Optimization and monitoring of IVF treatments requires good data on the effect and magnitude of clinical factors affecting treatment outcome. Many factors have been known to affect IVF outcomes. Currently there are still no data to predict whether a patient who undergoes In Vitro Fertilization (IVF) cycles can be considered a good candidate for oocyte freezing. The aim of this study was therefore to evaluate which biological and biochemical factors can be predictive of oocyte survival and fertilization, as well as of clinical pregnancy in oocyte thawing cycles. This study showed that none of the factors available on the day of the pick-up is able to predict (in case of oocyte cryopreservation) the success of a subsequent oocyte thawing cycle. Only the transfer of at least one Grade 1 embryo after oocyte thawing cycle has a statistically significant impact on pregnancy. Unfortunately, this cannot be considered an elective factor to guide the clinician and/or the embryologist in choosing patient's treatment as it is not available on the day of the oocyte pick up but it is a result of oocyte thawing.

**Keywords:** Oocyte thawing; Biological and biochemical markers; Fertilization rate; Ongoing pregnancy rate

---

**Biological and Biochemical factors Predictive of Oocyte survival, Fertilization, Pregnancy in oocyte thawing cycles**

Enrica Capitanio*, Alessia Galimberti, Laura Zanga, Federica Paternostro, Sara Melis, Antonella Di Pasqua, Ilario Candeloro and Francesco Maria Fusi

UOSD Centro PMA ASST Papa Giovanni XXIII Bergamo, Piazza OMS 1, 24127 Bergamo, Italy

*Corresponding Author: Enrica Capitanio, UOSD Centro PMA ASST Papa Giovanni XXIII Bergamo, Piazza OMS 1, 24127 Bergamo, Italy, Tel: +390352674479; Email: ecapitanio@asst-pg23.it

All authors contributed equally to this work.

**Abstract**

Optimization and monitoring of IVF treatments requires good data on the effect and magnitude of clinical factors affecting treatment outcome. Many factors have been known to affect IVF outcomes. Currently there are still no data to predict whether a patient who undergoes In Vitro Fertilization (IVF) cycles can be considered a good candidate for oocyte freezing. The aim of this study was therefore to evaluate which biological and biochemical factors can be predictive of oocyte survival and fertilization, as well as of clinical pregnancy in oocyte thawing cycles. This study showed that none of the factors available on the day of the pick-up is able to predict (in case of oocyte cryopreservation) the success of a subsequent oocyte thawing cycle. Only the transfer of at least one Grade 1 embryo after oocyte thawing cycle has a statistically significant impact on pregnancy. Unfortunately, this cannot be considered an elective factor to guide the clinician and/or the embryologist in choosing patient's treatment as it is not available on the day of the oocyte pick up but it is a result of oocyte thawing.

**Keywords:** Oocyte thawing; Biological and biochemical markers; Fertilization rate; Ongoing pregnancy rate

---

**Introduction**

Mature oocyte cryopreservation (OC) is a method to preserve reproductive potential in women of reproductive age. Clinical applications of oocyte cryopreservation include fertility preservation in cancer patients [1,2], social freezing [3], egg donation programs [4,5], minimization of ovarian hyperstimulation syndrome risk, oocyte accumulation in low-responder patients [6], and surplus oocyte storage after controlled ovarian stimulation when embryo cryopreservation is not feasible [7]. Optimization and monitoring of IVF treatments requires good data on the effect and magnitude of clinical factors affecting...
outcomes. It is known that several factors affect IVF outcomes including age, sperm quality, fertilization rate, embryo quality, frequency of transferred embryos, and endometrial thickness [8]. If any factor in oocyte thawing cycles could potentially influence the likelihood for a successful IVF treatment, this would enable clinicians and physicians to make better decisions in order to apply IVF depending on patients’ characteristics. Therefore, it is important to assess biological and biochemical markers available the day of oocyte retrieval which could reveal which patients who undergo In Vitro Fertilization are good candidates for oocyte freezing. This may be helpful to optimize IVF treatment in order to enhance good pregnancy outcome. At present there are no available data about this selection. The aim of this study was to evaluate which biological and biochemical factors available at the day of the oocyte pick-up can be considered predictive in survival and oocyte fertilization, as well as in the achievement of pregnancy in oocyte thawing cycles.

Methods

Patients

This retrospective study was performed at the fertility department of ASST Papa Giovanni XXIII. The inclusion criteria were female patients who had cryopreserved supernumerary oocytes at the day of pick-up and subsequently underwent oocyte thawing cycles. Exclusion criteria applied: patients who had cryopreserved the oocytes for fertility preservation or did not consent to embryo cryopreservation, ICSI performed with testicular sperm extraction (TESE). This study met and is in compliance with all ethical standards in medicine, and informed consent was obtained from all patients according to the Declaration of Helsinki and to the Italian legislation (Authorization of the Privacy Guarantor No. 9, December 12, 2013).

Hormone dosage

Antimullerian hormone (AMH) was measured by AMH Gen II ELISA (Beckman-Coulter) immunoassay. Serum androstenedione was dosed with the IMMULITE 2000 system at a competitive immunoenzymatically dosage in chemiluminescence and solid phase. Free serum testosterone and serum estradiol were dosed using a direct chemiluminescent competitive assay (ADVIA Centaur).

Ovarian stimulation

Patients were treated with two types of ovarian stimulation protocols to obtain an acceptable number of oocytes avoiding the risk of ovarian hyperstimulation syndrome (OHSS). The standard long stimulation protocol with GnRH agonist was started during the luteal phase (21-23 days) of the cycle. After pituitary down-regulation, ovarian stimulation was carried out with daily doses of recombinant follicle-stimulation hormone (FSH). The dose of gonadotropins was adjusted according to the follicular growth that was monitored by transvaginal ultrasonography and serum estradiol concentrations. The GnRH antagonist protocol was performed using recFSH from day 3 of the cycle, and the addition of an antagonist started from day 6 of gonadotropins administration or when a follicle of at least 14mm diameter was observed. The daily administration of GnRH antagonist was carried out until the trigger with hCG. When the lead follicle achieved 18 mm diameter, the hCG was given to trigger the ovulation. Oocyte retrieval was carried out 36 h later by ultrasound-guided transvaginal puncture.

Oocyte Vitrification/Warming

Oocytes were denuded within 38 hours after trigger and classified according to their maturation stage. The vitrification and thawing were performed according to the protocol by Kuwayama [9]. Briefly, Oocytes were treated at room temperature. Up to three oocytes were gradually equilibrated into equilibration
solution for 12-15 min, until the oocyte volume recovered almost completely. Oocytes were then exposed for 1 min to vitrification solution, aspirated within a capillary and put on the distal end of the Cryotop sheet with minimum amount of versus solution (0.1 lI or less) in a planar droplet. The Cryotop was plunged immediately into liquid nitrogen, capped and transferred to the storage tank. For warming, the Cryotop end was quickly submerged into 1 ml of warming solution containing 1.0 mol/l sucrose at 37C. After 60 s, oocytes were moved to 0.5 mol/l sucrose solution for 3 min at room temperature. Oocytes were finally washed with basic solution (0 mol/l sucrose) for a total time of 6 min at room temperature. After warming the oocytes were incubated in culture medium for 2 hours before Intracytoplasmic Sperm Injection (ICSI).

**Embryos transfer**

Embryo transfer was performed 2-5 days after ICSI. Embryos were assessed according to the ESHRE consensus guidelines [10]. Hormonal therapy (HT) protocol was used for endometrial preparation:

- Estroprogestinic therapy or GnRh-a suppression;
- At the beginning of new cycle, administration of Progynova 2mg starting from 1 to 3 cp per day;
- Administration of Progynova 2mg 3 cp per day from the 8th day of the cycle;
- Ultrasound check between day 8 and day 10 with estrogen and progesterone venous sampling;
- Starting so ministration of progesterone 600 mg per day the day after oocyte thawing.

The serum BHCG test was determined by two separate blood samples (two occasions), 10 days and 15 days post fresh ET or cryopreserved ET. In case of pregnancy, estrogen and progesterone therapy was suspended for 70-90 days after embryo transfer. Clinical pregnancy was defined as the ultrasound visualization of at least one intrauterine gestational sac. Delivery refers to viable infants born after 24-weeks’ gestation. First pregnancy ultrasound was performed in the participating infertility units whereas the course of pregnancy was assessed through telephone contact.

**Statistical analysis**

Basal characteristics were collected for every couple and IVF cycle in order to analyze possible predictors of the following outcomes: oocyte survival at thawing, fertilization rate of thawed oocytes, positive βhCG and clinical pregnancy rate. Based on each of these outcomes, subgroups of couples with unfavorable results were identified and basal characteristics were compared to those of couples with favorable results. A multi-variable logistic analysis was performed in order to identify possible predictors of success. Data analysis was performed using SPSS Software Statistics Desktop V 22.0 per MAC OS X (© IBM, International Business Machines Corp. New York, USA). p-values < 0.05 were considered statistically significant.

**Results**

A total of 237 patients aged between 22 to 42 who received IVF treatment were included.

**Oocytes survival after thawing**

The mean oocyte survival rate after thawing cycle was 88.9%. Therefore, it was used 80% as indicative value for a good oocyte survival in the thawing cycles. 187 out of 237 patients showed an oocyte survival rate greater than or equal to 80%. In 2 cases out of 237 no oocytes survived after thawing (data not shown).
Table 1: Oocyte’s survival ≥80% after thawing: parameters of the patients considered.

| Parameter                        | OOCYTE SURVIVAL ≥80% | P     |
|----------------------------------|----------------------|-------|
| **AGE mean (years)**             | 35.1±3.6             | 34.3±3.9 | 0.202 |
| **BMI mean (Kg/m²)**             | 22.1±3.5             | 22.9±3.7 | 0.197 |
| **ANDROSTENEDIONE mean (ng/mL)** | 2.9±1.6              | 3.1±1.6  | 0.627 |
| **AMH mean (µg/L)**              | 4.7±3.4              | 5.5±4.3  | 0.316 |
| **TESTOSTERONE mean (ng/mL)**    | 0.6±0.5              | 0.7±1.2  | 0.509 |
| **ESTRADIOL pg/mL**              | 2701.5±1336.9        | 2688.9±1148.7 | 0.952 |
| **ESTRADIOL/Nº OOCYTE RECOVERED**| 165.6±89.5           | 162.6±87.5 | 0.835 |

Table 2: Oocyte’s survival ≥80% after thawing: parameters of the patients considered for each group. P-value of each subgroup has been calculated respect to the total of the categories themselves.

| Parameter                        | OOCYTE SURVIVAL ≥80% | P     |
|----------------------------------|----------------------|-------|
| **AGE**                          |                      |       |
| <35 years                        | 84/108(77.8%)        | 0.698 |
| 35-39 years                      | 82/104(78.8%)        | 0.985 |
| >40 years                        | 21/25(84.0%)         | 0.509 |
| **BMI**                          |                      |       |
| <18 Kg/m²                        | 5/5(100%)            | 0.587 |
| 18-25 Kg/m²                      | 156/193(80.8%)       | 0.128 |
| 25-30 Kg/m²                      | 20/29(69.0%)         | 0.161 |
| >30 Kg/m²                        | 6/10(60.0%)          | 0.225 |
| **ANDROSTENEDIONE**              |                      |       |
| < 2.6 ng/mL                      | 31/42(73.8%)         | 0.697 |
| 2.6-4.9 ng/mL                    | 26/33(78.8%)         | 0.582 |
| 5-7.2 ng/mL                      | 2/3(66.7%)           | 1     |
| >7.2 ng/mL                       | 3/4(75%)             | 1     |
| **AMH**                          |                      |       |
| <1.1 µg/L                        | 4/4(100%)            | 1     |
| 1.1-5 µg/L                       | 95/116(81.9%)        | 0.609 |
| >5 µg/L                          | 48/62(77.4%)         | 0.410 |
| **TESTOSTERONE**                 |                      |       |
| <0.5 ng/mL                       | 47/62(75.8%)         | 0.548 |
| 0.5-1.85 ng/mL                   | 24/29(82.8%)         | 0.428 |
| 1.85-3.2 ng/mL                   | 1/1(100%)            | 1     |
| >3.2 ng/mL                       | 1/2(50%)             | 0.399 |
| **ANTAGONIST VS AGONIST**        | 144/186(77.4%)       | 0.285 |
| **INFERTILITY CAUSES**           |                      |       |
| Idiopathic                       | 20/24(83.3%)         | 0.575 |
| Ovulatory                        | 14/19(73.7%)         | 0.561 |
| Female Multiple                  | 8/11(72.7%)          | 0.607 |
| Male +Female                     | 86/104(82.7%)        | 0.206 |
| Male                             | 43/59(72.9%)         | 0.191 |
| Endometriosis                    | 6/6(100%)            | 0.347 |
| Tubal                            | 10/14(71.4%)         | 0.502 |
| Operator A                       | 72/91(79.1%)         | 0.927 |
| Operator B                       | 56/67 (74.6%)        | 0.322 |
| Operator C                       | 64/78(82.1%)         | 0.392 |
Some parameters were divided into subgroups and other factors were considered like the stimulation protocol and the cause of infertility. Moreover, the operator performing oocyte thawing was analyzed as a possible confounding element in the study (Table 2).

### Table 3: Fertilization rate $\geq 80\%$ of oocyte thawing: parameters of the patients considered.

| Parameter                              | OOCYTE FERTILIZATION $\geq 80\%$ | P     |
|----------------------------------------|----------------------------------|-------|
| AGE mean (years)                       | 35.3±3.5                         | 34.5±3.9 | 0.121 |
| BMI mean (Kg/m$^2$)                    | 21.9±2.9                         | 22.8±4.2 | 0.066 |
| ANDROSTENEDIONE mean (ng/mL)           | 2.9±1.64                         | 2.9±1.4  | 0.931 |
| AMH mean (µg/L)                        | 5.0±3.7                          | 4.7±3.5  | 0.531 |
| TESTOSTERONE mean (ng/mL)              | 0.66±0.89                        | 0.59±0.42 | 0.635 |
| ESTROGEN pg/mL                         | 2702.9±1264.6                    | 2696.8±1362.7 | 0.972 |
| ESTROGEN/N$^\circ$ OOCYTE RECOVERED    | 166.6±91.7                       | 163.1±86.0 | 0.773 |
| Spermatozoa capacitated (mil/ML)       | 7.9±11.5                         | 5.0±9.0  | 0.040 |
| Progressive motility                   | 34.5±19.3                        | 29.0±18.8 | 0.043 |

The mean of BMI of patients who had a fertilization rate $\geq 80\%$ is 21.9 ± 2.9 kg/m$^2$, whereas those who had a fertilization $<80\%$ have a mean BMI of 22.8 ± 4.2 kg/m$^2$. This result approaches the significance but is not (P = 0.066) (Table 3). The percentage of women who had a fertilization rate $\geq 80\%$ after oocyte thawing appears to be lower (20%, P = 0.016) in case of a BMI over 30 kg/m$^2$. Considering the causes of infertility, statistically significant differences were found only in the group of patients with a cause of tubal infertility (P = 0.043). We considered confounding elements: the operator performing the technique, the number of capacitated spermatozoa (7.9 ± 11.5 vs 5.0 ± 9.0, P = 0.040) and the progressive motility of the spermatozoa (34.5 ± 19.3 vs 29.0 ± 18.8; P = 0.043). They were all statistically significant in the group of patients who had a fertilization rate $\geq 80\%$ in oocyte thawing cycles.

### Pregnancy rate after oocyte thawing

57/238 patients undergoing oocyte thawing cycles had positive hCG after embryo transfer and in 47 patients' pregnancy had a positive evolution.
### Table 4: Oocyte’s fertilization ≥80% after thawing: parameters of the patient considered for each group. P-value of each subgroup has been calculated respect to the total of the categories themselves.

| Parameter              | OOCYTE FERTILIZATION ≥80% | P     |
|------------------------|---------------------------|-------|
| **AGE**                |                           |       |
| <35 years              | 58/107 (54.2%)            | 0.097 |
| 35-39 years            | 67/104 (64.4%)            | 0.218 |
| >40 years              | 16/24 (66.7%)             | 0.482 |
| **BMI**                |                           |       |
| <18 Kg/m²              | 5/5 (100%)                | 0.160 |
| 18-25 Kg/m²            | 119/192 (62.0%)           | 0.191 |
| 25-30 Kg/m²            | 15/28 (53.6%)             | 0.459 |
| >30 Kg/m²              | 2/10 (20%)                | 0.016 |
| **ANDROSTENEDIONE**    |                           |       |
| < 2.6 ng/mL            | 27/41 (65.9%)             | 0.585 |
| 2.6-4.9 ng/mL          | 19/33 (57.6%)             | 0.405 |
| 5-7.2 ng/mL            | 2/3 (66.7%)               | 1     |
| >7.2 ng/mL             | 3/4 (75%)                 | 1     |
| **AMH**                |                           |       |
| <1.1 µg/L              | 4/4 (100%)                | 0.299 |
| 1.1-5 µg/L             | 71/115 (61.7%)            | 0.800 |
| >5 µg/L                | 38/62 (61.3%)             | 0.819 |
| **TESTOSTERONE**       |                           |       |
| <0.5 ng/mL             | 39/61 (63.9%)             | 0.312 |
| 0.5-1.85 ng/mL         | 15/29 (51.7%)             | 0.260 |
| 1.85-3.2 ng/mL         | 0/1 (0%)                  | 0.398 |
| >3.2 ng/mL             | 2/2 (100%)                | 0.516 |
| **ANTAGONIST VS AGONIST** | 111/184 (60.3%)       | 0.846 |
| **INFERTILITY CAUSES** |                           |       |
| Idiopathic             | 15/24 (62.5%)             | 0.792 |
| Ovulatory              | 11/19 (57.9%)             | 0.845 |
| Female Multiple        | 5/11 (45.5%)              | 0.313 |
| Male +Female           | 58/102 (56.9%)            | 0.390 |
| Male                   | 35/59 (59.3%)             | 0.902 |
| Endometriosis          | 5/6 (83.3%)               | 0.406 |
| Tubal                  | 12/14 (85.7%)             | 0.043 |
| Operator A             | 56/91 (50.5%)             | 0.021 |
| Operator B             | 64/66 (69.7%)             | 0.054 |
| Operator C             | 48/77 (62.3%)             | 0.584 |
Table 5: Positive βhCG and ongoing pregnancy after embryo transfer in oocyte thawing cycles: parameters of the patients considered.

|                        | positive βhCG | P      |
|------------------------|--------------|--------|
| **AGE mean (years)**   | 34.7±3.4     | 35.00±3.8 | 0.617 |
| **BMI mean (Kg/m²)**   | 22.8±4.4     | 22.2±3.2 | 0.332 |
| **ANDROSTENEDIONE mean (ng/mL)** | 3.1±2.0  | 2.9±1.3 | 0.760 |
| **AMH mean (µg/L)**    | 5.5±4.3      | 4.7±3.3 | 0.213 |
| **TESTOSTERONE mean (ng/mL)** | 0.69±0.72  | 0.60±0.75 | 0.647 |
| **ESTROGEN pg/mL**     | 2816.0±1568.5 | 2684.5±1210.0 | 0.514 |
| **ESTROGEN/N° OOCYTE RECOVERED** | 168.3±95.1 | 164.0±87.2 | 0.567 |
| Spermatozoa capacitated (mil/ML) | 6.9±11.9 | 6.8±10.3 | 0.919 |
| N° transferred embryos | 2.4 ±0.5 | 2.1 ±0.7 | 0.014 |

**ONGOING PREGNANCY**

|                        | P      |
|------------------------|--------|
| **AGE mean (years)**   | 34.9±3.01 | 34.9±3.8 | 0.929 |
| **BMI mean (Kg/m²)**   | 22.7±4.5  | 22.2±3.3 | 0.519 |
| **ANDROSTENEDIONE mean (ng/mL)** | 2.7±1.4  | 2.9±1.5 | 0.776 |
| **AMH mean (µg/L)**    | 5.3±4.2  | 4.7±3.4 | 0.492 |
| **TESTOSTERONE mean (ng/mL)** | 0.8±0.9  | 0.6±0.7 | 0.438 |
| **ESTROGEN pg/mL**     | 2672.1±1433.5 | 2726.6±1278.7 | 0.809 |
| **ESTROGEN/N° OOCYTE RECOVERED** | 155.4±78.0 | 167.2±91.3 | 0.441 |
| Spermatozoa capacitated (mil/ML) | 5.7±11.5 | 7.1±10.6 | 0.450 |
| N° transferred embryos | 2.4±0.5  | 2.2±0.7 | 0.031 |

Tables 5 and 6 show that the considered parameters had no influence on the outcome in terms of positive βhCG and ongoing pregnancy.

By analyzing the confounding factors, both the number of transferred embryos and the transfer of almost one good quality embryo statistically influence the positive outcome (Tables 5 and 6). A percentage of 36.4 patients who transferred at least one Grade 1 embryo had positive βhCG compared to 9.9% of patients who did not transfer Grade 1 embryos. In the group of patients with an ongoing pregnancy, 20% of the patients had transferred at least one Grade 1 embryo while 5% had transferred only Grade 2 and 3 embryos (data not shown). In order to define which could be considered a real prognostic factor in oocyte thawing we considered all factors that were statistically significant for an increased success in oocyte thawing in terms of oocyte survival, fertilization and pregnancy achievement (P-value <0.10) and confounding factors that could interfere with the results obtained. Therefore, a multivariate analysis was performed. The results of the analysis are shown in Table 7.
Table 6: βhCG positive and pregnancy rate after embryo transfer in oocyte thawing cycle: characteristics of the patients considered for each group. P-value of each subgroup has been calculated respect to the total of the categories themselves.

|                | βhCG positive | P     | Ongoing pregnancy | P     |
|----------------|--------------|-------|-------------------|-------|
| **AGE**        |              |       |                   |       |
| <35 years      | 25/104(24%)  | 0.812 | 18/104(17.3%)     | 0.734 |
| 35-39 years    | 29/103(28.2%)| 0.286 | 22/103(21.4%)     | 0.273 |
| >40 years      | 3/23(13%)    | 0.169 | 2/23(8.7%)        | 0.211 |
| **BMI**        |              |       |                   |       |
| <18 Kg/m²      | 3/5(60.0%)   | 0.099 | 2/5(40%)          | 0.226 |
| 18-25 Kg/m²    | 42/187(22.5%)| 0.089 | 30/187(16%)       | 0.069 |
| 25-30 Kg/m²    | 8/28(28.6%)  | 0.620 | 7/28(25%)         | 0.325 |
| >30 Kg/m²      | 4/10(40%)    | 0.269 | 3/10(30%)         | 0.395 |
| **ANDROSTENEDIONE** |      |       |                   |       |
| < 2.6 ng/mL    | 10/41(24.4%) | 0.314 | 7/41(17.1%)       | 0.205 |
| 2.6-4.9 ng/mL  | 4/33(12.1%)  | 0.140 | 2/33(6.1%)        | 0.144 |
| 5-7.2 ng/mL    | 1/3(33.3%)   | 0.493 | 1/3(33.3%)        | 0.334 |
| >7.2 ng/mL     | 1/3(33.3%)   | 0.493 | 0/3(0%)           | 1     |
| **AMH**        |              |       |                   |       |
| <1.1 µg/L      | 1/4(25%)     | 1     | 0/4(0%)           | 1     |
| 1.1-5 µg/L     | 25/112(22.3%)| 0.908 | 20/112(17.9%)     | 0.487 |
| >5 µg/L        | 14/61(23.0%) | 0.935 | 9/61(14.8%)       | 0.671 |
| **TESTOSTERONE** |          |       |                   |       |
| <0.5 ng/mL     | 13/61(21.3%) | 0.627 | 9/61(14.8%)       | 0.862 |
| 0.5-1.85 ng/mL | 7/28(25%)    | 0.742 | 4/28(14.3%)       | 0.869 |
| 1.85-3.2 ng/mL | 0/1(0%)      | 1     | 0/0(0%)           | 1     |
| >3.2 ng/mL     | 1/2(50%)     | 0.406 | 1/2(50%)          | 0.283 |
| **ANTAGONIST VS AGONIST** | |       |                   |       |
|                | 40/181(22.1%) | 0.070 | 29/181(16%)       | 0.091 |
| **INFERTILITY CAUSES** | |       |                   |       |
| Idiopathic     | 6/24(25%)    | 0.979 | 5/24(20.8%)       | 0.730 |
| Ovulatory      | 7/19(36.8%)  | 0.204 | 5/19(26.3%)       | 0.343 |
| Female Multiple| 1/11(9.1%)   | 0.217 | 0/11(0%)          | 0.108 |
| Male +Female   | 24/101(23.8%)| 0.751 | 21/101(20.8%)     | 0.379 |
| Male           | 15/55(27.3%) | 0.624 | 10/55(18.2%)      | 0.986 |
| Endometriosis  | 1/6(16.7%)   | 1     | 0/6(0%)           | 0.595 |
| Tubal          | 3/14(21.4%)  | 0.764 | 1/14(7.1%)        | 0.267 |
| Operator A     | 20/90(22.2%) | 0.452 | 15/90(16.7%)      | 0.598 |
| Operator B     | 20/65(30.8%) | 0.195 | 14/65(21.5%)      | 0.431 |
| Operator C     | 17/74(23%)   | 0.643 | 13/74(17.6%)      | 0.835 |
| ET in 3th day  | 31/120(25.8%)| 0.907 | 25/120(20.8%)     | 0.516 |
| Almost one Grade 1 transferred embryo | 47/129(36.4%) | 0.000 | 37/129(28.7%)     | 0.000 |
Table 7: Multivariate analysis of the possible factors correlated with the success of oocyte thawing. The factors that have a significance are highlighted.

|                      | Sig. | OR  | 95% CI per OR |
|----------------------|------|-----|---------------|
|                      | Inf  | Sup |                |
| ONGOING PREGNANCY    |      |     |                |
| Almost one Grade 1 transferred embryo | 0.000 | 6.733 | 2.415 | 18.772 |
| N° embryos transferred | 0.141 | 1.581 | 0.859 | 2.909 |
| Progressive motility  | 0.023 | 0.976 | 0.956 | 0.997 |
| Antagonist            | 0.379 | 0.691 | 0.303 | 1.575 |
| BMI 18-25 Kg/m²       | 0.185 | 0.555 | 0.232 | 1.325 |
| POSITIVE BhCG         |      |     |                |
| Almost one Grade 1 transferred embryo | 0.000 | 4.135 | 1.924 | 8.887 |
| N° embryos transferred | 0.236 | 1.363 | 0.817 | 2.276 |
| Antagonist            | 0.239 | 0.640 | 0.305 | 1.346 |
| BMI18-25 Kg/m²        | 0.227 | 0.602 | 0.264 | 1.371 |
| BMI18 Kg/m²           | 0.584 | 1.742 | 0.238 | 12.728 |
| FERTILIZATION ≥80%    |      |     |                |
| Age <35 years         | 0.515 | 0.820 | 0.451 | 1.491 |
| BMI                  | 0.687 | 0.978 | 0.876 | 1.091 |
| BMI >30 Kg/m²         | 0.053 | 0.091 | 0.008 | 1.036 |
| Tubaric cause of infertility | 0.200 | 2.794 | 0.580 | 13.446 |
| Operator A            | 0.214 | 0.646 | 0.325 | 1.286 |
| Operator B            | 0.252 | 1.577 | 0.723 | 3.442 |
| Progressive motility  | 0.350 | 1.009 | 0.991 | 1.027 |
| N° spermatozoa capacitated | 0.572 | 1.010 | 0.977 | 1.044 |

Discussion

The morphological selection of oocytes before cryopreservation is important to ensure a good survival rate, a development of good quality embryos and to achieve pregnancy in thawing cycles. There are several factors that affect oocyte quality, including the physiological and psychophysical state of the patient and the hormonal stimulation protocol to which she is subjected. Nowadays there are no data about what could predict whether a patient who undergoes In Vitro Fertilization (IVF) cycles can be considered a good candidate for oocyte freezing. In the present study we considered as possible predictive parameters age, cause of infertility, AMH, BMI, androstenedione, testosteron, estrogen, stimulation protocol, ratio between estrogen and number of recovered oocytes. All these parameters have been compared with oocyte survival, fertilization rate, total and ongoing pregnancy rate in oocytes thawing cycle. Moreover, we considered some possible confounding factors (operator, number of capacitated spermatozoa, sperm progressive motility, number of transferred embryos, day of transfer, almost one Grade 1 transferred embryo). About oocyte survival after thawing, none of the considered factors was predictive as shown in Table 1. On the contrary, it has been observed that a BMI ≥ 30 kg/m² has a statistically significant influence on fertilization and that only 20% of patients with BMI above 30 kg/m² have a fertilization rate greater than or equal to 80% in thawing cycle. In fact, several studies have observed that an increase in BMI is associated to a decrease in oocyte quality as well as to lower birth rate [11,12].

By grouping patients according to infertility causes, it was observed that the percentage of patients with a fertilization rate above or equal to 80% after oocyte thawing is higher in case of tubal infertility. This result confirms previous data present in the literature, where a higher
A fertilization rate was observed in patients with a cause of tubal infertility compared to patients with endometriosis or unexplained infertility [13]. Sperm motility and concentration play a key role in the fertilization process. In fact, the percentage of mobile spermatozoa is correlated with fertility in vivo. There is also evidence of fertility improvement using pharmacological approaches (e.g., the use of antioxidant) to increase sperm motility in vitro [14]. To better understand the influence of the BMI > 30 kg/m² and tubal infertility cause on the fertilization rate in thawing cycles, we considered the progressive motility of spermatozoa, the concentration of spermatozoa after capacitation and the operator of oocyte thawing to understand if they could affect the result as confounding factors. All three have a statistically significant effect on the oocyte fertilization rate after thawing (Tables 3 and 4). Then we decided to perform a multivariate analysis including all the factors that are statistically correlated to a fertilization rate ≥ 80% in oocyte thawing cycles (P≤0.1). All subgroups were not statistically significant (Table 7). This data indicates that neither the BMI nor the tubal infertility cause can be prognostic factors in obtaining a fertilization rate ≥ 80% after oocyte thawing.

Finally, analyzing the pregnancy rate, it was observed that the achievement of the result does not depend on any factor studied. Considering the possible confounding factors, it has been observed that the number of embryos transferred after oocyte thawing and the transfer of at least one Grade 1 embryo have a statistically significant impact on the achievement of pregnancy (Tables 5 and 6). By transferring a larger number of embryos, there is an increased likelihood of achieving pregnancy in patients undergoing IVF cycles. In this study we observed that the transfer of at least one Grade 1 embryo results in 36.4% of patients with positive βhCG and in 20% of patients achieving an evolutionary pregnancy. Not transferring any Grade 1 embryo only 9.9% and 5% of patients can achieve positive βhCG and evolutionary pregnancy respectively (data not shown). This data is explained by the fact that Grade 1 embryos have a higher implantation rate than Grade 2 and Grade 3 embryos; therefore, the more Grade 1 embryos are transferred the higher is the probability of pregnancy [15,16]. To clarify whether confounding factors alone could affect pregnancy, we decided to perform multivariate analysis including all the factors that are statistically correlated with success in oocyte thawing (P<0.1). Thus, stimulation with GnRH antagonist, BMI between 18-25 kg/m² and BMI <18 kg/m² were correlated to positive βhCG, whereas stimulation with the GnRH antagonist and BMI between 18-25 kg/m² were correlated to evolutionary pregnancy. In both groups none of the factor had a statistically significant effect on the positive outcome. Only transferring at least one Grade 1 embryo seemed to influence the result (Tables 5 and 6). The present study showed that none of the considered parameter among those available before pick-up is able to predict the outcome of oocyte thawing cycle. Only the transfer of at least one top quality embryo influences the pregnancy outcome. However, this result cannot be considered an elective factor to decide if the oocytes should be cryopreserved or not because it is not available before oocyte vitrification.

Reference

1. Noyes N1, Knopman JM, Melzer K, et al. 2011. Oocyte cryopreservation as a fertility preservation measure for cancer patients. Reprod Biomed Online. 23: 323-333. Ref.: https://pubmed.ncbi.nlm.nih.gov/21570353/ Doi: https://doi.org/10.1016/j.rbmo.2010.11.011
2. Cobo A, Domingo J, Pérez S, et al. 2008. Vitrification: an effective new approach to oocyte banking and preserving fertility in cancer patients. Clin Transl Oncol. 10: 268-273. Ref.: https://pubmed.ncbi.nlm.nih.gov/18490243/ Doi: https://doi.org/10.1007/s12094-008-0196-7
3. Stoop D, Nekkebroeck J, Devroey P. 2011. A survey on the intentions and attitudes towards oocyte cryopreservation for non-medical
Biological and Biochemical factors Predictive of Oocyte survival, Fertilization, Pregnancy in oocyte thawing cycles

DOI: https://doi.org/10.36811/ojgor.2021.110018

OJGOR: August-2021: Page No: 01-11

reasons among women of reproductive age. Hum Reprod. 26: 655-661. Ref.: https://pubmed.ncbi.nlm.nih.gov/21212052/ Doi: https://doi.org/10.1093/humrep/deq367

4. Cobo A1, Kuwayama M, Pérez S, et al. 2008. Comparison of concomitant outcome achieved with fresh and cryopreserved donor oocytes vitrified by the Cryotop method. Fertil Steril. 89: 1657-1664. Ref.: https://pubmed.ncbi.nlm.nih.gov/17889865/ Doi: https://doi.org/10.1016/j.fertnstert.2007.05.050

5. Cobo A, Meseguer M, Remohi J, et al. 2010. Use of cryo-banked oocytes in in ovum donation programme: a prospective, randomized, controlled, clinical trial. Hum Reprod. 25: 2239-2246. Ref.: https://pubmed.ncbi.nlm.nih.gov/20591872/ Doi: https://doi.org/10.1093/humrep/deq146

6. Milán M, Cobo AC, Rodrigo L, et al. 2010. Redefining advanced maternal age as an indication for preimplantation genetic screening. Reprod Biomed Online. 21: 649-657. Ref.: https://pubmed.ncbi.nlm.nih.gov/20864410/ Doi: https://doi.org/10.1016/j.rbmo.2010.06.020

7. Cobo A, Bellver J, Domingo J, et al. 2008. New options in assisted reproduction technology. the Cryotop method of oocyte vitrification. Reprod Biomed Online. 17: 68-72. Ref.: https://pubmed.ncbi.nlm.nih.gov/18616893/ Doi: https://doi.org/10.1016/s1472-6483(10)60295-7

8. Vaegter KK, Lacke TG, Olovsson M, et al. 2017. Which factors are most predictive for live birth after in vitro fertilization and intracytoplasmic sperm injection (IVF/ICSI) treatments? Analysis of 100 prospectively recorded variables in 8,400 IVF/ICSI single-embryo transfers. Fertil Steril. 107: 641-648. Ref.: https://pubmed.ncbi.nlm.nih.gov/28108009/

9. KuwayamaM. 2007. Highly efficient vitrification for cryopreservation of human oocytes and embryos: the Cryotop method. Theriogenology. 67: 73-80. Ref.: https://pubmed.ncbi.nlm.nih.gov/17055564/ Doi: https://doi.org/10.1016/j.theriogenology.2006.09.014

10. Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology 2011 The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. Hum Reprod. 2011. 26: 1270-1283. Ref.: https://pubmed.ncbi.nlm.nih.gov/21502182/ Doi: https://doi.org/10.1093/humrep/der037

11. Wittemer C, Ohl J, Bailly M, et al. 2000. Does body mass index of infertile women have an impact on IVF procedure and outcome? J Assist Reprod Genet. 17: 547-552. Ref.: https://pubmed.ncbi.nlm.nih.gov/11209534/

12. Loveland JB, McClamrock HD, Malinow AM, et al. 2001. Increased body mass index has a deleterious effect on in vitro fertilization outcome. J Assist Reprod Genet. 18: 382-386. Ref.: https://pubmed.ncbi.nlm.nih.gov/11499322/ Doi: https://doi.org/10.1023/a:1016622506479

13. Bergendal A, Naffah S, Nagy C, et al. 1998. Outcome of IVF in patients with endometriosis in comparison with tubal-factor infertility. J Assist Reprod Genet. 15: 530-534. Ref.: https://pubmed.ncbi.nlm.nih.gov/9822979/

14. Auger J1, Serres C, Wolf JP, et al. 1994. Sperm motility and fertilization. Contracept Fertil Sex. 22: 314-318. Ref.: https://pubmed.ncbi.nlm.nih.gov/8032387/

15. Erenus M, Zouves C, Rajamahendran P, et al. 1991. The effect of embryo quality on subsequent pregnancy rates after in vitro fertilization. Fertil Steril. 56: 707-710. Ref.: https://pubmed.ncbi.nlm.nih.gov/1915946/ Doi: https://doi.org/10.1016/s0015-0282(16)54603-2

16. Taşdemir M, Taşdemir I, Kodama H, et al. 1995. Two instead of three embryo transfers in in-vitro fertilization. Hum Reprod. 10: 2155-2158. Ref.: https://pubmed.ncbi.nlm.nih.gov/8567858/ Doi: https://doi.org/10.1093/oxfordjournals.humrep.a136252