Zygote

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Impact of the number of retrieved oocytes on IVF outcomes: oocyte maturation, fertilization, embryo quality and implantation rate

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Research Article

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Summary

The process of oocyte retrieval represents a key phase during the cycles of *in vitro* fertilization (IVF). It involves controlled ovarian stimulation to retrieve the highest number of oocytes possible. According to many previous studies, the higher the number of oocytes the higher the chances of obtaining embryos for multiple transfers. In this study, in total, 1987 patients were retrospectively reviewed to investigate the correlations between the number of retrieved oocytes and the subsequent IVF outcomes. Patients were divided into three groups according to the number of retrieved oocytes (Group 1: ≤5 oocytes; Group 2: 6–15 oocytes; Group 3: ≥15 oocytes). The results showed a significant negative correlation between oocyte number and maturation rate as well as fertilization rate. However, a significant positive correlation was found between oocyte number and the blastulation rate. The implantation rate after fresh embryo transfers was higher in group 2 (6–15 oocytes) compared with group 1 (≤5 oocytes). According to our findings, we conclude that oocyte numbers between 6 and 15 oocytes can result in the highest chances of positive IVF outcomes in terms of embryo quality and fresh embryo transfers with lower risks of ovarian hyperstimulation.

Introduction

During the process of assisted reproductive technology (ART), controlled ovarian stimulation is the first important step towards *in vitro* fertilization (IVF) success. Multiple follicles are stimulated using different gonadotrophins and protocols in a single cycle for the collection of multiple oocytes (Macklon *et al*., 2006; Vermey *et al*., 2019). The general aim of this collection was to obtain as many oocytes as possible to increase the chance of good quality embryos. A high number of good quality embryos increases the chances of having more than one transfer, as many of the embryos can be vitrified for upcoming cycles. However, there is considerable heterogeneity in the published studies on the effect of the number of retrieved oocytes on IVF outcomes (Timeva *et al*., 2006; Cai *et al*., 2013). While some studies showed that an optimum number increases the live birth rates (Ji *et al*., 2013), others found that clinical pregnancies and live birth rate (LBR) do not decrease with a high number of oocytes (Briggs *et al*., 2015).

Also, it has been shown that poor ovarian reserve does not necessarily means poor pregnancy rates, as there are other factors that are involved such as female age (Klinkert *et al*., 2004; Hendriks *et al*., 2008; Yin *et al*., 2019). However, reports on poor responders with lower pregnancy rates have also been published (Biljan *et al*., 2000; De Sutter and Dhont, 2003; Timeva *et al*., 2006; Zhen *et al*., 2008). Before the phases of implantation and live birth, there are many key stages that can be affected by oocyte competence. Oocyte maturation, fertilization competence, cleavage quality and blastulation are known to be the first *in vitro* indicators of chances of implantation and live birth during IVF cycles. The aim of this study was to elucidate the association between the number of oocytes after retrieval and the following results of each step of the IVF process.

Materials and methods

Patients

This retrospective study was approved by the IRIFIV Fertility Centre, Casablanca (Morocco) informing the selected patients with consent. The study includes 1987 women, aged between 24 and 40 years old, who were eligible for oocyte retrieval, with primary and secondary infertility, and who underwent an intracytoplasmic sperm injection (ICSI) cycle between January 2015 and January 2020.

For the purpose of this study, cycles involving oocyte cryopreservation and frozen oocyte thawing were excluded from the analysis. Patients were divided into three groups according to the number of oocytes retrieved from each patient. Group 1: ≤5 oocytes, group 2: 6–15 oocytes, group 3 ≥15 oocytes. These categories were defined according to previous research (please refer to the Discussion).

Ovarian stimulation

Women underwent controlled ovarian stimulation with the flexible gonadotrophin-releasing hormone (GnRH) antagonist protocol. A daily subcutaneous injection of recombinant follicle-stimulating hormone (rFSH; Gonal-F, Merck-Serono) was used alone or in combination with human menopausal gonadotrophin (HMG; Menopur; Ferring). The follicle-stimulating hormone (FSH) dose was based on the woman's age, anti-müllerian hormone (AMH) concentration, in addition to prior history of ovarian stimulation and was adjusted according to the usual parameters of follicle growth, determined using serum estradiol (E2) concentration and ultrasound monitoring.

A daily dose of GnRH antagonist (Cetrotide, Merck-Serono or Orgalutran, MSD) was injected subcutaneously, starting from day 6 of FSH administration. The ovulation trigger was performed with 10,000 IU of human chorionic gonadotrophin (rHCG, Ovitrelle; Merck-Serono) and gonadotrophin-releasing hormone (Decapeptyl, Ferring), after obtaining follicles that reached dimensions of 17 mm or greater in diameter and adequate serum E2 levels. Oocytes were retrieved 34–36 h after hCG administration.

Oocyte and sperm preparation

The retrieved cumulus–oocyte complexes were isolated from follicular fluid, rinsed and cultured in culture medium (SAGE 1-Step, Origio). At 2–3 h after retrieval, the oocyte–corona–cumulus complexes were placed in a HEPES-buffered medium (Ferticult Flushing medium, Fertipro) containing hyaluronidase (Hyaluronidases in Ferticult Flushing medium, Fertipro) and were mechanically denudated using a 20–200-µl micropipette. The nuclear maturation grades were classified as metaphase II, metaphase I and germinal vesicle.

Sperm samples were collected in a sterile container from the male partner by masturbation, after 3–4 days of abstinence, and washed by centrifugation. All metaphase II oocytes underwent ICSI after denudation.

The temperature inside the incubators (IVF-Cube AD3100, ASTEC; Thermo Scientific HeraCell 150) was controlled using a certified thermometer and remained at 37 ± 0.2°C. Oxygen levels inside the incubators were at 5% and the cultivation medium was pH 7.3 ± 0.02 with CO_2 ~5.6%.

Assessment of the rate of maturation, fertilization, embryo quality and implantation

The rate of fertilization was calculated by the number of 2PN embryos on day 1 divided by the total number of metaphase II (MII) oocytes. The rate of 8-cell embryos on day 3 was calculated by the number of 8-cell embryos divided by the total number of embryos. The blastulation rate was calculated by the number of blastocysts obtained on day 5 divided by the total number of embryos of extended culture. On day 2, embryos were not taken out of the incubator for evaluation.

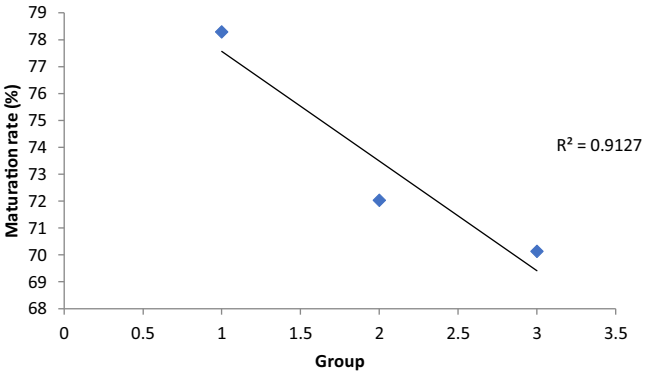

Figure 1. Association between the number of retrieved oocytes and the maturation rate. Group 1: (≤5 oocytes); Group 2: (6–15 oocytes); Group 3: (≥16 oocytes).

The embryo transfer policy was double embryo transfer (DET) on day 3. The implantation rate was calculated as the number of implanted embryos divided by the number of embryos transferred per group of patients.

Statistical analysis

The results are expressed as the mean ± standard deviation or percentage of the total. Data were obtained using Student's *t*-test and Statistical Package for the Social Sciences (SPSS) software. Statistical significance was defined as a P-value < 0.05. Correlation coefficient values were used to determine the significance of the correlations found in all data. In this analysis, the relationship was assessed for each possible confounding factor.

Results

Relationship between the number of retrieved oocytes and their maturation rate

Our data showed a significant negative correlation between the maturation rate and the number of retrieved oocytes ($r = -0.95$; $P = 4.79 \times 10^{9}$) (Figure 1). The highest maturation rate (78.2%) was observed in group 1 (≤5 oocytes) and the lowest maturation rate was observed in group 3 (≥16 oocytes) (Table 1).

Relationship between the number of retrieved oocytes and embryo quality

As shown in Table 1, we found a significant negative correlation ($r = -0.99$; $P = 1.03 \times 10^{6}$) between fertilization rate and the number of retrieved oocytes. The rate of fertilization significantly decreased from group 1 to group 3 (Figure 2).

Regarding the rate of 8-cell embryos on day 3, no correlation but a significant difference ($P = 1147 \times 10^{-13}$) was found between the groups. The group with the highest rate was group 2 (6–15 oocytes), followed by group 3 (≥ 16 oocytes) and finally group 1 (≤ 5 oocytes).

A significant positive correlation ($r = 1$; $P = 0.03$) was found between blastulation rates and the number of retrieved oocytes (Table 1). As seen in Table 1, there were no extended cultures on day 5 in group 1, which was due to the low numbers of oocytes and day 3 embryos in that group.

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Table 1. Comparison of maturation rates, fertilization and 8-cell embryos on day 3 according to the number of retrieved oocytes

	Group 1: (≤5 oocytes) (*N* = 966)	Group 2: (6–15 oocytes) (*N* = 877)	Group 3: (≥16 oocytes) (*N* = 144)	Coefficient of correlation	*P*-value
Maturation rate (%)	78.2 ± 35.47	72.03 ± 20.01	70.14 ± 18.98	−0.95	4.79E-09
Fertilization rate (%)	77.25 ± 35.47	71.36 ± 23.31	66.42 ± 21.13	−0.99	1.03E-06
Rate of 8-cell embryos on day 3 (%)	73.04 ± 41.35	85.21 ± 23.70	79.26 ± 25.18	–	1.147E-13
Blastulation rate (%)	–	30.33 ± 35.10 (*n* = 446)	37.84 ± 31.13 (*n* = 116)	1	0.03

Values are reported as the mean ± standard deviation (SD).

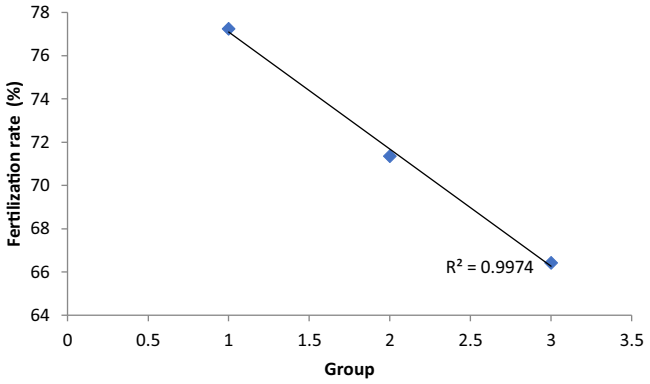

Figure 2. Association between the number of oocytes and the fertilization rate. Group 1: (≤5 oocytes); Group 2: (6–15 oocytes); Group 3: (≥16 oocytes).

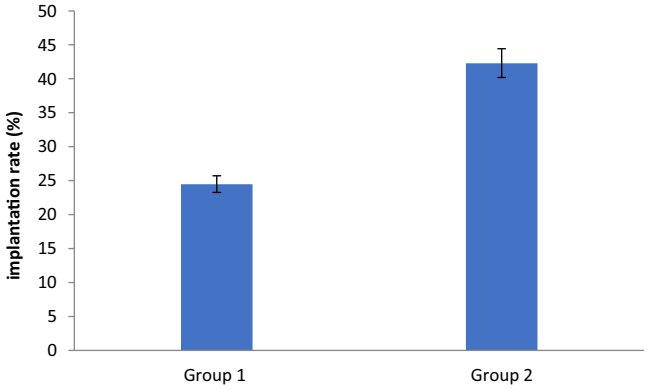

Figure 3. Association between the number of oocytes and the implantation rate. Group1: (≤5 oocytes); Group 2: (6–15 oocytes); Group 3: (≥16 oocytes).

Relationship between the number of retrieved oocytes and implantation rate after fresh day 3 embryo transfer

The implantation rates after fresh embryo transfer were 24.9% and 42.3% in groups 1 and 2, respectively (Figure 3). No implantation rate was included in group 3, all embryos were frozen. This group included a high cancelled cycle transfer rate due to ovarian hyperstimulation syndrome (OHSS).

Discussion

Ovarian stimulation is the important first step during IVF cycles and plays an important role in IVF outcomes. Many studies have been interested in the effect of the number of retrieved oocytes on implantation success, however many conflicting results have been found. In fact, some studies have suggested that the optimum number of oocytes to generate positive IVF outcomes can be ~6–10 oocytes (Sunkara *et al.*, 2011; Ji *et al.*, 2013; Steward *et al.*, 2014; Thaker *et al.*, 2020). Other researchers have shared that a higher number of retrieved oocytes increases the LBR (Zhou *et al.*, 2017), while other studies found that oocyte number did not significantly affect IVF outcomes (Briggs *et al.*, 2015). To date, only a few studies have analyzed the relationship between maturation, fertilization, as well as embryo quality and oocyte number (Sigala *et al.*, 2015; Decanter, 2018; Lin *et al.*, 2018; Agrawal *et al.*, 2020; Nikbakht *et al.*, 2021). Our data have shown a significant association between retrieved oocytes per patient and IVF success, from oocyte maturation to implantation.

Initially, our results showed a significant negative correlation between the number of retrieved oocytes and the rate of maturation and fertilization. In line with our findings, many studies have found a lower fertilization rate and lower oocyte quality in patients with high oocyte numbers. As most of these patients were known to have polycystic ovary syndrome (PCOS) with high AMH levels, the alteration in oocyte quality can be explained by an increased expression of reactive oxygen species (ROS) levels by granulosa cells (GCs) and follicular fluid (Arya *et al.*, 2012; Yilmaz *et al.*, 2016; Lai *et al.*, 2018; Sun *et al.*, 2021). The high oocyte number resulting from excessive follicle number is associated with folliculogenesis disturbance that is known to be a consequence of PCOS (Jonard and Dewailly, 2004; Homburg, 2009).

The metabolic activity of GCs is related to oocyte competence and development from maturity to implantation (Uyar *et al.*, 2013; Huang *et al.*, 2015; Lai *et al.*, 2018) through the cross-talk between oocyte and GCs (Rienzi *et al.*, 2012; Decanter, 2018). Intact GCs already produce a considerable amount of ROS by electron transport, which is maintained by enzyme antioxidants (Behl and Pandey, 2002; Migdal and Serres, 2011). In PCOS patients, the percentage of ROS in GCs has been shown to increase by 20-fold compared with the normal rate (Das *et al.*, 2008; Lai *et al.*, 2018), which can alter DNA and mitochondrial metabolism prior to the inadequate dialogue between GCs, cumulus cells (CC) and oocytes (Kenigsberg *et al.*, 2009; Kwon *et al.*, 2010). All this cellular disturbance can lead to high rates of immature oocytes, as well as a significantly low rate of fertilization and embryonic development (Plachot *et al.*, 2003; Dumesic and Abbott, 2008; Homburg, 2009; Qiao and Feng, 2011; Zhang *et al.*, 2017; Lai *et al.*, 2018; Jamil *et al.*, 2020). In accordance with these studies, our highest fertilization and maturation rates were observed in group 1 (1–5 oocytes). Moreover, some studies have shown that poorer ovarian responses can be due to altered mitochondrial RNA (mit-RNA) expression in CC and can also lead to negative oocyte and embryo quality (Karakaya *et al.*, 2015; Yin *et al.*, 2019). However, the poor ovarian response usually includes some oocytes that are less than or equal to two oocytes. In our study, in the group including

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1–5 oocytes, not all patients had abnormally low AMH levels, and therefore had a better chance of higher quality oocyte competence (Cohen *et al.*, 2018).

Although in our data, fertilization and maturation rates were the highest in group 1 (≤ 5 oocytes), the rate of 8-cell embryos on day 3 was the highest in group 2 (6–15 oocytes).

This interesting result can be explained by the reduced embryo quality resulting from mit-RNA deletions in CC. According to Gross *et al.* (2017), the role of maternal miRNA in embryo development is still unclear, but they might play a role in producing 'robust' embryos.

In addition, Thaker and colleagues showed that lipid peroxidation was at an optimum level in the 6–10 oocyte group, compared with lower and higher retrieved oocyte number, which can confirm the importance of maintaining a balance during ovarian stimulations. Even though the physiological basis for the association between AMH levels and embryo quality is still not well understood (Melado Vidales *et al.*, 2017; Shrikhande *et al.*, 2020), it is worth mentioning that an optimum AMH level results in an optimum oocyte number that can result in a higher quality embryo with lower OHSS risk.

In group 3 (≥16 oocytes), although the rate of 8-cell embryos on day 3 embryos was not the highest, it appeared to be higher compared with group 1. This can be explained by the higher number of oocytes that were prone to produce 8-cell embryos. In fact, when comparing embryo quality between PCOS patients and the control group, Fernández-González and colleagues (2019) found no significant difference between the two groups. However, the number of retrieved oocytes in the PCOS group was higher.

A high rate of 8-cell embryos on day 3 is considered a more promising result compared with lower rates, with the exception of aneuploidy, in which morphological characteristics are not a significant predictor of conception (Dolgushina *et al.*, 2015; Minasi *et al.*, 2016; Lee *et al.*, 2019; Munné *et al.*, 2019). Moreover, it has been shown that embryo morphology can help to increase the percentage of chromosomally normal embryos (Ziebe *et al.*, 2007). According to our data, as well as all the previous studies mentioned, we can confirm that, for a higher quality embryo competence, in terms of development and kinetics, an optimum number of retrieved oocytes can be adequate. The rate of fertilization and maturation is a very important factor in IVF success, however embryo kinetics and the subsequent cleavage quality in time are more impactful for quality embryo transfers and also cryopreservation.

For blastulation, we could not establish a rate for group 1 due to the low rate of prolonged culture to day 5 for such a low number of oocytes. Nevertheless, the blastulation rate of group 3 was significantly higher when compared with group 2. Cycle characteristics alone may not be an accurate predictor for the rate of blastulation, as there have been many conflicting results reported in previous literature (Jones *et al.*, 2020). It has been shown that better quality blastocysts are mostly generated by oocytes originating from a follicular volume of between 13 and 23 mm (Agrawal *et al.*, 2020). However, smaller follicles still have the capacity to produce blastocysts, which increases their number for higher chances of implantation. Most importantly, 8-cell embryos on day 3 can increase the rate of blastocyst formation, however embryos with a greater cell number on day 3 are not always predictive of a greater likelihood of blastocysts. In fact, 6–7-cell embryos may also result in blastulation, as Langley *et al.* (2001) have previously shown that 54% of 6-cell embryos formed blastocysts on day 5. Simply put, a large number of embryos in extended culture can increase the chances of obtaining blastocysts on day 5, irrespective of whether the corresponding day 3 embryos are of better quality (seven or eight cells) or lower quality (six cells) (Graham *et al.*, 2000; Langley *et al.*, 2001). This might explain the higher rate of blastulation in group 3 compared with group 2, regardless of the higher quality of day 3 embryos in group 2.

Regarding the most important indicator of IVF success, in our data, we noted that the implantation rate after the fresh transfer of day 3 embryos doubled from group 1 to group 2 (24–42%). Group 3 was not included in the implantation rates due to a lack of fresh embryo transfers in this group, as all transfers from OHSS are postponed to other cycles. The same trends in the results were observed with a higher LBR in oocyte retrievals of 10–14 oocytes (Zhou *et al.*, 2017). The higher rate of implantation in group 2 can be explained by the concordance with the highest rate of 8-cell embryos on day 3 in the same group. During our DET policy, the chances of transferring two 8-cell embryos on day 3 were higher in cultures that had a higher number of 8-cell embryos. In group 1 (≤ 5 oocytes), the rate of 8-cell embryos on day 3 was quite acceptable, nevertheless DET may involve two 8-cell embryos or it may also involve only one 8-cell embryo paired with another embryo with a lesser number of cells. As there are more good quality embryos available in group 2 compared with group 1, we can speculate that it might be one of a variety of factors that could affect the chances of implantation. A high number of retrieved oocytes may not necessarily mean a higher chance of implantation, however a high number of good quality embryos can increase the chances of implantation (Bosch *et al.*, 2016; Lin *et al.*, 2018; Polyzos *et al.*, 2018).

To conclude, our results showed that the quality of embryo cleavage plays an important role in IVF outcomes, as the quality of retrieved oocytes can be more impactful than their number. Also, some oocytes between 6 and 15 oocytes can result in the highest chances of positive IVF outcomes in terms of embryo quality and fresh embryo transfers with lower risks of ovarian hyperstimulation.

Conflict of interest. The authors declare that they have no conflict of interest.

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References

Agrawal, S., Panchal, S. and Nagori, C. (2020). Relationship between follicular volume, oocyte competence, and blastocyst development in ART. *Donald School Journal of Ultrasound in Obstetrics and Gynecology*, **14**(2), 136–143. doi: 10.5005/jp-journals-10009-1636

Arya, B. K., Haq, A. U. and Chaudhury, K. (2012). Oocyte quality reflected by follicular fluid analysis in poly cystic ovary syndrome (PCOS): a hypothesis based on intermediates of energy metabolism. *Medical Hypotheses*, **78**(4), 475–478. doi: 10.1016/j.mehy.2012.01.009

Behl, R. and Pandey, R. S. (2002). FSH induced stimulation of catalase activity in goat granulosa cells *in vitro*. *Animal Reproduction Science*, **70**(3–4), 215–221. doi: 10.1016/s0378-4320(02)00006-4

Biljan, M. M., Buckett, W. M., Dean, N., Phillips, S. J. and Tan, S. L. (2000). The outcome of IVF-embryo transfer treatment in patients who develop three follicles or less. *Human Reproduction*, **15**(10), 2140–2144. doi: 10.1093/humrep/15.10.2140

Bosch, E., Labarta, E., Kolibianakis, E., Rosen, M. and Meldrum, D. (2016). Regimen of ovarian stimulation affects oocyte and therefore embryo quality. *Fertility and Sterility*, **105**(3), 560–570. doi: 10.1016/j.fertnstert.2016.01.022

Briggs, R., Kovacs, G., MacLachlan, V., Motteram, C. and Baker, H. W. G. (2015). Can you ever collect too many oocytes? *Human Reproduction*, **30**(1), 81–87. doi: 10.1093/humrep/deu272

Cai, Q., Wan, F., Huang, K. and Zhang, Hanwang. (2013). Does the number of oocytes retrieved influence pregnancy after fresh embryo transfer? *PLOS ONE*, **8**(2), e56189. doi: 10.1371/journal.pone.0056189

Cohen, Y., Tannus, Samer, Alzawawi, N., Son, W. Y., Dahan, M. and Buckett, W. (2018). Poor ovarian response as a predictor for live birth in older women undergoing IVF. *Reproductive Biomedicine Online*, **36**(4), 435–441. doi: 10.1016/j.rbmo.2018.01.008

Das, M., Djahanbakhch, O., Hacihanefioglu, B., Saridogan, E., Ikram, M., Ghali, L., Raveendran, M. and Storey, A. (2008). Granulosa cell survival and proliferation are altered in polycystic ovary syndrome. *Journal of Clinical Endocrinology and Metabolism*, **93**(3), 881–887. doi: 10.1210/jc.2007-1650

De Sutter, P. and Dhont, M. (2003). Poor response after hormonal stimulation for *in vitro* fertilization is not related to ovarian aging. *Fertility and Sterility*, **79**(6), 1294–1298. doi: 10.1016/s0015-0282(03)00264-4

Decanter, C. (2018). Oocyte quality in PCOS. In: Palomba, S. (eds) *Infertility in Women with Polycystic Ovary Syndrome*. Springer, Cham. Available at https://doi.org/10.1007/978-3-319-45534-1_4

Dolgushina, N. V., Syrkasheva, A. G., Makarova, N. P., Kovalskaya, E. V., Kalinina, E. A. and Sukhikh, G. T. (2015). Correlation between oocyte morphology and the embryo aneuploidy rate in IVF cycles. *Gynecological Endocrinology*, **31**(Suppl. 1), 61–64. doi: 10.3109/09513590.2015.1086511

Dumesic, D. A. and Abbott, D. H. (2008). Implications of polycystic ovary syndrome on oocyte development. *Seminars in Reproductive Medicine*, **26**(1), 53–61. doi: 10.1055/s-2007-992925

Fernández-González, R., Laguna, R., Ramos-Ibeas, P., Pericuesta, E., Alcalde-Lopez, V., Perez-Cerezales, S. and Gutierrez-Adan, A. (2019). Successful ICSI in mice using caput epididymal spermatozoa. *Frontiers in Cell and Developmental Biology*, **7**, 346. doi: 10.3389/fcell.2019.00346

Graham, J., Han, T., Porter, R., Levy, M., Stillman, R. and Tucker, M. J. (2000). Day 3 morphology is a poor predictor of blastocyst quality in extended culture. *Fertility and Sterility*, **74**(3), 495–497. doi: 10.1016/s0015-0282(00)00689-0

Gross, N., Kropp, J. and Khatib, H. (2017). MicroRNA signaling in embryo development. *Biology*, **6**(3), 34. doi: 10.3390/biology6030034

Hendriks, D. J., Te Velde, E. R., Looman, C. W. N., Bancsi, L. F. J. M. M. and Broekmans, F. J. M. (2008). Expected poor ovarian response in predicting cumulative pregnancy rates: A powerful tool. *Reproductive Biomedicine Online*, **17**(5), 727–736. doi: 10.1016/s1472-6483(10)60323-9

Homburg, R. (2009). Androgen circle of polycystic ovary syndrome. *Human Reproduction*, **24**(7), 1548–1555. doi: 10.1093/humrep/dep049

Huang, B., Qian, K., Li, Z., Yue, J., Yang, W., Zhu, G. and Zhang, Hanwang. (2015). Neonatal outcomes after early rescue intracytoplasmic sperm injection: An analysis of a 5-year period. *Fertility and Sterility*, **103**(6), 1432–7.e1. doi: 10.1016/j.fertnstert.2015.02.026

Jamil, M., Debbarh, H., Aboulmaouahib, S., Aniq Filali, O., Mounaji, K., Zarqaoui, M., Saadani, B., Louanjli, N. and Cadi, R. (2020). Reactive oxygen species in reproduction: Harmful, essential or both? *Zygote*, **28**(4), 255–269. doi: 10.1017/S0967199420000179

Ji, J., Liu, Y., Tong, X. H., Luo, L., Ma, J. and Chen, Z. (2013). The optimum number of oocytes in IVF treatment: An analysis of 2455 cycles in China. *Human Reproduction*, **28**(10), 2728–2734. doi: 10.1093/humrep/det303

Jonard, S. and Dewailly, D. (2004). The follicular excess in polycystic ovaries, due to intra-ovarian hyperandrogenism, may be the main culprit for the follicular arrest. *Human Reproduction Update*, **10**(2), 107–117. doi: 10.1093/humupd/dmh010

Jones, C. A., Acharya, K. S., Acharya, C. R., Raburn, D. and Muasher, S. J. (2020). Patient and *in vitro* fertilization (IVF) cycle characteristics associated with variable blastulation rates: A retrospective study from the Duke Fertility Center (2013–2017). *Middle East Fertility Society Journal*, **24**(1), 2–7. doi: 10.1186/s43043-019-0004-z

Karakaya, C., Guzeloglu-Kayisli, O., Uyar, A., Kallen, A. N., Babayev, E., Bozkurt, N., Unsal, E., Karabacak, O. and Seli, E. (2015). Poor ovarian response in women undergoing *in vitro* fertilization is associated with altered microRNA expression in cumulus cells. *Fertility and Sterility*, **103**(6), 1469–76.e1. doi: 10.1016/j.fertnstert.2015.02.035

Kenigsberg, S., Bentov, Y., Chalifa-Caspi, V., Potashnik, G., Ofir, R. and Birk, O. S. (2009). Gene expression microarray profiles of cumulus cells in lean and overweight-obese polycystic ovary syndrome patients. *Molecular Human Reproduction*, **15**(2), 89–103. doi: 10.1093/molehr/gan082

Klinkert, E. R., Broekmans, F. J. M., Looman, C. W. N. and Te Velde, E. R. (2004). A poor response in the first *in vitro* fertilization cycle is not necessarily related to a poor prognosis in subsequent cycles. *Fertility and Sterility*, **81**(5), 1247–1253. doi: 10.1016/j.fertnstert.2003.10.030

Kwon, H., Choi, D. H., Bae, J. H., Kim, J. H. and Kim, Y. S. (2010). MRNA expression pattern of insulin-like growth factor components of granulosa cells and cumulus cells in women with and without polycystic ovary syndrome according to oocyte maturity. *Fertility and Sterility*, **94**(6), 2417–2420. doi: 10.1016/j.fertnstert.2010.03.053

Lai, Q., Xiang, W., Li, Q., Zhang, H., Li, Y., Zhu, G., Xiong, C. and Jin, L. (2018). Oxidative stress in granulosa cells contributes to poor oocyte quality and IVF-ET outcomes in women with polycystic ovary syndrome. *Frontiers of Medicine*, **12**(5), 518–524. doi: 10.1007/s11684-017-0575-y.

Langley, M. T., Marek, D. M., Gardner, D. K., Doody, K. M. and Doody, K. J. (2001). Extended embryo culture in human assisted reproduction treatments. *Human Reproduction*, **16**(5), 902–908. doi: 10.1093/humrep/16.5.902

Lee, C. I., Chen, C. H., Huang, C. C., Cheng, E. H., Chen, H. H., Ho, S. T., Lin, P.-Y. Y., Lee, M. S. and Lee, T. H. (2019). Embryo morphokinetics is potentially associated with clinical outcomes of single-embryo transfers in preimplantation genetic testing for aneuploidy cycles. *Reproductive Biomedicine Online*, **39**(4), 569–579. doi: 10.1016/j.rbmo.2019.05.020

Lin, L. T., Vitale, S. G., Chen, S. N., Wen, Z. H., Tsai, H. W., Chern, C. U. and Tsui, K. H. (2018). Luteal phase ovarian stimulation may improve oocyte retrieval and oocyte quality in poor ovarian responders undergoing *in vitro* fertilization: Preliminary results from a single-center prospective pilot study. *Advances in Therapy*, **35**(6), 847–856. doi: 10.1007/s12325-018-0713-1

Macklon, N. S., Stouffer, R. L., Giudice, L. C. and Fauser, B. C. J. M. (2006). The science behind 25 years of ovarian stimulation for *in vitro* fertilization. *Endocrine Reviews*, **27**(2), 170–207. doi: 10.1210/er.2005-0015

Melado Vidales, L., Fernández-Nistal, A., Martínez Fernández, V., Verdú Merino, V., Bruna Catalán, I. and Bajo Arenas, J. M. (2017) Anti-Müllerian hormone levels to predict oocyte maturity and embryo quality during controlled ovarian hyperstimulation. *Minerva Ginecologica*, **69**(3), 225–232. doi: 10.23736/S0026-4784.16.03958-7

Migdal, C. and Serres, M. (2011). Reactive oxygen species and oxidative stress. *Medecine Sciences*, **27**(4), 405–412. doi: 10.1051/medsci/2011274017.

Minasi, M. G., Colasante, A., Riccio, T., Ruberti, A., Casciani, V., Scarselli, F., Spinella, F., Fiorentino, F., Varricchio, M. T. and Greco, E. (2016). Correlation between aneuploidy, standard morphology evaluation and morphokinetic development in 1730 biopsied blastocysts: A consecutive case series study. *Human Reproduction*, **31**(10), 2245–2254. doi: 10.1093/humrep/dew183

Munné, S., Kaplan, B., Frattarelli, J. L., Child, T., Nakhuda, G., Shamma, F. N., Silverberg, K., Kalista, T., Handyside, A. H., Katz-Jaffe, M., Wells, D., Gordon, T., Stock-Myer, S., Willman, S. and STAR Study Group. (2019). Preimplantation genetic testing for aneuploidy versus morphology as selection criteria for single frozen–thawed embryo transfer in good-prognosis patients: a multicenter randomized clinical trial. *Fertility and Sterility*, **112**(6), 1071–1079.e7. doi: 10.1016/j.fertnstert.2019.07.1346

Nikbakht, R., Mohammadjafari, R., Rajabalipour, M. and Moghadam, M. T. (2021). Evaluation of oocyte quality in polycystic ovary syndrome patients undergoing ART cycles. *Fertility Research and Practice*, **7**(1), 2. doi: 10.1186/s40738-020-00094-z

Plachot, M., Belaisch-Allart, J., Mayenga, J. M., Chouraqui, A., Tesquier, A., Serkine, A. M., Boujenah, A. and Abirached, F. (2003). Oocyte and embryo quality in polycystic ovary syndrome. *Gynecologie, Obstetrique et Fertilite*, **31**(4), 350–354. doi: 10.1016/s1297-9589(03)00059-6

Polyzos, N. P., Drakopoulos, P., Parra, J., Pellicer, A., Santos-Ribeiro, S., Tournaye, H., Bosch, E. and Garcia-Velasco, J. (2018). Cumulative live

birth rates according to the number of oocytes retrieved after the first ovarian stimulation for *in vitro* fertilization/intracytoplasmic sperm injection: a multicenter multinational analysis including ~15,000 women. *Fertility and Sterility*, **110**(4), 661–670.e1. doi: 10.1016/j.fertnstert.2018.04.039

Qiao, J. and Feng, H. L. (2011). Extra- and intra-ovarian factors in polycystic ovary syndrome: Impact on oocyte maturation and embryo developmental competence. *Human Reproduction Update*, **17**(1), 17–33. doi: 10.1093/humupd/dmq032

Rienzi, L., Balaban, B., Ebner, T. and Mandelbaum, J. (2012). The oocyte. *Human Reproduction*, **27**(Suppl. 1), i2–21. doi: 10.1093/humrep/des200.

Shrikhande, L., Shrikhande, B. and Shrikhande, A. (2020). AMH and its clinical implications. *Journal of Obstetrics and Gynaecology of India*, **70**(5), 337–341. doi: 10.1007/s13224-020-01362-0

Sigala, J., Sifer, C., Dewailly, D., Robin, G., Bruyneel, Aude, Ramdane, N., Lefebvre-Khalil, V., Mitchell, V. and Decanter, C. (2015). Is polycystic ovarian morphology related to a poor oocyte quality after controlled ovarian hyperstimulation for intracytoplasmic sperm injection? Results from a prospective, comparative study. *Fertility and Sterility*, **103**(1), 112–118. doi: 10.1016/j.fertnstert.2014.09.040

Steward, R. G., Lan, L., Shah, A. A., Yeh, J. S., Price, T. M., Goldfarb, J. M. and Muasher, S. J. (2014). Oocyte number as a predictor for ovarian hyperstimulation syndrome and live birth: An analysis of 256,381 *in vitro* fertilization cycles. *Fertility and Sterility*, **101**(4), 967–973. doi: 10.1016/j.fertnstert.2013.12.026

Sun, B., Ma, Y., Li, L., Hu, L., Wang, F., Zhang, Y., Dai, S. and Sun, Y. (2021). Factors associated with ovarian hyperstimulation syndrome (OHSS) severity in women with polycystic ovary syndrome undergoing IVF/ICSI. *Frontiers in Endocrinology*, **11**, 615957. doi: 10.3389/fendo.2020.615957.

Sunkara, S. K., Rittenberg, V., Raine-Fenning, N., Bhattacharya, S., Zamora, J. and Coomarasamy, A. (2011). Association between the number of eggs and live birth in IVF treatment: an analysis of 400 135 treatment cycles. *Human Reproduction*, **26**(7), 1768–1774. doi: 10.1093/humrep/der106

Thaker, R., Mishra, V., Gor, M., Agarwal, R., Sheth, H., Kapadia, P. and Kumar, S. (2020). The role of stimulation protocol, number of oocytes retrieved with respect to follicular fluid oxidative stress and IVF outcome. *Human Fertility*, **23**(1), 23–31. doi: 10.1080/14647273.2018.1551630

Timeva, T., Milachich, T., Antonova, I., Arabaji, T., Shterev, A. and Omar, H. A. (2006). Correlation between number of retrieved oocytes and pregnancy rate after *in vitro* fertilization/intracytoplasmic sperm infection. *TheScientificWorldJournal*, **6**, 686–690. doi: 10.1100/tsw.2006.145

Uyar, A., Torrealday, S. and Seli, E. (2013). Cumulus and granulosa cell markers of oocyte and embryo quality. *Fertility and Sterility*, **99**(4), 979–997. doi: 10.1016/j.fertnstert.2013.01.129

Vermey, B. G., Chua, S. J., Zafarmand, M. H., Wang, R., Longobardi, S., Cottell, E., Beckers, F., Mol, B. W., Venetis, C. A. and D'Hooghe, T. (2019). Is there an association between oocyte number and embryo quality? A systematic review and meta-analysis. *Reproductive Biomedicine Online*, **39**(5), 751–763. doi: 10.1016/j.rbmo.2019.06.013

Yilmaz, N., Inal, H. A., Gorkem, U., Sargin Oruc, A., Yilmaz, S. and Turkkani, A. (2016). Follicular fluid total antioxidant capacity levels in PCOS. *Journal of Obstetrics and Gynaecology*, **36**(5), 654–657. doi: 10.3109/01443615.2016.1148683

Yin, H., Jiang, H., He, R., Wang, C., Zhu, J. and Cao, Z. (2019). Cumulative live birth rate of advanced-age women more than 40 with or without poor ovarian response. *Taiwanese Journal of Obstetrics and Gynecology*, **58**(2), 201–205. doi: 10.1016/j.tjog.2019.01.006

Zhang, Y., Liu, L., Yin, T. L., Yang, J. and Xiong, C. L. (2017). Follicular metabolic changes and effects on oocyte quality in polycystic ovary syndrome patients. *Oncotarget*, **8**(46), 80472–80480. doi: 10.18632/oncotarget.19058

Zhen, X. M., Qiao, J., Li, R., Wang, L. N. and Liu, P. (2008). The clinical analysis of poor ovarian response in in-vitro-fertilization embryo-transfer among Chinese couples. *Journal of Assisted Reproduction and Genetics*, **25**(1), 17–22. doi: 10.1007/s10815-007-9187-9

Zhou, J., Wang, B., Hu, Y. and Sun, H. (2017). Association between the number of oocytes retrieved and cumulative live birth rate in women aged 35–40 years undergoing long GnRH agonist IVF/ICSI cycles. *Archives of Gynecology and Obstetrics*, **296**(5), 1005–1012. doi: 10.1007/s00404-017-4503-9

Ziebe, S., Lundin, K., Janssens, R., Helmgaard, L., Arce, J. C. and MERIT (Menotrophin vs Recombinant FSH In Vitro Fertilisation Trial) Group. (2007). Influence of ovarian stimulation with HP-HMG or recombinant FSH on embryo quality parameters in patients undergoing IVF. *Human Reproduction*, **22**(9), 2404–2413. doi: 10.1093/humrep/dem221

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