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Is early embryo cleavage a factor to increase success in all types of ICSI indications?

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Summary

Purpose: The authors aimed to compare early embryo cleavage with pregnancy rates in intracytoplasmic sperm injection/embryo transfer (ICSI/ET) cycles due to male infertility or tubal factor infertility (TFI). *Materials and Methods:* 412 ICSI/embryo transfer cycles undergoing treatment for over two years were prospectively analyzed; 337 of the cycles were due to male infertility, whereas 75 suffered from tubal factors. Non-early cleaved (NEC) embryos were used for ET in 271 male factor and 67 tubal factor cycles, whereas early cleavage embryos were used for embryo transfer in 66 male factor and eight tubal factor cycles. *Results*: In 66 out of 337 cycles (19.58 %) in male factor group and in eight out of 75 tubal factor cycles (10.66%), early cleavage (EC) embryos were obtained (p = 0.069). The clinical pregnancy rate was significantly elevated in EC subgroup (34.8%) compared to NEC subgroup (20.6%) (p = 0.015) in the male factor infertility group. The clinical pregnancy rate was non-significantly elevated in EC subgroup (37.5%) compared to NEC subgroup (23.8%) (p = 0.410) in the TFI group. *Conclusions:* The authors found that the implantation and pregnancy success of EC embryos vary with the therapeutic indication. The success rate would be low even with usage of EC embryos in untreated cycles of TFI.

Key words: Early cleavage; Tubal factor infertility; Male factor infertility; Pregnancy rate.

Introduction

In recent years, selection of the embryo with the highest chance of implantation success has become one of the most favorite topics in intracytoplasmic sperm injection (ICSI) studies. Several reports have indicated that embryos showing early cleavage (EC) at 25-26 hours after microinjection have higher viability and implantation rates [1-4]. EC has also shown to be important for prediction of pregnancy rates [5, 6]. The study by Edwards *et al.* [7] was one of the earliest publications to report approximately 30% higher rates of implantation in EC embryos compared to non-early cleaved (NEC) ones. In many studies, EC embryos were reported to have better biochemical pregnancy and clinical pregnancy rates [8,9].

Single embryo transfer (ET), in particular, needs special attention on the evaluation of all clinical and laboratory related factors that could influence the development of embryo directly and obliges to device selection criterion that the chosen embryos should possess the highest implantation potential [10,11].

Although various classifications based on the morphology of embryos at different stages of development may point to the best appearing embryo, but the clinical settings, in which these embryos develop, are poorly understood [12, 13].

To the best of the present authors' knowledge, there are no studies that relate EC cycles with pregnancy results regarding specific intracytoplasmic sperm injection (ICSI) indications. The environment of oocytes which will eventually contribute to formation of EC embryos is extremely critical, as does the conditions nearby the implantation area. The causes of tubal factor related infertility, such as tubal dysfunction and tubal obstruction, decrease successful pregnancy by disrupting the growth of the oocyte, cleavage of embryos and implantation [14-16]. In tubal factor infertility (TFI) cycles, the conditions that cause the tubal factor itself also influence the success of treatment with ICSI/ET.

This study was designed to investigate the advantages of using EC embryos to achieve implantation and pregnancy in various indications of ICSI/ET cycles. An additional focus was established on the comparative developmental rate of EC embryos in tubal and male factor infertility, concerning the fact that TFI patients have an undesirable ovarian environment.

Materials and Methods

The study has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of

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Table 1. — *Cycle characteristics and outcomes in the groups.*

Parameters n	Male EC 66	Male NEC 271	Tubal EC 8	Tubal NEC 67
Age	32.10 ± 4.32	32.9 ± 4.5	31.0 ± 5.3	32.4 ± 4.0
Duration of infertility (years)	9.3 ± 5.3	9.2 ± 5.2	6.5 ± 6.0	8.3 ± 5.3
Day 3 FSH (mIU/mL)	6.9 ± 2.3	7.0 ± 2.5	7.8 ± 3.7	7.5 ± 2.7
Day 3 LH (mIU/mL)[a]	4.8 ± 2.0	4.6 ± 2.2	4.5 ± 2.0	5.0 ± 2.0
Day 3 E2 (pg/mL)[b]	38.6 ± 15.4	40.2 ± 20.0	39.5 ± 17.4	32.3 ± 17.9
Day 3 PRL	15.9 ± 5.2	15.9 ± 6.7	12.7 ± 3.5	15.4 ± 5.7
Day 3 TSH	1.8 ± 0.1	1.7 ± 0.1	1.4 ± 0.4	1.8 ± 0.9
BMI (kg/m²)[c]	25.9 ± 4.2	25.3 ± 4.0	24.5 ± 4.4	25.1 ± 4.3
Total gonadotropin dose, IU	2314.8 ± 806.6	2457.9 ± 968.2	2715.6 ± 980.0	2461.9 ± 951.9
Stimulation day	8.6 ± 1.5	8.8 ± 1.7	9.2 ± 1.3	8.6 ± 1.7
Endometrium thickness on hCG day (mm)	9.5 ± 1.9	9.4 ± 1.9	9.2 ± 2.3	9.3 ± 1.8
Mean no. of oocytes (metaphase II) retrieved	7.4 ± 3.5	6.1 ± 3.7	5.3 ± 2.1	6.6 ± 4.1
Number of cleavage embryos	5.6 ± 2.4	3.9 ± 2.7	5.0 ± 2.2	5.0 ± 3.7
Fertilization rate (%)	79.2 ± 18.9	67.5 ± 26.6	92.8 ± 10.4	77.1 ± 23.9
Number of grade 1 embryos transferred/cycle	1.8 ± 0.8	1.7 ± 0.8	1.5 ± 0.5	1.6 ± 0.8
Number of grade 2 embryos transferred /cycle	1.5 ± 0.6	1.7 ± 0.6	1.3 ± 0.5	1.8 ± 0.6
Number of transferred embryos/ ET	2.9 ± 0.7	2.7 ± 1.0	2.8 ± 0.6	2.9 ± 1.0
Implantation rate (%)	47.8	40.9	36.4	39.7
Biochemical pregnancy, n (%)	6/66 (9.0)	14/271 (5.1)	0/8 (0.0)	2/67 (2.98)
Clinical pregnancy rate, n (%)	23/66 (34.8)	56/271 (20.6)	3/8 (37.5)	16/67 (23.8)

Data are expressed as mean ±SD, unless otherwise indicated. [a] LH: luteinizing hormone; [b]E2: estradiol; [c] BMI: body mass index.

Helsinki) for experiments involving human and the approval was granted by the local ethics committee. In this single-center study, 412 ICSI cycles, carried out between 2005 and 2007 in Istanbul Cerrahpaşa University School of Medicine, Department of Reproductive Endocrinology, were analyzed prospectively. Seven patient were excluded due to lack of informed consent (n=3) and follow up loss (n=4). Among them, 337 of the cycles were treated for male factors [17] and 75 for tubal infertility. Only the ones verified with negative laparoscopic chromopertubation cases were included in tubal infertility. Only NEC embryos were used for ET in 271 male factor and 67 tubal factor cycles, whereas only EC embryos were used for ET in 66 male factor and eight tubal factor cycles. The inclusion criteria were as follows: 1) women aged below 39 years; 2) successful imaging of both ovaries by transvaginal ultrasonograhy (USG); 3) no history of previous ovarian surgery; 4) normal prolactin (PRL) and thyroid stimulating hormone (TSH) levels; and 5) follicle stimulating hormone (FSH) < 15 mIU/mL. The exclusion criteria were as follows: 1) history of systemic or pelvic disease that affects the ovaries; 2) history of pelvic irradiation; 3) history of cytotoxic treatments; and 4) hormonal therapy in the last six months before treatment.

The 66 EC cycles were compared with 271 NEC in male infertility group; and eight EC cycles were compared with 67 NEC ones in TFI group. EC rates were evaluated in male and TFI. The rate of pregnancy resulted by the use of EC or NEC subgroup embryos were determined and compared in tubal factor and male factor infertility groups individually.

The human chorionic gonadotropin (hCG) value > 5 mIU/ml on day 14 following the ET was accepted as a biochemical pregnancy, whereas the detection of fetal heart beat or the fetus itself on USG after four to five weeks of ET was accepted as a clinical pregnancy.

Stimulation protocols

Maturation of oocytes was achieved by administration of 10,000 units of human chorionic gonadotropin (hCG 10000 IU), when at least three dominant follicles (mean diameter 18 mm) were detected in women. These women were earlier administered gonadotropin releasing hormone (GnRH) agonist from day 21 of menstrual cycle causing a downregulation followed by reduction of GnRH dose to half and stimulation with gonadotropins. For ovarian stimulation, recombinant FSH (either follitropin-β or follitropin-α was used. Oocyte aspiration was performed after 36 hours. Fresh spermatozoa were used for ICSI and day 3 transfer was performed in each case.

Semen collection and preparation

Fresh semen collected by masturbation was prepared by density gradient centrifugation method. Liquefied semen was layered onto gradient solution and centrifuged at 1,500 rpm for ten minutes. The pellet was then washed and centrifuged twice at 1,500 rpm for ten minutes each time in a physiological salt based solution (EBSS) supplemented with 2% human serum albumin.

Embryo culture and transfer

After retrieval, oocytes were placed in human tubal fluid and cultured for two to four hours (day 0). After ICSI, embryo culture was performed at 37°C in 5% CO_2 incubators. Fertilization was assessed 16-18 hours after the ICSI procedure. Fertilized oocytes were then cultured for 48 hours in 30 µL of cleavage stage culture medium each having two embryos. On day 3, culture medium was replaced with fresh medium and just before ET; embryos were placed in blastocyst stage culture medium.

Intrauterine ET were performed under abdominal USG (five MHz). Soft transfer catheters were used to transfer the embryos. All the subjects were administered 50 mg intramuscular progesterone in oil for luteal phase support. On the second and third days of cultivation, embryos were evaluated for their number and equality of blastomers as well as the degree of fragmentation according to Veeck's embryo grading scale [18].

Table 2. — *P-value in the cycle characteristics and outcomes in the groups.*

Parameters	Male EC/NEC	Tubal EC/NEC	Male EC/Tubal EC	Male NEC/Tubal NEC
Age	0.163	0.368	0.507	0.361
Duration of infertility (years)	0.878	0.364	0.165	0.218
Day 3 FSH (mIU/mL)	0.828	0.786	0.401	0.229
Day 3 LH (mIU/mL)	0.488	0.539	0.697	0.190
Day 3 E2 (pg/mL)	0.563	0.317	0.881	0.005
Day 3 PRL	0.924	0.251	0.140	0.657
Day 3 TSH	0.529	0.304	0.400	0.364
BMI (kg/m²)	0.295	0.715	0.376	0.773
Total gonadotropin dose, IU	0.268	0.480	0.199	0.976
Stimulation day	0.332	0.377	0.282	0.495
Endometrium thickness on hCG day (mm)	0.571	0.910	0.666	0.726
Mean no. of oocytes (metaphase II) retrieved	0.101	0.415	0.104	0.418
Number of cleavage embryos	0.000	0.948	0.460	0.004
Fertilization rate (%)	0.000	0.004	0.053	0.008
Number of grade 1 embryos transferred/cycle	0.223	0.695	0.241	0.573
Number of grade 2 embryos transferred/cycle	0.138	0.072	0.481	0.321
Number of transferred embryos/ET	0.077	0.807	0.789	0.123
Implantation rate (%)	0.319	0.560	0.086	0.885
Biochemical pregnancy, n (%)	0.226	1.000	1.000	0.747
Clinical pregnancy rate, n (%)	0.015	0.410	1.000	0.565

Determination of EC and selection of the embryo

First mitotic division was controlled at 25 to 26 hours after ICSI. Embryos that reached the two-cell stage during this interval were accepted as EC embryos. EC embryos were cultured in separate droplets and were selected to be transferred preferably. Embryos that bear intact nuclei without nuclear membrane breakdown during first mitotic division control at 25 to 26 hours after ICSI were considered as NEC embryos. Patients, who received only EC embryo, were considered in the EC group.

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (release 16.0). Normally distributed (Kolmogorov-Smirnov test) parametric variables were tested by independent Student's *t*-test. Non-normally distributed metric variables were analyzed by Mann-Whitney U-test. Chi-square or Fisher's exact test was used for comparison of proportions. $P < 0.05$ was considered as statistically significant.

Results

In 66 of 337 cycles (19.58 %) of male factor group and in eight of 75 tubal factor cycles (10.66%), EC embryos developed ($p = 0.012$). The clinical pregnancy rate was significantly elevated in EC subgroup (34.8%) compared to NEC subgroup (20.6%) ($p = 0.015$) in the male factor infertility group (Table 1). The biochemical pregnancy rates were also higher in the EC group (9.0%) than that were in NEC group (5.1%), with no statistical significance ($p = 0.226$). The clinical pregnancy rate was non-significantly elevated in EC subgroup (37.5%), compared to NEC subgroup (23.8%) ($p = 0.410$) in the TFI group (Table 2).

Although there was no significant difference between EC and NEC subgroups regarding the semen parameters in the male factor infertility (Table 3), the pregnancy rates were significantly higher in the male factor EC group.

In TFI group, no difference in sperm parameters was noticed between EC and NEC subgroups, although the fertilization rates were statistically significant in favor of EC group. There was no statistical difference between EC and NEC subgroups in TFI.

The sperm parameters which showed a statistically significant difference between EC subgroups of male and tubal factors group were not different after the sperm wash step (Table 3). Border-line significance determined about fertilization rates was found particularly in favor of tubal EC group. Nevertheless, this advantage in tubal NEC group was not reflected in the pregnancy rates

Except for the sperm count after sperm wash, the other parameters compared between male NEC and tubal NEC groups were significantly different in favor of TFI. This fact can explain the significant increase in fertilization rates in tubal group. However this advantage in tubal NEC group did not cause a significant difference regarding pregnancy.

Discussion

The authors compared early EC with pregnancy rates in ICSI/ ET cycles due to male infertility or TFI in a prospective clinical study to investigate the advantages of using EC embryos for implantation and pregnancy for various indications in ICSI/ET cycles and found that EC embryos were obtained in 66 of 337 cycles (19.58 %) in male factor group

Table 3. — *Semen analysis and p-value in the groups*.

Parameters	Male EC	Male NEC	Tubal EC	Tubal NEC
Sperm count [b]	40.0 ± 43.7	42.2 ± 38.4	73.8 ± 20.1	81.9 ± 40.7
Sperm motility [b]	24.8 ± 17.8	27.0 ± 16.9	40.6 ± 11.0	38.5 ± 38.3
Sperm morphology [c]	2.3 ± 3.1	2.7 ± 2.7	3.7 ± 2.1	4.0 ± 4.2
Sperm count [a]	13.6 ± 15.1	22.0 ± 15.1	19.0 ± 6.3	21.8 ± 16.6
Sperm motility [a]	76.6 ± 18.8	67.5 ± 22.4	76.2 ± 9.1	78.6 ± 15.0

	Male EC/NEC	Tubal EC/NEC	Male EC/Tubal EC	Male NEC/Tubal NEC
Sperm count [b]	0.752	0.367	0.038	0.000
Sperm motility [b]	0.455	0.880	0.021	0.001
Sperm morphology [c]	0.426	0.848	0.219	0.020
Sperm count [a]	0.481	0.361	0.340	0.978
Sperm motility [a]	0.058	0.530	0.960	0.000

[a] after wash; [b] before wash; [c] Kruger strict criteria. Sperm counts are evaluated in millions/mL; Sperm morphology and motility are in %.

and in eight out of 75 tubal factor cycles (10.66%), (p = 0.012). Moreover, the clinical pregnancy rate was significantly higher in EC subgroup (34.8%) compared to NEC subgroup (20.6%) (p = 0.015) in the male factor infertility group, whereas it was non-significantly higher in EC subgroup (37.5%) compared to NEC subgroup (23.8%) (p = 0.410) in the TFI group. Therefore these findings suggest that the implantation and pregnancy success of early EC embryos vary with the therapeutic indication and the success rate would be low even with usage of early cleavage embryos in untreated cycles of TFI.

In Turkey, it is a legal obligation to transfer only one embryo to a woman under 35 years of age since March 2010 in order to prevent multiple pregnancies. To select the embryo of the highest quality, standard criteria must be determined. In addition, treatable factors which can arise from both male and female subjects should be determined for their effect in the quality of the embryo produced.

In some earlier studies, mean number of embryos transferred has usually been documented without special emphasis on the embryo quality, even though the quality of embryo is of critical importance [10]. Both the groups showed no statistical difference with reference to embryo grades. On the other hand, higher pregnancy rates were observed in the EC subgroup, compared to NEC subgroup in the male factor group. None of the previous studies subclassified their findings in concordance with the causes of infertility [1-3, 5]. To the best of the present authors' knowledge, this is the first study to investigate EC embryo pregnancies classified based on causes of infertility.

In contrast to previous studies, higher pregnancy rates were not observed in the EC subgroup compared to NEC subgroup in the tubal factor group (p = 0.410)(Table 2).

TFI has been shown to arise due to tubal disturbances such as infections (Chlamydia trachomatis (CT) and pelvic tuberculosis), endometriosis, and hydrosalpinx; all of which have been shown to affect the ovarian reserve along with the oocyte and embryo development [14-16].

Infections by CT can cause irreversible damages and tubal occlusion leading to TFI which may result in declined implantation rates and spontaneous abortions [19, 20]. Better pregnancy outcomes are reported on the use of doxycycline in patients who have antibodies raised against recent or former CT infections [19-23].

In TFI patients who suffer from genital tuberculosis, ovaries are also affected causing a poor response to ICSI treatment. In infertile women with genital tuberculosis, tubal adhesions are the most common lesions leading to bilateral (60%) or unilateral (40%) tubal occlusions [14]. Genital tuberculosis reduces the ovarian reserve, which consequently decreases the number of oocytes retrieved, the number of embryos transferred and, eventually, the pregnancy rates [20]. In addition to tubal adherences and disruption in transport, endometriosis has negative effects on the ovarian reserve, number of oocytes retrieved, and cleavage rates in patients with TFI. Initiation of ICSI treatment upon completion of endometriosis treatment would boost the pregnancy rates remarkably [15].

Daftary *et al.* (2007) showed that the presence of endometrial HOXA10 is crucial for implantation. Furthermore, it has been documented that HOXA10 expression decreases in response to hydrosalpinx fluid, while salpingectomy increases endometrial HOXA10. Once diagnosed via USG, pre-IVF treatment of hydrosalpinx by salpingectomy improves the success of IVF therapy with increase in implantation and live birth rates [16, 24, 25]. Therefore, hydrosalpinx, as a cause of TFI, should be treated by salpingectomy before IVF/ICSI to achieve better implantation and live birth rates.

In earlier studies, the issues that were found to cause TFI provide an undesirable environment for acquisition of good quality oocytes and embryos. These factors, unless treated, would have a direct negative impact on im-

plantation and intrauterine embryo development. As documented in previous studies, the evidence that EC embryos have higher implantation and pregnancy rates over non-EC ones was also supported in the male factor group in this study. However the advantages of using EC embryos were not found to be true for the tubal factor group. The lower number of pregnancies in EC subgroup of tubal factor cycles necessitates the need for the further study in a wider context.

Paternal effect should also not be ignored in EC embryos, as sperm motility is important for selection. Sakkas *et al.*[1] claimed that, semen characteristics (sperm concentration, motility, and morphology) have no effect on pregnancy rates, as well as semen parameters important for fertilization success in IVF cycles, may not be valid for ICSI cycles. Hammadeh *et al.* [26] showed that in both IVF and ICSI cycles, there is a negative correlation between reactive oxygen species (ROS) and sperm parameters, as ROS concentrations are negatively correlated with fertilization rates. Many studies also claim that DNA fragmentation rates and sperm motility have negative correlations [27-29]. When poor semen samples are used, the probability of using sperms with DNA fragmentation increases. Hence, ICSI performed with those sperms can result in poor fertilization and/or cleavage rates [30].

EC has been studied in a multitude of studies, most recently by time lapse monitoring and showed to be of some significance in development potential, but as with many other single morphological and morphokinetic characteristics, this relationship is tentative and far from absolute, especially in tubal factor patients. In addition, the timing of pronuclei breakdown should be considered on embryo selection parameters [31, 32]. Second polar body extrusion, pronuclear fading, and length of S-phase can be used in a better embryo selection [33].

Due to the present study design, the fertilization rates were significantly higher in TFI group compared to male factor group. In addition, higher fertilization rates were achieved in tubal factor EC subgroup. Despite high fertilization rates, no significant difference was observed between pregnancy results of EC and non-EC subgroups in both male and tubal factor groups.

With no discrimination of specific ICSI indication, previous studies have shown that EC embryos have higher implantation and pregnancy rates. However, the negative effects of tubal factors on EC embryos as a specific ICSI treatment indication were never studied before. The present authors showed that compared to male factor infertility group in TFI group, less amount of EC embryos were obtained ($p = 0.012$). Furthermore, it is concluded that the use of EC embryos will not help successfully increase implantation and pregnancy rates, unless the negative effects of tubal factors are treated. It is recommended that in patients suffering from TFI, instead of opting for ICSI treatment alone, complementary management of tubal factor pathology can increase both the number of EC embryos obtained and successful implantation of those embryos, ultimately leading to successful pregnancy rates.

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References

[1] Sakkas D., Shoukir Y., Chardonnens D., Bianchi P.G., Campana A.: "Early cleavage of human embryos to the two-cell stage after intracytoplasmic sperm injection as an indicator of embryo viability". *Hum. Reprod.*, 1998, *13*, 182.

[2] Shoukir Y., Campana A., Farley T., Sakkas D.: "Early cleavage of in-vitro fertilized human embryos to the 2-cell stage: a novel indicator of embryo quality and viability". *Hum. Reprod.*, 1997, *12*, 1531.

[3] Giorgetti C., Hans E., Terriou P., Salzmann J., Barry B., Chabert-Orsini V., *et al.*: "Early cleavage: an additional predictor of high implantation rate following elective single embryo transfer". Reprod. Biomed. Online, 2007, *14*, 85.

[4] Petersen C.G., Mauri A.L., Ferreira R., Baruffi R.L., Franco Júnior J.G.: "Embryo selection by the first cleavage parameter between 25 and 27 hours after ICSI". *J. Assist. Reprod. Genet.*, 2001, *18*, 209.

[5] Fancsovits P., Toth L., Takacs Z.F., Murber A., Papp Z., Urbancsek J.: "Early pronuclear breakdown is a good indicator of embryo quality and viability". *Fertil. Steril.*, 2005, *84*, 881.

[6] Sakkas D., Percival G., D'Arcy Y., Sharif K., Afnan M.: "Assessment of early cleaving in vitro fertilized human embryos at the 2-cell stage before transfer improves embryo selection". *Fertil. Steril.*, 2001, *76*, 1150.

[7] Edwards R.G., Fishel S.B., Cohen J., Fehilly C.B., Purdy J.M., Slater J.M., *et al.*: "Factors influencing the success of in vitro fertilization for alleviating human infertility". *J. In Vitro Fert. Embryo Transf.*, 1984, *1*, 3.

[8] Brezinova J., Oborna I., Svobodova M., Fingerova H.: **"**Evaluation of day one embryo quality and IVF outcome--a comparison of two scoring systems". *Reprod. Biol. Endocrinol.*, 2009, *3*, 7.

[9] Salumets A., Hydén-Granskog C., Mäkinen S., Suikkari A.M., Tiitinen A., Tuuri T.: "Early cleavage predicts the viability of human embryos in elective single embryo transfer procedures". *Hum. Reprod.*. 2003, *18*, 821.

[10] Fauque P., Léandri R., Merlet F., Juillard J.C., Epelboin S., Guibert J., *et al.*: "Pregnancy outcome and live birth after IVF and ICSI according to embryo quality". *J. Assist. Reprod. Genet.*, 2007, *24*, 159.

[11] Alter L., Boitrelle F., Sifer C.: "How can we nowadays select the best embryo to transfer?" *Gynecol. Obstet. Fertil.*, *42*, 515.

[12] Vergouw C.G., Al Nofal M., Kostelijk E.H., Rooth H., Hompes P.G., Schats R., Lambalk C.B.: "The association of the blastomere volume index (BVI), the blastomere symmetry index (BSI) and the mean ovality (MO) with ongoing implantation after single embryo transfer". *J. Assist. Reprod. Genet.*, 2013, *30*, 587.

[13] Steinberg M.L., Boulet S., Kissin D., Warner L., Jamieson D.J.: "Elective single embryo transfer trends and predictors of a good perinatal outcome--United States, 1999 to 2010". *Fertil. Steril.*, 2013, *99*, 1937.

[14] Gurgan T., Urman B., Yarali H.: "Results of in vitro fertilization and embryo transfer in women with infertility due to genital tuberculosis". *Fertil. Steril.*, 1996, *65*, 367.

[15] Barri P.N., Coroleu B., Tur R., Barri-Soldevila P.N., Rodríguez I.: **"**Endometriosis-associated infertility: surgery and IVF, a comprehensive therapeutic approach". *Reprod. Biomed. Online*, 2010, *21*, 179.

[16] Pfeifer, S., Fritz, M., Dale, R., Adamson, G., Barnhart, K., Catherino, W., *et al.*: "Committee opinion: role of tubal surgery in the era of assisted reproductive technology". *Fertil. Steril.*, 2012, *97*, 539.
[17] Jungwirth A., Giwercman A., Tournaye H., Diemer T., Kopa Z., Dohle G., *et al.*: "European Association of Urology guidelines on Male Infertility: the 2012 update". *Eur. Urol.*, 2012, *62*, 324.
[18] Veeck L.: "Preembryo grading and degree of cytoplasmic fragmentation". *In:* Veeck L. (ed). *An atlas of human gametes and conceptuses: an illustrated reference for assisted reproductive technology.* New York, USA: Parthenon, 1999, 46.
[19] Jakus S., Neuer A., Dieterle S., Bongiovanni A.M., Witkin S.S.: "Antibody to the Chlamydia trachomatis 60 kDa heat shock protein in follicular fluid and in vitro fertilization outcome". *Am. J. Reprod. Immunol.*, 2008, *59*, 85.
[20] Witkin S.S., Kligman I., Grifo J.A., Rosenwaks Z.: **"**Chlamydia trachomatis detected by polymerase chain reaction in cervices of culture-negative women correlates with adverse in vitro fertilization outcome". *J. Infect. Dis.*, 1995, *171*, 1657.
[21] Sharara F.I., Queenan J.T. Jr., Springer R.S., Marut E.L., Scoccia B., Scommegna A.: "Elevated serum Chlamydia trachomatis IgG antibodies. What do they mean for IVF pregnancy rates and loss?" *J. Reprod. Med.*, 1997, *42*, 281.
[22] Svenstrup H.F., Fedder J., Kristoffersen S.E., Trolle B., Birkelund S., Christiansen G.: "Mycoplasma genitalium, Chlamydia trachomatis, and tubal factor infertility--a prospective study". *Fertil. Steril.*, 2008, *90*, 513.
[23] Mårdh P.A.: **"**Tubal factor infertility, with special regard to chlamydial salpingitis". *Curr. Opin. Infect. Dis.*, 2004, *17*, 49.
[24] Dechaud H., Anahory T., Aligier N., Arnal F., Humeau H., Hedon B.: "Salpingectomy for repeated embryo nonimplantation after in vitro fertilization in patients with severe tubal factor infertility". *J. Assist. Reprod. Genet.*, 2000, *17*, 200.
[25] Strandell A., Lindhard A., Waldenström U., Thorburn J.: "Hydrosalpinx and IVF outcome: cumulative results after salpingectomy in a randomized controlled trial". *Hum. Reprod.*, 2001, *16*, 2403.
[26] Hammadeh M.E., Radwan M., Al-Hasani S., Micu R., Rosenbaum P., Lorenz M., Schmidt W.: "Comparison of reactive oxygen species concentration in seminal plasma and semen parameters in partners of pregnant and non-pregnant patients after IVF/ICSI". *Reprod. Biomed. Online*, 2006, *13*, 696.
[27] Lin M.H., Kuo-Kuang Lee R., Li S.H., Lu C.H., Sun F.J., Hwu Y.M.: "Sperm chromatin structure assay parameters are not related to fertilization rates, embryo quality, and pregnancy rates in in vitro fertilization and intracytoplasmic sperm injection, but might be related to spontaneous abortion rates". *Fertil. Steril.*, 2008, *90*, 352.
[28] Irvine D.S., Twigg J.P., Gordon E.L., Fulton N., Milne P.A., Aitken R.J.: "DNA integrity in human spermatozoa: relationships with semen quality". *J. Androl.*, 2000, *21*, 33.
[29] Giwercman A., Richthoff J., Hjollund H., Bonde J.P., Jepson K., Frohm B., *et al.*: "Correlation between sperm motility and sperm chromatin structure assay parameters". *Fertil. Steril.*, 2003, *80*, 1404.
[30] Sun J.G., Jurisicova A., Casper R.F.: "Detection of deoxyribonucleic acid fragmentation in human sperm: correlation with fertilization in vitro". *Biol. Reprod.*, 1997, *56*, 602.
[31] Lemmen J.G., Agerholm I., Ziebe S.: "Kinetic markers of human embryo quality using time-lapse recordings of IVF/ICSI-fertilized oocytes". *Reprod. Biomed. Online*, 2008, *17*, 385.
[32] Azzarello A., Hoest T., Mikkelsen A.L.: "The impact of pronuclei morphology and dynamicity on live birth outcome after time-lapse culture". *Hum. Reprod.*, 2012, *27*, 2649.
[33] Aguilar J., Motato Y., Escribá M.J., Ojeda M., Muñoz E., Meseguer M.: "The human first cell cycle: impact on implantation". *Reprod. Biomed. Online*, 2014, *28*, 475.

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