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International Journal of Physiological Sciences
RESEARCH ARTICLE

Cryopreservation as a strategy for prevention of Ovarian Hyperstimulation Syndrome in a Public Assisted Reproduction Service in São Paulo – Brazil

Luma Caroline Gomes Mattos de Macedo[1], Mario Cavagna Neto[2], Artur Dzik[2], Andressa do Rosário Rocha[2] and Sônia Maria Rolim Rosa Lima[1]

[1]Department of Obstetrics and Gynecology, Santa Casa de Sao Paulo School of Medical Sciences, São Paulo/SP, Brazil. 01221-020.
[2]CRSM Hospital Perola Byington, Human Reproduction, São Paulo/SP, Brazil. 01317-000

***Corresponding author:** Luma Caroline Gomes Mattos de Macedo, Rua Caio Prado, 275, apto. 1008, CEP: 01303-001, São Paulo/SP, Brasil, Tel: +55 11 95483-9491, Email: luma_karol@hotmail.com

Citation: Luma Caroline Gomes Mattos de Macedo, Mario Cavagna Neto, Artur Dzik, Andressa do Rosário Rocha, Sônia Maria Rolim Rosa Lima (2022) Cryopreservation as a strategy for prevention of Ovarian Hyperstimulation Syndrome in a Public Assisted Reproduction Service in São Paulo – Brazil. I J Physiol Sci 1: 1-9.

ABSTRACT

Introduction: In order to overcome infertility, some couples resort to Assisted Reproductive Techniques to achieve a pregnancy. However, these techniques can lead to side effects, such as Ovarian Hyperstimulation Syndrome (OHSS). This can occur iatrogenically, and may present mild, moderate or severe forms, and can lead to death. One form of prevention is cryopreservation of all embryos.

Objective: To evaluate the prevalence of OHSS and associated risk factors in patients undergoing fertilization cycles at risk of OHSS (≥ 15 antral follicles or ≥ 15 oocytes aspirated) and submitted to cryopreservation of all embryos.

Methods: A cross-sectional, institutional descriptive study of secondary data from charts of patients enrolled in the Assisted Reproduction Service of the Pérola Byington Hospital at risk for OHSS after controlled ovarian stimulation and submitted to cryopreservation of all embryos was conducted between January 2015 and September 2017.

Results: OHSS occurred in 47.5% of cycles, all of mild severity, and there were no moderate or severe cases of OHSS.

Conclusion: The cryopreservation of all embryos prevented moderate and severe forms of OHSS. Risk factors for OHSS should be evaluated prior to initiation of treatment, with less intense stimulation protocols accordingly.

Keywords: Ovarian Hyperstimulation Syndrome; Cryopreservation; Fertilization in Vitro

List of Abbreviations:

ART: Assisted Reproduction Technology; IVF: *In vitro* fertilization; OHSS: Ovarian Hyperstimulation Syndrome; VEGF: Vascular endothelial growth factor; GnRHa: Gonadotropin-releasing hormone agonist; BMI: Body mass index; ICSI: Intracytoplasmic sperm injection; AFC: Antral follicle count; PCOS: Polycystic ovarian syndrome

Background

Culturally, human reproduction not only represents perpetuation of the species, but is linked to personal realization, where inability to procreate can be perceived as a failure to achieve our biological destiny, besides being a social stigma [1]. One in 6 couples experience fertility problems and, for 20% of this group, the only way to achieve a pregnancy is by using Assisted Reproduction Technology (ART) [2]. These techniques, such as *in vitro* fertilization (IVF), seek to attain pregnancy by replacing or facilitating the defective stage in the reproduction process [3].

IVF is a technique in which fertilization and initial development of the embryos occurs outside the body, and the resultant embryos are subsequently implanted back into the uterus. This approach can help resolve male and female infertility problems. The rate of successful IVF pregnancies at qualified laboratories is around 20-60%, depending on the age of the woman [4]. The ART, however, can lead to side effects, such as Ovarian Hyperstimulation Syndrome (OHSS).

The syndrome can occur iatrogenically due to the high hormone dose administered to the patient during the oocyte stimulation phase. Human chorionic gonadotropin is one of the hormones used in the stimulation process. The greater number of oocytes produced increases the chance of fertilization and success of the technique. However, this boosting of hormone level to increase success is associated with a 2-3% incidence of moderate and severe forms of OHSS in ARTs [5]. By comparison, OHSS incidence in a Referral Hospital for Assisted Reproduction in São Paulo, Brazil was 1.9% [6].

The syndrome affects 6020 patients annually in the USA and Europe, with an estimated mortality of 1 in 450,000-500,000 [7]. Risk factors for OHSS include younger age, history of polycystic ovary syndrome (PCOS), personal history of high response during a previous IVF fertilization cycle and on evaluation of biomarkers, such as anti-Müllerian hormone level and follicles using ultrasound [8].

The physiology of OHSS is complex and not yet fully understood. However, the syndrome involves increased vascular permeability of the mesothelial layer of ovaries and leakage of protein-rich fluids into the interstitial or "third" space. Clinical symptoms reflect the degree of third-spacing and hemoconcentration resulting from the depletion in intravascular volume [9].

Pro-inflammatory vasoactive mediators, such as vascular endothelial growth factor (VEGF) are believed to be involved in this pathogenesis [10]. When stimulated under supraphysiological conditions, ovaries oversecrete VEGF to above normal levels, promoting excessive vascular permeability with leakage to the third space, leading to reduced perfusion of organs [11]. Some studies have shown stronger association of VEGF with human chorionic gonadotrophin (hCG) and higher VEGF in peritoneal fluid of patients who used hCG when compared to the GnRH agonist [12].

The signs and symptoms vary with syndrome severity. Mild symptoms occur in roughly 30% of patients, such as slight discomfort and distended abdomen due to increased volume of one or both ovaries [5, 13]. More severe forms can present with ascitis of varying severity, plural effusion, oliguria secondary to renal failure, thromboembolism, and death can occur as a result of hemoconcentration and reduced perfusion of other organs such as the kidneys, heart and brain [14].

The two main types of OHSS are late and early onset. In early OHSS, symptom onset takes place 7 days after the application of hCG (administered for final oocyte maturation), whereas late OHSS manifests 10 days after hCG application and is triggered by endogenous hCG produced by trophoblasts following pregnancy. The late form of OHSS, compared with the early form, has a higher probability of becoming severe (72.2% versus 42%) [15].

One method of preventing OHSS is by performing cryopreservation of embryos. The technique entails freezing embryos and implanting them during a later cycle, when ovarian response has normalized after previous hyperstimulation for follicle production. In addition, the use of an antagonist protocol and final follicle maturation with an Gonadotropin-releasing hormone agonist (GnRHa) trigger, followed by the freeze-all strategy, constitutes an effective option for the prevention of OHSS with high live birth rate [16, 17].

Taken together, this evidence suggests that cryopreservation represents one of the best prevention approaches for patients at high risk of OHSS. Therefore, the objective of the present study was to assess cryopreservation as a strategy for the prevention of OHSS and to identify the risk factors associated with the syndrome.

Materials and Methods

A cross-sectional, institutional descriptive study of secondary data from charts of patients enrolled in the Assisted Reproduction Service of the Pérola Byington Hospital at risk for OHSS (≥ 15 antral follicles or ≥ 15 oocytes aspirated) after controlled ovarian stimulation and submitted to cryopreservation of all embryos, was conducted between January 2015 and September 2017.

Exclusion Criteria

Patients undergoing cryopreservation for preservation of fertility, genetic factors, recipients and/or with excess embryos were excluded.

Variables

The variables evaluated were age, body mass index (BMI – weight/height² (kg/m²) categorized according to the World Health Organization criteria (WHO)); infertility factors (tubal factor, polycystic ovarian syndrome, endometriosis, male factor); levels of anti-Müllerian, follicle-stimulating and luteinizing hormones; antral follicle count by ultrasound; number of aspirated oocytes, number of mature oocytes; fertilization rate; fertilization technique employed: intracytoplasmic sperm injection (ICSI) or in vitro fertilization (IVF); embryo transfer; occurrence of OHSS and related complications using the classification of Golan *et al.* [18], for mild, moderate and severe forms; type of OHSS treatment; occurrence of biochemical pregnancy (detection of human gonadotropin hormone – hCG in maternal plasma 14 days after embryo transfer, with cut-off value = 5 mIU/ml) and clinical pregnancy (pregnancies confirmed by β-hCG and detection of gestational sac on ultrasound from 7 weeks gestation).

The protocols for controlled ovarian stimulation for IVF/ICSI employed in the human reproduction service studied were assessed. The protocol used was dictated by

Protocol A (AFC ≤10): stimulation with 300 IU FSH/hMG daily; GnRH antagonist (Cetrorelix or Ganirelix) 0.25mg; trigger with hCG (Choriomon 5000 IU) or GnRH agonist (Gonapeptyl 0.2 mg daily) when risk of OHSS.

Protocol B (AFC =11-15): long block with GnRH agonist (1.875 mg Triptorelin); stimulation with 300 IU FSH/ hMG daily; trigger with hCG (Choriomon 5000 IU).

Protocol C (AFC ≥ 15): stimulation with150 IU FSH/ hMG daily; GnRH antagonist (Cetrorelix or Ganirelix) 0.25mg; trigger with GnRH agonist (Gonapeptyl 0.2 mg daily).

Sample calculation, data collection and data analysis

The sample size was calculated using a Confidence Interval adopting an initial prevalence of 3% based on the article *Incidence and prediction of ovarian hyperstimulation syndrome in women undergoing gonadotropin-releasing hormone antagonist in vitro fertilization cycles* [19]. A 95% level of confidence and 4.5% error margin yielded an n = 55.

Data collection was carried out based on a review of medical charts using a data collection instrument containing the variables of interest for the study. A database was created using the statistical software SPSS (Statistical Package for Social Sciences) for Windows version 17.0. 5, where data were tabulated and analyzed. Continuous variables were expressed as the summary measures mean, median, standard deviation, minimum and maximum. Categorical variables were expressed as absolute (n) and relative (%) frequencies. The prevalence of OHSS and respective confidence interval were calculated for a 95% confidence level. Associations

between categorical variables were explored with bivariate analysis using chi-square or Fisher´s Exact tests, adopting a p-value < 0.05 as significant.

Ethical Aspects

The study was approved by the Plataforma Brasil by the Research Ethics Committee under CAAE: 46741721.6.0000.0069 and Permit no. 4.735.050, for report dated 26/05/2021. The study complied with the National Board of Health resolution CNS 196/96 on research involving humans.

Given this was a retrospective Care Protocol study based on secondary data from medical charts, involving no intervention, the level of risk was deemed minimal. The main potential ethical conflicts concerned information confidentiality and secrecy. In this regard, anonymity was guaranteed and data was to be used only for the purposes of the study. The researchers agreed not to disclose identity or violate any data on identity of patients which could violate their individuality.

Results

A total of 64 fertilization cycles of patients at risk of developing OHSS undergoing cryopreservation of all embryos were assessed. The mean age of patients was 32 years, range 23-40 years. and 56.1% were never-pregnant. The results of continuous variables are given in Table 1.

	Mean	SD[1]
Age	32.0	3.8
BMI[2]	26.6	4.9
AFC[3]	16.9	6.1
No. of aspirated oocytes	19.0	8.6
No. of mature oocytes	15.6	7.9

1. Standard Deviation; 2. Body Mass Index; 3. Antral follicle count

Table 1: Distribution of continuous variables for cycles of patients undergoing cryopreservation of all embryos

With regard to Body Mass Index (BMI), the mean value was 26.6 (overweight), 32.8% of patients were of healthy weight, and 2 had class 3 obesity.

Six cycles had 2 or more infertility factors. Of these cases, 43.8% had tubule factor, 28.1% polycystic ovarian syndrome (PCOS), 17.2% male factor,15.6% endometriosis and 7.8% had unexplained infertility.

Anti-müllerian hormone level averaged 5.1 ng/ml, and was ≥ 3.5 ng/ml in 48.4% of cases. Mean FSH level was 5.9 mIU/ml and LH level was 6.4 mIU/ml.

Protocol C was the most used (50.0%), followed by B (42.2%) and A (7.8%). All patients underwent cryopreservation of embryos to prevent OHSS. Mean number of antral follicles was 16.9, and 64.1% of cases had ≥ 15 follicles. Mean number of aspirated follicles was 19, and mean mature oocytes was 15.6. Mean fertilization rate was 72.8%.

The results of categorical variables are given in Table 2.

	Categories	N	%
Protocol	A	5	7.8
	B	27	42.2
	C	32	50
Fertilization technique	IVF[1]	14	21.9
	ICSI[2]	50	78.1
Embryo Transfer	Yes	56	87.5
Occurrence of Pregnancy (Total N = 56)	Biochemical	28	50.0
	Clinical	22	39.2
BMI[3] (Kg/m²)	Healthy weight (18.5-24.9)	21	32.8
	Overweight (25–29.9)	16	25
	Class 1 Obesity (30-34.9)	7	10.9
	Class 3 Obesity (>40)	2	3.1
AMH[4] (Total N = 31)	< 3.5 ng/ml	16	51.6
	≥ 3.5 ng/ml	15	48.4
Antral Follicle Count (AFC)	< 15	23	35.9
	≥ 15	41	64.1

1. In vitro fertilization; 2. Intracytoplasmic sperm injection; 3. Body mass index; 4. Anti-müllerian hormone

Table 2: Distribution of categorical variables for cycles of patients undergoing cryopreservation of all embryos

Intracytoplasmic sperm injection (ICSI) was the most commonly used fertilization technique (78.1% of cases). Embryo transfer was performed in 87.5% of the cycles. Biochemical pregnancy occurred in 50% of these cycles and clinical pregnancy in 39.2%. There were a total of 22 pregnancies, 4 of which were twin pregnancies.

OHSS occurred in 47.5% (CI = 22.7–68.2) of cycles, all with mild symptoms. There were no cases of moderate or severe OHSS. The results for risk factors associated with OHSS are given in Table 3.

		Mild OHSS[1]		P[2]	95% CI[3]	
		Yes	No		LB[4]	UB[5]
No. of aspirated oocytes	≤ 15	8 (42.1)	11 (57.9)	0.567	0.53	4.58
	> 15	21 (50.0)	21 (50.0)			
Trigger	Gonapeptyl	13 (39.4)	20 (60.6)	0.212	0.69	5.39
	Choriomon	15(55.6)	12 (44.4)			
AMH[6]	≤ 3.4	5 (31.3)	11 (68.8)	0.213	0.09	1.72
	> 3.4	8 (53.3)	7 (46.7)			
BMI[7]	< 25	10 (45.5)	12 (54.5)	0.654	0.41	4.10
	≥ 25	13(52.0)	12 (48.0)			
PCOS[8]	No	21 (46.7)	24 (53.3)	0.819	0.36	3.58
	Yes	8 (50.0)	8 (50.0)			
Age	> 35	5 (55.6)	4 (44.4)	0.724	0.35	6.05

1. Ovarian hyperstimulation syndrome; 2. Significance level < 0.05; 3. Confidence interval; 4. Lower bound; 5. Upper bound; 6. Anti-müllerian hormone; 7. Body Mass Index; 8. Polycystic ovarian syndrome

Table 3: Factors associated with OHSS in cycles of patients undergoing cryopreservation of all embryos

Discussion

This study showed that cryopreservation of all embryos prevented moderate and severe forms of ovarian hyperstimulation syndrome (OHSS) in patients at risk for OHSS, in that there were no moderate or severe cases of the syndrome. This evidence supports embryo freezing as a key strategy for reducing risk in susceptible patients, given that fresh embryo transfer triggers a further hCG surge following implantation.

These findings are consistent with previous results reported in the literature. A randomized clinical trial describing the use of *freeze-all* compared elective cryopreservation of all embryos with a new fresh embryo transfer in patients at risk of OHSS. These authors found a reduced risk of moderate/severe OHSS, where 6% of patients in the fresh embryo transfer group developed severe OHSS versus zero cases in the freeze-all group. Also, there was no significant difference in live birth rates between the 2 groups [20].

Several principal clinical parameters have been established, such as number of follicles on the day of the trigger, to help attenuate the risk of moderate and severe OHSS [21]. Thus, the use of *freeze-all* after a GnRH agonist trigger is the gold standard strategy for patients at risk of OHSS. With regard to clinical aspects, it is important to note that embryo freezing is a well-established technique with similar pregnancy rates as fresh embryo transfer [22, 23].

In the present study, mild OHSS occurred in 47.5% of the patients assessed. Other studies report mild syndrome in around 30% of IVF cycles [5]. One of the determinants of this rate is the fact that total prevention of OHSS is not possible until the pathogenesis of the syndrome has been fully elucidated. Thus, although it can prevent late OHSS (moderate and severe forms), cryopreservation is not totally effective for the prevention of the syndrome because the strategy cannot prevent early onset OHSS (mild form) [9].

Another factor which may influence the outcome of this study for mild cases of OHSS is the protocol employed for the cycle. The choice of protocol was dictated by antral follicle count (AFC) and risk factors, where women with AFC>15 underwent the protocol with antagonist and GnRH agonist trigger (Protocol C). However, 42.2% of cycles were performed using Protocol B (use of agonist and hCG trigger) because AFC count was between 11 and 15. Nevertheless, this number of follicles subsequently rose following ovarian stimulation to over 15 follicles aspirated, a factor which may have influenced the development of OHSS.

Generally, there is a tendency for fewer moderate and severe cases of OHSS. This reduction is due to the greater screening of risk factors, with ovarian marker studies that predict supraphysiological response and allow the use of individualized ovarian stimulation protocols. In addition, wider use of GnRH antagonists for the prevention of premature release of LH, and expansion of freeze-all procedures, are factors contributing to a reduction in complications related to OHSS [24].

The specific risk factors (markers of ovarian reserve) are anti-Müllerian (AMH) level and antral follicle count (AFC) [6]. Randomized prospective studies have shown that a basal AMH level ≥ 3.5 ng/ mL can predict hyper-response with high sensitivity (90.5%) and specificity (81.3%) [25]. Women with levels exceeding 5 ng/mL are at 3 times greater risk of developing OHSS [26]. Studies suggest that an AFC >16 has 89% sensitivity and 92% specificity of predicting high ovarian response [27].

Risk factors associated with patient profile include women who are young, underweight, have presented the syndrome in previous cycles and have a history of PCOS [8]. Moreover, a study identified a high risk for OHSS in patients with ≥15 follicles measuring ≥ 11mm on the day of the trigger [28]. In the present study, risk factors were evaluated and no statistically significant association with OHSS was found, possibly explained by the small sample size, since this was not a primary objective of the study.

This study has some limitations, such as the final pregnancy rate, where some patients had not undergone embryo transfer and were still being treated at the assisted reproduction service during the study period. Other patients also went on to receive a further stimulation cycle. The retrospective design of the study represents another limitation. Further studies involving larger casuistics will allow investigation of other aspects related to this outcome, improving the success of assisted reproduction techniques and preventing complications such as severe forms of OHSS.

Conclusion

In summary, cryopreservation of all embryos was found to prevent moderate and severe forms of OHSS. Risk factors for OHSS should be evaluated before commencing treatment, with less intense stimulation protocols adopted accordingly. Thus, efforts should be made in OHSS prevention, given that once the syndrome has developed, there is no reliable form of treatment, particularly in severe cases.

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