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ARTICLE

Collecting semen samples at home for IVF/ICSI does not negatively affect the outcome of the fresh cycle

BIOGRAPHY

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KEY MESSAGE

Collecting semen at home for IVF/ICSI treatment resulted in higher sperm concentration and motility compared with semen collected at the clinic. Cycle outcomes were similar between the two groups, which suggests that collecting semen samples at home is not detrimental to treatment outcome.

ABSTRACT

Research question: Does the site of semen collection influence IVF/intracytoplasmic sperm injection (ICSI) cycle outcome?

Design: Retrospective study performed at the University Medical Centre Ljubljana, including all stimulated and modified natural IVF/ICSI cycles (with at least one oocyte retrieved) performed in 2019 with fresh ejaculated semen samples. IVF/ICSI cycle outcomes, in terms of oocytes, embryos and pregnancy rates according to site of semen sample collection (at home or at clinic) were evaluated.

Results: Samples collected at clinic had significantly lower sperm concentration (median [interquartile range, IQR], 50 [20–100] million/ml versus 70 [30–100] million/ml, adjusted odds ratio [OR] 0.001, 95% confidence interval [CI] 1.574×10^{-6} to 0.196, $P = 0.012$) and motility (60 [50–70]% versus 70 [50–70]%, adjusted OR 0.034, 95% CI 0.002 to 0.563, $P = 0.018$, adjusted for age). There was no difference in total sperm count, semen volume or sperm morphology, or women's age (36 [32–39] versus 36 [33–39] years) and men's age (37 [34–41] versus 38 [34–42] years), between semen samples collected at clinic versus at home. When all IVF/ICSI cycles were analysed together using generalized estimating equation analysis, no significant difference in cycle outcomes attributed to site of semen sample collection was observed. There were also no significant differences in cycle outcomes when only first cycles were analysed.

Conclusions: Collecting semen samples at home has a positive effect on sperm quality (sperm concentration and motility were higher), but no significant differences in cycle outcomes are observed when these samples are used in IVF/ICSI cycles. Therefore, it is suggested that collecting semen samples at home for IVF/ICSI procedures is safe and has no negative effect on treatment outcomes.

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INTRODUCTION

Coronavirus disease 2019 (COVID-19) is forcing people in professional and private life to adhere to strict preventive measures to minimize the possibility of spreading the virus. The medically assisted reproduction field is no exception, and guidelines from various professional societies (*American Society for Reproductive Medicine, 2020*; *ESHRE COVID-19 Working Group, 2020*; *Veiga et al., 2020*) have been prepared on how to restart treatments or how to alter the work to make it as safe as possible. The guidelines suggest that patients spend the shortest possible time at the clinic, indicating that as much communication as possible should be conducted by telephone, e-mail, etc. The aim is not to have patients spend unnecessary time in waiting rooms, and another way to achieve this is to have them bring in semen samples collected at home. With regard to semen sample collection, the World Health Organization (WHO) recommends that in normal situations, semen samples should be collected at a clinic near the laboratory to reduce the chances of negative external influences on semen quality (*World Health Organization, 2010*). The WHO manual suggests that semen should be collected at home only when the patient has difficulty providing a sample via masturbation at the clinic or when there is no appropriate venue for semen collection near the laboratory. The use of such an approach should take into consideration that even short exposure of spermatozoa to seminal plasma might negatively influence spermatozoa quality (*Björndahl and Kvist, 2003*). The reason for this impact is that seminal plasma is rich in various endogenous compounds, whose concentrations can vary from man to man, and these compounds can have various impacts on spermatozoa quality (*Björndahl and Kvist, 2003*). Another issue is the presence of somatic cells in seminal plasma. These cells are, besides abnormal spermatozoa, the main source of reactive oxygen species (ROS) (*Aitken and Baker, 1995*). Small concentrations of ROS are essential for normal sperm functioning, but when the amount exceeds the capacity of cellular defensive mechanisms, there can be oxidative stress, with a serious detrimental effect on sperm DNA (*Agarwal et al., 2014*). Another important aspect is the temperature at which the semen is transported to the laboratory. The WHO manual suggests that semen should be kept at temperatures of 20–37°C during transport. It was shown more than 40 years ago that if any delay in semen analysis is expected, the semen should be kept at room temperature (20°C), because the motility and viability decrease significantly faster at 37°C (*Appell and Evans, 1977*). In addition, *Appell and Evans (1977)* showed that if semen is kept at 4°C, then most of the spermatozoa are immotile after 6 h, although viability decreases more slowly and remains high after 18 h. Similarly, *Esfandiari et al. (2002)* confirmed that motility significantly decreases if semen is kept at 4°C but, in contrast, suggested that motility is the highest and ROS levels are the lowest if semen is kept at 37°C. If semen is incubated for only 2 h at 37°C, morphological impairment of sperm nuclei (large vacuoles) can also be observed, while if incubated at 21°C, no such negative effect is observed (*Peer et al., 2007*). Furthermore, the influence of temperature on spermatozoa quality was also studied in native semen as a function of the semen preparation method (density gradient centrifugation [DGC] versus the swim-up technique) and long-term incubation (*Thijssen et al., 2014*). It was revealed that after 24 h of incubation at room temperature (23°C), the motility, viability and morphology were significantly higher than they were in samples incubated at 35°C, but the study could not draw any conclusions regarding which semen preparation method was better in these conditions.

Based on available data, there is still no firm conclusion regarding whether collecting semen samples at home has any influence on sperm quality or on IVF/ICSI cycle outcome. The study centre is currently using this approach on a daily basis, so its data is potentially useful in this discussion. A few years ago, when the clinic was being renovated, patients were strongly encouraged to collect semen samples at home and bring them to the laboratory for IVF/ICSI procedures. Because no negative influence on the outcome of IVF/ICSI cycles following this approach was observed, this practice was continued. Today, due to the COVID-19 situation, this approach is widely advised, but because data regarding its success are limited, it was decided to analyse data from the study centre and present the outcome of the ICSI/IVF cycles in terms of oocytes, embryos and pregnancy rates according to the semen sample collection approach (at home or at clinic).

MATERIALS AND METHODS

This retrospective study was carried out at the Department of Human Reproduction, Division of Obstetrics and Gynaecology, University Medical Centre Ljubljana. All stimulated and modified natural IVF/ICSI cycles performed from January 2019 to December 2019 were included, where fresh ejaculated semen samples (collected at the clinic or at home) were used for conventional IVF or ICSI procedures and at least one oocyte was retrieved. The data for these cycles were collected from the study centre's institutional database of assisted reproductive technology (ART) procedures. The collection and analysis of these data in anonymized form is allowed by the Personal Data Protection Act (Article 17, Official Gazette of the Republic of Slovenia No 94/07, 2004) and by the Healthcare Databases Act (Official Gazette of the Republic of Slovenia No 65/00, 2000; No 47/15, 2015; 31/18, 2018). The National Medical Ethics Committee of Slovenia (0120-174/2018/6) also allows the collection of anonymized data for observational study in standardized treatments in the usual management of patients. Before starting the treatment, each patient signs an informed consent for the procedures and to allow data collection and analysis in anonymized form for research purposes.

The clinic at the study centre offers all patients the option of collecting semen samples at home if they prefer. If the patient collects semen at the clinic, all instructions are given just before collection, but if the patient collects semen at home, all instructions are given in advance in written form, and sterile containers are provided on the day on which the follicles are large enough and women receive instructions about when to administer human chorionic gonadotrophin (HCG). In this case, men are advised to ejaculate on that day, not to have too long an abstinence and are instructed to carry out the semen collection for the IVF/ICSI procedure just before the couple is about to leave home and go to the clinic for oocyte retrieval. This means that ejaculation abstinence is similar for all patients, i.e. approximately 2 days. Usually, the semen is delivered to the IVF laboratory within 1–1.5 h after collection. Patients are instructed to keep the container with the semen sample close to body temperature during transport.

Ovarian stimulation protocols

All women underwent ovarian stimulation, which was achieved with a gonadotrophin-releasing hormone (GnRH) antagonist or a GnRH agonist protocol. In the GnRH antagonist protocol, ovarian stimulation started on day 2 of the cycle. The daily dose of recombinant or urinary gonadotrophin was tailored according to the patient's clinical parameters and ranged from doses of 150 IU to 300 IU. GnRH antagonist cetrorelix acetate (Cetrotide; ASTA Medica AG, Frankfurt, Germany) or ganirelix (Orgalutran; Organon, Dublin, Ireland) were co-administered subcutaneously daily at a dose of 0.25 mg when the dominant follicle measured ≥13 mm in diameter. When at least three follicles measured ≥17 mm in diameter, 5.000 IU of human chorionic gonadotropin (hCG) (Pregnyl; Organon, Dublin Ireland) or 6.500 IU (250 mcg) of recombinant hCG alpha (Ovitrelle; Merck Serono Europe, London, UK) was administered. Ultrasound-guided transvaginal oocyte retrieval was carried out 36 h after HCG administration.

In the GnRH agonist protocol, daily administration of 0.6 mg of GnRH agonist buserelin acetate (Suprefact; Sanofi-Aventis, Frankfurt, Germany) was started on the 22nd day of the previous menstrual cycle. After 15 days of treatment or when the endometrium was thin and the ovaries were dormant, a daily dose of gonadotrophin was added. The daily dose of recombinant or urinary gonadotrophin was tailored according to the patient's clinical parameters and ranged from 200 to 300 IU. When at least three follicles measured ≥ 20 mm in diameter, 5.000 IU of hCG (Pregnyl; Organon) or 6.500 IU (250 mcg) of recombinant hCG alpha (Ovitrelle; Merck Serono Europe) was administered. Ultrasound-guided transvaginal oocyte retrieval was carried out 36 h after HCG administration.

Few patients underwent the IVF procedure in a modified natural cycle. They underwent transvaginal ultrasonographic examination from day 9 of the cycle onward. Serum oestradiol was measured daily, and urine samples were tested for the presence of LH surge with a commercially available kit (RapiTest LH; Morwell Diagnostics GmbH, Egg, Switzerland). When the dominant follicle measured 16 mm in diameter, serum oestradiol exceeded 0.39 nmol/l, and no LH surge was detected in the urine sample, >>5000 IU of hCG (Pregnyl; Organon) or 6.500 IU (250 mcg) of recombinant hCG alpha (Ovitrelle; Merck Serono Europe) was administered, and aspiration of the follicle was performed 31–32 h after HCG administration.

Semen preparation

If a semen sample was collected at the clinic, it was incubated for 30 min at room temperature to allow liquefaction, and then it was assessed for volume, concentration and motility. If the sample was collected at home, the assessment was performed immediately after the laboratory received the sample. All assessments were performed at room temperature. The volume was assessed using a graduated disposable pipette. Sperm concentration and total motility were assessed using 20µm 10 × 10 grid disposable counting slides (CellVision, Heerhugowaard, The Netherlands) according to the manufacturer's instructions. Briefly, 5 µl of semen was added to slide, left for 5–10 min to stabilize, and then, where possible, at least 200 spermatozoa were counted per slide using a phase contrast microscope (400 × magnification). Sperm counting was performed once unless the count was obviously different to the sperm count from previous cycles or from the spermiogram, in which case the counting was repeated. The same approach was applied to sperm motility assessment, which was evaluated from the same sample as sperm counting and calculated as all counted motile spermatozoa divided by count of all spermatozoa. Sperm motility was evaluated under a phase contrast microscope (400 × magnification) and spermatozoa were classified only as motile or immotile. The spermatozoa morphology was not evaluated on the day of oocyte retrieval, although it was evaluated in previous samples when the diagnostic spermiogram was performed. All samples for diagnostic spermiograms were collected at the clinic. At that point, semen smears were stained using a Papanicolaou method to evaluate the morphology (using strict Tygerberg criteria). Where possible, at least 200 spermatozoa were assessed under 1000 × magnification. Sperm vitality and antisperm antibodies were not examined on the day of oocyte aspiration, but only when a diagnostic spermiogram was performed.

In all cases, after initial assessment, the samples were prepared using DGC (100% layer and 40% layer of Pure Sperm [Nidacon, Mölndal, Sweden]) for 20 min at 225*g* at room temperature. Then, the 100% layer was washed in 4 ml of Sperm Preparation Medium (Origio, Måløv, Denmark), which was followed by centrifugation for 10 min at 300*g* at room temperature. After centrifugation, the supernatant was discarded, and 0.3–1 ml of Sperm Preparation Medium was added to enable the swim-up. The samples were then put into an incubator at 37°C. After approximately 2 h of incubation, these samples were either prepared for ICSI or a conventional insemination of cumulus–oocyte complexes (COC) was performed.

Embryo culture and embryo transfer

The morning after insemination, the oocytes were examined for the presence of pronuclei. Normally fertilized oocytes (with two pronuclei [2PN]) were further cultured in continuous culture medium, SAGE 1-Step (Origio), or in sequential G-series media (Vitrolife, Västra Frölunda, Sweden). In cases of sequential media use, embryos were cultured in G1 Plus medium (Vitrolife) until the third day, and then they were transferred to G-2 Plus medium (Vitrolife). In cases where there were only one or two embryos, they were transferred into the uterus on the third day of development, at the cleavage stage. In the majority of other cases, when there were more than two embryos, embryo transfer was performed on day 5 at the blastocyst stage. Embryo transfers were performed using a Guardia™ Access Embryo Transfer Catheter (Cook Medical, Bloomington, IN, USA), and in all embryo transfers, only one or a maximum of two embryos were transferred. Supernumerary embryos reaching the blastocyst stage were vitrified.

Definitions

To determine how many embryos were indeed transferred or had the potential to be transferred in the future, the utilization rate, defined as the proportion of all transferred and cryopreserved embryos per number of all obtained embryos (regardless of the day of transfer or of cryopreservation), was calculated. When calculating the pregnancy rate, the embryo transfer on day 3 and day 5 were combined and were not analysed separately. Pregnancy was defined as a positive beta-HCG test 15 days after embryo transfer.

Statistical analysis

To determine the differences between the groups for the age data of included IVF/ICSI cycles and for cycle outcomes

TABLE 1 RESULTS OF GEE ANALYSES FOR THE BASELINE SEMEN CHARACTERISTICS

			Unadjusted OR		Adjusted OR for men's age	
	Collected at home	Collected at clinic	Collected at home	Collected at clinic OR (95% CI)	Collected at home	Collected at clinic OR (95% CI)
Semen volume (ml)[a]	2.5 (2–3)	2.5 (2–3)	1 (ref)	0.967 (0.752, 1.244)	1 (ref)	0.964 (0.744, 1.247)
Concentration in million/ml[a]	70 (30–100)	50 (20–100)	1 (ref)	0.001 (1.834×10^{-6}, 0.224)*	1 (ref)	0.001 (1.574×10^{-6}, 0.196)**
Total sperm count (in million)[a]	140 (60–210)	100 (30–210)	1 (ref)	1.690×10^{-7} (4.260×10^{-16}, 67.033)	1 (ref)	2.302×10^{-7}, (6.043×10^{-16}, 87.671)
Motility (%)[a]	70 (50–70)	60 (50–70)	1 (ref)	0.035 (0.002, 0.593)***	1 (ref)	0.034 (0.002, 0.563)****
Morphology (percentage of normal spermatozoa)[b]	6 (3–12)	5.5 (2–10)	1 (ref)	1.090 (0.483, 2.461)	1 (ref)	1.020 (0.450, 2.310)

Values reported as median (IQR).

CI = confidence interval; GEE = generalized estimating equation; IQR = interquartile range; OR = odds ratio.

[a] Assessed on the day of oocyte retrieval based on 837 cycles (694 men) in the collected at home group and 244 cycles (210 men) in the collected at clinic group.

[b] Assessed in a diagnostic spermiogram using a different semen sample collected prior to the day of oocyte retrieval; samples collected at the clinic in all cases (n = 904). Data are based on 837 cycles and 244 cycles included in the collected at home group and the collected at clinic group. In the collected at home group there were 694 men and in the collected in clinic group 210 men.

* Statistically significant difference with P = 0.014.

** Statistically significant difference with P = 0.012.

*** Statistically significant difference with P = 0.020.

**** Statistically significant difference with P = 0.018.

for first cycles only, Pearson's chi-squared and Mann–Whitney U-tests were used to analyse the data as appropriate (the normality of data was analysed with the Shapiro–Wilk test). Where appropriate, numerical data are presented as median and interquartile range, and P-values <0.05 were recognized as statistically significant. The G*Power program, version 3.1.9.4 (*Faul et al., 2007*) was used to calculate the statistical power of analysis of first cycles only. To consider the dependence between cycle outcomes and baseline semen characteristics from the same couple, generalized estimating equations (GEE) analysis was performed. Odds ratios (OR) and 95% confidence intervals (CI) were estimated in unadjusted and adjusted models, in which the following covariates were included: women's and men's age, dose of gonadotrophins, number of retrieved oocytes and sperm quality.

RESULTS

This study retrospectively analysed the outcome of IVF/ICSI cycles from 2019, when fresh ejaculated semen collected at the clinic or at home was used to fertilize oocytes. Altogether, there were 1081 cycles with at least one oocyte retrieved. Most of the cycles were stimulated (n = 1062); only 19 were modified natural cycles. The median age of the women was 36 (32–39) years, and the median number of oocytes obtained per cycle was 7 (4–11.5). The fertilization rate per all COC retrieved was 52.3% and 63.7% if the estimated number of metaphase II (MII) oocytes was used for calculation. The cleavage rate of fertilized oocytes was 97.9%, which resulted in 3 (2–6) embryos per cycle. Some embryos were transferred at the cleavage stage on day 3, but most were cultured until day 5/6, and 50.9% of them developed to blastocysts. When the utilization rate of embryos was calculated, 55.8% of them were usable. Embryo transfer was performed in 78.2% of cycles, while the rest of the cycles were either freeze-all (11.5%), or no appropriate embryo for embryo transfer or cryopreservation was obtained in the cycle (10.3%). The median number of transferred embryos was 1 (1–2), and the pregnancy rate per embryo transfer was 34.0%.

For the analysis of cycles according to the site of sperm sample collection, there were 837 cycles (694 couples) where semen samples were collected at home and 244 cycles (210 couples) where semen samples were collected at the clinic. Samples collected at clinic had significantly lower median concentration of spermatozoa per ml (values expressed as median [IQR], 50 [20–100] million/ml versus 70 [30–100] million/ml, adjusted OR 0.001, 95% CI 1.574×10^{-6} to 0.196, P = 0.012), and motility (60 [50–70]% versus 70 [50–70]%, adjusted OR 0.034, 95% CI 0.002 to 0.563, P = 0.018). There was no difference in total sperm count or semen volume. Furthermore, no difference was found in sperm morphology, in a different semen sample used for the diagnostic spermiogram collected at the clinic in all cases, between the collected at home and collected at clinic groups (TABLE 1).

When baseline characteristics of included IVF/ICSI cycles according to the site of semen sample collection were analysed, no significant difference was observed in women's age (at clinic versus at home, 36 [32–39] versus 36 [33–39] years) and men's age (37 [34–41] versus 38 [34–42] years), in median number of COC and embryos, or of the proportion of immature oocytes. To analyse all other outcomes of IVF/ICSI cycles, GEE analysis was conducted and it revealed that most of the outcomes were similar between the groups, although a few differences were observed (TABLE 2). In the group of samples collected at clinic, a significantly lower proportion of polyploidies was observed (6.2% versus 7.5%, adjusted OR 0.976, 95% CI 0.953–1.000, P = 0.046) and a significantly higher proportion of cycles with cryopreservation, but without embryo transfer (freeze-all) (13.1% versus 11.1%, adjusted OR 1.080, 95% CI 1.003–1.163, P = 0.041). While the GEE analysis considers the dependence between cycles from the same couple, an alternative way to assess the outcomes is to analyse first cycles only. There were 379 first cycles in the semen collected at home group and 87 in the collected

TABLE 2 RESULTS OF GEE ANALYSES FOR THE ASSOCIATION OF THE SITE OF SEMEN COLLECTION AND THE IVF/ICSI CYCLE CHARACTERISTICS AND OUTCOMES

			Unadjusted OR		Adjusted OR for women's and men's age, dose of gonadotrophins, number of oocytes retrieved and sperm quality	
	Collected at home	Collected at clinic	Collected at home	Collected at clinic OR (95% CI)	Collected at home	Collected at clinic OR (95% CI)
Number of cycles	837	244				
Number of COC	7215	2221				
Median COC number per cycle	7 (4–11)	8 (4–12)	1 (ref)	1.524 (0.595, 3.905)	1 (ref)	2.932 (0.780, 11.016)
Number of immature oocytes (%)	1247 (17.3)	436 (19.6)	1 (ref)	1.018 (0.991, 1.047)	1 (ref)	1.018 (0.979, 1.058)
Number of MII oocytes	5968	1785				
% of fertilized oocytes (% calculated on MII oocytes number)	3803 (63.7)	1135 (63.6)	1 (ref)	0.999 (0.964, 1.036)	1 (ref)	1.020 (0.969, 1.074)
Fertilized oocytes (% calculated on COC number)	52.7	51.1	1 (ref)	0.988 (0.953, 1.023)	1 (ref)	0.999 (0.949, 1.051)
Polyploidies (% calculated on MII oocytes number)	449 (7.5)	110 (6.2)	1 (ref)	0.983 (0.965, 1.002)	1 (ref)	0.976 (0.953, 1.000)*
Embryos (%)	3721 (97.8)	1110 (97.8)	1 (ref)	1.035 (1.004, 1.067)**	1 (ref)	1.043 (0.999, 1.089)
Embryos per cycle (median number)	3 (2–6)	4 (2–6)	1 (ref)	1.085 (0.611, 1.926)	1 (ref)	1.648 (0.726, 3.738)
Number of embryos cultured until day 5/6 (median number per cycle)	3516 (3 [1–6])	1044 (4 [1–6])	1 (ref)	1.043 (0.568, 1.916)	1 (ref)	1.689 (0.712, 4.008)
Blastocyst rate (rate per embryos cultured until day 5/6)	1835 (52.2)	484 (46.4)	1 (ref)	0.977 (0.932, 1.025)	1 (ref)	1.025 (0.956, 1.099)
Embryo utilization rate[a]	2112 (56.7)	582 (52.4)	1 (ref)	0.998 (0.952, 1.047)	1 (ref)	0.998 (0.932, 1.070)
Number and proportion of embryos transferred/cryopreserved on day 3 with respect to the number of all utilized embryos	207 (9.8)	66 (11.3)	1 (ref)	1.012 (0.956, 1.070)	1 (ref)	0.961 (0.890, 1.037)
Number of cycles with at least one blastocyst	531 (63.4)	155 (63.5)	1 (ref)	1.072 (0.783, 1.466)	1 (ref)	1.064 (0.465, 1.239)
Embryo cryopreservation rate (rate per all embryos)	1293 (34.7)	333 (30.0)	1 (ref)	0.983 (0.946, 1.022)	1 (ref)	1.000 (0.956, 1.184)
Number of cycles with cryopreservation	376 (44.9)	106 (43.4)	1 (ref)	0.927 (0.904, 1.045)	1 (ref)	0.987 (0.885, 1.080)
Number of cycles with cryopreservation/ without ET	93 (11.1)	32 (13.1)	1 (ref)	1.020 (0.971, 1.072)	1 (ref)	1.080 (1.003, 1.163)**
Number of cycles without cryopreservation/ without ET	91 (10.9)	20 (8.2)	1 (ref)	0.973 (0.935, 1.014)	1 (ref)	0.954 (0.897, 1.015)
Number of ET	653 (78.0)	192 (78.7)	1 (ref)	1.009 (0.950, 1.071)	1 (ref)	0.974 (0.888, 1.068)
Median number of transferred embryos	1 (1–2)	1 (1–2)	1 (ref)	1.035 (0.954, 1.123)	1 (ref)	1.068 (0.930, 1.226)
Number of ET with single embryo transferred	487 (74.6)	135 (70.3)	1 (ref)	0.966 (0.890, 1.048)	1 (ref)	1.380 (0.690, 2.761)
Pregnancies (rate per ET)[b]	221 (33.8)	66 (34.4)	1 (ref)	1.001 (0.926, 1.081)	1 (ref)	1.035 (0.924, 1.160)

Values reported as median (IQR).

CI = confidence interval; COC = cumulus–oocyte complex; ET = embryo transfer; GEE = generalized estimating equation; MII = metaphase II; OR = odds ratio.

[a] Embryo utilization rate was defined as the proportion of transferred/cryopreserved embryos per number of all obtained embryos.

[b] Pregnancy was defined as a positive beta-HCG test 15 days after embryo transfer.

* Statistically significant difference with $P = 0.046$.

** Statistically significant difference with $P = 0.028$.

***Statistically significant difference with $P = 0.041$.

at clinic group, which represents 45.3% and 35.7% of all included cycles in each group, respectively (TABLE 3). In this case, the results did not reveal any statistically significant difference between the groups.

Further, to check whether any difference can be observed in subgroups of patients, some additional GEE analyses were performed. Analysis of pregnancy rates accounting for the methods used to fertilize oocytes (conventional IVF versus ICSI) did not reveal any significant difference (TABLE 4). To determine whether a significantly higher proportion of immature oocytes in the group of patients who collected semen at the clinic influenced preimplantation embryo development, only cycles where the number of retrieved oocytes was greater than the overall mean number were analysed, and patients who had a proportion of immature oocytes that was higher than the average overall

TABLE 3 IVF/ICSI CYCLE OUTCOMES ACCORDING TO THE SITE OF SEMEN SAMPLE COLLECTION FOR FIRST CYCLES ONLY

	Collected at home	Collected at clinic	*P*-value
Number of first cycles	379	87	
Median female age, years	35 (31–38)	35 (31–39)	0.944
Number of COC	3549	874	
Median COC number	8 (4–12)	8 (4–13)	0.435
Number of immature oocytes	550	143	
Embryos	1932	479	
Median number of embryos per cycle	4 (2–6)	4 (2–7)	0.347
Number of embryos cultured until day 5/6	1854	465	
Number of blastocysts (rate per embryos cultured until day 5/6)	1031 (55.6)	241 (51.8)	0.143
Embryo utilization rate (the proportion of transferred/cryopreserved embryos per number of all obtained embryos)	1124 (58.2)	259 (54.1)	0.104
Number of cryopreserved embryos (rate per all embryos)	789 (40.8)	187 (39.0)	0.471
Number of ET	295	59	
Number of ET with single embryo transferred	255 (86.4)	46 (78.0)	0.096
Pregnancies (rate per ET)[a]	102 (34.6)	25 (42.4)	0.254

Values reported as median (interquartile range) or *n* (%).

P-value <0.05 indicates a statistically significant difference.

COC = cumulus–oocyte complex; ET = embryo transfer; ICSI = intracytoplasmic sperm injection

[a] Pregnancy was defined as a positive beta-HCG test 15 days after embryo transfer.

proportion from this group were excluded. This means that only cycles with nine or more retrieved oocytes and with a proportion of immature oocytes that was less than 17.8% were included.

The results presented in TABLE 5 show that the median number of embryos per cycle was significantly higher in the samples collected at clinic group (9 [6–12] versus 8 [6–11], adjusted OR 2.996, 95% CI 2.476–3.625, $P < 0.001$), as well as the number of embryos cultured until day 5/6 (9 [6–12] versus 8 [6–11], adjusted OR 4.710, 95% CI 1.609–13.783, $P = 0.005$). Furthermore, a significantly higher OR

TABLE 4 PREGNANCY RATE PER ET IN IVF COMPARED WITH ICSI CYCLES ACCORDING TO THE SITE OF SEMEN COLLECTION

			Unadjusted		Adjusted OR for women's and men's age, dose of gonadotrophins, number of retrieved oocytes and sperm quality	
	Collected at home	Collected at clinic	Collected at home	Collected at clinic OR (95% CI)	Collected at home	Collected at clinic OR (95% CI)
	Conventional IVF					
Number of ET	338	69				
Median number of transferred embryos	1 (1–2)	1 (1–2)	1 (ref)	0.994 (0.872, 1.132)	1 (ref)	0.998 (0.856, 1.162)
Number of ET with single embryo transferred	253 (74.9)	50 (72.5)	1 (ref)	1.029 (0.607, 1.744)	1 (ref)	1.194 (0.671, 2.124)
Pregnancies[a]	118 (34.9)	27 (39.1)	1 (ref)	0.853 (0.500, 1.455)	1 (ref)	0.763 (0.438, 1.330)
	ICSI					
Number of ET	305	122				
Median number of transferred embryos	1 (1–2)	1 (1–2)	1 (ref)	1.124 (0.972, 1.299)	1 (ref)	1.116 (0.963, 1.293)
Number of ET with single embryo transferred	226 (74.1)	84 (68.9)	1 (ref)	0.768 (0.469, 1.255)	1 (ref)	0.759 (0.463, 1.244)
Pregnancies[a]	102 (33.4)	38 (31.1)	1 (ref)	0.919 (0.577, 1.463)	1 (ref)	0.942 (0.590, 1.503)

Values reported as median (interquartile range) or *n* (%).

P-value <0.05 indicates a statistically significant difference.

CI = confidence interval; ET = embryo transfer; ICSI = intracytoplasmic sperm injection; OR = odds ratio.

[a] Pregnancy was defined as a positive beta-HCG test 15 days after embryo transfer. The presented data represent 834 embryo transfers in which only conventional IVF or ICSI was used for fertilization. Eleven ET in which both methods were used to fertilize oocytes were excluded from this analysis.

TABLE 5 OUTCOMES IN A SUBGROUP OF CYCLES WITH AT LEAST NINE RETRIEVED OOCYTES AND LESS THAN 17.8% IMMATURE OOCYTES

			Unadjusted OR		Adjusted OR for women's and men's age, dose of gonadotrophins, number of retrieved oocytes, and sperm quality	
	Collected at home	Collected at clinic	Collected at home	Collected at clinic OR (95% CI)	Collected at home	Collected at clinic OR (95% CI)
Number of cycles	178	51				
Number of COC	2699 13 (11–17)	782 13 (11–19)				
Number of immature oocytes	190 (7.0)	50 (6.4)	1 (ref)	0.991 (0.974, 1.008)	1 (ref)	0.993 (0.969, 1.018)
Embryos	1617	468				
Median number of embryos per cycle	8 (6–11)	9 (6–12)	1 (ref)	1.233 (0.254, 5.989)	1 (ref)	2.996 (2.476, 3.625)*
Number of embryos cultured until day 5/6	1613 8 (6–11)	466 9 (6–12)	1 (ref)	1.203 (0.243, 5.960)	1 (ref)	4.710 (1.609, 13.783)**
Number of blastocysts (rate per embryos cultured until day 5/6)	923 (57.2)	235 (50.4)	1 (ref)	1.000 (0.999, 1.001)	1 (ref)	1.000 (0.999, 1.000)
Embryo utilization rate (proportion of transferred/cryopreserved embryos per number of all obtained embryos)	911 (56.3)	235 (50.2)	1 (ref)	0.721 (0.243, 2.144)	1 (ref)	0.707 (0.238, 2.100)
Number of cryopreserved embryos (rate per all embryos)	747 (46.2)	188 (40.2)	1 (ref)	1.000 (0.999, 1.001)	1 (ref)	1.000 (0.999, 1.000)
Number of ET	133	39				
Number of ET with single embryo transferred	102 (76.7)	31 (79.5)	1 (ref)	1.034 (0.889, 1.202)	1 (ref)	1.198 (1.035, 1.387)***
Pregnancies (rate per ET)[a]	53 (39.8)	9 (23.1)	1 (ref)	0.861 (0.730, 1.015)	1 (ref)	0.724 (0.597, 0.878)****

Values reported as median (interquartile range) or *n* (%).

CI = confidence interval; COC = cumulus–oocyte complex; ET = embryo transfer; IQR = interquartile range; OR = odds ratio.

[a] Pregnancy was defined as a positive beta-HCG test 15 days after embryo transfer.

* Statistically significant difference with $P < 0.001$.

** Statistically significant difference with $P = 0.005$.

*** Statistically significant difference with $P = 0.015$.

**** Statistically significant difference with $P = 0.001$.

in the samples collected at clinic group was also observed for the proportion of embryo transfers with single embryo transfer (adjusted OR 1.198, 95% CI 1.035–1.387, $P = 0.015$), although it was significantly lower for pregnancy rate (adjusted OR 0.724, 95% CI 0.597–0.878, $P = 0.001$).

DISCUSSION

The results of this study indicate that IVF laboratory outcomes are at least comparable if semen samples for IVF/ICSI procedures are collected at home and not at the clinic. Because only data for the previous year (2019) were analysed, it was not possible to determine whether there was any influence on the miscarriage and birth rate, although the current data suggest that pregnancy rates in the overall population were similar between the compared groups.

Because there are several possible external influences that can negatively affect the quality of spermatozoa and consequently the outcome of the IVF/ICSI procedure, these results are somewhat surprising. Furthermore, no studies were found addressing the question of whether there is any influence on the outcome of IVF/ICSI procedures if semen samples are collected at home. On the other hand, two studies explored whether the location of semen collection exerted any influence on intrauterine insemination (IUI) outcome (*Song et al., 2007*; *Yavas and Selub, 2004*). *Yavas and Selub (2004)* concluded that semen collection at the clinic led to a better pregnancy rate, but this occurred only when women were stimulated with human menopausal gonadotrophin. When women were stimulated with clomiphene citrate, there was no difference in pregnancy rates based on the site of semen collection. Furthermore, they suggested that if the start of semen preparation is delayed from 30 min to 1 h or more, irrespective of collection site, then the pregnancy rate can be impaired. They suggested that the pregnancy rate can also be impaired if IUI is performed more than 90 min after the semen is prepared for treatment. On the other hand, a study by *Song et al. (2007)* did not find any difference in the ongoing pregnancy rate when patients collecting semen at the clinic were compared with those who collected semen at home. There was also no difference in semen parameters, although the time from semen collection to the IUI procedure was significantly longer in patients who performed semen collection at home. No data are available from patients in this study regarding the time from semen collection at home to semen processing. It is estimated that it is probably longer than the time lapse for patients who performed semen collection at the clinic; nevertheless, it was observed that semen parameters, in terms of concentration and motility, were better when semen was collected at home.

The observation that some semen parameters were better when semen

was collected at home is consistent with the results of a study by *Elzanaty and Malm (2008)*. Their study showed that home-collected semen samples had a significantly higher spermatozoa concentration, total sperm count and motility, while there was no difference in the morphology or the concentration of biochemical substances (neutral alpha-glucosidase, prostate-specific antigen, zinc and fructose). Furthermore, significantly fewer home-collected samples had abnormal sperm concentration or abnormal rapid progressive motility. While this study showed a positive effect on semen quality when samples were collected at home, a study by *Shetty Licht (2008)* did not find any statistically significant difference in sperm count, sperm motility, total count or total motile count between those samples collected at home and those collected at clinic. The reason for such an observation may be derived from the study design, because the patients could decide for themselves if they preferred to perform the collection at home or at the clinic. In this way, semen was collected at the clinic only by patients who were comfortable with this option, while patients who could be under elevated stress performed semen collection at home. The first studies evaluating the relationship between psychological stress and semen quality suggested that stress negatively affects the quality of semen in patients who are involved in IVF treatment (*Clarke, 1999*; *Harrison et al., 1987*; *Ragni and Caccamo, 1992*), but this conclusion was later challenged by *Nouri et al. (2014)*, who suggested that semen quality indeed declines during IVF, but this is probably not due to subjective stress. Despite this, they showed that in couples with subjective male stress, there were more poor responders, more miscarriages and fewer live births. *Haidl (2014)* further suggested that poorer outcome of IVF treatment may occur due to acute stress in males and because functional parameters of semen quality could be impaired in addition to basic parameters. For instance, *Vellani et al. (2013)* showed that male stress was associated with increased sperm DNA fragmentation in IVF patients.

An additional factor that could potentially influence the quality of semen is the period of sexual abstinence. The WHO recommends an abstinence of 2–7 days (*World Health Organization, 2010*), but the optimal sexual abstinence period is still debatable. Some semen quality parameters can improve with longer periods, but others improve with shorter periods (*Hanson et al., 2018*). A meta-analysis by *Hanson et al. (2018)* suggests that longer abstinence increases semen volume and sperm count, but motility, morphology and DNA fragmentation seem to improve with shorter periods of abstinence. These studies mostly focused only on sperm quality parameters, so there is not much data in the literature on how the sexual abstinence period influences the outcome of an ART cycle. *Periyasamy et al. (2017)* showed that clinical pregnancy rates were significantly higher if the abstinence period was less than 8 days; furthermore, the positive effect of a shorter abstinence period was also revealed in a higher live birth rate per embryo transfer. There are other studies showing that a shorter abstinence period positively influenced ART cycle outcomes. *Jurema et al. (2005)* suggested that pregnancy rates in IUI are improved if abstinence is less than 4 days, while *Colturato et al. (2007)* further suggested that in ICSI cycles, the best embryo quality and the highest pregnancy rate are achieved after an abstinence period of 1 day; a similar result was confirmed by *Borges et al. (2019)*. Furthermore, recurrent ejaculation for 4 days with a final abstinence period of 12 h was shown to improve the pregnancy rate in ICSI cycles (*Sánchez-Martín et al., 2013*). Because we want to avoid a long sexual abstinence period in our IVF programme, which can potentially impair the quality of semen, we aimed to 'synchronize' the abstinence period with the planned oocyte retrieval. This can be optimally carried out by advising the patients to ejaculate on the day on which the follicles are large enough to administer HCG, meaning that usually about 2 days pass before men collect semen for the IVF/ICSI procedure. Therefore, we can say that the abstinence period is probably similar for all patients and does not influence the results of the current analysis; this is also one of the strengths of this study. Another strength is the large number of included cycles, which are distributed throughout the entire year. This means that the influence of external factors (e.g. temperature) or other factors (fluctuations in the outcome of IVF/ICSI cycles) is minimized. On the other hand, because this is a retrospective study and all patients were included regardless of their diagnosis, the results could be biased. Another bias could be the analysis of all cycles and not just the first cycles. The prognosis is better for the first cycle than subsequent cycles and an unbalanced distribution of first-cycle patients between the two arms may lead to bias. To avoid this bias, an analysis was also done of the first cycles only. The results did not reveal any significant difference in cycle outcomes according to the site of semen analysis, meaning that results are similar to the case where all cycles were analysed, but as revealed by statistical power analysis, the first cycles only analysis was underpowered. The statistical power for analysis of blastocyst rate, embryo cryopreservation rate and embryo utilization rate were 0.314, 0.110 and 0.734, but this low power may also arise because of different group sizes. Namely, in the semen collected at home group there were 379 first cycles and in the semen collected at clinic group only 87 cycles. Therefore, it is suggested that a prospective randomized trial taking all these limitations into account should be performed in the future.

In conclusion, this study retrospectively analysed whether collecting semen samples at home influences sperm quality or IVF/ICSI cycle outcome in terms of oocytes, embryos and pregnancy rates. The results revealed that collecting semen at home has a positive effect on sperm quality (sperm concentration and motility were higher), although no other significant difference was observed in terms of oocytes, embryos or pregnancies. When all cycles were analysed, a lower proportion of polyploidies and higher proportion of freeze-all cycles were observed in the group of semen samples collected at home, but these differences cannot be attributed to the site of semen sample collection. Based on these data, it is suggested that collecting semen at home for IVF/ICSI procedures is safe and has no negative effect on treatment outcomes.

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