount of bioactive elements, growth factors and cytokines, these cells "sense" and "signal" changes in the microenvironment where they reside[18,19]. The use of ASCs, either culture expanded or obtained by enzymatic digestion, created a huge interest in regenerative medicine and *in vitro* and *in vivo* studies demonstrated their anti-inflammatory and regenerative potentials[16,20]. Nevertheless, cell expansion and/or enzymatic manipulation have complex regulatory constraints[21-23]. Hence, availability of a minimally manipulated, autologous adipose tissue rich in these naturally occurring regenerative cells would have remarkable clinical relevance. Techniques for harvesting and processing the adipose tissue have rapidly evolved in the last years[24,25], and published data shows both the safety and efficacy of using fat and its derivatives[26]. We took advantage of a commercially available technique that intra-operatively provides micro-fragmented adipose tissue in a short time, without expansion and/or enzymatic treatment. By the aid of this technology, the adipose tissue is micro-fragmented and washed until free of pro-inflammatory oil and blood residues, while preserving an intact stromal vascular niche [27]. The resulting product, which has been shown to be effective in the treatment of different pathologies[28-36], provides the key elements to support a natural regenerative response: the scaffold (the adipose tissue structure), the cells (ASCs), and growth factors (secreted cytokines and chemokines)[37]. For these reasons, we decided to test its regenerative potential in three women affected by post-menopausal urogenital atrophy. The informed consent was obtained by all the patients.

Cases Presentation

Three women (mean age 59 years-old) in menopause from over 5 years presented with urogenital atrophy. Only one patient took HRT (estriol 0.5 mg/die for vaginal use) 6 years before. Standard basal clinical evaluation included the assessment of symptoms related to urogenital atrophy, with particular reference to vaginal dryness, burning, itching and stranguria. In order to establish the subjective level for each individual symptom, women were asked to classify their symptoms as mild, moderate, or severe. Vaginal and clitoral sensitivity was also assessed to evaluate whether it was normal, slightly reduced or very reduced over time. Since the three women were sexually active, the intensity of dyspareunia was also recorded using the Numerical Rating Scale for pain. The site of pain – mons pubis, labia majora, urethral orifice, prepuce, clitoris, frenulum, vestibulum, labia minora, hymen, fossa navicularis, and fourchette – was reported on a topographical map of the vulvovaginal area. A biopsy was taken in the post-hymen vaginal area for immunohistochemical analysis and vaginal discharge was collected to measure pH and detect the characteristics of bacteria flora.

The three patients resulted symptomatic for all the considered symptoms. Vaginal dryness, arisen more than 4 years before, was reported as severe and increasing, with little or no benefits from topical treatments (gels, moisturizers, lubricants). Burning and itching, arisen 2-3 years before, were felt as severe and increased after sexual intercourse. Dyspareunia was also increasing. In particular, patients suffered for moderate pain in the urethral orifice, prepuce, clitoris, frenulum, and fourchette, and strong pain in vestibulum, labia minora, hymen, and fossa navicularis. No pain in mons pubis and labia majora. The sensitivity of clitoris and vagina was reported as reduced in one patient and very reduced in the other two. Background data of the patients are reported in Supplementary Table I. All women agreed to undergo to injections of autologous and micro-fragmented adipose tissue and signed their informed consent. Patients underwent the procedure in day surgery under slight sedation and were followed-up at 3, 6, 9, 12, 18, 24 and 36 months after the procedure.

Patients and Methods

Harvesting of the Adipose Tissue

The lower or the lateral abdomen was chosen as donor site for adipose tissue harvesting. Before

Supplementary table I. Descriptive characteristics of the population.

ID patient	Age	Pregnancies	VL	N° of VL	Age at M	HT	TA	Dryness	Burning	Itching	Sens C.	Sens V.	Stranguria
1	64	yes	yes	2	52 y.o	yes	yes	severe	severe	severe	very reduced	very reduced	severe
2	58	yes	yes	1	52 y.o	no	yes	severe	severe	severe	very reduced	very reduced	moderate
3	54	yes	yes	1	48 y.o	no	yes	severe	severe	severe	reduced	reduced	none

VL: vaginal lacerations; M: menopause; HR: hormonal therapy; TA: topical agents; Sens C: sensitivity clitoris; Sens V: sensitivity vagina

harvesting the fat, 100 cc of Klein Solution (500 cc saline, 1 cc epinephrine 1/1000 IU, and 40 cc lidocaine 2%) was injected in the periumbilical area using a disposable 17G blunt cannula connected to a luer-lock 60-cc syringe. The fat (120 cc) was then harvested using a 13G blunt cannula, for a fast and a-traumatic suction, connected to a Vaclock® 20-ml syringe.

Processing of the Adipose Tissue with Lipogems® Device

The harvested fat was immediately processed in the Lipogems® processing kit, a disposable device that progressively reduces the size of the adipose tissue clusters while eliminating oily substances and blood residues with pro-inflammatory properties. The entire process, carried out in one surgical step, was performed in complete immersion in physiological solution minimizing any traumatic action on cell products. The resulting micro-fragmented adipose tissue was collected in a 60-cc syringe and positioned for decanting the excess of saline solution.

Micro-fragmented Adipose Tissue Injection

Micro-fragmented fat (15 cc) was gently injected with a 20G blunt cannula in small amounts into the subcutaneous and subepithelial vaginal space in a homogeneous and uniform manner following the topographic map indicating the painful and sensible sites. In addition, because two patients suffered from stranguria, with urine leakage when coughing, the para-urethral areas were also injected for about 3 cm along the urethra course.

Total surgical time, from lipoaspiration to injection was about 1 hour. Patients were discharged with the prescription of medicated intimate lavage, antibiotic for 3 days (azithromycin 500 mg/die), and refrain from sexual intercourse for the first 4 weeks.

Immunohistochemical Analysis

Biopsies were collected 15 days before the procedure and 36 months post-op. The tissue was fixed in 10% formalin and processed for routine histopathology evaluation, sectioned to a thickness of 4-μm and stained with haematoxylin and eosin (median 10 slides per case). Immunoperoxidase studies were performed on 5-μm thick formalin-fixed, paraffin embedded tissue sections. Tissue sections were placed on a Ventana BenchMark XT (Ventana Medical Systems, Inc., Oro Valley, AZ, USA) for staining. The staining pro-

tocol included online deparaffinization, heat-induced epitope retrieval with Ventana Cell Conditioning 1 (CC1) followed by primary antibody incubation at 37°C for 32 minutes. Antigen-antibody reactions were visualized using the ultraView Universal DAB Detection Kit by Ventana (Ventana Medical Systems, Inc.). Counterstaining was performed on the Ventana BenchMarkXT using Ventana Haematoxylin II for 8 min, followed by bluing reagent for 4 minutes.

Immunohistochemical antibodies sources and conditions: anti-CD34 (QBEnd-10; Dako, Carpinteria, CA, USA); anti-Desmin clone DE-U-10 (Sigma-Aldrich, St. Louis, MO, USA); Monoclonal Anti-Actin, α-Smooth Muscle clone 1A4 (Sigma-Aldrich, St. Louis, MO, USA); Monoclonal Mouse Anti-Human CD20cy clone L26/1R604 (Dako, Glostrup, Denmark); Polyclonal Rabbit Anti-Human CD3 clone IR503 (Dako, Glostrup, Denmark); Monoclonal Mouse Anti-Human Ki-67 antigen, clone MIB/IR626 (Dako, Glostrup, Denmark).

Tests on Vaginal Discharge

Physical-chemical and microscopic examination of vaginal discharge on fresh or after Gram stain was executed to measure vaginal pH and determine the characteristics of bacteria flora.

Results

Neither intra- nor post-operative complications occurred. Three months after the procedure, vaginal dryness, itching and burning decreased from severe to moderate in all patients, and at 6 months one patient reported the decrease to mild. At 9 months, the symptoms disappeared in all the three women and this condition of wellbeing remained stable up to 36 months. Only the 64-year-old woman referred a mild occasional post-coital burn.

Vaginal and clitoral sensitivity, which was very reduced in 2 women and reduced in the other, improved at 3 months and returned to normal condition at 6 months, remaining stable until 36 months. In one case, vaginal and clitoral sensitivity returned normal 4 months after the procedure and the patient reported that it was even greater than what she experienced before the menopause (Table II). Dyspareunia was evaluated by clinical examination of the vulvovaginal sites. Three months after the procedure, pain significantly decreased from strong to moderate or from moderate to mild. Interestingly, between 6 and 9 months, the two younger women perceived no pain in all the evaluated sites, while the 64-year-old woman reported a mild persistence of pain (NRS score 1-2) at the fossa navicularis and fourchette (Figure 1).

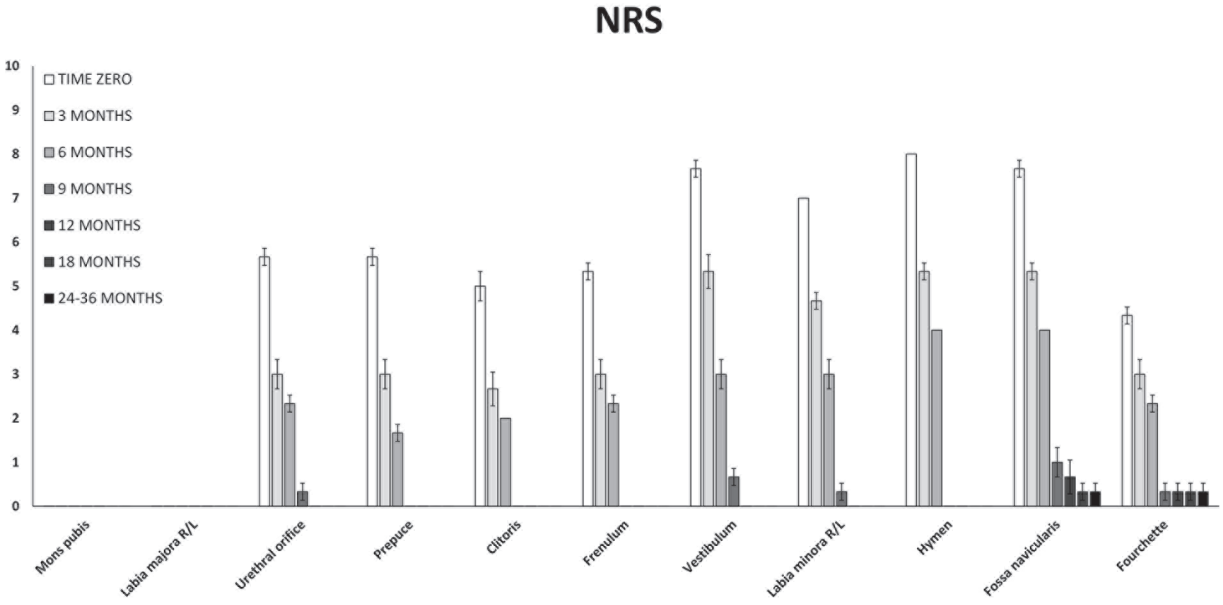

Figure 1. Intensity of dyspareunia reported with the Numerical Rating Scale (NRS) for pain (0-10) from baseline to 36 months' follow-up. Results are expressed as mean and standard error.

Supplementary table II. Summary of the results.

ID patient	follow up time	dryness	burning	itching	sensitivity clitoris	sensitivity vagina	stranguria
01		severe	severe	severe	very reduced	very reduced	severe
02	Baseline	severe	severe	severe	very reduced	very reduced	moderate
03		severe	severe	severe	reduced	reduced	none
01	3 months	moderate	moderate	moderate	reduced	reduced	moderate
02		moderate	moderate	moderate	reduced	reduced	none
03		moderate	moderate	moderate	normal	normal	none
01	6 months	moderate	moderate	moderate	normal	normal	mild
02		moderate	moderate	mild	normal	normal	none
03		mild	mild	absent	normal	normal	none
01	9 months	mild	mild	mild	normal	normal	none
02		mild	absent	absent	normal	normal	none
03		absent	absent	absent	normal	normal	none
01	12 months	absent	absent	absent	normal	normal	none
02		absent	absent	absent	normal	normal	none
03		absent	absent	absent	normal	normal	none
01	18 months	absent	absent	absent	normal	normal	none
02		absent	absent	absent	normal	normal	none
03		absent	absent	absent	normal	normal	none
01	24 months	absent	mild	absent	normal	normal	none
02		absent	absent	absent	normal	normal	none
03		absent	absent	absent	normal	normal	none
01	36 months	absent	mild	absent	normal	normal	none
02		absent	absent	absent	normal	normal	none
03		absent	absent	absent	normal	normal	none

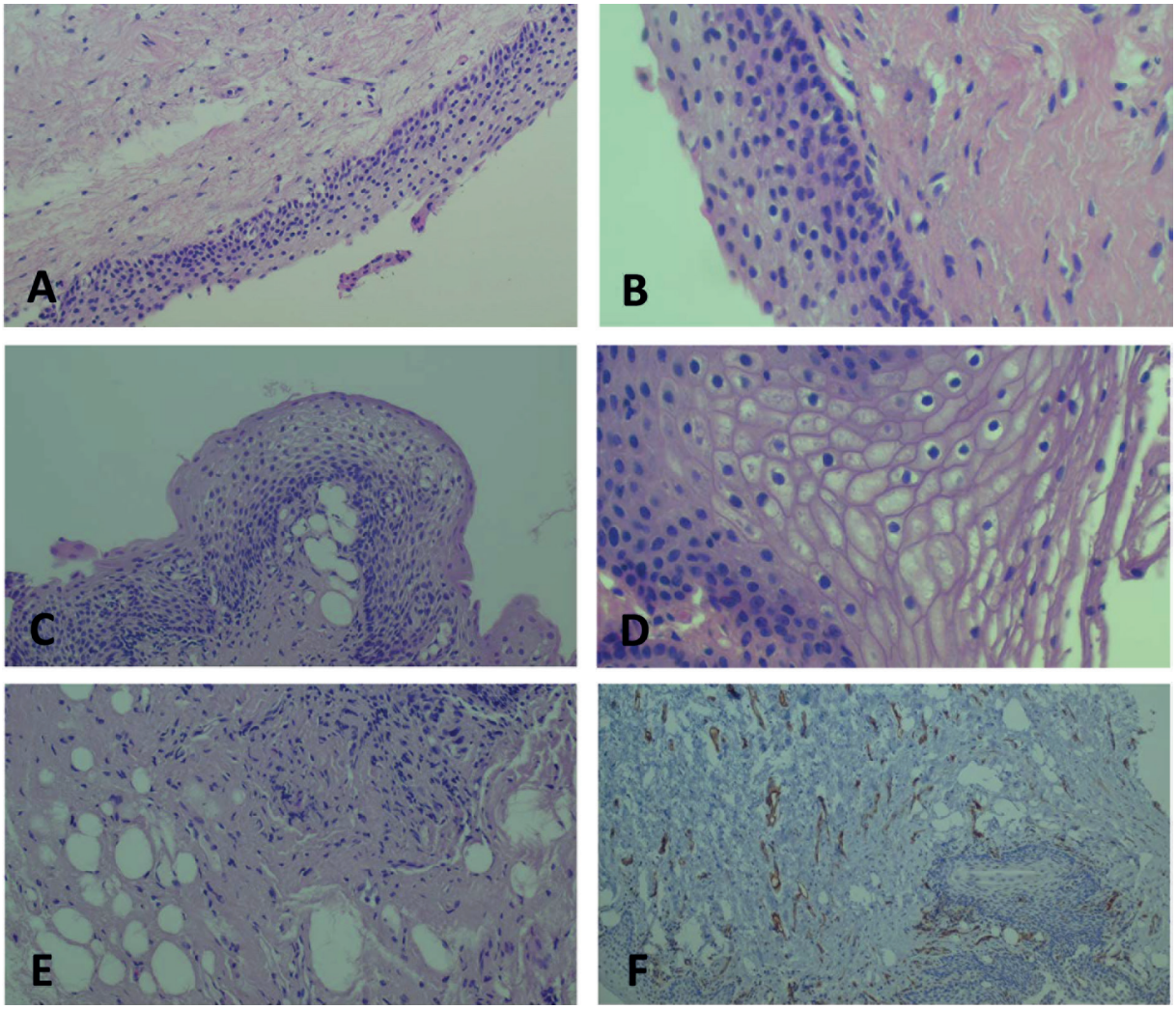

Figure 2. Immunohistochemical evaluations of patient 2. ***A-B,*** Pre-operative. ***C-F,*** 36-months post-op histology. ***A,*** Squamous vaginal epithelium flattened with a noticeable reduction of the stratum corneum. ***B,*** Detail of picture A showing considerable reduction in the thickness of the squamous vaginal epithelium at the level of the Malpighian with marked superficial hypo-glycogenesis. ***C,*** Squamous vaginal normotrophic epithelium of regular cellular thickness, composed of clear, hyper-glycogenic Malpighian cells, with regular basal layer. ***D,*** Detail of the squamous epithelium with a Malpighian and superficial layer of hyper-glycogenic cells. ***E,*** Unusual adipose tissue composed of medium and large asymmetric adipocytes in chorion oedematosus. ***F,*** Hyper-vascularity with small arterial-venous vessels.

Stranguria, reported by two women, disappeared between 6 and 9 months, while urine leakage has ceased starting from 3 months (Table II). Interestingly, also the aesthetic appearance benefited from the treatment, with the external genitals florid and turgid as in fertile period starting from the 6th month, and the vaginal mucosa from pale to rosy colour.

Histology at 36 months revealed a total recovery of vaginal vitality with production of glycogen, vasculature hyperplasia and regeneration of the epithelium and subcutaneous tissue in all the patients. The squamous vaginal epithelium appeared undamaged, with regular cellular thickness composed of clear and hyper-glycogenic Malpighian cells and regular basal layer (Figure 2).

The analysis of vaginal discharge showed the restoration of the physiologic acid pH (range 4.0-4.2 compared to 6.0-6.2 pre-op) with the colonization of lactobacilli in all the three women as in the fertile age (data not shown).

Discussion

Genitourinary atrophy is an alteration of the tissues of the genital apparatus and of the urethra that occurs in menopause when the production of ovarian steroid hormones ceases. Estrogen deficiency induces morphological and structural changes in the vagina, vulva and urethra, causing vaginal dryness, itch, burning, dyspareunia, stranguria, decreased sensitivity and urine leakage. These disorders inevitably arise with the end of the fertile age with subjective variability and with ingravescent course. Although not disabling, these symptoms are certainly capable of damaging physical and mental wellbeing, reducing women self-confidence and undermining sentimental life.

Hormone replacement therapy is the only pharmacological treatment able to block and slow down this degenerative process, but it requires ongoing therapy to maintain its effectiveness.

Our hypothesis that genitourinary atrophy can be counteracted by regenerative medicine has been confirmed, beyond the hypothesized expectations, in our first three cases treated with autologous and micro-fragmented adipose tissue injection. The innovative technique we utilized intra-operatively provides micro-fragmented adipose tissue in short time, without expansion or enzymatic treatment. The adipose tissue is micro-fragmented and washed until free of pro-inflammatory oil and blood residues, while preserving viable elements with pericyte identity within an intact stromal vascular niche[27]. The integrity of the niche entails the release of regenerative factors and bioactive molecules (immunomodulatory, anti-scarring, anti-apoptotic, angiogenic, mitotic, and anti-microbial), which make the transplanted tissue acting as a "timely releasing medium" of such elements in the site of injection[18,19]. The main structural and morphological adipose unit, the adipose niche, maintained after the processing, protects activated ASCs, strengthening their effectiveness in the recipient environment, and behaving as a large-scale tool to supply the damaged tissue with a regenerative environment. The three women reported significant improvements 6 months after treatment and a complete resolution of their symptoms at 9 months, with no complications or adverse events. This benefit, subjectively reported and confirmed by clinical evaluation, remained constant without recurrence at least until the follow-up of 36 months. Noteworthy, the treatment was not repeated and no adjuvant topical hormone therapy was required. Histology at 36 months revealed a total recovery of vaginal vitality with production of glycogen, vasculature hyperplasia and regeneration of the epithelium and subcutaneous tissue in all the patients.

Conclusions

The results of these three first cases show the safety, feasibility and efficacy of using autologous micro-fragmented and minimally manipulated adipose tissue injection for the treatment of urogenital atrophy. The procedure is simple, sustainable, quick, minimally invasive, one-step, and safe. After 36 months, the results are very satisfactory and promising. A similar study on 16 women is now ongoing, and, if the results will be confirmed, this approach will open the doors to a safe, viable and effective alternative therapy for the treatment of post-menopausal genitourinary atrophy.

Conflict of Interest

G.A. Casarotti and P. Chiodera declare having no competing interests. C. Tremolada is Founder and President of Lipogems International Spa and Inventor of the Lipogems® System.

References

1) Semmens JP, Wagner G. Estrogen deprivation and vaginal function in postmenopausal women. JAMA 1982; 248: 445-448.
2) Bachmann GA, Nevadunsky NS. Diagnosis and treatment of atrophic vaginitis. American family physician 2000; 61: 3090-3096.
3) Heinemann C, Reid G. Vaginal microbial diversity among postmenopausal women with and without hormone replacement therapy. Can J Microbiol 2005; 51: 777-781.
4) Pabich WL, Fihn SD, Stamm WE, Scholes D, Boyko EJ, Gupta K. Prevalence and determinants of vaginal flora alterations in postmenopausal women. J Infect Dis 2003; 188: 1054-1058.
5) Caillouette JC, Sharp CF, Zimmerman GJ, Roy S. Vaginal pH as a marker for bacterial pathogens and menopausal status. Am J Obstet Gynecol 1997; 176: 1270-1277; discussion 1275-1277.
6) Pandit L, Ouslander JG. Postmenopausal vaginal atrophy and atrophic vaginitis. Am J Med Sci 1997; 314: 228-231.
7) Moalli PA, Talarico LC, Sung VW, Klingensmith WL, Shand SH, Meyn LA, Watkins SC. Impact of menopause on collagen subtypes in the arcus tendineous fasciae pelvis. Am J Obstet Gynecol 2004; 190: 620-627.
8) Tinelli A, Malvasi A, Rahimi S, Negro R, Vergara D, Martignago R, Pellegrino M, Cavallotti C. Age-related pelvic floor modifications and prolapse risk

factors in postmenopausal women. Menopause 2010; 17: 204-212.

9) Bergman A, Karram MM, Bhatia NN. Changes in urethral cytology following estrogen administration. Gynecol Obstet Invest 1990; 29: 211-213.

10) Semmelink H, De Wilde P, Van Houwelingen J, Vooijs GP. Histomorphometric study of the lower urogenital tract in pre-and post-menopausal women. Cytometry 1990; 11: 700-707.

11) Jackson S, James M, Abrams P. The effect of oestradiol on vaginal collagen metabolism in postmenopausal women with genuine stress incontinence. BJOG 2002; 109: 339-344.

12) Robinson D, Cardozo L. Urogenital effects of hormone therapy. Best Pract Res Clin Endocrinol Metab 2003; 17: 91-104.

13) Nappi RE, Nijland EA. Women's perception of sexuality around the menopause: outcomes of a European telephone survey. Eur J Obstet Gynecol Reprod Biol 2008; 137: 10-16.

14) Giuseppina Onesti M, Carella S, Ceccarelli S, Marchese C, Scuderi N. The use of human adipose-derived stem cells in the treatment of physiological and pathological vulvar dystrophies. Stem Cells Int 2016; 2016: 2561461.

15) Gimble JM, Guilak F, Bunnell BA. Clinical and preclinical translation of cell-based therapies using adipose tissue-derived cells. Stem Cell Res Ther 2010; 1: 19.

16) Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H, Alfonso ZC, Fraser JK, Benhaim P, Hedrick MH. Human adipose tissue is a source of multipotent stem cells. Mol Biol Cell 2002; 13: 4279-4295.

17) Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, Benhaim P, Lorenz HP, Hedrick MH. Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Eng 2001; 7: 211-228.

18) Caplan AI, Correa D. The MSC: an injury drugstore. Cell Stem Cell 2011; 9: 11-15.

19) Caplan AI, Dennis JE. Mesenchymal stem cells as trophic mediators. J Cell Biochem 2006; 98: 1076-1084.

20) Chamberlain G, Fox J, Ashton B, Middleton J. Concise review: mesenchymal stem cells: their phenotype, differentiation capacity, immunological features, and potential for homing. Stem Cells 2007; 25: 2739-2749.

21) Ährlund-Richter L, De Luca M, Marshak DR, Munsie M, Veiga A, Rao M. Isolation and production of cells suitable for human therapy: challenges ahead. Cell Stem Cell 2009; 4: 20-26.

22) Arcidiacono JA, Blair JW, Benton KA. US Food and Drug Administration international collaborations for cellular therapy product regulation. Stem Cell Res Ther 2012; 3: 38.

23) Sensebé L, Bourin P, Tarte K. Good manufacturing practices production of mesenchymal stem/stromal cells. Hum Gene Ther 2010; 22: 19-26.

24) Oberbauer E, Steffenhagen C, Wurzer C, Gabriel C, Redl H, Wolbank S. Enzymatic and non-enzymatic isolation systems for adipose tissue-derived cells: current state of the art. Cell Regen (Lond) 2015; 4: 7.

25) Raposio E, Bertozzi N. How to isolate a ready-to-use adipose-derived stem cells pellet for clinical application. Eur Rev Med Pharmacol Sci 2017; 21: 4252-4260.

26) Gutowski KA, Force AFGT. Current applications and safety of autologous fat grafts: a report of the ASPS fat graft task force. Plast Reconstr Surg 2009; 124: 272-280.

27) Bianchi F, Maioli M, Leonardi E, Olivi E, Pasquinelli G, Valente S, Mendez AJ, Ricordi C, Raffaini M, Tremolada C, Ventura C. A new nonenzymatic method and device to obtain a fat tissue derivative highly enriched in pericyte-like elements by mild mechanical forces from human lipoaspirates. Cell Transplant 2013; 22: 2063-2077.

28) Benzi R, Marfia G, Bosetti M, Beltrami G, Magri AS, Versari S, Tremolada C. Microfractured lipoaspirate may help oral bone and soft tissue regeneration: a case report. CellR4 2015; 3: e1583.

29) Cestaro G, De Rosa M, Massa S, Amato B, Gentile M. Intersphincteric anal lipofilling with micro-fragmented fat tissue for the treatment of faecal incontinence: preliminary results of three patients. Wideochir Inne Tech Maloinwazyjne 2015; 10: 337-341.

30) Franceschini M, Castellaneta C, Mineo G. Injection of autologous micro-fragmented adipose tissue for the treatment of post-traumatic degenerative lesion of knee cartilage: a case report. CellR4 2016; 4 e1768.

31) Giori A, Tremolada C, Vailati R, Navone SE, Marfia G, Caplan AI. Recovery of function in anal incontinence after micro-fragmented fat graft (Lipogems®) injection: two years follow up of the first 5 cases. CellR4 2015; 3: e1544.

32) Grossi P, Giarratana S, Cernei S, Grossi S, Doniselli FM. Low back pain treated with disc decompression and autologous micro-fragmented adipose tissue: a case report. CellR4 2016; 4: e1772.

33) Raffaini M, Tremolada C. Micro fractured and purified adipose tissue graft (Lipogems®) can improve the orthognathic surgery outcomes both aesthetically and in postoperative healing. CellR4 2014; 2: e1118.

34) Saibene A, Pipolo C, Lorusso R, Portaleone SM, Felisati G. Transnasal endoscopic microfractured fat injection in glottic insufficiency. B-ENT 2014; 11: 229-234.

35) Striano R, Chen H, Bilbool N, Azatullah K, Hilado J, Horan K. Case Study: Non-responsive knee pain with osteoarthritis and concurrent meniscal disease treated with autologous microfragmented adipose tissue under continuous ultrasound guidance. CellR4 2015; 3: e1690.

36) Bianchi F, Olivi E, Baldassarre M, Giannone FA, Laggetta M, Valente S, Cavallini C, Tassinari R, Canaider S, Pasquinelli G, Tremolada C, Ventura C. Lipogems, a new modality of fat tissue handling to enhance tissue repair in chronic hind limb ischemia. CellR4 2014; 2: e1289.

37) Tremolada C, Colombo V, Ventura C. Adipose tissue and mesenchymal stem cells: state of the art and Lipogems® technology development. Curr Stem Cell Rep 2016; 2: 304-312.