The Impact of Assisted Reproduction on First Trimester Biochemical Markers in Singleton and Twin Pregnancies

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The objective was to determine the effect of in vitro fertilization (IVF) on nuchal translucency, free beta-human chorionic gonadotropin (Free β-HCG) and pregnancy-associated plasma protein-A (Papp-A) measurements for singleton and twin pregnancies. This was a retrospective population-based register study that included 2921women with 11-13 weeks of gestation singleton and twin pregnancies. All patients were evaluated for the first trimester Down syndrome combined screening. We identify the effect of assisted reproductive technologies (ART) such as in vitro fertilization (IVF) on the nuchal translucency (NT), free beta-human chorionic gonadotropin (β-HCG) and pregnancy-associated plasma protein-A (PAPP-A) values. The study included 2921 patients: 2526 with spontaneous conception (86.5%) and 395 following IVF (13.5%); in our study singleton pregnancies were 2660 (91.1%), dichorionic twin pregnancies 232 (7.9%) and 12 (0.4%) monochorionic twin pregnancies. The mean β-HCG were about 56.67 IU/l (95%CI: 54.98-58.36) in singleton pregnancies and 128.08 IU/l (95%CI: 115.72-140.45) in twin pregnancies. PAPP-A mean values in singleton pregnancies were 3.54 IU/l (95%CI: 3.42-3.66) and 5.25 IU/l (95%CI: 4.46-6.08) in twin pregnancies. The results were statistically significant with p value $p \leq 01$. There were also significant differences between β-HCG values in spontaneous pregnancies HCG 63.35 IU/l (95%CI: 60.14-64.10) and IVF pregnancies HCG 99.9 IU/l (95%CI: 91.8-107.99) with p value $p \leq 0.01$. In our study the IVF had no impact on nuchal translucency values. Free β-HCG levels were higher in twin pregnancies and IVF pregnancies. PAPP-A concentrations were exceeded in twin pregnancies.

Keywords: in vitro fertilization, pregnancy-associated plasma protein-A,beta-human chorionic gonadotropin
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Assisted reproductive technologies (ART) and IVF have significantly increased since the first baby was born 35 years ago [1].Nowadays studies are concerned on ART long term consequences and children perinatal outcomes and risks[2].The most important complication of ART is the increased prevalence of multiple pregnancies after IVF. Twin pregnancies are associated with poorer perinatal and infant outcome [3]. An important problem related to IVF and multiple pregnancies is based on prenatal diagnosis including chromosomal risks evaluation and obstetrics relates issues.

The objective of our study was to determine if IVF has an impact on the most important parameters from the first trimester screening: nuchal translucency, free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A in singleton and twin pregnancies.

Experimental part

This was a retrospective population-based register study that included 2921women with singleton and twin pregnancies between 11-13 weeks of pregnancy. This is an unselected population of consecutively recruited patients. All patients were evaluated in our unit from July 2011 to January 2014 for first trimester Down syndrome combined screening.

The evaluation protocol consisted in first trimester ultrasound scan performed by our FMF certified physicians. Crown -lump length, nasal bone presence, NT value were evaluated and recorded in our data base. On the same day or within 24 h period the patient had blood test for free beta-human chorionic gonadotropin (β-HCG) and pregnancy-associated plasma protein-A (PAPP-A). The results were recorded in the data base and the risk assessment was performed using Kryptor method.

In order to evaluate the impact of the ART on the nuchal translucency (NT), free beta-human chorionic gonadotropin (β-HCG) and pregnancy-associated plasma protein-A (PAPP-A) two groups were defined: the control one (spontaneous conceptions) and the IVF pregnancy group.We adjusted the measurements of β-HCG, PAPP-A and NT by known maternal factors and gestation at screening before evaluating the impact of IVF in singleton and twin pregnancies. Individual MoM values were calculated for PAPP-A and HCG after correction for gestational age as well as for maternal and pregnancy factors, except for IVF conception. Our statistical analysis

refers to the corrected MoM values and absolute mean values.

The data were analyzed using SPSS version 19.0 (statistical packages for social sciences). As first step we analyzed the characteristics of the whole group, then each group separately: singleton pregnancies, twin pregnancies, spontaneous or IVF pregnancies. Pearson's correlation as appropriate and two sided P values of <0.05 were considered to indicate statistical significance. Anova test was used to compare and evaluate statistical significance between and within groups.

The study included 2921 patients: 2526 with spontaneous conception (86.5%) and 395 following IVF (13.5%); our study singleton pregnancies were 2660 (91.1%), dichorionic twin pregnancies 232 (7.9%) and 12 (0.4%) monochorionic twin pregnancies. Although the study contained only 12 cases of monochorionic twin pregnancies, it was possible to evaluate the differences in â-HCG, PAPP-A and NT between monochorionic and dichorionic twin pregnancies using Mann-Whitney U test which evaluated the distribution form within each group and permitted to identify the differences between the groups with the same distribution form.

Results and discussions

We realized a retrospective study with data since 2011-2014. The study included 2921 pregnant patients with gestational age between 11-13 weeks of gestation. All patients were Caucasian. In our unit we performed only intrauterine insemination and IVF (with or without ICSI). We didn't perform embryo transfer, oocyte donations doing inconsistence of legal regulations. The intrauterine inseminations were not realized on natural menstrual cycles. All procedures implied ovarian stimulation protocols. Even if it was a population study to eliminate the bias we excluded cases patient with other ART than IVF and multiple pregnancies with more than 2 embryos.

The majority of pregnancies 2526 (86.5%) were obtained spontaneous and 395 using IVF (13.5%). The incidence of trisomy 21 for our study was 0.3%(9 cases). The study included 2921 pregnant patients: 2660 (91.1%) with singleton pregnancies, 232 (7.9%) with dichorionic twin pregnancies and 12 cases with monochorionic twin pregnancies (0.4%). Mean CRL (crown-rump length) for the group study was 63.18 mm (mean 45, max 84, std deviation 8.186), mean NT (nuchal translucency) was 1.66 mm (min 0-max 23 mm, std deviation 0.824) mean PAPP-A was 3.70 IU/l (IU/l) (min 0, max 60, std deviation 3.685) mean PAPP-A(MoM) was 1.26 (min 0, max 28, std deviation 0.929) and mean HCG 63.35 (min 0, max 444, std deviation 53.84).

We found the following direct correlations within the whole study group: CRL with NT and PAPP-A (IU/l), NT with PAPP-A (IU/l) and PAPP-A (MoM) all with p value ≤ 0.01. The correlations were significant at the 0.01 level.

Within spontaneous pregnancies we identified the following parameters mean values CRL 63.15 mm (mean 45, max 84, std deviation 8.28), NT 1.64 mm (min 0-max 23mm, std deviation 0.805), PAPP-A (IU/l) 3.75 (min 0, max 60 mean, std deviation 3.54) mean 1.27 PAPP-A (MoM) (min 0, max 9 , std deviation 0.779).

Direct correlations for spontaneous pregnancies: CRL with NT and PAPP-A (IU/l) p value ≤0.01. CRL didn't correlate with PAPP-A (MoM) p= 0.822. NT and PAPP-A (IU/l) p= 0.331, NT and PAPP-A (MoM) p=0.574. The correlations were significant at the 0.01 level.

Studying the group with pregnancies conceived using IVF we obtained that CRL mean value was 63.36 mm (mean 45, max 84, std deviation 7.565) and NT mean value was 1.75 mm (min 1-max 15 mm, std deviation 0.932).

Direct correlations for IVF patients CRL with NT p= 0.016 and CRL with PAPP-A (IU/l) p= 0.014, CRL didn't correlate with PAPP-A (MoM). The correlations were significant at the 0.05 level. NT correlated with PAPP-A (IU/l) p value ≤0.01. The correlations were significant at the 0.01 level.

The twin group was characterized by mean CRL 63.16 mm (min 45, max 84, std deviation 7.74), mean NT of 1.71 mm (min 1-max 5 mm, std deviation 0.605), PAPP- A (IU/l) (total 212)- min 0, max 60 mean 5.51, std deviation 7.64, PAPP- A (MoM) (total 195) min 0, max 8, mean 1.15, std deviation 0.921.

Direct correlations for all twin patients with: CRL with NT p value ≤0.01.

We selected the cases of spontaneous twin and we identified that CRL was 63.11 mm (mean 45, max 84, std deviation 9.71), NT mean was 1.6 mm (min 1-max 4 mm, std deviation 0.623), PAPP- A (IU/l) mean was 9.95 (min 0, max 60, std deviation 13.078, PAPP- A (MoM) min 0, max 8, mean 1.55, std deviation was 1.67, β-HCG (IUI) , mean 124.4 IUI (min 22-max 431, std deviation 119.33), β-HCG (MoM) min 0, max 5, mean 1.63, std deviation 1.40.

Correlations for all spontaneous twin patients: CRL and NT p=0.011, CRL and PAPP-A, Free HCG 0.027. The correlations were significant at the 0.01 level.

We separated the cases of IVF twin. The values for CRL were 63.18 mm (mean 45, max 84, std deviation 7.22), NT mean value was 1.73 mm (min 1-max 4 mm, std deviation 0.600), PAPP- A (IU/l) mean value was 4.48 (min 0, max 44, std deviation 5.23), PAPP- A (MoM) mean 1.05 (min 0, max 8, std deviation 0.58), β-HCG (IUI) , mean 130.95 IUI (min 31-max 444, std deviation 78.994), β-HCG (MoM) (min 0, max 3, mean 1.18, std deviation 0.59).

Correlations for all IVF twin patients: CRL and NT, CRL and PAPP-A (IU/l), CRL and PAPP-A (MoM), p value ≤0.01 NT and PAPP-A (MoM) p=0.025.

We observed that there are statistically significant differences between β-HCG and PAPP-Avalues in singleton and in twin pregnancies, with increased values for twin pregnancies. Considering the conception mode as comparison factor there were identified statistically significant differences for β-HCG between spontaneous and IVF pregnancies with higher values for IVF pregnancies.

After analysing the twin pregnancies group we identified that in IVF pregnancies only PAPP-A had higher values for IVF pregnancies, but not PAPP-A MoM or β-HCG.

Considering IVF the comparing factor between singleton and twin pregnancy, we observed that β-HCG and β-HCG MoM were elevated in IVF singleton pregnancies than spontaneous singleton pregnancies. Finally, comparing only by IVF pregnancies the significant difference was observed in β-HCG values. This is the reason why it should be discussed about a correction factor for β-HCG values in IVF singleton pregnancies.

The results were statistically significant with *p* value ≤0.01.

In the past 20 years, maternal age was associated with first trimester ultrasonographic examination of the fetus and biochemical measurement of different proteins or hormones in the maternal circulation to identify high risk pregnancies for trisomies or obstetrical conditions such as fetal growth restriction or preeclampsia. This approach of screening can nowadays identify over 90% of affected fetuses, but there is still a need for invasive testing in 3–5% of population [4].

The initial traditional method of identifying the high risk pregnancies was advanced maternal age, but this type of

	Total singleton pregnancies		Total twin pregnancies		P value
	Mean value	CI 95%	Mean value	CI 95%	
CRL	63.165	62.85-63.48	63,49	62.39-64.59	0.081
NT	1.65	1.62-1.68	1.72	1.64-1.80	0.82
β-HCG	56.67	54.98-58.36	128.08	115.72-140.45	0.001
β-HCG (MoM)	1.17	1.05-1.29	1.24	1.13-1.36	0.498
PAPP-A	3.54	3.42-3.66	5.25	4.46-6.08	0.001
PAPP-A(MoM)	1.27	1.24-1.31	1.07	0.99-1.16	0.141

Table 1
PARAMETERS FOR SINGLETON AND TWIN PREGNANCIES (MARKED IN BLUE- STATISTICALLY SIGNIFICANT DIFFERENCES BETWEEN GROUPS)

	Spontaneous singleton pregnancies		IVF singleton pregnancies		P value
	Mean value	CI 95%	Mean value	CI 95%	
CRL	63.62	62.46-64.78	63.47	62.18-64.77	0.155
NT	1.75	1.66-1.84	1.77	1.58-1.95	0.58
β-HCG	130.65	117.99-143.31	68.43	60.78-76.07	0.001
β-HCG (MoM)	1.19	1.09-1.28	1.17	1.05-1.29	0.001
PAPP-A	4.71	3.85-5.57	2.43	1.86-3.00	0.119
PAPP-A(MoM)	1.04	0.95-1.14	1.43	1.08-1.79	0.91

The results were statistically significant with p value ≤ 0.01.

	Spontaneous pregnancies		IVF pregnancies		P value
	Mean value	CI 95%	Mean value	CI 95%	
CRL	63.14	62.81-63.47	63.55	62.69-64.41	0.64
NT	1.63	1.6-1.66	1.75	1.65-1.86	0.014
β-HCG	63.35	55.07-58.80	99.9	91.8-107.99	0.001
β-HCG (MoM)	1.21	1.18-1.24	1.18	1.10-1.25	0.47
PAPP-A	3.72	3.59-3.84	3.55	3.03-4.07	0.06
PAPP-A (MoM)	1.26	1.23-1.29	1.24	1.06-1.42	0.65

Table 2
PARAMETERS FOR SPONTANEOUS AND IVF PREGNANCIES (MARKED IN BLUE- STATISTICALLY SIGNIFICANT DIFFERENCES BETWEEN GROUPS)

The results were statistically significant with p value ≤ 0.01.

screening requires invasive testing in about 5% of pregnant women and it can identify 30% of affected fetuses [5-10].

The majority of IVF research is concerned on singletons, and data on development of IVF twins are limited [6].

The data about the effect of assisted reproductive technologies (ART) on the ultrasound and biochemical components of first trimester combined screening for trisomy are controversial. A systematic search of the literature was performed to identify the effect of ART, particularly in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) with fresh embryo transfer, on the nuchal translucency, free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A measurements. Furthermore, it was performed a meta-analysis that established the ratios between ART and spontaneous pregnancies values of median of the multiple of median (m0 MoM). Free beta-human chorionic

	IVF twin pregnancies		IVF singleton pregnancies		P value
	Mean value	CI 95%	Mean value	CI 95%	
CRL	63.14	62.8-63.48	63.47	62.18-64.77	0.52
NT	1.62	1.59-1.66	1.77	1.58-1.95	0.013
β-HCG	55.84	54.07-57.61	68.43	60.78-76.07	0.001
β-HCG (MoM)	1.20	1.17-1.24	1.17	1.05-1.29	0.61
PAPP-A	3.65	3.52-3.77	2.43	1.86-3.00	0.025
PAPP-A(MoM)	1.26	1.23-1.29	1.43	1.08-1.79	0.61
	Spontaneous twin pregnancies		IVF twin pregnancies		P value
	Mean value	CI 95%	Mean value	CI 95%	
CRL	62.94	59.81-66.08	63.62	62.46-64.78	0.96
NT	1.61	1.39-1.83	1.75	1.66-1.84	0.21
β-HCG	116.97	78.92-155.03	130.65	117.99-143.31	0.67
β-HCG(MoM)	1.5	1.05-1.95	1.19	1.09-1.28	0.003
PAPP-A	7.69	5.63-9.75	4.71	3.85-5.57	0.001
PAPP-A(MoM)	1.19	0.97-1.42	1.04	0.95-1.14	0.002

Table 3
PARAMETERS FOR IVF PREGNANCIES (MARKED IN BLUE- STATISTICALLY SIGNIFICANT DIFFERENCES BETWEEN GROUPS)

gonadotropin test showed slightly higher values in the ICSI group than controls (RR = 1.09, 95%CI: 1.03-1.16), but not in the IVF group (RR = 1.03, 95%CI: 0.94-1.12). Pregnancy-associated plasma protein-A values for IVF/ICSI, IVF and ICSI showed lower values in comparison with controls (RR, 95%CI 0.85, 0.80-0.90; 0.82, 0.74-0.89 and 0.83, 0.79-0.86, respectively). The nuchal translucency measurement did not show any statistical differences between study groups (IVF and ICSI) and controls (RR = 1.00, 95%CI: 0.94-1.08 and RR = 1.01, 95%CI: 0.97-1.05, respectively). These results may be due to alterations in the placentation of ART pregnancies [7].

In our study measurements in singleton and twin pregnancies for mean β-HCG were about 56.67 IU/l (95% CI: 54.98-58.36) in singleton pregnancies and 128.08 IU/l (95% CI: 115.72-140.45) in twin pregnancy. PAPP-A recorded mean values in singleton pregnancies about 3.54 IU/l (95% CI: 3.42-3.66) and 5.25 IU/l (95% CI: 4.46-6.08) in twin pregnancies. The results were statistically significant with p value p≤0.01. There were also significant differences between -HCG values in spontaneous pregnancies -HCG 63.35 IU/l (95% CI: 60.14-64.10) and IVF pregnancies -HCG 99.9 IU/l (95% CI: 91.8-107.99) with p value ≤0.01.

Studies comparing first trimester maternal screening in IVF women with those that conceived spontaneously remarked a decreased serum PAPP-A level and a higher nuchal translucency after IVF, this theoretically being associated with an increased risk of Down syndrome. But we should also take into consideration the fact that in IVF singleton pregnancies the incidence of vanishing twin is higher than in spontaneously pregnancies and that could influence biochemical first trimester markers (Lanes). Anckaert found in his study that IVF pregnancies are associated with lower serum PAPP-A level and that particularly in non-male infertility there are higher serum level of HCG than in spontaneous pregnancies. The hypothesis for this result is similar with the explanation of higher HCG in Down syndrome: a weakly, abnormally glicosilated HCG, with a lower clearance by placenta, or a lower predisposition for LH/HCG receptor leading to a lack of hormone used. This higher level of HCG in non male infertility may suggest a relationship between abnormal placenta and infertility. Bellver in his study found that NT is not influenced by the conception mode. However, in ART pregnancies with nondonor oocytes there are lower serum level of PAPP-A, but the false positive pregnancy rate (FPR) is increased only in ICSI patients. In donor cycles there are higher levels of serum HCG but this could not influence FPR. In frozen cycles serum PAPP-A level is lower in patients which are taken estrogens for endometrial preparation, but not in natural cycles. There are different explanations regarding this lower serum of PAPP-A in ART patients. One is related to the infertility itself, being observed that patients with more than two years to pregnancy had lower serum PAPP-A in comparison to control. Also the delayed in placental maturation in infertility, the drugs used for ovarian stimulation, the laboratory manipulation or all these together could contribute to this finding [8,9].

Low PAPP-A in twin pregnancy, <5th % percentile is associated with low body weight and could be an useful tool for screening for growth disorders in twin pregnancies. Another interesting characteristic of ART twin pregnancies biochemical first trimester screening is that they present level of free β-HCG higher than in spontaneous twin pregnancies [10,11].

A systematic MESH-term search in MEDLINE using PubMed and the Cochrane Library was performed until May 2011, with no earlier date limit retrieved 562 citations, 96 of which were evaluated in detail and 57 were then excluded for not meeting the selection criteria. A total of 61 articles were finally selected for review. The data shows that, for IVF/ICSI singletons, combined first trimester prenatal screening based on maternal age, nuchal translucency scan and biomarkers is appropriate. However, biomarkers seem to be altered, causing a higher false-positive rate, in IVF/ICSI singleton gestations. Correction factors have been developed and should be used when screening for Down's syndrome in singleton pregnancies. With regard to IVF/ICSI twin pregnancies, biomarker values seem to be dependent on chorionicity as well as gestational age. Whether the use of a correction factor for mode of conception in the risk calculations for Down's syndrome in twin pregnancies is valid has not been fully elucidated. First trimester prenatal screening after IVF/ICSI treatment requires specific precautions in both singleton and twin[12]. However, a Canadian consensus made recommendations on prenatal screening for and diagnosis of fetal aneuploidy (e.g., Down syndrome and trisomy 18) in twin pregnancies [13].

We found that in IVF singleton pregnancies there is a significant higher value for free β-HCG. This parameter can influence independently the risk result for Down syndrome and the impact is even more important when both of those parameters are modified, also in high risk pregnancy[14- 17] In order to provide an accurate risk for IVF singleton pregnancies there are required further studies to estimate the correction factor. Mothers with IVF pregnancies tend to be older and the percentage of twin pregnancies is higher with IVF[18].Alternative methods of screening need further validation in both IVF and twin pregnanciestesting such as cffDNA that can be feasible and can enhance the diagnostic spectrum of non-invasive prenatal testing[19].

Conclusions

In our study the IVF had no impact on nuchal translucency values. Free β-HCG levels were higher in twin pregnancies and IVF pregnancies. PAPP-A concentrations were exceeded in twin pregnancies.Accurate conventional first trimester screening is especially useful in IVF pregnancies.

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