The Journal of Maternal-Fetal & Neonatal Medicine

ISSN: (Print) (Online) Journal homepage: www.tandfonline.com/journals/ijmf20

Mesenchymal stem cell secretome ameliorates over-expression of soluble fms-like tyrosine kinase-1 (sFlt-1) and fetal growth restriction (FGR) in animal SLE model

Muhammad Adrianes Bachnas, Gustaaf Albert Dekker, Ambar Mudigdo, Bambang Purwanto, Sri Sulistyowati, Erry Gumilar Dachlan, Muhammad Ilham Aldika Akbar, Angliana Chouw, Cynthia Retna Sartika & Widjiati Widjiati

To cite this article: Muhammad Adrianes Bachnas, Gustaaf Albert Dekker, Ambar Mudigdo, Bambang Purwanto, Sri Sulistyowati, Erry Gumilar Dachlan, Muhammad Ilham Aldika Akbar, Angliana Chouw, Cynthia Retna Sartika & Widjiati Widjiati (2023) Mesenchymal stem cell secretome ameliorates over-expression of soluble fms-like tyrosine kinase-1 (sFlt-1) and fetal growth restriction (FGR) in animal SLE model, The Journal of Maternal-Fetal & Neonatal Medicine, 36:2, 2279931, DOI: 10.1080/14767058.2023.2279931

To link to this article: https://doi.org/10.1080/14767058.2023.2279931

THE JOURNAL OF MATERNAL-FETAL & NEONATAL MEDICINE
2023, VOL. 36, NO. 2, 2279931
https://doi.org/10.1080/14767058.2023.2279931

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

OPEN ACCESS Check for updates

Mesenchymal stem cell secretome ameliorates over-expression of soluble fms-like tyrosine kinase-1 (sFlt-1) and fetal growth restriction (FGR) in animal SLE model

Muhammad Adrianes Bachnas[a], Gustaaf Albert Dekker[b], Ambar Mudigdo[c], Bambang Purwanto[d], Sri Sulistyowati[a], Erry Gumilar Dachlan[e], Muhammad Ilham Aldika Akbar[f], Angliana Chouw[g], Cynthia Retna Sartika[g] and Widjiati Widjiati[h]

[a]Department of Obstetrics and Gynecology, Division of Maternal-Fetal Medicine, Faculty of Medicine, Universitas Sebelas Maret/Dr. Moewardi Hospital, Solo, Indonesia; [b]Obstetrics and Gynaecology Department, Lyell-McEwin Hospital, The University of Adelaide, Adelaide, Australia; [c]Department of Pathology Anatomy, Faculty of Medicine, Universitas Sebelas Maret/Dr. Moewardi Hospital, Solo, Indonesia; [d]Department of Internal Medicine, Faculty of Medicine, Universitas Sebelas Maret/Dr. Moewardi Hospital, Solo, Indonesia; [e]Department of Obstetrics and Gynecology, Division of Maternal-Fetal Medicine, Faculty of Medicine, Universitas Airlangga/Dr. Soetomo Hospital, Surabaya, Indonesia; [f]Department of Obstetrics and Gynecology, Division of Maternal-Fetal Medicine, Faculty of Medicine, Universitas Airlangga/Airlangga University Hospital, Surabaya, Indonesia; [g]ProSTEM, Prodia StemCell Indonesia Laboratory, Jakarta, Indonesia; [h]Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia

ABSTRACT

Introduction: In the near future, stem cell research may lead to several major therapeutic innovations in medical practice. Secretome, a "by-product" of stem cell line cultures, has many advantages. Its easiness of storage, usage, and fast direct effect are some of those to consider. Fetal growth restriction (FGR) remains one of the significant challenges in maternal-fetal and neonatal medicine. Placentation failure is one of the most profound causal and is often related to increasing sFlt-1 in early pregnancy. This study aimed to investigate hUC-MSC secretome in ameliorating sFlt-1 and how to improve outcomes in preventing FGR in an animal model.

Materials and methods: Pristane-induced systemic lupus erythematosus (SLE) in a mouse model was used to represent placentation failure and its consequences. Twenty-one mice were randomized into three groups: (I) normal pregnancy, (II) SLE, and (III) SLE with secretome treatment. Pristane was administered in all Groups four weeks prior mating period. Secretome was derived from human umbilical cord mesenchymal stem cells (hUC-MSC) conditioned medium on the 3rd and 4th passage, around day-21 until day-28 from the start of culturing process. Mesenchymal stem cell was characterized using flow cytometry for CD105+, CD90+, and CD73+ surface antigen markers. Immunohistochemistry anlysis by using Remmele's Immunoreactive Score (IRS) was used to quantify the placental sFlt-1 expression in each group. Birth weight and length were analyzed as the secondary outcome. The number of fetuses obtained was also calculated for pregnancy loss comparison between Groups.

Results: The administration of secretome of hUC-MSC was found to lower the expression of the placental sFlt-1 significantly in the pristane SLE animal model (10.30 ± 1.40 vs. 4.98 ± 2.57; $p < 0.001$) to a level seen in normal mouse pregnancies in Group I (3.88 ± 0.49; $p = 0.159$). Secretome also had a significant effect on preventing fetal growth restriction in the pristane SLE mouse model (birth weight: 354.29 ± 80.76 mg vs. 550 ± 64.03 mg; $p < 0.001$ and birth length: 14.43 ± 1.27 mm vs. 19.00 ± 1.41 mm), comparable to the birth weight and length of the normal pregnancy in Group I (540.29 ± 75.47 mg and 18.14 ± 1.34 mm, $p = 0.808$ and $= 0.719$). Secretome administration also showed a potential action to prevent high number of pregnancy loss as the number of fetuses obtained could be similar to those of mice in the normal pregnant Group (7.71 ± 1.11 vs. 7.86 ± 1.06; $p = 0.794$).

Conclusions: Administration of secretome lowers sFlt-1 expression in placenta, improves fetal growth, and prevents pregnancy loss in a mouse SLE model.

ARTICLE HISTORY

Received 3 January 2023
Revised 26 October 2023
Accepted 31 October 2023

KEYWORDS

Human umbilical cord; mesenchymal stem cell; secretome; sFlt-1; placenta; fetal growth restriction (FGR)

CONTACT Muhammad Adrianes Bachnas adrianesbachnas@staff.uns.ac.id Department of Obstetrics and Gynecology, Division of Maternal-Fetal Medicine, Faculty of Medicine, Dr. Moewardi Hospital, Sebelas Maret University, Solo, Indonesia. Address: Jalan Kol. Sutarto No.132, Jebres, Surakarta, Jawa Tengah, 57126, Indonesia

Supplemental data for this article can be accessed online at https://doi.org/10.1080/14767058.2023.2279931.

1. Introduction

Auto-immune disorders, in particular systemic lupus erythematosus (SLE), are associated with a markedly increased rate of fetal growth restriction (FGR), pregnancy loss and preeclampsia [1]. The adverse pregnancy outcome in SLE is related to placental damage as demonstrated by the histopathological findings [2,3]. Other pathophysiologic mechanisms contributing to the adverse pregnancy outcome include the systemic inflammatory state, endothelial vasculitis, and thrombosis [3]. All of these could potentially cause preeclampsia, fetal growth restriction, and fetal loss [3].

The other manifestation of placental damage in SLE is the increasing production of soluble Fms-like tyrosine kinase type 1 (sFlt-1), an anti-angiogenic factor [4]. High expression of sFlt-1 in the placenta is a strong predictor for adverse pregnancy outcomes, including FGR and preeclampsia with primary antiphospholipid syndrome [5]. The overexpression of sFlt-1 in the placenta is believed as the consequence of the increased synthesis of type I interferons that turn to suppress the production of angiogenic factors (particularly fibroblast growth factor, interleukin-8 and VEGF) and trigger anti-angiogenic gene expression, lessening endothelial cell migration and suppressing endothelial progenitor cell proliferation [6]. This mechanism could explain why SLE, mainly with primary antiphospholipid syndrome, becomes a decisive risk factor for a pregnancy developing preeclampsia, especially early onset of preeclampsia in conjunction with FGR, in which placentation failure is the main culprit [7].

We chose the SLE mouse model as the linkage representation of SLE with placental insufficiency that leads to FGR [8]. Various technic has been established to represent SLE in mice model, one of which is pristane-induced lupus (PIL) [9–11]. The PIL model, in particular, has proven to develop SLE with its complex immune dysfunctions and full-blown clinical and laboratory representations, which many have not [12,13]. In this animal model, sFlt-1 is increasing in both placenta and blood as one of the substantial component of preeclampsia with placentation failure and also related with FGR [8,14,15]. In line with this, a previous study has shown us that human sFLt-1 exposure to a mouse model could lead to severe changes in placental differentiation and vascularization that induced preeclampsia with fetal growth restriction started from vascular remodeling failure of main stem villous vessel of trophoblast and spiral decidual artery [8,14]. Another study resulted in a similar finding, sFlt-1 over-expression in mouse model consistently related to the development of preeclampsia accompanied by FGR and even late-onset preeclampsia [16]. These results provide data to verify the *in vivo* pathologic effects of sFlt in primate-specific, predominant placental sFlt-1 isoform [16]. They can induce the full spectrum of symptoms in this obstetrical syndrome [16]. The increasing overexpression of sFlt-1 also attracts complement production and attachment to the maternal-fetal interface and causes damage to its junctions, resulting in placental ischemia and remodeling failure [17]. Some of the complements that previous studies have highlighted are C3b, c4d c5b-9, and c1q [17]. Those could complement the dots between SLE, Preeclampsia, FGR, and sFlt-1. Overall, High expression sFlt-1 is among the most influential players for these conditions, so it is one of the best markers in study to predict adverse outcomes, picture the severity of placental damage, and evaluate the effectiveness of therapy or management [18,19].

To overcome these problem, we see the opportunity for using a new treatment option in medical fields instead of using drugs, which is stem cell [20,21]. Stem cell, particularly mesenchymal stem cell (MSC) including its metabolites, has been widely and extensively studied in many fields of medical science and clinical settings [22]. Its capability is shown by prior studies of promoting angiogenesis by triggering the production and activation of angiogenic factors simultaneously with suppressing anti-angiogenic factors, reducing inflammation by its immunomodulatory attitude, and supporting cells proliferation and inhibiting cell proliferation senescence and cell death from aging and apoptotic process [21,23]. It can potentially rejuvenate an already injured tissue and has been studied on heart ischemia [23,24]. We want to see this when we apply it to the broken placenta. However, as valuable consideration is warranted, the worries of cell interference on cell differentiation of organs in fetal development are very concerning. Some prior studies have shown some perturbed data that possible uncontrolled proliferation could happen, for instance, triggering the cancer process [25]. It is very challenging to get the benefit from stem cells, but it could hinder its side effect, mainly in its administration during pregnancy. An inspiring idea has come recently the thought of cell-free stem cell therapy by using only its metabolite [26–28]. The full spectrum of stem cell metabolites is known as secretome, which contains wide varieties of growth hormones either as soluble proteins or enclosed in extracellular vesicles (EVs) and

also exosomes, microvesicles, and immunomodulatory molecules [27,28].

Research of MSC-derived secretomes has drawn much attention due to their ability to mimic all the therapeutic effects produced by the MSCs (i.e. endogenous tissue repair and regulation of the immune system) [26]. The secretome of mesenchymal stem cells could be more beneficial than the parental cells because their specific cargo contains mRNAs, miRNAs, and proteins that can be biologically transferred to recipient cells without worrying about uncontrolled negative cell attitude after cell chimerism with the host [29]. Secretome also has other advantages in storage, transfer, and production easiness, and their administration is also less worrisome than MSC injection [20,22,30]. In particular, the secretome of MSC represents promising candidates for cell-free-based preventing placental injury in the SLE process by facilitating anti-angiogenic suppression and promoting good fetal growth as the endpoint.

2. Materials and methods

Twenty-one BALB/c mice were enrolled in the study and divided randomly into normal pregnant mouse Group (I), SLE pregnant mouse Group (II), and SLE pregnant mouse with secretome treatment Group (III). We used Federer's formula to calculate proper sample size [31,32]. Pristane (2,6,10,14-tetramethylpentadecane) was used to trigger SLE in mice. This typical mouse model has been widely used and is known to be associated with poor placentation and maternal-fetal outcomes [13]. Pristane injections were administered 0.5 ml intraperitoneal to group II and III, four weeks before mating. Group I was a normal pregnancy group, which did not receive any special treatment. All mice in three groups were mated and soon as pregnancy was confirmed by seeing copulatory plug, only Group III received intraperitoneal injection of secretome 0.5 ml (day-1 of pregnancy). Secretome was taken from conditioned media used in the culture process of human umbilical cord stem cells (hUC-MSC). The hUC-MSC was processed using an explant method under normoxic conditions using conditioned media of Dulbecco's modified eagle medium high glucose supplemented with 5% human platelet lysate and L-glutamine. The hUC-MSCs were confluent at the 3rd and 4th passage and demonstrated character of Mesenchymal Stem Cell by positivity on surface antigen markers which are CD90+, CD105+, and CD73+ using flow-cytometry as can be seen in Figure 1. Conditioned media then was taken and used as the secretome material, which was on day-21 until day-28 from the start of the culture process. On the day-16 of the pregnancy, mice were terminated, placenta and babies were collected and analyzed. The study was approved by the ethical committee of Airlangga University with registry number: 3.KE.167.10.2018 and Sebelas Maret University with

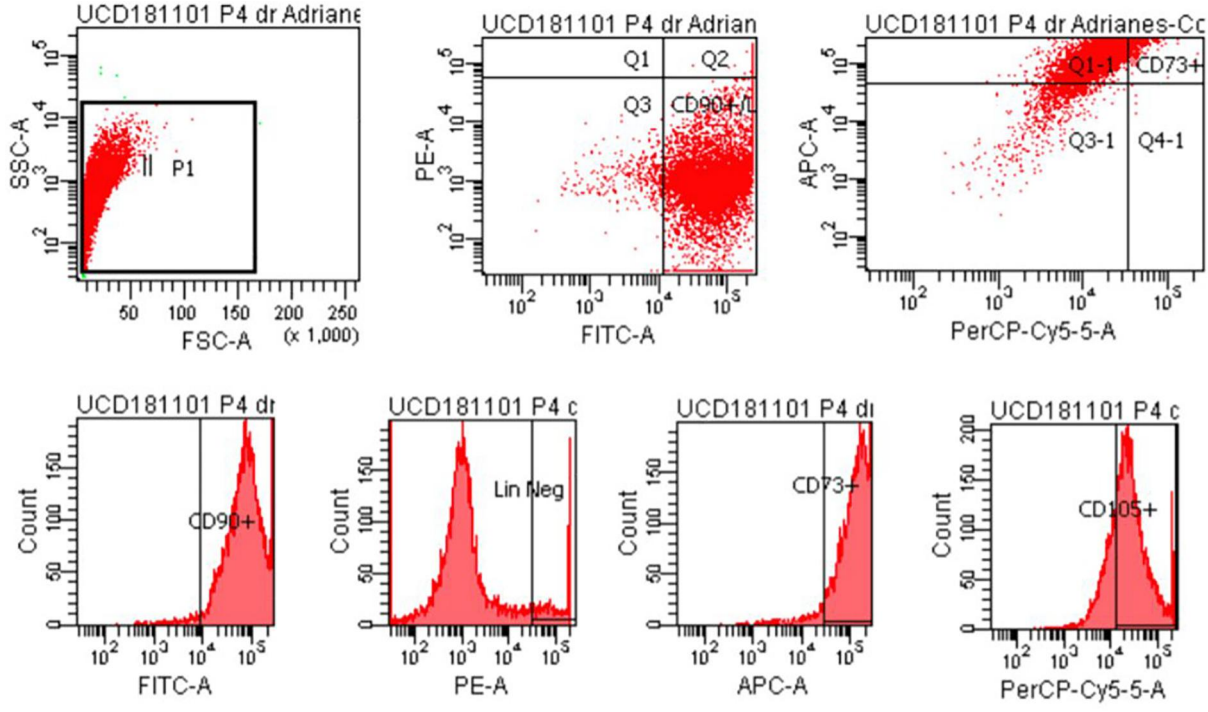

Figure 1. The characteristic profile of Mesenchymal stem cell using flow cytometry.

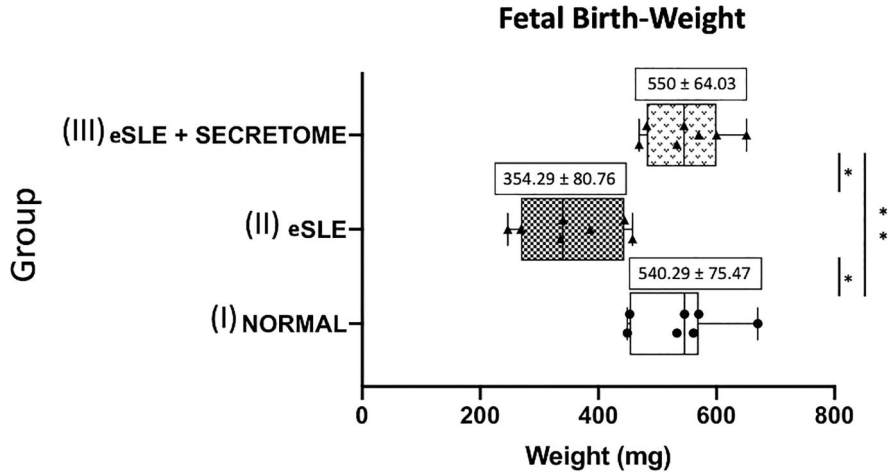

Figure 2. Effect of hUC-MSC secretome normalized sFlt-1 expression in placenta of SLE pregnant mice. *Significant; **not significant.

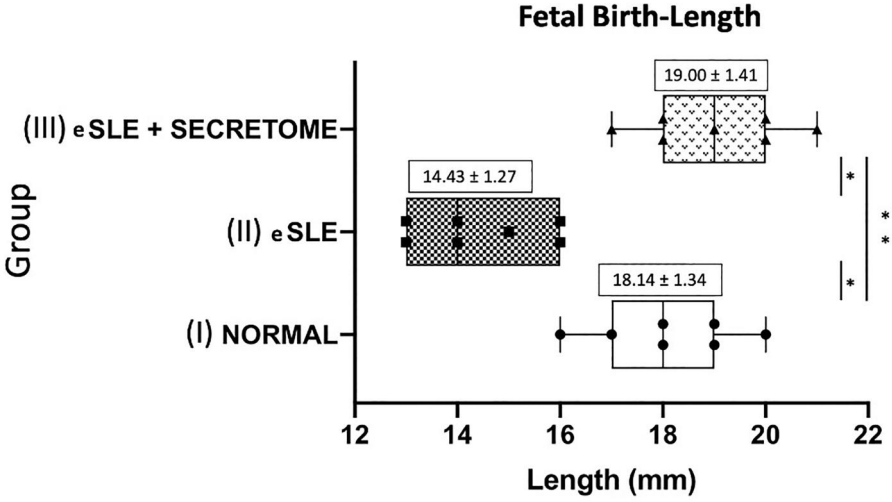

Figure 3. Effect of hUC-MSC secretome prevents fetal growth restriction as seen on birth weight. *Significant; **not significant.

registry number: 7/2/X/HREC/2018 and fulfilled ARRIVE and PREPARE criteria [33–35].

3. Results

In this study, pristane administration caused poor placentation markedly with significant over-expression of sFlt-1 and fetal growth restriction (SLE group). The type of fetal growth restriction was symmetrical, reflecting poor placentation since the early pregnancy. Birth weight was 75% lower and birth length was 30% shorter in SLE group compared to the normal pregnancy (354.29 ± 80.76 mg and 14.43 ± 1.27 mm vs. 540.29 ± 75.47 mg and 18.14 ± 1.34 mm; $p < 0.001$). These data can be seen in Figures 2 and 3. An additional interesting data was the pregnancy loss reduction, which can be seen on Figure 4. SLE condition has significantly causing higher rate of pregnancy loss as has been shown by significantly lower number of fetuses obtained in the day of termination, compared to those in normal pregnant group (4.00 ± 0.81 vs. 7.86 ± 1.06; $p < 0.001$). The data also showed that by giving secretome the number of fetuses obtained could be similar to those of normal pregnant group (7.71 ± 1.11 vs. 7.86 ± 1.06; $p = 0.794$). Soluble Flt-1 expression found on cytotrophoblast layer and perivascular area increased significantly to levels about 2.6 times higher in the SLE group compared to normal pregnancy (10.30 ± 1.40 vs. 3.88 ± 0.49; $p < 0.001$) as seen on Figure 5. The quantification was done by using immunoreactive score (IRS) scoring system as seen in Table 1 and the microscope reading could be seen in Figure 6 [37]. Secretome injection resulted in significant amelioration of the placental sFlt-1 overexpression (4.98 ± 2.57; $p < 0.001$) and becoming equal to the normal pregnancy ($p = 0.159$). It has also

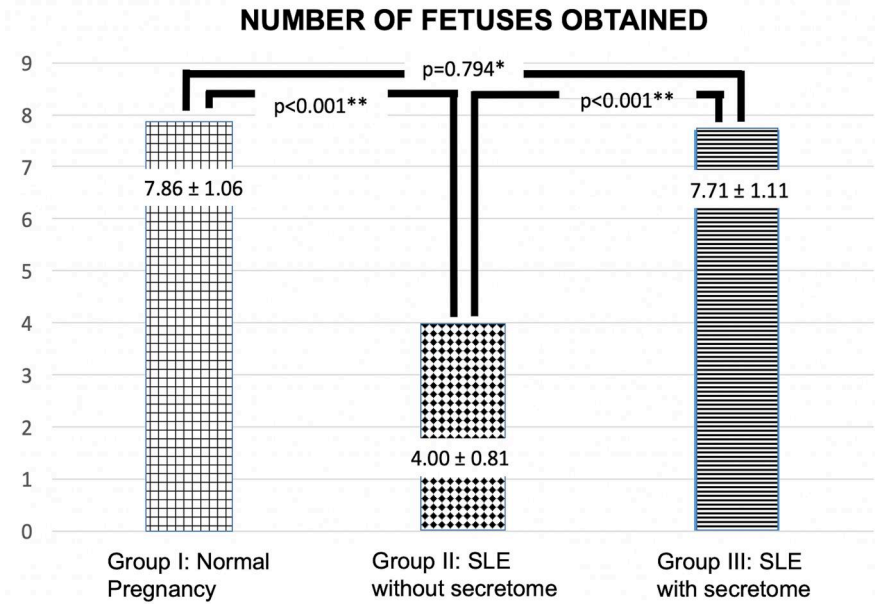

Figure 4. Effect of hUC-MSC secretome prevented pregnancy loss as seen on number of fetuses obtained. *Significant; **not significant.

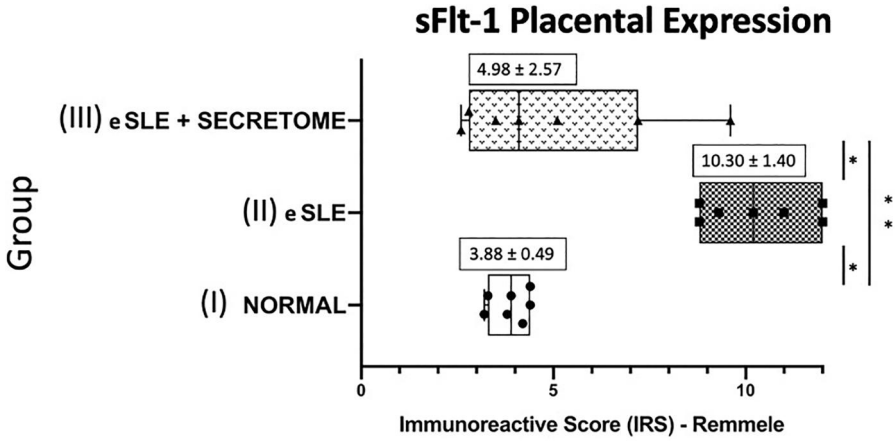

Figure 5. Effect of hUC-MSC secretome prevents fetal growth restriction as seen on birth length. *Significant; **not significant. The image was obtained using a digital light microscope: Nikon Eclipse Ci with Nikon DSR21 16 Megapixels in 800× magnification. Positive staining of immunohistochemistry reaction on the sFlt-1 expression of trophoblasts cell can be seen as a brown-gold color indicated with orange arrows. Higher expression of sFlt-1 resulted in more intense coloration.

showed major significant effect on providing optimal fetal growth even after placenta was damaged by using pristane (550 ± 64.03 mg and 19.00 ± 1.41 mm; $p < 0.001$) and made it comparable to the normal pregnant group on birth weight ($p = 0.808$) and birth length ($p = 0.719$) this also can be seen on Figure 5.

4. Discussion

Secretome of hUC-MSCs in this study has showed a positive effect on normalizing sFlt-1 and preventing fetal growth restriction as seen in the results. Its easiness to get and to administer, and the fact that it was previously under-valued as bio-waste of hUC-MSCs culture process make it more attractive as a potential near future novel therapy modality [38]. We used a model of damaged placenta by using pristane to trigger an immunological process, closely mimicking the natural pathological process of a placentation failure [12]. Pristane is a chemical substance that contains high levels of isoprenoid alkane causing intraperitoneal flood of monoclonal antibodies such as antinuclear antibody (ANA) and anti-double-stranded DNA (anti-dsDNA) [39,40]. Those biomarkers are specific for SLE, and related to over-production of anti-angiogenic factors [39]. Many studies have indicated

Table 1. Immunoreactive score of remmele and stegner (IRS) [36].

Percentage of positive cells (P)	Intensity of staining (I)	IRS = P × I (0–12)
0 = No positive cells	0 = No color reaction	0 – 1 = negative
1 = <10% Of positive cells	1 = Mild reaction	2 – 3 = mild
2 = 10–50% Of positive cells	2 = Moderate reaction	4 – 8 = moderate
3 = 50–80% Of positive cells	3 = Intense reaction	9 – 12 = strongly positive
4 = >80% Of positive cells		

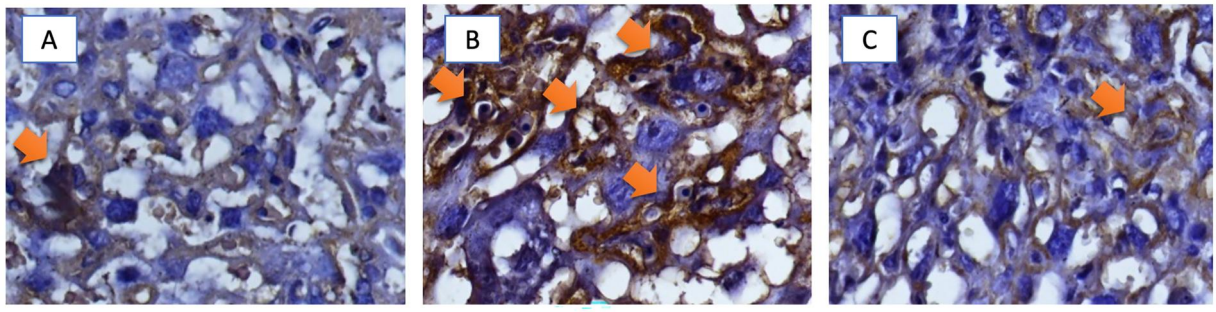

Figure 6. Microscope reading on immunohistochemistry staining of sFlt-1 expression in placenta of the three Groups. (A) Normal pregnancy, (B) SLE without secretome treatment, (C) SLE with secretome treatment. Group B shows significant IHC coloring, representing high expression of sFlt-1. IHC coloring between group A and C shows comparable strength of sFlt-1 expression.

that the overproduction of anti-angiogenic factors in animal model or even human, can cause both preeclampsia and fetal growth restriction, mainly for the early onset [4]. This has been also demonstrated in the current study.

Vascular endothelial growth factor (VEGF) and Placental Growth Factor (PLGF) is diminished in the pregnancy with SLE. This decrease in VEGF and PLGF from early pregnancy could adversely affect the vascular remodeling, with spiral arteries retaining their small diameter and thick vessel wall [41,42]. Meanwhile, the chronic inflammatory state produced in SLE, exacerbates the effects of the hypoxemia affecting the trophoblast leading to oxidative-stress and as such overproduction of sFlt-1 [43,44]. Soluble Flt-1 will adhere to the receptor binding domain of placental growth factor (PLGF) and also vascular endothelial growth factor (VEGF) receptors [45]. This blockade would antagonize the angiogenesis signaling pathway, and leads to endothelial dysfunction, hypertension, and proteinuria, characteristic of early onset preeclampsia; which will simultaneously associated with the development of fetal growth restriction [46–48]. The vascular endothelial growth factor is responsible for trophoblast invasion to the uterine vessels and transforms spiral artery into wide flaccid channel to promote low resistance blood flow to the placenta and provides adequate perfusion [43,44]. Insult to this process, will cause low to moderate chronic hypoxic state and result in later trophoblast stress, poor placentation, and fetal growth restriction. Meanwhile, in cases with severe response will cause abortion or pregnancy loss [44,48].

Secretome originates from conditioned media contains a high level of VEGF and other important factors supporting angiogenesis [49]. Angiogenesis plays a crucial part in placental development [43,44]. Any insults causing disturbance of the placentation process may need therapy that enhances the angiogenesis process and for that reason hUC-MSC secretome is attractively suitable for the therapy [50]. Secretome also has other positive capabilities, such as immunomodulation, anti-inflammation, and also has a clear anti-apoptotic effect [49,51]. Our data clearly demonstrate that administration of secretome basically normalizes the sFlt-1 expression in placenta. On the other hand, there are some limitations in this study in which proteomics markers related to de clinical development of the disease, such as interleukins and complements, were not examined. We also did not measure the mice blood pressure, so the development of preeclampsia was not noticeable. This should be covered in further research.

Secretome may also directly "rescue" the placentation process and maternal endothelium by its rich amount of other growth factors, for instance hepatocyte growth factor [43]. Secretome may not only reduce trophoblast stress (and as such reduce sFlt-1, inflammatory cytokines, etc.) but may also result in improved vascular remodeling. A normal placentation process would guarantee good oxygenation and good nutrition supply to the fetus [41,43–45]. This mechanism may

explain the beneficial effects on fetal growth observed after secretome administration. Unfortunately, our animal model was not fully representing the failure of the vascular remodeling process. It needs further research with another animal model that directly suits the vascular remodeling failure with more sample size.

The exciting finding of significant pregnancy loss in the SLE mouse group could explain that, somehow, the pristane-induced mouse model also developed antiphospholipid syndrome. This should be investigated further in the following research. However, at least we can see that secretome has also given a preventive action on reducing pregnancy loss by giving a similar number of fetuses obtained in the SLE mouse group with secretome provision. This could be comparable to what we see in LMWH and LDA treatment for preventing pregnancy loss in SLE with antiphospholipid syndrome [52].

Compared to stem cells, secretome has many advantages [49,53,54]. It is far cheaper, more stable, and easier to handle and store [38,55]. These advantages make hUC-MSC secretome able to be developed in mass production with easiness in distribution chain and with a price reasonably reachable by most patients. Unlike stem cells, secretome is cell-free, make it considerably safer, with fewer concerns about tumorigenicity, carcinogenesis, possible effects on organogenesis [55,56].

5. Conclusions

Administration of secretome lowers sFlt-1 expression in the placenta, improves fetal growth, and prevents pregnancy loss in a mouse SLE model. These promising findings represent the potential advantage of using secretome for preventing placental damage mainly due to SLE pathology and even more profound with antiphospholipid syndrome. Extensive studies are needed before this novel future management for FGR-related placental damage can be used in clinics.

6. Limitations

This study, unfortunately, did not measure quantitatively serum levels of sFlt-1 and PlGF, as we know from the recent studies that show the importance of the sFlt-1/PlGF ratio of maternal serum levels to predict and manage FGR [57]. Low PlGF in maternal serum level has also been found to correlate more with FGR rather than sFlt-1, and sFlt-1 is a better predictor for preeclampsia than PlGF [58]. This is a very substantial finding for the future direction of our following studies. Addressing the potential harmful interference of secretome during embryo and fetal development is another critical point that should be elaborated in the upcoming study, even though theoretically, secretome is cell-free and thoughtfully is better in safety concerns than administering cells in such stem cell therapy [59]. Clinical implication for the future therapy of FGR with secretome is emerging since many new treatment modalities have been extensively studied [60]. However, no firm, robust evidence has been discovered until now. Only a combination of LMWH and LDA for pregnancy complicated with SLE and antiphospholipid syndrome or thrombophilia and LDA for high-risk preeclampsia can prevent FGR well [52]. Timely delivery remains the only best clinical option for FGR in general [36]. FGR in the SLE mouse model is not entirely the same condition as in human FGR without SLE. The results from this study could only be thoughtful new evidence for FGR-related placental infarct due to SLE.

Acknowledgements

The author would like to acknowledge "Prodia ProSTEM" that has provided laboratory space, equipment, and materials for producing hUC-MSC secretome. The author would also like to acknowledge Postgraduate Program of Medical Science of Sebelas Maret University for its contribution on language processing and statistical analysis and The Faculty of Veterinary Medicine of Airlangga University for its contribution on providing working places and materials for the animal study. RSUD Dr. Moewardi General Hospital for providing support on ethical issues and human resources for developing the research. The authors report no funding source to be declared.

Author contributions

Author schematized the concept of the study (hypothesis, methodology, data collection and analysis). All the coauthors shared the same involvement in evaluating the study process, providing the relevant literatures, and polished the writing.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

The author(s) reported there is no funding associated with the work featured in this article.

ORCID

Muhammad Adrianes Bachnas http://orcid.org/0000-0002-1710-3909

Data availability statement

The authors confirm that the data supporting the findings of this study are available within the article or its supplementary materials.

References

[1] Carp HJA, Selmi C, Shoenfeld Y. The autoimmune bases of infertility and pregnancy loss. J Autoimmun. 2012;38(2–3):J266–274. doi: 10.1016/j.jaut.2011.11.016.

[2] Ogishima D, Matsumoto T, Nakamura Y, et al. Placental pathology in systemic lupus erythematosus with antiphospholipid antibodies. Pathol Int. 2000; 50(3):224–229. doi: 10.1046/j.1440-1827.2000.01026.x.

[3] Ostensen M, Clowse M. Pathogenesis of pregnancy complications in systemic lupus erythematosus. Curr Opin Rheumatol. 2013;25(5):591–596. doi: 10.1097/BOR.0b013e328363ebf7.

[4] Qazi U, Lam C, Karumanchi A, et al. Soluble fms-like tyrosine kinase associated with preeclampsia in pregnancy in systemic lupus erythematosus. J Rheumatol J Rheumatol Sept. 2008;35:631–634.

[5] Mayer-Pickel K, Nanda M, Gajic M, et al. Preeclampsia and the antiphospholipid syndrome. Biomedicines. 2023;11(8):11. doi: 10.3390/biomedicines11082298.

[6] Ugolini-Lopes MR, Torrezan GT, Gândara APR, et al. Enhanced type I interferon gene signature in primary antiphospholipid syndrome: association with earlier disease onset and preeclampsia. Autoimmun Rev. 2019;18(4):393–398. doi: 10.1016/j.autrev.2018.11.004.

[7] Castellanos Gutierrez AS, Figueras F, Morales-Prieto DM, et al. Placental damage in pregnancies with systemic lupus erythematosus: a narrative review. Front Immunol. 2022;13:941586. doi: 10.3389/fimmu.2022.941586.

[8] Li W, Titov AA, Morel L. An update on lupus animal models. Curr Opin Rheumatol. 2017;29(5):434–441. doi: 10.1097/BOR.0000000000000412.

[9] Perry D, Sang A, Yin Y, et al. Murine models of systemic lupus erythematosus. J Biomed Biotechnol. 2011;2011:271694. doi: 10.1155/2011/271694.

[10] Moore E, Reynolds JA, Davidson A, et al. Promise and complexity of lupus mouse models. Nat Immunol. 2021;22(6):683–686. doi: 10.1038/s41590-021-00914-4.

[11] Celhar T, Fairhurst A-M. Modelling clinical systemic lupus erythematosus: similarities, differences and success stories. Rheumatology. 2017;56(suppl_1):i88–i99. doi: 10.1093/rheumatology/kew400.

[12] Reeves WH, Lee PY, Weinstein JS, et al. Induction of autoimmunity by pristane and other naturally occurring hydrocarbons. Trends Immunol. 2009;30(9):455–464. doi: 10.1016/j.it.2009.06.003.

[13] Freitas EC, de Oliveira MS, Monticielo OA. Pristane-induced lupus: considerations on this experimental model. Clin Rheumatol. 2017;36(11):2403–2414. doi: 10.1007/s10067-017-3811-6.

[14] Gatford KL, Andraweera PH, Roberts CT, et al. Animal models of preeclampsia: causes, consequences, and interventions. Hypertension. 2020;75(6):1363–1381. doi: 10.1161/HYPERTENSIONAHA.119.14598.

[15] Ahmed A, Singh J, Khan Y, et al. A new mouse model to explore therapies for preeclampsia. PLOS One. 2010;5(10):e13663. doi: 10.1371/journal.pone.0013663.

[16] Szalai G, Romero R, Chaiworapongsa T, et al. Full-Length human placental sFlt-1-e15a isoform induces distinct maternal phenotypes of preeclampsia in mice. PLOS One. 2015;10(4):e0119547. doi: 10.1371/journal.pone.0119547.

[17] Kim MY, Guerra MM, Kaplowitz E, et al. Complement activation predicts adverse pregnancy outcome in patients with systemic lupus erythematosus and/or antiphospholipid antibodies. Ann Rheum Dis. 2018; 77(4):549–555. doi: 10.1136/annrheumdis-2017-212224.

[18] Ramma W, Ahmed A. Therapeutic potential of statins and the induction of heme oxygenase-1 in preeclampsia. J Reprod Immunol. 2014;101–102(100):153–160. doi: 10.1016/j.jri.2013.12.120.

[19] Hendrix M, Bons J, van Haren A, et al. Role of sFlt-1 and PlGF in the screening of small-for-gestational age neonates during pregnancy: a systematic review. Ann Clin Biochem. 2020;57(1):44–58. doi: 10.1177/0004563219882042.

[20] Ding D-C, Chang Y-H, Shyu W-C, et al. Human umbilical cord mesenchymal stem cells: a new era for stem cell therapy. Cell Transplant. 2015;24(3):339–347. doi: 10.3727/096368915X686841.

[21] Kim N, Cho S-G. Clinical applications of mesenchymal stem cells. Korean J Intern Med. 2013;28(4):387–402. doi: 10.3904/kjim.2013.28.4.387.

[22] Eleuteri S, Fierabracci A. Insights into the secretome of mesenchymal stem cells and its potential applications. Int J Mol Sci. 2019;20(18):4597. doi: 10.3390/ijms20184597.

[23] Hou L, Kim JJ, Woo YJ, et al. Stem cell-based therapies to promote angiogenesis in ischemic cardiovascular disease. Am J Physiol Heart Circ Physiol. 2016; 310(4):H455–H465. doi: 10.1152/ajpheart.00726.2015.

[24] Bian X, Ma K, Zhang C, et al. Therapeutic angiogenesis using stem cell-derived extracellular vesicles: an emerging approach for treatment of ischemic diseases. Stem Cell Res Ther. 2019;10(1):158. doi: 10.1186/s13287-019-1276-z.

[25] Dittmar T, Nagler C, Niggemann B, et al. The dark side of stem cells: triggering cancer progression by cell fusion. Curr Mol Med. 2013;13(5):735–750. doi: 10.2174/1566524011313050005.

[26] Xia J, Minamino S, Kuwabara K, et al. Stem cell secretome as a new booster for regenerative medicine. Biosci Trends. 2019;13(4):299–307. doi: 10.5582/bst.2019.01226.

[27] Ahangar P, Mills SJ, Cowin AJ. Mesenchymal stem cell secretome as an emerging Cell-Free alternative for improving wound repair. Int J Mol Sci. 2020;21(19): 7038. doi: 10.3390/ijms21197038.

[28] Sandonà M, Di Pietro L, Esposito F, et al. Mesenchymal stromal cells and their secretome: new therapeutic perspectives for skeletal muscle regeneration. Front Bioeng Biotechnol. 2021;9:652970. doi: 10.3389/fbioe.2021.652970.

[29] Gwam C, Mohammed N, Ma X. Stem cell secretome, regeneration, and clinical translation: a narrative

review. Ann Transl Med. 2021;9(1):70–70. doi: 10.21037/atm-20-5030.
[30] Li F, Zhang J, Yi K, et al. Delivery of stem cell secretome for therapeutic applications. ACS Appl Bio Mater. 2022;5(5):2009–2030. doi: 10.1021/acsabm.1c01312.
[31] Ko MJ, Lim C-Y. General considerations for sample size estimation in animal study. Korean J Anesthesiol. 2021;74(1):23–29. doi: 10.4097/kja.20662.
[32] Charan J, Kantharia ND. How to calculate sample size in animal studies? J Pharmacol Pharmacother. 2013;4(4):303–306. doi: 10.4103/0976-500X.119726.
[33] Kilkenny C, Browne W, Cuthill IC, et al. Editorial: animal research: reporting *in vivo* experiments-The ARRIVE guidelines. J Cereb Blood Flow Metab. 2011;31(4):991–993. doi: 10.1038/jcbfm.2010.220.
[34] Kilkenny C, Browne WJ, Cuthill IC, et al. Improving bioscience research reporting: the arrive guidelines for reporting animal research. Animals. 2014;4(1):35–44. doi: 10.3390/ani4010035.
[35] Smith AJ, Clutton RE, Lilley E, et al. PREPARE: guidelines for planning animal research and testing. Lab Anim. 2018;52(2):135–141. doi: 10.1177/0023677217724823.
[36] McCowan LM, Figueras F, Anderson NH. Evidence-based national guidelines for the management of suspected fetal growth restriction: comparison, consensus, and controversy. Am J Obstet Gynecol. 2018;218(2S):S855–S868. doi: 10.1016/j.ajog.2017.12.004.
[37] Kaemmerer D, Peter L, Lupp A, et al. Comparing of IRS and Her2 as immunohistochemical scoring schemes in gastroenteropancreatic neuroendocrine tumors. Int J Clin Exp Pathol. 2012;5:187–194.
[38] Konala VBR, Mamidi MK, Bhonde R, et al. The current landscape of the mesenchymal stromal cell secretome: a new paradigm for cell-free regeneration. Cytotherapy. 2016;18(1):13–24. doi: 10.1016/j.jcyt.2015.10.008.
[39] Satoh M, Reeves WH. Induction of lupus-associated autoantibodies in BALB/c mice by intraperitoneal injection of pristane. J Exp Med. 1994;180(6):2341–2346. doi: 10.1084/jem.180.6.2341.
[40] Kalim H. Low birth weight and maternal and neonatal deaths are complications of systemic lupus erythematosus in pregnant pristane induced lupus mice. Arch Rheumatol. 2015;30(4):285–291. doi: 10.5606/ArchRheumatol.2015.5505.
[41] Polizzi S, Mahajan VB. Intravitreal anti-VEGF injections in pregnancy: case series and review of literature. J Ocul Pharmacol Ther. 2015;31(10):605–610. doi: 10.1089/jop.2015.0056.
[42] Bujold E, Romero R, Chaiworapongsa T, et al. Evidence supporting that the excess of the sVEGFR-1 concentration in maternal plasma in preeclampsia has a uterine origin. J Matern Neonatal Med. 2005;18(1):9–16. doi: 10.1080/14767050500202493.
[43] Eddy AC, Bidwell GL, George EM. Pro-angiogenic therapeutics for preeclampsia. Biol Sex Differ. 2018;9(1):36. doi: 10.1186/s13293-018-0195-5.
[44] Sulistyowati S, Bachnas MA, Anggraini N, et al. Recombinant vascular endothelial growth factor 121 injection for the prevention of fetal growth restriction in a preeclampsia mouse model. J Perinat Med. 2017;45:245–251.
[45] Burton GJ, Jauniaux E. Pathophysiology of placental-derived fetal growth restriction. Am J Obstet Gynecol. 2018;218(2S):S745–S761. doi: 10.1016/j.ajog.2017.11.577.
[46] Dekker GA, Sibai BM. Etiology and pathogenesis of preeclampsia: current concepts. Am J Obstet Gynecol. 1998;179(5):1359–1375. doi: 10.1016/s0002-9378(98)70160-7.
[47] Aviram A, Sherman C, Kingdom J, et al. Defining early vs late fetal growth restriction by placental pathology. Acta Obstet Gynecol Scand. 2019;98(3):365–373. doi: 10.1111/aogs.13499.
[48] Garcia-Manau P, Mendoza M, Bonacina E, et al. Soluble fms-like tyrosine kinase to placental growth factor ratio in different stages of early-onset fetal growth restriction and small for gestational age. Acta Obstet Gynecol Scand. 2021;100(1):119–128. doi: 10.1111/aogs.13978.
[49] Ferreira JR, Teixeira GQ, Santos SG, et al. Mesenchymal stromal cell secretome: influencing therapeutic potential by cellular pre-conditioning. Front Immunol. 2018;9:2837. doi: 10.3389/fimmu.2018.02837.
[50] Baschat AA, Hecher K. Fetal growth restriction due to placental disease. Semin Perinatol. 2004;28(1):67–80. doi: 10.1053/j.semperi.2003.10.014.
[51] Cunningham CJ, Redondo-Castro E, Allan SM. The therapeutic potential of the mesenchymal stem cell secretome in ischaemic stroke. J Cereb Blood Flow Metab. 2018;38(8):1276–1292. doi: 10.1177/0271678X18776802.
[52] Abheiden CNH, Blomjous BS, Kroese SJ, et al. Low-molecular-weight heparin and aspirin use in relation to pregnancy outcome in women with systemic lupus erythematosus and antiphospholipid syndrome: a cohort study. Hypertens Pregnancy. 2017;36(1):8–15.
[53] Polacek M, Bruun JA, Elvenes J, et al. The secretory profiles of cultured human articular chondrocytes and mesenchymal stem cells: implications for autologous cell transplantation strategies. Cell Transplant. 2011;20(9):1381–1393. doi: 10.3727/096368910X550215.
[54] Gallina C, Turinetto V, Giachino C. A new paradigm in cardiac regeneration: the mesenchymal stem cell secretome. Stem Cells Int. 2015;2015:765846. doi: 10.1155/2015/765846.
[55] Vizoso FJ, Eiro N, Cid S, et al. Mesenchymal stem cell secretome: toward cell-free therapeutic strategies in regenerative medicine. Int J Mol Sci. 2017;18(9):1852–1859. doi: 10.3390/ijms18091852.
[56] Rygaard K, Lindenberg S. Stem cells for obstetricians and gynecologists. Acta Obstet Gynecol Scand. 2002;81(5):383–388. doi: 10.1034/j.1600-0412.2002.810502.x.
[57] Chen W, Wei Q, Liang Q, et al. Diagnostic capacity of sFlt-1/PlGF ratio in fetal growth restriction: a systematic review and meta-analysis. Placenta. 2022;127:37–42. doi: 10.1016/j.placenta.2022.07.020.
[58] Gaccioli F, Sovio U, Gong S, et al. Increased placental sFLT1 (soluble fms-Like tyrosine kinase receptor-1) drives the antiangiogenic profile of maternal serum preceding preeclampsia but not fetal growth restriction. Hypertension. 2023;80(2):325–334. doi: 10.1161/HYPERTENSIONAHA.122.19482.

[59] Kim MJ, Kim ZH, Kim SM, et al. Conditioned medium derived from umbilical cord mesenchymal stem cells regenerates atrophied muscles. Tissue Cell. 2016;48(5):533–543. doi: 10.1016/j.tice.2016.06.010.

[60] Groom KM, David AL. The role of aspirin, heparin, and other interventions in the prevention and treatment of fetal growth restriction. Am J Obstet Gynecol. 2018;218(2S):S829–S840. doi: 10.1016/j.ajog.2017.11.565.