The effect of day 2 versus day 3 embryo transfer on early pregnancy outcomes in women with a low yield of fertilized oocytes

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Abstract

Purpose To evaluate the differences in implantation and pregnancy rates when embryo transfer occurs on D2 versus D3 in women with a low yield of fertilized oocytes.

Methods A total of 156 IVF/ICSI cycles from 141 women at an academic fertility center were analyzed in a retrospective fashion. Women with a low number of fertilized oocytes (≤ 2 two pronuclei (2PN) stage zygotes) who had their fresh embryo transfer on D2 or D3 were included in the study. Positive pregnancy test per IVF cycle (PPT), clinical pregnancy rate (CPR), spontaneous abortion rate (SABR), and implantation rate (IMPR) were the main outcome measures assessed. Mann-Whitney *U* test and χ^2 test were used as appropriate. A generalized linear mixed effect model adjusted for relevant covariates was conducted. $P < 0.05$ was considered significant.

Results Patients having their embryo transfer on D2, when compared to those who had a D3 embryo transfer, experienced similar PPT [30.8 vs. 28.2%, respectively; adjusted OR (95%CI): 0.49 (0.16, 1.52)], CPR [26.9 vs. 25.6%, respectively; adjusted OR (95%CI): 0.44 (0.12, 1.67)], and IMPR [17.3 vs. 16.7%, respectively; adjusted β (95%CI) – 5.6% (– 15.0, 3.9)].

Conclusion Our findings suggest that transferring embryos on D2 versus D3 in women with a limited number of 2PN stage zygotes does not affect early pregnancy outcomes. These results indicate that there can be some flexibility in scheduling the day of transfer at the convenience of both the patient and the center.

Keywords IVF · Embryo transfer · 2PN · Implantation rate · Pregnancy outcomes

Introduction

Day of embryo transfer must balance the time spent in culture with replicating the in vivo migration of an embryo into the uterus. Although embryos were traditionally transferred on Day 2 (D2) to minimize time spent in culture, Day 3 (D3) fresh embryo transfers became increasingly common in order to more closely mimic the in vivo process in which embryos typically enter the uterus at the morula stage [1]. Later transfer also has the added benefit of giving the embryologist more time to select the most promising embryos for transfer.

Early studies comparing D2 versus D3 embryo transfers produced conflicting results regarding the impact on implantation and clinical pregnancy rates [2–7]. A prospective, randomized study performed by Laverge et al. found that implantation and pregnancy rates were similar for D2 and D3 embryo transfers in women with at least seven normally fertilized embryos, with a possible deleterious effect on embryo quality when left in culture until day 3 [1]. Baruffi and colleagues found similar pregnancy outcomes when D2 and D3 transfers were compared for women undergoing intracytoplasmic

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sperm injection (ICSI) with on average >2 embryos transferred and at least one embryo left for cryopreservation [7]. A Cochrane Database Review published in 2016 concluded that there are no significant differences in the known outcomes reported such as clinical pregnancy, miscarriage, or multiple gestation rates between D2 and D3 embryo transfers [8]. However, 12 studies were assessed with what was described as an overall inconsistency and imprecision in reporting and methodology.

Importantly, further questions have been raised regarding whether the timing of embryo transfer impacts outcomes for specific populations, for example those with poor ovarian response, as the increased ability to select better quality embryos on day 3 may compensate for reduced oocyte quantity and quality in these women. While Bahceci et al. found that increased time in culture negatively impacted clinical pregnancy rates in women of all ages with poor ovarian response (five or fewer follicles identified after ovarian stimulation), another study by Xin et al. showed that patients under age 35 with poor ovarian response (normal ovarian reserve but fewer than six oocytes retrieved) may benefit from a D3 embryo transfer in terms of clinical pregnancy rates [9, 10]. However, ovarian response was defined differently in these studies. Therefore, there are questions remaining regarding whether D2 or D3 embryo transfers offer advantages for specific patient populations.

To our knowledge, no studies have examined whether timing of embryo transfer is significant in the setting of patients with a low yield of fertilized oocytes, which may be due to poor ovarian response to stimulation yielding few mature oocytes, or intrinsic abnormalities in either the oocyte or the sperm impacting fertilization [11]. The aim of our study was to retrospectively assess whether women with two or fewer 2PN stage zygotes after IVF or ICSI benefit in implantation and clinical pregnancy rates from a D2 or D3 embryo transfer. We hypothesized that day of transfer would not significantly impact outcomes in this population of patients, thus affording infertility clinics greater flexibility in scheduling embryo transfers for these women.

Methods

Study design

Women undergoing D2 or D3 fresh embryo transfer between April 27, 2015, and March 11, 2017, who had a low number of fertilized oocytes (≤ 2 2PN stage zygotes), were included in the study. Prior to April 4, 2016, all women with two or fewer fertilized oocytes were transferred on D2 at Massachusetts General Hospital (MGH), after which the protocol changed to performing D3 transfers, with no other changes in laboratory or clinical protocols. This study design eliminated any

provider or laboratory selection bias. Exclusion criteria included donor oocyte, pre-implantation genetic testing, frozen embryo transfer, and gestational carrier cycles, as well as women who yielded >2 normally fertilized oocytes. Cycles were categorized into D2 or D3 transfers. Demographic data and cycle characteristics were collected for all women from electronic medical records.

This study was conducted with approval from the Partners Healthcare Internal Review Board at MGH.

Clinical and embryo transfer protocols

All study patients completed a standard infertility work-up at the Massachusetts General Hospital Fertility Center prior to IVF/ICSI [12]. Treatment included standard controlled ovarian hyperstimulation protocols as previously described [12]: (1) low-dose, luteal phase down-regulation protocol with GnRH agonist, (2) antagonist protocol with oral contraceptive pill preparation, (3) flare protocol, and (4) antagonist protocol with patch preparation (patch protocol). Final follicular maturation was achieved with human chorionic gonadotropin (hCG; Serono, Norwell, MA) when a minimum of three follicles with a diameter of ≥ 16 mm and a serum estradiol level of ≥ 600 pg/mL were attained. Transvaginal ultrasound-guided oocyte retrieval was performed in the standard fashion 35–37 h later.

Fertilization was assessed between 16 and 18 h after conventional insemination or intracytoplasmic sperm injection (ICSI). Normally fertilized, 2PN stage zygotes were placed in individual 25 μ L drops of Continuous Single Culture media supplemented with 10% SSS (Irvine Scientific), overlaid with mineral oil and cultured in a bench top incubator set at 37° with an atmosphere of 5% O₂ and 6.5% CO₂ for 2 (prior to April 4, 2016) or 3 (after April 4, 2016) days. All women in our population were eligible to have all embryos transferred, and thus no embryo selection was required unless women opted specifically for single embryo transfer, in which case, the top-quality embryo based on blastomere cell number, fragmentation, and symmetry was selected at the time of transfer.

Outcomes of interest

Positive pregnancy test (serum β -hCG concentration > 6 IU/L) per IVF cycle (PPT), clinical pregnancy rate (at least one gestational sac identified on transvaginal ultrasound per initiated cycles; CPR), spontaneous abortion (SAB) rate (number of SABs per clinical pregnancy; SABR), and implantation rate (number of sacs seen on transvaginal ultrasound per number of embryos transferred; IMPR) were assessed in all women after D2 or D3 transfer. Subanalyses to further assess the impact of D2 vs. D3 transfer by age (< and ≥ 38 years), AMH (< and ≥ 1.0 ng/mL), and fertilization rate (< and $\geq 50\%$) were also performed.

Statistics

Mann-Whitney *U* test and χ^2 tests were used as appropriate. PPT, CPR, SABR, and IMPR outcomes for D3 vs. D2 transfers were additionally compared with generalized linear mixed effect models adjusted for age, body mass index (BMI), anti-Müllerian hormone (AMH) level, and endometrial thickness, fertilization method (PPT, CPR, and IMPR), assisted hatching (PPT and IMPR), number of embryos transferred (PPT), and infertility diagnosis (SABR). *P* value < 0.05 was considered significant. STATA version 14.2 (College Station, TX; StataCorp LP) was used for the analyses.

Results

Patient demographic characteristics

The study population included 156 IVF/ICSI cycles (78 D2 transfers and 78 D3 transfers) from 141 women. Their baseline demographic and clinical characteristics are shown in Table 1. Age and markers of ovarian reserve were similar between the D2 and D3 groups. BMI was slightly higher on average in the D3 transfer group (*P* = 0.027). Infertility diagnosis was most often a combination of male factor, idiopathic, anovulatory, tubal factor, endometriosis, or polycystic ovarian syndrome (PCOS). Median number of embryos transferred in both groups was 2.0 (*P* = 1.0, Table 1).

Cycle characteristics

A higher proportion of D3 transfers had undergone ICSI (82.1 vs. 53.8%, *P* < 0.001, Table 1). Stimulation protocols also varied significantly amongst the two groups, including low-dose luteal, antagonist, flare, and patch regimens (*P* = 0.026). Endometrial lining and peak estradiol were similar on average between the D2 and D3 transfer groups. In addition, the median number of oocytes retrieved (5.0 oocytes in both groups, *P* = 0.26) and the median number of mature metaphase II (MII) oocytes obtained (3.5 and 3.0 in D2 vs. D3 groups, respectively, *P* = 0.79) were comparable between the two groups. Either one or two embryos were transferred in all patients.

Implantation and pregnancy outcomes

PPT, CPR, SABR, and IMPR were not significantly different between the D2 and D3 transfer groups: PPT 30.8% (*n* = 24) in D2 vs. 28.3% (*n* = 22) in D3 group, *p* = 0.86; CPR 26.9% (*n* = 21) in D2 vs. 25.6% (*n* = 20) in D3 group, *P* = 0.86; SABR 14.3% (*n* = 3) in D2 vs. 30.0% (*n* = 6) in D3 group, *P* = 0.28; and IMPR 17.3% in D2 vs. 16.7% in D3 group, *P* =

0.90. One patient in the cohort underwent an elective termination due to trisomy XYY.

When adjusted for possible confounders, no differences in PPT, CPR, SABR, or IMPR were observed (Table 2). Furthermore, when the data were further stratified by age (< and ≥ 38 years), AMH (< and ≥ 1.0 ng/ml), and fertilization rate (< and ≥ 50%), there were again no significant differences in outcomes between D2 and D3 transfers (Table 2). In particular, women with AMH < 1.0 ng/ml had a similar IMPR [adjusted OR − 5.8% (95% CI − 20.8, 9.2); *P* = 0.453] and CPR [adjusted OR 0.53 (95% CI 0.11, 2.65); *P* = 0.439]. Fertilization rate had no impact on outcomes as well (Table 2). However, a non-significant trend appeared in the age < 38 years group revealing that in the D3 transfers compared to D2 transfers, the odds of a PPT was 0.30 (95% CI 0.09, 1.00; *P* = 0.051), and the odds of a clinical pregnancy was 0.37 (95% CI 0.13, 1.11; *P* = 0.077).

Discussion

Optimizing embryo time in culture for pregnancy outcomes is the primary consideration in scheduling embryo transfers. While longer time in culture may aid in identifying higher quality embryos, it can also lead to greater manipulation in vitro, which may in turn negatively impact outcomes. Prior literature suggests that D2 or D3 transfer does not impact pregnancy outcomes, although some studies indicate that certain maternal characteristics like ovarian reserve or response to stimulation may influence D2 versus D3 transfer outcomes. Notably, several previous studies noting no differences in day 2 versus day 3 transfer focused on patients with a “sufficient” number of fertilized oocytes obtained, described variably as 5–7 oocytes [1, 13].

Given the inconsistent findings reported in the literature, our goal in this study was to clarify whether there is an advantage in pregnancy outcomes of D2 or D3 fresh embryo transfers in women with a low yield fertilized oocytes. To our knowledge, this is the first study to selectively analyze D2 vs. D3 transfer outcomes for this population of women. Importantly, we demonstrate that in women with one or two 2PN zygotes, embryo transfer on D2 or D3 yields similar outcomes in terms of PPT, IMPR, CPR, and SABR, consistent with our hypothesis. This finding notably allows clinical practices flexibility in planning transfers for these women without jeopardizing pregnancy outcomes.

Subpopulations of women with low ovarian reserve (AMH < 1.0 ng/ml) and low fertilization rate (< 50%) did not appear to differ with regard to D2 vs. D3 embryo transfer. Additionally, women with a fertilization rate ≥ 50%, corresponding to those with fewer than five oocytes retrieved based on our study inclusion criteria, had similar outcomes regardless of transfer. Interestingly, prior studies have suggested that

Table 1 Characteristics^a of 156 IVF cycles^b (141 women) with D2 and D3 embryo transfer

Characteristic	D2 <i>N</i> = 78	D3 <i>N</i> = 78	<i>P</i> value
Age, years	38.2 (35.1, 39.8)	37.0 (33.5, 39.9)	0.148
Body mass index, kg/m ²	23.6 (21.0, 26.2)	25.1 (22.1, 28.5)	0.027
Basal FSH, IU/L	7.8 (6.2, 9.6)	7.1 (6.0, 9.1)	0.372
AMH, ng/ml	1.4 (0.7, 3.0)	1.4 (0.8, 3.0)	0.974
Infertility diagnosis, <i>n</i> (%)			0.089
Male factor	8 (10.3)	9 (11.5)	
Idiopathic	18 (23.1)	8 (10.3)	
Anovulatory	2 (2.6)	0 (0.0)	
DOR	12 (15.4)	12 (15.4)	
Tubal factor	3 (3.8)	1 (1.3)	
Endometriosis	5 (6.4)	2 (2.6)	
PCOS	1 (1.3)	3 (3.8)	
Combined factors	29 (37.2)	43 (55.1)	
Fertilization method, <i>n</i> (%)			< 0.001
Intracytoplasmic sperm injection	42 (53.8)	64 (82.1)	
Conventional insemination	36 (46.2)	14 (17.9)	
Stimulation protocol, <i>n</i> (%)			0.026
Low-dose luteal	26 (33.3)	21 (26.9)	
Antagonist	22 (28.2)	19 (24.4)	
Flare	27 (34.6)	23 (29.5)	
Patch/antagonist	3 (3.8)	15 (19.2)	
Assisted hatching, <i>n</i> (%)	28 (35.9)	28 (35.9)	1.000
Endometrial lining, mm	10.7 (9.1, 12.0)	10.0 (9.0, 11.6)	0.443
Peak estradiol, pg/ml	1428.5 (1044.3, 1962.3)	1460.0 (1056.5, 1975.3)	0.910
Oocytes retrieved, <i>n</i>	5.0 (3.0, 7.0)	5.0 (3.8, 9.0)	0.262
Mature oocytes (MII), <i>n</i>	3.5 (2.0, 6.0)	3.0 (2.0, 5.0)	0.792
Two pronuclear zygotes (2PN), <i>n</i>	2.0 (1.0, 2.0)	2.0 (1.0, 2.0)	0.480
Embryos transferred, <i>n</i>	2.0 (1.0, 2.0)	2.0 (1.0, 2.0)	1.000

IQR, interquartile range; *n*, number; *DOR*, diminished ovarian reserve; *PCOS*, polycystic ovaries syndrome

^a Values are presented as median (*IQR*) unless otherwise noted

^b Thirteen women contributed for 2 cycles. One woman contributed for 3 cycles

poor ovarian response may impact clinical pregnancy rates depending on D2 or D3 transfer [9, 10]. Xin and colleagues further suggested a possible benefit to D3 transfer specifically amongst younger women with fewer than six oocytes retrieved [10]. Our data in contrast illustrate that neither ovarian response (given the low number of oocytes retrieved in the fertilization rate $\geq 50\%$ subgroup) nor ovarian reserve (determined by AMH) impact clinical outcomes for D2 vs. D3 embryo transfers. When the study population was restricted to women younger than 38 years and controlled for other covariates, a non-significant trend towards improved CPR after D2 embryo transfer appeared. Whether this is due to a benefit of greater in vivo time for embryo development cannot be determined with the present data, though with a larger cohort this trend may be delineated further.

A major strength of this retrospective study was the selection of subjects before and after a transfer day protocol

change, with no other changes in laboratory or clinical protocols or procedures that might impact pregnancy outcomes. There were no changes in clinic patient recruitment or selection for IVF/ICSI, and demographic factors such as age and BMI were controlled in our analyses. The median number of embryos transferred in each group was the same. Furthermore, our population is narrowly defined by number of 2PN, with markers of ovarian reserve (AMH) and fertilization rate taken into consideration, avoiding the inconsistent terminology plaguing prior studies on this subject. Therefore, we believe our results are generalizable to the broader infertility population with few 2PNs. Stimulation protocols differed between the groups, but this did not impact our findings (data not shown). Fertilization methods did differ significantly between the D2 group (primarily IVF) and D3 group (primarily ICSI), although male factor infertility rates were similar (Table 1). Although we controlled for fertilization method in our

Table 2 Odds ratio for positive pregnancy test (PPT), clinical pregnancy rate (CPR), spontaneous abortion rate (SABR), and implantation rate (IMPR) outcomes for total cohort and subpopulations. D2 group was used as the reference group

	Crude OR (95%CI)	Adjusted OR (95%CI)	P value (adjusted model)
PPT^a			
Total cohort	0.81 (0.35, 1.92)	.49 (0.13, 1.84)	0.290
Age < 38 years	0.53 (0.22, 1.31)	.30 (0.09, 1.00)	0.051
Age ≥ 38 years ^b	1.46 (0.42, 5.07)	1.33 (0.22, 7.95)	0.751
AMH < 1.0 ng/ml	1.16 (0.26, 5.30)	0.31 (0.05, 1.94)	0.211
AMH ≥ 1.0 ng/ml	0.71 (0.30, 1.71)	0.41 (0.13, 1.34)	0.140
Fertilization rate < 0.5 ^b	0.45 (0.15, 1.40)	0.50 (0.11, 2.23)	0.367
Fertilization rate ≥ 0.5	1.25 (0.46, 3.37)	0.76 (0.22, 2.63)	0.665
CPR^c			
Total cohort	0.82 (0.27, 2.48)	0.44 (0.12, 1.68)	0.231
Age < 38 years	0.53 (0.17, 1.70)	0.37 (0.13, 1.11)	0.077
Age ≥ 38 years ^b	1.72 (0.44, 6.77)	1.51 (0.27, 8.42)	0.638
AMH < 1.0 ng/ml ^b	1.27 (0.34, 4.74)	0.53 (0.11, 2.65)	0.439
AMH ≥ 1.0 ng/ml	0.70 (0.28, 1.71)	0.42 (0.14, 1.25)	0.120
Fertilization rate < 0.5 ^b	0.70 (0.23, 2.14)	0.42 (0.11, 1.61)	0.208
Fertilization rate ≥ 0.5	1.24 (0.45, 3.41)	0.81 (0.23, 2.83)	0.737
SABR^d			
Total cohort ^b	2.57 (0.54, 12.14)	1.78 (0.22, 14.61)	0.590
Fertilization rate ≥ 0.5 ^b	1.33 (0.23, 7.74)	0.10 (0.003, 3.85)	0.219
IMPR^e			
Total cohort	−2.0% (−12, 7.5%)	−5.6% (−15.0, 3.9%)	0.250
Age < 38 years	−9.3% (−23.5, 4.8%)	−11.1% (−25.1, 2.8%)	0.118
Age ≥ 38 years ^f	6.8% (−7.2, 21.0%)	5.2% (−11.0, 21.6%)	0.519
AMH < 1.0 ng/ml	1.7% (−14.4, 17.8%)	−5.8% (−20.8, 9.2%)	0.453
AMH ≥ 1.0 ng/ml	−4.8% (−16.5, 7.0%)	−8.0% (−19.3, 3.2%)	0.163
Fertilization rate < 0.5 ^f	−5.6% (−18.5, 7.4%)	−7.0% (−19.7, 5.7%)	0.275
Fertilization rate ≥ 0.5	−4.7% (−16.8, 7.3%)	−7.2 (−19.0, 4.5%)	0.226

^a Covariates in adjusted model: Age, BMI, AMH, and endometrial thickness (continuous), fertilization method, assisted hatching, number of embryos transferred

^b Generalized linear mixed effect model did not concave. A multiple logistic regression model using only the 1st cycle was conducted instead

^c Covariates in adjusted models: Age, BMI, AMH, and endometrial thickness (continuous), fertilization method

^d Covariates in adjusted models: Age, BMI, AMH, and endometrial thickness (continuous), infertility diagnosis

^e Estimates are presented as β coefficient (95%CI). Covariates in adjusted model: Age, BMI, AMH, and endometrial thickness (continuous), fertilization method, assisted hatching

^f Generalized linear mixed effect model did not concave. A multiple linear regression model using only the 1st cycle was conducted instead

analyses, the discrepancy in fertilization method may reflect underlying bias in the distribution of infertility diagnoses that we could not fully address. Notably, our inclusion criteria also selected for women at higher risk for poor outcomes given that they were not candidates for D5 transfer. Although our data suggest no differences in D2 and D3 transfer for these women, the small number of subjects in our study limited its power, warranting a larger prospective study to definitively address pregnancy outcomes.

Day of transfer has been studied extensively and found overall to have no impact on women's outcomes whether performed on D2 or D3. This study specifically examines this

question in a well-defined group of women not previously addressed in the literature: those with two or fewer normally fertilized oocytes. We conclude that even for these women, regardless of age, BMI, AMH, endometrial thickness, or fertilization method, there is no difference in clinical outcomes between D2 and D3 transfer. This was also true for women in our population with poor ovarian reserve or fertilization rates. Our findings support clinics' use of either D2 or D3 transfers for this population of women, which affords them additional flexibility in scheduling.

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Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflicts of interest.

References

1. Laverge H, De Sutter P, Van der Elst J, Dhont M. A prospective, randomized study comparing day 2 and day 3 embryo transfer in human IVF. *Hum Reprod.* 2001;16(3):476–80.
2. Carillo AJ, Lane B, Pridham DD, Risch PP, Pool TB, Silverman IH, et al. Improved clinical outcomes for in vitro fertilization with delay of embryo transfer from 48 to 72 hours after oocyte retrieval: use of glucose- and phosphate-free media. *Fertil Steril.* 1998;69(2):329–34.
3. Dawson KJ, Conaghan J, Ostera GR, Winston RM, Hardy K. Delaying transfer to the third post-insemination to select non-arrested embryos, increases development to the fetal heart stage. *Hum Reprod.* 1995;10(1):177–82.
4. Ertzeid G, Dale P, Tanbo T, Storeng R, EKjekshus E, Abyholm T. Clinical outcome of day 2 versus day 3 embryo transfer using serum-free culture media: a prospective randomized study. *J Assist Reprod Genet.* 1999;16(10):529–34.
5. Pantos K, Makrakis E, Stavrou D, Karantzis P, Vaxevanoglou T, Tzigounis V. Comparison of embryo transfer on day 2, day 3, and day 6: a prospective randomized study. *Fertil Steril.* 2004;81(2):454–5. <https://doi.org/10.1016/j.fertnstert.2003.07.016>.
6. Bastu E, Celik C, Keskin G, Buyru F. Evaluation of embryo transfer time (day 2 vs day 3) after imposed single embryo transfer legislation: when to transfer? *J Obstet Gynaecol.* 2013;33(4):387–90. <https://doi.org/10.3109/01443615.2012.761186>.
7. Baruffi R, Mauri A, Petersen C, Felipe V, Franco Junior J. Day 2 vs. day 3 embryo transfer after intracytoplasmic sperm injection. A prospective, randomized study. *J Reprod Med.* 2003;48(8):631–4.
8. Brown J, Daya S, Matson P. Day three versus day two embryo transfer following in vitro fertilization or intracytoplasmic sperm injection. *Cochrane Database Syst Rev.* 2016;12 <https://doi.org/10.1002/14651858.CD004378.pub3>.
9. Bahceci M, Ulug U, Ciray H, Akman M, Erden H. In vitro fertilization: efficiency of changing the embryo transfer time from day 3 to day 2 among women with poor ovarian response: a prospective randomized trial. *Fertil Steril.* 2006;86(1):81–5.
10. Xin ZM, Xu B, Jin HX, Song WY, Sun YP. Day 3 embryo transfer may have better pregnancy outcomes in younger than 35-year-old patients with poor ovarian response. *J Assist Reprod Genet.* 2012;29(10):1077–81. <https://doi.org/10.1007/s10815-012-9830-y>.
11. Georgiadak K, Khoury N, Spandidos D, Zoumpourlis V. The molecular basis of fertilization (Review). *Int J Mol Med.* 2016;38(4):979–86. <https://doi.org/10.3892/ijmm.2016.2723>.
12. Souter I, Baltagi LM, Kuleta D, Meeker JD, Petrozza JC. Women, weight, and fertility: the effect of body mass index on the outcome of superovulation/intrauterine insemination cycles. *Fertil Steril.* 2011;95(3):1042–7. <https://doi.org/10.1016/j.fertnstert.2010.11.062>.
13. Jones GM, Trounson AO, Gardner DK, Kausche A, Lolatgis N, Wood C. Evolution of a culture protocol for successful blastocyst development and pregnancy. *Hum Reprod.* 1998;13(1):169–77.