Journal of Experimental and Clinical Medicine
https://dergipark.org.tr/omujecm

Research Article

J Exp Clin Med
2022; 39(4): 1008-1012
doi: 10.52142/omujecm.39.4.16

The effects of maternal anticoagulant therapy on cord blood levels of VEGF-A and soluble Flt-1 in women with recurrent miscarriage

Şahin TAKCI[1,*], Ayşe KORKMAZ TOYGAR[2], Şule YİĞİT[3], Murat YURDAKÖK[3]

[1]Department of Neonatology, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Türkiye
[2]Department of Neonatology, Faculty of Medicine, Acibadem University, İstanbul, Türkiye
[3]Department of Neonatology, Faculty of Medicine, İhsan Doğramacı Children's Hospital, Hacettepe University, Ankara, Türkiye

Received: 04.04.2022 • **Accepted/Published Online:** 05.07.2022 • **Final Version:** 29.10.2022

Abstract

Angiogenic imbalance of the placenta is one of the prominent pathophysiologic mechanisms leading to pregnancy complications like recurrent miscarriages. Low molecular weight heparin and low dose aspirin are frequently used to manage recurrent miscarriages. Vascular endothelial growth factor (VEGF) and its soluble receptor-sFlt-1-plays a major role in angiogenesis. This study investigates cord blood VEGF-A and sFlt-1 levels of women with recurrent miscarriages who receive anticoagulant treatment. We included term newborns whose mothers were treated with LMWH and low-dose aspirin due to recurrent miscarriages. The control group consisted of healthy gestational age-matched infants born in the same period without an adverse perinatal outcome. We assayed the concentrations of VEGF-A and sFlt 1 in umbilical cord blood by ELISA and compared the study and control groups. We included in the study forty-four infants with a maternal LMWH and low dose aspirin treatment during pregnancy and 42 healthy infants as the control group. There were no significant differences between the groups' demographics and cord blood VEGF-A and sFlt-1 levels. There was also no correlation between the cumulative LMWH dosage and serum levels of these angiogenic factors. Cord blood VEGF-A and sFlt-1 levels were comparable in women with recurrent miscarriage under anticoagulant treatment and healthy subjects. Further studies are needed to compare women with recurrent miscarriages with or without heparin treatment to understand better the effects of anticoagulant treatment on the circulatory profile of cord blood angiogenic factors.

Keywords: cord blood, recurrent miscarriage, heparin, VEGF-A, sFlt-1

1. Introduction

Recurrent miscarriage is a primary health problem for women of reproductive age, with at least two consecutive miscarriages before 20 weeks of gestation. It affects approximately 1% to 5% of couples trying to conceive (1). Several conditions are involved in recurrent miscarriages, including antiphospholipid syndrome, acquired thrombophilia, and heritable thrombophilia, such as factor V Leiden and the prothrombin G20210A mutation, but half of the pregnancy losses remain unexplained (2).

It is likely that unexplained recurrent pregnancy loss may reflect a prothrombotic situation and that miscarriage in affected women may be caused by thrombosis in decidual vessels. However, beyond the "thrombosis hypothesis", angiogenic imbalance is an important cause of insufficient utero-placental circulation in the pathophysiology of recurrent miscarriage (3). Despite various discrepancies about the pathophysiology in the literature, many studies show that the treatment with heparin, aspirin or both is associated with a significantly higher rate of live births in women with a history of recurrent miscarriage (4, 5). Heparin has been reported to organize angiogenesis and support endovascular communication between the trophoblast and endothelial cells besides the anticoagulant effects (6). Regulation of angiogenesis is mainly controlled by the pro-angiogenic and anti-angiogenic factors released from the placental unit, such as placenta growth factor (PGF), vascular endothelial growth factor (VEGF), soluble FMS-like tyrosine kinase-1 (sFlt-1) and soluble endoglin. Heparin could demonstrate its effectiveness through complex interactions with pro-and anti-angiogenic factors (7). The literature includes several in-vivo and in-vitro studies evaluating the effects of anticoagulation therapy on angiogenesis; however, little is known about the fetal side (7-9). It is well known that the abnormality of angiogenesis plays a critical role in certain prematurity complications such as retinopathy of prematurity and bronchopulmonary dysplasia (10).

*Correspondence: stakci@gmail.com

In the current case-controlled prospective study, we aimed to investigate cord blood VEGF-A and sFlt-1 levels of women with recurrent miscarriages receiving anticoagulant treatment.

2. Materials and Methods

We conducted this study prospectively over 11 months (April 2012–March 2013) at Hacettepe University School of Medicine. Our institution's ethics committee (12-HEK-033) approved the study, and we obtained informed consent from all families before including the infants in the study.

2.1. Study population

We included term infants (≥ 37 weeks of gestation) in the study. We prospectively included newborns whose mothers were treated with LMWH and low dose aspirin due to recurrent miscarriages. The control group consisted of healthy gestational age-matched infants born in the same period without an adverse perinatal outcome. We excluded infants with a history of antenatal cigarette or alcohol exposure, maternal infection, hypertension, pre-eclampsia, chronic renal or cardiac disease, thyroid disease, epilepsy active asthma. We also excluded infants with congenital heart disease and other congenital and chromosomal abnormalities if determined after birth.

Two or more failed pregnancies before 20 weeks of gestation are considered recurrent miscarriages. We did not include biochemical, ectopic, and molar pregnancies. We excluded women who had uterine anatomic abnormalities, endocrine or chromosomal disorders, history of venous or arterial thromboembolism, and an exact indication for anticoagulant treatment during pregnancy rather than a recurrent miscarriage. We tested all participating women with recurrent miscarriages for acquired thrombophilia, antiphospholipid syndrome and heritable thrombophilia such as factor V Leiden, prothrombin G20210A, plasminogen activator inhibitor mutations and plasma activity levels of Protein C, Protein S and antithrombin.

2.2. Treatment protocol

We treated pregnant women diagnosed with recurrent miscarriages with enoxaparin (Clexane; Rhone-Poulet Rorer, France) subcutaneously in combination with low dose aspirin administered orally. We started both LMWH and aspirin treatments before six weeks of gestation in all cases. We maintained heparin treatment throughout delivery while stopping aspirin treatment approximately one week before delivery and noted the daily and cumulative doses of LMWH within the initiation time of the treatment. We calculated cumulative LMWH dosage between the multiplication of daily drug dose and total days of treatment.

2.3. Analytic procedure

We obtained mixed arterial-venous cord blood samples and temporarily stored them on ice upon delivery. We centrifuged all the samples at 3000 rpm within 15 min of collection. A technician, unaware of the patient's condition until assayed, aliquoted and froze serum at -80°C. We assayed the concentrations of VEGF-A and sFlt 1 in cord blood by ELISA (eBioscience, Bender MedSystems, Vienna, Austria), according to the protocol recommended by the manufacturer. The minimum detectable concentrations in the assays for VEGF-A and sFlt1 were 7.9 pg/ml and 30 pg/ml, respectively. The calculated overall inter-assay and intra-assay coefficients of variation were 4.3% and 6.2% for VEGF-A and 5.1% and 5.5% for sFlt-1, respectively.

2.4. Statistical analysis

We carried out statistical analysis using Statistical Package for Social Sciences (SPSS) version 19 (IBM SPSS Statistics for Windows, Armonk, NY: IBM Corp; 2010) and calculated statistical differences between the studied groups using the Student t-test for the data normally distributed. We used the Mann-Whitney U-test for the data not normally distributed and the chi-square or Fisher's exact tests to compare categorical variables. We performed correlation between studied parameters using Spearman's coefficient of correlation. We considered differences to be statistically significant at $P<0.05$.

3. Results

We included Forty-four infants with a maternal LMWH and low dose aspirin usage during pregnancy in the study group and 42 healthy infants in the control group. There were no significant differences between the two groups' mean gestational ages, birth weight, gender and maternal ages (Table I). Median gravity was 3.5 (3-7) in the study group and 2 (1-5) in the control group (p=0.000). Median parities were 1 (1-3) and 1 (1-4) in the study and control groups, respectively (p=0.75). Mean first and fifth minute Apgar scores were significantly lower in the study group than in the control group (p=0.000).

Table 1. Comparison of the characteristics of the study and control group

Gestational age (weeks)*	37.2±0.6	37.4±0.7	0.18
Birth weight (g)*	3013±358	3106±344	0.22
Maternal age (years)*	30.4±5	30.7±4.7	0.79
Gender M/F (%)	30/14 (68.2/31.8)	25/17 (59.5/40.5)	0.54
Type of birth (NVD/CS)	6/38 (13.6/86.4)	9/33 (21.4/78.6)	0.40
Apgar 1.minute*	8.3±0.9	8.9±0.4	0.00
Apgar 5.minute*	9.2±1	9.9±0.3	0.00
Gravida (min-max)**	3.5 (3-7)	2 (1-5)	0.00
Parity (min-max)**	1 (1-3)	1 (1-4)	0.70
VEGF (pg/ml)*	301.4±220.9	360.5±174.9	0.16
sFlt-1 (pg/ml)*	530±610	380±520	0.52
Cumulative LMWH dosage (mg)*	6009±2067	-	

*Mean±standard deviation, **Median, M: male, F: female, NVD: Normal Vaginal Delivery, CS: Cesarean Section, VEGF: Vascular Endothelial Growth Factor, sFlt-1: soluble FMS-like tyrosine kinase-1, LMWH: Low Molecular Weight Heparin

We found the detectable thrombophilia etiologies of recurrent miscarriages as the following: Factor V Leiden heterozygous mutation in 8, plasminogen activator inhibitor heterozygous mutation in 2, Prothrombin G20210 A heterozygous mutation in 3 women, Protein C deficiency in 1, and Protein S deficiency in 1 woman (some women had two diagnoses)

We measured the serum VEGF-A levels in all samples. The mean cord blood VEGF-A level was 301.4±220.9 pg/ml in the study group and 360.5±174.9 pg/ml in the control group (p=0.16). Twenty-nine of 86 samples were not within the detectable limit for sFlt-1 (18 in the study group and 11 in the control group). Cord blood sFlt-1 levels were 530 ± 610 pg/ml and 380 ± 520 pg/ml in study and control groups, respectively (p=0.52).

Mothers received LMWH with a range of 20 to 60 mg per day. The cumulative mean maternal heparin dosage was 6009±2067 mg. Women received 100 mg of aspirin per day and stopped approximately before one week of delivery. There was no correlation between the cumulative heparin dosage and serum VEGF-A level (r=-0.082 and p=0.59). Although it did not reach statistical significance, there was a trend of negative correlation between the cumulative heparin dose and serum sFlt-1 (r=-0.41 and p=0.051).

4. Discussion

This study shows that the circulating VEGF-A and sFlt-1 levels do not alter in women with recurrent miscarriages receiving anticoagulant treatment. While there is no correlation between the cumulative dose of enoxoparine and cord blood VEGF-A, there is a negative correlation between total enoxoparine dose and cord blood sFlt-1.

Adequate uteroplacental blood flow during healthy pregnancies is necessary to maintain optimal fetal growth. Angiogenic growth factors play an essential role in cytotrophoblast differentiation, the remodeling of the spiral arteries, and the vascular development of the fetus (11). Angiogenic imbalance of the placenta is one of the prominent pathophysiologic mechanisms underlying pregnancy complications like recurrent miscarriage, pre-eclampsia, intrauterine growth restriction, and death (2, 12).

VEGF and sFlt-1 appear to be promising biomarkers for understanding the pathophysiology of angiogenesis in such conditions (7). VEGF is a pro-angiogenic key mediator of other angiogenic pathways with mitogenic, anti-antiapoptotic, and vascular permeability-enhancing activities primarily for vascular endothelium. VEGF family consists of VEGF-A, VEGF-B, VEGF-C, VEGF-D and PGF. VEGF-A is the most important member of this family (13, 14). The effects of VEGF are mediated by binding to tyrosine kinase receptors, the two main ones being FMS-like tyrosine kinase 1 (Flt-1) and the fetal liver kinase-1 (Flk-1). Flt-1 is a weak transmembrane tyrosine kinase-type VEGF receptor expressed in vascular endothelial cells, placental trophoblast cells, macrophages and monocytes. The soluble form of the Flt-1 receptor (a variant with a ligand-binding domain) is generated through alternative splicing of the same pre mRNA that encodes Ft-1 and proteolytic cleavage of Flt's ectodomain sFlt-1. It captures free VEGF with high affinity and functions as a circulating VEGF antagonist. Thus, sFlt can inhibit the pro-angiogenic role of VEGF competitively (15, 16).

It is known that VEGF and its receptor, sFlt-1, play a key role in cytotrophoblast differentiation and survival during placentation (17). In normal pregnancies, sFlt-1 increases excessively during gestation and decreases sharply soon after delivery. This condition suggests that the placenta is the primary origin of this circulating factor (18). Growing evidence shows that an altered balance between the levels of VEGF and sFlt-1 has been proposed as an indicator of placental hypoxia-ischemia and endothelial dysfunction leading to recurrent miscarriage (19). There are conflicting results on the levels and mRNA expressions of VEGF and sFlt-1 in women with recurrent miscarriage. In some studies, decreased sFlt-1 levels in maternal blood are proposed as a potential marker for predicting the risk of recurrent miscarriage (20-22). On the other hand, other reports found placental expressions of sFLt-1 and VEGF mRNA significantly higher in women with recurrent miscarriage than in the control group (19, 23). Wide variation of the study design and relatively low sample size might be the reasons for inconsistent results.

A recent metanalysis showed that LMWH could decrease recurrent miscarriage with a history of women with three or more miscarriages (24). Although more solid and assured results are needed to clarify LMWH treatment's benefit, it is increasingly used to prevent recurrent miscarriage (25, 26).

Favorable effects of heparin on trophoblast implantation and apoptosis inhibition suggest this treatment is a significant potential therapeutic agent in managing recurrent miscarriage (27). Most angiogenic proteins bind heparin and depend on heparan sulfate for their biological activities. Systemic heparin treatment may affect these proteins and vascular endothelial cells (28). Searle et al. suggested that heparin mobilized the sFlt-1 bound to heparan sulfate proteoglycans of the extracellular matrix into the circulation (2011). It has been shown that LMWH (enoxoparine) increases circulating levels of sFlt-1 and enhances urinary elimination in preeclamptic mothers (29). Also, a couple of other studies demonstrated that heparin elevates serum levels of sFlt-1 in vivo and in vitro (8, 9, 30, 31). Again, in the study by Searle et al. (8), heparin strongly induced sFlt-1 release in patients who underwent coronary angiography and sFlt-1 returned to baseline values 6 to 10 hours after heparin administration. In the study of Rosenberg et al. (9), sFlt-1 levels increased, and VEGF levels did not alter in women receiving heparin treatment in the third trimester compared to healthy controls.

The same study evinced that upregulation in the levels of

sFlt-1 is generated from heparin-induced shedding of Flt-1 receptor ectodomain both in vivo and in vitro (9). Recently, Moore et al. (32) clearly demonstrated that the placenta is a source of a large amount of sFlt-1, and chronic unfractionated heparin can display matrix-bound sFlt to the maternal circulation. In our study, cord blood sFlt-1 and VEGF-A levels did not alter within the maternal LMWH and low dose aspirin usage. Although numerous studies investigated the serum or plasma levels of sFlt-1 and VEGF in heparin-treated pregnant women, the circulatory profile of these factors has not been studied in the fetal site yet. As LMWH does not cross the placenta, non-altered cord blood levels may be a common condition. However, complex molecular interactions between the fetal and maternal side of the placenta under maternal anticoagulant therapy could not be ignored. The recognized or unrecognized causes of recurrent miscarriage and heparin usage may have different effects on circulating cord blood VEGF-A and sFlt-1 levels. Thus, further studies comparing women who have recurrent miscarriages with or without heparin treatment may be helpful to understand better the effects of anticoagulant treatment on the circulatory profile of cord blood angiogenic factors. It is common knowledge that heparin exerts its effects on VEGF and VEGF receptors in a dose and time manner (9, 33). Therefore, we calculated the cumulative LMWH dose for each woman during pregnancy and did not find any correlation between the heparin dose and the levels of both factors. Although the result did not reach statistical significance, the results indicate that there may be an association between the cumulative heparin dose and sFlt-1 level ($p=0.051$ and $r=-0.46$).

In conclusion, cord blood VEGF-A and sFlt-1 levels are not affected in women with recurrent miscarriage by maternal LMWH and low-dose aspirin treatment. These findings will improve our understanding of the complex interactions between maternal anticoagulant therapy and angiogenic factors on the fetal side. On the other hand, it is difficult to make a strong comment on the cord blood sFlt-1 level due to the low sample size.

Conflict of interest

The authors have no conflicts of interest relevant to this article to disclose.

Funding

No external funding for this manuscript.

Acknowledgments

None to declare.

Authors' contributions

Concept: Ş.T., M.T., Design: A.K.T, Ş.Y., Data Collection or Processing: Ş.T., A.K.T, Ş.Y., M.T., Analysis or Interpretation: Ş.T., Literature Search: Ş.T., Writing: Ş.T.

References

1. Allison JL, Schust DJ. Recurrent first trimester pregnancy loss: revised definitions and novel causes. Curr Opin Endocrinol Diabetes Obes 2009; 16: 446-50. http://doi.org/10.1097/MED.0b013e3283327fc5
2. Matthiesen L, Kalkunte S, Sharma S. Multiple pregnancy failures: an immunological paradigm. Am J Reprod Immunol 2012; 67 :334-340. http://doi.org/ 10.1111/j.1600-0897.2012.01121.x
3. Rana S, Burke SD, Karumanchi SA. Imbalances in circulating angiogenic factors in the pathophysiology of pre-eclampsia and related disorders. Am J Obstet Gynecol 2020; S0002-9378 :31196-0. http://doi.org/10.1016/j.ajog.2020.10.022
4. Elmahashi MO, Elbareg AM, Essadi FM, Ashur BM, Adam I. Low dose aspirin and low-molecular-weight heparin in the treatment of pregnant Libyan women with recurrent miscarriage. BMC Res Notes 2014; 7: 23. http://doi.org/10.1186/1756-0500-7-23.
5. Shaaban OM, Abbas AM, Zahran KM, Fathalla MM, Anan MA, Salman SA. Low-Molecular-Weight Heparin for the Treatment of Unexplained Recurrent Miscarriage With Negative Antiphospholipid Antibodies: A Randomized Controlled Trial. Clin Appl Thromb Hemost 2017; 23: 567-572. http://doi.org/10.1177/1076029616665167
6. Kwak-Kim J, Agcaoili MS, Aleta L, Liao A, Ota K, Dambaeva S, et al. Management of women with recurrent pregnancy losses and antiphospholipid antibody syndrome. Am J Reprod Immunol. 2013 Jun;69(6):596-607. doi: 10.1111/aji.12114.
7. Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu KF, et al. Circulating angiogenic factors and the risk of pre-eclampsia. N Engl J Med 2004; 350: 672– 683. http://doi.org/10.1056/NEJMoa031884.
8. Searle J, Mockel M, Gwosc S, Datwyler SA, Qadri F, Albert GI, et al. Heparin strongly induces soluble fms-like tyrosine kinase 1 release in vivo and in vitro--brief report. Arterioscler Thromb Vasc Biol 2011; 31: 2972-2974. http://doi.org/10.1161/ATVBAHA.111.237784.
9. Rosenberg VA, Buhimschi IA, Lockwood CJ, Paidas MJ, Dulay AT, Ramma W, et al. Heparin elevates circulating soluble fms-like tyrosine kinase-1 immunoreactivity in pregnant women receiving anticoagulation therapy. Circulation 2011; 124: 2543-2553. http://doi.org/10.1161/CIRCULATIONAHA.111.046821.
10. Levesque BM, Kalish LA, Winston AB, Parad RB, Hernandez-Diaz S, Phillips M, et al. Low urine vascular endothelial growth factor levels are associated with mechanical ventilation, bronchopulmonary dysplasia and retinopathy of prematurity. Neonatology 2013; 104: 56-64. http://doi.org/ 10.1159/000351040.
11. Catarino C, Rebelo I, Belo L, Rocha S, Castro EB, Patrício B, et al. Fetal and maternal angiogenic/anti-angiogenic factors in normal and preeclamptic pregnancy. Growth Factors. 2009 ;27: 345-351. http://doi.org/10.3109/08977190903184670
12. Osol G, Moore LG. Maternal uterine vascular remodeling during pregnancy. Microcirculation 2014; 21: 38-47. http://doi.org/10.1111/micc.12080
13. Shibuya M. Vascular Endothelial Growth Factor (VEGF) and Its Receptor (VEGFR) Signaling in Angiogenesis: A Crucial Target for Anti- and Pro-Angiogenic Therapies. Genes Cancer 2011; 2: 1097-1105. http://doi.org/10.1177/1947601911423031
14. Andraweera PH, Dekker GA, Roberts CT. The vascular endothelial growth factor family in adverse pregnancy outcomes. Hum Reprod Update. 2012; 18: 436-457. http://doi.org/10.1093/humupd/dms011
15. Kendall RL, Thomas KA. Inhibition of vascular endothelial cell growth factor activity by an endogenously encoded soluble receptor. Proc Natl Acad Sci U S A 1993; 90: 10705-10709. http://doi.org/10.1073/pnas.90.22.10705.

16. Kappas NC, Zeng G, Chappell JC, Kearney JB, Hazarika S, Kallianos KG, et al. The VEGF receptor Flt-1 spatially modulates Flk-1 signaling and blood vessel branching. J Cell Biol 2008; 181: 847-858. http://doi.org/10.1083/jcb.200709114

17. Brockelsby JC, Anthony FW, Johnson IR, Baker PN. The effects of vascular endothelial growth factor on endothelial cells: a potential role in pre-eclampsia. Am J Obstet Gynecol 2000; 182: 176-183. http://doi.org/10.1016/s0002-9378(00)70510-2

18. Clark DE, Smith SK, He Y, Day KA, Licence DR, Corps AN, et al. A vascular endothelial growth factor antagonist is produced bythe human placenta and released into the maternal circulation. Biol Reprod 1998; 59: 1540-1548. http://doi.org/10.1095/biolreprod59.6.1540

19. Pang L, Wei Z, Li O, Huang R, Qin J, Chen H, et al. An increase inb vascular endothelial growth factor (VEGF) and VEGF soluble receptor-1 (sFlt-1)are associated with early recurrent spontaneous abortion. PLoS One 2013; 8: e75759. http://doi.org/10.1371/journal.pone.0075759

20. Daponte A, Pournaras S, Polyzos NP, Tsezou A, Skentou H, Anastasiadou F, et al. Soluble FMS-like tyrosine kinase-1 (sFlt-1) and serum placental growth factor (PlGF) as biomarkers for ectopic pregnancy and missed abortion. J Clin Endocrinol Metab 2011; 96: E1444-51. , http://doi.org/10.1210/jc.2011-0037

21. Kaitu'u-Lino TJ, Whitehead CL, Ngian GL, Permezel M, Tong S. Serum concentrations of soluble Flt-1 are decreased among women with a viable fetus and no symptoms of miscarriage destined for pregnancy loss. PLoS One 2012; 7: e32509. http://doi.org/10.1371/journal.pone.0032509

22. Muttukrishna S, Swer M, Suri S, Jamil A, Calleja-Agius J, Gangooly S, et al. Soluble Flt-1 and PlGF: new markers of early pregnancy loss? PLoS One 2011; 6: e18041. http://doi.org/ 10.1371/journal.pone.0018041

23. Pang LH, Li MJ, Li Yang DM, Shi L. Vascular endothelial growth factor (VEGF) and the VEGF soluble receptor-1 (sFlt-1) in chorionic villus tissue from Chinese women with early recurrent spontaneous abortion. J Int Med Res 2011; 39: 830-837. http://doi.org/10.1177/147323001103900316

24. Wang G, Zhang R, Li C, Chen A. Evaluation of the effect of low molecular weight heparin in unexplained recurrent pregnancy loss: a meta-analysis of randomized controlled trials. J Matern Fetal Neonatal Med. 2021: 1-8. http://doi.org/10.1080/14767058.2021.1957819

25. Kaandorp SP, Goddijn M, van der Post JA, Hutten BA, Verhoeve HR, Hamulyák K, et al. Aspirin plus heparin or aspirin alone in women with recurrent miscarriage. N Engl J Med 2010; 362: 1586-1596. http://doi.org/ 10.1056/NEJMoa1000641

26. Clark P, Walker ID, Langhorne P, Crichton L, Thomson A, Greaves M, et al. SPIN (Scottish Pregnancy Intervention) study: a multicenter, randomized controlled trial of low-molecular-weight heparin and low-dose aspirin in women with recurrent miscarriage. Blood 2010; 115: 4162-4167. http://doi.org/10.1182/blood-2010-01-267252

27. Nelson SM, Greer IA. The potential role of heparin in assisted conception. Hum Reprod Update 2008; 14: 623-645. http:doi.org/10.1093/humupd/dmn031

28. Norrby K, Nordenhem A. Dalteparin, a low-molecular-weight heparin, promotes angiogenesis mediated by heparin-binding VEGF-A in vivo. APMIS 2010; 118: 949-957. http://doi.org/10.1111/j.1600-0463.2010.02635.x

29. Hagmann H, Bossung V, Belaidi AA, Fridman A, Karumanchi SA, Thadhani R, et al. Low-molecular weight heparin increases circulating sFlt-1 levels and enhances urinary elimination. PLoS One 2014; 9: e85258. http://doi.org/ 10.1371/journal.pone.0085258

30. Sela S, Natanson-Yaron S, Zcharia E, Vlodavsky I, Yagel S, Keshet E. Local retention versus systemic release of soluble VEGF receptor-1 are mediated by heparin-binding and regulated by heparanase. Circ Res 2011; 108: 1063-1070. http://doi.org/10.1161/CIRCRESAHA.110.239665.

31. Denizot Y, Leguyader A, Cornu E, Laskar M, Orsel I, Vincent C, et al. Release of soluble vascular endothelial growth factor receptor-1 (sFlt-1) during coronary artery bypass surgery. J Cardiothorac Surg 2007; 2: 38. http://doi.org/10.1186/1749-8090-2-38

32. Moore KH, Chapman H, George EM. Unfractionated heparin displaces sFlt-1 from the placental extracellular matrix. Biol Sex Differ 2020; 11: 34. http://doi.org/10.1186/s13293-020-00311-w

33. Park M, Lee ST. The fourth immunoglobulin-like loop in the extracellular domain of FLT-1, a VEGF receptor, includes a major heparin-binding site. Biochem Biophys Res Commun 1999; 264: 730-734. http://doi.org/10.1006/bbrc.1999.1580