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Original article

Predictive factors for biochemical pregnancy in intracytoplasmic sperm injection cycles

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ABSTRACT

The aim of this study was to investigate which factors contribute to the incidence of biochemical pregnancy (BP) in intracytoplasmic sperm injection (ICSI) cycles. This cohort study included cycles performed from June 2010 to September 2016 in a private, university-affiliated IVF centre. Cycles were split into four groups, depending on the pregnancy outcomes: Clinical Pregnancy (CP, n = 903), Biochemical Pregnancy (BP, n = 55), Miscarriage (MI, n = 142) and Negative Pregnancy (NP, n = 2034). The effects of ovarian stimulation, laboratory data and seminal parameters on pregnancy outcomes were evaluated using adjusted general linear models. Discriminant analyses were conducted to construct a model for pregnancy prediction and to establish cut-offs for BP. The total sperm count ($p = 0.035$), total and progressive sperm motility ($p = 0.001$ and $p = 0.023$, respectively), total motile sperm count (TMSC, $p = 0.029$) and the endometrial thickness ($p < 0.001$) were lower among BP group cycles. Lower rates of high-quality cleavage-stage embryos were observed in the BP group compared to CP and MI groups ($p < 0.001$). In discriminant analyses, cut-offs for BP prediction were established for the following factors: endometrial thickness < 11 mm, sperm motility < 55.5% and total dose of follicle-stimulating hormone (FSH) > 2400 IU. The incidence of biochemical pregnancy was four times higher when the aforementioned factors did not meet the defined cut-offs. The combination of suboptimal endometrial development and poor seminal and embryo quality contribute to an increased incidence of biochemical pregnancy in ICSI cycles.

1. Introduction

Human reproduction is marked by its inefficiency. It is estimated that 70% of all pregnancies are lost prior to live birth; among these, 25%–50% end up as biochemical pregnancies (BP) [1,2]. BP is defined as very early loss where the initial serum or urine beta human chorionic gonadotropin (β-hCG) pregnancy test is positive, but it does not progress into a clinical pregnancy, confirmed by the presence of an embryonic sac and a foetal heartbeat on ultrasound at 6–7 weeks of gestation [2,3].

Most BP losses go unrecognised in natural pregnancies, because the menstrual cycle is not significantly altered. Nevertheless, in patients undergoing assisted reproduction technology (ART) treatments, in which β-hCG levels after embryo transfer are actively monitored, BP is diagnosed in up to 20% of the cycles [2,4,5].

A positive β-hCG test is indeed evidenced that at least one embryo reached the advanced preimplantation phase of development and attempted to implant [6]. Moreover, a positive test, as opposed to a previous negative β-hCG test, has traditionally been recognised as a better indication of successful pregnancy in future ART cycles [7–10]. Nevertheless, recent studies showed poorer ART outcomes after recurrent BP, and a number of losses correlated with unsuccessful outcomes [11–13].

Very early pregnancy loss is believed to be influenced by endometrial receptivity [14] and embryo quality [13,15–17]. Even though poor semen quality, sperm chromosomal abnormalities and sperm DNA fragmentation have been associated with increased risk for recurrent pregnancy loss [18–20], the predictive value of semen parameters for BP outcome has never been established.

Despite the high incidence of BP, its predictive factors and precise aetiologies remain unknown [16]. Therefore, the objective of this study was to investigate those factors that contribute to the incidence of biochemical pregnancy in intracytoplasmic sperm injection (ICSI) cycles.

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2. Materials and methods

2.1. Study design

This cohort study included data from 3134 intracytoplasmic sperm injection (ICSI) cycles performed from June 2010 to September 2016, in a private university-affiliated in vitro fertilisation centre.

Cycles were split into four groups according to their pregnancy outcome: Clinical Pregnancy (CP, n = 903), Biochemical Pregnancy (BP, n = 55), Miscarriage (MI, n = 142) and Negative Pregnancy (NP, n = 2034). The effects of seminal parameters (ejaculatory abstinence, seminal volume and concentration, total sperm count, total and progressive sperm motility), ovarian stimulation response (total follicle-stimulating hormone (FSH) dose administered, estradiol level on recombinant human chorionic gonadotrophin (hCG) trigger day, numbers of follicles, oocytes and mature oocytes) and laboratory data (fertilisation rate, number of obtained and transferred embryos, high-quality embryo rates, blastocyst rate and endometrial thickness) on pregnancy outcomes were evaluated.

2.2. Inclusion and exclusion criteria

Patients meeting the following inclusion criteria participated in this study:

- Couples undergoing first ICSI cycle
- Controlled ovarian stimulation performed by the combination of gonadotropin-releasing hormone (GnRH) antagonist, recombinant follicle-stimulating hormone (FSH) and recombinant human chorionic gonadotrophin (hCG)
- Cycles with fresh embryo transfer at day five

We excluded the following cycles:

- Abnormal karyotype for male and / or female partner
- Stage III or IV endometriosis
- Hidrosalpinx
- Important systemic disease
- Positive screening for sexually transmitted diseases
- Cycles performed with vitrified/thawed oocytes or embryos
- Cycles performed with donated oocytes or embryos
- Frozen sperm cycles
- Surgical sperm retrieval
- Cycles in which preimplantation genetic testing was performed
- Cycles cancelled before fresh embryo transfer
- Cycles that resulted in ectopic pregnancy

All patients signed a written informed consent form, and the study was approved by the local institutional review board.

2.3. Controlled ovarian stimulation

Controlled ovarian stimulation was achieved by the administration of daily doses of recombinant follicle-stimulating hormone (r-FSH, Gonal-F®, Merck KGaA, Geneva, Switzerland), beginning with 225 IU on the third day of patients' menstrual cycles. The first ultrasound control and the oestradiol (E2) plasma dosage tests were performed on the seventh cycle day. Depending on the response of each patient as controlled by transvaginal ultrasound monitoring of the follicle size, the dose of recombinant-FSH was adjusted. Pituitary suppression was performed using a gonadotropin-releasing hormone (GnRH) antagonist (GnRHa, Cetrotide®; Merck KGaA Geneva, Switzerland), beginning when at least one follicle ≥ 14 mm was visualised. When at least three follicles reached 18 mm in diameter and serum estradiol level reached more than 600 pg/ml, the final follicular maturation was triggered with recombinant human chorionic gonadotrophin (hCG, Ovidrel™, Merck KGaA, Geneva, Switzerland). Oocyte retrieval was performed 35 h later through transvaginal ultrasound ovum pick-up.

2.4. Oocyte preparation

Retrieved oocytes were maintained in culture media (Global for fertilisation, LifeGlobal, Guilford, CT, USA) supplemented with 10% protein supplement (LGPS, LifeGlobal, Guilford, CT, USA) and covered with paraffin oil (Paraffin oil P.G., LifeGlobal, Guilford, CT, USA) for 2–3 h before cumulus cell removal. Surrounding cumulus cells were removed after exposure to a HEPES-buffered medium containing hyaluronidase (80 IU/ml, LifeGlobal, Guilford, CT, USA). The remaining cells were then mechanically removed by gently pipetting with a hand-drawn Pasteur pipette (Humagen Fertility Diagnostics, Charlottesville, VA, USA) [21].

Oocyte morphology was assessed using an inverted Diaphot microscope with a Hoffmann modulation contrast system (Eclipse TE 300 microscope, Nikon, Tokyo, Japan) under 400x magnification, just before sperm injection (5 h after retrieval). Oocytes that had released the first polar body were considered mature and were used for ICSI [22].

2.5. Semen analysis

Semen samples were obtained for laboratory use by masturbation. After liquefaction for 30 min, semen samples were evaluated for sperm concentration, sperm count, motility and morphology. Sperm counting and motility assessment were performed according to the manufacturer's instructions (Leja® slide, Gynotec Malden, Nieuw-Vennep, the Netherlands). The volume of the ejaculate was determined by aspirating the liquefied sample into a graduated disposable pipette. Sperm concentrations were expressed as 10^6 spermatozoa/ml, and total sperm count was expressed as 10^6 spermatozoa. Sperm motility was assessed in 200 random spermatozoa by characterising them as having progressive motility, non-progressive motility or being immotile. The motility was expressed as a percentage. Total motile sperm count (TMSC) was calculated by multiplying total sperm count by progressive motility divided by 100 [23]. Sperm samples were prepared for ICSI using a two-layered density gradient centrifugation technique (50% and 90% isolate, Irvine Scientific, Santa Ana, CA, USA) prior to ICSI [21].

2.6. Intracytoplasmic sperm injection

Intracytoplasmic sperm injection was performed according to Palermo et al. [21]. Sperm selection was performed at 400x magnification (Eclipse TE 300 microscope, Nikon, Tokyo, Japan). The injection was performed in a micro-injection dish prepared with 4-μl droplets of buffered medium (Global w/HEPES, LifeGlobal, Guilford, CT, USA), and covered with paraffin oil on a heated stage at 37.0 ± 0.5 °C on an inverted microscope. Fertilisation was confirmed by the presence of two pronuclei and the extrusion of the second polar body approximately 16 h after ICSI [22].

2.7. Embryo quality and embryo transfer

Embryos were morphologically evaluated on days two, three and five of development using an inverted microscope under 400x magnification (Eclipse TE 300 microscope, Nikon, Tokyo, Japan). High-quality cleavage-stage embryos were defined as those with all of the following characteristics: 3 − 5 cells on day two or 8 − 10 cells on day three, < 15% fragmentation, symmetric blastomeres, the absence of multinucleation, colourless cytoplasm with moderate granulation and no inclusions, the absence of perivitelline space granularity and the absence of zona pellucida dimorphisms. The blastocyst rate was defined as the number of embryos that reached blastocyst stage on day five over the number of embryos with normal fertilisation.

Embryos were placed in a 50-μL drop of culture medium (Global,

LifeGlobal, Guilford, CT, USA) supplemented with 10% protein supplement and covered with paraffin oil in a humidified atmosphere under 7.5% CO_2 at 37 °C for five days. Embryo transfer was performed on day five of development using a soft catheter with transabdominal ultrasound guidance. One to two embryos were transferred per patient [24].

2.8. Clinical follow-up

A pregnancy test (serum β-hCG) was performed ten days after embryo transfer. All women with a positive test underwent transvaginal ultrasound scan after two weeks. Biochemical pregnancy was defined as a positive pregnancy test that resolved spontaneously, as evidenced by the absence of either an intrauterine or ectopic embryonic sac in ultrasound screening. Clinical pregnancy was diagnosed when a foetal heartbeat was detected before 20 weeks. Miscarriage was defined as a pregnancy loss before 20 weeks. Negative pregnancy was defined as a negative serum β-hCG test.

2.9. Data analysis and statistics

Data were expressed as the mean ± standard deviation for continuous variables, while percentages were used for categorical variables. The analyses were performed using SPSS Statistics 20 (IBM, New York, New York, USA). The sample size was determined by considering effect size of 10%, α of 5%, β of 80% and 11 covariates for 1634 subjects using G*Power 3.1.7 (Franz Faul, Universität Kiel, Germany).

To assess the effects of ICSI characteristics on pregnancy outcomes, a general linear model (GLM) was used, followed by a Bonferroni post hoc test. Potential confounder variables were used as covariates, which include: maternal age, paternal age, total FSH dose, estradiol peak, oocytes retrieved, obtained embryos, high-quality embryo rates on days two and three, blastocyst rate, number of transferred embryos and endometrial thickness. All post hoc significances were labelled with different letters.

Discriminant analyses were conducted by the stepwise method for ICSI cycle prediction of biochemical or clinical pregnancy outcomes. Predictor variables were maternal and paternal ages, total FSH doses administered, estradiol levels on the day of hCG administration, retrieved oocytes, obtained embryos, transferred embryos, endometrial thickness, high-quality embryo rates on days two and three, blastocyst rates, sperm counts, total and progressive sperm motilities and TMSC. The total sperm motility, endometrium thickness and total FSH dose administered cut-offs were calculated using the maximum likelihood technique from weighted BP and CP means. The data were grouped according to the established BP cut-off, and analyses of selected cases were performed using the general linear model followed by the Bonferroni post hoc test, adjusted for the same confounding variables described above.

3. Results

Patients' demographics are shown in Table 1. Most cycles that ended up as biochemical pregnancy were due to male factor infertility indication, either isolated or in conjunction with female factors. Patient's ovarian response to controlled ovarian stimulation was similar in the BP and other groups, regarding total dose of FSH administered, estradiol level at hCG trigger day and the number of follicles, oocytes and mature oocytes obtained (Table 2). The total sperm counts were nearly two-fold lower ($p = 0.035$) in BP cycles than in the other groups. The total and progressive sperm motility ($p = 0.001$ and $p = 0.023$, respectively) and TMSC ($p = 0.029$) were also lower in cycles ending in a BP (Table 3).

Comparing ICSI outcomes, lower rates of high-quality embryos on day two were observed in the BP group compared to the CP and MI groups, although they were higher than those of the NP group ($p < 0.001$). On day three, high-quality embryo rates were still lower in the BP group compared to the CP and MI groups, but similar to those of the NP group ($p < 0.001$). Blastocyst rates were similar between the BP and CP groups, but were higher than those of the MI and NP groups ($p < 0.001$). The number of transferred embryos was similar among the BP, CP and MI groups, but higher than in the negative group ($p < 0.001$). Endometrial thicknesses were lower in the BP group than in the other groups ($p < 0.001$, Table 4).

Discriminant analyses were conducted to predict whether ICSI cycles resulted in clinical or biochemical pregnancies. Endometrial thickness ($p < 0.001$), total sperm motility ($p = 0.005$) and total FSH dose ($p = 0.007$) were the variables that could correctly classify 93.8% of the original cases. Based on the discriminant analyses, cut-offs for BP prediction were established as follows: endometrial thickness < 11 mm, sperm motility < 55.5% and total dose of FSH > 2400 IU. The incidence of biochemical pregnancy was four times higher when the aforementioned factors did not meet the defined cut-offs (Table 5).

4. Discussion

Biochemical pregnancy arises possibly because of failures in the complex embryo-uterine dialogue during the implantation process, thus decreasing reproductive success. Nevertheless, risk factors for BP after ART have not been comprehensively studied. Our evidence suggests that the combination of lower endometrial thickness, higher dose of FSH administered and poorer semen motility leads to an increased chance of BP outcomes in ICSI cycles.

Our study did not find significant differences in the demographical characteristics of the different pregnancy outcomes: paternal and maternal age, body mass index and duration of infertility were similar among all groups; however, we noticed a higher incidence of BP in cycles with male factor infertility. In a larger case sample, Yang [13] also reported a higher prevalence of male infertility in BP, although its implications were not further discussed.

Cycles resulting in BP showed two-fold lower sperm count, 20% lower total and progressive motility and 60% lower TMSC compared to all other groups. The significance of seminal quality was shown by discriminant analysis, in which total sperm motility was important for the biochemical pregnancy prediction. As far as we know, this is the first study to correlate a traditional sperm parameter with BP outcomes; hence there is no reported cut-off to compare with. Nevertheless, sperm motility importance for pregnancy outcomes has been demonstrated for testicular extracted sperm [25], intrauterine insemination [26,27] and recurrent pregnancy loss [28].

Despite the fact that the total dose of FSH administered in BP cycles did not differ from those of others, this variable became relevant when analysed in combination with endometrial and sperm parameters by the discriminant model prediction, indicating that ovarian stimulation can interfere with the implantation process and lead to a higher chance of biochemical pregnancy outcome. In fact, the use of mild ovarian stimulation in high responders has been associated with increased implantation rates [29]. The dose of FSH used for ovarian stimulation has been associated with displacement of the window of implantation, hindering the endometrium-embryo dialogue, and resulting in increased risk of spontaneous abortion [13,30] and biochemical pregnancy [13,31].

From early implantation, there is a natural selective pressure operating to reject embryos of poor viability from progressing beyond the peri-implantation period [32–35]. Endometrial decidualized stromal cells not only have a broad function in terms of vascular remodelling and immune activity regulation, but they also act as biosensors for signals derived from pre-implanted embryo [32,35]. In the present study, cycles that resulted in biochemical pregnancy presented poorer embryo quality at cleavage-stage, indicating that embryo quality may, in fact, be associated with BP. Indeed, cleavage-stage embryo quality has previously been associated with early pregnancy loss [36,37], while

Table 1
Descriptive analysis of patient demographical characteristics.

	Clinical pregnancy (n = 903)	Biochemical pregnancy (n = 55)	Miscarriage (n = 142)	Negative pregnancy (n = 2034)	p
Maternal age (years)	34.37 ± 0.15	35.24 ± 0.59	36.12 ± 0.36	36.57 ± 0.97	0.156
Maternal BMI (kg/m^2)	24.37 ± 0.13	25.29 ± 0.55	24.92 ± 0.33	24.38 ± 0.09	0.175
Paternal age (years)	37.48 ± 0.22	37.39 ± 0.88	38.55 ± 0.53	38.84 ± 0.14	0.383
Duration of infertility (years)	2.63 ± 1.72	2.44 ± 1.84	3.04 ± 2.50	2.72 ± 2.15	0.844
Type of infertility (%)					< **0.001**
Male factor	28.2	32.8	20.1	18.2	
Ovarian factor	8.9	9.0	16.7	17.9	
Tubal factor	7.8	5.4	6.0	6.4	
Endometriosis	9.2	9.1	7.4	9.8	
PCOS	3.6	3.6	2.9	1.9	
Male and female factors	20.0	23.6	24.1	23.5	
Others	22.3	16.5	22.8	22.3	

Note: BMI: body mass index, PCOS: polycystic ovary syndrome.

embryo chromosomal abnormalities have been associated in up to 70% of sporadic spontaneous losses [38,39]. Recently, Bartolacci et al [40] reported that abnormal sperm parameters concentration and motility can in fact compromise early embryonic development but not blastulation rates, in agreement to our results.

Furthermore, embryo quality and chromosomal status are directly impacted by sperm quality, influencing embryogenesis from a very early stage [41,42]. Poor seminal parameters observed in the BP group may indicate that early paternal effects must have influenced embryo development. In addition, embryo implantation potential could also be altered in BP cycles due to late paternal effects. Perhaps, the attempt of the embryo to implant fails after full activation of paternal inheritance, causing embryo chromosomal aberrations to become more evident and susceptible to negative endometrial selection, resulting in pregnancy loss [41,28,43].

In a preimplantation genetic diagnostic setting, Troncoso [31] showed that the incidence of BP was related to factors altering the process of implantation at the endometrial level, as opposed to only the chromosomal status of the embryo. Knowing that normal process of implantation consists of a series of events between the embryo and the endometrium, altered endometrial receptivity may be the ultimate cause of BP in ART cycles. Endometrial thickness on the day of hCG trigger has been regarded as a good indication of endometrial receptivity [44–46] and pregnancy after IVF and embryo transfer [47,48], even though there is no established cut-off value for proper endometrial development [49,50].

In this study, we observed that endometrial thickness in the biochemical pregnancy group was significantly lower than in the miscarriage, clinical and negative pregnancy groups. The endometrial thickness difference observed between CP and BP (11.1 mm vs. 9.7 mm) might indicate compromised endometrial development. In fact, an endometria of around 9 mm had been correlated to higher incidence of biochemical pregnancy [44,51,52]. A cut-off of 8.75 mm has been established for live birth prediction [48] and 7 mm for miscarriage [47]. Recently, Liu et al [53] analysed 40,000 embryo transfer (ET) cycles and demonstrated that clinical pregnancy and live birth rates decline as the endometrial thickness decreases below 8 mm in fresh ET and below 7 mm in frozen–thaw ET cycles. We could suggest that endometrial thickness around 9 mm is associated with an impaired implantation process, leading to BP.

Even though isolated endometrial thickness may be a poor predictor of IVF outcomes [54], the discriminant analysis highlighted that special attention should be given to cycles with endometrium thickness lower than 11 mm, in the presence of poor sperm motility and high dose of FSH administered.

Surprisingly, we found that seminal parameters and endometrial thickness were lower in cycles ending in BP than in negative pregnancies. In fact, while biochemical pregnancies are indeed conceptions followed by very early miscarriage, in negative cycles, no trace of implantation attempt can be observed. Our evidence suggests that cycles with combined poor embryo quality and suboptimal endometrial development are not fated for negative outcomes; however, they have a higher chance of embryo-uterus interaction displacement and consequently, inability to support embryo implantation thus ending in biochemical pregnancies. Given that the analysed data refers to cycles with fresh embryo transfer, the biochemical pregnancy risk could be mitigated by frozen/thawed unstimulated embryo transfer, which has been extensively correlated with better embryo-endometrium synchrony [15,55–58].

As for endometrial thickness, it could be argued that the poor embryo quality observed in the NP group was the primary reason for the negative result. Embryo implantation depends on the acquisition of a receptive endometrium. In addition, proper embryo development is crucial for adequate dialogue between the endometrium and the embryo.

In conclusion, the incidence of biochemical pregnancy in ICSI cycles is attributed to factors that interfere with the implantation process, including poor endometrial receptivity, supraphysiological hormone levels and poor seminal parameters related to embryo quality. The main limitations of the present study are its retrospective nature, the reduced number of BP cycles, the difference in male factor in demographics, the adjustment of the dose of FSH and the non-use of live birth as the primary outcome measure.

Despite that, the study was able to analyse a variety of factors that

Table 2
Descriptive analysis of patient responses to controlled ovarian stimulation.

	Clinical pregnancy (n = 903)	Biochemical pregnancy (n = 55)	Miscarriage (n = 142)	Negative pregnancy (n = 2034)	p
Total FSH dose (IU)	2,391.58 ± 29.49	2,473.13 ± 114.41	2,433.46 ± 71.44	2,448.70 ± 19.36	0.440
Estradiol level at hCG trigger day (pg/ml)	1,919.94 ± 66.12	2,162.38 ± 236.29	2,012.48 ± 156.23	1,715.66 ± 42.94	0.063
Follicles (n)	15.21 ± 0.34	15.92 ± 1.21	15.19 ± 0.80	15.42 ± 0.22	0.091
Oocytes retrieved (n)	10.71 ± 0.25	11.61 ± 0.91	11.05 ± 0.60	10.45 ± 0.16	0.440
Oocyte yield (%)	71.92 ± 0.83 [a]	73.52 ± 2.96 [ab]	73.11 ± 1.95 [ab]	68.85 ± 0.54 [b]	**0.004**
MII oocytes (n)	8.45 ± 0.20 [a]	8.36 ± 0.74 [ab]	8.63 ± 0.49 [ab]	7.81 ± 0.13 [b]	0.038

Note a ≠ b ≠ c (GLM Bonferroni post hoc $p < 0.05$). MII: Metaphase II (mature) oocytes.

Table 3
Descriptive analysis of patient seminal parameters.

	Clinical pregnancy (n = 903)	Biochemical pregnancy (n = 55)	Miscarriage (n = 142)	Negative pregnancy (n = 2034)	p
Ejaculatory Abstinence (days)	4.36 ± 0.19	4.09 ± 0.66	3.49 ± 0.45	4.24 ± 0.12	0.369
Seminal volume (ml)	3.03 ± 0.76	2.93 ± 0.29	3.21 ± 0.18	3.16 ± 0.05	0.473
Seminal concentration (x 10^6/ml)	43.26 ± 2.43	23.94 ± 5.21	36.55 ± 4.99	40.66 ± 1.49	0.067
Total sperm count (x 10^6)	123.91 ± 7.18 [b]	62.95 ± 14.17 [a]	106.41 ± 15.03 [b]	119.26 ± 4.54 [b]	**0.035**
Total sperm motility (%)	56.55 ± 0.87 [b]	44.02 ± 3.39 [a]	57.66 ± 2.09 [b]	57.57 ± 0.56 [b]	**0.001**
Progressive motility (%)	42.47 ± 0.84 [b]	33.78 ± 3.26 [a]	42.59 ± 2.01 [b]	43.58 ± 0.54 [b]	**0.023**
TMSC	74.16 ± 4.29 [b]	31.17 ± 15.58 [a]	71.78 ± 9.89 [b]	77.97 ± 2.72 [b]	**0.029**

Note: a ≠ b ≠ c (GLM Bonferroni post hoc $p < 0.05$). TMSC: total motile sperm count.

Table 4
Descriptive analysis of ICSI laboratory and clinical outcomes.

	Clinical pregnancy (n = 903)	Biochemical pregnancy (n = 55)	Miscarriage (n = 142)	Negative pregnancy (n = 2034)	p
Fertilisation rate (%)	84.72 ± 0.47	85.01 ± 2.94	83.71 ± 1.94	82.80 ± 0.53	0.074
Obtained embryos (n)	7.05 ± 0.11 [a]	6.57 ± 0.40 [ab]	7.17 ± 0.27 [ab]	6.53 ± 0.74 [b]	**0.009**
High-quality embryo rate day two (%)	29.98 ± 1.04 [a]	27.94 ± 1.71 [b]	31.53 ± 2.45 [a]	24.79 ± 0.70 [c]	< **0.001**
High-quality embryo rate day three (%)	54.41 ± 1.16 [a]	49.34 ± 4.16 [b]	55.65 ± 2.75 [a]	47.01 ± 0.75 [b]	< **0.001**
Blastocyst rate (%)	52.11 ± 1.30 [a]	50.12 ± 4.65 [a]	41.97 ± 3.07 [b]	37.08 ± 0.08 [c]	< **0.001**
Transferred embryos (n)	1.25 ± 0.04 [b]	1.05 ± 0.15 [b]	1.07 ± 0.09 [b]	0.97 ± 0.03 [a]	< **0.001**
Endometrial thickness (mm)	11.06 ± 0.76 [b]	9.74 ± 0.34 [a]	10.97 ± 0.22 [b]	10.75 ± 0.06 [b]	< **0.001**

Note a ≠ b ≠ c (GLM Bonferroni post hoc $p < 0.05$).

Table 5
Descriptive analysis of COS and ICSI outcomes defined by BP cut-offs.

	Selected (n = 221)	Remaining (n = 2030)	p
COS outcomes			
Total FSH dose (IU)	2,979.14 ± 56.33	2,382.80 ± 18.60	< **0.001**
Estradiol peak (pg/ml)	1,718.48 ± 110.55	1,781.23 ± 38.71	0.592
Follicles (n)	15.58 ± 0.58	15.11 ± 0.20	0.450
Oocytes retrieved (n)	10.73 ± 0.43	10.31 ± 0.14	0.362
Oocyte yield (%)	69.06 ± 1.43	69.71 ± 0.49	0.667
MII oocytes (n)	8.32 ± 0.36	7.80 ± 0.12	0.169
Laboratorial outcomes			
Fertilisation rate (%)	82.89 ± 1.43	83.09 ± 0.49	0.892
Obtained embryos (n)	6.95 ± 0.19	6.57 ± 0.06	0.067
High-quality embryo rate at day two (%)	24.22 ± 0.01	26.75 ± 0.01	0.181
High-quality embryo rate at day three (%)	48.00 ± 0.02	49.59 ± 0.07	0.450
Blastocyst rate (%)	44.03 ± 0.02	42.14 ± 0.09	0.420
Clinical outcomes			
Transferred embryos (n)	0.97 ± 0.07	1.06 ± 0.02	0.208
Endometrial thickness (mm)	9.38 ± 0.16	11.08 ± 0.05	< **0.001**
Positive β-hCG (%)			< **0.001**
CP	74.3	81.9	
BP	15.7	3.9	
MI	10	14.2	

could interfere with pregnancy outcomes, and punctuate the most relevant ones in a robust predictive model. Herein, biochemical pregnancy can be predicted by utilising combined cut-offs of: endometrial thickness < 11 mm, sperm motility < 55.5% and total dose of FSH > 2400 IU. This approach can both improve the understanding concerning mechanisms responsible for biochemical pregnancy and assist in the management of cases of previous pregnancy loss.

Declarations of interest

None.

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