UNIVERSITY OF MEDICINE AND PHARMACY OF CRAIOVA
DOCTORAL UNIVERSITY

OPTIMIZATION OF OVARIAN RESPONSE IN PROCEDURES OF IN VITRO FERTILIZATION FOR PATIENTS WITH LOW OVARIAN RESERVE

DOCTORAL STUDY

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CRAIOVA 2019

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CAP III. HISTORY OF IN VITRO FERTILIZATION

In vitro fertilizaton, commonly known as IVF has captured public attention since its sensational introduction in 1978. Assisted reproduction technology, nowadays is available in most of the civilized cities of the world.

HISTORY OF IN VITRO FERTILIZATION IN THE WORLD

Since 1980, the first embryo transfer has been done by Walter Heape, professor and physician at the University of Cambridge, England. In 1978, the first in vitro fertilization in England, was done by Patrick Steptoe and Robert Edwards.

First in vitro maturation of oocytes extracted by vaginal ecoguided puncture taking place in 1994.

HISTORY OF IN VITRO FERTILIZATION IN ROMANIA

In Romania, Acad. Ioan Munteanu established the first in vitro fertilization clinic in 1995. The 2nd Clinic was established by Acad.Prof Dr. Bogdan Marinescu at Obstetrics Gynecology Hospital Prof. Dr.Panait Sârbu, Bucharest.

In 2005, there was a premiere, the oldest woman who gave birth to fetus at 66-year-old . She followed an IVF protocol with donated oocytes and donated sperm.

CAP IV.INFERTILITY

Infertility represents the incapacity of a couple to procreating after 1 year of unprotected sex life.

The most important causes of female infertility are age, ovarian reserve, and oocyte quality.

DIAGNOSTIC ALGORITHM OF FEMALE INFERTILITY

Anamnesis, clinical examination, transvaginal ultrasound, HSG, laparoscopy, MRI, as well as AFC, FSH, AMH, are key elements indispensable for establishing the diagnosis of infertility and the causes that have determined this pathology.

LOW OVARIAN RESERVE

In the current practice, POR patients have reduced ovarian reserve (AMH <1.2ng / ml, AFC <7), poor response to maximal stimulation with exogenous gonadotropins (proven by previous stimulation) and most often advanced age.

The Bologna criteria.

The POSEIDON clasification.

CAP V.OVARIAN STIMULATION FOR IN VITRO FERTILIZATION

Controlled ovarian stimulation represents the key manipulation process of the ovarian function, the first objective is to achieve an increased number of follicles and oocytes.

FSH, LH gonadotrophic hormones are indispensable in the development of antral follicles. For FIV, reaching and exceeding the FSH threshold as well as maintaining the FSH window opening are key elements in controlled ovarian stimulation.

CONTROLLED OVARIAN STIMULATION

Gonadotropins

GnRH

GnRH agonists

PROTOCOLS TYPES

MINIMAL STIMULATION
AGONIST PROTOCOLS

Long agonist protocol
Short agonist protocol
SHORT ANTAGONIST PROTOCOL
DUAL STIM
ADJUVANT THERAPIES

OVARIAN PUNCTURE

First used in 1987, vaginal ecoguided puncture is the most used method of oocytes retrieval.

EMBRYOS TRANSFER

Embryo transfer is basically the final stage and it is imperative to make a precise pre-transfer balance.

Elective single embryotransfer-sET has become more and more common. Embryo quality is the main factor that directly influences pregnancy rate. Complex embryo / endometrial compatibility ensures success of implantation. Intrauterine platelet transfusion (PRP) has had good results in improving endometrium quality.

BIRTH RISKS AND OBSTETRIC COMPLICATIONS IN IN VITRO FERTILIZATION PREGNANCY

Pregnancies obtained by human reproductive techniques are associated with an increased incidence of obstetric complications. The risk of obstetric complications increases directly in proportion to age.

These pregnancies have an increased risk of associating HTA, preeclampsia, gestational diabetes, abnormal insertion of placenta, retroplacental hematoma, increased maternal and perinatal mortality.

CAP VI.MATHERIAL AND METHOD

My study is retrospective and prospective based of 665 patients who performed an in vitro fertilization procedure between January 2015 and May 31, 2019 with a total of 1511 punctures. This study was done by collaborating with the Department of In Vitro Fertilization of the Clinical Obstetrics and Gynecology Hospital, Prof.Dr.Panait Sârbu.

The data were introduced into the Excel and SPSS tables where they were analyzed and processed statistically. I have especially identified that patients who had a poor response to in vitro fertilization procedures.

I have analysed parameters such as: age (age range), AMH, AFC, FSH, type of stimulation protocol followed, type of medication, number of days of stimulation, type of IVF / ICSI procedure, number of oocytes retrieved, growth rates of embryos - day 3 or blastocysts, the type of fresh or thawed embryo transfer, biochemical pregnancies and the live birth rates.

All of the patients performed the in vitro fertilization procedures: hormonal profile on day 2-3 of the menstrual cycle, laboratory analysis, serology, Babeș-Papanicolau exam, cervical and vaginal cultures , ultrasonography for AFC measurement in the follicular phase, AMH, hysterosalpingography or hysteroscopy, diagnostic hysteroscopy, laparoscopy, psychological examination of the infertile couple.

For the short protocol, the start of stimulation was achieved only if the progesterone dosed on day 2 was less than 0.8 ng / ml. The starting day of stimulation was day 2-3 of the menstrual cycle, the patient being recalled in the clinic for ultrasound monitoring.

For the long agonist protocol, inhibition was started on day 21 of the menstrual cycle for 14 days. After setting the down regulation status (estradiol <0.50 pg / ml, LH <2.5 IU / ml, endometrium <6mm) and progesterone <0.8ng / ml, gonadotrophin stimulation was started with daily agonist (Triptotelin 0.1 mg / day) administration. The trigger was performed with 10.000UI urinary HCG or 6500UI recombined HCG.

The doses of gonadotropins used, varied according to the age of the patients ,and started from the 150 IU FSH classic dose in patients under 35 years of age, or 225 IU FSH after 35year until the dose of 450 IU FSH.

Ovarian puncture was performed under transvaginal ultrasound by aspiration without flushing. There were no postoperative complications after performing the vaginal puncture in the analyzed group of patients.

Embryo transfer was performed at 72 hours or day 5 (blastocysts) with Wallace catheter under transabdominal ultrasound guide. There were no cases of retransfer in the analyzed patients. The transfer of thawed embryos was performed on the natural cycle, and in the case of anovulation, the artificial cycle was induced.

Patients whose endometrial thickness was under 7 mm performed 2 PRP procedures in days 7 and 12 of the following menstrual cycle. A significant increase in the thickness of the endometrium following these procedures was observed. Two ERA tests were performed to determine the optimal uterine receptivity for embryo transfer.

In order to support the classical luteal phase they used progesterone 200mg intravaginal 3 cpr / day, estrogen cpr between 6-8 mg / day cotinuously until 10 weeks of pregnancy.

Beta HCG was performed 10-12 days after embryo transfer. Ultrasound confirmation of pregnancy was performed 3-4 weeks after embryo transfer.

CAP VII.RESULTS AND CONCLUSIONS

I have identified a total of 129 patients as poor responder during the 5 years , representing 19,39% of a total of 665 patients that have done an in vitro fertilization procedure between January 2015 and May 2019. The annual responder rate of patients with poor ovarian response was 8.33% in 2015, 11.85% in 2016, 20% in 2017, 41% in 2018 and 12.2% in 2019.

A percentage of 20.2% patients where included in the group below 35 years and 79.8% over 35 years. Age is the most important prognostic factor for the success rate of assisted human reproduction. I have identified an increased number of patients over 35 years in studied group.

AMH comparative analysis revealed an increased percentage of 78.64% of patients group over 35-year-old age with AMH of less than 1.2ng / ml compared to patients below 35 years of age old where the majority of 57.69% was for of AMH above 1.2 ng / ml. This shows a correlation between AMH and age of the patients.

AFC is correlated with age. As higher the age is, the number of antral follicles is decreasing. For the group below 35 years an AFC of 0-4 follicles was 7.69% compared to 37.83% for the age over 35 years. AFC of 5-8 follicles is 61.55% in patients under 35 years versus 43.68% in patients over 35 years . The higher the AMH value is, the higher is the AFC .

The average number of oocytes retrieved for the patients below of 35 years old was 3.66 oocytes compared to an average of 4 oocytes obtained in the group over 35 years old.

The average number of embryos obtained after fertilization was 3 embryos for the group of patients below 35 years versus 2 embryos obtained in patients over 35 years old.

By correlating the average of the retrieved oocytes and the mean of embryos obtained, a higher success rate is observed for the age group below 35 years.

I analyzed each type of ovarian stimulation protocol used for the in vitro fertilization. So, i have noticed that the long agonist protocol was used in 33.3%, the short antagonist protocol in 55% and the short agonist protocol in 11.6% of cases.

On age groups, a superior use of the short antagonist protocol was observed at both age categories: 50.48% over 35 years versus 73.07% under 35 years. The long agonist protocol was used only for patients over 35 years with incidence of 41.74%, while the short agonist protocol was used mostly in 26.93% of cases in patients between 35 years old.

The average number of stimulation days for patients under age of 35 years was 9.31 days versus 10.10 days for patients over 35 years with a total average of 9.94 stimulation days per puncture.

I was interested in how the embryos where fertilized and so i analyzed the frequency of each type of IVF / ICSI procedure. I observed that for patients under 35 years IVF had the majority with 96.15% versus 65.04% for patients over 35 years.

Transfer of fresh embryos predominated with 58.8% compared to 41.17% for thawed embryos.

The rate of biochemical pregnancy was 69.2% for patients under 35 years versus 33.9% for patients over 35 years . The total rate of biochemical pregnancies in the analyzed group was 38.8%. For 27.96% of the patients the embryo transfer was not performed.

Analyzing the pregnancy rate in relation to the number of oocytes obtained by puncture, i have noticed that the biochemical pregnancy rate increases depending on the number of oocytes and embryos. The implantation rate for the analyzed patient group was 57.6%, with an implantation rate of 11.76% for the 3 days embryos versus 45.88% for the blastocysts. By analyzing the pregnancy rate corelated to the fertilization type of FIV / ICSI , 54% of biochemical pregnancies were obtained after standard IVF and 13% were obtained after ICSI.

Out of the total number of biochemical pregnancies , 41 had a gestational duration over 37 weeks with live fetus, 3 pregnancies had gestational under 37 weeks, and 3 pregnancies have stopped evolution.

The statistical analysis of each protocol type according to different parameters highlighted the effectiveness of each in relation to optimization of ovarian response.

The long agonist protocol was used in 33.3% of stimulation cycles, respectively 43 stimulation cycles.

Reported to AMH, this protocol type was used in cases where the AMH <1.2ng / ml.

At 86.04% of cycles a daily dose (0.1 mg of Triptorelinum) was used to achieve down-regulation versus 6 stimulation cycles benefiting from a single inhibitory dose (Triptorelin 3.75 mg). The number of days inhibition was 12.32 days.

Based on the number of exogenous gonadotropin ovarian stimulation days, we noticed that the average number of days of stimulation was 12 days, with 51.16%.

The average oocytes retrieved by puncture was 3 (23%). Considering the use of this protocol type at an AMH of less than 1.2 ng / ml and a 100% use for AFC 0-4 follicles, the yield per oocyte number obtained under the long protocol was successful.

The average number of embryos obtained was 1.65 embryos. However, the use of the long agonist protocol for ovarian stimulation achieved for 48.8% of 2 embryos. The rate of cleavage was 56.8%.

The embryo fertilization method for this type of protocol was 53.55% for standard IVF versus 32.55% for ICSI. A percentage of 13.9% of patients who received this type of protocol did not get oocytes in the ovarian puncture

Regarding the quality of embryos obtained after oocyte fertilization in vitro, the ratio was 75.67% blastocysts versus 24.32% day 3 embryos.

The embryo transfer performed in 52.38% of the cases was with cryopreserved and thawed embryos and in 47.61% of the cases with fresh embryos. Thawed embrio transplant was performed with rate of 72.7% per natural cycle and 27.3 % per artificial cycle.

The rate of pregnancy achieved was 25.58% related to the number of patients benefiting of this protocol type and 12.9% related to the total number of embryo transfers .

The short antagonist protocol was used in 55.03% of cases.

By AMH, the ratio was 53.52% for the group of patients with AMH> 1.2ng / ml and 46.47% for AMH <1.2ng / ml.

It was utilized in 10.85% of cases in patients with low responder risk in ovarian stimulation, 27.90% in patients with a high risk of poor response to ovarian stimulation, and 16.27% in patients with normal ovarian reserve .

The mean number of stimulation days for the short antagonist protocol was 8.8 days.

The stimulating duration for the short antagonist protocol was 8-9 days with a maximum of 65.07%.

The oocyte average retrieved by ovarian pounce was 3.5 oocytes, the maximum being 4 in 40.84% of the cases.

The average embryos were 2.59. Embryo quality was 71.83% for blastocysts versus 22.54% for 3 days embryos.

The cleavage rate was 83.5% and the total transfer rate for this type of protocol was 71.83%. Major transfers were 71.83% of fresh embryos versus 43.13% of thawed embryo transfers.

The pregnancy rate for the short antagonist protocol was 42.35%.

Concydering the embryo quality, embryo transfer, pregnancy rate, and pregnancy with normal gestation over 37 weeks, I have noticed an increased number of high-quality embryo transfers with very good quality .The normal gestational pregnancy rate was 50.7%.

The short agonist protocol was used in 11.6% of the total stimulation procedures.

For the age group under 35 years was used in 26.9% of cases versus 7.76% for the patients over 35 years.

According to the number of patients who received this type of ovarian stimulation, the percentage was 46.6% between 35 years versus 53.3% over 35 years.

Within the analyzed group, this type of protocol was not used in patients with normal ovarian reserve and it was used for the patients with AFC 0-4 category for 93.33%.

The use of the short protocol for AMH was 93.33% for AMH above 1.2 ng / ml versus 6.6% for AMH above 1.2 ng / ml.

The maximum number of oocytes obtained by using the short agonist protocol was 5 oocytes with 86.6%. The average of oocytes was 4,66.

The average embryo obtained by short agonist protocol was 2.33 with a minimum of 0 embryos and a maximum of 4 embryos, the frequency of 2 embryos accounting for 46.66% of the total number of embryos. The cleavage rate was 50%.

Analyzing the achieved pregnancy rate, only 2 pregnancies where obtained with a success rate of 2.35% , the transferred embryos beeing in the third day.

There is a close correlation between pregnancy and age, AMH and AFC. The pregnancy rate was even higher as the age was lower.

The highest incidence of pregnancy i identified in patients with 4 oocytes (26%) and the lowest frequency being only 2% for single oocyte.

Depending on the type of fertilization of the standard IVF / ICSI , the highest was 54% for standard FIV as compared to 13% ICSI, and for the quality of embryos, the highest rate of pregnancy was observed for blastocysts with a majority percentage of 78%.

Of the total of 50 pregnancies obtained by in vitro fertilization, 20 where associated with obstetric complications: 7.3% associated with placenta praevia, 9.7% associated with metrorrhagia and premature cleavage of the normal inserted placenta , 12.19% HTA and 19.51% were complications due to gestational diabetes.

Concerning AMH, the short antagonist protocol had a frequency of use of 2.53% for the group of patients with AMH> 1.2 and 46.47% for AMH <1.2.

Analyzing based on the value of the AFC evaluated at the start of each stimulation cycle, a 55.03% use rate was observed in favor of the short antagonist protocol.

The highest pregnancy rate was obtained using the 72% antagonist protocol compared to the lower 4% rate obtained using the agonist protocol.

At the same time, the percentage of use of the short antagonist protocol was increased for all variables analyzed: 55.03% by AMH, 55.03% AFC, 72% for pregnancy rate and 55% for age. This demonstrates the efficiency of using it and the increased success rate it has maintained.

There was clearly a much higher percentage of pregnancy rates in the age range of under 35 years. Regarding age-related pregnancy rate, we observed a higher rate in the age range below 35 years 69.2% versus 33.9% over 35 years, the total pregnancy rate being 38.8% relative to the total of patients analyzed. The failure rate of obtaining a pregnancy was 61.2%.

Relative to the number of oocytes obtained the highest frequency of pregnancy we identified it in patients with 4 oocytes with 26% and the lowest frequency being only 2% for single ovulated patients.

Interestingly, from the statistical analysis of the rate of pregnancy obtained according to the type of fertilization of the standard IVF / ICSI embryos, the highest share was 54% in favor of IVF versus 13% ICSI considering the increased group of patients over 35 years