A prospective randomized study comparing two commercially available types of human embryo culture media: G1-PLUS™/G2-PLUS™ sequential medium (Vitrolife) and the GL BLAST™ sole medium (Ingamed)

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ABSTRACT

Objective: To check the efficacy of two types of commercially available embryo culture medium: G1-PLUS™/G2-PLUS™ sequential (Vitrolife, Gothenburg, Sweden) and GV BLAST™ sole (Ingamed, Maringá, Brazil) with regards to fertilization, cleavage, blastocyst and pregnancy rates.

Methods: Prospective and randomized study conducted from March to July 2015, using the medical records of 60 patients submitted to Intracytoplasmic Sperm Injection techniques (ICSI). Data regarding the age of patients, together with fertilization, cleavage, blastocyst and pregnancy rates, were collected and compared in relation to the: G1-PLUS™/G2-PLUS™ sequential and GV BLAST™ sole mediums. The data were tabulated and compared using the Pearson's Chi-Square test (95% CI).

Results: There was no significant difference when comparing patients divided into higher and lower fertility age. No significant statistical difference was noted between the fertilization rates ($P=0.59$), cleavage ($P=0.91$), evolution to blastocyst ($P=0.33$) and total pregnancy ($P=0.83$) when comparing the embryos cultured in the different media analyzed.

Conclusion: We conclude that the G1-PLUS™/G2-PLUS™ sequential and GV BLAST™ sole mediums are equally effective with regards to fertilization, cleavage, blastocyst development and total pregnancy rates.

Keywords: *In vitro* fertilization, Embryonic culture medium, ICSI, Pregnancy rate.

INTRODUCTION

Highly complex procedures employed in Intracytoplasmic Sperm Injection techniques (ICSI) require knowledge of human embryo physiological needs, from zygote through to the blastocyst formation. Using the knowledge acquired from embryonic manipulation, it was possible to create artificial embryo culture media, based on the compositions of tubal and uterine secretions (Cossello, 2009). These mediums influence both embryo quality and pregnancy rates, and may differ according to the nature or concentration of their components (Cossello, 2009; Almodin *et al.*, 2001). Various culture mediums have been compared and have presented different results relating to *in vitro* fertilization success (table 1).

This paper aims at comparing the efficacy of two types of human embryo culture medium: G1-PLUS™/G2-PLUS™ sequential (Vitrolife, Gothenburg, Sweden) and the GV BLAST™ sole (Ingamed, Maringá, Brazil) through a prospective, randomized study comparing fertilization, cleavage, blastocyst and pregnancy rates.

MATERIALS AND METHODS

Experimental groups

A prospective, randomized study was carried out from March to July 2015 in a clinic of assisted human reproduction in Curitiba/PR. Data from 60 patients who were submitted to the Intracytoplasmic Sperm Injection (ICSI) technique in fresh cycles, was tabulated and compared. Patients were randomly divided into Group 1 ($n=34$) and Group 2 ($n=26$). Group 1 patient embryos were cultivated with G1-PLUS™/G2-PLUS™ sequential (Vitrolife) medium, whereas Group 2 patient embryos were cultivated in GV BLAST™ sole (Ingamed) medium. Comparisons of the groups regarding: patient age, rates of normal fertilization, embryo cleavage, blastocyst development and pregnancy were conducted.

Culture media

G1-PLUS™ and G2 PLUS™ are sequential embryonic media. G1 PLUS™ provides carbohydrates, amino acids and hyaluronan to support the first stages of development (until D3), while the G2 PLUS™ medium contains a quantity of amino acids and hyaluronan for cultivation until the blastocyst. In addition, both mediums contain human serum albumin. The GV BLAST™ medium is a bicarbonate buffering system, made to promote early embryonic development to the blastocyst stage. It consists of a single culture medium, without any protein components. The general protocols of assisted human reproduction laboratories include protein supplements when working with the medium. Current recommendations are to employ 10% Ingamed serum, and not to use human albumin.

Ovulation induction, ICSI, embryo cultivation and pregnancy verification

The hormonal stimulation cycle was initiated following clinical, biochemical and imaging examinations in order to obtain fresh oocytes (Dzik *et al.*, 2010).

Freshly collected oocytes were denuded, and classified according to their maturity. Only oocytes in metaphase II were submitted to ICSI, 3 to 4 hours after follicular aspiration (Dzik *et al.*, 2010). Once injected, oocytes were randomly divided into Group 1 (G1-PLUS™/G2-PLUS™) and Group 2 (GV BLAST™). The oocytes injected in Group 1 were grown in G1-PLUS™ medium until Day 3 of development. The embryos that were not transferred until Day 3 were cultured in the G2-PLUS™ medium until the fifth day. Those from Group 2 were cultured in the GV BLAST™ medium until the third day of development. Similarly, embryos that were not transferred until the third day had their medium renewed and were grown until the fifth day.

Oocyte fertilization was checked between 16 and 18 hours post-ICSI. Embryos were evaluated on the second

Table 1: Compared ICSI/IVF media including data here described.

Media Compared	ICSI/IVF	Results/ Conclusions	References
BM1 (Ellios Bio-Media) and IVF-50 (Scandinavian IVF Science)	ICSI	BM1 yielded embryos with superior quality	Parinaud <i>et al.</i> , 1998
P-1 (Irvine Scientific) and IVF-50 (Scandinavian IVF Science)	ICSI	There was no difference between the media	Mauri <i>et al.</i> , 2001
Sydney IVF (Cook, Queensland) and Quinn's Advantage (Cooper Surgical)	ICSI	Culture medium Quinn's Advantage showed better rates in embryo quality and pregnancy	Hoogendijk <i>et al.</i> , 2007
GV BLAST (Ingamed) and P-1 (Irvine Scientific)	ICSI	There was no difference in blastocyst and pregnancy rates between the media	Lopes <i>et al.</i> 2008
G5 (G-1 PLUS v5, Vitrolife AB, Kungsbac-ka.) and Global IVF (Guilford, CT)	ICSI	There was no difference between the media	Reed <i>et al.</i> , 2009
Sydney IVF (Cook, Queensland) and GM501 (Gynemed, Lensahn)	ICSI	Pregnancy rates were significantly higher with sole medium	Paternot <i>et al.</i> , 2010
GM501 (Gynemed, Lensahn) and ISM1 (MediCult, Jyllinge)	IVF	There was a higher rate of fragmentation of embryos with medium GM501	Campo <i>et al.</i> , 2010
Vitrolife G3 (Goteborg, Sweden) and Sydney IVF (Cook, Queensland)	IVF	Medium G3 had better pregnancy rates	Dumoulin <i>et al.</i> , 2010
G5 (G-1 PLUS v5, Vitrolife AB, Kungsbac-ka.) and Embryo Assist (Universal IVF, MediCult A / S, Jyllinge)	ICSI	Culture medium G5 had better blastocyst and pregnancy rates	Hambiliki <i>et al.</i> , 2011
Maria Research Center (MRC) and Sydney IVF (Cook, Queensland)	IVF	Medium MRC was as effective as medium Sydney IVF on embryo quality and pregnancy rates	Yoon <i>et al.</i> , 2011
Global IVF (Guilford, CT) and Quinn'sAdv (Cooper Surgical)	ICSI	The embryos in sole medium had higher blastocyst, pregnancy, and implantation rates	Khoury <i>et al.</i> , 2012
Global IVF (Guilford, CT) and Quinn'sAdv (Cooper Surgical)	ICSI	There was no difference between the media	Summers <i>et al.</i> , 2013
G-TL (Vitrolife Sweden AB) and G1/G2 (Vitrolife Sweden AB)	ICSI	No significant differences were found in embryo quality or pregnancy rates	Hardarson <i>et al.</i> , 2015
G1-PLUS™/G2-PLUS™ sequential (Vitro-life) and the GL BLAST™ sole (Ingamed)	ICSI	There was no difference between the media	* Data obtained in the present study

(D2) and third (D3) days of culture based on the number of cleaved cells, which corresponds to the day of development. The cleavage of the embryos was categorized as per Ben-Yosef; 2004. The classification was divided into three groups: fast cleaving (> 4 cells on D2 or > 8 cells on D3); normal cleaving (2-4 cells on D2 or 6-8 cells on D3) or delayed cleaving (< 2 cells on D2 or < 6 cells on D3).

Embryos were transferred on the second, third or fifth day of embryonic culture according to the age of the patient, the number of mature oocytes obtained and embryonic quality (Dzik *et al.*, 2010).

After 12 to 14 days of embryo transfer, pregnancy was evaluated by quantitative Beta hCG examination (testing for the beta fraction of chorionic gonadotropin) (Basirat *et al.*, 2010). Pregnancy rates were assessed according to the day of embryo transfer: D2, D3 and D5.

Statistical analysis

The data were tabulated and statistical comparison between groups performed using the Pearson's Chi-Square test (Social Science Statistics: <http://www.socscistatistics.com/tests/chisquare/Default2.aspx>). Analyses resulting in a $P < 0.05$ were considered statistically significant.

Ethics Committee Approval

This project received approval from the Research Ethics Committee of Universidade Positivo, under number 45767215.7.0000.0093.

RESULTS

In order to ensure age homogeneity in the sample, which is important to account for the effect of maternal age on oocyte quality, the patients in both groups were divided into age groups: 26-30; 31-35; 36-40; 40-45 and 45-50. Statistical analyses revealed equivalent age distribution in both groups ($P=0.40$).

Of the total 311 mature oocytes subjected to ICSI, 171 (55%) were from Group 1, while 140 (45%) were from Group 2. According to table 2, there was no significant difference between the groups with regards to: fertilization rate ($P=0.59$); embryo cleavage rate ($P=0.91$); the rate of embryos that developed to blastocyst ($P=0.33$), and to the pregnancy rate ($P=0.83$).

We also found no significant difference when comparing media at the embryonic stage according to the number of cells in each day ($P=0.69$), in accordance with table 3.

The pregnancy rates were similar on all days of embryo transfer: on D2 ($P=0.07$), D3 ($P=0.08$) and D5 ($P=0.04$) according to table 4.

Regarding the overall pregnancy rate, 41.17% of the women from Group 1 became pregnant, while 38.46% in Group 2 achieved the same goal which was determined to be statistically equivalent.

DISCUSSION

Since different human embryonic cultivation media are commercialized, it is important that they are compared in

Table 2: Comparison between G1-PLUS™/G2-PLUS™ and GV BLAST™

	G1-PLUS™/G2-PLUS™	GV BLAST™
Fertilization Rate (% embryo 2PN/ oocytes MII)	67%	67%
Cleavage Rate (%)	91%	92%
Blastocyst Rate (%)	47%	56%
Total Pregnancy Rate (%)	41%	38%

2PN: 2 pronucleus; MII: metaphase II

Table 3: Embryo development comparison

	G1-PLUS™/G2-PLUS™	GV BLAST™
Fast-cleaving embryos (%)	15%	11%
Normal-cleaving embryos (%)	68%	72%
Delayed-cleaving embryos (%)	17%	17%

Table 4: Pregnancy rate according to the day of embryo transfer

	G1-PLUS™/G2-PLUS™	GV BLAST™
DAY 2	18%	25%
DAY 3	50%	44%
DAY 5	67%	50%

terms of their efficacy, especially those from new market entry companies, which offer products to compete with those deemed to be international.

In a study of 182 patients, Mauri *et al.*, 2001 compared the P-1 medium (Irvine Scientific) and the IVF-50 medium (Scandinavian IVF Science) for fertilization, cleavage, transfer, abortion and pregnancy rates and found no difference between the media. However, other studies have reported the advantages and disadvantages of tested media (Ben-Yosef *et al.*, 2004), comparing the P-1 (Irvine Scientific) and IVF (COOK) media. In this case, it was concluded that the P-1 medium afforded advantages regarding implantation and embryo development rates. The supporting data was obtained from the average embryo cell number counts on each developmental day in comparison with what is expected.

Embryos in normal-cleaving and fast-cleaving rates are indicative of good quality culture as they have greater potential for implantation and pregnancy (Ziebe *et al.*, 1997; Giorgetti *et al.*, 1995). There are no previous reports of studies comparing the media used in our study with any other commercially available medium. We observed no significant difference in the efficacy of the tested media regarding the average embryo cell number on each day.

Hambiliki *et al.*, 2011, compared the G-IVF™ v5 PLUS/ G-1™ v5 PLUS media and the Universal IVF medium/EmbryoAssist™ for embryo morphology and development on D2 and D3. The study showed an increased fertility rate in the G-IVF™ v5 PLUS/ G-1™ v5 PLUS medium, but there was no difference in ploidy and embryo quality. Lopes *et al.*, 2008 compared the GV BLAST™ (Ingamed) medium and the HTF/P1P-1 medium (Irvine Scientific). The results showed similarity between mediums in fertilization, cleavage and pregnancy rates. In the present study, we also used the GV BLAST™ medium, and similarly there was no change in embryo quality in comparison with the G1-PLUS™/G2-PLUS™ medium. We did not observe any statistical difference in relation to the comparison of overall pregnancy rates.

Mantikou *et al.*, 2013, in his systematic review,

evaluated 20 different methods of cultivation from 11 different companies and concluded that it is still uncertain as to which is the best medium to cultivate embryos to improve pregnancy rates for in vitro fertilization. As observed in the previously mentioned studies, there remains uncertainty as to which culture medium facilitates the highest pregnancy rate.

Although the compared mediums have different components and are from separate companies, the result indicates that both the G1-PLUS™/G2-PLUS™ sequential medium and the GV BLAST™ sole medium are equally effective in relation to fertilization, cleavage, blastocyst formation and pregnancy rates.

CONFLICT OF INTERESTS

No conflict of interest have been declared.

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