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Use of dimethylxanthine theophylline (SpermMobil®) does not affect clinical, obstetric or perinatal outcomes

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Abstract

Purpose To evaluate whether the use of a commercially available dimethylxanthine theophylline compound (SpermMobil®) for artificial sperm activation would negatively affect clinical, obstetric and perinatal outcomes.

Methods Artificial sperm activation (ASA) was used when sperm motility after preparation was low or absent in our clinical standard procedure practice. ICSI cycles using either testicular or ejaculated sperm with concentration smaller than 5 million/ml from August 2012 to January 2018 were retrospectively analyzed ($n = 815$) and divided into two groups, a control group where no ASA was needed and the SpermMobil® group with ASA.

Results The fertilization rate was significantly higher in the control group, but pregnancy and implantation rates did not differ significantly. Number of embryos transferred, good quality embryos for ET and number of frozen blastocysts were similar in both groups. Clinical pregnancy loss was significantly reduced in the SpermMobil® group, which was reflected in slightly better live birth rates than in the control group. Furthermore, there were no significant differences regarding gestational age, weight, height and z score for singletons or multiples in the SpermMobil® ($n = 27$ and $n = 10$) or control ($n = 144$ and $n = 67$) groups. There were no reports of malformation, perinatal mortality or intensive therapy in the SpermMobil® group, whereas in the control group, 12 babies needed intensive care, besides one intrauterine death.

Conclusion The use of SpermMobil® in samples with mostly immotile sperm not only facilitates the embryologists work but also optimizes the treatment outcomes for those patients with a bad prognosis. This is the first report of obstetric and perinatal outcomes after applying a theophylline derivative in human clinical use.

Keywords SpermMobil® · ICSI · Dimethylxanthine theophylline · Perinatal outcomes · Obstetric outcomes · Artificial sperm activation

Introduction

With the implementation of intracytoplasmic sperm injection (ICSI), severe male infertility could be promptly treated [1], as all anatomic steps are bypassed by depositing one single sperm in the ooplasm. Even though ICSI can be performed with spermatozoa of any origin (ejaculated, testicular or epididymal, for example), it requires a viable sperm to be identified. Nagy and colleagues reported that the only factor strongly having a negative effect on ICSI outcomes was the injection of immotile spermatozoa [2].

In cases of absolute asthenozoospermia, several approaches have been described to overcome this problem [3]. The hypo-osmotic swelling test (HOST), the mechanical touch technique, the laser-assisted immotile sperm selection, birefringence-polarization microscopy

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and exposure to chemical stimulants have been reported by several authors with variable amount of success to improve clinical outcomes in cases of absolute asthenozoospermia, nicely reviewed by Ortega and colleagues [3]. Among the chemical stimulants, several compounds have been tested to improve sperm motility, such as relaxin [4], caffeine [5–7], 2-deoxyadenosine [8], being theophylline [5, 9–11] and pentoxifylline [8, 12–16] the most studied.

Pentoxifylline and theophylline are 3′5′-nucleotidase phosphodiesterase (PDE) inhibitors of the methylxanthine group [17] that increase intracellular levels of cyclic adenosine monophosphate (cAMP) [15] by preventing its breakdown. This increase in cAMP will result in a higher energy-producing metabolic activity and oxygen uptake [18], thus enhancing sperm motility [3, 9] and even initiating the capacitation process [18]. Pentoxifylline has been also successfully applied orally to improve seminal parameters in patients with varicocele [19]. Otherwise it has mostly been applied in situ to improve the fertilizing capacity of samples with poor motility [5, 9, 12].

Spermatozoa with poor motility or metabolically quiescent become active in response to increased cAMP levels after artificial sperm activation (ASA) with pentoxifylline [8]. Cryopreservation and subsequent thawing is one factor strongly affecting motility and, therefore, several studies have applied pentoxifylline or other compounds to recover sperm motility after cryopreservation [8, 13]. Sperm motility is also mostly absent in testicular sperm and use of pentoxifylline has been successfully applied in the past years [11, 14, 20].

Once exposed to theophylline derivatives, spermatozoa have forward acceleration, augmentation of lateral head excursions and intensification of flagellar beat, resulting in an increase of energy consumption in the cells [12]. It is not clear if the effect can hold for several hours after exposure due to the persistence of phosphodiesterase inhibition, high cAMP concentration or other cAMP-unrelated mechanisms [12]. Clinically, the use of theophylline has been prioritized due to its increased half-life (4–8 h) and, therefore, allowing adequate pre-warming before its use.

Although several studies have extensively studied the effects of theophylline or other similar derivatives on sperm function and embryo viability, there are no reports if ASA with theophylline might be associated with risks for the offspring. Early studies in the field have expressed concerns about its clinical use in humans, as sperm motility-enhancing agents have serious detrimental effects on mouse embryo development [21, 22], its implantation [23] and in higher doses even embryocidal [24]. Therefore, we decided to evaluate if use of ASA would result in negative obstetric or perinatal outcomes in the offspring.

Materials and methods

Study design

Theophylline treatment of immotile spermatozoa was first tested in our center for testicular spermatozoa. After observing positive effects on fertilization and pregnancy rates [20], we started applying theophylline treatment routinely to all patients presenting no motile sperm for ICSI, either with testicular or ejaculated sperm. Approval of the Ethical Medical Committee was not necessary, as SpermMobil® (GM501 SpermMobil, Gynemed) is a CE-certified product that has been used routinely in our center and all data have been anonymously analyzed.

ICSI cycles using either testicular or fresh ejaculated sperm with concentrations smaller than 5 million/ml from August 2012 to January 2018 were retrospectively analyzed ($n = 815$). This concentration was set internally as threshold for a worse ICSI outcome based on the older version of the WHO guideline for severe oligoasthenoteratozoospermia (OAT) [25].

SpermMobil® was used in 113 cycles, from which 61 used testicular sperm (53.98%) and 89 received an embryo transfer (ET, 78.76%), whereas 702 cycles, from which 62 used testicular sperm (8.83%) and 567 received an ET (80.77%), did not require its use (control). Donor and frozen sperm, as well as retrograde and after electrostimulation ejaculation, were excluded from the analysis.

Sperm sample collection

The testicular sperm extractions (TESE, $n = 123$) were performed at the "Urologische Gemeinschaftspraxis Ulm". In short, a small incision in the scrotal skin was made and the tunica vaginalis was opened sharply. The seminiferous tubules were freed and exposed from an opening in the tunica albuginea before being excised [26]. The biopsies were placed in sperm washing media (Quinn's Advantage™ Sperm Washing Medium, SAGE, or Sperm Rinse™, Vitrolife) for the transport to the fertility clinic and further manipulation. The samples were transported in a thermic dewar (32–36 °C) and transport did not last longer than 30 min. The samples were mechanically disaggregated under sterile conditions in a Petri dish with fresh sperm washing media and further digested with a commercially available collagenase (GM501 Collagenase, Gynemed) [27] according to the manufacturer's instructions. All samples presenting enough sperm for an ICSI treatment were frozen with an automatic slow-freezing device (CTE2104, Cryo-Technik-Erlangen) and glycerol was used as cryoprotectant (SpermCryo™ All-round, Gynemed).

On the day of oocyte pickup, depending on the number of collected oocytes and quality of the frozen testicular sperm sample, appropriate number of straws were rapidly thawed at 37 °C and washed with sperm washing media followed by a centrifugation step (350 g for 10 min). The pellet was resuspended in sperm washing media and incubated at room temperature until ICSI.

Ejaculated sperm were analyzed and prepared according to the WHO guidelines with modifications [28]. Briefly, semen analyses were performed on a heated stage (HT300, Minitüb, Tiefenbach, Germany) after the samples were liquefied (10–60 min) depending on its appearance over time. After liquefaction, pH was measured using paper strips (Macherey-Nagel GmbH & Co. KG, Düren, Germany) and then total volume, viscoelastic properties and overall appearance were estimated with a transfer pipette (Sarstedt). Spermatozoa motility and concentration were estimated using 10 µl of native semen in a Makler Chamber (Sefi-Medical Instruments, Haifa, Israel) under 20-fold magnification (Olympus BX 51, Hamburg, Germany) with a phase-contrast microscope (Olympus, 20x/0.40 Ph1). A pre-warmed two-step gradient (PureCeption™, SAGE, or SpermGrad™, Vitrolife) was overlaid with liquified semen in a 15-+ml centrifuge tube. Samples were centrifuged for 20 min at 350 g (Megafuge 1.0 R, Heraeus, Langenselbold, Germany). After carefully discarding the supernatant, the pelleted spermatozoa were resuspended in fresh pre-warmed sperm washing medium in a new centrifuge tube to maximize recovered motility. Sperm were again centrifuged at 250 g for 10 min for washing, and then the supernatant was discarded and fresh medium (0.1–0.3 ml) was laid carefully over it.

Controlled ovarian stimulation (COS)

Female partners were mostly stimulated according to an antagonist protocol ($n = 810$), as shown in Table 1. For the long agonist protocol ($n = 5$), down-regulation was achieved with Triptorelin (Decapeptyl®, Ferring) or Nafarelin (Synarel®, Pfizer). The gonadotropins used were either recombinant (Gonal-f, Merck; Pergoveris, Merck; Puregon, MSD; Rekovelle, Ferring; Ovaleap, TEVA; Luveris, Merck; Bemfola, Gedeon Richter; Elonva, MSD) or urinary (Menogon or Bravelle, Ferring). For triggering final oocyte maturation, human chorionic gonadotropin (recombinant, Ovitrelle®, Merck, or Brevactid®, Ferring) or Triptorelin (Decapeptyl®, Ferring, or Triptofem, Karmed) were administered 36 h (h) before oocyte pickup.

Ovum pickup was performed transvaginal in a flushing media (FertiCult™ Aspiration medium, FertiPro) and cumulus–oocyte complexes (COCs) were collected in an appropriate buffered media (Quinn's Advantage™ Medium with HEPES, SAGE, or G-MOPS PLUS™, Vitrolife). COCs were washed and incubated in a fertilization media (Quinn's Advantage™ Protein Plus Fertilization Medium, SAGE, or G-IVF PLUS™, Vitrolife) until denudation with hyaluronidase (SAGE) and ICSI. Only mature metaphase II oocytes were injected.

ICSI

Sperm suspensions were pipetted into the ICSI dishes in a large swim-out drop, covered with pre-warmed mineral oil (Oil for Tissue Culture, SAGE, or OVOIL™, Vitrolife) and incubated for some period (30 min to 2 h) at 37 °C before checking for motility. If enough motile spermatozoa were found and collected in a polyvinylpyrrolidone (PVP) drop (SAGE), oocytes were then transferred into the ICSI dish to minimize its manipulation time outside the incubator. If after this incubation period no motile sperm were observed, SpermMobil® was added to the droplet according to the manufacturer's instructions.

As previously described [12], the effects of pentoxifylline on spermatozoa motility appear very fast (10 min) and the observed accelerated movement pattern can be seen for another 2 h in the absence of the compound. Therefore, ICSI could be performed without any further delay once SpermMobil® was added to the droplet, as motile spermatozoa were often found immediately. When enough motile sperm were collected in a PVP drop, oocytes were then added to the ICSI dish as described for the control.

For both groups, morphologically normal and fast progressive motile spermatozoa were used whenever possible. Spermatozoa were immobilized with the ICSI pipette (K-MPIP-3135, Cook) just before injection as described elsewhere [1]. Injected oocytes were cultured for 16–20-h post-ICSI (Quinn's Advantage™ Cleavage Medium, SAGE, or G-TL PLUS™, Vitrolife) until fertilization check. Normal fertilized zygotes (presence of two polar bodies and two pronuclei) were either cultured until embryo transfer (ET) or cryopreserved at the zygote stage.

Embryo culture and assessment

Cleavage embryo morphology was assessed on day 2 (44 ± 1 h post-ICSI) or 3 (68 ± 1 h) according to the Istanbul consensus [29] for number of cells, symmetry of blastomeres and multinucleation. If the patient had three or more zygotes, blastocyst culture was planned and culture media was replaced on day 3 until August 2014 (Quinn's Advantage™ Blastocyst Medium, SAGE), as after August 2014, a single-step culture media was used (G-TL PLUS™, Vitrolife). Embryo assessment on day 5 was performed 115 ± 2 h post-ICSI and blastocysts showing a prominent and discernible inner cell mass (ICM) and a trophectoderm (TE) comprising many cells and tightly adhered were preferred for ET. Morphologically ideal embryos for ET were

Table 1 Baseline characteristics of the study population and outcomes of the ICSI cycles in all patients

	SpermMobil® ($n=113$)		Control ($n=702$)			
	Mean ± SD or *n* (%)	95% CI	Mean ± SD or *n* (%)	95% CI	OR (95% CI)	*p* value
Patients						
Maternal age (years)	34.21 ± 5.25	33.23–35.19	34.05 ± 4.53	33.71–34.38	–	NS
Paternal age (years)	38.97 ± 7.19	37.63–40.31	37.83 ± 6.30	37.36–38.3	–	NS
Testicular sperm	61 (53.98)	–	62 (8.83)	–	12.11 (7.74–18.71)	< 0.0001
Controlled ovarian stimulation						
Days of stimulation	9.80 ± 4.25	9.004–10.59	9.56 ± 3.18	9.33–9.8	–	NS
Total gonadotropin dose (IU)	1636 ± 820.5	1483–1789	1732 ± 966.7	1661–1804	–	NS
Antagonist protocol	110 (97.35)		700 (99.72)		0.10 (0.02–0.52)	0.021
Number of oocytes	11.34 ± 7.08	10.02–12.66	10.72 ± 7.39	10.18–11.27	–	NS
Number of MII oocytes	8.41 ± 5.43	7.40–9.42	7.95 ± 5.70	7.53–8.37	–	NS
Fertilization						
Number of 2PN	3.96 ± 3.31	3.35–4.6	4.68 ± 4.06	4.34–4.98	–	NS
Fertilization rate (%)	50.22 ± 28.66	44.88–55.57	59.33 ± 28.42	57.22–61.43	–	0.0009
Number of cultured 2PN	2.75 ± 2.072	2.37–3.14	2.95 ± 2.19	2.79–3.11	–	NS
Number of frozen 2PN	1.21 ± 2.72	0.70–1.72	1.73 ± 3.77	1.45–2.01	–	NS
Number of 0PN	2.86 ± 2.75	2.35–3.37	1.92 ± 2.33	1744–2.09	–	0.0002
Number of 1PN	0.23 ± 0.52	0.13–0.33	0.23 ± 0.52	0.19–0.27	–	NS
Number of 3PN	0.19 ± 0.46	0.11–0.28	0.17 ± 0.46	0.14–0.20	–	NS
Number of degenerated after ICSI	1.16 ± 2.39	0.71–1.6	0.96 ± 1.18	0.83–1.09	–	NS
Embryo transfer						
Number of embryos for ET	1.25 ± 0.78	1.10–1.40	1.29 ± 0.77	1.23–1.34	–	NS
Number of ideal embryos for ET	0.88 ± 0.82	0.72–1.03	0.90 ± 0.83	0.84–0.96	–	NS
Number of not ideal embryos for ET	0.37 ± 0.62	0.25–0.49	0.39 ± 0.64	0.341–0.44	–	NS
Day 2/3 transfers	46 (51.69)	–	220 (38.80)	–	0.59 (0.38–0.93)	0.027
Day 5 transfers	43 (48.31)	–	347 (61.20)	–	0.59 (0.38–0.93)	0.027
Number of frozen blastocysts	0.34 ± 0.85	0.18–0.49	0.34 ± 0.85	0.27–0.40	–	NS
Reason for no embryo transfer						
Freeze all	13 (11.50)	–	74 (10.54)	–	1.10 (0.59–2.03)	NS
No fertilization	8 (7.08)	–	50 (7.12)	–	0.99 (0.47–2.10)	NS
Embryo arrest	3 (2.65)	–	11 (1.57)	–	1.71 (0.50–5.62)	NS

Data presented as mean ± standard deviation (SD) or *n* (%)

IU international units, *MII* metaphase II, *PN* pronuclei, *ET* embryo transfer, *OR* odds ration, *CI* confidence interval, *NS* not significant

considered those with grade 1 (good) for cleavage stage embryos and grade 1 for both ICM and TE for blastocysts. Surplus blastocysts were cryopreserved on day 5 or 6 and ET was performed with intravaginal ultrasound guidance.

Pregnancy and live birth documentation

The concentration of human chorionic gonadotropin (hCG) in blood was measured 14–16 days after the ET. Biochemical pregnancy was defined as hCG levels higher than 20 mU/mL. Clinical pregnancy was confirmed 4 weeks after ET via ultrasound, where the existence of gestational sacs with heartbeats was observed. All pregnancies were further monitored by the patients' own gynecologists after 8 weeks of gestation.

The patients were asked to inform the clinic if they had an abort, any complication or once they have delivered. Among the information required, patients were asked to send us the birth date, delivery method, weight and length at birth, gender and healthy status of the child and any complications there might have occurred until or after the 20th week of pregnancy. Intensive care treatment after birth, malformations or any other complications was also reported.

As birthweight is subject to physiological variations and not only risks, we also calculated the standard deviation scores (z scores) for birthweight considering the most important physiological variables affecting this parameter [30]. Gestational age and gender of the child were taken into account when calculating the z score based on Fenton Preterm Growth Chart [31]. Unfortunately, we could not

account if it were a singleton or part of a multiple in this growth chart. Preterm birth was considered to all infants delivered before the 37 weeks of gestation and very preterm birth to those under 34 weeks of gestation. Low weight at birth was applied to all infants who weighed less than 2500 g and very low weight at birth was defined as less than 1500 g. Perinatal mortality included both stillbirths after the 28th pregnancy week and neonatal deaths up to 7 days of life.

Statistical analyses

Statistical analyses were performed by Fisher's exact test for categorical variables, such as fertilization, pregnancy and implantation rates. Fisher's exact test was also applied for verifying differences among perinatal and obstetric outcomes, as well as proportion of TESE samples in each group. Odds ratios (OR) with 95% confidence interval (CI) were calculated when applicable.

Mann–Whitney test was used for continuous or ordinal variables, as those did not follow a normal distribution (tested with D'Agostino and Pearson normality test). These variables were expressed as means ± standard deviation (SD) and 95% CI. A p value smaller than 0.05 was considered to be significant. Mean, standard deviation (SD) and 95% CI were also computed.

Results

Baseline characteristics of the study population and ICSI cycles

This study included 815 ICSI cycles, resulting in the delivery of 248 babies, 37 in the SpermMobil® group and 211 in the control group. As shown in Table 1, no significant differences were observed in the baseline characteristics of the study population, except for the percentage of cycles using testicular sperm or oligozoospermic samples (<0.0001, Supplemental Fig. 1). Controlled ovarian stimulation was also comparable between the groups in terms of days of stimulation and total gonadotropin dose used. However, there were significantly more agonist protocols in the SpermMobil than in the control group ($p=0.021$). Numbers of oocytes and metaphase II oocytes were similar in both groups.

From the 815 ICSI cycles, 159 did not receive an ET due to complete fertilization failure ($n=58$), embryo arrest ($n=14$) or freeze-all strategy ($n=87$) to avoid an ovarian hyperstimulation syndrome (OHSS). The proportions for each subcategory were comparable for both groups, as shown in Table 1.

All baseline characteristics of the patients and ICSI cycles were also analyzed for those patients receiving an ET and the same differences were observed as for all patients in the study (Supplemental Table 1). Total sperm count and sperm motility were also significantly higher in the control group, both in the ejaculate and after preparation (Supplemental Table 2).

Fertilization and embryo development

Fertilization rate was higher in the control group than in the SpermMobil® group, but number of zygotes obtained, cultured or frozen did not vary among the groups (Table 1). Number of abnormal fertilization (1 or > 3 pronuclei) or degenerated cells after ICSI did not vary between the groups when considering all patients in the study, but there were significantly more degenerated oocytes after ICSI in the control group when analyzing only patients who received an ET ($p=0.032$), as shown in Supplemental Table 1. The number of embryos for ET and their quality on ET day were similar among the groups, as well as the number of surplus blastocysts frozen after ET. Patients in the control group had significantly more day 5 transfer than patients in the SpermMobil® group ($p=0.027$), as shown in Table 1.

Pregnancy, delivery and neonatal outcomes

Positive ß-hCG levels could be detected in 39.78% of all patients receiving an ET and the corresponding implantation rate was found to be 29.57%. When looking at pregnancy and implantation rates in each group, there were no significant differences among the groups regarding initial pregnancy loss or clinical pregnancy per ICSI cycle or per ET (Table 2). Nevertheless, live birth rates (at least one live birth per women), both per ICSI cycle or ET, tended to be slightly higher in the SpermMobil® group, but no statistical significant difference could be shown (Table 2).

Clinical pregnancy loss was significantly higher in the control than in the SpermMobil® group ($p=0.0098$). If looking at early clinical pregnancy losses (where a gestational sac was observed but no fetal heartbeat), there were more aborts in the control group, although not statistically significant. The same trend was observed for late clinical pregnancy losses (positive heartbeat was observed at least once) but did not reach significant difference among the groups, as shown in Table 2.

From the 32 pregnant patients in the SpermMobil® group, 27 patients had a singleton pregnancy and five revealed a twin pregnancy, resulting in the live birth of 27 singletons and ten twins. In the control group, among the 177 pregnant patients, 144 patients had a singleton pregnancy resulting in the live birth of 143 children, whereas 33 patients had a multiple pregnancy (1 triplet and 32 twin pregnancies) resulting in the live birth of 67 children (all comprised in the twin group). There were no reports of malformations and there was one perinatal death in the control group.

Table 2 Pregnancy outcomes

	SpermMobil®	Control	OR (95% CI)	*p* value
Number of ET	89	567		
Positive β-hCG	34 (38.20)	227 (40.04)	0.93 (0.58–1.46)	NS
Initial pregnancy loss	0 (0.0)	3 (1.32)	NA	NS
Clinical pregnancy/ICSI	34 (30.36)	224 (31.91)	0.93 (0.61–1.44)	NS
Clinical pregnancy/ET	34 (38.20)	224 (39.51)	0.95 (0.59–1.49)	NS
Live birth/ICSI	32 (28.32)	177 (25.21)	1.17 (0.75–1.81)	NS
Live birth/ET	32 (35.96)	177 (31.22)	1.24 (0.79–1.97)	NS
Multiple pregnancies	5 (15.63)	33 (18.64)	1.24 (0.46–3.14)	NS
Implantation rate	39 (27.66)	270 (29.87)	0.90 (0.60–1.34)	NS
Clinical pregnancy loss	2 (5.13)	60 (22.22)	5.29 (1.45–22.80)	0.0098
Early clinical pregnancy loss (negative heart beat)	2 (5.13)	44 (16.30)	3.60 (0.97–15.64)	NS
Late clinical pregnancy loss (positive heart beat)	0 (0.0)	16 (7.08)	NA	NS
Children born	37	210		

Shown as *n* (%)

ET embryo transfer, *OR* odds ratio, *NS* not significant

The data corresponding to delivery and neonatal outcomes for singletons are shown in Table 3. There were no significant differences among the groups regarding gestational age, weight or height at delivery, nor type of delivery or gender of the babies. Preterm and very preterm birth, as well as low and very low birth weight rates tended to be higher in the SpermMobil® group but they did not reach statistical significance. Oppositely, z scores tended to be higher in the SpermMobil® group and no neonatal intensive care (NIC) was needed in this group. As shown in Table 4, obstetric and perinatal outcomes for multiple pregnancies also did not show any significant differences among the groups. As for singletons, babies in the SpermMobil® group tended to have higher z scores and no NIC was needed in this group.

Table 3 Obstetric and perinatal outcome in singleton pregnancies

	SpermMobil® (*n* = 27)	95% CI	Control (*n* = 143)	95% CI	OR (95% CI)	*p* value
Delivery outcomes						
Weeks at delivery	38.41 ± 2.98	37.23–39.59	39.24 ± 1.96	38.91–39.56	–	NS
Preterm births (< 37 weeks)	3 (11.11)	–	14 (9.79)	–	1.161 (0.33–3.97)	NS
Very preterm birth (< 34 weeks)	1 (3.70)	–	1 (0.70)	–	5.5 (0.28–105.0)	NS
Cesarean section	6 (22.22)	–	49 (34.26)	–	0.55 (− 0.03–0.34)	NS
Neonatal outcomes						
Female neonates	10 (37.03)	–	69 (48.25)	–	0.64 (0.28–1.43)	NS
Z score	0.11 ± 0.77	− 0.20–0.42	− 0.19 ± 1.01	− 0.36 to − 0.02	–	NS
Birth weight (g)	3239 ± 672.6	2967–3510	3265 ± 515.1	3176–3353	–	NS
Low birth weight (< 2500 g)	2 (7.41)	–	7 (4.89)	–	1.57 (0.31–7.89)	NS
Very low birth weight (< 1500 g)	1 (3.70)	–	1 (0.70)	–	5.5 (0.28–105.0)	NS
Neonatal height (cm)	49.8 ± 4.68	47.87–51.73	50.87 ± 2.75	50.37–51.36	–	NS
Malformations	0 (0.00)	–	0 (0.00)	–	NA	NA
Perinatal mortality	0 (0.00)	–	1 (0.70)	–	0 (0.00–48.33)	NS
Admission to NICU	0 (0.00)	–	4 (2.79)	–	0 (0.00–5.59)	NS

Data presented as mean ± standard deviation or *n* (%)

NICU neonatal intensive care unit, *OR* odds ratio, *CI* confidence interval, *NS* not significant, *NA* not applied

Table 4 Obstetric and perinatal outcome in multiple pregnancies

	SpermMobil® ($n=10$)	95% CI	Control ($n=67$)	95% CI	OR (95% CI)	p Value
Delivery outcomes						
Weeks at delivery	36.2 ± 2.94	34.1–38.3	35.6 ± 4.07	34.6–36.6	–	NS
Preterm births (< 37 weeks)	3 (30.00)	–	35 (52.24)	–	0.61 (0.18–2.18)	NS
Very preterm birth (< 34 weeks)	2 (20.00)	–	9 (13.43)	–	1.61 (0.30–7.31)	NS
Cesarean section	4 (40.00)	–	41 (61.19)	–	0.4228 (0.13–1.52)	NS
Neonatal outcomes						
Female neonates	3 (30.00)	–	41 (61.20)	–	0.27 (0.073–1.16)	NS
Z score	− 0.85 ± 1.14	− 1.66 to − 0.04	− 0.94 ± 1.0	− 1.2 to − 0.68	–	NS
Birth weight (g)	2398 ± 570	1990–2805	2300 ± 703.8	2117–2484	–	NS
Low birth weight (< 2500 g)	6 (60.00)	–	33 (49.25)	–	1.54 (0.43–5.18)	NS
Very low birth weight (< 1500 g)	0 (0.00)	–	7 (10.44)	–	0 (0.0–3.18)	NS
Neonatal height (cm)	47.4 ± 3.50	44.9–49.9	47.04 ± 2.91	46.2–47.8	–	NS
Malformations	0 (0.00)	–	0 (0.00)	–	NA	NA
Perinatal mortality	0 (0.00)	–	0 (0.00)	–	NA	NA
Admission to NICU	0 (0.00)	–	8 (11.94)	–	0 (0.0–3.52)	NS

Data presented as mean ± standard deviation or n (%)

NICU neonatal intensive care unit, *OR* odds ratio, *CI* confidence interval, *NS* not significant, *NA* not applied

Discussion

To our knowledge, this is the first study to evaluate obstetric and perinatal outcomes after ASA. As far as we are concerned, no other publication has compared or reported neonatal outcome measures for children born after ASA.

In summary, we could not observe any clinically relevant increase in obstetric or perinatal risks among the pregnancies achieved after ASA, suggesting that theophylline treatment of immotile spermatozoa does not exert any harmful effect on embryo development. Contrastingly, we found that ASA resulted in a decrease in clinical pregnancy losses, which was reflected on slightly higher live birth rates than in the control group.

It has been shown that the only parameter strongly affecting ICSI outcome is the injection of an immotile spermatozoon, as it is not possible to assess its viability [2]. Therefore, several methods have been described to overcome this issue and assess sperm viability before injection [3]. We found that pharmacological stimulation of immotile spermatozoa to be the most practical and less invasive method in our setting, as we can immediately observe several spermatozoa simultaneously and select the best ones for injection. Furthermore, the use of a commercially available, CE-certified and quality-tested product makes its application in the ART laboratory very easy since the European Tissue Directives rule out the self-blending of solutions. We did not assess how much time was saved for each ICSI once SpermMobil® was applied, but others have shown a significant decrease in the time needed to find a viable sperm [11].

Use of chemical stimulants for motility also raises safety questions. Early studies in animal models have shown that even short exposures of embryos to pentoxifylline could impair embryo development and reduce cell number in blastocysts [22]. This negative effect in embryo development was also reflected in an impaired implantation after exposure of zygotes for 30 min to 3.6 and 7.2 mM pentoxifylline [23]. Contrastingly, theophylline and caffeine, for example, have been shown to improve cleavage and embryo development rates in bovines [5]. Nonetheless, selected spermatozoa exposed to theophylline were extensively washed in a theophylline-free medium before being injected into the oocytes, as recommended previously [16, 23] and the theoretical risk of a biological hazard was thus considered negligible [11].

For humans, there have been different reports on effectiveness of the ASA treatment. Some studies have not observed an increase in the number of motile spermatozoa for normozoospermic samples [12] but beneficial effects for oligospermic and asthenospermic samples [32]. On the other hand, it has been shown that the effects of pentoxifylline on asthenozoospermic specimens are shorter and less effective than in normozoospermic ones, possibly due to a faster exhaustion of energetic resources in those samples [12]. Despite the several applications of pentoxifylline and theophylline, methylxanthines cannot resolve all cases of complete asthenozoospermia, as axonemal defects or functional sperm tail defects will not result in any motility and, therefore, the HOS test is still an option to be considered [14, 33].

Another important fact to mention is that in the SpermMobil® group, the majority of the samples were

of testicular origin (53.98%), which may also favor later embryo development. A recent meta-analysis has shown that testicular sperm led to better embryo quality and higher pregnancy and implantation rates than ejaculated sperm in cryptozoospermic samples [34]. Kang and colleagues suggest that the oxidative stress to which the ejaculated sperm are exposed may pose a higher threat to ICSI outcome than the immaturity of the testicular sperm. Additionally, sperm DNA damage in cryptozoospermic samples may also be associated with pregnancy losses [35] and could explain the higher abort rates we found in the control group, as only 8.83% of sperm samples were of testicular origin.

We are aware that our study has some limitations. First, this is a retrospective analysis and allocation of patients has not been done randomly but selectively based on the absence of sperm motility on the day of therapy. Second, the control group does not truly represent the best control group, as total sperm count and motility varied significantly among the groups. However, we found inadequate not to use SpermMobil® in cases of complete asthenozoospermia, as we have observed higher fertilization and pregnancy rates after ASA [20], in accordance with other studies [10, 11, 33]. If we would not have applied SpermMobil® to the samples showing no spontaneous motility, we would have had even lower fertilization rates, as reported by Nagy et al. [2], and consequently lower pregnancy and implantation rates. Third, the statistical power may be limited to detect a significant decrease in clinical pregnancy losses, as the study was not designed to detect such an outcome.

In conclusion, our data suggest that ASA does not seem to provoke a major increase in the risk of obstetric and perinatal negative outcomes in the offspring. However, a much bigger sample size is necessary to ensure that no negative impact on the health parameters of the offspring is occurring after ASA. Additionally, pediatric follow-ups should still be performed to evaluate the psychomotor development, scholastic and prevalence of disease, to assess the lack of long-term consequences after ASA.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest and no funding was received.

Ethical approval Ethical approval was not considered necessary by our Ethics Committee, as we retrospectively analyzed the outcomes of our standard clinical practice with a CE-certified compound and there was neither allocation of treatment nor patient information disclosed to third parties.

Informed consent No informed consent was obtained.

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