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Comparative Study of Follicular Fluid Inflammatory Cytokines and Post-IVF Pregnancy Outcomes in Women with Polycystic Ovary Syndrome (PCOS)

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ABSTRACT

The process of reproduction is complex and requires optimum functioning of interconnected physiological mechanism. However, any disconnect or failure in ovarian cellular communication between the key cells involved in follicular development may result in infertility. Cytokines, which are important in cellular signaling, play an important role in mechanisms that results in a follicular development, implantation and also in the progression of a pregnancy to live birth. In other to understand the effect of chronic inflammation on follicular development in endocrine diseases such as polycystic ovary syndrome (PCOS), it seems logical to evaluate follicular fluid inflammatory biomarkers which is in direct contact with the follicular cells. This study aims to compare follicular fluid cytokine levels and post In-vitro Fertilization (IVF) pregnancy outcomes. Embryo development was assessed, revealing that women with PCOS had a higher number of oocytes retrieved on day 0, but a lower number of fertilized oocytes and embryos progressing to the cleavage stage and blastocyst stage compared to the control group. Cytokine analysis showed no significant difference in IL-6, IL-12, and TNFα concentrations between subjects with less than 50% oocyte fertilization rate, although IL-18 concentrations were higher in this group. Conversely, IL-12 and TNFα were significantly higher in women with more than 50% oocyte fertilization rate. Further analysis indicated that IL-12 and TNFα concentrations were associated with blastocyst development, with higher levels observed in women with more than two blastocysts. IL-12 was also identified as a discriminating cytokine for positive pregnancy outcomes, with significantly elevated levels in women who achieved pregnancy. No significant differences were found in IL-6, IL-18, and TNFα concentrations between women with positive and negative pregnancy outcomes. These findings suggest that specific cytokines, particularly IL-12 and TNFα, may play a crucial role in oocyte fertilization and embryo development in women undergoing fertility treatment.

Keywords

Follicular Fluid, Inflammatory Cytokines, Polycystic Ovary Syndrome (PCOS), IVF Outcomes, Pregnancy Rates, Cytokine Levels.

Introduction

Infertility is a disease of the male or female reproductive system defined by the failure to achieve a pregnancy after 12 months or more after regular unprotected sexual intercourse [1]. In females, infertility can be caused by many factors including uterine, tubal or ovarian disorders, some of these disorders can be inflammatory disorders such as those seen in polycystic ovary syndrome (PCOS) and endometriosis. Cytokines are important protein mediators of immunity, inflammation, cell proliferation, differentiation, and fibrosis [2]. With reference to inflammation, cytokines are mainly categorized as anti-inflammatory and pro-inflammatory cytokines according to their inflammatory responses and reactions in vivo, these cytokines are known to be produced by Th2 and Th1 cells [3]. Th1 cytokines including, IL-15, TNF-α, IFN-γ, IL-12 and IL-2 are cytokines that encourage cell-mediated immunity, these cytokines are also known to support chronic or acute inflammation and are associated with pregnancy loss [4]. Type-2 cytokines including, IL-6, IL-5, IL-4, IL-13 and IL-10 are cytokines that mainly enhance humoral immunity they are also anti-inflammatory in their actions and

are known to contribute to a successful pregnancy. Cytokines have also been associated with natural ovarian function, there are evidence showing that the ovary is a site of natural inflammatory response and that cytokines are constantly involved in the normal physiological functions of ovarian cells. Immune regulatory proteins including cytokines, growth factors and chemokines are generated in various cells including ovarian cells, this usually happens after the adaptive or innate immune activation but in the case of the ovary, a body of evidence suggests that cytokines may not necessarily be produced following innate or adaptive immune activation but that cytokines constitute part of the normal modulators of ovarian functions and may be produced through local secretions independent of the presence of leukocytes [5]. A body of evidence has revealed that cytokines are necessary for normal ovarian function and can play a major role in controlling the production of local ovarian steroids and also in the progression and retrogression of follicles in humans [6]. More so, Cytokines have been shown to also exhibit an important role in embryo division and growth, blastocyst implantation, aiding endometrial cell differentiation, forming of new cappilaries and ultimately, trophoblast invasion to form a healthy early pregnancy [7]. Follicular fluid is an important constituent within the intra follicular microenvironment, reflecting local secretory activities of the resident oocytes and its surrounding granulosa and theca cells and their resulting metabolite [8]. During *in vitro* fertilization (IVF), oocytes pickup procedure, follicular fluid is recovered as a by-product. Follicular fluid is an interesting body fluid to investigate given its proximity to oocytes. To the best of existing knowledge, there is paucity of data establishing the relationship between follicular fluid cytokines in PCOS and post IVF pregnancy rate. Therefore, this study aims to compare follicular fluid inflammatory cytokine levels with post-IVF pregnancy outcomes.

Study Area

This study was carried out in selected fertility Clinics across Southern Nigeria, namely Divine Trinity Specialist Hospital and Fertility Center Benin City, University of Benin teaching Hospital (UBTH) Benin City and Life International Hospital Awka. These facilities were independent private or public organizations and were equipped with needed facilities and expertise for in-vitro Fertilization treatment and PCOS diagnosis.

Study Population

The study group was taken from patients receiving *in-vitro* fertilization treatment in the selected fertility clinics. Women in this study were grouped according to their clinical history of infertility which includes polycystic ovary syndrome (PCOS), male factor infertility and idiopathic infertility. Women with idiopathic and/or male factor infertility were grouped as control group.

Selection Criteria

Inclusion Criteria

This study included women with PCOS and apparently healthy women whose partner were diagnosed with male infertility. The inclusion criteria for PCOS were based on the definition of PCOS adopted at the joint consensus meeting of the American Society for Reproductive Medicine and the European Society of Human Reproduction and Embryology (ASRM/ESHRE), namely the presence of two out of the following three criteria:

1. Oligo- and/or anovulation
2. Hyperandrogenism (clinical and/or biochemical) and
3. Polycystic ovaries with the exclusion of other etiologies [9].

Exclusion Criteria

This study excluded women who had any of the following conditions:

- History of, chronic hypertension
- Known autoimmune disorder
- Women who did not give consent
- Diabetes mellitus or treatment with oral glucocorticoids.
- Congenital adrenal hyperplasia
- Intake of exogenous hormone medications

Ethical Clearance and Consent

Ethical clearance was obtained from the University of Benin Teaching Hospital Ethics and Research Committee. Consent was obtained from study participants before collection of samples. Participants were informed that participation is voluntary, and the confidentiality and privacy of all participants was respected they were assured that there was no penalty for refusal or withdrawal from participation.

Follicular Fluid Sample Collection

Follicular fluid samples were collected from the first punctured follicles during oocyte retrieval after ovarian hyper stimulation with the short antagonist protocol. Ovulation was triggered by recombinant human chorionic gonadotropin (Pregnyl, Baxter USA) when at least three follicles measure larger than 17 mm. Oocytes were retrieved 36 hrs later aided by trans-vaginal ultrasound guidance. Aspirated follicular fluid were aseptically transferred into a sterile plate for oocyte pick-up, the residual follicular fluid was aseptically transferred into sterile 15ml Falcon tubes for storage at -20°C until used for cytokine assay

Quantitative estimation of follicular fluid cytokines

Follicular fluid cytokines (IL-6, IL-12, IL-18 and TNF-alpha) levels were measured using (Abcam, Massachusetts USA) ELISA kits. Briefly, FF samples and standards were added to wells in a 96 well plates coated with monoclonal antibody specific for each cytokine, Biotinylated labelled antibody specific for each cytokine were added and incubated, the wells were washed thoroughly then the enzyme Streptavidin Horseradish Peroxide was added to the wells and incubated. After the final wash, TMB substrate solution was added for a colour reaction, the intensity of this coloured products were read at 450 nm using the BioTek 800 TS Absorbance plate reader.

Oocyte Insemination

Following the oocyte retrieval procedure, oocyte-cumulus complex (COC) was routinely incubated for at least 2 hours in an organ dish, containing Human Tubal Fluid (HTF) media (LifeGlobal, LLC, Gilford, CT) with 10% of Human Serum Albumin (HSA, LifeGlobal, LLC, Gilford, CT) after retrieval. Semen samples produced from the subject's pathner were washed under aseptic conditions using the gradient centrifugation method (Origio Gradient Solution. Copenhagen, Denmark), Depending on the sperm count, the COC were inseminated with appropriate volume of washed sperm or injected using intracytoplasmic sperm injection (ICSI). COCs were cultured under mineral oil with Embryo culture media (SAGE 1 Step, Origio, Copenhagen, Denmark) with 10% HSA.

Fertilization Assessment

A first fertilization check was performed at approximately 18 hours after the cumulus-oocyte complex (coc) *in-vitro* insemination procedure on day-1 post-fertilization by checking under the stereo zoom microscope for presence of two pronuclei (PN). Using a 135Um diameter micropipette, fertilised oocytes were separated from the digested coc and transfered into a fresh cell culture dish containing fresh embryo culture media, following the numerical order in the culture dish, pictures of and notes on each oocyte were taken. The culture dish containing the zygotes was then incubated uninterrupted until day-5, the day of the embryo transfer.

Blastocyst Development Assessment

The Gardner blastocyst grading system was utilized in this study to assigns blastocyte quality score. Three separate quality scores to each blastocyst embryo these qualities include:

Blastocyst development stage – expansion and hatching status

Inner cell mass (ICM) score, or quality

Trophectoderm (TE) score, or quality

Blastocysts were given a quality grade for each of the 3 components and the score was expressed with the expansion grade listed first, the inner cell mass grade listed second and the trophectoderm grade third. For example, a blastocyst quality grade of 4AB means that the blastocyst is expanded (grade 4), has many tightly packed cells in the inner cell mass (grade A), and has a trophectoderm with few cells forming a loose epithelium (grade B).

Embryo Transfer

Embryo were transfered to each subject on day 5 after oocyte development, the embryo transfer catheter was loaded (Embryo replacement catheter, Wallace, Germany) with the embryos, the physician passed the loaded embryo through the cervical opening of the subjects up to the middle of the uterine cavity. Abdominal ultrasound was used simultaneously to guide the catheter tip advance to the proper location. When the catheter tip reached the ideal location, the embryos were then "transferred" (squirted out of the catheter) to the lining of the uterine cavity (endometrial lining) by the infertility specialist physician. The catheter was slowly withdrawn and checked under a microscope for any retained embryos.

ß-HCG assay for pregnancy confirmation

Pregnancies were confirmed by quantitative measurement of serum beta-human chorionic gonadotropin on day 14 following embryo transfer, serum concentrations of B-HCG were measured using fluorescent immunosorbent assay meter (Finecare Wondfo, Japan). ß-HCG levels greater than 25MIU/ml were considered as viable pregnancy.

Statistical Analysis

Statistical Package for Social Sciences (SPSS) was used for statistical analysis. Most of the data are shown as mean ± standard error of mean (SEM) and a p-value <0.05 is considered as significant. Statistical differences were calculated with Fisher test and $\chi 2$ test. Correlation was calculated by Pearson's correlation test or Spearman's test. A two-way ANOVA was used to compare corresponding factors.

Results

A total of 206 women attending fertility clinics in 4 facilities across the study area were recruited for this study, study subjects were divided into two groups according to the Rotterdam diagnostic criteria for PCOS. 101 (49%) women who met the Rotterdam criteria for diagnosis of PCOS were grouped as the PCOS group while, 106 (51%) apparently healthy women who had male factor infertility were recruited and grouped as the control group. The study group age ranged from 26 to 53 yrs, there was no significant difference between the mean ages of the PCOS and the control group with mean SD (29.46 ± 4.3) for PCOS group vs (30.44 ± 3.1) for the control group. The mean body mass index (BMI) in the PCOS (25.37 ± 5.9) group were significantly higher than mean BMI of the control group (24.29 ± 3.8) on the other hand, the mean number of menstrual cycle/yr in the PCOS group were significantly lower than that of the control group (5 ± 4.63 and 9 ± 3.04) respectively.

Figure 1 represents a graphical representation of embryo development in the study population. A higher no of oocytes were retrieved from women with PCOS compared to the control group on day 0. Day one

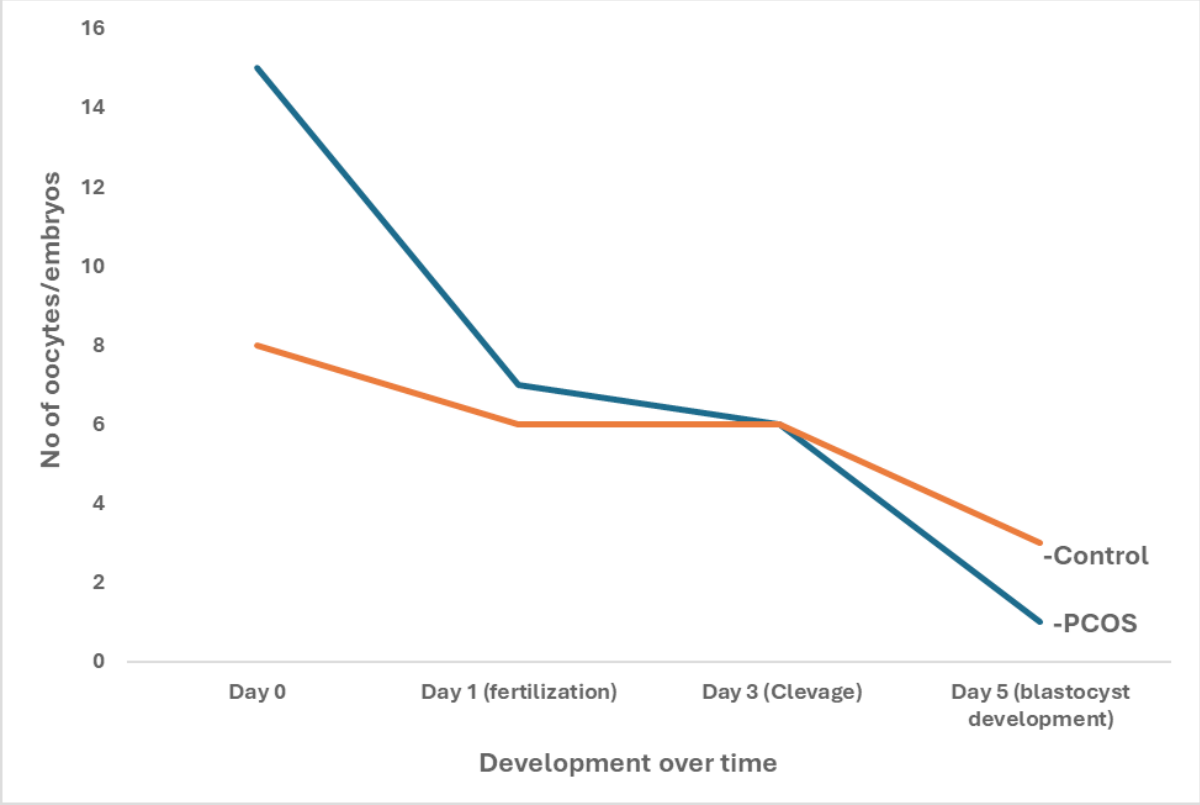

Figure 1: Embryo development in study subjects.

Table 1: Effect of cytokine concentrations on oocyte fertilization.

	<50% Fertilization rate			>50% Fertilization rate		
Cytokine	**PCOS Mean ± SEM (pg/ml)**	**Control Mean ± SEM (pg/ml)**	**Sig**	**PCOS Mean ± SEM (pg/ml)**	**Control Mean ± SEM(pg/ml)**	**Sig**
IL-6	6.291±1.117	6.370±1.513	>0.05	7.043±1.683	6.328±1.973	>0.05
IL-12	1.174±0.109	1.035±0.271	>0.05	1.224±0.801	1.977±0.193	**<0.05***
IL-18	48.298±5.052	37.172±6.391	**<0.05***	42.618±7.364	44.045±6.921	>0.05
TNF	25.124±2.402	24.057±2.382	>0.05	29.118±2.657	23.901±1.582	**<0.05***

SEM= Standard Error of Mean; χ^2=chi-square value; *significant < 0.05

Table 2: Cytokine concentrations and blastocyst development.

Cytokine	0 Blastocyst (Mean ± SD)	1-2 Blastocyst (Mean ± SD)	> 2 Blastocyst (Mean ± SD)	Significance
IL-6	8.303±5.332	7.374±6.631	7.950±7.523	>0.05
IL-12	0.474±1.791	1.354±1.762	2.835±1.547	**<0.05***
IL-18	40.337±10.167	39.801±13.012	43.661±8.436	>0.05
TNFα	18.389±5.618	25.930±3.501	29.227±2.488	**<0.05***

SEM= Standard Error of Mean; χ^2=chi-square value; *significant < 0.05

Table 3: Evaluation of pregnancy outcome in relation to cytokine levels.

Pregnancy				
Cytokine	**Positive (Mean ± SEM)**	**Negative (Mean ± SEM)**	**χ^2**	**SIG**
IL-6	11.03±1.753	9.65±1.121	0.4885	P>0.05
IL-12	2.036±0.153	1.624±0.082	0.0114	P<0.05*
IL-18	64.24± 12.41	60.13±15.97	0.891	P >0.05
TNFα	25.27±9351	20.63±9.059	0.6013	P>0.05

SEM= Standard Error of Mean; χ^2=chi-square value; *significant < 0.05

reveals the number of embryos fertilized following IVF insemination with partner's washed sperm. The PCOS group shows a steep drop in the number of oocytes fertilized whereas the control group had a higher number of fertilized oocytes. On day 3, the control group graph plateaued maintaining the same number of oocytes developing into embryos. On the other hand, there was a drop in the number of oocytes progressing to cleavage stage in the PCOS group. Finally, there was a higher number of embryos that developed into blastocyst in the control group compared to the PCOS group on day 5.

The effect of follicular fluid cytokine concentration on oocyte fertilization is revealed in table 1. There was no significant difference in IL-6, IL-12 and TNFα concentrations between study subjects with <50% oocyte fertilization rate. However, IL-18 concentrations were significantly higher in women with <50% oocyte fertilization rate. On the other hand, IL-12 and TNFα were significantly higher in women with >50% oocyte fertilization rate.

Table 2 shows the relationship between follicular fluid cytokine concentration and blastocyst development in the study subjects. IL-12 and TNFα were discriminatory in the development of blastocyst, the mean concentrations of IL-12 and TNFα increased with increasing blastocyst number, women with more than 2 blastocyst had elevated concentrations of follicular fluid IL-12 and TNFα. On the other hand, there was no significant difference between the concentrations of IL-6 and IL-18 in women with no blastocyst development and women with more than 2 blastocyst.

Discussion

In recent years, researchers have focused on evaluating the role of serum cytokines in pregnancy outcomes following medically assisted reproduction in healthy fertile females, these studies revealed that certain cytokines were necessary for successful IVF outcomes including higher fertilization rates, higher embryo progression and increased pregnancy rates, these cytokines include (IL)-1, IL-6 and vascular endothelial growth factor (VEGF) [10], on the other hand, certain cytokines were associated with adverse post IVF outcomes including reduced pregnancy rate and failed pregnancy , cytokines identified for poor pregnancy outcome were IL-15, IL-12 and VEGF [10]. Other studies found that cytokine profiles present in the follicular fluid were altered according to the cause of infertility [11]. The effect of follicular fluid cytokine concentrations on fertilization and embryo development was evaluated in this study, we evaluated the expression of four (4) different cytokines in the follicular fluid of women attending fertility clinics in relation to oocyte fertilization rate and progression to blastocyst embryo. Ultimately, we found some cytokines to be discriminatory in the fertilization and development of oocytes retrieved from the study subjects. The mammalian ovulation event can be considered from the perspective of an inflammatory reaction, with pro-inflammatory cytokines produced and functionally interacting throughout the process. TNF-α in IVF has already been the subject of much study by infertility researchers. Some authors have concluded that follicular TNF-α might deteriorate the microenvironment in the follicle, thereby negatively affecting oocyte and embryo quality [12]. Still others have proposed a positive role of TNF-α regarding oocyte quality, and ovulation. Overall, the roles of TNF-α in female reproduction are likely to be complex and dynamically involved in the different stages of folliculogenesis, however, this study observed a significant elevation in the mean levels of TNF- alpha in the follicular fluid of women with better embryo development. Another cytokine observed to significantly influence the maturation of embryos in this study is IL-12, IL-12 has been suggested to favor ovarian folliculogenesis, a positive correlation has been reported between the level of follicular IL-18 and the number

of retrieved oocytes and implantation success in women with different etiologies of infertility [13]. Previous studies examining IL-12 in the follicular fluid have yielded contradictory results. Nevertheless, a majority of the findings have indicated that IL-12 is associated with a positive effect on folliculogenesis, oocyte quality and implantation, this study observed a significant difference in the levels of IL-12 in women with good fertilization rate and women with poor fertilization rate, IL-12 was also significantly elevated in women with greater than 50% blastocyst development compared to women with poor blastocyst development, this also resulted in a significant difference in pregnancy rate. The general model on the biological role of IL-12 predicts that this cytokine is required for the establishment of organ-specific autoimmunity [14]. According to such a model, IL-12, produced by activated hematopoietic phagocytic cells (monocytes, macrophages, and neutrophils), by dendritic cells (DC), and by the recently identified IFN-producing killer DC lineage, acts as a critical regulator of cell-mediated responses. IL-18 was found to have a positive effect in oocyte fertilization as there was a significant elevation of follicular fluid IL-18 in women that had better oocyte fertilization rate.

Conclusion

In conclusion, this study highlights the significant role of IL-12 in the maturation of embryos and its positive correlation with ovarian folliculogenesis, oocyte quality, and implantation success. The findings demonstrate that higher levels of IL-12 are associated with better fertilization rates and increased blastocyst development, ultimately leading to higher pregnancy rates. Despite some contradictory results in previous studies, the majority of evidence supports the beneficial effects of IL-12 in reproductive outcomes. Additionally, the study underscores the importance of IL-18 in enhancing oocyte fertilization, further contributing to successful reproductive processes. These insights into the roles of IL-12 and IL-18 provide valuable information for improving fertility treatments and understanding the mechanisms underlying successful embryo development and implantation.

References

1. Segal TR, Giudice LC. Before the beginning: environmental exposures and reproductive and obstetrical outcomes. Fertility and Sterility. 2019; 112: 613-621.
2. Abdulkhaleq LA, Assi MA, Abdullah R, et al. The crucial roles of inflammatory mediators in inflammation: A review. Vet World. 2018; 11: 627-635.
3. Wautier JL, Wautier MP. Pro- and Anti-Inflammatory Prostaglandins and Cytokines in Humans: A Mini Review. Int J Mol Sci. 2023; 24: 9647.
4. Yockey LJ, Iwasaki A. Interferons and Proinflammatory Cytokines in Pregnancy and Fetal Development. Immunity. 2018; 49: 397-412.
5. Field SL, DasguptaT, Cummings M, et al. Cytokines in ovarian folliculogenesis, oocyte maturation and luteinisation. Mol Reprod Dev. 2014; 81: 284-314.
6. Richards JS, Pangas SA. New insights into ovarian function. Handb Exp Pharmacol. 2010; 198: 3-27.
7. Vujisić S, Lepej SZ, Aksamija A, et al. B- and T-cells in the follicular fluid and peripheral blood of patients undergoing IVF/ET procedures. Am J Reprod Immunol. 2004; 52: 379-385.
8. Ibadin Ngozi Ogbenyalu, Okojie Rachael O, Ibadin Kennedy Osegua. Microbiology of Follicular Fluid Aspirated in Women with Polycystic Ovarian Syndrome (PCOS) Attending Fertility Clinic on Southern Part of Nigeria. Gynecol Reprod Health. 2023; 7: 1-6.
9. Azziz R, Carmina E, Dewailly D, et al. Positions statement: criteria for defining polycystic ovary syndrome as a predominantly hyperandrogenic syndrome: an Androgen Excess Society guideline. J Clin Endocrinol Metab. 2006; 91: 4237-4245.
10. Rao VA, Kurian NK, Rao KA. Cytokines, NK cells and regulatory T cell functions in normal pregnancy and reproductive failures. Am J Reprod Immunol. 2023; 89: e13667.
11. Ngozi O Ibadin, Rachael O Okojie, Kennedy O Ibadin. The Determination and Comparative Study of Basic Hormonal Assessment in Polycystic Ovary Syndrome (PCOS) Women Undergoing Assisted Reproduction Treatment in Southern Part of Nigeria. IJSBAR. 2023; 72: 53-61.
12. Romanowska-Próchnicka K, Felis-Giemza A, Olesińska M, et al. The Role of TNF-α and Anti-TNF-α Agents during Preconception, Pregnancy, and Breastfeeding. Int J Mol Sci. 2021; 22: 2922.
13. Nisari M, Ulger H, Unur E, et al. Effect of interleukin 12 (IL-12) on embryonic development and yolk sac vascularisation. Bratisl Lek Listy. 2014; 115: 532-537.
14. Liu J, Cao S, Kim S, et al. Interleukin-12: an update on its immunological activities, signaling and regulation of gene expression. Curr Immunol Rev. 2005; 1: 119-137.