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Perinatal outcomes, risk perception and psychological adjustment in twin and singleton pregnancies: does assisted reproduction technologies makes the difference?

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Aos “meus amores pequeninos”, P&S,

por serem a fonte de inspiração deste trabalho.

Title

Perinatal outcomes, risk perception and psychological adjustment in twin and singleton pregnancies: does assisted reproduction technologies makes the difference?

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Functions and responsibilities

The design and execution of the study are student's responsibility.

The advisers had the function of guiding in the design and study execution.

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RESUMO

Introdução: O número crescente de gravidezes gemelares é, em parte, devido, às técnicas de procriação medicamente assistida (PMA). A gravidez gemelar é uma condição de alto risco, geralmente associada a idade materna avançada e significativa morbilidade e mortalidade perinatal. O impacto da PMA nos resultados perinatais, perceção de risco e ajustamento psicológico não é, ainda, muito claro e os estudos existentes não são consensuais. Além disso, alguns resultados associados à PMA (ex. parto pré-termo) podem ser consequência de um elevado número de gravidezes gemelares e não da PMA *per se*. Assim, é importante compreender o impacto da PMA e distingui-lo do impacto causado por gravidez gemelar.

Objetivos: Este estudo pretende comparar as diferenças nos resultados perinatais, perceção de risco e ajustamento psicológico entre gravidezes PMA e espontâneas e gravidezes gemelares e de feto único.

Metodologia: Realizou-se num hospital universitário, um estudo prospetivo longitudinal com 32 gravidezes gemelares (17 espontâneas e 15 pós PMA) e 52 gravidezes de feto único (25 espontâneas e 27 pós PMA).

Ansiedade, depressão e perceção de risco foram avaliadas através de questionários de autorresposta, adaptados para a população portuguesa. Dados relativos aos resultados perinatais foram colhidos dos processos clínicos. A colheita de dados ocorreu em quarto momentos diferentes: primeiro, segundo e terceiro trimestres de gravidez e um mês após o parto.

Resultados: A diferença da média da idade materna nos diferentes grupos não foi estatisticamente significativa (p=0.665). O tempo de duração da relação conjugal foi maior no grupo PMA (gemelar) (p=0.031). O grupo PMA (gemelar e feto único) tem um número significativamente mais elevado de consultas obstétricas e ecografias. O aumento de peso durante a gravidez, também foi maior no grupo PMA (gemelar e espontâneo) (p=0.001). Os

resultados mostram que não há diferenças estatisticamente significativas na maioria dos resultados perinatais e na ansiedade, entre os grupos PMA e espontânea ao longo da gravidez. Houve diferenças estatisticamente significativas na depressão, entre os grupos de gravidez gemelar e de feto único e na preocupação com a relação nos grupos PMA (gemelar e feto único) e no grupo de gravidez gemelar espontânea (p=0.001).

Conclusões: No nosso estudo não encontrámos diferenças estatisticamente significativas entre os grupos de gravidezes PMA e espontâneas para a maioria dos resultados perinatais. A perceção de risco e preocupação é maior no grupo PMA. Os níveis de ansiedade são similares em ambos os grupos, PMA e espontâneas e os níveis de depressão são diferentes não entre PMA e espontâneas, mas entre gemelar e feto único. Os nossos resultados sugerem que a PMA não tem um efeito negativo na saúde materna ou do feto, mas a gravidez gemelar, quer PMA quer espontânea requer particular atenção.

PALAVRAS-CHAVE Gravidez gemelar; Procriação medicamente assistida; Resultados perinatais; Perceção de risco; Ajustamento psicológico

ABSTRACT

Introduction: The growing number of twin pregnancies is due, in part, to the use of assisted reproduction technologies (ART). Twin pregnancy is a high-risk condition, generally associated with relevant maternal and perinatal morbidity and mortality. The impact of ART in perinatal outcomes, risk perception and psychological adjustment of twin pregnancies is not yet clear, as existing studies are not consensual. In addition, some findings associated with the use of ART (e.g. preterm birth) may be a consequence of the higher number of twin pregnancies, rather than the use of ART *per se*. Therefore, it is important to understand the impact of using ART and to distinguish this impact from the one caused by twin pregnancy.

Objectives: This study aims to compare the differences in perinatal outcomes, risk perception and psychological adjustment between spontaneous and ART pregnancies and twin and singleton pregnancies.

Methodology: A prospective longitudinal study with 32 twin (17 spontaneous and 15 ART pregnancies) and 52 singleton pregnancies (25 spontaneous and 27 ART pregnancies) was conducted in a university hospital.

Anxiety, depression and risk perception were assessed using self-report questionnaires adapted for the Portuguese population. Data on perinatal outcomes was collected from the clinical file. Data collection occurred at four different times: first, second, and third trimester of pregnancy and at one month *postpartum*.

Results: Difference in maternal mean age was not statistically significant (p=0.665). The duration of the couple relation was longer in ART twin group (p=0.031). ART group (twin and singleton) has significant higher number of obstetric appointments and ultrasounds. The weight gain during pregnancy was also higher in ART group (twin and singleton) (p=0.001). Results showed that there were no statistically significant differences in the majority of perinatal

outcomes and anxiety between ART (twin and singleton) and spontaneous groups (twin and singleton) along the pregnancy. There are significant differences in depression between twin and singleton groups and in worries with relation in the ART group (twin and singleton) and spontaneous twin (p=0.001).

Conclusions: In our study, we have not found statistically significant differences between ART and spontaneous pregnancies groups for the major perinatal outcomes. The perception of risk and worries are higher in the ART group. The anxiety levels are similar in both ART and spontaneous groups. Depression levels are different between twin and singleton groups, regardless of the method of conception. Our results suggest that ART does not have negative effect in fetal and maternal health, but twin pregnancy, either spontaneous or after ART has higher risk of pregnancy complications and emotional burden.

KEY WORDS

Twin pregnancies; Assisted reproductive technologies (ART); Perinatal outcomes, Risk perception; Psychological adjustment.

1. INTRODUCTION

Infertility is "a disease of reproductive system defined by failure in achieving a clinical pregnancy after 12 months or more of unprotected sexual intercourse".[1] It affects about 10% of the population worldwide and this is similar in Portugal[2, 3].

Because the decline in fertility is associated with ageing and decreasing in the number of oocytes in ovaries, it can be expected that infertility problems will increase in the next years.[4-6] Some studies show that fertility starts decreasing after 25 years of age and attribute that decline to the decreasing quality of oocytes.[5, 7]

In our present society, infertility is generally associated to age and, once it is more common for women to get pregnant each time later, the infertile couples look for help to achieve pregnancy through ART, which frequently results in multiple pregancies[8-11]

Therefore, it is important to understand if whether undergoing ART and specifically having a twin pregnancy after ART, has a negative impact in maternal and fetal health.

The literature describes women who undergo ART as frequently nulliparous and with advanced age, what might be, by itself, a group in higher risk of obstetric complications and consequently perinatal complications.[9, 12, 13] However, studies are still inconclusive and more information on the impact of ART and twin pregnancies after ART is needed.

Previous studies that compare the perinatal outcomes between ART twin pregnancies and spontaneous twin pregnancies are inconsistent.[9,11] Some found similar perinatal outcomes[10-18] while others found that ART twin pregnancy is associated with worst perinatal outcomes.[9, 19-22] On the contrary, studies that focused on singleton pregnancy after ART consistently reported that singleton pregnancies after ART are associated with higher risk of preterm birth, low birth

weight, pregnancy hypertension and *C*-section delivery.[23,24]

The perception that twin pregnant women have from pregnancy risk as to possible complications and problems that might occur is believed to be similar in both groups, ART and spontaneous. According to some studies, the anxiety and expectations are higher in ART pregnancy due to all the inherent conditions and, as so, ART pregnant women look for more information.[25] These women are usually warned about the possibility of having a twin pregnancy being, due to that, more informed about the risks associated with that condition. [26-28] Most of the times, there is a discrepancy between pregnant women and health professionals' risk perception.[27, 28]

Finally, pregnancy status by itself already causes some anxiety, which seems subjectively increased when that pregnancy is the result of ART. The impact of ART in the anxiety level during pregnancy is not consensual in the several studies.

Confronted with a twin pregnancy, pregnant women after ART are more anxious than spontaneous pregnant. This difference may attenuate or even disappear in the *postpartum*.[25, 29]

2. STUDY AIM

As documented above, there are still some inconsistencies regarding the effect of undergoing ART in the perinatal outcomes, risk perception and psychological adjustment of pregnant women and specifically in the case of twin pregnancy. This clinical research work aimed at filling this gap by studying the differences on perinatal outcomes, risk perception and psychological adjustment between spontaneous pregnancies, twin and singleton, ART twin and ART singleton pregnancies. This study goes beyond previous published work by adopting a multidisciplinary approach and a prospective design, studying spontaneous and ART pregnancies from the first trimester to first month *postpartum*.

3. MATERIALS AND METHODS

3.1. Participants

Participants were pregnant women in pre-natal surveillance at Centro Hospitalar Universitário de Coimbra. Inclusion criteria were twin (bichorionic and biamniotic) or singleton pregnancy, both spontaneous and after ART.

The exclusion criteria were monochorionic and monoamniotic twin pregnancy, adolescent pregnancy, maternal infectious pathology (CMV, toxoplasmosis, rubella, hepatitis, HIV) and mental pathology.

3.2. Procedures

This prospective study was approved by the Ethics Committee of Faculty of Medicine, University of Coimbra.

Twin pregnant women who conceived through ART and spontaneously were recruited while attending their first multiple pregancy appointment at Daniel de Matos Maternity.

Singleton pregnancies following ART were recruited at the Reproductive Medicine Unit in their first obstetric appointment after pregnancy was confirmed. Singleton spontaneous pregnancies were recruited in the first trimester reference appointment at Daniel de Matos Maternity.

Eligible pregnant women were invited to participate by the researcher, who presented the study objectives, warranted the confidentiality of the data, informed about the participants' rights and researchers' obligations and asked for their consent to participate. All women who agreed to participate signed an informed consent form. After, they were contacted to fill out the

questionnaires when attending the obstetric appointment at first, second and third trimesters of pregnancy and one month *postpartum*. All questionnaires were delivered before and handed back during the appointment. Clinical data was collected from the clinical files.

In order to assure absolute confidentiality of the data, all questionnaires were coded with a code number (one for each participant) and a letter, to assign each participant to the cooresponding group (A, for ART pregnant group and B for spontaneous pregnant group).

3.3. Measures

The assessment protocol included a sociodemographic questionnaire, a clinical information sheet and self-report questionnaires on anxiety, depression and perceived pregnancy risks.

The sociodemographic questionnaire assessed personal information relevant for sample description (e.g. education, profession, marital status, socioeconomic status (SES)).

The clinical information sheet included clinical information regarding current pregnancy, namely maternal age, obstetric complications (hypertension, preeclampsia, gestational diabetes, intra uterine growing restriction (IUGR), premature rupture of membranes (PROM), pre-term birth threat, type of delivery (vaginal *versus C*-section), gestational age at delivery, number of appointments, weight gain during pregnancy, Apgar index and need of neonatal intensive care unit (NICU) admission.

Anxiety, depression and perceived pregnancy risks were assessed with validated self-report questionnaires.

Hospital Anxiety and Depression Scale: The Portuguese version of HADS it is an instrument used to identify possible and probable cases of anxiety and depression in patients in a hospital context. This scale presents 14 items, each one is answered by the individual in an ordinal scale

of four positions (0-3). The value 0 refers to less serious and 3 to most serious, making a total of 21 points to each scale (HADS-A (anxiety) and HADS-D (depression). According to the original version authors, one must adopt the cut off recommended to both scales: without anxiety/depression from 0-8 and with anxiety/depression equal or above 9[30, 31]. In our study, the Cronbach alpha is 0.79 to anxiety and 0.74 to depression.

Cambridge Worries Scale: The CWS intends to measure the intensity of the worries of pregnant women. It includes the sociomedical area, relationship, reproductive loss, health and socioeconomic area. The Portuguese version has 13 items. It is used a self-assessment Likert scale of 6 points from 0 (it is not a worry) to 5 (it is a big worry). In the end of the questionnaire, there is a space for other worries that pregnant might have at the moment and that are not in the questionnaire items[32, 33]. In our study, the Cronbach alpha of the total scale was 0.78.

4. STATISTICAL ANALYSIS

All the data were registered in an *Excel* database designed to this study. The statistical analysis was performed using the program *SPSS*® 22.0 *Statistical Software (SPSS, Inc. Chicago, IL).*

Dichotomous variables were coded using No (0) and Yes (1) according to the existence or not of obstetrics comorbidities and the need or not of NICU admissions. The answer "yes" to the existence of obstetric co-morbidities and to the need of NICU admissions was coded as "1"; the answer "no" to these questions was coded as "0". The type of delivery was coded as "1" to vaginal delivery, "2" to *C*-section delivery and "3" to vaginal instrumented delivery.

As to categorical variables, these were organized in tables of contingency and the chi-square test was used, being that the intensity of the association, when existed, it was measured through

the measure of contingency of Pearson. A value of $p < 0.05$ was considered significant.

To examine changes in psychological adjustment and risk perception along time and between the four groups, we performed analysis of variance using the General Linear Model (GLM) for Repeated Measures, with time as a within subject factor and group as a between subject group. Interactions that affects Time X Group were examined. Time measurements only included the measurement during pregnancy, as few of them gave birth by the time this study was concluded.

Post hoc power calculations demonstrated that the achieved sample size was sufficient to detect only medium to large effects [f=.20. p<.05, power = .80, G*Power 3].[34] Significance level used was 0.05. However, because small to medium effects would not be detected, marginally significant differences (p<0.01) were also reported and discussed.

5. RESULTS

Participants were 84 pregnant women undergoing prenatal surveillance at Centro Hospitalar Universitário de Coimbra.

The following tables summarize the sample description (table 1), pregnancy outcomes (table 2), obstetric complications (table 3) and perinatal outcomes in delivery (table 4) in the four groups (ART twin (AT), Spontaneous twin (ST), ART singleton (AS), Spontaneous singleton (SS)).

Table 1 - Sample description

	Twins		Singletons		
	ART (*n*= 15)	**Spontaneous (*n*=17)**	**ART (*n*=25)**	**Spontaneous (*n*=27)**	***Significance level p***
Age	34.67±3.90	33.47±4.70	33.80±4.20	32.90±4.60	.665
Years of education	14.20 ±2.90	14.12±2.91	12.63±4.47	13.72 ±3.23	.334
SES (% Medium)	86.7	76.5	40.7	64.0	**.014**
Employment status (% Employed)	93.33	88.23	96.2	92	.786
Residence area (% Urban)	46.7	52.9	66.7	40.0	.277
Years of couple relationship	8.66±5.62	6.00±6.31	5.84±2.72	5.25±2.86	**.031**

Values refer to *mean*
The *p* refers to the differences between the *means* of the four groups

There were no statistically significant differences between the four groups, except for years of couple relationship and SES. Significant differences between four groups were regarding duration of couple relationship (p= 0.031). Bonferroni *Post-hoc* tests revealed that women from singleton spontaneous pregnancy were significantly younger than women from the ART twin pregnancy group (p = .025). In the four groups, most of the pregnant had a medium SES and most of them were employed and lived in an urban area. More women with singleton pregnancies had a higher SES than women with twin pregnancy.

The groups were compared in terms of obstetric outcomes. Table 2 and Table 3 report data on pregnancy and newborn outcomes for the four groups.

Table 2 –– Comparison of pregnancy outcomes between groups

	Twin		Singletons		
	ART (*n*= 16)	**Spontaneous (*n*=15)**	**ART (*n*=25)**	**Spontaneous (SS) (*n*=27)**	***p***
Total of pregnancy appointments	15.80±1.75	15.83±0.40	7.77±0.83	4.87±0.35	**<.001[1]**
Total of ultrasounds	9.70±1.25	10.33±0.51	4.77±0.44	4.5±0.92	**<.001[2]**
Total weight gain (Kg)	15.70±2.26	14.83±0.40	9.77±1.71	10.00±2.39	**<.001[3]**
Gestational age at delivery (weeks)	36.00±2.05	36.80±0.40	37.77±2.63	36.62±4.13	.567
Type of delivery	5 caesarean	4 caesarean	6 vaginal	5 vaginal	2.27

Notes:

[1] SS<AS<AT;ST

[2]SS;AS<AT;ST

[3]SS;AS<AT;ST

According to table 2, statistically significant differences were found regarding number of total appointment during pregnancy, total number of ultrasounds and average weight gain. Results show that differences occur mainly between singleton and twin group, with the twin pregnancy group (either ART or spontaneous) having more appointments, more ultrasounds and more weight gain than in the singleton group (*p*=.001).

There was no statistically significant difference in gestational age at delivery and in type of delivery between ART and spontaneous groups.

In the twin group, most of the deliveries were *C*-sections while in the singleton group it was vaginal birth.

Table 3 – Obstetric complications

	Twins				Singleton		
	ART (*n*= 16)	**ART (*n*= 16)**	**Spont (*n*= 17)**	**Spont (*n*=15)**	**ART (*n*=25)**	**Spont (*n*=27)**	***p***
	Fetus 1	**Fetus 2**	**Fetus 1**	**Fetus 2**			
HTA (%)	0		0		7.4	12	.097
PE (%)	6.7		0		7.4	4	.630
DM (%)	6.7		5.9		5.9	12	.482
IUGR (%)	13.3	33.3	11.8	35.3	7.4	4	Singleton .50 Fetus 1 .48 **Fetus 2 .05**
PROM (%)	20		5.9		7.4	4	.824
PTBT (%)	13.3		5.9		11.1	0	.620
PTB (%)	26.7		5.9		7.4	4	.540

P refers to comparison between ART and spontaneous groups.
Spont, Spontaneous; HTA, Hypertension; PE, Preeclampsia; DM, Diabetes *mellitus*; IURG, Intra uterine growth restriction; PROM, Premature rupture of membranes; PTBT, Preterm birth threat; PTB, Preterm birth.

In relation to obstetric complications (table 3), in twins, the ART pregnancy group had a higher prevalence of preterm birth and PROM, but not statistically significant. In singletons the ART group had a higher prevalence of preeclampsia and the spontaneous group, had a higher prevalence of hypertension and diabetes *mellitus* in pregnancy even though this was not statically significant.

The ART twin group had a higher prevalence of IUGR (for fetus 2, p=0.05).

Table 4 – Perinatal outcomes in delivery

	Twins				Singletons				
	ART (*n*= 15)	**ART (*n*= 15)**	**Spontaneous (*n*= 17)**	**Spontaneous (*n*=17)**	**ART (*n*=25)**	**Spontaneous (*n*=27)**	***P singleton***	***P F₁***	***P F₂***
	Fetus 1	**Fetus 2**	**Fetus 1**	**Fetus 2**					
Sex (F %)	20	33.3	23.5	23.5	18.5	16	.019	.152	.515
Birth weight (g)	2370.00±366.75	2081.70±424.02	2575.33±394.56	2581.66±247.02	2758.88± 695.00	3023.75±551. 80	.402	.309	.100
Apgar5	9.20±1.03	9.30±1.05	9.83±0.40	9.83±0.40	9±0.86	8.00±3.29	.39	.390	.104
Apagar10	9.60±0.69	9.60±0.69	10.00±0.00	10.00±0.00	9.6±0.5	8.5±3.46	.33	.178	.104
NICU (%)	13.3	20	0	0	7.4	0	.156	.242	.137

Data in the table refer to the new born

There is no statistically significant difference between twin and singleton groups concerning the newborn sex, as the majority are male.

Concerning birth weight, no statistically significant difference is shown, but singleton newborn weights, in average, more than twins as shown in table 4.

Apgar index shows no statistically significant difference, but in the group of spontaneous singletons, it is lower.

As for the need for NICU admission, even though there are no statistically significant differences, the group of ART twin has a higher percentage of newborns who needed NICU admission.

Table 5 summarizes the evolution of anxiety, depression and worry evolution over time

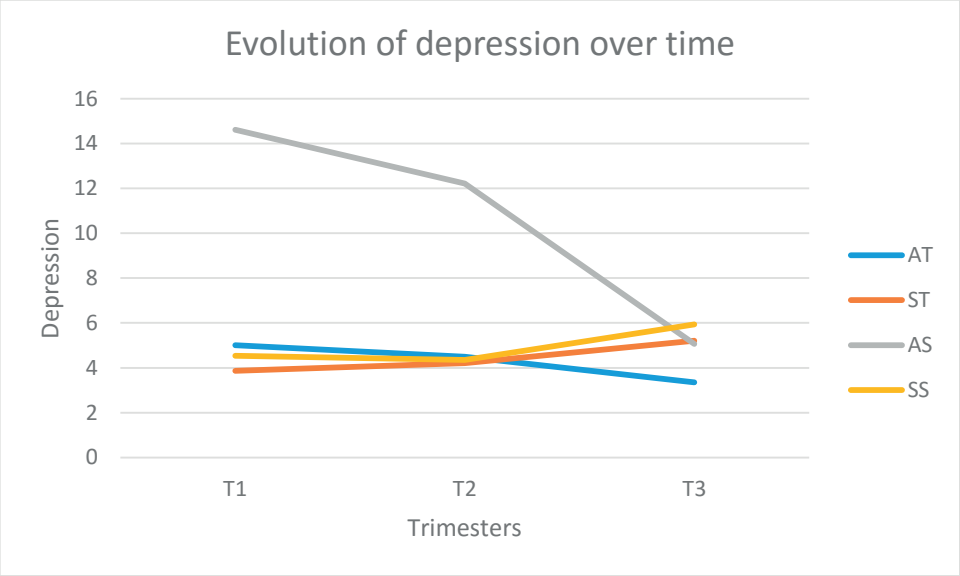
Table 5 – Evolution of anxiety, depression and worries along pregnancy

	Twin ART (*n*=15)			**Spont twin (*n*=17)**			**Singleton ART (*n*=27)**			**Spont singleton (*n*= 25)**		
	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3
	(N=15)	(N=14)	(N=14)	(N=17)	(N=14)	(N=10)	(N=27)	(N=27)	(N=27)	(N=25)	(N=20)	(N=15)
Adjusment												
Anxiety	8.60±3.24	6.14±2.34	7.92±4.17	7.35±3.51	6.21±4.72	7.30±2.35	8.62±2.43	8.14±2.78	7.70±2.39	7.92±3.58	6.80±4.88	7.80±2.11
Depression	5.00±3.02	4.50±1.74	3.35±1.95	3.87±2.96	4.21±2.99	5.20±2.85	14.61±1.47	12.22±5.33	5.07±2.98	4.54±3.10	4.35±2.88	5.93±2.78
Worries												
Others	1.69±0.88	1.59±0.65	1.17±0.74	0.68±0.97	0.85±0.94	1.15±0.57	0.82±0.95	1.11±0.92	0.90±0.89	1.55±0.75	1.46±0.61	0.80±0.86
Loss	3.75±1.04	3.57±0.89	3.28±1.10	3.55±0.98	3.35±1.02	4.45±0.68	4.29±0.98	4.09±0.69	3.94±0.46	3.83±1.12	3.40±0.89	3.86±0.48
Pregnancy	2.67±1.03	2.48±0.67	2.65±0.78	1.85±0.89	2.21±1.02	2.28±1.31	2.90±0.89	2.74±0.77	2.61±0.59	2.72±0.91	2.38±0.71	2.66±0.49
Relation	0.78±0.99	0.82±0.82	0.42±0.51	0.60±1.10	1.00±1.14	0.60±0.65	0.88±1.18	0.90±1.20	1.03±1.12	0.86±1.08	0.36±0.61	0.86±1.15

Analysis of repeated measures (Table 5) reveled that that were no differences in anxiety along the three assessment points between the four groups (F6, 124 = 0.46 p= 0.833).

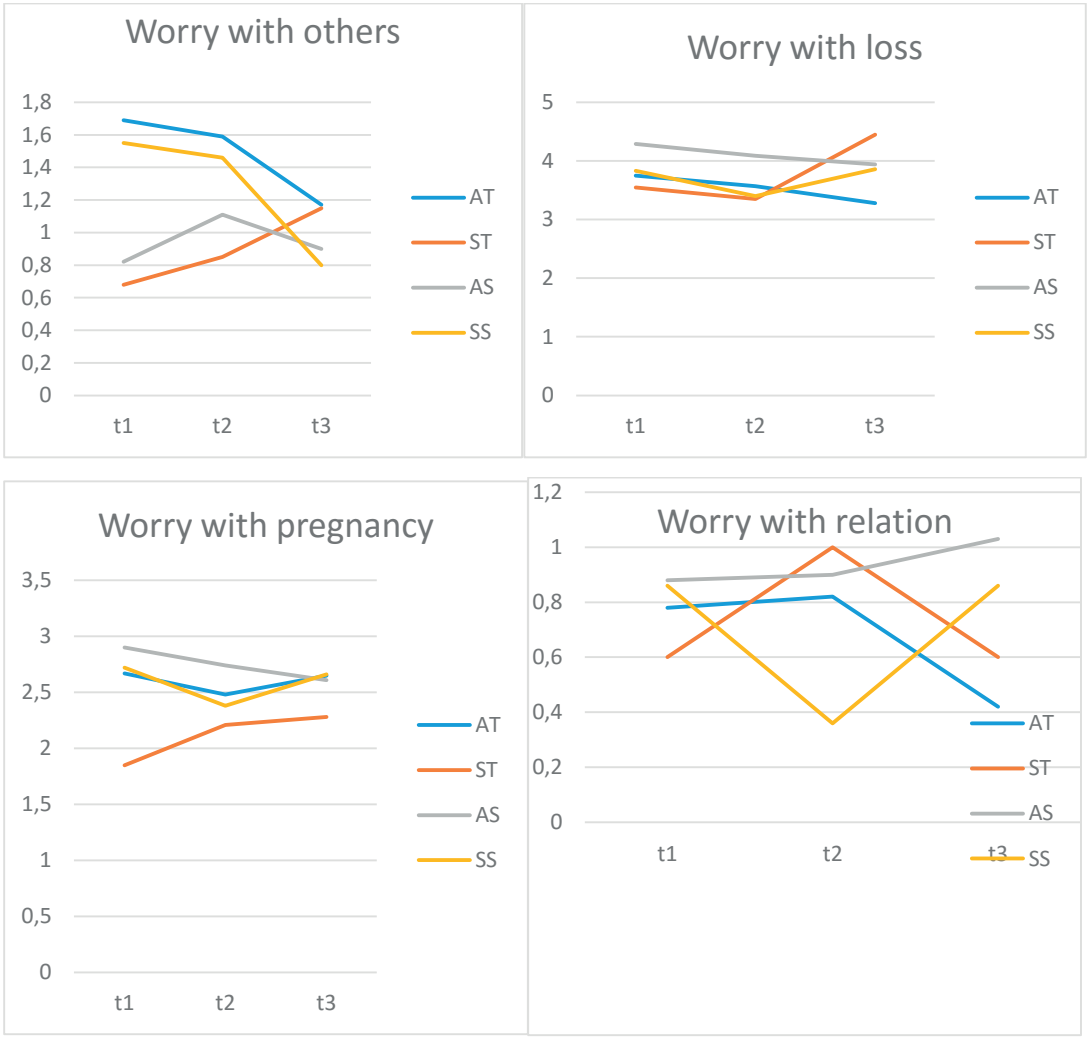
A significant interaction effect was found regarding depression, as it is shown in graphic 1 (F6.118 = 9.87 p<0.001). Women from ART singleton group reported higher levels of depression in the first and second trimester than the other groups, but no differences were found in the third trimester.

Graphic 1 – Graphic representation of depression evolution over the three moments



AT: ART twin; ST: spontaneous twin; AS: ART single; SS: spontaneous single;

Figure 1 – Representation of worries' evolution over the three moments



AT: ART twin; ST: spontaneous twin; AS: ART single; SS: spontaneous single;

Regarding worry over the time, as shown in the table 5 and figure 1, there are only statistically significant differences for worries with loss and relation.

In the twin group, worry with loss decreases and in the singleton group it increases after the second trimester.

Worry with relation increases up to the second trimester in twin group and ART singleton and then it decreases for twin group in the third trimester and it increases for ART singleton group. As for spontaneous singleton, it is the inverse. It decreases until the second trimester and it increases after that.

6. DISCUSSION

The main aim of this study was to compare perinatal outcomes, risk perception and psychosocial adjustment between spontaneous and ART pregnancies, both single and twin.

First, the four groups studied were similar regarding their sociodemographic variables. This was also true for age. Indeed, although several studies refer that women undergoing ART are older[9, 12, 13], our results show, the average age of women in ART and spontaneous groups was very similar.

Being older, these women are in higher risk for obstetric complications such as hypertension, preeclampsia, gestational diabetes *mellitus* and preterm delivery [9, 12, 13]. This was not verified in our study, maybe because the average age of women is lower.

Our results showed that the ART twin group had higher prevalence of premature rupture of membranes. This is in contrast with past literature, that reports the smaller incidence of premature rupture of membranes in ART group, which would result from tighter surveillance[13, 16, 20]. We believe that this discrepancy may be because these studies refer to singletons, and in our study, the highest value of premature rupture of membrane was found in the twin ART group only.

Conversely, when examining the presence of comorbidities between the four groups, no statistically significant differences were found, contrary to what is referred in the scientific literature[9, 19-22]. This may be because these studies do not take into account the chorionicity of pregnancies as we have done. We have exclude monochorionic and monoamniotic twin pregnancies, as they tend to have higher morbidity and mortality.

Our results also showed that most of the deliveries in the ART group were *C*-sections. This is in accordance with the literature, which argues that this may be due to higher parental and obstetric anxiety in these pregnancies[23, 24]. We verified this in our results. As for perinatal

outcomes, in our study, there were no significant differences in the birth weight and Apgar index, which is in accordance to some studies[10-18]. As for the need of NICU admission, we have verified that the ART twin group needed it more often than spontaneous twin or singleton groups, probably because they had, on average, lower weight and were born at earlier gestational age.

As for the risk perception, we found that the ART group has a better risk perception and worries more about the pregnancy. According to the literature, ART pregnant women are more informed about risks and look for more information, being aware of the risks and tending to be more worried.[26, 27, 28]

Another interesting finding was that worries with loss tended to decrease from first trimester to the second trimester and then increased from the second to the third in the ART group (twin and singleton). This might be because in the second trimester, women tend to feel safer about not having a miscarridge, as the first trimester, the period of higher miscarridge risk, is over. The subsequent increase may be related to fears highlighted by the approximation of the *partum*. Nonetheless, our results also showed that there were no differences in anxiety over the time, which was not expected, even though the impact of ART in anxiety levels during pregnancy is not consensual in several studies[25,29].

Finally, significant differences were found in depression scores along time between the study groups. The ART (twin and singleton) group reported lower values of depression in the second moment. In our opinion, this may be because these couples have achieved the so desired pregnancy and they feel relieved. Contrary, the fact that women with the spontaneous twin pregnancy increased their level of depression may be the reflect adjustment to difficulties of the twin pregnancy, which was most likely not expected.

Conclusions: This clinical research work aimed at filling the gap on the effect of ART in the perinatal outcomes, risk perception and psychological adjustment of pregnant women and specifically in the case of twin pregnancy, by studying the differences in perinatal outcomes, risk perception and psychological adjustment between spontaneous and ART twin and ART singleton and spontaneous pregnancies.

Our study showed that although there were some differences between the study groups, these were more similar than different in perinatal outcomes.

However, differences were mainly between twin and single pregnancy and not between ART and spontaneous group.

Strengths and limitations

This study has several important strengths. First of all, we included four groups of participants, which is a strength, as most of the studies only compare two groups, singleton *versus* twin or ART *versus* spontaneous. Having the four groups, allows studying the influence of all these factors. It was a prospective study, which allowed us to examine the evaluation of obstetric outcomes along the pregnancy. In addition, we used a multidisciplinary approach, considering medical and psychological measures, which have better described the experience of women along the pregnancy. Moreover, the fact that the study did not include monochorionic and monoamniotic twin pregnancies, less frequent after ART and associated with higher morbidity and mortality homogenizes the groups.

However, some limitations are also worth to mention. The most important is the small sample size in each group. Although the global sample is size relevant, some groups, namely in the *postpartum* period, are small because the women are still pregnant by the time this preliminary study was concluded. Nevertheless, the sample size was enough to detect large effects in most of our analysis. In addition, the fact that it was done in a tertiary care center to where all the high-risk pregnancies are referred, does not give us a sample of general population but a particular part of it.

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