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Hair growth stimulated by allogenic adipose-derived stem cells supplemented with ATP in a mouse model of dihydrotestosterone-induced androgenetic alopecia

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Abstract

Background Androgenetic alopecia (AGA), also known as male or female pattern hair loss, is the most prevalent form of alopecia worldwide. Current treatments are based on hormone drugs, topical vasodilators and hair transplants. Newer options include stem cell therapy targeted at recovering the capacity for hair follicle regeneration. This study examines the effects of intradermally administering allogenic adipose-derived stem cells (ASCs) per se or supplemented with ATP in a mouse model of dihydrotestosterone (DHT)-induced AGA.

Methods Male and female C57BL6-strain mice were treated with DHT to induce AGA and then given injections of treatment solution in a defined area of the depilated back skin, and the same injections three days later. The treatments tested were several concentrations of ASCs combined with two ATP formulations. Photographs of the treated zones were taken on days 7, 10, 14, 17 and 21 and subjected to Image J analysis. On day 21, skin samples were also obtained for histological analysis. The main outcome measure was percentage treated surface area showing hair regrowth on treatment days 17 and 21 expressed as five categories: null, poor, moderate, intense and complete (20, 40, 60, 80 and 100% respectively).

Results The experimental groups found to show the highest number of male individuals with intense/complete hair regrowth on day 21 were those in which mice received low dose ASCs ($1 \cdot 10^6$) combined either with liposomal ATP or non-liposomal ATP. Both these groups showed significant differences compared to controls. In females, while low dose ASC treatments and the high dose ASC + liposomal ATP treatment led to no hair regrowth improvement over the control treatment, medium dose ASC (2×10^6) + non-liposomal ATP gave rise to greater regrowth scores.

Conclusions Hair regrowth was improved in all experimental groups in which male mice were administered stem cell solutions supplemented with ATP. In female mice, the highest hair regrowth scores were observed for the medium dose ASC + liposomal ATP treatment.

Keywords Androgenetic alopecia, Mouse model, Adipose derived stem cells, ATP, Hair growth

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Background

Androgenetic alopecia (AGA) is the most prevalent type of hair loss disorder that affects men and women worldwide. In individuals with a genetic predisposition to AGA, dihydrotestosterone (DHT) binding to the androgen receptor (AR) present on dermal papilla cells has been identified as a key responsible mechanism. As the conversion of free testosterone to DHT is driven by 5α-reductase, systemic 5α-reductase inhibitors like finasteride have long been used to treat AGA and, along with topical minoxidil, are currently approved by the European Medicines Agency (EMA) for this purpose [1, 2].

Similarly, the hair shaft miniaturization observed in AGA is often associated with the loss and senescence of hair follicle stem cells [3–5] and dermal papilla cells [6]. To recover these cell populations, emerging cell regenerative therapies such as PRP (platelet-rich plasma), PRF (platelet-rich fibrin), and also hair follicle stem cell- [7] and mesenchymal stem cell-based therapies are popular options [8]. In effect, cell therapies are currently dominating the evolving landscape of hair restoration and outcomes include greater hair density and thickness along with improved patient satisfaction scores [9–18].

Mesenchymal stem cells (MSCs) and their secretoma have been widely used in the field of regenerative medicine. Derived products such as MSC-conditioned media and extracellular vesicles are a growing focus of investigation with applications in the treatment of various medical conditions. Both conditioned media and exosomes (nanomembranous vesicles) contain cytokines and growth factors that play a role in hair restoration.

The effectiveness of MSC-based treatments arises from their main functions in organ and tissue homeostasis. Additionally, these cells have the ability to secrete growth factors and anti-inflammatory cytokines thereby participating in immunomodulation within the follicle niche and in the reduced lymphocyte infiltration generally observed in these patients [19, 20]. While MSCs are found in different anatomical locations such as periosteum, bone trabeculae, synovial membrane, muscle tissue, dermis and bone marrow [21, 22], their extraction from adipose tissue is less complex and they can be obtained through a non-invasive procedure with a high cell yield [23]. These benefits have meant that adipose-derived stem cells (ASCs) are being widely investigated for their use in the field of dermatology, particularly in tissue regeneration to treat psoriasis and alopecia.

Most literature reports of MSC-conditioned medium for AGA treatment describe it as a growth factor-enriched secretome produced by MSC metabolism, but also refer to its stromal vascular fraction as an easily extractable heterogeneous adipose cell population composed of adipocytes, preadipocytes, adipose stem cells, endothelial progenitor cells, hematopoietic progenitors, monocytes, leukocytes and pericytes [25]. In the present study, we examine whether isolated ASCs promote anagen onset in an AGA microenvironment.

Adenosine triphosphate (ATP) synthesis is known to be essential for the normal hair cycle and proper formation of the hair shaft. Consistent with this, several mitochondrial disorders have been linked to hair abnormalities [26]. One of the most ATP-demanding processes is cytoskeleton configuration and disintegration during mitosis [27]. Further, filamentous mitochondria, which produce the greatest amounts of ATP and least number of reactive oxygen species, are known to benefit dermal papilla cell functionality during the hair growth cycle [28, 29].

Specifically, MSCs play an important role in mesodermal adult tissue maintenance [30] by communicating with the microenvironment through the secretion of signalling molecules such as ATP, ADP and their metabolites. This signalling pathway is called purinergic [31] and, by means of P2 receptors, proliferation and differentiation processes are regulated [32]. In this context, the mitogenic effect of ATP has been described by Riddle (2007) [33] to increase bone marrow mononuclear cell and brown preadipocyte proliferation [33, 34]. This cell division enhancement was promoted by an intracellular Ca^{2+} increase, which activates calcineurin through nuclear NFATc1 translocation [35]. Cooley [36] observed beneficial effects on hair transplant grafts of postoperative topical liposomal ATP treatment given in conjunction with a commercially available extracellular matrix and hypothermosol. In addition, the use of liposomal ATP has been shown to enhance free ATP penetrability avoiding hydrolysis caused by ectoenzymes [37].

The aim of the present study was to assess the hair growth effects of different concentrations of ASCs or ASCs + ATP solutions in a mouse model of androgen-induced AGA.

Methods

Mice

Experimental animals were 200 male and female 6-week-old mice purchased from Charles River Laboratories. The study protocol was approved by the Experimental Animal Ethics Committee of the Hospital Clínico San Carlos of Madrid and was in line with ARRIVE guidelines 2.0.

Male mice were housed individually. All animals were kept in controlled conditions of temperature (21 ± 2 °C) and relative humidity (55 ± 10%), and a standard 12 h light/dark cycle. Water and food were provided ad libitum. Wheels, cardboard tubes and polycarbonate igloos were used to enrich the animals' environment. The mice were anaesthetized by isoflurane 2% inhalation during

the experimental procedures, and euthanized through CO_2 exposure at the end of the study.

To establish the AGA model, animals were subcutaneously administered DHT (5α-androstan-17β-ol-3-one SIGMA dissolved in DMSO (Merck)/isotonic saline solution 1:1 (1 mg/animal)) five times per week. Four days after the start of DHT treatment (day 0), mice were depilated with an electric shaver and hair removal cream using a 4.5 × 2 cm template to frame the study zone. In all mice, hair removal induced hair growth conversion from the telogen to anagen phase.

Isolation and expansion of ASCs

Adipose stem cell lipoaspirates were obtained from abdominal adipose tissue (~ 500 mL) in two healthy subjects undergoing liposuction. Lipoaspirates were treated with collagenase and the stromal vascular fraction obtained after centrifugation. These were then transferred to DMEM culture medium and filtered. After another centrifugation, the supernatant was discarded and cells were seeded in culture plates. After 48 h, cells that adhered to the plates were preserved, expanded through three passages, and finally cryopreserved.

Preparation of ASCs and ASC/ATP solutions

After 48 h of culture, cells were harvested and resuspended in three different solutions: hypothermosol FRS (HTS); HTS plus non-liposomal ATP (Sigma) [100 µM] (adenosine 5′-triphosphate (ATP) disodium salt hydrate PM: 551.19 g/mol); and HTS plus a commercial ATP solution [100 µM] (Energy Delivery Solutions-Liposomal ATP Zavimed Medical Equipment). The liposomal ATP concentration in this commercial solution is 1.8 mg/mL and total ATP concentration is 5.5 mg/mL. Hereafter, this commercial solution will be designated liposomal ATP. ASCs were resuspended in 180 µL of each solution. Cells were also characterized by cytometry according to typical stem cell markers (CD90, CD73, CD10, CD166, and CD44).

Study groups

The 200 animals were divided into two sets of treatment groups: AGA model (30 animals) (Fig. 1) in which the groups WT and SHAM did not receive DHT and experimental (170 animals), in which all groups were DHT-administered (Fig. 2).

Administration of solutions

Animals were intradermally administered 180 µL of each treatment solution. The assessment zone on the dorsal skin was divided into three 3 cm^2 rectangles, and six 10-µL equidistant injections were then made through a 30G syringe in each one. This procedure was repeated three days later.

Macroscopy

Photographs of animals were taken on days 11, 14, 18 and 21 in the AGA model group, and on days 7, 10, 14, 17 and 21 in the experimental groups. Individual study zones on the backs of the mice were also photographed. Assessment was blinded by assigning five hair regrowth

Group	n
WT	10 (♀ 5 ♂ 5)
SHAM (DMSO/saline solution)	10 (♀ 5 ♂ 5)
DHT (AGA model control)	10 (♀ 5 ♂ 5)

Fig. 1 AGA model groups

Excipient	ATP	ASC dose	Group	n
Hypothermosol	None	None	Control	10 (♀ 5 ♂ 5)
	None	$4 \cdot 10^6$	HighASC	20 (♀ 10 ♂ 10)
	Liposomal	None	LipoATP	10 (♀ 5 ♂ 5)
		ASC $1 \cdot 10^6$	LowASC/LipoATP	20 (♀ 10 ♂ 10)
		ASC $2 \cdot 10^6$	MediumASC/LipoATP	20 (♀ 10 ♂ 10)
		ASC $4 \cdot 10^6$	HighASC/LipoATP	20 (♀ 10 ♂ 10)
	Non-liposomal	None	NonLipoATP	10 (♀ 5 ♂ 5)
		ASCs $1 \cdot 10^6$	LowASC/nonLipoATP	20 (♀ 10 ♂ 10)
		ASCs $2 \cdot 10^6$	MediumASC/nonLipoATP	20 (♀ 10 ♂ 10)
		ASCs $4 \cdot 10^6$	HighASC/nonLipoATP	20 (♀ 10 ♂ 10)

Fig. 2 Experimental treatment groups

levels classified as null, poor, intense, moderate and complete representing 20, 40, 60, 80, and 100% of regrowth, respectively, across the established zone on days 17 and 21. Experimental groups were compared to the control group (HTS).

Image analysis

A defined 1.5 × 3.9 (5.85 cm^2) surface area on the backs of the mice was assessed by FIJI Image J software providing the following grey scale and hair regrowth measures:

- Raw Integrated Density (Raw Int Den) (on days 14, 17 and 21): inverse variable of the intensity resulting from the sum of the grey values of the pixels in the selected area.
- Modal Grey Value (on days 7 and 10): the most frequent greyscale value in the surface evaluation inversely proportional to skin colour intensity. This variable corresponds to the highest peak of the image histogram. The modal grey value in dark shades is lower than in light ones.
- Hair growth (on days 14, 17 and 21): area of hair regrowth expressed in relation to the study zone (hair growth area (pixels)/depilation area) × 100.

Histological analysis

Skin samples were collected on day 21 in all treatment groups. The dorsal skin was fixed in 4% formaldehyde, embedded in paraffin, and sectioned to a 5 µm-thickness along the longitudinal and vertical plane of the hair follicles. In this study, haematoxylin–eosin staining was used for histology which was blindly assessed by a dermatopathologist. To assess hair density, the stained skin tissues were observed and captured under a light microscope.

Statistical analysis

Optimal sample size was determined based on statistical analysis of the results of similar hair growth studies. Data from the AGA model and experimental treatments were analysed as independent blocks using Statistical Package for the Social Sciences (SPSS) version 26, a software package developed by IBM for statistical analysis. The Shapiro–Wilk normality test indicated that most variables were non-normally distributed, so median and interquartile range (IQR) were calculated. To detect associations between the two data sets (model and experimental), a Mann Whitney U test was carried out between the treatments DHT (AGA control) and HTS (experimental control) for a modal grey value recorded on day 7, hair growth rate on days 14 and 21 and raw integrated density on days 14 and 21.

To compare image-related variables, we used the Kruskal–Wallis test with Bonferroni correction. Data for all the experimental groups were compared to HTS as the control group. Hair regrowth percentages were compared using the Chi-square test with Bonferroni correction.

Results

Cytometry

No changes in the mesenchymal stem cell phenotype were detected after ATP [100 µM] supplementation using both formulations (Fig. 3).

Macroscopy

AGA model

Throughout the study, hair regrowth progressed in a similar manner in the male mouse groups WT and SHAM, and by day 21, the entire test zone was covered in hair. Mice in the DHT group showed a relatively reduced amount of hair (Fig. 4).

In Fig. 5 it may also be observed that the administration of DHT gave rise to less hair regrowth in the female mice.

Male mice

In the SHAM and WT groups, hair regrowth on the backs of mice was complete on day 21 whereas in the HTS control group, regrowth was graded as moderate/intense respectively. In all the experimental groups, a beneficial effect on anagen onset was observed. As expected, similar hair regrowth percentages were recorded in the HTS and DHT groups, indicating moderate/intense regrowth (Fig. 6a, b).

In the groups Low ASC/LipoATP and Low ASC/non-LipoATP, the highest numbers of animals with intense/complete regrowth were observed on day 21. The High-ASC group also showed good outcomes (70% of mice showed intense/complete regrowth) as did the MediumASC/non-LipoATP-treated animals, followed by MediumASC/LipoATP, which showed similar treatment outcomes to the LipoATP group (60%) (Fig. 6c, d).

Better outcomes were observed for the low-dose ASC plus ATP groups than in the liposomal and non-liposomal ATP groups, suggesting a synergistic effect of ASCs and ATP.

Female mice

Hair regrowth assessment in the female mice was performed on day 17, as complete regrowth occurs earlier in females than males and it was not possible to detect differences between treatments on day 21. Some of the groups of female mice did not show more hair regrowth than the control HTS-treated mice, as was effectively observed in male mice. Specifically, a low ASC dose plus either non-liposomal or liposomal ATP, or non-liposomal

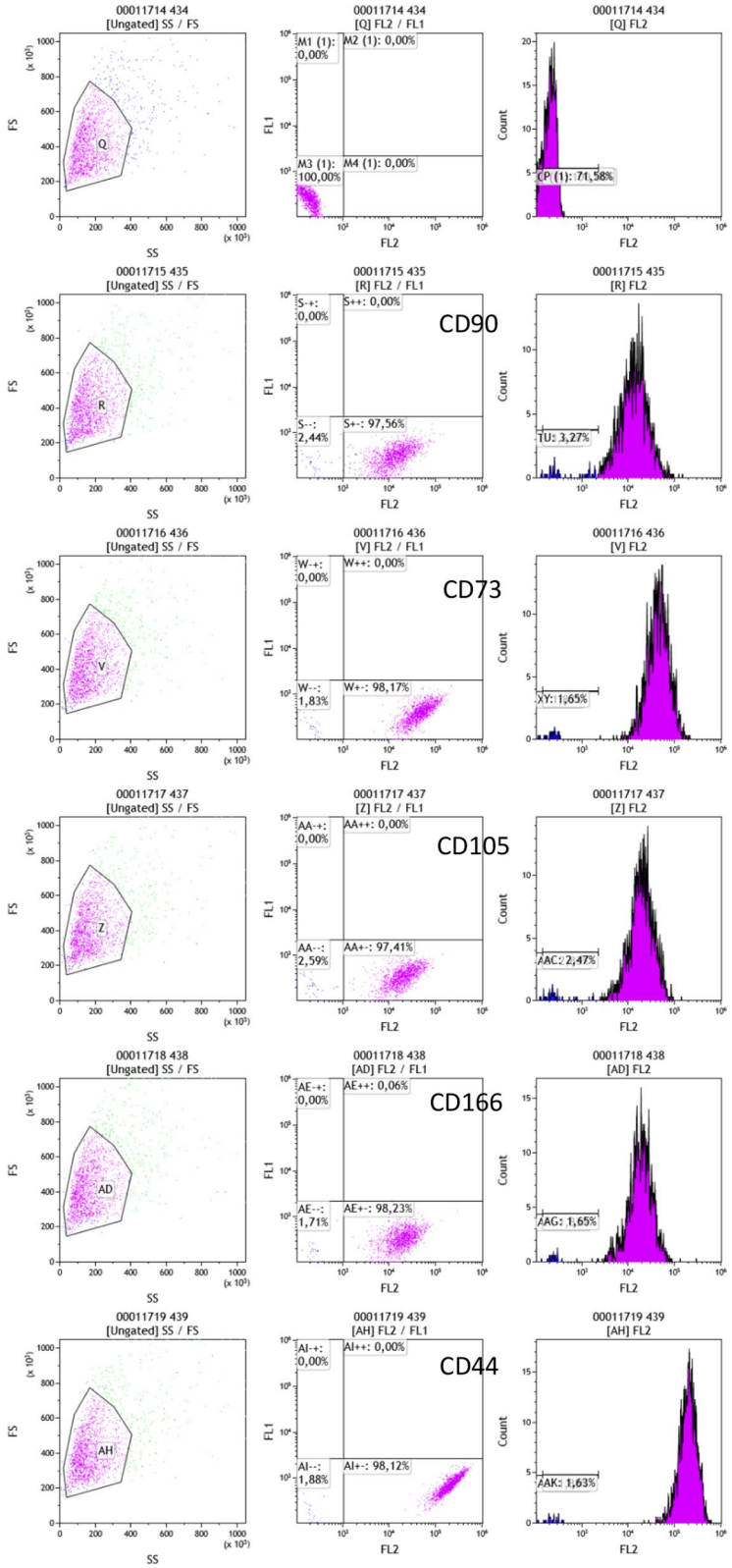


Fig. 3 Immunophenotype of $2x10^6$ ASCs incubated in HTS + liposomal ATP solution. Through flow cytometry, the immunophenotype of the stem cells was assessed according to several markers (CD90; CD73; CD166; CD105 and CD44) 24 h after incubation of the ASCs with ATP [100 µM] in refrigerated conditions. Representative image

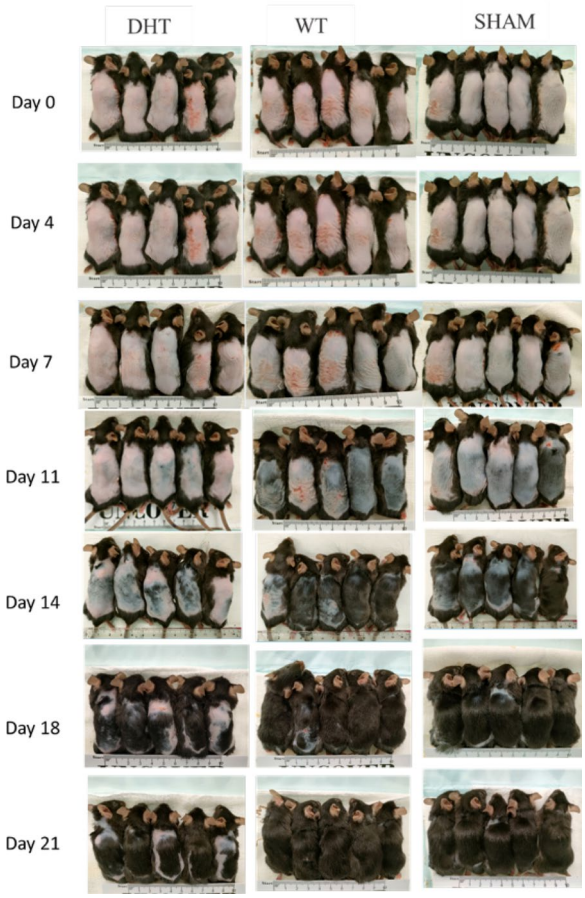


Fig. 4 Male AGA model assessment. Hair regrowth photographs taken on days 0, 4, 7, 11, 14, 18 and 21 after depilation in male C57BL6 mice

ATP alone, did not result in improved regrowth outcomes (intense/complete regrowth proportions) over those obtained with HTS, indicating no hair growth promoting effects.

Further, in female mice treated with liposomal ATP, 100% showed complete regrowth on day 17, similar to the WT and MediumASC/nonLipoATP group showing 90% intense/complete regrowth. All groups of female mice treated with a high ASC dose alone or in combination with either ATP formulation showed intense/complete regrowth values of 80% with the exception of the MediumASC/LipoATP group returning values equivalent to the HTS group (60%) (Fig. 7a, b). No significant differences were detected between the treatment and control HTS groups.

Image analysis

Modal grey value

During AGA model testing, the WT group showed a 22.58% lower modal grey value on day 11 than the DHT and SHAM groups, consistent with the delayed anagen onset observed by visual inspection in the DHT group. The bald patches observed in the SHAM groups of both sexes are likely explained by the actions of DMSO at room temperature. No significant differences were detected between the model treatment groups.

On day 7, the experimental groups of male mice showed higher variability in modal grey values than those of female mice. The groups High ASC/LipoATP showed the highest modal grey values, which were similar to the values recorded for HTS, followed by those recorded in the High ASC/nonLipoATP and Low ASC/LipoATP groups. These last groups showed a greater delay in skin darkening compared to the other experimental groups (Fig. 8a).

Significant differences were detected between several of the experimental groups (nonLipoATP, LipoATP, MediumASC/LipoATP, MediumASC/nonLipoATP and LowASC/nonLipoATP), and the HTS group on day 7, and between nonLipoATP, LipoATP, MediumASC/LipoATP, and MediumASC/nonLipoATP and the HTS group on day 10 (Fig. 8b).

The treatment groups of female mice: LowASC/LipoATP, HighASC/nonLipoATP and HighASC/LipoATP showed lower modal grey values than HTS and therefore an earlier anagen onset. Significant differences were detected between HighASC/LipoATP and HTS ($P=0.009$).

On day 10, female anagen onset occurred earlier than in males. Only in males were significant differences in grey scale values detected in the groups nonLipoATP, MediumASC/nonLipoATP and HighASC/LipoATP. All the male experimental groups showed lower values than HTS, suggesting that different treatments could accelerate hair shaft elongation.

In females, none of the treatments produced lower values than HTS except the LowASC/LipoATP group (Fig. 8b).

Hence most of the female mice groups showed greater darkening of the skin than males, in agreement with the earlier occurrence of anagen onset in the female mouse AGA model.

Raw integrated density

Our raw integrated density data confirmed the efficacy of the animal model in both sexes and an earlier anagen onset in the female mouse AGA model. In males, significant differences were observed between the treatments LowASC/LipoATP, HighASC/LipoATP, HighASC/nonLipoATP and LowASC/nonLipoATP and HTS (Fig. 9). These data also support the hair growth rate results described below.

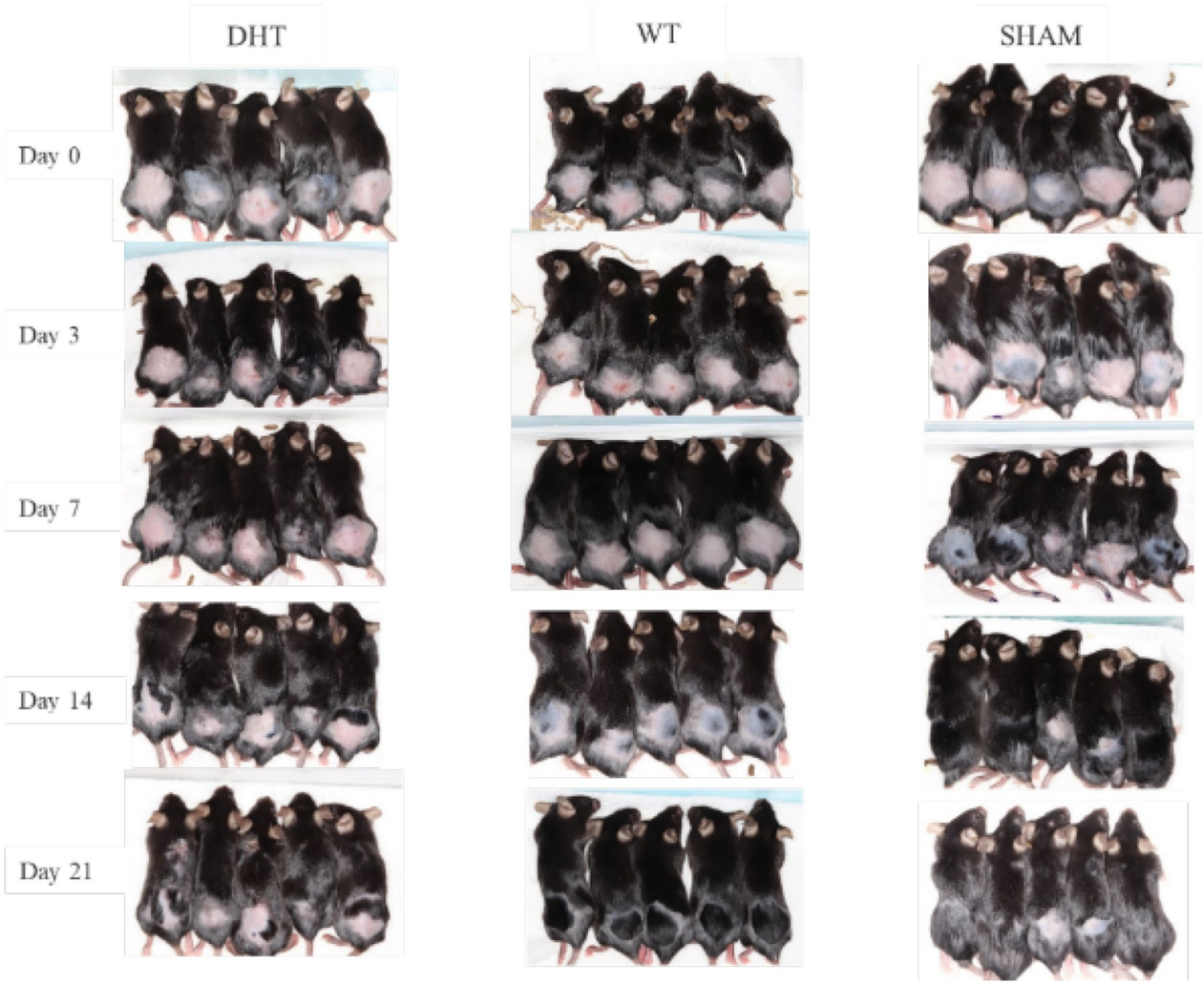


Fig. 5 Female AGA model assessment. Hair regrowth on days 0, 4, 7, 14 and 21 recorded in female C57BL6 mice after depilation

Hair growth rate

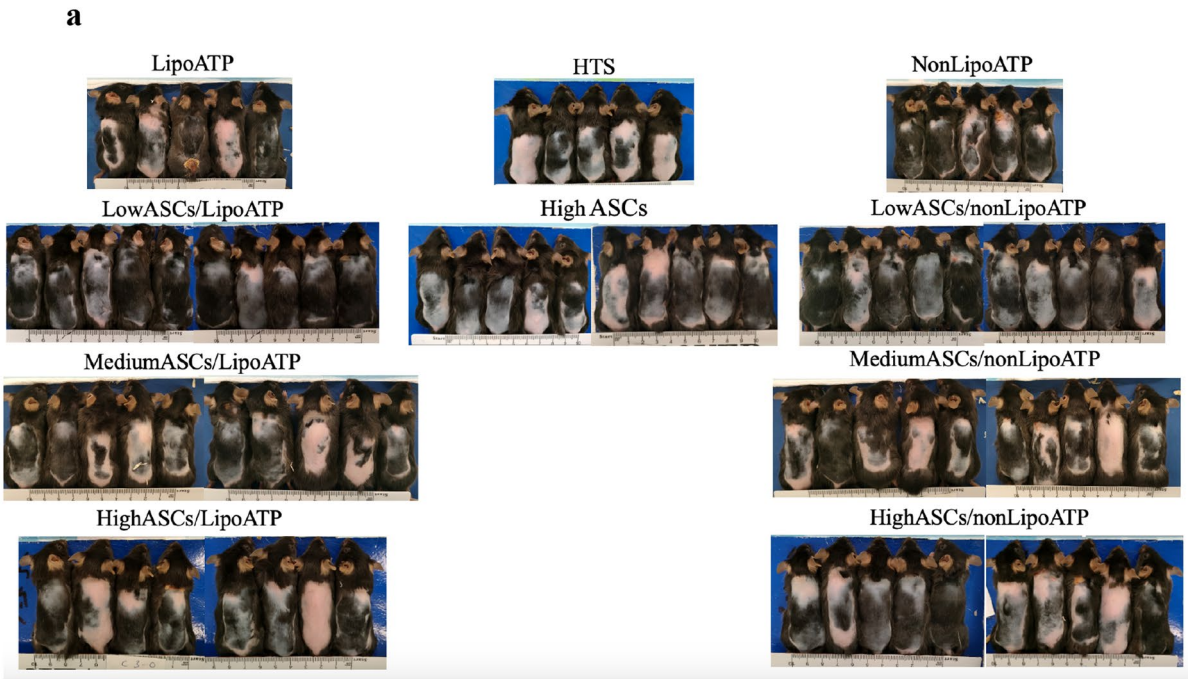
On day 14, the male mouse HTS group showed no hair regrowth, whereas the female group showed a 61.36% repopulated surface area. Taking into account AGA model anagen onset differences according to sex, most of the male experimental groups showed a 0–20% hair growth rate whereas greater variability was observed in female mice. Significant differences were detected in male mice between the NonLipoATP (P= 0.045), LowASC/LipoATP (P= 0.000) and LowASC/nonLipoATP (P= 0.000) groups and HTS (Fig. 10a).

Upon visual inspection, all male experimental groups showed an earlier anagen onset than the HTS group. A priori, all treatments seemed to have a beneficial effect on hair regrowth. In contrast, in female mice, the treatments LowASC/nonLipoATP and HighASC/LipoATP did not improve on the HTS treatment. In both sexes, the WT treatment group showed almost complete hair regrowth at this time point.

On day 17, the male mice groups showed greater intergroup variability in hair growth and considerably more in the NonLipoATP group. Significant differences were detected between the groups LowASC/LipoATP (P= 0.000) and NonLipoATP (P= 0.009) versus HTS.

Female mice in the groups HighASC, NonLipoATP, LipoATP, MediumASC/LipoATP and HighASC/nonLipoATP showed higher hair growth rates than the HTS group, although differences were not significant. According to our macroscopy assessment, growth rates were between 80% and 90% in the DHT-administered female mice groups in contrast to males, who did not exceed 50% (Fig. 8b).

On day 21, the differences between sexes remained. While the female HTS group displayed complete



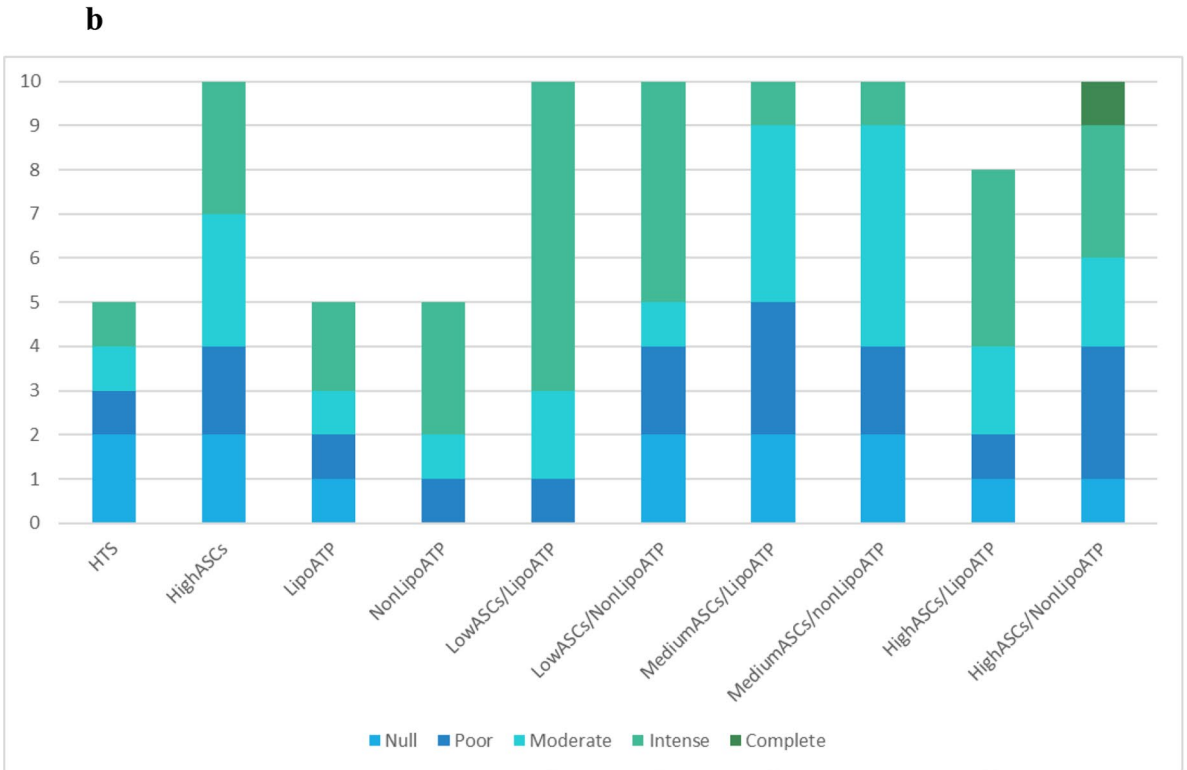
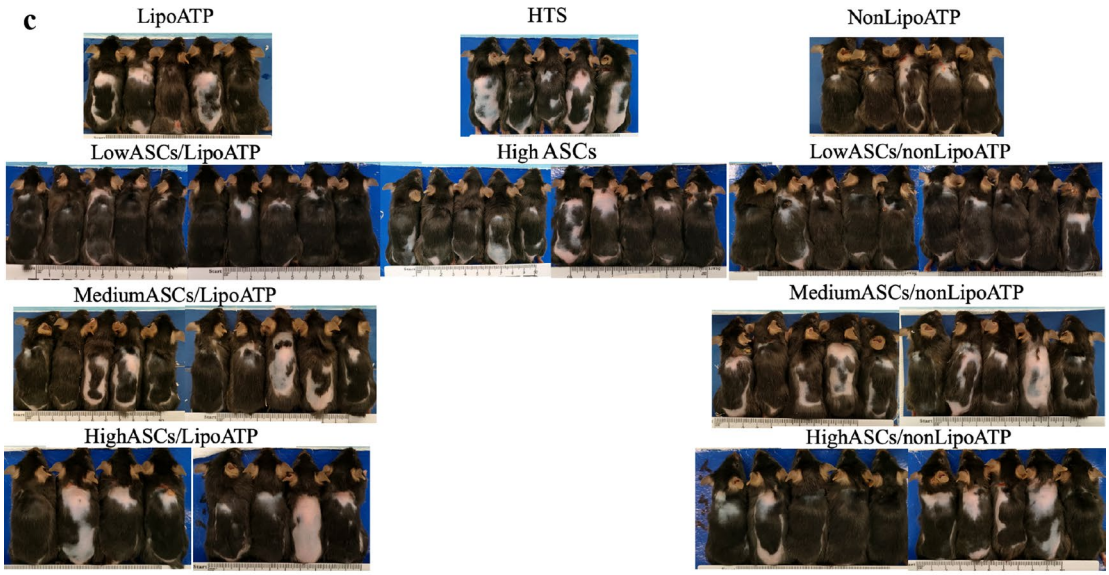


Fig. 6 Male mice experimental groups. **a** Pictures of hair regrowth taken on day 17. **b** Visual classification into 5 levels of hair regrowth on day 17. **c** Pictures of hair regrowth taken on day 21. **d** Visual classification into 5 levels of hair regrowth on day 21 (AGA model groups included)



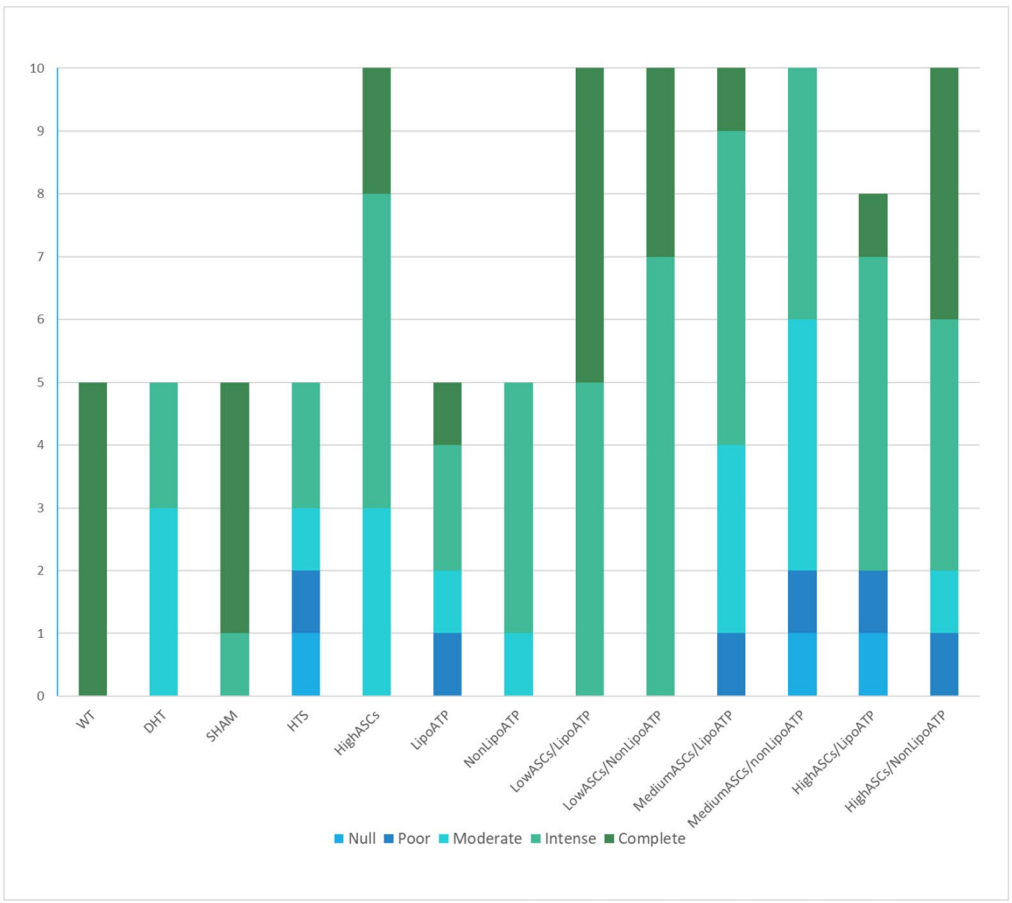
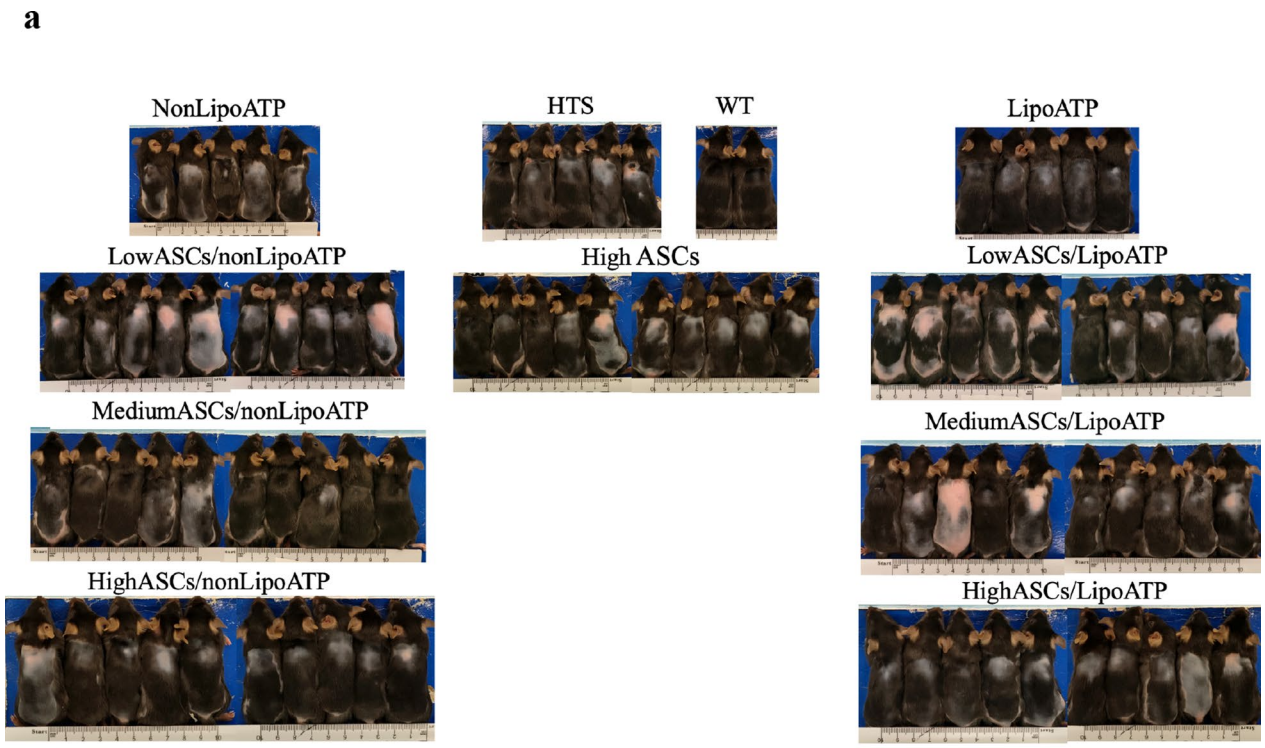


Fig. 6 continued



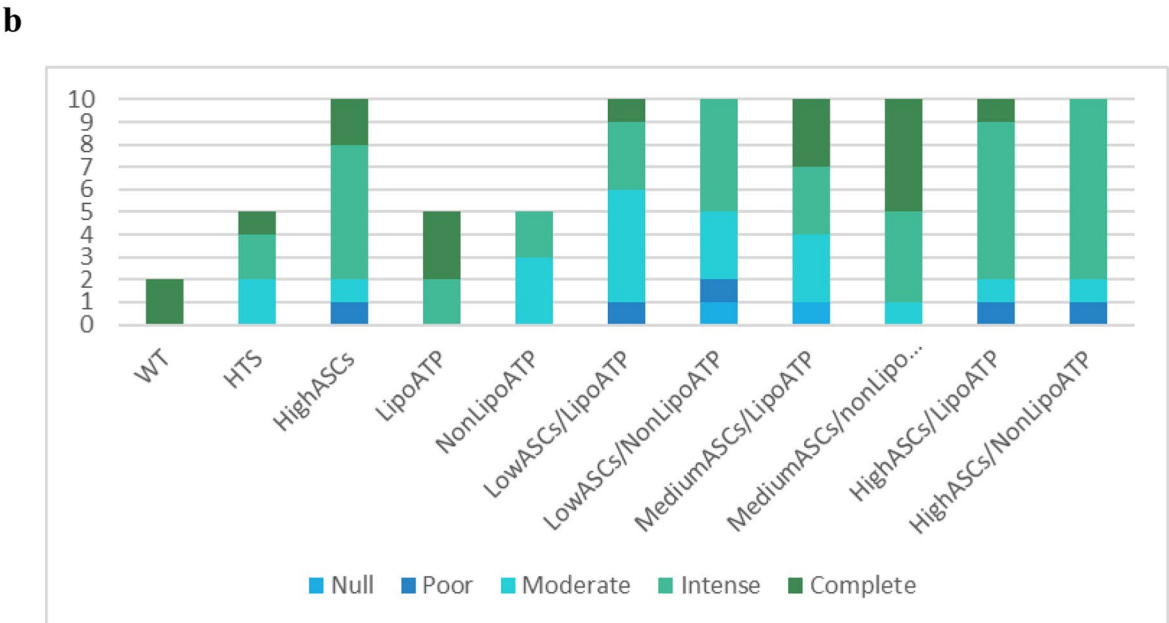
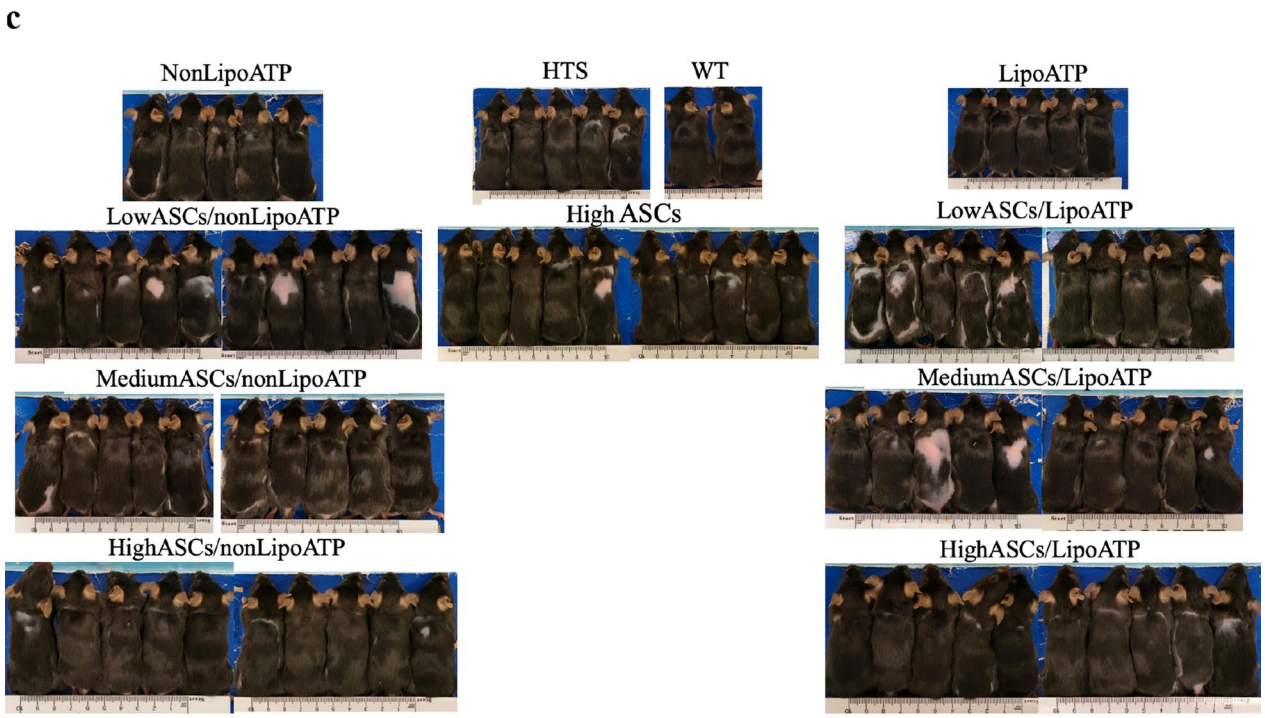


Fig. 7 Female mice experimental groups. **a** Pictures of hair regrowth taken on day 17. **b** Visual classification into 5 levels of hair regrowth on day 17. **c** Pictures of hair regrowth taken on day 21. **d** Visual classification into 5 levels of hair regrowth on day 21 (AGA model groups included)



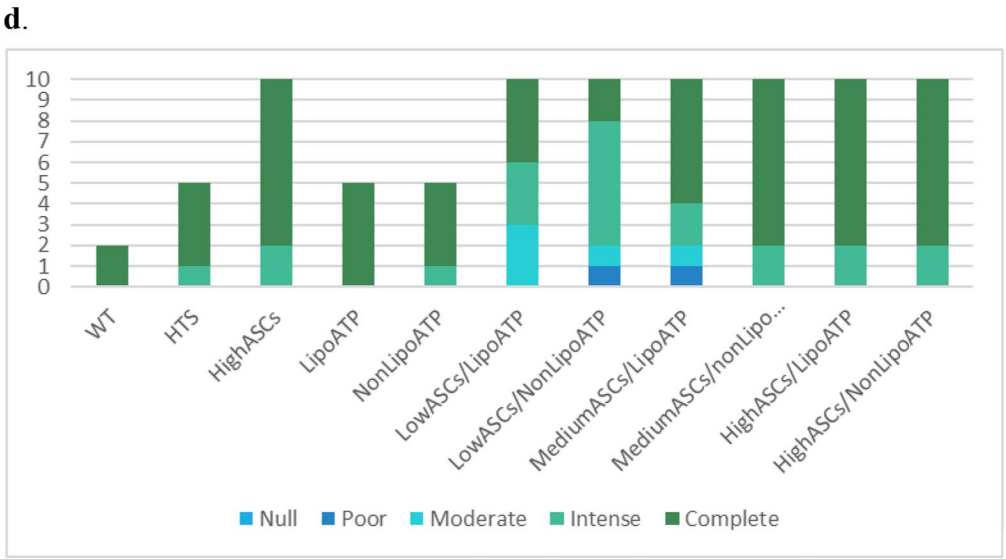


Fig. 7 continued

regrowth, in males the rate was about 63.72%, similar to females on day 14 (61.36%).

Male mice in the LowASC/LipoATP group showed complete regrowth and, along with those in the LowASC/nonLipoATP group, showed significant differences compared to HTS ($P = 0.009$ and $P = 0.045$, respectively) (Fig. 8c).

Histological analysis

A greater hair density and bulb diameter were recorded in the male mouse LowASC/LipoATP group and female mouse MediumASC/nonLipoATP group compared to their respective controls (Figs. 11 and 12).

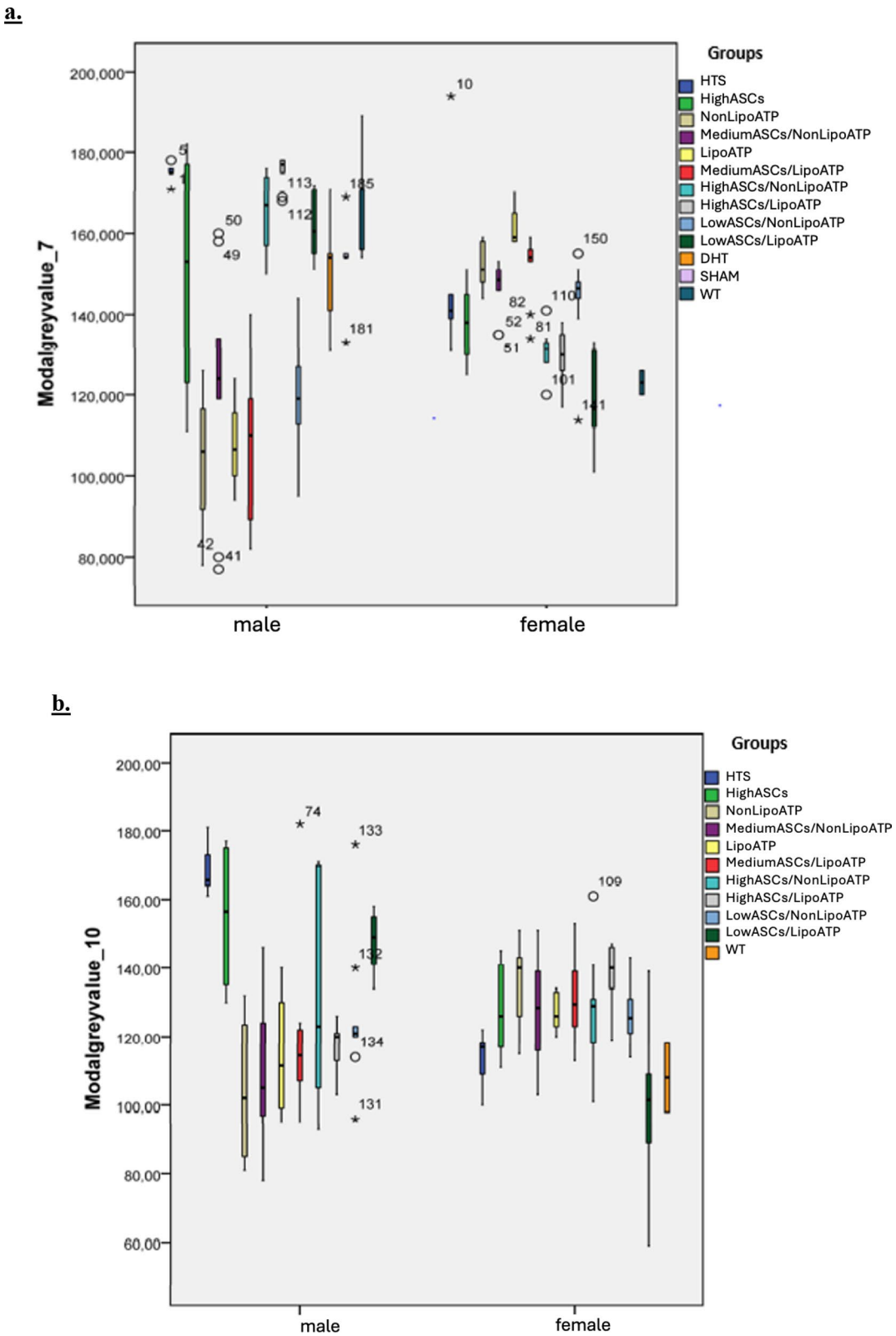


Fig. 8 Box-and-whisker plots of modal grey values on day 7 (**a**) and day 10 (**b**) recorded in the experimental treatment groups of female and male mice

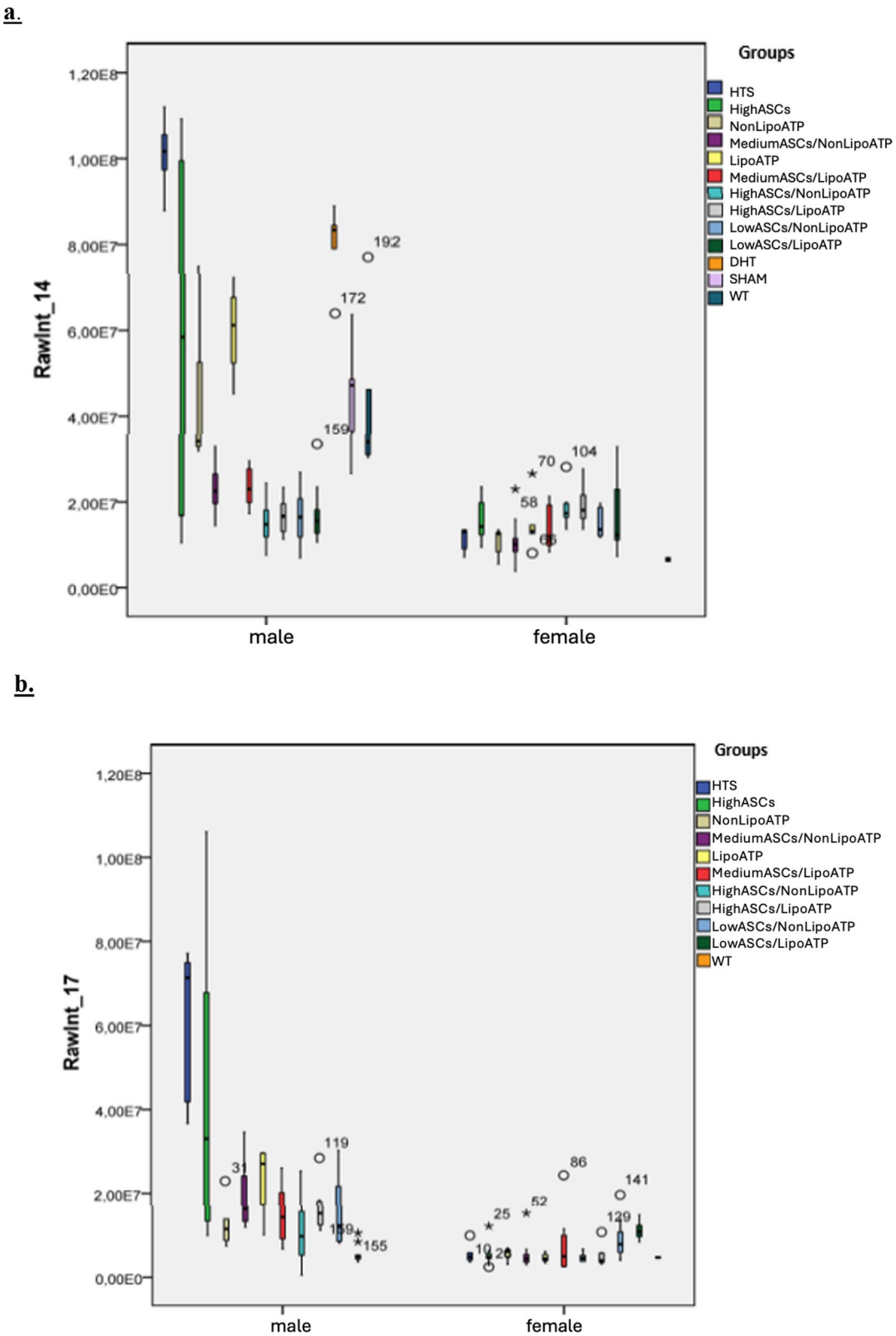


Fig. 9 Box-and-whisker plots of raw integrated density on days 14 (**a**), 17 (**b**) and 21 (**c**) recorded in the experimental treatment groups of female and male mice

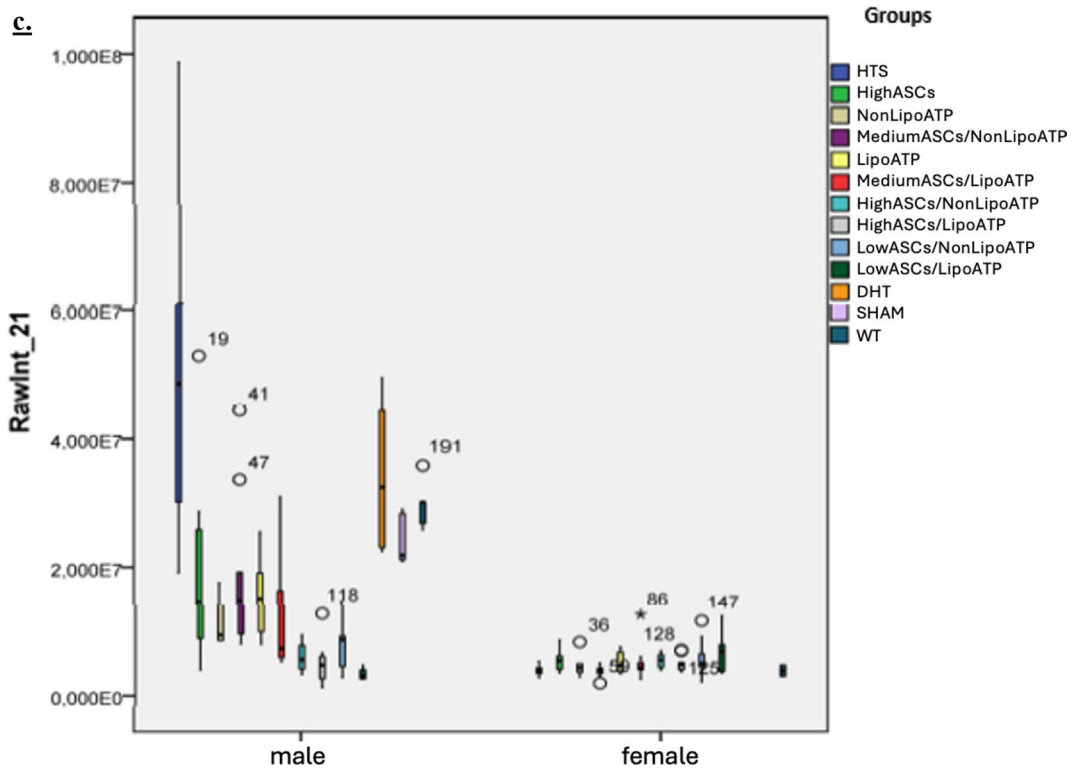


Fig. 9 continued

Discussion

The present study was designed to assess the efficacy of several treatments for AGA based on the intradermal injection of ASCs and ATP in male and female mice. As expected, the AGA model established showed a delay in hair growth and skin darkening in both sexes. In males, this effect was more marked probably because of a greater number of ARs in male than female hair follicles, as observed in humans [38]. Our results effectively confirm the earlier onset of anagen in DHT-administered females than males, likely due to lesser actions of the androgen in the former.

Consistent with these findings, the intense/complete hair regrowth rates in the male mouse HTS group were 40% on day 21, whereas in females this rate was 60% on day 17. These results are hardly comparable considering the different optimal assessment time points in the two sexes.

In both sexes, the ASC + ATP treatments were beneficial. Specifically, in male mice, the LowASC/LipoATP and LowASC/nonLipoATP treatments led to the higher hair regrowth percentages. Hence, these components appeared to have a synergistic effect in male mice and this effect was observed for both types of ATP formulations. We propose that a physiological concentration of ATP may be acting as an active promoter of ASCs, increasing their potential to supply the follicle niche with the necessary energy. As a mitogenic molecule and energy supplier, ATP could promote the proliferation and functionality of dermal papilla cells, enhancing angiogenesis and thus avoiding hypoxia.

According to our hair growth results, it seems that all the treatments were beneficial in male mice, and most of them in female mice. To achieve in females on day 17 the same results as those observed on day 21 in the male mice with low dose ASCs and liposomal ATP, a double dose of ASCs (i.e., medium dose) supplemented with non-liposomal ATP would be needed. In female mice, most of the preparations gave rise to faster hair growth compared to control HTS treatment.

Although both sexes showed improvements in hair regrowth when treated with a high dose of ASCs, ATP supplementation could be a therapeutic option, enabling a reduction in the ASC dose without affecting the outcome.

Conclusions

– DHT administration served to properly mimic AGA in C57BL6 male and female mice causing a delayed anagen onset which was more marked in males.

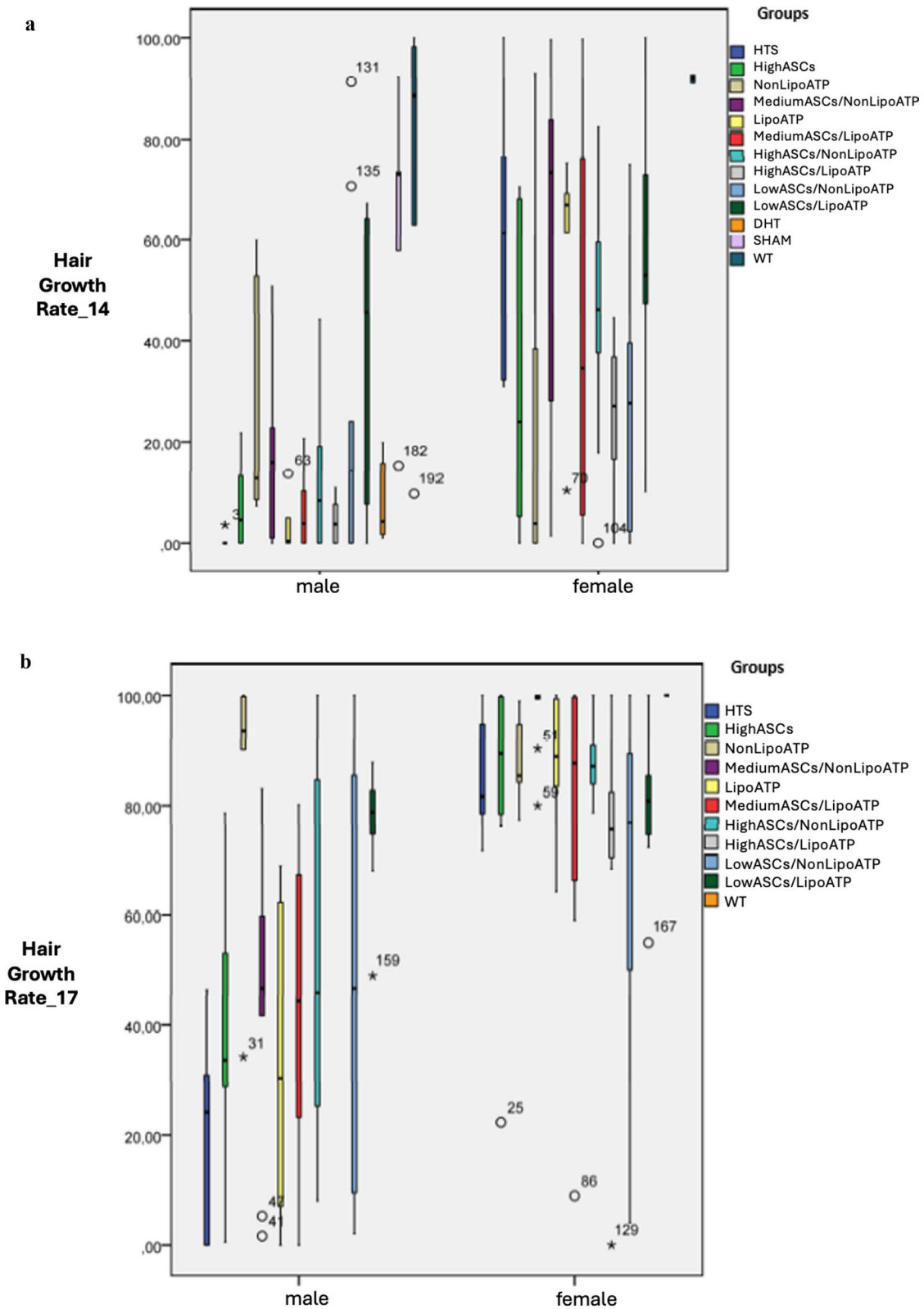


Fig. 10 Box-and-whisker plots of hair growth rates on days 14 (**a**), 17 (**b**) and 21 (**c**) recorded in the experimental groups of male and female mice

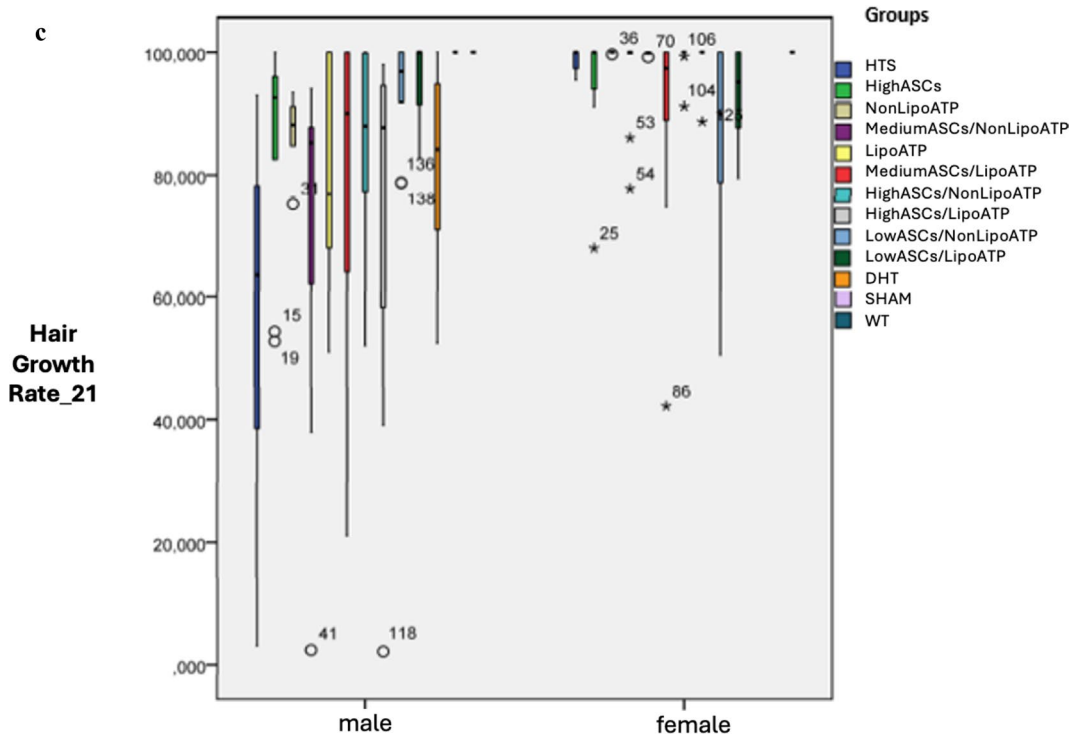


Fig. 10 continued

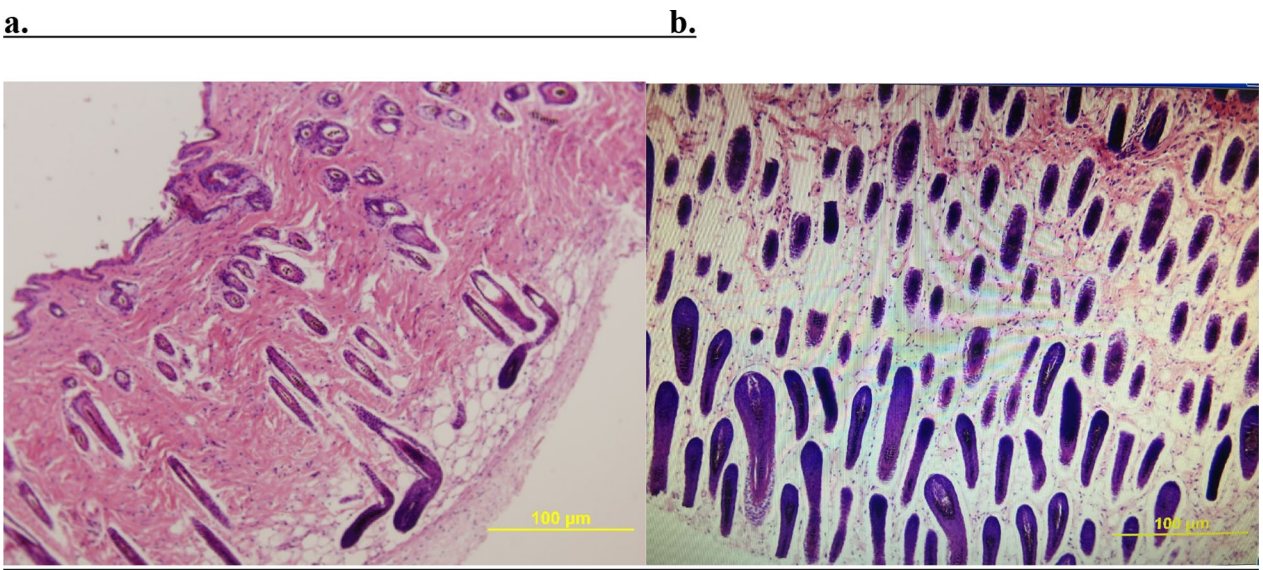


Fig. 11 Representative images of histological findings in the skin of male mice in the control (**a**) and experimental (**b**) treatment groups. The experimental group showed greater hair follicle density and bulb diameter

– All the treatments tested increased the hair growth rate in males. Mice treated with low-dose ASCs plus ATP showed the highest proportions of intense/complete regrowth.

– An unexpected synergistic effect of ASCs and liposomal/non-liposomal ATP was observed, which exceeded the beneficial effects of high dose ASCs or ATP alone in male mice.

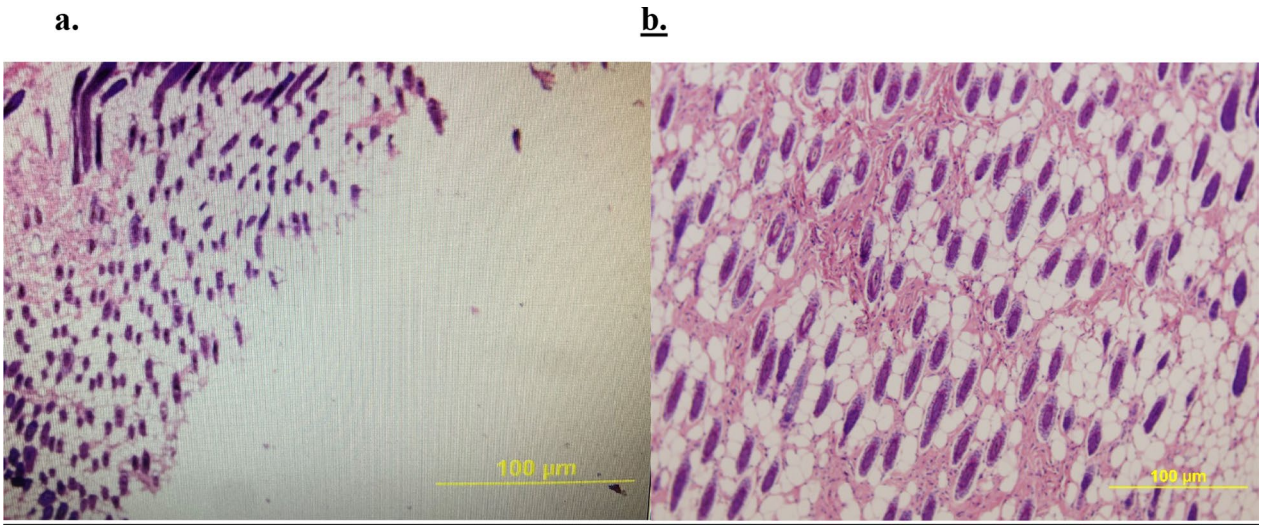


Fig. 12 Representative images of histological findings in the skin of female mice in the control (**a**) and experimental (**b**) treatment groups. The experimental group showed a higher hair follicle density

- In male mice, the treatments liposomal ATP alone or non-liposomal ATP combined with medium and high concentrations of ASCs gave rise to a higher number of individuals with intense/complete hair regrowth than the control HTS treatment.
- In female mice, most of the preparations induced faster hair growth compared to HTS. The highest number of mice showing intense/complete hair regrowth was noted for the medium- and high dose ASC solutions supplemented with non liposomal ATP.
- In female mice, no synergistic effect was observed such that liposomal ATP treatment led to improved outcomes compared to medium-dose ASCs were added. Conversely, the non-liposomal ATP treatment effect seemed to be similar to the medium dose ASC/LipoATP treatment.
- Our findings suggest that ATP supplementation of cell preparations might be a good therapeutic option to enhance the beneficial impacts of MSCs.

Abbreviations

ASCs	Adipose-derived stem cells
MSCs	mesenchymal stem cells
AR	androgen receptor
DHT	dihydrotestosterone
HTS	hypothermosol

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Author contributions
ELB: conceptualization, idea, study design, funding acquisition, methodology, formal analysis, project administration, research, supervision, resources, review and editing. LPP: formal analysis methodology, writing—original draft, project administration, data curation, project administration, investigation, resources and visualization. PTE: methodology, project administration, investigation, resources, writing—review and editing. MLGM: histological analysis.

Availability of data and materials
Not applicable.

Declarations

Ethics approval and consent to participate
The project "Terapia celular en el tratamiento de la alopecia androgenética inducida en ratón mediante el uso de células madre mesenquimales expandidas derivadas de tejido adipose ES280790000088" was approved by the Experimental Animal Ethical Committee of the Hospital Clínico San Carlos of Madrid (Jul 16, 2020) and the General Administration of Agriculture and Livestock of the Community of Madrid (Nov 18, 2021).

Consent for publication
Not applicable.

Competing interests
LPP, PTE and MLGM state no conflict of interest. ELB is an adviser for MMC INTERNATIONAL HEALTH HOLDING.

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