IRAQI JOURNAL OF EMBRYOS AND INFERTILITY RESEARCHES (IJEIR)
Zainab et al., Vol. 11, Issue 1, Pp. 64-77 (2021) Original Research
DOI: http://doi.org/10.28969/IJEIR.v11.i1.r6.21 E-ISSN: 2616-6984 P-ISSN: 2218-0265

Effect of Hyaluronic Acid Vaginal Pessary on Endometrial Receptivity and Pregnancy Outcome through assessment of Serum and Follicular fluid Lymphatic Vessel Endothelial Hyaluronan Receptor 1 (LYVE1) in Women Undergoing ICSI.

Zainab H. Ali[1], Hayder A. L. Mossa[1], Muayad S. Abbood[1]

[1]**High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al Nahrain University, Baghdad, Iraq.**

ABSTRACT

Successful implantation needs a receptive endometrium, a functional and normal embryo at the stage of blastocyst developmental and a synchronized dialogue between embryonic tissues and maternal tissues. The current study was planned and conducted to investigate the potential role of vaginal pessary of hyaluronic acid on intracytoplasmic sperm injection (ICSI) outcome. This study included 63 infertile women (32 women as study group receiving hyaluronic acid and 31 control group) who were subjected to ovulation induction programs with gonadotropin releasing hormone (GnRH) antagonist protocols for in vitro fertilization (IVF)/ intracytoplasmic sperm injection (ICSI) procedures. Blood samples were taken by Vacutainer tubes and centrifuged according to the manufacturer's instructions for the preparation of serum samples. Serum samples for Lymphatic vessel endothelial hyaluronan receptor 1 (LYVE1) were obtained from each patient when the basal hormonal status was determined. The second time point is when the samples were obtained on the day of ova pickup, after that the G1 embryos will be transferred inside the uterine cavity. Then performance of endometrial receptivity parameters or patterns evaluation and patients were followed up to do pregnancy test after14 days after embryo transfer. On the day of human chorionic gonadotropin (hCG), there was no significant difference in mean serum Lymphatic vessel endothelial hyaluronan receptor 1 (LYVE1) between hyaluronic acid group and reference group, respectively (p = 0.824). There was no significant difference in mean follicular fluid Lymphatic vessel endothelial hyaluronan receptor 1 (LYVE1) between the hyaluronic acid group and reference group, 0.92 ±0.36 pg/ml versus 0.82 ±0.22 pg/ml, respectively (p = 0.223). Treatment with the hyaluronic acid resulted in non-significant increase in serum and follicular fluid Lymphatic vessel endothelial hyaluronan receptor 1 (LYVE1), and this increment is not related to Lymphatic vessel endothelial hyaluronan receptor 1 (LYVE1) improvement in local blood endometrial blood flow as reflected by reduction in resistive index and pulsatility index with eventual improvement in pregnancy outcome.

Received: 26-April-2021 Accepted: 30-May-2021 Published: 06-Jun-2021

How to cite:

Zainab H. Ali; Hayder A. L. Mossa; Muayad S. Abbood; Effect of Hyaluronic Acid Vaginal Pessary on Endometrial Receptivity and Pregnancy Outcome through assessment of Serum and Follicular fluid Lymphatic Vessel Endothelial Hyaluronan Receptor 1 (LYVE1) in Women Undergoing ICSI; Iraqi Journal of Embryos and Infertility Researches (IJEIR), (2021); 11 (1): 80-42.
Doi: http://doi.org/10.28969/IJEIR.v11.i1.r6.21

KEYWORD

Endometrial Receptivity, Pregnancy Outcome, LYVE1, Hyaluronic Acid Vaginal Pessary

1. Introduction

The problem of infertility is a common issue in daily clinical practice, with a prevalence rate in the range of 9 to 18 % worldwide. It is very promising to know that 61,430 neonates were born in 2008 with the aid of assisted reproductive technology. This fact sheds light on the vast number of infertile women seeking medical advice and assisted reproduction worldwide **(Hanson et al.,2017 [1])**. The process of pregnancy and delivering a healthy baby is a complex biological process that assumes a complex pathway passing through the availability of healthy male and female gametes, healthy male and female reproductive systems, normally growing embryo, receptive endometrium, normal placental function and so much many complicated physiological events **(Aldujaily et al.2015 [2], Ivell, R., & Anand-Ivell, R.,2021 [3]).** Therefore, improving fertility potential in couples who are infertile requires the improvement of many individual steps involved in the physiology of reproduction **(Aldujaily et al.2015 [2], Ivell, R., & Anand-Ivell, R.,2021 [3]) .**

The process of embryo implantation is a unique physiological process, and it is regarded as the most critical step of the reproductive process. The success of implantation is in need for a receptive endometrium, a normal and functional embryo at the stage of blastocyst maturation, and a dialogue that is synchronized between maternal tissues and embryonic tissues. The receptivity of the endometrium to blastocyst implantation is limited to a critical transient and spatially restricted window, known as the window of implantation. The period of implantation lies between 6 and 10 days following the LH surge and remains for about 48 hours **(Simon et al.2020 [4])**. Local angiogenesis is a necessary step for pregnancy and implantation **(Kupesic et al., 2001**

[5]). A number of diagnostic tools have been proposed to "estimate" or "measure" the receptivity of endometrium .

These tools comprise ultrasound measures (endometrial thickness and sub-endometrial and uterine blood flows) and local production of molecules that are vasoactive, both based on the process of angiogenesis, which occurs locally around the implantation period **(El-Zenneni et al. 2016 [6])**. The function of LYVE-1 has just recently been identified as that of a lymphatic docking receptor for dendritic cells, selectively engaging with their surface HA glycocalyx to regulate entry to peripheral lymphatics and migration to downstream lymph nodes for immune activation. Furthermore, LYVE-1 mediates the trafficking of macrophages **(Jackson et al.,2018 [18])**.

Hyaluronic acid, also called hyaluronan, is an anionic, non-sulfated glycosaminoglycan distributed widely throughout connective, epithelial, and neural tissues **(Saylan et al.,2016 [7]).** It is unique among glycosaminoglycans in that it is a nonsulfated synthesis of Hyaluronic acid, and its organization into an extracellular matrix are processes involved in successful ovulation and fertilization in most mammals, including humans **(Mohiyiddee et al.,2005 [8], Petersen et al.,2010 [9]).** Hyaluronic acid (HA) has been reported to participate in the regulation of vascular development in a number of physiological processes **(Ghose et al.,2018 [10])**. The novelty of the current study is the evaluation of the potential role of hyaluronic acid on the implantation and receptivity of endometrial following local administration. Therefore, the current study was planned and conducted to investigate the potential role of vaginal pessary of hyaluronic acid on ICSI outcome**.**

2. Patients and Methods

The present study proposal was conducted with infertile female patients that were admitted to the High Institute for Infertility Diagnosis and ART's Clinics, other IVF centers, and candidates for IVF/ICSI. After the approval of the ethical committee, that must be obtained prior to the initiation of the study. Inclusion criteria were: age of women between 18 and 39 years at the time of screening; basal serum follicle stimulating hormone level (FSH) <10 mIU/mL in the early follicular phase; good physical and mental health and recurrent implantation failure. The exclusion criteria were ovarian cysts, endocrinopathies such as hyperprolactinemia, congenital adrenal hyperplasia, thyroid disease, and clinically suspected Cushing's syndrome, uterine pathology such as leiomyoma, adenomyosis, congenital uterine anomalies and tubal hydrosalpinx, endometriosis cases, hematological diseases, old age above 40 years, azoospermic cases (Testicular Biopsy). This study included 63 infertile women (32 women as the study

group receiving hyaluronic acid and 31 in the control group) who were subjected to ovulation induction programs with GnRH antagonist protocols for IVF/ICSI procedures. The hyaluronic acid was given in the form of once-daily 5 mg pessary from cycle day five till the day of hCG triggering. Blood samples were taken by Vacutainer tubes and centrifuged according to the manufacturer's instructions for the preparation of serum samples. Serum samples for LYVE1 were obtained from each patient when the basal hormonal status was determined. The second time point is when the samples were obtained on the day of ova pickup; after that, the G1 embryos will be transferred inside the uterine cavity. Then, the performance of endometrial receptivity parameters

or patterns evaluation and patients were followed up to do a pregnancy test after 14 days after embryo transfer.

3. Statistical analysis

Data were analyzed using SPSS version 16 and Microsoft Office Excel 2007. Categorical variables were presented as numbers and percentages, while numeric variables were expressed as mean, range, and standard deviation. Independent samples t-test was used to compare means of numeric variables between study groups. The level of significance was set at $\leq p\ 0.05$.

4. Results

In the current study, 63 infertile women were included, and they were categorized randomly into two groups: the hyaluronic acid group (n = 32) and the reference group (n = 31). The mean age of all participating women was 32.51 ±5.61 years, and the mean body mass index of all participating women was 28.02 ±2.76 kg/m2; there was no significant difference in mean age and mean BMI between the hyaluronic acid group and reference group (p> 0.05), **(Table 1).**

The comparisons of serum and follicular fluid levels of vascular markers between the hyaluronic acid group and the reference group are shown in **(Table 2).** There was no significant difference in mean serum LYVE1 between the hyaluronic acid group and the reference group (p> 0.05) **(Figure 1)**. On the day of hCG, there was no significant difference in mean serum LYVE1 between the hyaluronic acid group and reference group, respectively (p = 0.824) **(Figure 2).** In addition, there was no significant difference in mean follicular fluid LYVE1 between the hyaluronic acid group and reference group, 0.92 ±0.36 pg/ml versus 0.82 ±0.22 pg/ml, respectively (p = 0.223) **(Figure 3).**

The comparison of ultrasound findings and biophysical profile between the hyaluronic acid group and reference group is shown in **(Table 3).** There was

a highly significant difference in mean endometrial thickness between the hyaluronic acid group and reference group, 8.03 ±0.75 mm versus 6.41 ±1.39 mm, respectively (p< 0.001), the thickness being greater in the hyaluronic acid group. There was a highly significant difference in the mean pulsatility index and resistive index between the hyaluronic acid group and reference group (p< 0.001), the level being less in the hyaluronic acid group.

The comparison of biochemical pregnancy rate between the study group and control group is shown in **(Table 4).** Overall, out of 63 infertile women, successful biochemical pregnancy was reported in 25 cases (39.7 %), and the rate of positive biochemical pregnancy of the study group was higher than that of the control group, 62.5 % versus 16.1 %, respectively in a highly significant manner (p< 0.00).

Table (1): Demographic characteristics of infertile women participating in the current study

Characteristic	**Total** ***n*** **= 63**	**Hyaluronic acid group** ***n*** **= 32**	**Control group** ***n*** **= 31**	***p***
Age (years)				
Mean ±SD	32.51 ±5.61	33.16 ±5.17	31.84 ±6.04	0.356 I
Range	20 -42	21 -40	20 -42	NS
BMI (kg/m2)				
Mean ±SD	28.02 ±2.76	27.83 ±2.97	28.22 ±2.56	0.581 I
Range	22.84 -35.8	22.84 -33.67	23.42 -35.8	NS
n: number of cases; SD: standard deviation; I: independent samples t-test; NS: not significant at p> 0.05				

Table (2): Comparison of serum levels of vascular markers between the hyaluronic acid group and reference group at cycle day 2

Characteristic	Total *n* = 63	Hyaluronic acid group *n* = 32	Reference group *n* = 31	*p*
LYVE1 (pg/ml) cycle day 2				
Mean ±SD	0.66 ±0.19	0.68 ±0.21	0.65 ±0.17	0.569 I
Range	0.36 -0.98	0.36 -0.98	0.4 -0.86	NS
LYVE1 (pg/ml) day of hCG				
Mean ±SD	0.80 ±0.22	0.80 ±0.23	0.79 ±0.20	0.824 I
Range	0.46 -1.26	0.46 -1.26	0.46 -0.99	NS
Follicular fluid LYVE1 (pg/ml)				
Mean ±SD	0.87 ±0.30	0.92 ±0.36	0.82 ±0.22	0.223 I
Range	0.48 -1.79	0.5 -1.79	0.48-1.07	NS
n: number of cases; SD: standard deviation; I: independent samples t-test; NS: not significant at p> 0.05				

Table (3): The comparison in Doppler study findings according to pregnancy outcome

Characteristic	Total *n* = 63	Study group *n* = 32	Control group *n* = 31	*p*
Endometrial thickness (mm)				
Mean ±SD	7.21 ±1.38	8.03 ±0.75	6.41 ±1.39	< 0.001 I
Range	4 -9	7 -9	4 -8	HS
Pulsatility index				
Mean ±SD	1.17 ±0.63	0.69 ±0.28	1.68 ±0.48	< 0.001 I
Range	0.4 -2	0.4 -2	1 -2	HS
Resistive index				
Mean ±SD	1.01 ±0.30	0.77 ±0.07	1.25 ±0.24	< 0.001 I

Range	0.6 -1.5	0.6 -1.5	0.7 -0.9	HS
n: number of cases; SD: standard deviation; I: independent samples t-test; NS: not significant at p> 0.05; S: significant at p ≤ 0.05; HS: highly significant at p ≤ 0.01				

Table (4): Comparison of biochemical pregnancy rate between hyaluronic acid group and reference group

Pregnancy outcome	**Total *n* = 63**	**Hyaluronic acid group *n* = 32**	**Reference group *n* = 31**	***p***
Positive, *n* (%)	25 (39.7 %)	20 (62.5 %)	5 (16.1 %)	< 0.001 C
Negative, *n* (%)	38 (60.3 %)	12 (27.5 %)	26 (83.9 %)	HS
n: number of cases; C: chi-square test; HS: highly significant at p ≤ 0.05				

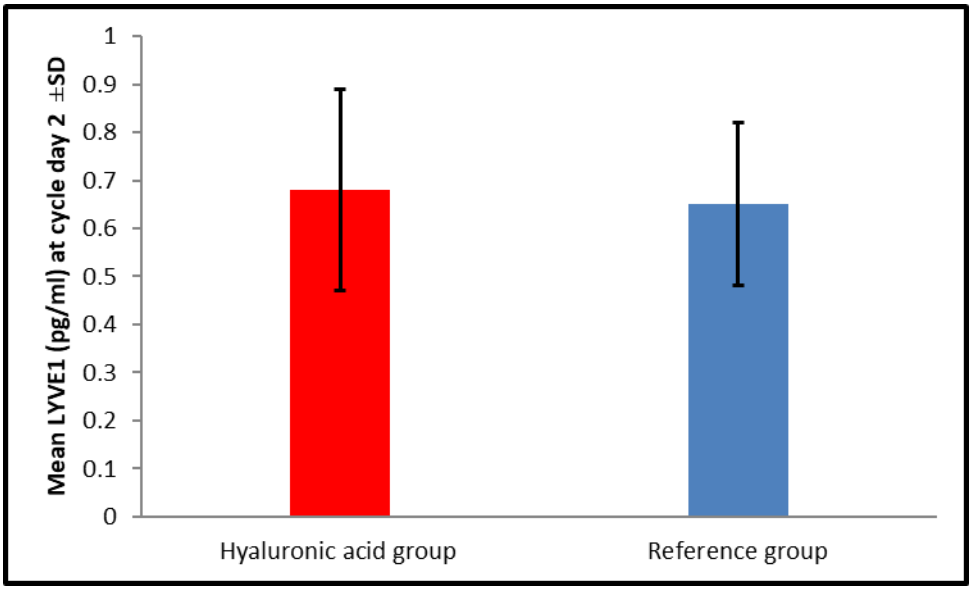

Figure (1): Comparison of serum LYVE1 at cycle day two between the study group and control group

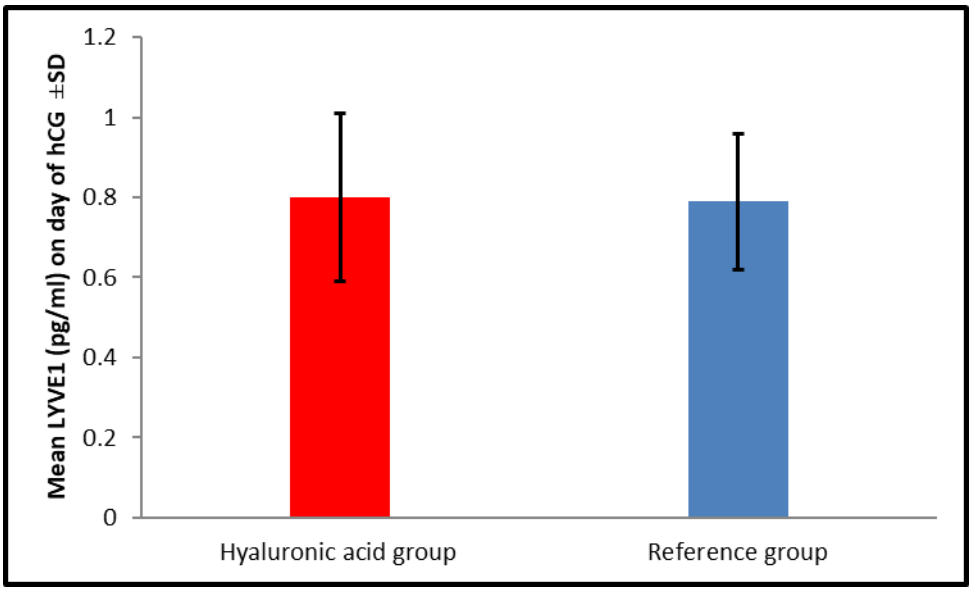

Figure (2): Comparison of serum LYVE1 on the day of hCG between the study group and control group

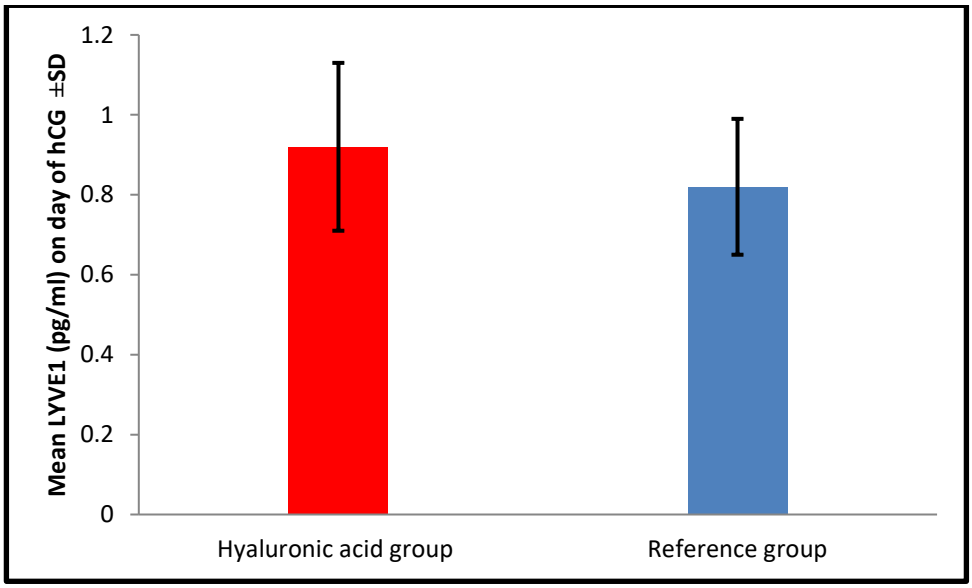

Figure (3): *Comparison of follicular fluid LYVE1 between the study group and control group*

5. Discussion

The current interventional study included 63 infertile women in order to see the effect of hyaluronic acid on ICSI outcome and specifically on implantation and endometrial receptivity. The lack of significant difference in mean age and mean BMI between hyaluronic acid and reference groups are mandatory in such interventional studies in order to avoid any bias in the results that may be attributed to the effect of the independent variables (age and BMI) since a lot of previous reports raised the issue of the correlation between women age or BMI and ICSI outcome and in particular the endometrial receptivity and implantation **(Bartmann et al.,2004 [11], Kung, et al.,2015 [12], Lan, et al.,2013 [13], Rittenberg, et al.,2011 [14], Kudesia, et al.,2015 [15], Rehman, et al.,2013 [16], Arabipoor, et al.,2019 [17], Aynekin, B et al.,2022[18]) .**

In the current study, at the beginning of the cycle (cycle day 2), there was no significant difference in mean serum LYVE1 between the hyaluronic acid group and reference group, and on the day of hCG, indicating that treatment with hyaluronic acid did not affect significantly the serum level of LYVE1. LYVE-1, a close relative of the leucocyte receptor CD44 is the main receptor for hyaluronan (HA) in lymphatic vessel endothelium and a widely used marker for distinguishing between blood and lymphatic vessels. Enigmatic for many years because of its anomalous HA-binding characteristics, the function of LYVE-1 has just recently been identified as that of a lymphatic docking receptor for dendritic cells, selectively engaging with their surface HA glycocalyx to regulate entry to peripheral lymphatics and migration to downstream lymph nodes for immune activation. Furthermore, LYVE-1 mediates the trafficking of macrophages **(Jackson et al.,2018 [18])**. Indeed, experimental

studies have shown a role for lymphangiogenesis in the process of embryo implantation and pregnancy recognition in experimental animals, but the role is still unclear **(Hayashi et al.,2019 [19])**. Based on our observation and the above-mentioned experimental data, we can suggest that vaginal pessary of hyaluronic acid did not result in the upregulation of LYVE-1 receptors, but it may act through these receptors to improve endometrial receptivity and embryo implantation through an unknown mechanism which is in need for further experimental work to be clarified.

In the current study, there was no significant difference in mean follicular fluid LYVE1 between the hyaluronic acid group and reference group, and therefore, the treatment with hyaluronic acid appears to have no additive effect on the level of LYVE1. Indeed, after a thorough search in the available published articles, we failed to find an article with a similar study design in order to compare our results with it, and therefore, our study provides a novel insight to the clinical value of using hyaluronan in improving embryo implantation and pregnancy outcome during ICSI cycles .

In the current study, there was a highly significant difference in mean endometrial thickness between the hyaluronic acid group and reference group, the thickness being greater in the study group. In this study in addition, there was highly significant difference in the mean pulsatility index and mean resistive index between hyaluronic acid group and the reference group; the level being less in hyaluronic acid group. Taking all these findings together, one can conclude that the significant improvement in vascular blood flow of endometrium, reflected by less impedance to vascular flow (significantly lower resistive index and significantly lower pulsatility index), resulted in significant increase in endometrial thickness. And the rise in blood flow in the study group in comparison with a control group is due

to hyaluronic acid treatment but not LYVE1 .

In line with this suggestion, a previous study has shown that Women of the Unexplained Infertility Group had thinner endometrial thickness, higher sub-endometrial flow resistance index (RI) and lower sub-endometrial flow pulsatility index (PI) in comparison with fertile women **(El-Zenneni, et al.,2015 [6])**.

In the current study, the rate of positive biochemical pregnancy of the study group was higher than that of control group, 62.5 % versus 16.1 %, respectively, in a highly significant manner ($p< 0.001$). The only possible explanation for this highly significant discrepancy in biochemical pregnancy rate in study group is attributable to treatment with hyaluronic acid with resulted in significant increase in endometrial thickness and better endometrial blood flow (less resistive index and less pulsatility index) and hence better endometrial receptivity and significantly higher embryo implantation rate.

6- Conclusion

Treatment with hyaluronic acid resulted in non-significant increase in serum and follicular fluid LYVE1, and this increment not related to LYVE1 improvement in local blood endometrial blood flow as reflected by reduction in the resistive index and pulsatility index with eventual improvement in pregnancy outcome.

Acknowledgment

We would like to acknowledge the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies/ Al Nahrain University, Baghdad, Iraq.

Funding

This work received no funding .

Author Contribution

Zainab H. Ali, performed the study, Hayder A. L. Mossa, and Muayad S. Abbood, supervised the work .

Conflict of Interest

The authors declare no conflict of interest .

Ethical Clearance

The study was approved by the Ethical Approval Committee.

Financial Disclosure

There is no financial disclosure.

Ethical Clearance

The study was approved by the Ethical Approval Committee

References

[1] Hanson, B., Johnstone, E., Dorais, J., Silver, B., Peterson, C. M., &Hotaling, J. (2017). Female infertility, infertility-associated diagnoses, and comorbidities: a review. Journal of assisted reproduction and genetics, 34 (2), 167–177. https://doi.org/10.1007/s10815-016-0836-8

[2] Al-Dujaily S. S., Mossa HAL, Alwachi S. N. Study of Calpain Activity, Sperm Penetration Assay in Unexplained Infertile Men in Relevance to Assisted Reproductive Technologies. World Journal of Pharmaceutical Research,2015;4 (2):100-117

[3] Ivell, R., & Anand-Ivell, R. (2021). The Physiology of Reproduction - Quo Vadis? Frontiers in physiology, 12, 650550. https://doi.org/10.3389/fphys.2021.650550

[4] Simon C., J.C. Martin, A. Pellicer. Paracrine regulators of implantation Baillieres Best Pract Res Clin Obstet Gynaecol, 14 (5) (2000), pp. 815-826

[5] Kupesic S, I. Bekavac, D. Bjelos, A. Kurjak. Assessment of endometrial receptivity by transvaginal fertilization procedures. J Ultrasound Med, 20 (2001), pp. 125-134

[6] El-Zenneni H, MoustafaR., Abdel-Hafeez M., El-Salally H., A., Abdel-Kader A. Assessment of uterine, sub endometrial blood flows and endometrial gland vascular endothelial growth factor (EG-VEGF) in women with unexplained infertility. Middle East Fertil Soc J, 20 (2015), pp. 119-126

[7] Saylan A, Duman S. Efficacy of Hyaluronic Acid in The Selection of Human Spermatozoa with Intact DNA by The Swim-up Method. Cell J. 2016 Spring;18 (1):83-8. doi: 10.22074/cellj.2016.3990. Epub 2016 Apr 4. PMID: 27054122; PMCID: PMC4819390

[8] Mohiyiddeen L, Hardiman A, Fitzgerald C, Hughes E, Mol BW, Johnson N, et al. Tubal flushing for subfertility. Cochrane Database Syst Rev. 2005; 5: CD003718.

[9] Petersen CG, Massaro FC, Mauri AL, Oliveira JB, Baruffi RL, Franco JG Jr. Efficacy of hyaluronic acid binding as¬say in selecting motile spermatozoa with normal morphol¬ogy at high magnification. Reprod Biol Endocrinol. 2010; 8: 149. https://. doi: 10.4103/jfmpc.jfmpc_212_19.

[10] Ghose S, Biswas S, Datta K, Tyagi RK. Dynamic Hyaluronan drives liver endothelial cells towards angiogenesis. BMC Cancer. 2018 Jun 11;18 (1):648. doi: 10.1186/s12885-018-4532-1. PMID: 29890947; PMCID: PMC5996548.

[11] Bartmann AK, Romão GS, Ramos Eda S, Ferriani RA. Why do older women have poor implantation rates? A possible role of the mitochondria. J Assist Reprod Genet. 2004;21 (3):79-83. doi:10.1023/b: jarg.0000027018.02425.15 **[12]** Kung A, Munne S, Bankowski B, Coates A, Wells D. Validation of next-generation sequencing for comprehensive chromosome screening of embryos. Reprod Biomed Online. 2015; 31:760-769.

[13] Lan KC, Lin PY, Chang YC, et al. Growth hormone supplementation may improve the pregnancy rate and endometrial receptivity among women aged more than 40 years undergoing in vitro fertilization. Biomed J. 2019;42 (6):411-416. doi:10.1016/j.bj.2019.05.003.

[14] Rittenberg V, Seshadri S, Sunkara SK, Sobaleva S, Oteng-Ntim E, El-Toukhy T. Effect of body mass index on IVF treatment outcome: an updated systematic review and meta-analysis. Reprod Biomed Online. 2011 Oct;23 (4):421-39. doi: 10.1016/j.rbmo.2011.06.018. Epub 2011 Jul 28. PMID: 21885344.

[15] Kudesia R, Wu H, Hunter Cohn K, et al. The effect of female body mass index on in vitro fertilization cycle outcomes: a multi-center analysis. J Assist Reprod Genet. 2018;35 (11):2013-2023. doi:10.1007/s10815-018-1290-6

[16] Rehman R, Hussain Z, Fatima SS. Effect of weight status on pregnancy outcome in intra cytoplasmic sperm injection. Iran J Reprod Med. 2013;11 (9):717-724 https:// doi:10.1186/s43055-021-00430-3

[17] Aynekin, B., Akalin, H., Muderris, I.I. et al. Biomarker potential of the GRP78 cell-free RNA in endometrial cancer. Egypt J Med Hum Genet 23, 143 (2022). https://doi.org/10.1186/s43042-022-00355-0