Ectopic pregnancy risk factors in infertile patients: a 10-year single center experience

Federico Cirillo
IRCCS Humanitas Research Hospital

Ilaria Paladino
Humanitas University

Camilla Ronchetti
IRCCS Humanitas Research Hospital

Andrea Busnelli
IRCCS Humanitas Research Hospital

Emanuela Morenghi
IRCCS Humanitas Research Hospital

Leonora Grilli
Humanitas University

Pasquale Patrizio
University of Miami

Paolo Emanuele Levi-Setti (✉ paolo.levi_setti@humanitas.it)
IRCCS Humanitas Research Hospital

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Abstract

The present retrospective study included both in vivo and in vitro assisted reproductive technologies (ART) procedures performed from January 2009 to December 2018 at a tertiary-care Fertility Centre. The purpose was to assess the incidence of ectopic pregnancy (EP) in infertile population who undergoes ART and to identify any risk factor impacting the occurrence of EP after ART. Among 27,389 cycles, 7,352 pregnancies were achieved, of which 132 were EPs, the 1.80% (95% CI 1.5-2.1) of all pregnancies. In fresh cycles, a history of prior pelvic adhesions showed the greatest impact on the incidence of EP (aOR 2.49 95% CI 1.53 – 4.07 p < 0.001). Other factors associated with EP incidence were also identified, such as female age, basal FSH, the transfer of blastocyst embryos and difficulties during the embryo transfer procedure. In frozen cycles, the only factor influencing the incidence of EP was Anti-Mullerian Hormone (AMH) serum concentration (aOR 0.81 95% CI 0.65 – 1.00, p = 0.048). To conclude, the incidence of EP observed was comparable to that reported after natural conception. On the other hand, pre-existing risk factors, traditionally more common in infertile population, appeared to influence the incidence of EP and should thus be modified if possible.

Introduction

Ectopic pregnancy (EP) is a leading cause of maternal death or morbidity during the first trimester of pregnancy [1, 2]. While EP represents 1–2% of all pregnancies in the general population [1], initial evidence demonstrated an increased incidence of EPs after assisted reproductive technologies (ART), reaching rates as high as 8.6% [3]. During the last years, significant improvements in ART techniques have been made and a decrease in the number of associated EPs has been observed. This is most likely due to a reduction in the number of transferred embryos [4]. Nonetheless, the incidence of EP still appears to exceed that observed after natural conception [5]. In addition, rates of EP are highly variable among centers of different countries (varying from 1.5%-2% in UK and USA up to 5% in China) [4, 6, 7] and in national registries [8–10]. It has thus been hypothesized that differences in ART procedures and protocols may play a role in justifying this incidence variability[11]. In particular, the stage of transferred embryos (cleavage versus blastocyst stage) and the impact of fresh versus frozen/thawed embryo transfer (ET) cycles [12–15] have been widely investigated. In this regard, some studies found frozen ET to be associated with a slightly lower incidence of EP compared to fresh ET [16, 17]. However, possible confounding factors cannot be ignored. In fact, EP and infertility notoriously share some risk factors, making it unclear whether ART procedures have a direct effect or if the observed association is due to the included population itself [18–20]. Furthermore, the specific role of uterine contraction during fresh or warmed cycles, uterine manipulation, progesterone for luteal phase support (LPS) [21, 22] and the operator performing the procedure still deserve to be investigated [23]. Considering the widespread use of ART worldwide and the high morbidity and mortality associated with EP, providing further insight into the risk factors associated with EPs after ART appears of utmost relevance. Against this background, the objective of the present study was to calculate the incidence of EPs in all medically assisted procreation
(MAP)[24] procedures (both in vivo and in vitro) at a single center throughout a 10-year period. In addition, we also attempted to identify risk factors impacting the occurrence of EP after MAP.

**Materials And Methods**

**Study design and population**

In the present retrospective study, we included all MAP cycles (i.e., both in vivo MAP and in vitro ART procedures) performed from January 1st, 2009 to December 31st, 2018 in a single, tertiary care, University-affiliated Infertility Unit (Fertility Center of the Humanitas Research Hospital, Rozzano, Milan, Italy).

Collected data were female age, female body mass index (BMI), smoking habits, duration of infertility, primary or secondary infertility, previous history of EP, positive history for recurrent miscarriage, markers of ovarian reserve (i.e., basal Follicle Stimulating Hormone (FSH), Anti Müllerian Hormone (AMH) and Antral Follicle count (AFC), positive history for pelvic adhesions (which refers to pelvic infections, endometriosis, pelvic adhesions and frozen pelvis), presence of uterine fibroids and cause of infertility. Additionally, for patients who underwent ART, we collected: the number of oocytes retrieved, the number of supernumerary frozen oocytes and embryos, the type of ET (i.e., fresh or frozen), the embryo stage at transfer (i.e., cleavage stage or blastocyst stage embryo), the number of fresh and frozen transferred embryos, and the difficulty of ETs (ETs were considered difficult if they required more than one attempt or cervical manipulation or were associated with pain or bleeding).

Considering the low number of EPs obtained during the study period, pelvic infections, endometriosis, pelvic adhesions and frozen pelvis were included in a single variable named positive history for pelvic adhesions.

Patients were divided in two sub-groups according to the MAP cycle outcome: positive outcome with EP and positive outcome with eutopic pregnancy according to The European Society of Human Reproduction and Embryology (ESHRE) terminology [25].

**Interventions**

**IUI**

Ovarian stimulation protocol for IUI cycles and procedures have already been described elsewhere [26]. Luteal phase was supported with 200 mg daily of micronized vaginal progesterone (Prometrium, Rottapharm S.pa. or Progeffik, Effik Italy S.p.a), starting on the same night of the IUI procedure.

**In vitro Fertilization (IVF): Ovarian stimulation, egg collection and ET**
COS was performed using four different protocols: GnRH agonist long protocol; GnRH agonist short protocol; GnRH antagonist protocol; Flare-up GnRH agonist protocol [27]. Transvaginal ultrasound, estradiol and progesterone determinations were performed from the 5th day of gonadotropin administration to monitor follicles and endometrial growth. When at least three follicles with a mean diameter of > 16 mm were observed and hormone levels were adequate, 250 mcg of recombinant hCG (Ovitrelle; Merck Serono S.p.A.) was administered subcutaneously, for final follicle maturation. Patients at high risk for ovarian hyperstimulation syndrome (OHSS) were triggered with a GnRH analogue (Decapeptyl, Ipsen, Milan, Italy or Fertipeptil, Ferring S.p.A, Milan, Italy) [28]. Oocyte retrieval was performed transvaginally 34–36 h after ovulation triggering. Fresh ET was performed either 3 or 5 days following oocyte retrieval according to the patient history and characteristics of the embryos. A standardized internal protocol for performing the ET was followed: freehand insertion of a preloaded soft catheter into the uterine cavity, under transabdominal ultrasound (US) guidance. The latter has always been performed throughout the study period, even prior to the NICE indication [29]. In cases of non-optimal endometrial growth, premature progesterone rise, increased risk of OHSS or pre-implantation testing, elective embryo cryopreservation was performed. Frozen-thawed ET was preceded by endometrial synchronization, using one of the following three protocols: natural cycle (NC-FET), modified natural cycle (mNC-FET) or artificial replacement cycle (AR-FET).

**EP diagnosis**

EP diagnosis was done by combining the clinical signs and symptoms, the beta-human Chorionic Gonadotropins (ß-hCG) assessment and the transvaginal high-resolution ultrasound (TVU) imaging.

ß-hCG measurement was performed, according to the stage of transferred embryos, 12–14 days after fresh ET, Frozen Embryo Transfer (FET) or IUI procedures, and if positive was repeated every 48 hours, until it reached at least 1,000 IU.

TVU was performed at 6 gestational weeks (4 weeks after the procedure) or earlier in case of patients presenting clinical signs and symptoms strongly suspicious for EP, such as abdominal pain, vaginal bleeding and abnormal rise of ß-hCG levels.

**Primary and secondary aims**

The primary aim of the present study was to detect the incidence of EP in patients who underwent in vivo and in vitro MAP procedures, after adjusting for potential multiple confounding factors. The secondary aim was to investigate the potential risk factors associated with EP occurrence after MAP procedures.

**Ethical issue**

The study was conducted according to the Helsinki Declaration as well as in accordance with the current Italian legal and regulatory requirements. The study protocol design was conducted in accordance with Good Clinical Practice (GCP) guidelines of our internal Research Hospital, it was approved by the Humanitas Institutional Review Board (Comitato Etico dell’IRCCS dell’Istituto Clinico Humanitas) and the
study protocol was registered in ClinicalTrials.gov (NCT04325854, 13/03/2020), prior to full variable extraction.

Patients who underwent these cycles signed an informed consent approving that their medical records could be used for research purposes, if their anonymity was protected. Hence no specific consent was needed for this study [23]. Patients provided an informed written consent to have data from their medical records used in research, as the internal IRB and ethics committee required.

Data used in this study were collected via an internal web-based database, which enables storage of information about any patient and provides easy access for data analysis. Data protection is warranted by advanced threat prevention, enterprise-class encryption, and authentication for any user with periodical need of password renewal [30]. All data were fully anonymized before the authors accessed them.

**Statistical analysis**

First, EP trends in a 10-year period were evaluated by column charts and trend lines.

Then, a logistic regression analysis considering only pregnant patients (0 for eutopic pregnancy, 1 for ectopic pregnancy) was carried out. The analysis was conducted for all ART procedures (IUI and IVF) and subsequently restricted to the IVF population. In particular, the IVF population analysis was further restricted according to fresh and frozen embryo transferred cycles. We considered variables related to the infertile population risk factors and to the risk related to the procedure performed.

Results were expressed as mean ± standard deviation (SD), or median with interquartile range (IQR) or number and percentage, as appropriate. Association of ectopic pregnancy with all considered variables were explored through univariable logistic regression. Variables with a p value less than 0.25 were then submitted to multivariable logistic regression analysis, to identify factors independently associated to outcomes.

Due to the strong impact of the "primary infertility" variable, and due to the not excessively high number of EPs, we decided to exclude this variable from the multivariable analysis, in order to better assess the impact of the others.

Results of the logistic regression analysis were expressed as odds ratio (OR), 95% confidence interval (CI) [31].

All analyses were made with Stata15 (StataCorp, 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LLC). Statistical significance was set at p < 0.05.

**Results**

**Baseline characteristics and demographics**
As shown in Fig. 1, a total of 27,389 cycles in 13,191 couples were evaluated. Of these, 16,125 were fresh ET cycles (58.9%), 6,106 were FET cycles (22.3%), 1,228 were oocyte warming cycles (4.5%) and 3,930 were IUI cycles (14.3%). A total of 20,024 cycles resulted in a negative outcome (73.1%, 95% CI 72.6–73.7%), 132 resulted in EP (0.5%, 95% CI 0.4–0.6% of all cycles and 1.8%, 95% CI 1.5–2.1% of all pregnancies) and 7,220 resulted in eutopic pregnancy (26.4%, 95% CI 25.9–26.9%).

Trends in EP rates over the years are reported in Fig. 2 (EP% per two-year period for pregnant women). Patients’ demographic and clinical characteristics, of the whole study population and of the two subgroups separately, are reported in Table 1. Basal characteristics did not differ between groups with the exception of primary infertility incidence which was significantly higher in the eutopic pregnancy group, the basal FSH level which resulted significantly lower in the EP group and the rate of women with a positive history for pelvic adhesions which resulted significantly higher in the EP group. As for the indication to treatment, only endometriosis showed a significantly higher rate in EP group (Table 1). MAP outcomes in the general population and in the two sub-groups are reported in Table 2. No significant differences emerged with the exception of the embryo stage at transfer in fresh cycles (i.e., the cleavage stage ET was observed to be significantly more frequent in the eutopic pregnancy group) and the rate of difficult ETs which resulted significantly higher in the EP group.
Table 1
Population Baseline Characteristics (BMI: Body Mass Index; FSH: Follicular Stimulating Hormone; AMH: Anti-Mullerian Hormone; AFC: Antral Follicular Count; ET: Embryo Transfer)

| Variable                        | All Pregnancies | Ectopic pregnancy | Eutopic pregnancy | p value |
|---------------------------------|-----------------|-------------------|-------------------|---------|
| Procedures                      | 7,352           | 132 (1.80%)       | 7,220 (98.07%)    |         |
| Fresh ET                        | 4,506 (61.29%)  | 87 (65.91%)       | 4,419 (61.20%)    |         |
| Frozen ET                       | 2,254 (30.65%)  | 32 (24.24%)       | 2,222 (30.78%)    |         |
| Frozen oocytes                  | 206 (2.80%)     | 3 (2.27%)         | 203 (2.81%)       |         |
| IUI                             | 386 (5.25%)     | 10 (7.58%)        | 376 (5.21%)       |         |
| Female age (years)              | 35.65 ± 3.85    | 35.86 ± 3.77      | 35.65 ± 3.86      | 0.516   |
| Female BMI (kg/m²)              | 21.45 (19.72–23.74) | 21.48 (20.13–23.44) | 21.45 (19.72–23.74) | 0.798 |
| Smoking (%)                     | 2,799 (38.07%)  | 56 (42.42%)       | 2,743 (37.99%)    | 0.299   |
| Infertility duration (years)    | 3.67 (2.50–5.42) | 3.88 (2.58–5.58)  | 3.58 (2.50–5.42)  | 0.581   |
| Primary infertility             | 3,953 (53.77%)  | 46 (34.85%)       | 3,907 (54.11%)    | < 0.001 |
| Previous EP                     | 344 (4.68%)     | 4 (3.03%)         | 340 (4.71%)       | 0.365   |
| Recurrent abortions             | 19 (0.26%)      | 0 (0.00%)         | 19 (0.26%)        | 0.555   |
| Basal FSH (mUI/mL)              | 6.80 (5.50–8.30) | 6.30 (5.25–7.60)  | 6.80 (5.50–6.80)  | 0.011   |
| AMH                             | 2.20 (1.20–3.87) | 2.00 (1.12–3.31)  | 2.20 (1.20–3.90)  | 0.099   |
| AFC                             | 10 (6–17)       | 10 (6–15)         | 10 (6–17)         | 0.334   |
| Positive history for pelvic adhesions a | 961 (13.07%) | 33 (25.00%) | 928 (12.85%) | < 0.001 |
| Uterine Fibroids                | 1,144 (15.46%)  | 28 (21.21%)       | 1,116 (15.45%)    | 0.071   |

Indication to treatment

|                         | All Pregnancies | Ectopic pregnancy | Eutopic pregnancy | p value |
|-------------------------|-----------------|-------------------|-------------------|---------|
| Male factor             | 3,044 (41.40%)  | 44 (33.33%)       | 3,000 (41.55%)    | 0.057   |
| Tubal factor            | 941 (12.80%)    | 22 (16.67%)       | 919 (12.73%)      | 0.180   |

a i.e. pelvic infections, endometriosis, pelvic adhesions and frozen pelvis

b i.e. genetic or recurrent pregnancy failures.
| Variable                            | All Pregnancies | Ectopic pregnancy | Eutopic pregnancy | p value |
|------------------------------------|-----------------|-------------------|-------------------|---------|
| Endometriosis                      | 319 (4.34%)     | 11 (8.33%)        | 308 (4.27%)       | 0.031   |
| Unexplained                        | 950 (12.92%)    | 19 (14.99%)       | 931 (12.89%)      | 0.611   |
| Male and female factor             | 987 (13.42%)    | 16 (12.12%)       | 971 (13.45%)      | 0.658   |
| Ovulatory                          | 225 (3.06%)     | 4 (3.03%)         | 221 (3.06%)       | 1.000   |
| Reduced ovarian reserve            | 659 (8.96%)     | 11 (8.33%)        | 648 (8.97%)       | 1.000   |
| Multiple female factors            | 210 (2.86%)     | 5 (3.79%)         | 205 (2.84%)       | 0.517   |
| Other indications b                | 17 (0.23%)      | 0 (0.00%)         | 17 (0.24%)        | 1.000   |

a i.e. pelvic infections, endometriosis, pelvic adhesions and frozen pelvis

b i.e. genetic or recurrent pregnancy failures.
Table 2
IVF/ICSI fresh and frozen cycles outcomes

| Variable                              | All Pregnancies | Ectopic pregnancy | Eutopic pregnancy | p value |
|---------------------------------------|-----------------|-------------------|-------------------|--------|
| Oocytes retrieved                     | 9.69 ± 4.79     | 9.89 ± 4.65       | 9.69 ± 4.79       | 0.518  |
| Cleavage stage transfers              | 4,298 (61.88%)  | 78 (63.93%)       | 4,220 (61.84%)    | 0.637  |
| Blastocyst stage transfers            | 2,648 (38.12%)  | 44 (36.07%)       | 2,604 (38.16%)    |        |
| Cycles with frozen oocytes            | 532 (11.81%)    | 10 (11.49%)       | 522 (11.81%)      | 0.927  |
| Cycles with frozen embryos            | 2,066 (29.66%)  | 40 (32.79%)       | 2,026 (29.60%)    | 0.445  |
| Frozen embryos                        | 1.89 ± 1.13     | 1.83 ± 1.17       | 1.89 ± 1.13       | 0.520  |
| Frozen Oocytes                        | 6.86 ± 2.91     | 7.90 ± 4.31       | 6.84 ± 2.88       | 0.960  |
| Number Fresh Embryos Transferred      | 2.11 ± 0.59     | 2.06 ± 0.51       | 2.11 ± 0.59       | 0.347  |
| Number Warmed Embryos Transferred     | 1.30 ± 0.55     | 1.49 ± 0.70       | 1.30 ± 0.55       | 0.089  |
| Cleavage stage fresh embryo transfers | 3,925 (87.14%)  | 69 (79.31%)       | 3,856 (87.30%)    | 0.027  |
| Blastocyst stage fresh embryo transfers | 579 (12.86%)  | 18 (20.69%)       | 561 (12.70%)      |        |
| Cleavage stage frozen embryo transfers | 373 (15.27%)  | 9 (25.71%)        | 364 (15.12%)      | 0.084  |
| Blastocyst stage frozen embryo transfers | 2,069 (84.73%) | 26 (74.29%)       | 2,407 (84.88%)    |        |
| Difficult transfers                   | 1,467 (21.47%)  | 35 (28.93%)       | 1,432 (21.34%)    | 0.044  |

Logistic regression analysis (ectopic pregnancy vs eutopic pregnancy)

First of all, we considered patients who underwent IUI and IVF (Table 3), and then we restricted our population exclusively to patients who underwent IVF, dividing them according to the type of ET cycle (i.e., fresh or frozen thawed) (Table 4). In supplementary Table 1 is shown the logistic regression (ectopic vs eutopic pregnancy) in only IUI cycles. Considering the entire population (i.e., patients who underwent IUI and IVF), both in univariable and multivariable logistic regression, factors which had a significant impact on EP incidence were the basal FSH concentration, (OR 0.91 95% CI 0.84–0.98, p = 0.012; aOR 0.90 95%
CI 0.84–0.97, p = 0.010) and a positive history for pelvic adhesions, (OR 2.26 95% CI 1.52–3.37 p < 0.001; aOR 2.24 95% CI 1.50–3.37, p < 0.001).
Table 3
Univariable and Multivariable logistic regression (EP vs Eutopic pregnancy) in IUI, IVF/ICSI

| Variable                               | Univariable OR (95% CI) | p value   | Multivariable OR (95% CI) | p value   |
|----------------------------------------|-------------------------|-----------|---------------------------|-----------|
| Female age                             | 1.01 (0.97–1.06)        | 0.543     |                           |           |
| Female BMI                             | 0.98 (0.93–1.03)        | 0.457     |                           |           |
| Smoking                                | 1.20 (0.85–1.70)        | 0.299     |                           |           |
| Years of infertility                   | 1.00 (0.98–1.02)        | 0.829     |                           |           |
| Primary infertility                    | 0.45 (0.32–0.65)        | < 0.001   |                           |           |
| Previous Ectopic Pregnancies           | 0.63 (0.23–1.72)        | 0.370     |                           |           |
| Recurrent pregnancy loss               | NC                      |           |                           |           |
| Basal FSH                              | 0.91 (0.84–0.98)        | 0.012     | 0.90 (0.84–0.97)          | 0.010     |
| Basal AMH                              | 0.93 (0.85–1.02)        | 0.124     |                           |           |
| AFC                                    | 0.98 (0.95–1.01)        | 0.119     |                           |           |
| Positive history for pelvic adhesions  | 2.26 (1.52–3.37)        | < 0.001   | 2.24 (1.50–3.37)          | < 0.001   |
| Uterine fibroids                       | 1.47 (0.97–2.25)        | 0.072     |                           |           |
| Indication to treatment                |                         |           |                           |           |
| Male factor                            | 0.70 (0.49–1.01)        | 0.059     |                           |           |
| Tubal factor                           | 1.37 (0.86–2.18)        | 0.181     |                           |           |
| Endometriosis                          | 2.04 (1.09–3.82)        | 0.026     |                           |           |
| Unexplained                            | 1.14 (0.70–1.86)        | 0.611     |                           |           |
| Male and female factor                 | 0.89 (0.52–1.50)        | 0.658     |                           |           |
| Ovulatory                              | 0.99 (0.36–2.70)        | 0.984     |                           |           |
| Reduced ovarian reserve                | 0.92 (0.49–1.72)        | 0.798     |                           |           |
| Multiple female factors                | 1.35 (0.55–3.33)        | 0.518     |                           |           |

(IUI: intrauterine insemination; Body Mass Index; FSH: Follicular Stimulating Hormone; AMH: Anti-Mullerian Hormone; AFC: Antral Follicular Count; ET: Embryo Transfer.)

a i.e. pelvic infections, endometriosis, pelvic adhesions and frozen pelvis

b i.e. genetic or recurrent pregnancy failures
| Other indications | NC |
|-------------------|----|
| Procedures        |     |
| Fresh ET          | 1  |
| Frozen ET         | 0.73 (0.48–1.10) | 0.133 |
| Frozen oocytes    | 0.75 (0.23–2.39) | 0.628 |
| IUI               | 1.35 (0.70–2.62) | 0.374 |

(IUI: intrauterine insemination; Body Mass Index; FSH: Follicular Stimulating Hormone; AMH: Anti-Mullerian Hormone; AFC: Antral Follicular Count; ET: Embryo Transfer.)

| a | i.e. pelvic infections, endometriosis, pelvic adhesions and frozen pelvis |
| b | i.e. genetic or recurrent pregnancy failures |
Table 4
Univariable and Multivariable logistic regression (EP vs Eutopic pregnancy) in IVF/ICSI. (BMI: Body Mass Index; FSH: Follicular Stimulating Hormone; AMH: Anti-Mullerian Hormone; AFC: Antral Follicular Count; ET: Embryo Transfer). Multivariable results are corrected by years.

| Variable                        | Fresh cycles |            |            | Frozen cycles |            |            |
|---------------------------------|--------------|------------|------------|---------------|------------|------------|
|                                 | Univariable  | Multivariable | Univariable | Multivariable | Univariable | Multivariable |
|                                 | OR (95% CI)  | p value    | OR (95% CI) | p value       | OR (95% CI) | p value    |
| Female age                      | 1.04 (0.99–1.11) | 0.134     | 1.07 (1.01–1.13) | **0.033** | 0.95 (0.87–1.03) | 0.226 |
| BMI                             | 0.98 (0.92–1.04) | 0.477     |             |               | 0.96 (0.85–1.07) | 0.421 |
| Smoking                         | 1.06 (0.69–1.64) | 0.783     |             |               | 1.31 (0.67–2.56) | 0.433 |
| Years of infertility            | 1.00 (0.99–1.02) | 0.785     |             |               | 0.95 (0.83–1.10) | 0.515 |
| Primary infertility             | 0.42 (0.27–0.66) | <0.001    |             |               | 0.61 (0.31–1.21) | 0.157 |
| Previous Ectopic Pregnancy      | 0.54 (0.13–2.23) | 0.398     |             |               | 0.89 (0.21–3.76) | 0.878 |
| Recurrent Pregnancy Failure     | NC           |            |            |               | NC          |            |
| Basal FSH (mIU/mL)              | 0.88 (0.80–0.96) | **0.005** | 0.87 (0.79–0.96) | **0.003** | 0.96 (0.82–1.12) | 0.598 |
| Basal AMH                       | 1.01 (0.90–1.14) | 0.820     |             |               | 0.77 (0.62–0.96) | 0.022 |
| AFC                             | 0.98 (0.95–1.02) | 0.336     |             |               | 0.97 (0.91–1.04) | 0.413 |

* a i.e. pelvic infections, endometriosis, pelvic adhesions and frozen pelvis.
|                                | Fresh cycles | Frozen cycles |
|--------------------------------|--------------|---------------|
| **Positive history for pelvic adhesions**<sup>a</sup> | 2.35 (1.45–3.81) | 2.49 (1.53–4.07) | < 0.001 | 2.21 (1.02–4.75) | 0.043 | - |
| **Presence of myoma/s** | 1.53 (0.91–2.55) | 0.108 | - | 1.28 (0.55–2.94) | 0.566 | - |
| **Indication to treatment** |              |               |               |               |               |               |               |
| Male factor                  | 0.71 (0.45–1.11) | 0.128 | - | 0.79 (0.40–1.57) | 0.503 | - |
| Tubal factor                 | 1.44 (0.82–2.53) | 0.206 | 1.55 (0.67–3.59) | 0.301 | - |
| Unexplained                  | 1.00 (0.50–2.00) | 0.990 | 0.97 (0.34–2.78) | 0.959 | - |
| Endometriosis                | 1.55 (0.67–3.61) | 0.304 | 3.03 (1.05–8.76) | 0.040 | - |
| Male and female factor       | 0.96 (0.52–1.77) | 0.889 | 0.81 (0.28–2.30) | 0.690 | - |
| Ovulatory                    | 0.65 (0.09–4.71) | 0.667 | 0.98 (0.13–7.22) | 0.980 | - |
| Reduced ovarian reserve      | 1.09 (0.58–2.07) | 0.781 | NC | - |
| Multiple female factors      | 1.57 (0.57–4.33) | 0.388 | 0.98 (0.13–7.22) | 0.980 | - |
| Oocytes retrieved            | 1.01 (0.97–1.05) | 0.703 | - | - |
| Embryos transferred          | 0.85 (0.59–1.22) | 0.376 | 1.64 (0.99–2.70) | 0.051 | - |

<sup>a</sup> i.e. pelvic infections, endometriosis, pelvic adhesions and frozen pelvis.
After limiting the analysis to patients who underwent IVF, we observed different results according to the type of ET (i.e., fresh ET vs frozen ET). In the fresh ET sub-group, factors found to have an impact on EP incidence both in univariable and multivariable logistic regression were basal FSH (OR 0.88 95% CI 0.80–0.96, p = 0.005; aOR 0.87 95% CI 0.79–0.96, p = 0.003), a positive history for pelvic adhesions (OR 2.35 95% CI 1.45–3.81, p = 0.001; aOR 2.49 95% CI 1.53–4.07 p < 0.001), blastocyst stage ET (OR 1.34 95% CI 1.03–1.74, p = 0.030; aOR 1.32 95% CI 1.01–1.72, p = 0.043) and a difficult transfer (OR 1.82 95% CI 1.16–2.85, p = 0.009; aOR 1.86 95% CI 1.18–2.93, p = 0.007). Female age resulted associated with an increased risk of EP only in the multivariable analysis (OR 1.04 95% CI 0.99–1.11, p = 0.134; aOR 1.07 95% CI 1.01–1.13, p = 0.033) (Table 4). On the other hand, the only factor which influenced EP incidence in the frozen ET sub-group was AMH (OR 0.77 95% CI 0.62–0.96, p = 0.022: aOR 0.81 95% CI 0.65–1.00, P = 0.048). Multivariable logistic regression analysis in Table 4 was adjusted per year.

## Discussion

The overall EP rate in our study conducted throughout a 10-year period, was 1.8% (95% CI 1.5–2.1) of all pregnancies. This rate is similar to the general population, where EPs account for 1–2% of all pregnancies [1]. Thus, our results do not suggest that patients who underwent MAP procedures have an increased risk of developing EP. The incidence noted in our study is also consistent with other recent contributions which evaluated EP rate in the ART population [4, 6, 32].
Importantly, over the years, we observed a decrease in EP incidence after frozen ET (Fig. 1). This trend could be the consequence of the changes in the Italian law regulating ART (i.e., law 40/2004) [33]. In fact, in 2009, the lawmaker revoked both the insemination limit to a maximum of three oocytes and the obligation to transfer all the obtained embryos at the same time [33]. In the first following years, a progressive increase in the number of frozen-thawed ET cycles occurred. However, the policy of elective single blastocyst transfer took a few more years to take hold. One can thus speculate that the higher incidence of EPs observed in the first part of the study period is due to the widespread practice of transferring two or more frozen-thawed embryos. In fact, the analysis of data from the Centers for Disease Control and Prevention’s United States ART Surveillance System showed a progressive increase in the risk of EP as the number of transferred embryos increases [4].

Having a positive history for pelvic adhesions was shown to have a relevant impact on the EP rate (aOR 2.24 95% CI 1.50–3.37, p < 0.001). The observed association was expected since tubal anatomy can be distorted after pelvic infections or endometriosis and it is well known that pelvic adhesions are a significant risk factor for EP in both natural and assisted conception [1, 4, 33]. In addition, the entity of this association was even more pronounced when the analysis was restricted to the fresh ET subgroup (aOR 2.49 95% CI 1.53–4.07 p < 0.001) (Table 4). This may be explained by the known modification of the hormonal balance. Indeed, the high estrogen levels may alter the uterine environment leading to a more pronounced uterine contractility and, as a consequence, to an increased risk of retrograde movement of the embryo in the fallopian tube [34, 35] [17, 32, 36], even if no significant impact of the serum dosage of estradiol at the trigger day on EP was detected in fresh cycles (calculated per 100 pg/ml ) (OR 1.01, 95% CI 1.00–1.03, p = 0.089; aOR 1.01, 95% CI 0.99–1.03, p = 0.228). Likewise, high progesterone levels are supposed to be responsible for a dysfunctional tubal peristalsis that may lead to a higher EP rate in fresh cycles [37].

In our study, embryo transferred at blastocyst stage in fresh cycles emerged as a possible risk factor for EP (OR 1.34 95% CI 1.03–1.74, p = 0.030: aOR 1.32 95% CI 1.01–1.72, p = 0.043). A blastocyst has a higher implantation potential than a cleavage-stage embryo [38]. The combined effect of the higher blastocyst implantation potential and the increased uterine contractility in the fresh cycle environment may be a possible explanation to this finding. On the other hand, previous studies reported a reduced risk of EPs in frozen/thawed blastocyst ET [12, 21, 39]. Moreover, as reported in supplementary table 2, notably if the number of transferred blastocysts was one, the p was not significant, so that it was possible to conclude that the transfer of blastocyst embryos in fresh cycles could significantly influence the risk of EP only if the number of transferred blastocysts is more than one. Single fresh blastocyst transfers did not increase the risk of ectopic pregnancy. The factors influencing the choice between cleavage or blastocyst stage embryo transfer greatly vary from one fertility clinic to another and may act as further confounding factors. Future studies considering possible covariates are thus warranted before drawing conclusion about the impact of embryo stage on the risk of EP.

Results about the association between biomarkers of ovarian reserve and EP risk are conflicting.
On the one hand, we observed a lower EP risk in women in the high serum FSH category in fresh ET cycles (OR 0.88 95% CI 0.80–0.96, p = 0.005; aOR 0.87 95% CI 0.79–0.96, p = 0.003). This finding is not in line with most of the current literature [40–42] showing either a higher risk of EP in women with high FSH serum concentration or the lack of an association. [43–45].

On the other hand, in the frozen ET group, high serum AMH concentration resulted associated with a reduction (OR 0.77 95% CI 0.62–0.96, p = 0.022; aOR 0.81 95% CI 0.65–1.00, p = 0.048). This finding agrees with the previous contribution on this issue [46]. Combining these contradictory findings into a unique explanation is a difficult task. Considering the non-negligible prevalence of this condition, more studies specifically designed to address this issue should be carried out.

A difficult ET (i.e., an ET which required more than one attempt, cervical manipulation or that was associated with pain or bleeding) emerged as a possible risk factor in fresh cycles (aOR 1.86 95% CI 1.18–2.93, p = 0.007). It is well known that a difficult ET is associated with an increased risk of failed transfer. This occurs most commonly with a traumatic deposition of the embryo resulting from a difficult manipulation of the catheter inside the uterus, which may stimulate uterine contractions [47, 48]. These uterine contractions have been linked to a relocation of the embryo once placed in the uterus [48, 49]. The enhancement of uterine combinations favored by the concomitant ovarian stimulation could justify the observed association.

Therefore, a relevant attention should be put on the embryo transfer procedure, and on the possibility of better outcomes when the procedure is performed by an expert operator. This is described by a previous study which shows how the expertise of the operator performing embryo transfer is a crucial factor affecting the outcome of the ART cycle. [23]

Surprisingly, we failed to identify an association between tubal factor infertility and EP risk. Although tubal infertility is a known and well established risk factor for EP [1], its evaluation may be difficult [50]. Tubal receptivity and abnormal tubal contractility cannot be assessed in every day clinical practice. Furthermore, according to our protocols, women who have an a priori indication to IVF don't routinely undergo tubal patency evaluation. Henceforth, an underestimation of tubal factor infertility cannot be excluded making the observed association poorly reliable.

Our results did not show any statistically significant difference in the association between fresh or frozen ET and EP rates in both univariable and multivariable analysis. These are in line with previous research which also failed to show a relationship between EP and the type of ET [6, 16].

**Strengths, limitations, need for future research and main conclusion of the study**

The strengths of our study include the large number of cycles analyzed and the length of the study period. In addition, thanks to our strict protocols, patients lost at follow-up were few, 13 over 7,365 pregnancies (0.17%). Moreover, differently from other large studies which used national ART registries,
our internal web-based database enabled us to collect information on established risk factors for EP, including previous obstetric and gynecological history, smoking habits, and uterine surgeries.

However, there are also some limitations. First, owing to the retrospective study design, several data in the dataset could be either conflicting or potentially incorrect. Previous ectopic pregnancies may be an instance of misleading data, due to missing information on the time of its occurrence.

In addition, we did not consider biochemical pregnancies in our study. Biochemical pregnancies are considered a very early pregnancy loss, i.e. a pregnancy that does not develop normally and that fails to progress [51]. Therefore, biochemical pregnancies may be regarded as an early EP which results in no implantation and miscarriage [52]. Lack of inclusion of biochemical pregnancies may have, thus, led to a possible EP incidence underestimation.

On the other hand, we might also have overestimated EP incidence due to our pregnancy follow-up protocol. In fact, we routinely perform ultrasonography four weeks after fresh ET, FET or IUI procedures (6th week of gestation). In contrast, in the general non-infertile population, first trimester obstetric ultrasound, in the absence of specific indications, is usually performed later. Several naturally conceived EPs which undergo spontaneous resolution are thus erroneously classified as spontaneous miscarriage or biochemical pregnancies. Furthermore, pregnancies conceived using ART may be monitored more closely, which may result in more frequent identification of EP than in spontaneously conceived pregnancies. It is also possible that some misclassification of ectopic pregnancies and miscarriages may have occurred, due to allocation of pregnancies of unknown location.

**Conclusions**

In conclusion, our study confirms that the EP prevalence of infertile women undergoing ART is comparable with that of naturally conceived pregnancies. On the other hand, pre-existing risk factors which are traditionally more common in the infertile population appear to influence the pregnancy outcomes. Pelvic infections, endometriosis, pelvic adhesions and frozen pelvis seem to play an important role in increasing the incidence of EP. In a clinical perspective, infertile patients should thus be evaluated for an EP risk factors assessment in order to actively reduce the modifiable risks factors. A special attention should be paid to women with a high a priori risk. In this subgroup, the embryo transfer procedure should be performed by an expert operator.

**Declarations**

**Acknowledgments**

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**Author Contributions**
Conceptualization: FC, IP and PELS. Data curation: EM. Formal analysis: EM. Investigation: EM, FC, IP and PELS.

Methodology: EM, FC and PELS. Supervision: PP, AB and PELS. Visualization: EM and IP. Writing – original draft: FC, IP and PELS. Writing – review & editing: CR, LG, FC, IP and PELS.

Data availability

The dataset underlying this article is available in Zenodo and can be accessed only upon request because it includes sensitive data. Please address your request to the corresponding author if interested.

Competing interests

The authors declare no competing interests.

References

1. Marion, L. L. & Meeks, G. R. Ectopic pregnancy: History, incidence, epidemiology, and risk factors. Clin Obstet Gynecol 55, 376–386, doi:10.1097/GRF.0b013e3182516d7b (2012).

2. Khan, K. S., Wojdyla, D., Say, L., Gülmezoglu, A. M. & Van Look, P. F. WHO analysis of causes of maternal death: a systematic review. Lancet 367, 1066–1074, doi:10.1016/S0140-6736(06)68397-9 (2006).

3. Clayton, H. B. et al. Ectopic pregnancy risk with assisted reproductive technology procedures. Obstet Gynecol 107, 595–604, doi:10.1097/01.AOG.0000196503.78126.62 (2006).

4. Perkins, K. M., Boulet, S. L., Kissin, D. M., Jamieson, D. J. & Group, N. A. S. N. Risk of ectopic pregnancy associated with assisted reproductive technology in the United States, 2001–2011. Obstet Gynecol 125, 70–78, doi:10.1097/AOG.0000000000000584 (2015).

5. Smith, L. P., Oskowitz, S. P., Dodge, L. E. & Hacker, M. R. Risk of ectopic pregnancy following day-5 embryo transfer compared with day-3 transfer. Reprod Biomed Online 27, 407–413, doi:10.1016/j.rbmo.2013.06.015 (2013).

6. Santos-Ribeiro, S., Tournaye, H. & Polyzos, N. P. Trends in ectopic pregnancy rates following assisted reproductive technologies in the UK: a 12-year nationwide analysis including 160 000 pregnancies. Hum Reprod 31, 393–402, doi:10.1093/humrep/dev315 (2016).

7. Huang, B. et al. Is frozen embryo transfer cycle associated with a significantly lower incidence of ectopic pregnancy? An analysis of more than 30,000 cycles. Fertil Steril 102, 1345–1349, doi:10.1016/j.fertnstert.2014.07.1245 (2014).

8. Centers for Disease Control and Prevention (CDC). Ectopic pregnancy–United States, 1990–1992. MMWR Morb Mortal Wkly Rep 44, 46–48 (1995).

9. Medicine, S. f. A. R. T. a. t. A. S. f. R. Assisted reproductive technology in the United States: 1998 results generated from the American Society for Reproductive Medicine/Society for Assisted
Reproductive Technology Registry. *Fertil Steril* **77**, 18–31, doi:10.1016/s0015-0282(01)02985-5 (2002).

10. Scaravelli G, D. L. R., Vigiliano V, Bolli S, Spoletini R, Fiaccavento S, Bertini A, Speziale L. (www.salute.gov.it, 2019).

11. Muller, V. *et al.* Ectopic pregnancy following in vitro fertilization: meta-analysis and single-center experience during 6 years. *Gynecol Endocrinol* **32**, 69–74, doi:10.1080/09513590.2016.1232550 (2016).

12. Fang, C., Huang, R., Wei, L. N. & Jia, L. Frozen-thawed day 5 blastocyst transfer is associated with a lower risk of ectopic pregnancy than day 3 transfer and fresh transfer. *Fertil Steril* **103**, 655–661.e653, doi:10.1016/j.fertnstert.2014.11.023 (2015).

13. Li, R. R. *et al.* Comparative study of pregnancy outcomes between day 3 embryo transfer and day 5 blastocyst transfer in patients with progesterone elevation. *J Int Med Res* **41**, 1318–1325, doi:10.1177/0300060513489480 (2013).

14. Bu, Z., Xiong, Y., Wang, K. & Sun, Y. Risk factors for ectopic pregnancy in assisted reproductive technology: a 6-year, single-center study. *Fertil Steril* **106**, 90–94, doi:10.1016/j.fertnstert.2016.02.035 (2016).

15. Shi, W. *et al.* Comparison of perinatal outcomes following blastocyst and cleavage-stage embryo transfer: analysis of 10 years' data from a single centre. *Reprod Biomed Online* **38**, 967–978, doi:10.1016/j.rbmo.2018.12.031 (2019).

16. Decler, W., Osmanagaoglu, K., Meganck, G. & Devroey, P. Slightly lower incidence of ectopic pregnancies in frozen embryo transfer cycles versus fresh in vitro fertilization-embryo transfer cycles: a retrospective cohort study. *Fertil Steril* **101**, 162–165, doi:10.1016/j.fertnstert.2013.10.002 (2014).

17. Londra, L. *et al.* Ectopic pregnancy after in vitro fertilization: differences between fresh and frozen-thawed cycles. *Fertil Steril* **104**, 110–118, doi:10.1016/j.fertnstert.2015.04.009 (2015).

18. Dubuisson, J. B. *et al.* Risk factors for ectopic pregnancy in 556 pregnancies after in vitro fertilization: implications for preventive management. *Fertil Steril* **56**, 686–690, doi:10.1016/s0015-0282(16)54600-7 (1991).

19. Talbot, P. & Riveles, K. Smoking and reproduction: the oviduct as a target of cigarette smoke. *Reprod Biol Endocrinol* **3**, 52, doi:10.1186/1477-7827-3-52 (2005).

20. Stewart, L. M., Stewart, C. J. R., Spilsbury, K., Cohen, P. A. & Jordan, S. Association between pelvic inflammatory disease, infertility, ectopic pregnancy and the development of ovarian serous borderline tumor, mucinous borderline tumor and low-grade serous carcinoma. *Gynecol Oncol*, doi:10.1016/j.ygyno.2020.01.027 (2020).

21. Zeng, M. F. & Li, L. M. Frozen blastocyst transfer reduces incidence of ectopic pregnancy compared with fresh blastocyst transfer: a meta-analysis. *Gynecol Endocrinol* **35**, 93–99, doi:10.1080/09513590.2018.1497154 (2019).

22. Xing, W., Ou, J. & Cai, L. Thawed embryo transfer and ectopic pregnancy: a meta-analysis. *Arch Gynecol Obstet* **297**, 1345–1352, doi:10.1007/s00404-018-4724-6 (2018).
23. Cirillo, F. *et al.* The human factor: does the operator performing the embryo transfer significantly impact the cycle outcome? Hum Reprod, doi:10.1093/humrep/dez290 (2020).

24. Zegers-Hochschild, F. *et al.* The International Glossary on Infertility and Fertility Care, 2017. *Fertil Steril* **108**, 393–406, doi:10.1016/j.fertnstert.2017.06.005 (2017).

25. Kirk, E. *et al.* Terminology for describing normally sited and ectopic pregnancies on ultrasound: ESHRE recommendations for good practice. Hum Reprod Open **2020**, hoaa055, doi:10.1093/hropen/hoaa055 (2020).

26. Immediata, V. *et al.* Twenty-one year experience with intrauterine inseminations after controlled ovarian stimulation with gonadotropins: maternal age is the only prognostic factor for success. J Assist Reprod Genet, doi:10.1007/s10815-020-01752-3 (2020).

27. Ovarian Stimulation, T. E. G. G. *et al.* ESHRE guideline: ovarian stimulation for IVF/ICSI. Hum Reprod Open **2020**, hoaa009, doi:10.1093/hropen/hoaa009 (2020).

28. Blumenfeld, Z. The Ovarian Hyperstimulation Syndrome. *Vitam Horm* **107**, 423–451, doi:10.1016/bs.vh.2018.01.018 (2018).

29. O'Flynn, N. Assessment and treatment for people with fertility problems: NICE guideline. *Br J Gen Pract* **64**, 50–51, doi:10.3399/bjgp14X676609 (2014).

30. Levi-Setti, P. E. *et al.* Appraisal of clinical complications after 23,827 oocyte retrievals in a large assisted reproductive technology program. *Fertil Steril* **109**, 1038–1043.e1031, doi:10.1016/j.fertnstert.2018.02.002 (2018).

31. Zhang, J. & Yu, K. F. What's the relative risk? A method of correcting the odds ratio in cohort studies of common outcomes. JAMA **280**, 1690–1691, doi:10.1001/jama.280.19.1690 (1998).

32. Jwa, S. C. *et al.* Ovarian stimulation increases the risk of ectopic pregnancy for fresh embryo transfers: an analysis of 68,851 clinical pregnancies from the Japanese Assisted Reproductive Technology registry. *Fertil Steril* **114**, 1198–1206, doi:10.1016/j.fertnstert.2020.06.032 (2020).

33. Chang, H. J. & Suh, C. S. Ectopic pregnancy after assisted reproductive technology: what are the risk factors? *Curr Opin Obstet Gynecol* **22**, 202–207, doi:10.1097/GCO.0b013e32833848fd (2010).

34. Fanchin, R. *et al.* Hormonal influence on the uterine contractility during ovarian stimulation. Hum Reprod **15 Suppl 1**, 90–100, doi:10.1093/humrep/15.suppl_1.90 (2000).

35. Mueller, A. *et al.* Role of estrogen and progesterone in the regulation of uterine peristalsis: results from perfused non-pregnant swine uterus. Hum Reprod **21**, 1863–1868, doi:10.1093/humrep/del056 (2006).

36. Zhang, Y. L., Sun, J., Su, Y. C., Guo, Y. H. & Sun, Y. P. Ectopic pregnancy in frozen-thawed embryo transfer: a retrospective analysis of 4,034 cycles and related factors. *Syst Biol Reprod Med* **59**, 34–37, doi:10.3109/19396368.2012.731470 (2013).

37. Paltieli, Y. *et al.* High progesterone levels and ciliary dysfunction— a possible cause of ectopic pregnancy. *J Assist Reprod Genet* **17**, 103–106, doi:10.1023/a:1009465900824 (2000).
38. Glujovsky, D., Blake, D., Farquhar, C. & Bardach, A. Cleavage stage versus blastocyst stage embryo transfer in assisted reproductive technology. Cochrane Database Syst Rev, CD002118, doi:10.1002/14651858.CD002118.pub4 (2012).

39. Li, Z., Sullivan, E. A., Chapman, M., Farquhar, C. & Wang, Y. A. Risk of ectopic pregnancy lowest with transfer of single frozen blastocyst. Hum Reprod 30, 2048–2054, doi:10.1093/humrep/dev168 (2015).

40. Patil, M. Ectopic pregnancy after infertility treatment. J Hum Reprod Sci 5, 154–165, doi:10.4103/0974-1208.101011 (2012).

41. Parashi, S., Moukhah, S. & Ashrafi, M. Main risk factors for ectopic pregnancy: a case-control study in a sample of Iranian women. Int J Fertil Steril 8, 147–154 (2014).

42. Kim, S. W. et al. Correlation between Ovarian Reserve and Incidence of Ectopic Pregnancy after. Yonsei Med J 60, 285–290, doi:10.3349/ymj.2019.60.3.285 (2019).

43. Malak, M., Tawfeeq, T., Holzer, H. & Tulandi, T. Risk factors for ectopic pregnancy after in vitro fertilization treatment. J Obstet Gynaecol Can 33, 617–619, doi:10.1016/S1701-2163(16)34910-6 (2011).

44. Weigert, M., Gruber, D., Pernicka, E., Bauer, P. & Feichtinger, W. Previous tubal ectopic pregnancy raises the incidence of repeated ectopic pregnancies in in vitro fertilization-embryo transfer patients. J Assist Reprod Genet 26, 13–17, doi:10.1007/s10815-008-9278-2 (2009).

45. Li, C. et al. Risk factors for ectopic pregnancy in women with planned pregnancy: a case-control study. Eur J Obstet Gynecol Reprod Biol 181, 176–182, doi:10.1016/j.ejogrb.2014.07.049 (2014).

46. Tan, C. W. et al. Endometriosis, endometrium, implantation and fallopian tube. Human Reproduction 28, i206-i226, doi:10.1093/humrep/det211 (2013).

47. Mains, L. & Van Voorhis, B. J. Optimizing the technique of embryo transfer. Fertil Steril 94, 785–790, doi:10.1016/j.fertnstert.2010.03.030 (2010).

48. Lesny, P., Killick, S. R., Tetlow, R. L., Robinson, J. & Maguiness, S. D. Embryo transfer—can we learn anything new from the observation of junctional zone contractions? Hum Reprod 13, 1540–1546, doi:10.1093/humrep/13.6.1540 (1998).

49. Bulletti, C. & de Ziegler, D. Uterine contractility and embryo implantation. Curr Opin Obstet Gynecol 18, 473–484, doi:10.1097/01.gco.0000233947.97543.c4 (2006).

50. Suresh, Y. N. The role of tubal patency tests and tubal surgery in the era of assisted reproductive techniques. The Obstetrician & Gynaecologist 16, 1467–2561, doi:10.1111/tog.12070 (2014).

51. Annan, J. J., Gudi, A., Bhide, P., Shah, A. & Homburg, R. Biochemical pregnancy during assisted conception: a little bit pregnant. J Clin Med Res 5, 269–274, doi:10.4021/jocmr1008w (2013).

52. Zeadna, A., Son, W. Y., Moon, J. H. & Dahan, M. H. A comparison of biochemical pregnancy rates between women who underwent IVF and fertile controls who conceived spontaneously†. Hum Reprod 30, 783–788, doi:10.1093/humrep/dev024 (2015).
Figures

Enrollment

Enrollable cycles (n) = 27,389

Excluded cycles (n) = 20,024
- Not meeting inclusion criteria (n=0)
- Negative outcome (n=20,024)

Follow-up

Patients lost to follow-up (n) = 13

Analysis

Analyzed (n) = 7,352
Excluded (n) = 0

Figure 1

Study flowchart.
Figure 2

**EP% per two-years periods for pregnant women.** EP: ectopic pregnancy; ET: Embryo Transfer; IUI: intrauterine insemination.

### Supplementary Files

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- Supplementarytables1and2.docx