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# Do the Number of Oocytes Retrieved and Mature Oocyte Rate in the Cohort Affect IVF Results?

# Toplanan Oosit Sayısı ve Olgun Oosit Oranı IVF Sonuçlarını Etkiler mi?

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## ÖZ

**Amaç:** Bu çalışmanın amacı, tek fresh embriyo transferinde elde edilen oosit sayısı ve olgun oosit oranının IVF sonuçları üzerindeki etkilerini araştırmaktır.

**Gereç ve Yöntemler:** Toplanan oosit sayısı ve matür oosit oranı açısından iki ana grup oluşturuldu. 561 IVF siklusunda elde edilen oosit sayısına göre 0-5 oosit grup 1 (n=175), 6-10 oosit grup 2 (n=214), 11-15 oosit (n=121) grup 3 ve 16 veya daha fazla oosit grup 4 (n=51) olarak belirlendi. Matür oosit (metafaz II, MII oosit) oranına göre; grup 1A (n=338) %76-100 matür oosit oranı, grup 2A (n=150) %75-50 matür oosit oranı ve grup 3A (n=73) %50'den az matür oosit oranı olmak üzere üç grup oluşturuldu.

**Bulgular:** Toplanan oosit sayısı ile fertilizasyon oranı negatif korelasyon gösterirken, 6-10 oosit grubunda B-hCG pozitifliği ve klinik gebelik oranlarının daha iyi olduğu gözlendi. Ortalama yaş, bazal LH ve ovulasyonun tetiklendiği gün E2 seviyeleri ve fertilizasyon oranı, üç olgun oosit oranı grubu arasında önemli ölçüde farklıydı.

**Sonuç:** Sonuç olarak, toplanan oosit sayısından bağımsız olarak kohorttaki MII oosit oranları IVF sonuçlarını etkilemedi.

**Anahtar Kelimeler:** Oosit sayısı, Olgun oosit oranı, Fresh tek embriyo transferi

## ABSTRACT

**Aim:** The aim of this study was to investigate the effects of the number of oocytes retrieved and rate of mature oocytes on IVF outcomes in single fresh embryo transfer.

**Materials and Medhods:** Two main groups were formed regarding number of oocytes retrieved and mature oocyte rate. According to the number of oocytes retrieved in 561 IVF cycles, 0-5 (n=175) oocytes were determined as group 1, 6-10 (n=214) oocytes as group 2, 11-15 (n=121) oocytes as group 3 and 16 or more oocytes as group 4 (n=51). Regarding mature oocyte (metaphase II, MII oocyte) rate, three groups were formed: group 1A (n=338) 76-100% mature oocytes, group 2A (n=150) 50-75% mature oocytes, and group 3A (n=73) less than 50% mature oocytes.

**Results:** The number of oocytes retrieved was negatively correlated fertilization rate, whereas B-hCG positivity and clinical pregnancy rates were observed to be better in the 6-10 oocytes group. The mean age, basal LH, and ovulation trigger day E2 levels, and fertilization rate were differed significantly between three groups of mature oocyte rate.

**Conclusion:** In conclusion, MII oocyte rates in the cohort, regardless of the number of oocytes retrieved, did not affect IVF outcomes.

**Keywords:** Number of oocyte, Mature oocyte rate, Fresh single embryo transfer.

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## INTRODUCTION

Assisted reproductive techniques (ART) aims to promote multiple follicular development and recruit embryos with the highest implantation potential (1). To obtain highest live birth rate, it is investigated to identify the optimum number of retrieved oocytes per stimulation cycle. Some researchers argued that a higher number of oocytes retrieved is associated with improved outcomes, where others suggested that higher oocyte yields are associated with reduced oocyte quality and lower developmental potential of embryos (2-4). Moreover, studies results on the relationship between oocyte number and pregnancy rates are controversial. It seems like an increase in oocyte number decreases oocyte quality and IVF outcomes, however, recommended oocyte number to attain the best pregnancy rates per cycle differs from one study to another. Some authors suggest best expected live birth rate obtained by using 10 to 15 oocytes whereas the others suggest 15, 7-16 or 12-18 (5). In addition, it has been suggested that there may be a continuous positive relationship between the number of oocytes retrieved and the cumulative live birth rate (6). Controlled ovarian stimulation (COS) protocols aims to recruit higher rate of mature metaphase II (MII) oocytes (7). Among all infertility patients, 8.6% to 15.2% of them have one oocyte that cannot complete nuclear maturation (8). The percentage of oocytes is 85% in metaphase II (MII), 11% in metaphase I (MI), and 4% in germinal vesicles (GV) at different nuclear maturation stages in routine ART cycles, respectively (9). Oocyte maturation is one of the most important steps for the oocyte to reach competence for successful fertilization and subsequent embryonic development. Oocyte competence depends on two main processes. The first is nuclear maturity which is morphologically visible by observing the extrusion of the first polar body. The second is cytoplasmic maturity; a molecular process without any obvious markers other than subtle cytosolic and membrane changes (10). It is accepted that correct changes must occur in the localization, morphology, and biochemical properties of the organelles and cytoskeleton for the oocyte to obtain developmental competence potential (11). The failure or incomplete oocyte maturation causes infertility (12). This study is designed to contribute to our knowledge on the field by using a fresh single embryo transfer. The first aim of this study is to retrospectively investigate the effect of the number of oocytes on IVF results in fresh single embryo transfer cycles. The second aim of this study is to investigate the effect of mature oocyte rates in the cohort on IVF outcomes.

## MATERIALS AND METHODS

### Patient population, stimulation protocol and oocyte retrieval

The study design was approved by local ethics committee of the Ankara City Hospital (reference number: E1/19/278). Five hundred sixty-one fresh single embryo transfer cycles, performed between 2018 to 2019, were examined respectively. The controlled ovarian stimulation protocol consisted of gonadotropin-releasing hormone (GnRH) antagonist or agonist administration for pituitary suppression. Recombinant FSH (r-FSH) or human menopausal gonadotropin (hMG) followed by (Human chorionic gonadotropin) hCG were used during the controlled ovarian hyperstimulation cycles. Oocyte retrieval was performed under the guidance of transvaginal ultrasound 36 h after 10,000 IU hCG injection to trigger ovulation. All ultrasonographically identifiable follicles, larger than 14 mm., were aspirated. Oocyte retrieval and embryology laboratory processes were performed by the same team. Five hundred sixty-one IVF cycles were divided into four groups regarding the total number of oocytes retrieved, and three groups regarding mature oocyte rate. The four groups regarding the number of oocytes were having cases as follows: group 1 with 0-5 oocytes (n=175), group 2 with 6-10 oocytes (n=214), group 3 with 11-15 oocytes (n=121), and group 4 with more than 16 oocytes (n=51). Three groups regarding mature oocytes (MII) rates were formed according to the criteria stated in the Eshre 2017 consensus report (15). Mature oocyte rate was 76-100% in group 1A, (n=338), 75-50 % in group 2A, (n=150), and less than 50% in group 3A (n=73). Age, body mass index (BMI), Antimullerian hormone (AMH), basal serum follicle stimulating hormone (FSH), basal serum luteinizing hormone (LH), and estradiol (E2) levels, ovulation trigger day E2, ovulation trigger day progesterone (P) levels (ng/mL), total gonadotropin dose, B-hCG poizitivity, and mature oocyte rate were recorded from the charts. Cycles with only single fresh embryo transfer were included in the retrospective study. The number of cycles of patients was ignored.

### Oocyte preparation, ICSI and embryo culture

Cumulus–oocyte complexes (COCs) were collected from the aspirated follicular fluid then cultured and transferred to fertilization medium. Following the incubation, denudation of the oocyte by removal of the surrounding cumulus and corona cells. Denuded oocytes were cultured at 37 C, 6% CO2, 5% O2, and 90% humidity until ICSI. Fertilization was checked 16-18 h.

after ICSI for signs of fertilization. Embryos were checked and graded every day by using appropriate scoring systems modified from previously defined systems (16-18). Day 2 and day 3 embryo scoring were performed respectively 42–44 h. and 64–65 h. after ICSI. Day 4 embryos were scored by using consensus scoring system for Day 4 embryos (15). Day 5 blastocyst evaluations were based on the degree of blastocoel expansion, visible inner cell mass, and continuous trophoectoderm with sufficient cells. Embryos were graded from 1 to 5 (best to worst). Grade 1 and grade 2 embryos were accepted as good quality on day 2, day 3, day 4, and day 5. Fresh single embryo transfer (ET) was performed on the second, third, or fifth day after ICSI. Luteal phase support was provided by vaginal progesterone (Crinone 8 % gel, Serono, UK), twice a day. Pregnancy was determined by β-hCG levels in blood 12 days after embryo transfer. Clinical pregnancy was defined as the presence of a gestational sac with accompanying fetal heartbeat by ultrasound 4 weeks following the ET procedure. The patients with β-hCG level more than 10 mIU/mL were considered pregnant. Live birth was defined as continuing live birth after 20 weeks of gestation and one month survival. The live birth rate was calculated by dividing number of live births by total number of cycles.

### Statistical Analysis

All statistical tests were conducted using Jamovi software version 1.6.23.0 (The Jamovi Project, 2021). Normality of data distribution was analyzed by either using Kolmogorov-Smirnov or Shapiro Wilk test. Since the data distribution was found to be non-parametric, numerical data were reported as median (minimum-maximum). Categorical data was reported as percentages. Correlation between variables were examined using Spearman's rank correlation (rho) test. All tests were two-sided and performed at 0.05 significance level.

## RESULTS

Five hundred sixty-one IVF cycles were divided into four groups according to the number of oocytes retrieved. There was no significant difference between the four groups regarding their BMI, basal LH and E2 levels, β hCG positivity, good quality cleavage embryo rate, and good quality blastocyst rate. The mean age, AMH, basal FSH levels, ovulation trigger day E2 and P levels, total gonadotropin dose, fertilization rate, B-hCG positivity, and clinical pregnancy rate were significantly differed between the four groups ($p<0.05$). The mean age, basal FSH level, total gonadotropin dose, and fertilization rate was higher in group 1 whereas AMH level, and ovulation trigger day E2 level were higher in group 4 ($p<0.05$) (Table 1 and 2).

**Table 1:** Clinical characteristics of the patients in the groups according to oocyte numbers.

| Variable | Group 1 (1-5 oocytes) n=175 | Group 2 (6-10 oocytes) n=214 | Group 3 (11-15 oocytes) n= 121 | Group 4 (16≥ oocytes) n=51 | p value # | Correlation coefficient (rs) |
|---|---|---|---|---|---|---|
| Age (Years) | 33 (19 - 43) †‡± | 29 (21 - 41) † | 28 (18 - 41) ‡ | 28 (20 - 38) ± | <0,001 | - 0,358@ |
| BMI (kg/m²) | 24 (16 - 36,5) | 24 (17 - 39,7) | 24 (17,2 - 36) | 24,7 (16,9 - 33) | 0,562 | - 0,003 |
| Anti-Mullerian Hormone (AMH) | 0,9 (0 - 7,8) †‡± | 2,1 (0,17 - 19,6) †$¶ | 3,2 (0,6 - 32) ‡$ | 4,1 (1,7 - 10,3) ±¶ | <0,001 | 0,407@ |
| Basal FSH (mIU/mL) | 8,1 (1,5 - 24) †‡± | 7 (0,3 - 23) †¶ | 6 (1 - 15) ‡ | 5,9 (4 - 10,9) ±¶ | <0,001 | - 0,356@ |
| Basal LH (mIU/mL) | 5,2 (1,7 - 15) | 5,4 (0,1 - 41) | 6 (1 - 23) | 5,9 (2 - 32,2) | 0,597 | 0,058 |
| Basal E2 (ng/mL) | 39 (6,2 - 185) | 39 (10 - 108) | 38 (11,8 - 87) | 40 (15 - 79) | 0,470 | - 0,076 |
| Ovulation Trigger day of E2 (ng/mL) | 1031 (137 - 11812) †‡± | 1646 (458 - 4369) †$¶ | 2371 (850 - 6248) ‡$§ | 2903 (1379 - 7233) ±¶§ | <0,001 | 0,521@ |
| Ovulation Trigger day of P (ng/mL) | 0,4 (0,1 - 7) †‡± | 0,5 (0,1 - 2,5) †$¶ | 0,6 (0,1 - 2,4) ‡$ | 0,7 (0,1 - 4,4) ±¶ | <0,001 | 0,195@ |
| Total gonadotropin dose (IU) | 2400 (150 - 6250) †‡± | 1956 (663 - 5250) †$¶ | 1725 (896 - 4950) ‡$ | 1721 (1000 - 3673) ±¶ | <0,001 | - 0,341@ |

| | |
|---|---|
| # Kruskal Wallis test. | *Dwass-Steel-Critchlow-Fligner test pairwise comparisons* |
| † Statistically significant difference between Group 1 and 2 | $ Statistically significant difference between Group 2 and 3 |
| ‡ Statistically significant difference between Group 1 and 3 | ¶ Statistically significant difference between Group 2 and 4 |
| ± Statistically significant difference between Group 1 and 4 | § Statistically significant difference between Group 3 and 4 |

@ Statistically significant correlation at 0,05 level (Spearman's rank correlation test)

The number of oocytes retrieved negatively correlated with age, total gonadotropin dose, and basal FSH (p< 0,05), and positively correlated with estrogen level on trigger day, progesterone level on trigger day, and AMH levels (p<0.05) (Table 1). In addition, B-hCG positivity and clinical pregnancy rates were observed to be better in the 6-10 oocytes group. (p<0.05) (Table 2).

**Table 2: F**ertilization, pregnancy, live birth and transfer days after ICSI of the groups according to the number of oocytes.

| Variable | Group 1 (1-5 oocytes) n=175 | Group 2 (6-10 oocytes) n=214 | Group 3 (11-15 oocytes) n= 121 | Group 4 (16≥ oocytes) n=51 | p value | Correlation coefficient ($r_s$) |
|---|---|---|---|---|---|---|
| **Fertilisation Rate (2PN)** | 66 (25 - 100) †‡± | 50 (11 - 100) † | 50 (8 - 100) ‡ | 50 (17 - 94) ± | <0.001# | - 0,224@ |
| B-**hCG (+)** | 23,5% ± | 39,2% | 24,7% | 12,7% ± | 0,035* | 0,121 |
| **Clinical Pregnancy Rate** | 22,8% ± | 41,4% | 22,8% | 13,1% ± | 0,039* | 0,109@ |
| **Live Birth Rate** | 24,8% | 37,6% | 26,5% | 11,1% | 0,155* | 0,098@ |
| **Good Quality Cleavage Embryo Rate** | 100 (0 - 100) | 100 (33 - 100) | 100 (50 - 100) | 100 (50 - 100) | 0,414# | 0,013 |
| **Good Quality Blastocyt Rate** | 5,4% | 51,4% | 16,2% | 27% | 0,064* | 0,065 |

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|---|---|
| # Kruskal Wallis test * Chi-square test. | *Dwass-Steel-Critchlow-Fligner test pairwise comparisons* |
| † Statistically significant difference between Group 1 and 2 | $ Statistically significant difference between Group 2 and 3 |
| ‡ Statistically significant difference between Group 1 and 3 | ¶ Statistically significant difference between Group 2 and 4 |
| ± Statistically significant difference between Group 1 and 4 | § Statistically significant difference between Group 3 and 4 |

@ Statistically significant correlation at 0,05 level (Spearman's rank correlation test)

As the five hundred sixty-one IVF cycles were divided into three groups according to the oocyte MII ratio, no significant differences were observed between groups in terms of AMH, BMI, basal FSH and E2, and ovulation trigger day P levels, hCG positivity, good quality cleavage embryo rate, good quality blastocyst rate, clinical pregnancy rate, and live birth rate. The mean age, basal LH, total gonadotropin dose, and fertilization rate were differed significantly between three groups. In group 3A, the mean age was higher whereas estrogen level on trigger day, and basal LH level was lower as compared to the other groups (p<0,05). Regardless of the number of oocytes, MII rates compared with other groups in the cohort did not positively affect IVF outcomes (Table 3 and 4).

**Table 3:** Clinical characteristics of the patients in the groups according to mature oocyte rate.

| Variable | Group 1A (76-100%) n=338 | Group 2A (75-50%) n=150 | Group 3A (50%↓) n=73 | p value # | Correlation coefficient ($r_s$) |
|---|---|---|---|---|---|
| **Age (Years)** | 29 (18 - 42) ‡ | 29 (20 - 41) $ | 31 (23 - 43) ‡$ | 0,003 | 0,107@ |
| **BMI (kg/m²)** | 23,9 (16 - 39,7) | 24 (17 - 36) | 24,3 (17 - 36,5) | 0,327 | 0,063 |
| **Anti-Mullerian Hormone (AMH)** | 2,1 (0 - 32) | 2,3 (0,1 - 19,6) | 1,5 (0,1 - 10,6) | 0,075 | - 0,003 |
| **Basal FSH (mIU/mL)** | 7 (0,3 - 17,6) | 7 (1 - 24) | 6 (1,5 - 16) | 0,392 | - 0,026 |
| **Basal LH (mIU/mL)** | 6 (0,1 - 41) ‡ | 5 (1 - 21) | 4,8 (1,7 - 15) ‡ | 0,001 | - 0,133@ |
| **Basal E2 (ng/mL)** | 39 (7,8 - 108) | 38 (10 - 101) | 37 (6,2 - 185) | 0,720 | - 0,002 |
| **Ovulation Trigger day of E2 (ng/mL)** | 1686 (137 - 7233) ‡ | 1806 (245 - 11812) $ | 1395 (289 - 6827) ‡$ | 0,002 | - 0,065 |
| **Ovulation Trigger day of P (ng/mL)** | 0,5 (0,1 - 7) | 0,5 (0,1 - 4,4) | 0,5 (0,1 - 2) | 0,687 | - 0,016 |
| **Total gonadotropin dose (IU)** | 2025 (150 - 4575) | 2024 (1084 - 5850) | 2250 (663 - 6250) | 0,073 | 0,151@ |
| **Mature Oocyte Rate** | 100 (0 - 100) †‡ | 67 (53 - 78) †$ | 43 (13 - 100) ‡$ | <0,001 | - 0,879@ |

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| # Kruskal Wallis test. |
| *Dwass-Steel-Critchlow-Fligner test pairwise comparisons* |
| † Statistically significant difference between Group 1 and 2 |
| ‡ Statistically significant difference between Group 1 and 3 |
| $ Statistically significant difference between Group 2 and 3 |

@ Statistically significant correlation at 0,05 level (Spearman's rank correlation test)

**Table 4:** Fertilization, pregnancy, live birth and transfer days after ICSI of the groups according to mature oocyte rate.

| Variable | Group 1A (76-100%) n=338 | Group 2A (75-50%) n=150 | Group 3A (50%↓) n=73 | p value | Correlation coefficient ($r_s$) |
|---|---|---|---|---|---|
| **Fertilisation Rate (2PN)** | 55 (8 - 100) ‡ | 50 (10 - 100) $ | 80,5 (17 - 100) ‡$ | <0,001# | 0,123@ |
| B-**hCG (+)** | 63,9% | 26,5% | 9,6% | 0,311* | - 0,060 |
| **Clinical Pregnancy Rate** | 64,8% | 26,2% | 9% | 0,231* | - 0,069 |
| **Live Birth Rate** | 67,5% | 23,9% | 8,5% | 0,163* | - 0,085 |
| **Good Quality Cleavage Embryo Rate** | 100 (0 - 100) | 100 (33 - 100) | 100 (0 - 100) | 0,969# | - 0,042 |
| **Good Quality Blastocyt Rate** | 73% | 21,6% | 5,4% | 0,933* | 0,009 |

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|---|
| # Kruskal Wallis test * Chi-square test. |
| *Dwass-Steel-Critchlow-Fligner test pairwise comparisons* |
| † Statistically significant difference between Group 1 and 2 |
| ‡ Statistically significant difference between Group 1 and 3 |
| $ Statistically significant difference between Group 2 and 3 |

@ Statistically significant correlation at 0,05 level (Spearman's rank correlation test)

## DISCUSSION

Advances in laboratory technology and clinical practice have been increased success rates in ART. It is unclear what the level of controlled ovarian stimulation should be to provide best results from in vitro fertilization (6). Number of studies suggest that high number of oocytes may be negatively associated with oocyte quality (19), fertilization rate (20), and live birth rates (5), however, there are studies suggest that a higher response is associated with increased pregnancy and live birth rates (LBR) (21, 22). Several studies have been conducted to determine the optimal number of oocytes for fresh LBR maximization. To contribute the efforts on determination of the effect of number of oocytes retrieved on IVF outcomes, we analyzed 516 cycles.

Although the number of oocytes retrieved in fresh cycles was found to be strongly associated with IVF success, significant clinical and methodological heterogeneities exist between studies. Recent studies have suggested that supraphysiological amounts of estradiol and progesterone in fresh cycles may reduce endometrial receptivity, which in turn results lower implantation rates (5, 23). According to our findings, we can suggest that the best results in terms of bhcg positivity and clinical pregnancy rates are in the 6-10 oocytes group.

Moreover, we investigated the effect of MII ratios on IVF results. Oocyte developmental competence is generally described as "the ability of a female gamete to mature into an egg with its potential to be fertilized and sustain embryo development to the blastocyst stage" (24). For developmental competence, oocytes must undergo correct nuclear and cytoplasmic maturation following luteinizing hormone (LH) surge (25). Oocyte developmental competence involves a large number of factors regulated by different signalling pathways at various stages prior to fertilization (12) When we analysed the effect of MII ratios in cohort on IVF results in the fresh single embryo transfer cycle, irrespective of the number of oocytes, we observed that MII ratios had no effect on IVF outcomes. ($p< 0.001$).

The definition of cytoplasmic maturation is still unclear since an early predictor of viability isn't available and it can only be inferred by the behaviour of cortical granules (26-28). However, it is known that oocytes may not have sufficient cytoplasmic maturation to complete embryonic development, even if successful nuclear or meiotic maturation into the metaphase II stage of meiosis (13). Normal fertilization requires cytoplasmic competence which includes metabolic and structural modifications leading to genetic activation and cell cycle progression from meiosis to mitosis to obtain embryos with full development potential 14, 29. Our findings support the literature as reported that incomplete cytoplasmic maturation of metaphase 2 oocyte despite nuclear maturation and limited number of cycles may be adversely effects pregnancy and live birth rates.

There is a positive correlation between the number of oocytes and the number of best/quality embryos (30). Findings of a meta-analysis by Vermey bg et al. also reveal that there is a positive correlation between the number of oocytes and the number of top/quality embryos, although there is considerable heterogeneity between the studies (31). In this study, the difference between number of oocytes and M2 oocyte rate did not affect the quality of the cleavage embryos and blastocyt. We think that due to insufficient cytoplasmic maturation or a limited number of cases, high rates of mature oocytes and increasing oocte number may not have affected embryo quality.

Another important factor is age in determining fertility potential of women. Fertility decreases with age, corresponding to the decrease in the quantity and quality of oocytes. The follicular depletion pattern, influential factors, timing, and mechanisms that determine both the decreasing oocyte number and oocyte quality are not fully understood (32). Studies have indicated that advanced female age increases the chance of immature oocyte recruitment in ART cycles (10). Similar to the previous reports, we observed that both the number of oocytes retrieved and the ratio of MII oocytes in the cohort decreased with increasing female age.

Results of the current study are consistent with previous studies: insufficient cytoplasmic maturation of metaphase 2 oocyte in the cohort adversely affects oocyte competence, pregnancy rates, and live births in fresh IVF cycles. In conclusion, we may suggest that evaluating oocytes on the basis of nuclear maturity without taking cytoplasmic maturation into account may be insufficient to predict IVF outcomes. Further research to define new biomarkers that identifies the oocyte nuclear and cytoplasmic maturation processes and even for technical developments that correct the disruptions is needed.

## REFERENCES


1. Haahr, T., Esteves, S. C., Humaidan, P. 2018. Individualized controlled ovarian stimulation in expected poor-responders: an update. Reprod Biol Endocrinol, 16, 20.

2. Baker, V.L., Brown, M.B., Luke, B., Conrad, K.P. 2015. Association of number of retrieved oocytes with live birth rate and birth weight: an analysis of 231,815 cycles of in vitro fertilization. Fertil. Steril. 103: 931–938.e932

3. Van der Gaast, M.H., Eijkemans, M.J., van der Net, J.B., de Boer, E.J., Burger, C.W., van Leeuwen, F.E., Fauser, B.C., Macklon, N.S. 2006. Optimum number of oocytes for a successful first IVF treatment cycle. Reprod Biomed Online 13: 476–480.

4. Valbuena, D., Martin, J., de Pablo, J.L., Remohi, J., Pellicer, A., Simon, C. 2001. Increasing levels of estradiol are deleterious to embryonic implantation because they directly affect the embryo. Fertil. Steril. 76: 962–968.

5. Sesh Kamal Sunkara, Vivian Rittenberg, Nick Raine-Fenning, Siladitya Bhattacharya, Javier Zamora, and Arri Coomarasamy. 2011. Association between the number of eggs and live birth in IVF treatment: an analysis of 400 135 treatment cycles. Human Reproduction Vol.26, No.7 pp. 1768–1774.

6. Law YJ, Zhang N, Kolibianakis EM, Costello MF, Keller E, Chambers GM et all, Is there an optimal number of oocytes retrieved at which live birth rates or cumulative live birth rates per aspiration are maximized after ART? A systematic review. Reprod Biomed Online. 2021;42(1):83-104.

7. Rubıno, P., Vıgano, P., Luddı, A. & Pıombonı, P. 2016. The ICSI procedure from past to future: a systematic review of the more controversial aspects. Human Reproduction Update, 22, 194-227.

8. Beall, S., Brenner, C., Segars, J. 2010. Oocyte maturation failure: A syndrome of bad eggs. Fertility and Sterility, 94, 2507–2513.

9. Avci B., Kasapoglu, I., Cakir C., Ozbay, A., Ata, B., Uncu, G. 2020. Fertilisation and early embryonic development of immature and rescue in vitro-matured sibling oocytes. Hum Fertil (Camb). Jan 17;1-10.

10. Halvaei, I., Ali Khalili, M., Razi, M. H., Nottola, S. A. 2012. The effect of immature oocytes quantity on the rates of oocytes maturity and morphology, fertilization, and embryo development in ICSI cycles. J Assist Reprod Genet, 29, 803-10.

11. Luna Mao, Hangying Lou, Yiyun Lou, Ning Wang, Fan Jin. 2014. Behaviour of cytoplasmic organelles and cytoskeleton during oocyte maturation Reproductive BioMedicine Online 28, 284–299.

12. Sen, A., Caiazza, F. 2013. Oocyte maturation: a story of arrest and release. Front Biosci (Schol Ed), 5, 451-77.

13. Alessandra P., Mohamad I., Derek K., Stephen C., Zev R., Gianpiero D. Palermo. 2019. High proportion of immature oocytes in a cohort reduces fertilization, embryo development, pregnancy and live birth rates following ICSI Reprod Biomed Online Oct;39(4):580-587.

14. Reichman, D. E., Politch, J., Ginsburg, E. S., Racowsky, C. 2010. Extended in vitro maturation of immature oocytes from stimulated cycles: an analysis of fertilization potential, embryo development, and reproductive outcomes. J Assist Reprod Genet, 27, 347-56.

15. ESHRE Special Interest Group of Embryology and Alpha Scientists in Reproductive Medicine. 2017. The Vienna consensus: report of an expert meeting on the development of art laboratory performance indicators. Human Reproduction Open, pp. 1–17.

16. Tesarik J., Greco E. 1999. The probability of abnormal preimplantation development can be predicted by a single static observation on pronuclear stage morphology. Human Reproduction 14(5): 1318-23.

17. 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. Human Reproduction, Vol.26, No.6 pp. 1270–1283, 2011

18. Baczkowski T, Kurzawa R, Głabowski W. Methods of embryo scoring in in vitro fertilization. Reprod Biol 2004;4:5–22.

19. Mitwally, M.F., Bhakoo, H.S., Crickard, K., Sullivan, M.W., Batt, R.E., Yeh, J. 2006. Estradiol production during controlled ovarian hyperstimulation correlates with treatment outcome in women undergoing in vitro fertilization–embryo transfer. Fertility and sterility; 86: 588–596.

20. Kok, J.D., Looman, C.W., Weima, S.M., Te Velde, E.R. 2006. A high number of oocytes obtained after ovarian hyperstimulation for in vitro fertilization or intracytoplasmic sperm injection is not associated with decreased pregnancy outcome. Fertility Sterility; 85: 918–924

21. Fatemi, H.M., Doody, K., Griesinger, G., Witjes, H., Mannaerts, B. 2013. High ovarian response does not jeopardize ongoing pregnancy rates and increases cumulative pregnancy rates in a GnRH-antagonist protocol. Human Reproduction; 28: 442–452.

22. Briggs, R., Kovacs, G., Maclachlan, V., Motteram, C., Baker, H.W. 2015. Can you ever collect too many oocytes? Human Reproduction 30: 81–87.

23. Venetis, C., Kolibianakis, E., Bosdou, J., Tarlatzis, B. 2013. Progesterone elevation and probability of pregnancy after IVF: a systematic review and meta-analysis of over 60 000 cycles. Human Reproduction Update 19: 433–457.

24. Conti, M., Franciosi, F. 2018. Acquisition of oocyte competence to develop as an embryo: integrated nuclear and cytoplasmic events. Human Reproduction Update, 24, 245-266.

25. Reader, K. L., Stanton, J.-A. L., Juengel, J. L. 2017. The Role of Oocyte Organelles in Determining Developmental Competence. Biology (Basel). Sep 18;6(3):35.

26. Sathananthan, A.H., Selvaraj, K., Girijashankar, M.L., Ganesh, V., Selvaraj, P., Trounson, A.O. 2006. From oogonia to mature oocytes: Inactivation of the maternal centrosome in humans. Microsc. Res. Tech.; 69: 396–407.

27. Arroyo, A., Kim, B., Yeh, J. 2020. Luteinizing Hormone Action in Human Oocyte Maturation and Quality: Signaling Pathways, Regulation, and Clinical Impact. Reproductive Sciences 27, 1223-1252.

28. Livia Z. Yanez, Jinnuo Han, Barry B. Behr, Renee A. Reijo Pera, David B. Camarillo. 2016. Human oocyte developmental potential is predicted by mechanical properties within hours after fertilization Nature Communications DOI: 10.1038/Ncomms10809 1-12

29. Balakier, H., Sojecki, A., Motamedi, G., Librach, C. 2004. Time dependent capability of human oocytes for activation and pronuclear formation during metaphase II arrest. Hum Reprod, 19, 982-7.

30. Labarta, E., Bosch, E., Mercader, A., Alama, P., Mateu, E., Pellicer, A. 2017. A Higher Ovarian Response after Stimulation for IVF Is Related to a Higher Number of Euploid Embryos. Biomed. Res. Int. 2017:5637923.

31. Vermey BG, Chua SJ, Zafarmand MH, Wang R, Longobardi S, Cottell E, Beckers F, Mol BW, Venetis CA, D'Hooghe 2019. Is there an association between oocyte number and embryo quality? A systematic review and meta-analysis. T.Reprod Biomed Online. Nov;39(5):751-763.

32. Vollenhoven B, Hunt S. Ovarian ageing and the impact on female fertility, F1000Research, 7, F1000Faculty Rev, 2018: 1835