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Inositol: effects on oocyte quality in patients undergoing ICSI. An open study

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Abstract. – OBJECTIVES: Nuclear and cytoplasmic competence of human oocyte is critical for future competence of the embryo upon which ultimately depends the outcome of an ART (Assisted Reproductive Technology) treatment. Follicular microenvironment in which the oocyte develops is crucial, and this must be taken into account particularly with the use of hormonal ovarian stimulation protocols. Inositol is an important element of the follicular environment and data support that its higher level in follicular fluid correlates with the development of a good oocyte.

Aim of this study is to understand the effects of treatment with inositol on oocyte quality in a sample of patients undergoing ICSI (Intracytoplasmic Sperm Injection).

PATIENTS AND METHODS: Assessment of oocyte/embryo quality and pregnancy rates in 149 patients divided, according to a controlled randomized pattern, into two groups: study group 1 treated with folic acid and inositol and control group 2 treated with folic acid alone.

RESULTS: The number of patients with excellent and good oocyte quality appears to be significantly higher in group 1 (p = 0.02), as shown, they significantly increased the number of embryos of grade A transferred in the group 1 (p = 0.02) compared to group 2, despite being completely similar averages of total embryos transferred (total mean ± SD = 2.4 ± 0.8, group 1 mean ± SD = 2.4 ± 0.8, group 2 mean ± SD = 2.4 ± 0.8). There is not any significant difference between groups 1 and 2 in the number of positivity to β-hCG and in the number of biochemical pregnancies detected although it has a tendency to increase in the first and to decrease in the second for group 1. The increase in percentage of clinical pregnancies in group 1 was statistically significant (p = 0.02), whereas there was no apparent significance in the difference between the biochemical and clinical pregnancies in the two groups despite the positive trend in the study group.

CONCLUSIONS: Relying on "inositol help" to solidify our efforts, seems to be an easy path to help to deepen the effectiveness of its use in all patients still under 40 but with prior failed attempts at ICSI or diagnosed with PCOS or as "poor responders".

Key Words:
Myo-inositol, D-chiro-inositol, Oocyte quality assessment, Embryo quality assessment.

Introduction

Inositol, although not a true vitamin, is a vitamin factor and considered part of B group. In organisms it is found in phospholipids, stimulate the endogenous production of lecithin, it is essential for the growth of hair and performs a check on the metabolism of fats and sugars as well as in cellular activities of the nervous system.

Myo-inositol (MI) is one of the most relevant isomeric forms of inositol present in nature and can be synthesized by the body in the follicular microenvironment; various natural forms of inositol also exist, among which the most studied is the D-chiro-inositol (DCI), which is not synthesized from precursors in the body but obtained from epimerization of MI.

The activity of the epimerase is reduced in the form of type 2 diabetes mellitus and in polycystic ovary syndrome (PCOS), one of the most frequent causes of endocrine disruption and infertility by chronic anovulation in women of childbearing age.

There is also evidence that DCI, in addition to increasing insulin sensitivity, is capable of improving ovarian function[1] for which the administration of inositol results in reduced quantities of the FSH necessary to ovarian stimulation and in recoveries to the best oocyte pick-up both in number and in quality[2].

The purpose of this study is to understand the effects of treatment with inositol, in addition to treatment with folic acid on oocyte quality in a sample of patients undergoing cycles of Assisted Reproductive Technology (ART) for ICSI (Intracytoplasmic Sperm Injection).

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The role of female gametes is crucial in determining the embryo competence and, therefore, the outcome of an ART treatment.

It is now widely recognized that the quality of the oocyte is determined not only by the nuclear and mitochondrial genome but also by the ovarian and follicular microenvironment that influence transcription and translation and, as a consequence, cytoplasmic maturation[3].

The process that governs the natural selection of the dominant follicle and, therefore, the single oocyte to ovulate is very complex. Using hormonal stimulation protocols for In Vitro Fertilization (IVF) cycles, many stages of this complicated process are skipped leading to the maturation of multiple oocytes but with differing degrees of quality. This is due mainly to the desynchronization of the nuclear and cytoplasmic maturation processes[4], caused by the different sensitivity of the oocyte and the cells of cumulus-corona radiata complex to stimulating agents[5].

Many of the so-called dysmorphic oocytes fertilize after ICSI and develop in an apparently normal manner during the early stages of embryonic development[6], while during the subsequent phases are matched by high rates of miscarriage that suggest a real possibility that defects inherent in the egg cell can have adverse consequences later[7].

Therefore, any impact on the subsequent pre-implantation development of the embryo is closely related to the presence, size and number of abnormalities that may be present in the oocyte.

Patients and Methods

A total of 149 patients undergoing ICSI cycles were included in the study in the "*Servizio di Diagnosi e Cura della Riproduzione Umana*", *Struttura Complessa di Ostetricia e Ginecologia*, *Azienda Ospedaliera di Perugia,* in the period between June 2012 and May 2013.

Each patient was included only once; therefore, the results for each patient refer to a single treatment cycle.

The recruitment criteria include being under 40 years old, at least one previous failed attempt with ICSI with low-quality oocyte recovery, diagnosis of PCOS (i.e., with oligomenorrhea, hyperandrogenism and pelvic ultrasonographic appearance characterized by multiple anechoic areas)[8], diagnosis of "poor responders" (i.e., with poor ovarian response to hormonal stimulation, an age greater than 37 years and the need for high doses of FSH stimulation in previous cycles).

Only ICSI treatments arrived to the transfer of embryos in the uterus (Embryo-Transfer) and carried out on Day +2/3 are included in the study.

Patients with a partner with a diagnosis of severe male infertility such as cryptozoospermia (i.e., retrieval of sperm in the semen after centrifugation) and azoospermia (i.e., eventual retrieval of sperm from the testicle or epididymis) were excluded from the study.

According to a randomized pattern, the total number of patients was divided into two groups, the study group consisting of 58 patients treated with folic acid and inositol (group 1), and the control group consists of 91 patients treated with folic acid alone (group 2).

Starting from 3 months before the ICSI cycle the patients in group 1 were prescribed 2000 mg/day of myo-inositol, D-chiro-inositol 400 mg/day and folic acid 400 mg/day while the patients in group 2 only folic acid 400 mg/day.

The technique of IVF using ICSI involves several steps, which include controlled ovarian stimulation, recovery, decumulation and the subsequent insemination of oocytes, culture and transfer of embryos in the uterus, accompanied respectively by ultrasound scans and hormone assays, and by assessment of oocyte quality, fertilization and embryos. Each of these phases is essential for the success of the entire treatment cycle.

Assessment of Oocyte Quality

The assessment of oocyte quality in routine activities in the laboratory is mainly morphological. Already at the time of recovery the observation is carried out under a stereomicroscope, assessing the degree of expansion of the cells of the cumulus-corona-oocyte complex (CCOC) (cumulus cells must be visibly well-separated from each other due to the accumulation of hyaluronic acid in the extracellular matrix and those immediately adjacent to the oocyte become less compact and radiate from it: this is a good predictor of getting a mature oocyte or MII at decumulation). Then, following the decumulation of such complexes, observations of the oocyte are made with an inverted microscope with particular regard to the following parameters:

1. *Stage of maturity:*
- presence of the first polar body (PBI) in the perivitelline space (PVS): MII stage
- presence of the germinal vesicle (GV): GV stage
- absence of both: MI stage

2. *Size and shape:*
- a critical dimension oocyte is essential for the resumption of meiosis even if the average diameter of a MII oocyte may vary. It is absolutely necessary, however, to distinguish and eliminate the giant oocytes because they have a double set of chromosomes
- there are many abnormal forms that can also fertilize and lead to pregnancy but it has been observed that if the deformation is very serious embryonic development is delayed[9]

3. *Cytoplasmic characteristics:*
- ooplasma: dense and homogeneous, grainy or degeneration
- presence of vacuoles
- presence of plaques of smooth endoplasmic reticulum (SER clusters): the worst dysmorphism observable due to the lethal consequences reported in subsequent development[10-12]
- presence of plaques of organelles (organelle clustering) that lead to a localized granulation in the cytoplasm
- presence of refractile bodies (RB)

4. *Extracytoplasmatic characteristics:*
- thickness and density of the pellucida zone (PZ)
- size, shape and number (sometimes there may be multiple PB) of PBI
- size and shape of the PVS
- presence of granules in the PVS

In the present study the assessment of oocyte quality is defined by the number of patients found:
- *Very good quality* (A) (Figure 1): > 50% of oocytes retrieved have normal characteristics, i.e., without abnormalities
- *Good quality* (B): > 50% of oocytes retrieved have a nonfatal fault
- *Poor quality* (C): > 50% of oocytes retrieved have two minor anomalies
- *Very poor quality* (D): > 50% of oocytes retrieved have more than two anomalies not serious or one severe.

Assessment of Embryo Quality

After assessing the fertilization of the oocytes for the proper presence of 2 PB and 2 pronuclei (PN) (17 ± 1 hours after insemination or day+1), assessment of embryo quality in the laboratory is based on the observation of the embryos being in cleavage, in our case after 44 ± 1 hours post-ICSI (day+2) and 68 ± 1 (day+3).

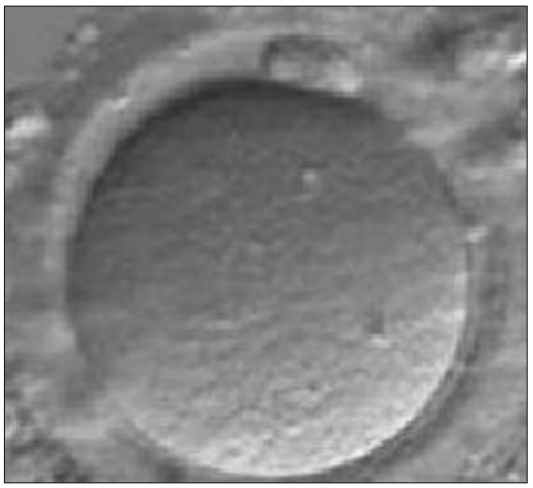

Figure 1. Good quality oocyte.

The observation is made under an inverted microscope with particular regard to the following parameters[7]:

1. *Number of blastomeres*: at each stage of development, the number of cells is determined and expected to be 4 in day+2 and 8 in day+3; on average, embryos that divide more slowly or faster than the expected rate have a reduced implantation potential

2. *Degree of fragmentation*: a fragment is defined as a cytoplasmic anucleate extracellular structure bound with a membrane diameter < 45 mm in embryos in day+2 and < 40 mm in day+3; the relative degrees of fragmentation are definitive as mild (< 10%), moderate (10-25%) and severe (> 25%); the presence of fragments may be associated with aneuploidy[13], however, being a dynamic phenomenon can not be the only morphological criterion evaluated

3. *Multinucleation*: refers to the presence of more than one interphase nucleus in one blastomere and its presence is associated with elevated levels of chromosomal aberrations[14]; the evaluation of multinucleation must be carried out on day+2 (in day+3 cells are too small to ensure a realistic assessment) and the observation of a single blastomere with more than one core is sufficient to consider the embryo multinucleated

4. *Size of the blastomeres:* the cell size of embryos at 4 and 8 cells should be roughly equal; the presence of blastomeres with different sizes derived from irregular cleavage[15] and its negative impact on the final outcome is confirmed by several authors[16,17].

In the present study the assessment of embryo quality is defined by the number of embryos found:

- *Very good quality* (A) (Figures 2, 3): 4/8 mononuclear blastomeres of equal size respectively in day+2/3, with < 10% of fragmentation
- *Good quality* (B): > 50% of the blastomeres with number and size appropriate to the stage of development, mononuclear cells, with 10-25% of fragmentation
- *Poor quality* (C): > 50% of the blastomeres where number and size do not fit for the stage of development, multinucleation, with > 25% of fragmentation.

Determination of Pregnancy

At 14 days from Embryo-Transfer execution, quantitative blood detection of beta-human chorionic gonadotropins (β-hCG) is performed (pregnancy test).

Among all the tests with β-hCG positive (+β-hCG), the biochemical pregnancies are those in which the pregnancy is interrupted with the subsequent fall of the serum concentration of gonadotropins.

There is a diagnosis of clinical pregnancy with ultrasound visualization of one or more chambers or definitive clinical gestational signs of pregnancy[18].

Statistical Analysis

The statistical comparison between groups 1 and 2 was performed using the two-tailed Fisher's exact test for qualitative data (evaluation of oocyte maturation and quality, embryo quality and pregnancy diagnosis). $p < 0.05$ was considered statistically significant.

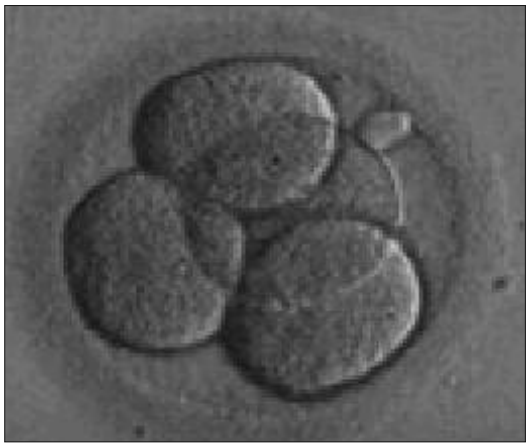

Figure 2. Good quality embryo in day +2.

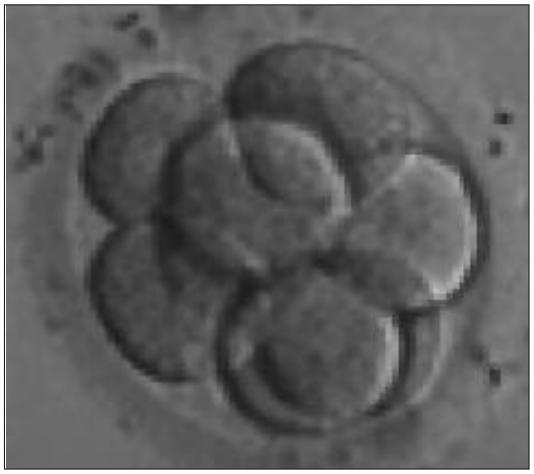

Figure 3. Good quality embryo in day +3.

Results

During the study, 149 patients meeting the criteria for recruitment were randomized into two groups: the study group 1 consisting of 58 patients treated with folic acid and inositol and the control group 2 consisting of 91 patients treated with folic acid alone.

The two groups were homogeneous within the parameters of inclusion adopted for the study.

Evenness in the average number of oocytes retrieved at ovum pick-up in groups 1 and 2 (total mean ± SD = 7.7 ± 4.2, group 1 mean ± SD = 8 ± 4, group 2 mean ± SD = 8 ± 4), no significant difference is found in the number of mature oocytes (MII) taken (Figure 4) and in the number of immature (MI and VG).

The number of patients with excellent and good oocyte quality (A+B) appears to be significantly higher in group 1 ($p = 0.02$) (Figure 5), as shown, they significantly increased the number of embryos of grade A transferred in the group 1 ($p = 0.02$) (Figure 6) compared to group 2, despite being completely similar averages of total embryos transferred (total mean ± SD = 2.4 ± 0.8, group 1 mean ± SD = 2.4 ± 0.8, group 2 mean ± SD = 2.4 ± 0.8).

There is not any significant difference between groups 1 and 2 in the number of positivity to β-hCG (Figure 7) and in the number of biochemical pregnancies detected (Figure 8) although it has a tendency to increase in the first and to decrease in the second for group 1.

The increase in percentage of clinical pregnancies in group 1 was statistically significant (p =

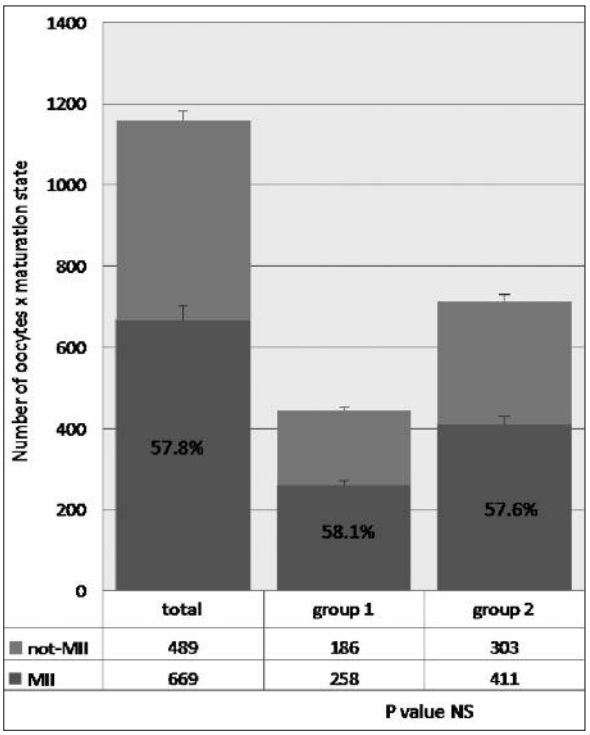

Figure 4. Number of oocytes retrieved and maturation state.

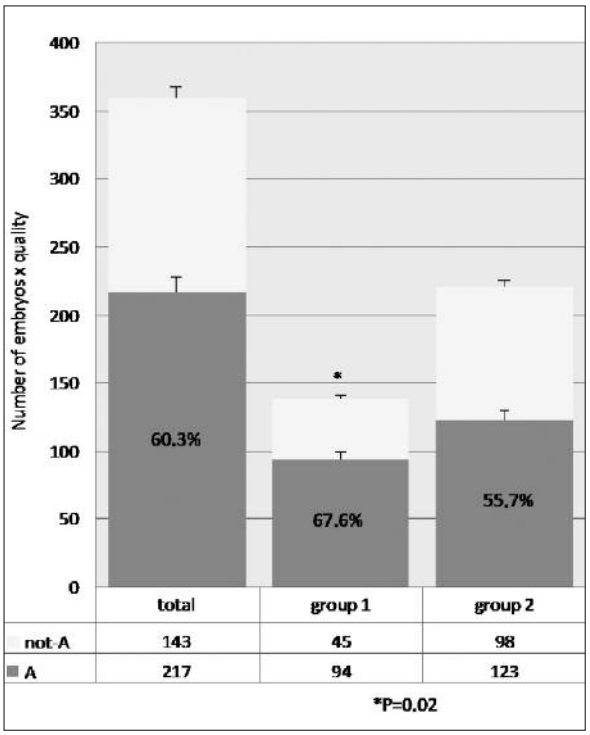

Figure 6. Number and quality of embryos transferred.

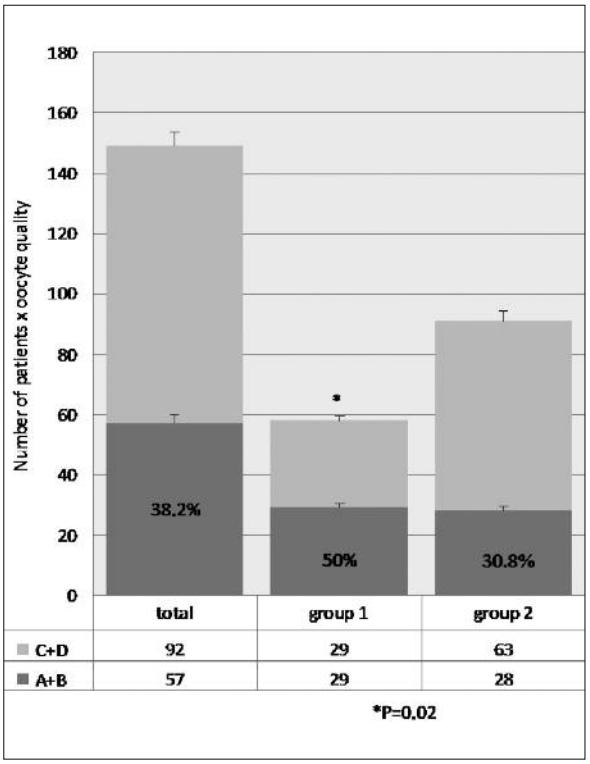

Figure 5. Number of patients and oocyte quality.

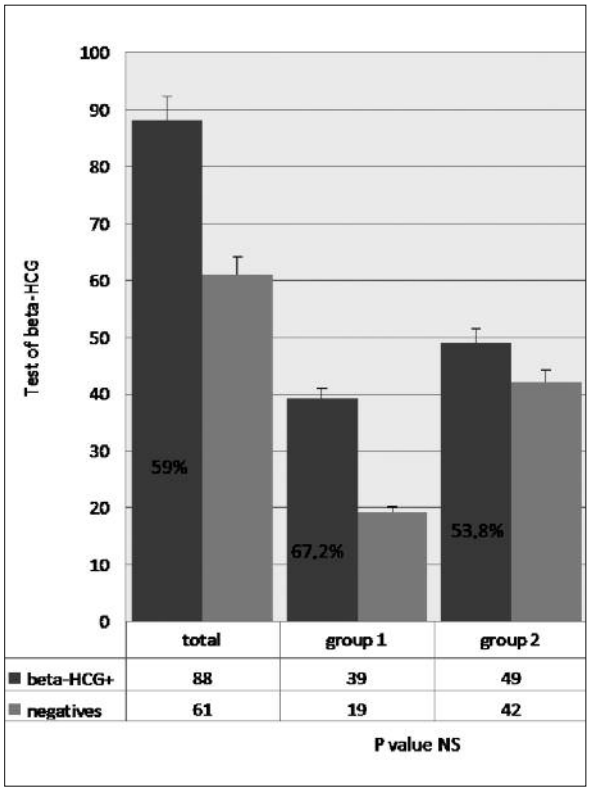

Figure 7. Number of positivity to β-hCG.

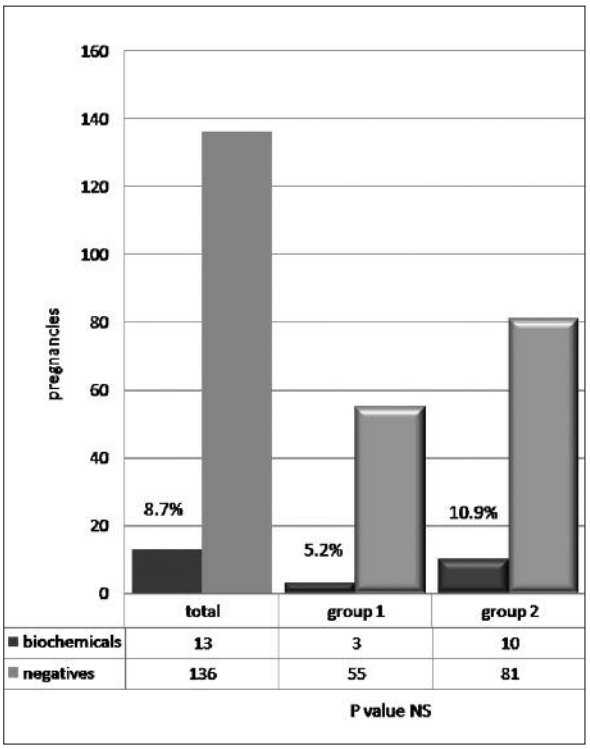

Figure 8. Number of biochemical pregnancies.

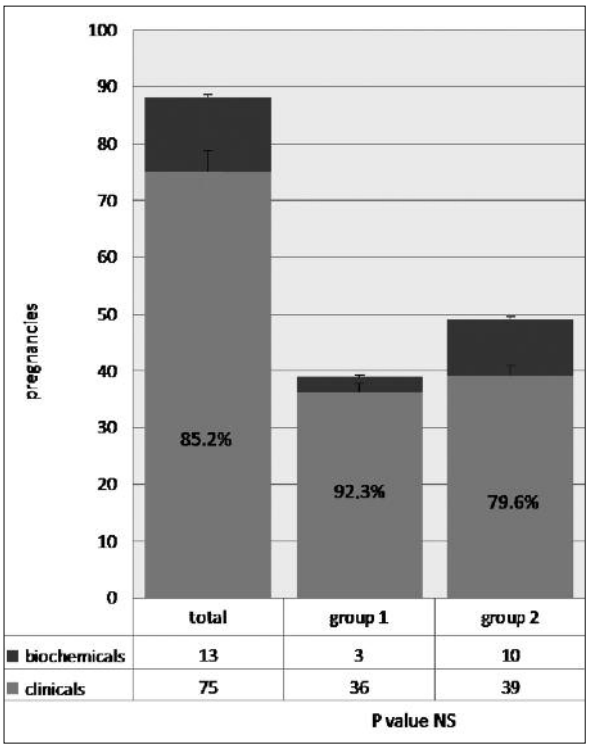

Figure 10. Comparison of clinical and biochemical pregnancies.

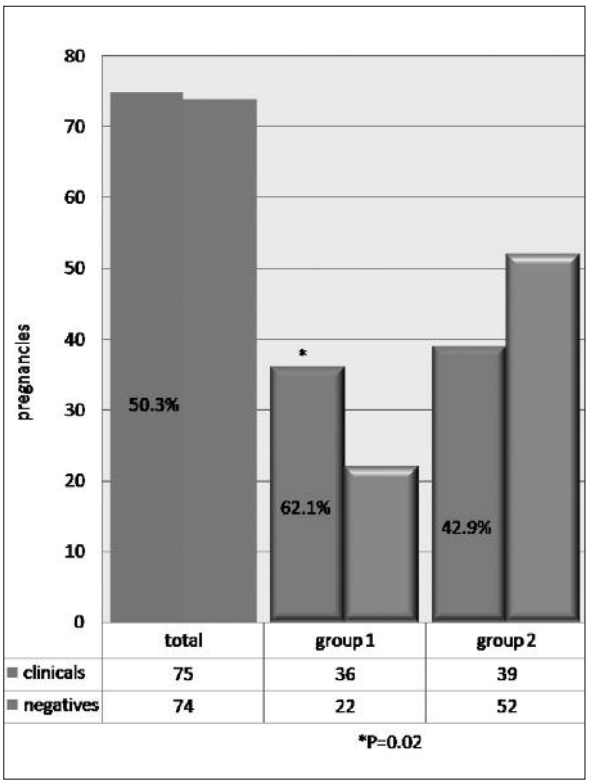

Figure 9. Number of clinical pregnancies.

0.02) (Figure 9), whereas there was no apparent significance in the difference between the biochemical and clinical pregnancies in the two groups despite the positive trend in the study group (Figure 10).

Discussion

The nuclear and cytoplasmic competence of human oocyte is critical for future competence of the embryo upon which ultimately depends the outcome of an ART treatment.

The follicular microenvironment in which the oocyte develops is crucial for its competence, and this must be taken into account particularly with the use of hormonal ovarian stimulation protocols in which many stages of the complex process of development of the egg cell are skipped, bringing about the formation of more oocytes with varying degrees of quality.

Inositol is an important element of the follicular environment and data are present in the literature to support the fact that the presence of higher levels of MI in follicular fluid correlates with a

higher quality oocyte[19] and that the supplementation with MI in IVF cycles is positively correlated to the progression of meiosis in mouse germ vesicles and to promote the oscillation of intracellular calcium[20].

Preliminary data of the few studies that have so far turned attention to this molecule in patients with PCOS indicate that their treatment with the addition of MI to folic acid reduces the number of germinal vesicles and degenerated oocytes at the time of pick-up oocyte, without compromising the total number of oocytes retrieved[21]. These results are in line with those of other less recent scientific papers, thus highlighting the positive effect of inositol on the development of a good oocyte[22].

Ours available data do not indicate an increase in the number of oocytes retrieved at the pick-up when patients are treated with inositol and folic acid instead of folic acid only, nor were any differences in the amount of mature oocytes and non-mature found.

Our work shows, however, that the overall quality of oocytes and, consequently, that of the embryos developed is significantly improved as a result of treatment with inositol and with them the number of clinical pregnancies obtained is increased (while without differences in the β-HCG positivities or in biochemical pregnancies).

In previously cited studies, reference is made to the possibility of using this type of approach to reduce the risk of hyperstimulation in some patients (after the administration of MI a significant reduction of estradiol levels at the time of ovulation induction is found) or to treat patients with PCOS[23,24] for both the insulin-sensitizing activity and for its role on ovarian function.

Conclusions

Based on our case load, relying on "inositol help" to solidify our efforts, seems to be an easy path to help to deepen the effectiveness of its use in all patients still under 40 but with prior failed attempts at ICSI or diagnosed with PCOS or as "poor responders".

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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