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## Research Article

### ***Effects of low LH serum levels on oocyte retrieval, fertilization rate and embryo quality during controlled ovarian stimulation: results from a prospective cohort analysis***

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## **Abstract**

### **Objectives:**

Luteinizing hormone plays a key role in normal follicular development and oocyte maturation in controlled ovarian stimulation. Luteinizing hormone stimulates the proliferation and differentiation of theca cells for the secretion of androgens, synergistically increasing estrogen production. This study aimed to investigate the effects of low luteinizing hormone concentrations on oocyte retrieval, fertilization and embryo development in patients undergoing in vitro fertilization/intracytoplasmic sperm injection.

### **Design:**

We prospectively (ClinicalTrials ID: NCT05755529) analyzed patients undergoing in vitro fertilization/intracytoplasmic sperm injection, subdividing them into three groups according to their age. Serum luteinizing hormone levels were evaluated on day 3, during stimulation (day 10) and before ovulation induction (day 12).

### **Participants/Materials, Setting, Methods:**

Forty-three consecutive women were scheduled for IVF and received ovarian stimulation with follitropin alfa (Gonal F, Merck Serono, Germany) and ganirelix (Fyremaldel, Sun Pharma, Italy). Statistical analysis was performed with InStat 3.10, GraphPad software, San Diego, CA. Normal distribution was tested by the Shapiro–Wilk test. Continuous variables were expressed as the mean and standard deviation (SD). Categorical variables are expressed as frequencies and percentages.

### **Results**

Our data analysis suggests that serum luteinizing hormone levels progressively decrease during controlled ovarian stimulation, and this effect is more evident in the early phase of this procedure. From this perspective, circulating luteinizing hormone levels may significantly decrease during the late follicular phase due to the negative feedback of ovarian hormones from multiple follicular developments or after the suppressive effects of gonadotropin-releasing hormone antagonists.

### **Limitations**

Although our study confirms that exogenous LH can be considered a strategy in women with reduced LH levels during ovarian stimulation to improve oocyte quality and reproductive outcome, the generalizability of the results is limited by the low number of participants enrolled.

### **Conclusions**

Exogenous luteinizing hormone may be considered a strategy in women with a decrease in luteinizing hormone levels during ovarian stimulation to improve oocyte quality and reproductive outcome.

## Introduction

Controlled ovarian stimulation for assisted reproduction treatments has significantly changed over the last decade [1]. Adequate ovarian stimulation using purified or recombinant gonadotrophins [2] is a crucial factor for the success rate of in vitro fertilization and embryo transfer (IVF-ET). Luteinizing hormone (LH) is essential for normal follicular development and oocyte maturation [3]. On the one hand, LH stimulates the proliferation and differentiation of theca cells for the secretion of androgens, which synergistically increases the production of estrogens [4]; on the other hand, LH helps produce small amounts of progesterone in the late follicular phase, thus promoting positive estrogen feedback, which is necessary for follicular development and maturation [5]. Fluctuations in LH levels during the follicular phase significantly impact the oocyte's morphological and functional changes and further influence its meiotic state and its ability to be fertilized [6].

Several factors may be involved in the modulation of circulating LH levels during the follicular phase, including gonadotrophin releasing hormone (GnRH), inhibin, estradiol, and gonadotropin growth attenuation factor (GnSAF), such as anti-Müllerian hormone (AMH) [7,8]. However, none of these substances fully explain why LH levels vary from individual to individual.

Vanetik et al. [9] showed an increase in LH levels after 5 days of stimulation, suggesting that an increase in LH levels occurs mainly in "high responses" or "low responses" during ovarian stimulation. However, the increase in LH in the middle follicular phase can lead to a sharp drop in LH level once a GnRH antagonist is administered, as well as the potential need for LH supplementation.

Adequate exposure to endogenous and/or exogenous LH ("threshold" concept), as well as not excessive exposure to LH (the "maximum" concept), appear to be mandatory for an optimal follicle. Indeed, previous studies (summarized elsewhere [10–13]) showed that the therapeutic benefit of exogenous LH at a standard daily dose of 75 IU is observed only when the endogenous serum LH is less than 1.2 IU/L, confirming the concept of the LH threshold; nevertheless, a combination of exogenous LH at a daily dose of 75 to 150 IU and recombinant follicle stimulating hormone (r-FSH) improved the results of ovarian stimulation in only a minority (5–17%) of the patients; finally, high doses of exogenous LH (more than 225 UI per day) have been found to potentially lead to secondary follicle atresia. Thus, a high daily dose of exogenous LH can deleteriously affect follicular growth. In particular, exogenous LH has been shown to play a key role, especially in women with moderate-severe scores for poor ovarian response [10], when the ovarian reserve is low, and this was further confirmed by a recent systematic review and meta-analysis of randomized controlled trials by Conforti et al. [12], who highlighted the efficacy of exogenous LH in women of advanced reproductive age who underwent assisted reproductive technology. Interestingly, exogenous LH supplementation was found to be more effective when initiated on D1 compared to later supplementation at D6 of ovarian stimulation [13].

This is a prospective cohort analysis (ClinicalTrials ID: NCT05755529) with the aim of evaluating the effect of LH levels on oocyte retrieval, fertilization rate and embryo quality during controlled ovarian stimulation.

## Materials and Methods

### 2.1 Patients and study protocol

We analyzed a total of 43 consecutive women (main characteristics are reported in Table 1) who were prospectively enrolled and scheduled for IVF and received ovarian stimulation with follitropin alfa (Gonal F, Merck Serono, Germany) and ganirelix (Fyremaldel, Sun Pharma, Italy). We included infertile women with ages between 18 and 40 years, body mass index 18–29 kg/m<sup>2</sup>, regular menstrual cycles, normal uterine cavity assessment by hysteroscopy, normal uterus and ovaries at transvaginal sonography, FSH < 10 IU/L, LH < 10 IU/L and estradiol (E<sub>2</sub>) < 50 pg/mL on days 1–2 of the menstrual cycle. We excluded women with abnormal cervical cytology and/or those affected by any other endocrinological, metabolic, or autoimmune diseases.

The design, analysis, interpretation of data, drafting, and revisions conformed to the Helsinki Declaration, the Committee on Publication Ethics guidelines (<http://publicationethics.org/>), and the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement [14],

validated by the Enhancing the Quality and Transparency of Health Research Network ([www.equator-network.org](http://www.equator-network.org)). The data collected were anonymized, taking into account the observational nature of the study, without personal data that could lead to formal identification of the patient. Each patient in this study was informed about the procedures and signed consent to allow data collection and analysis for research purposes. The study was not advertised. No remuneration was offered to the patients to give consent to be enrolled in this study.

## 2.2. Ovarian stimulation protocol

The treatment (shown in Fig. 1) started from day 2 or 3 of the menstrual cycle (equal to day 1 of the treatment cycle) onward with a daily s.c. gonadotrophin (Gonal F, Merck Serono, Germany) injection in the abdominal wall.

Treatment with a GnRH antagonist (Fyremaldel, Sun Pharma, Italy) was started from days 6-8. The starting dose was based on the patient's characteristics and history. On days 6-8, the dose was re-evaluated and eventually adjusted, depending on the individual ovarian response assessed by ultrasound. Both follitropin alfa and ganirelix were continued until (and including) the day of ovulation induction. On the day when at least three follicles  $\geq 18$  mm were observed by ultrasound, ovulation was triggered using two i.m. injections of triptorelin acetate 0.1 mg/ml (Fertipeptil, Ferring, Switzerland). We used the GnRH agonist to minimize the risk of ovarian hyperstimulation syndrome (OHSS) because, in some cases, pharmacological stimulation was performed with maximum dosages to increase oocyte retrieval and to reach 15 oocytes, defined as optimum for the pregnancy rate. Intracytoplasmic sperm injection (ICSI) was performed 36 hours after recombinant human chorionic gonadotropin (R-HCG) administration, and luteal phase support was started in all patients according to the preference of the treatment center (progesterone 8% vaginal gel, twice daily, or progesterone 200 mg vaginal suppositories, twice daily).

## 2.3. Ultrasound and hormonal assessment

At baseline (before starting ovarian stimulation), transvaginal ultrasound was performed, and blood samples were taken for hormonal assessments. Validated immunoassays were performed to measure serum concentrations of FSH, LH, estradiol, and progesterone (MINI VIDAS-Biomerieux). From day 1 of treatment, the subjects returned to the clinic at least every 2 days for ultrasonography. When one or more follicles  $\geq 14$  mm were seen by ultrasound, daily blood samples were taken for hormonal analysis until (and including) the day of ovulation induction.

## 2.4. Oocyte preparation and ICSI procedure

The aspirated follicular fluid was passed to the adjoining laboratory during oocyte retrieval. Cumulus–corona–oocyte complexes were identified in sterile plastic dishes (cat. no. 1029, Falcon; Becton-Dickinson Labware, Franklin Lakes, NJ, USA), rinsed, transferred to IVF-50 medium (Scandinavian IVF Science AB) and incubated at 37°C in an atmosphere of 5% CO<sub>2</sub> in the air until ICSI. Immediately before ICSI, the cumulus and corona cells were removed by brief exposure to Gamete 100 (HEPES-buffered medium) containing 80 IU/ml hyaluronidase Fraction VIII (Hyase-10X; Scandinavian IVF Science AB). To enhance enzymatic removal of the cumulus and corona cells, the oocytes were aspirated in and out of a hand-drawn Pasteur pipette with an approximate inner diameter of 130  $\mu$ m (Laboratory Pipette art. no. 11130; Swemed Lab International AB, Sweden). Denudation was performed in a four-well culture dish (cat. no. 45-176740; Nunc Bround Products, Kamstrup, Denmark). Denuded oocytes were washed twice and incubated in IVF-50 medium until ICSI was performed.

Oocytes were then examined under an inverted microscope at 200x magnification to assess the integrity and maturation stage. Only morphologically normal mature oocytes with a visible first polar body were microinjected. ICSI was performed in microdroplets under oil (Ovoil-150; Scandinavian IVF Science AB) using plastic culture dishes (cat. no. 1006, Falcon; Becton-Dickinson Labware) under a microscope at 400x magnification.

## 2.5. Embryo culture

After ICSI, embryos were placed in 4-well Petri dishes (Nalge Nunc, Naperville, IL, USA) and cocultured at 37°C in a 5% CO<sub>2</sub> incubator. Pronucleus formation was verified 16–20 hours post-ICSI, and the progression of embryo growth was recorded daily. Pronucleus formation and/or the timely

cleavage of nucleated embryos were used as fertilization measures for oocytes subjected to ICSI. Morula and blastocyst rates were used to assess embryo development for each replicate and were determined relative to the number of fertilized oocytes. Blastocyst formation was defined as the expansion of the embryo after compaction and cavitation to include both the trophoctoderm and inner cell mass.

## 2.6. Statistical analysis

Statistical analysis was performed with InStat 3.10, GraphPad software, San Diego, CA. Normal distribution was tested by the Shapiro–Wilk test. Continuous variables were expressed as the mean and standard deviation (SD). Categorical variables are expressed as frequencies and percentages. All histograms represent the means  $\pm$  standard deviations of the data obtained. Statistical significance was determined by one-way analysis of variance (ANOVA) and paired two-tailed Student's t test. A p value  $<0.05$  was considered to indicate statistical significance.

## Results

### 3.1. LH levels in different age ranges

First, overall serum LH values significantly decreased from day 3, during stimulation (day 10) and before ovulation induction (day 12), as described in Figure 2. Therefore, considering the decreasing trend of serum LH levels in the entire population, we performed a subanalysis (shown in Fig. 3), dividing our population into three age groups: 24–29, 30–35 and 36–40 years. Interestingly, we found a similar decrease in median serum LH values during the first 12 days of stimulation.

In the first group (24–29 years; 14 women), the mean serum LH value on day 3 was  $6.09 \pm 1.3$  IU/L, on day 10 was  $2.7 \pm 1.17$  IU/L, and on day 12 was  $1.04 \pm 0.71$  IU/L (day 3 vs day 10:  $p < 0.05$ ; day 10 vs day 12:  $p < 0.05$ ; day 3 vs day 12:  $p < 0.01$ ). In the second group (30–34 years; 14 women), we observed a similar trend: in detail, mean LH serum levels decreased from  $5.62 \pm 2.07$  IU/L on day 3 to  $1.97 \pm 1.46$  IU/L on day 10 and reached  $0.83 \pm 0.51$  IU/L on day 12 (day 3 vs day 10:  $p < 0.001$ ; day 3 vs day 12:  $p < 0.0001$ ; day 10 vs day 12: n.s.). Finally, in the third group (35–40 years; 15 women), mean LH serum levels decreased from  $7.05 \pm 0.81$  IU/L on day 3 to  $3.80 \pm 1.37$  IU/L on day 10 of stimulation and reached  $1.93 \pm 1.14$  IU/L on day 12 (day 3 vs day 10:  $p < 0.0001$ ; day 10 vs day 12:  $p < 0.001$ ; day 3 vs day 12:  $p < 0.0001$ ).

### 3.2 Oocyte retrieval, fertilization and embryonic development

Considering oocyte retrieval, we found significant differences among the three groups, as shown in Figure 4. In detail, a mean of  $7.07 \pm 2.49$  oocytes were retrieved in the first group (age 20–29 years) and  $5.69 \pm 3.03$  oocytes in the second group (age 30–34 years), whereas in the last group (age 35–40 years),  $3.71 \pm 2.40$  oocytes were retrieved ( $p < 0.05$  for age 20–29 years vs age 35–40 years; n.s. for age 20–29 years vs other age ranges, and for age 30–34 years vs age 35–40 years).

When it comes to embryos obtained after fertilization of retrieved oocytes, the question arises as to how to classify them most properly. The commonly accepted method for classifying and selecting embryos remains morphological and developmental rate assessment, which today is increasingly augmented with morpho-kinetic data from timelapse measurements provided by the latest technologies.

Despite numerous attempts put in place with the goal of identifying new variables to classify embryonic morphology, the only predictive variables accepted today remain those that are subjectively assessed by the individual operator and therefore cannot be standardized. The ability of embryologists to score embryo morphology "correctly" with minimal subjectivity and high intra- and inter-observer concordance is a key goal to be achieved in developing consensus among practitioners and can only depend on the competence, accuracy, and consistency of embryologists.

In 2010, the Society for Assisted Reproductive Technology (SART) decided to make a standardized scoring system for embryo morphology mandatory and to collect these data in the databases of the various centers involved in ART. The system is based on the evaluation of cell number, fragmentation and symmetry for early-stage embryos, compaction and fragmentation for morulae and expansion, Inner cell mass and trophoctoderm for blastocysts. Operating in this way, proper and standardized documentation of each embryonic variable should allow for proper

classification of embryos, thus using the same language everywhere in the same way. This documentation not only ensures traceability, but also allows future studies to evaluate accumulated data and determine the predictive value of single and/or combined embryonic variables.

With this aim, the classification derived from the results of Lundini et al. [15] in 2015 was adopted, and as shown in Figure 5a, in the first group (age 20-29 years), only 66% of the embryos were of good quality (“*grade a*”); the percentage of “*grade a*” embryos was 54.5% in the second group (age 30-34 years) and 45.5% in the last group (age 35-40 years).

In detail (shown in Fig. 5b), in the first group (age 20-29 years), 7,7% of oocytes were not fertilized, 30,8% of the embryos reached the blastocyst stage, and the remaining 61,5% were transferred on day 3 because they were classified as poor-quality embryos.

In the second group (age 30-34 years), 15,4% of the oocytes were not fertilized, 23,7% of the embryos reached blastocyst age, and the remaining 61,5% were transferred on day 3 because they were classified as poor-quality embryos.

In the third group (age 35-40 years), 23,07% of the oocytes were not fertilized, 7,7% of the embryos reached the blastocyst stage, and the remaining 69,34% were transferred on day 3 because they were classified as poor-quality embryos.

### Discussion/Conclusion

LH is essential for normal follicular development and oocyte maturation [3]. In particular, LH can promote the proliferation and differentiation of theca cells for androgen secretion, synergistically increasing estrogen production [16]. In the late follicular phase, LH helps to produce small amounts of progesterone, promoting positive estrogen feedback, which is necessary for follicular development and maturation [5].

Many studies have highlighted the importance of LH levels during controlled ovarian stimulation for adequate follicular development and successful clinical outcomes [17,18]. Indeed, fluctuations in LH levels during the follicular phase have a significant impact on morphological and functional changes in the oocyte and further affect its meiotic status and its ability to be fertilized [6].

Benmachiche and colleagues investigated the association between the LH level on the day of GnRH agonist trigger and reproductive outcomes in a large cohort of GnRH antagonist cotreated IVF/ICSI treatment cycles and observed that patients with  $LH > 1.60$  mIU/ml exhibited significantly better reproductive outcomes than those with  $LH < 1.60$  mIU/ml [19]. In addition, other authors have observed a robust association between low serum LH levels and poor oocyte retrieval as well as low reproductive outcomes [20].

Our data analysis suggests that serum LH levels progressively decrease during controlled ovarian stimulation, and this effect is more evident in the early phase of this procedure. From this perspective, circulating LH levels may significantly decrease during the late follicular phase due to the negative feedback of ovarian hormones from multiple follicular developments or after the suppressive effects of GnRH antagonists [21–24]. Namely, treatment with the antagonist from the 8<sup>th</sup> day of stimulation or when 2-3 follicles reach 13-14 mm or in a fixed pattern starting from the 6<sup>th</sup> day of stimulation could block the release of LH from the pituitary gland, relegating the maturation of the oocyte to the effect of the LH that is already available in the circulation. Under these conditions, the follicles that have reached 12-13 mm in diameter and that have externalized and amplified the receptors for LH bind the greatest quantity of LH. Smaller follicles that express low levels of LH receptors may not complete the maturation process, reducing the number of mature oocytes retrieved at harvest. By using the GnRH antagonist, the follicles could be of different sizes and receptivity to the LH depending on the stage of maturity, and therefore, the largest will use the circulating LH to complete maturation.

In this scenario, our data confirm that low serum LH levels negatively affect oocyte quality, despite a good number of oocytes retrieved. In contrast, other authors have shown an increase in LH, rather than the expected decrease, after 5 days of stimulation [9]; however, the increase in LH in the middle follicular phase leads to a sharp drop in LH once a GnRH antagonist is administered and the possible need for LH supplementation.



The potential benefit of LH supplementation over the sole use of FSH during controlled ovarian stimulation remains controversial, although a retrospective multicenter controlled study on women with poor ovarian response found better reproductive outcomes using exogenous LH supplementation [10]. In this context, recombinant luteinizing hormone (r-hLH) has high specific activity and may be indicated in combination with FSH to stimulate follicular development in women with LH and FSH deficiency. Based on these findings, exogenous LH may be used as an emergency drug in women with a decrease in LH levels observed during stimulation. In addition, LH supplementation would also decrease the need for r-FSH, which could lead to lower doses of gonadotrophin necessary to achieve adequate controlled ovarian stimulation.

According to a previous study (NCT00328926), supplementation with exogenous LH was found to play a positive role in patients with initial LH deficiency who underwent COS; nevertheless, it may be possible that supplementation with exogenous LH could also play a key role in women with a sudden drop in LH levels during COS (regardless of initial LH deficiency).

In our work, we found LH levels lower than 1.2 (0.3 maximum lower) at the terminal phase of stimulation: at this stage, the potential supplementation of exogenous LH would be too late to achieve adequate oocyte quality. In this scenario, our data could be considered a basis to evaluate, in future investigations, whether supplementation with exogenous LH may play a positive role in oocyte quality even in women with a sudden drop in LH levels during COS.

Our results conclude that low LH levels may also be among the causes of low oocyte and embryo quality. However, we acknowledge that several other possible factors could explain low oocyte/embryo quality, such as suboptimal initial gonadotropin dose, suboptimal dose adjustment, and suboptimal ovulation triggering with a GnRH agonist where triggering with HCG should have been used [25–29].

Our study confirms that serum LH levels progressively decrease during controlled ovarian stimulation, and this effect is more evident in the early phase of this procedure. Based on these findings, exogenous LH may be considered a strategy in women with a decrease in LH levels during ovarian stimulation to improve oocyte quality and reproductive outcome.

## **Statements**

### **Statement of Ethics**

The study was approved by the Institutional Review Board of Centro Aster (approval ID 22/2021). Written informed consent was obtained for participation in this study.

### **Conflict of Interest Statement**

The authors have no conflicts of interest to declare.

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### **Author Contributions**

Conceptualization, J.D. and G.Br. (Giovanni Bracchitta); methodology, G.Bu. (Giovanni Buzzaccarini); validation, R.V., B.B. and P.C.; formal analysis, S.T.; investigation, M.D.; data curation, C.M.S.; writing—original draft preparation, J.D.; writing—review and editing, A.S.L. and A.E.; visualization, V.U.; supervision, B.B.; project administration, G.Br. All authors have read and agreed to the published version of the manuscript.

### **Data availability statement**

The full dataset of the study will be available from the first author (Jessica Dragotto) upon reasonable request.

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## Figure Legends

### Fig 1. Schematic summary of the treatment schedule.

LH and PRG assessment (red arrow) and GnRH antagonist administration (day 6-8, blue arrow) in women undergoing stimulation with r-FSH (start on day 2-3, until ovulation induction on day 12, blue arrows).

### Fig 2. LH levels from day 3 to day 12 of ovarian stimulation in the whole cohort.

Data are reported as the mean  $\pm$  SD.

Day 3:  $6,11 \pm 1,64$  IU/L; day 10:  $3,68 \pm 1,72$  IU/L; day 12:  $1,66 \pm 1,16$  IU/L.

\*\*\*\*  $p < 0.0001$  for each time point vs the other time points (day 3 vs day 10; day 3 vs day 12; day 10 vs day 12).

### Fig 3. LH levels from day 3 to day 12 of ovarian stimulation in the three different groups.

Data are shown as the mean and standard deviation.

Age 20-29 years: 14 women; age 30-34 years: 14 women; age 35-40 years: 15 women.

Age 20-29 years: day 3:  $6,09 \pm 1,3$  UI/L; day 10:  $2,7 \pm 1,17$  UI/L; day 12:  $1,04 \pm 0,71$  UI/L.

Age 30-34 years: day 3:  $5,62 \pm 2,07$  UI/L; day 10:  $1,97 \pm 1,46$  UI/L; day 12:  $0,83 \pm 0,51$  UI/L.

Age 35-40 years: day 3:  $7,05 \pm 0,81$  UI/L; day 10:  $3,80 \pm 1,37$  UI/L; day 12:  $1,93 \pm 1,14$  UI/L.

\* $p < 0.05$ ; \*\*  $p < 0,01$ ; \*\*\*  $p < 0.001$ ; \*\*\*\*  $p < 0.0001$ .

### Fig 4. Oocyte retrieval in the three age groups.

Data are shown as the mean and standard deviation.

Age 20-29 years: 14 women; mean oocyte retrieval:  $7.07 \pm 2.49$  oocytes.

Age 30-34 years: 14 women; mean oocyte retrieval:  $5.69 \pm 3.03$  oocytes.

Age 35-40 years: 15 women; mean oocyte retrieval:  $3.71 \pm 2,40$  oocytes.

\* $p < 0.05$ .

### Fig 5. a) Embryo quality in the three groups.

Data are presented as a percentage, considering the number of embryo grade a or d according to Lundin et al. [15].

Age 20-29 years: 14 women; "grade a" embryo: 66%; "grade d" embryos: 34%.

Age 30-34 years: 14 women; "grade a" embryo: 54,5%; "grade d" embryos: 45,5%.

Age 35-40 years: 15 women; "grade a" embryo: 45,5%; "grade d" embryos: 54,5%.

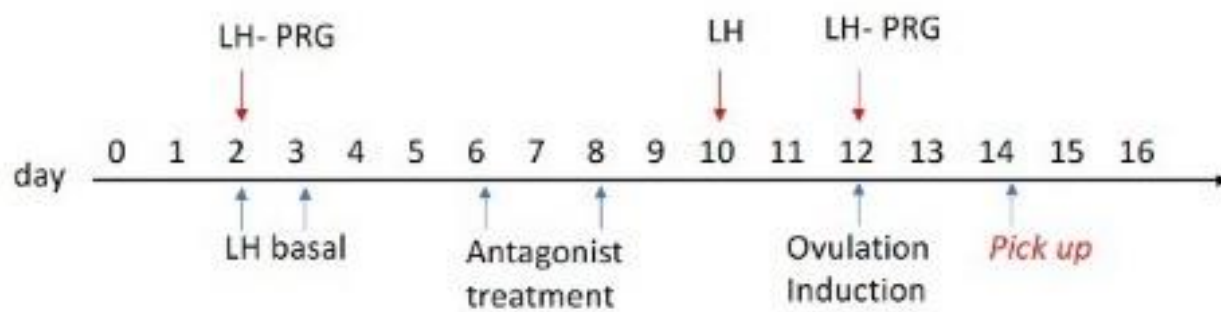
### b) Oocyte-embryo development in the three groups.

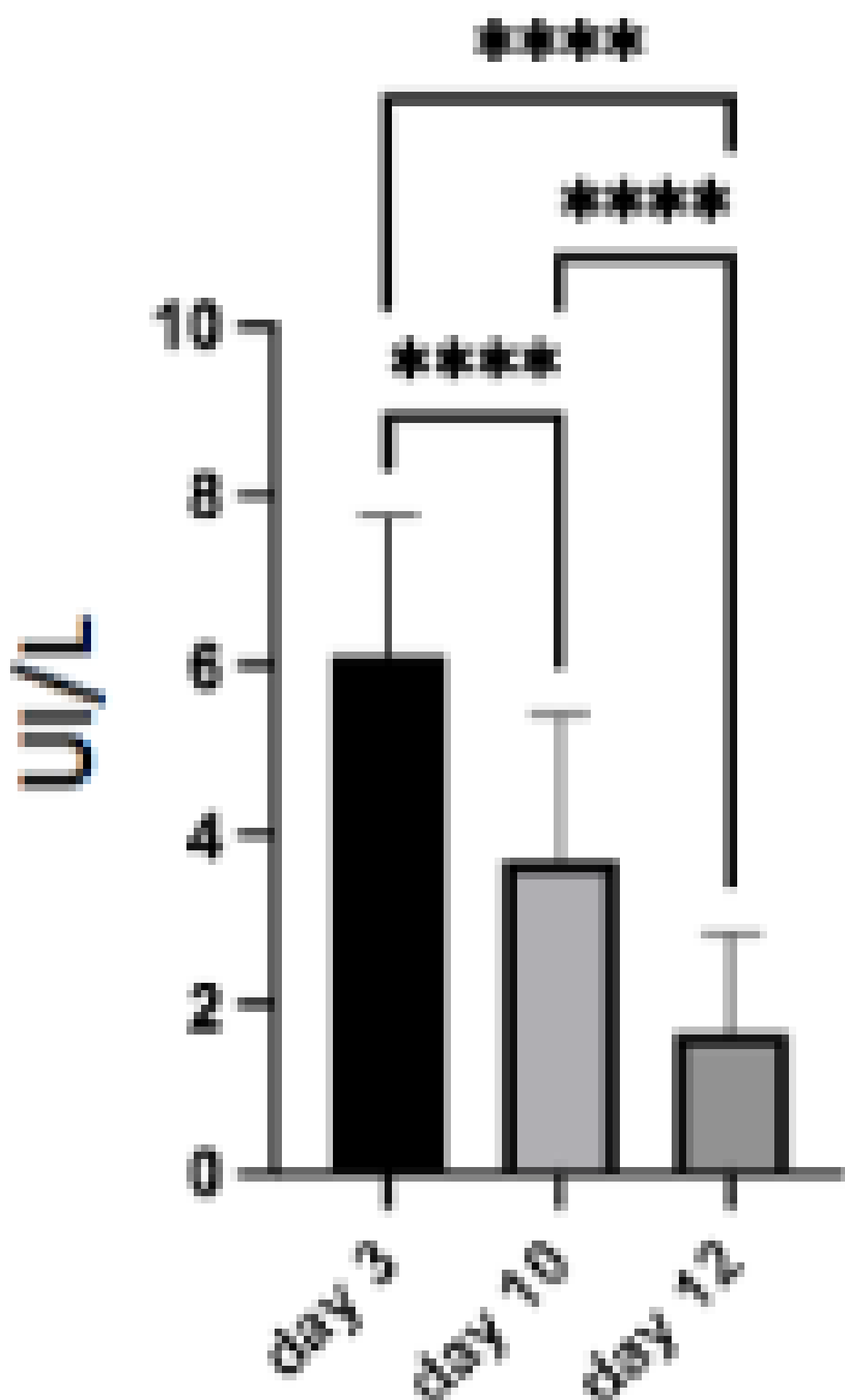
Data are presented as percentages, considering the number of nonfertilized oocytes, blastocysts, and embryos (day 3) for each group.

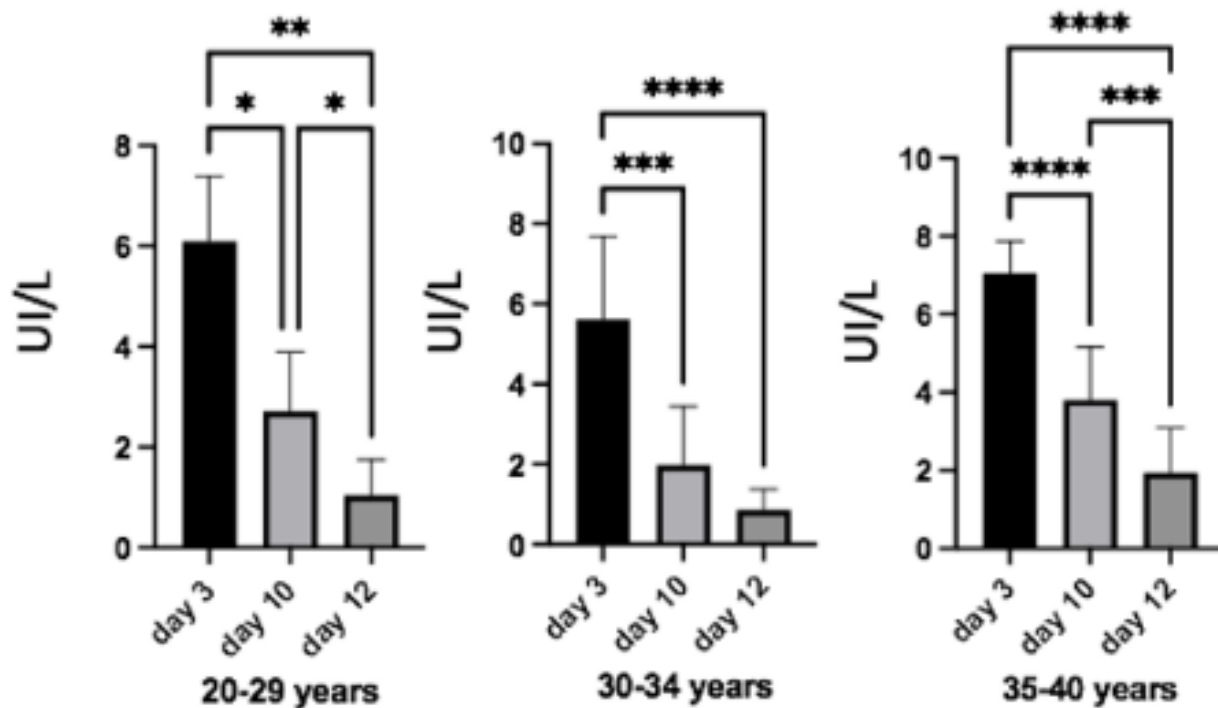
Age 20-29 years: 14 women; not fertilized: 7,7%; blastocysts: 30,8%; embryos 61,5%.

Age 30-34 years: 14 women; not fertilized: 15,4%; blastocysts: 23,7%; embryos 61,5%.

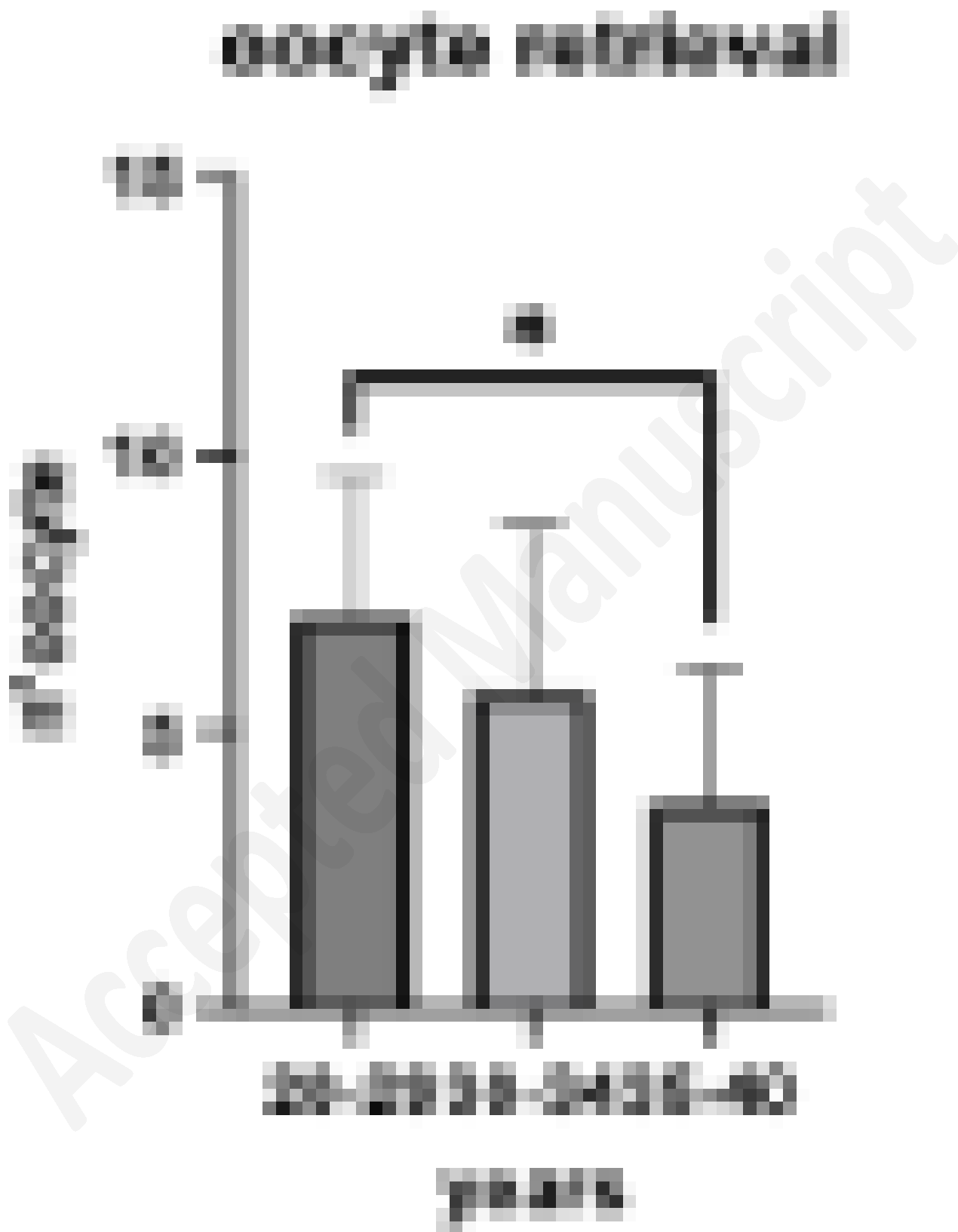
Age 35-40 years: 15 women; not fertilized: 23,07%; blastocysts: 7,7%; embryos 69,34%.

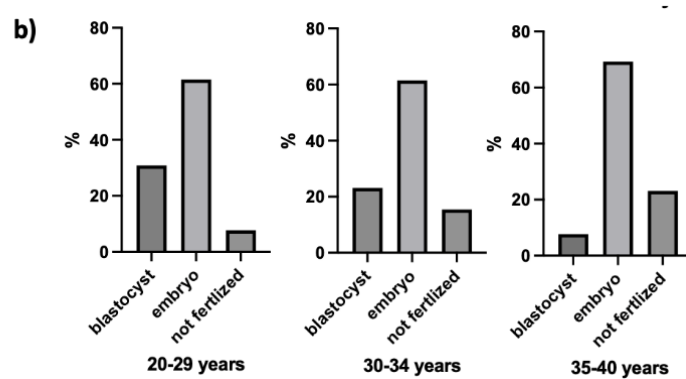
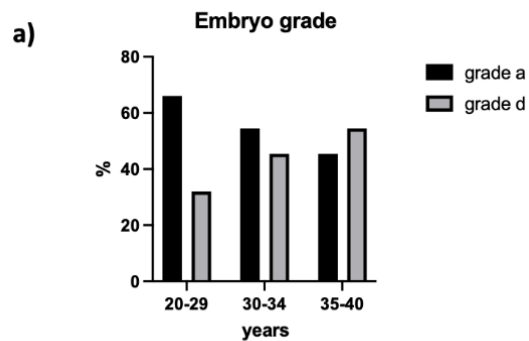














<b>Characteristics of the patients</b>	
Patients (n)	43
Age (years)	35 ± 0.78
BMI (Kg/m <sup>2</sup> )	26 ± 3.20
Basal FSH concentration (IU/l)	8.62 ± 3.66
Smoking status (%)	39.53%
AFC	9.95 ± 3.77
AMH, ng/ml	1.51 ± 0.98
<b>Characteristics of infertility</b>	
Duration (years)	2.98 ± 1.37
Primary (%)	90.7%
Secondary (%)	9.3%
Ovulatory – endocrine factor (%)	27.9%
Tubal factor (%)	7%
Male factor (%)	9.3%
Advanced Reproductive Age* (%)	37.2%
Endometriosis (%)	2.3%
Unexplained (%)	9.3%
Mixed (%)	7%
<b>Number of previous ICSI cycles</b>	<b>1.53 ± 0.96</b>
<b>(average)</b>	
<b>Number of previous pregnancies (%)</b>	<b>9.3%</b>
<b>Number of previous miscarriages (%)</b>	<b>23.3%</b>
<b>Number of previous live birth (%)</b>	<b>25.6%</b>

Table 1. Characteristics of the patients.