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ASSISTED REPRODUCTION TECHNOLOGIES

# Sperm count affects cumulative birth rate of assisted reproduction cycles in relation to ovarian response

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## Abstract

**Purpose** To explore the possible influence of sperm quality, as assessed by prewash total sperm count (TSC), on cumulative success rates in assisted reproduction cycles.

**Methods** Retrospective study carried out in private IVF centre. Seven hundred sixty-five couples undergoing complete ICSI cycles, i.e. whose all embryos were transferred or disposed of. Couples were characterised by male infertility and female age younger than 36 years. Couples with a combination of female and male infertility factors were excluded. The primary outcome measure was cumulative live birth rate. Secondary outcomes were cumulative pregnancy and miscarriage rates. No specific interventions were made.

**Results** Higher TSC values have a positive impact on cumulative success rates in cycles characterised by few retrieved oocytes (1 to 5), while does not influence the outcome of cycles with a normal (6 to 10) or high (> 10) number of retrieved oocytes.

**Conclusions** The study highlights the importance of sperm quality for the efficacy of assisted reproduction treatments. This influence may remain relatively cryptic in association with normal or high ovarian response, but emerge decisively in cases of reduced ovarian response, suggesting a relationship between ovarian response and oocyte ability to compensate for paternal-derived deficiencies.

**Keywords** Sperm count · Ovarian stimulation · Assisted reproduction · Pregnancy · Live birth

## Introduction

The clinical outcome of assisted reproduction technology (ART) treatments is influenced by a myriad of intrinsic and extrinsic factors. Crucially, oocyte, but not sperm, developmental competence is largely jeopardised by chromosomal aneuploidy. Sustained rates of aneuploidy are in fact observed at all maternal ages, unfortunately with an exponential increase after the age of 35 years [1, 2]. For such a reason, the female gamete is recognised as the single most important factor affecting the ability of the preimplantation embryo to implant and develop to term, in vitro as well as in vivo [3]. Oocyte legacy has therefore overshadowed the perception of sperm role in embryogenesis and, more generally, reproduction. However, sperm actively participates in the genetic, epigenetic and cellular make-up of the embryo [4, 5]. A major part of sperm function unfolds at fertilization. This emerged rather clearly from the early history of ART when, in the absence of effective micromanipulation approaches, in vitro fertilization (IVF) was almost unachievable in cases of very severe male factor infertility or, worse, azoospermia. The advent of ICSI marked an epochal progress in the treatment of such cases [6]. With ICSI, in principle only, very few motile sperm are needed to maximise the generation of embryos from a cohort of oocytes, with obvious implications for treatment success [7]. However, the disarming ease by which ICSI overcomes otherwise untreatable sperm/semen dysfunctions has generated a paradox. The male gamete has been progressively perceived by many fertility specialists as an element essential to compose a biparental zygote genome, but with little, if any,

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role in developmental processes before and after implantation. Consistent with this notion, purely male factor infertility cases are believed to be associated with a favourable clinical outcome after ART treatment [8]. Such a prognostic prediction has some rationale for the above explained dominant role of the oocyte in development. Nevertheless, it is largely based on outcome measures, e.g. implantation rates and pregnancy rates in fresh embryo transfers (ET), that by definition are inadequate to express the reproductive outcome of an entire cohort of oocytes derived from a single cycle of treatment. Hence, especially in situations in which a specific factor is suspected to have a significant impact on embryo developmental competence and ultimately reproductive outcome, broader, more comprehensive outcome measures should be adopted to appraise clinical success. From intense debates stirred across decades, cumulative live birth rate (CLBR) per treatment cycle has emerged as one of the most reliable and inclusive quantitative assessments by which clinical outcome can be expressed in ART [9–12].

In this retrospective study, we explored the possible influence of sperm quality on CLBR. To this end, we focused on the specific impact of prewash total sperm count (TSC), a parameter adopted because previously described being crucially associated with male fecundity, infertility and health [13]. Importantly, and specific to this study, TSC was observed in dependence of different patterns of ovarian response to hormone stimulation in young patients. Overall, the study data indicate that, while TSC is an important determinant of cumulative clinical success, its relative impact depends on ovarian response, suggesting new hypotheses on the mutual interaction between the quality of male and female gametes in the establishment of a viable pregnancy.

## Materials and methods

### Study population and groups

Reported data concern a retrospective cohort study carried out between January 2009 and December 2013 on 765 couples undergoing complete ICSI cycles, i.e. whose all embryos were transferred or disposed of. Pregnancy rates were calculated in relation to the number of started cycles. Approval for the study was obtained from the local Institutional Review Board. Informed consent was obtained from selected patients.

Couples included were characterised by male infertility diagnosis and female age younger than 36 years. Couples with a combination of female and male infertility factors were excluded. The cohort was grouped according to male partner's TSC into five groups: (1) $< 0.1 \times 10^6$ (189/765 cycles, 24.7%); (2) $0.1 \times 10^6$ to $1 \times 10^6$ (144/765 cycles, 18.8%); 3) $1 \times 10^6$ to $5 \times 10^6$ (150/765 cycles, 19.6%); (4) $5 \times 10^6$ up to $10 \times 10^6$ (103/765 cycles, 13.5%); (5) $10 \times 10^6$ to $39 \times 10^6$ TSC, (179/765 cycles, 23.4%).

These TCS intervals were identified on the basis of collective information or experience; we have chosen 39 million sperm total per ejaculate as maximum because the WHO manual for sperm analysis indicates this value as cut-off below which a semen sample is defined as oligospermic. In men with lowest TSC, the percentage of motile sperm was 19%, while in those with highest TSC this value was 29%. Groups were also analysed according to the number of oocytes retrieved and therefore, indirectly, ovarian response. We chose the intervals of 1–5, 6–10, and > 10 because, on the basis of existing literature, represent cases of poor, average, and good/high ovarian response. Preimplantation genetic testing (PGT) treatments and cycles in which spermatozoa were recovered surgically were excluded from this study.

### Semen analysis and preparation

Semen samples were collected after 3–5 days of sexual abstinence by masturbation on the day of oocyte pick-up (OPU). After liquefaction for 30 min, semen samples were analysed, and volume, concentration, motility, agglutination, presence of round cells and morphology were evaluated before semen preparation according to the World Health Organization guidelines [13]. Sperm count were evaluated with the use of counting chambers improved Neubauer (IMN).

On the day of OPU, fresh sperm samples were prepared using a discontinuous PureSperm gradient (Nidacon, Flöjelbergsgatan, Sweden), and subsequently, the bottom fraction was aspirated and washed 10 min as previously described [14].

Sperm sample was layered upon a 40:80% PureSperm density gradient and processed by centrifugation at 600 g for 15 min and washed in 2 mL of sperm culture medium (PureSperm wash; Nidacon). The 40:80% PureSperm gradient volumes were changed according to the total number of motile spermatozoa as previously described [15]. After sperm preparation, a second evaluation of concentration, morphology and motility was carried out.

### Ovarian stimulation, oocyte and embryo handling procedures

Ovarian stimulation and oocyte retrieval were carried out as previously described [16]. In women, follicular stimulation was performed with highly purified FSH (IBSA, Lodi, Italy) with starting dose ranging from 100 to 300 IU per day, according to hormonal and anthropometric parameters. OPU was performed 35–36 h after induction of final oocyte maturation with hCG administration (Gonasi; Amsa, Rome, Italy), via transvaginal ultrasound–guided aspiration. After 2 h from oocyte retrieval, cumulus cells were removed from companion oocytes [17]. Denuded oocytes were then assessed for nuclear

status and oocytes showing the extrusion of the first polar body were considered mature and used for ICSI as previously described [18]. Fertilization was assessed 16–18 h after insemination. Oocytes displaying two pronuclei and a second polar body were considered to be normally fertilised and cultured further. The embryos obtained were then cultured and transferred and/or cryopreserved until day 3 at cleavage stage. The number of transferred embryos was decided according to patient needs and national guidelines. Embryos were cryopreserved using a Kitazato vitrification protocol (BioPharma Co., Japan) with a closed system device (HSV straw, Cryo Bio System, France) as previously described [11]. Vitrification and warming procedures were carried out as previously described by Cobo et al. [19]. Subsequent frozen embryo transfer (FET) was performed in a supplemented cycle. In supplemented FET cycles, estrogen (Climara, Bayer) and vaginal progesterone (Crinone, Merck Serono) were administered in a sequential regimen aimed at mimicking the endocrine exposure of the endometrium in the physiologic cycle. Assessment of clinical pregnancy was achieved by ultrasound detection of the gestational sac and visualisation of foetal heartbeat. Miscarriage was defined as pregnancy loss after ultrasound confirmation of embryo implantation and detection of foetal heartbeat. Live birth was defined as the birth of at least one newborn after 24 weeks' gestation that exhibited signs of viability.

### Statistical analysis

Continuous data were presented as absolute values, mean ± SD. The comparison between different study groups, with regard to maternal age and number of oocytes retrieved was carried out using ANOVA test to evaluate the statistical significance of differences among age groups and oocytes retrieved. Categorical variables were presented as absolute values and percentages. The chi square test was used to compare cumulative clinical pregnancy, miscarriage and cumulative live birth rates. The primary outcome measure was cumulative live birth rate, while the secondary outcomes were cumulative pregnancy and miscarriage rate. Differences in outcome measures between groups were compared using the chi squared test. $P$ values < 0.05 were considered to be statistically significant or highly significant if < 0.01.

## Results

Data were collected from 765 completed stimulation cycles, i.e. in which all embryos were transferred in fresh or frozen ET or disposed of, if deemed non-viable. Treatments of women older than 35 were not included to minimize the well-documented maternal age effect on treatment outcome. In a preliminary analysis, cycles were divided into five groups according to TSC values (expressed in millions): i.e. $\leq 0.1 \times 10^6$, $> 0.1 \times 10^6$ to $1 \times 10^6$, $> 1 \times 10^6$ to $5 \times 10^6$, $> 5 \times 10^6$ to $10 \times 10^6$; $> 10 \times 10^6$ to $39 \times 10^6$ (Table 1). Such groups were identified according to TSC values discussed in the World Health Organization guidelines [13]. Thirty-five cycles (4.6%) were interrupted since no embryos for transfer or cryopreservation were obtained. Mean age and number of retrieved oocytes were comparable among groups. The mean number of embryos across the different TSC groups was also similar ($P = 0.066$). Cumulative pregnancy rates (CPR) were progressively higher with increasing TSC, reaching a plateau in groups with higher TSC ($P = 0.025$). The same trend was observed in CLBR ($P = 0.01$). On the contrary, miscarriage rates were comparable ranging from 14.7 to 25% ($P = 0.5$).

In order to ascertain possible differences between specific groups regarding CPR, miscarriage rate and CLBR, we performed a post hoc test (Supplementary Table 1).

### Outcome in individual TSC groups

Cumulative outcome rates were comparatively assessed in individual TSC groups in relation to different oocyte yield (1 to 5; 6 to 10, > 10). As expected, in the most severe TSC condition ($\leq 0.1 \times 10^6$), average number of embryos were progressively higher ($P < 0.01$) as the number of retrieved oocytes increased (Supplementary Table 2). Equally, CPR (0.0001) (Fig. 1a) and CLBR ($P = 0.008$) (Fig. 1b) also increased in relation to oocyte number. Similar trends were observed in the outcome of groups with TSC values of $> 0.1 \times 10^6$ to $1 \times 10^6$ and $> 1 \times 10^6$ to $5 \times 10^6$. In groups characterised by TSC of $> 5 \times 10^6$ to $10 \times 10^6$ and $> 10 \times 10^6$ to $39 \times 10^6$, CPR and LBR also seemed to increase in association with a higher number of retrieved oocytes, but such differences were not statistically significant.

### Outcome in cycles with different oocyte yield as a function of TSC

Cumulative outcome rates were further investigated, by performing separate analyses in groups of patients with different oocyte yield (1 to 5; 6 to 10, > 10) and adopting TSC as independent variable. In the group where 1 to 5 oocytes were collected, the average number of embryos was not statistically different in the sperm categories $\leq 0.1 \times 10^6$, $> 0.1 \times 10^6$ to $1 \times 10^6$, $> 1 \times 10^6$ to $5 \times 10^6$, $> 5 \times 10^6$ to $10 \times 10^6$; $> 10 \times 10^6$ to $39 \times 10^6$ ($P = 0.73$) (Fig. 2 and Supplementary Table 2). On the contrary, CPR ($P = 0.02$) and CLBR ($P = 0.02$) increased significantly with higher TSC values (Fig. 3 a and b and Supplementary Table 2). In the other groups with oocyte yield of oocyte (6 to 10 and > 10) average number of embryos did not vary in dependence of TSC ($P = 0.62$ and $P = 0.57$, respectively) (Supplementary Table 2). Likewise, differences in CPR ($P = 0.48$ and $P = 0.52$, respectively) and CLBR ($P = 0.41$ and $P = 0.28$, respectively) were not significantly different (Fig. 3 a and b).

**Table 1** Number of embryos, CPR, and CLBR in cycles selected for female age < 36 years and classified according to TSC

| | | TSC | | | | | *P* |
|---|---|---|---|---|---|---|---|
| | | $< 0.1 \times 10^6$ | $> 0.1 \times 10^6$–$1 \times 10^6$ | $1 \times 10^6$–$5 \times 10^6$ | $5 \times 10^6$–$10 \times 10^6$ | $10 \times 10^6$–$39 \times 10^6$ | |
| No. of cycles | 765 | 189 | 144 | 150 | 103 | 179 | |
| Patient age (y), mean ± SD | 31.6 ± 2.7 | 31.5 ± 2.9 | 31.6 ± 2.9 | 31.7 ± 2.5 | 31.9 ± 2.9 | 31.8 ± 2.7 | 0.75 |
| No. of retrieved oocytes, mean ± SD | 10.1 ± 5.1 | 10.4 ± 5.5 | 9.3 ± 5.1 | 10.5 ± 5.6 | 10.1 ± 4.6 | 9.6 ± 4.9 | 0.185 |
| No. of viable embryos, mean ± SD | 3.1 ± 1.9 | 3.0 ± 1.6 | 2.8 ± 1.7 | 3.4 ± 2.2 | 3.1 ± 1.8 | 3.2 ± 1.9 | 0.066 |
| No. of cumulative pregnancies (%) | 288 (37.6) | 56 (29.6) | 48 (33.3) | 61 (40.7) | 45 (43.7) | 78 (43.6) | 0.025 |
| No. of miscarriages (%) | 51 (17.7) | 14 (25) | 9 (18.7) | 9 (14.7) | 7 (15.6) | 12 (15.4) | 0.5 |
| No. of cumulative live births (%) | 237 (31.0) | 42 (22.2) | 39 (27.1) | 52 (34.7) | 38 (36.9) | 66 (36.9) | 0.01 |

## Discussion

Total sperm count has been described as being highly predictive of male health in general [20] and reproduction in particular [21]. Its relative importance in determining the efficacy of IVF treatments remains uncertain and therefore demands thorough assessment, especially in the light of an alarming trend towards a decline observed over the last decades [20]. In this study, we explored the implications of different values of prewash sperm TSC for the clinical outcome in assisted reproduction treatments. To this end, we analysed ICSI cycles performed in women younger than 36 years, to minimise the impact of female age on cycle outcome, while, crucially, TSC was also crossed-analysed with oocyte yield. The overall picture derived from such analysis suggests that better sperm quality, as assessed by higher TSC values, has a positive

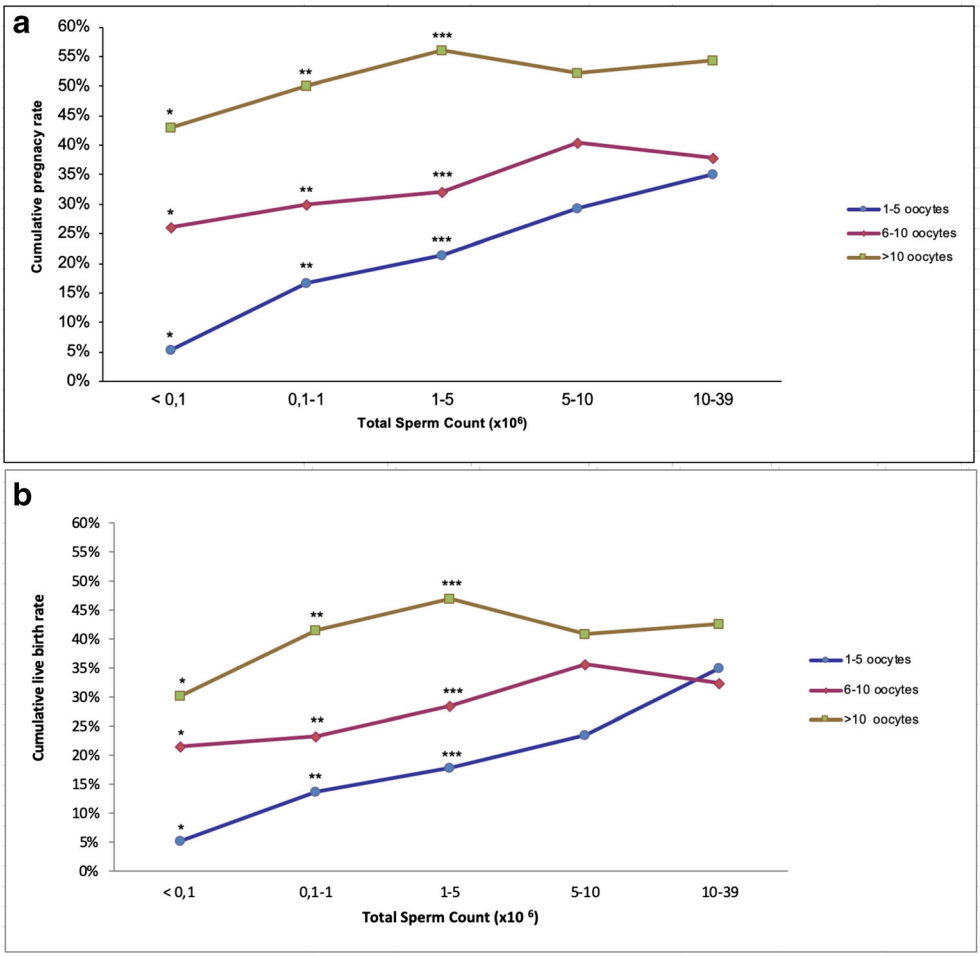


**Fig. 1** Cumulative outcome rates comparatively assessed in individual TSC groups in relation to different oocyte yield. **a** $^*P < 0.0001$, $^{**}P < 0.0045$, $^{***}P < 0.0019$. **b** $^*P < 0.0085$, $^{**}P < 0.012$, $^{***}P < 0.0121$

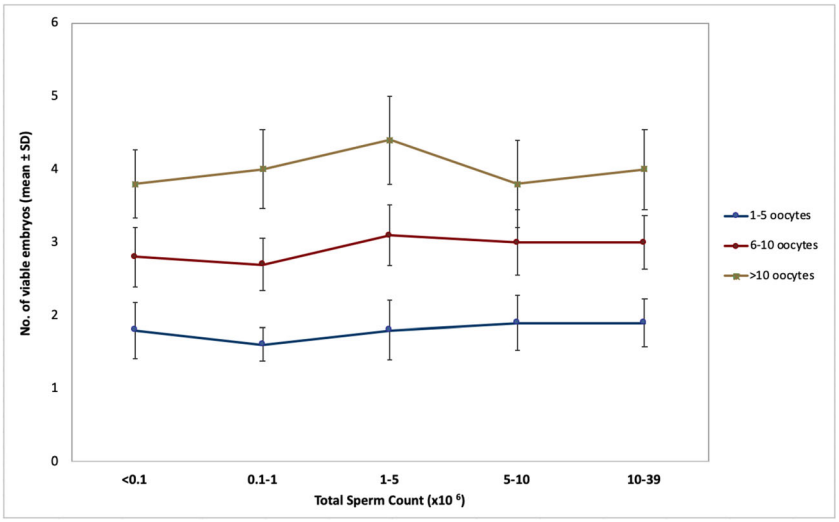


**Fig. 2** Mean (± SD) number of embryos in cycles classified according to TSC and subanalysed based on number of retrieved oocytes

impact on cumulative success rates in cycles characterised by few retrieved oocytes, while does not seem to influence the outcome of cycles with a normal or high number of retrieved oocytes. This highlights the importance of the male gamete for the outcome of assisted reproduction treatments, suggesting also a relationship between ovarian response and ability of the oocyte to compensate for paternal-derived deficiencies.

In the perception of reproductive specialists, the overwhelming role of the oocyte in development has overshadowed the importance of the male gamete. This view has been reinforced by the success of ICSI by which virtually all male factors cases, even the most extreme, have become treatable [22], bypassing the first steps of sperm–oocyte interaction at fertilization. Nevertheless, the sperm contribution to

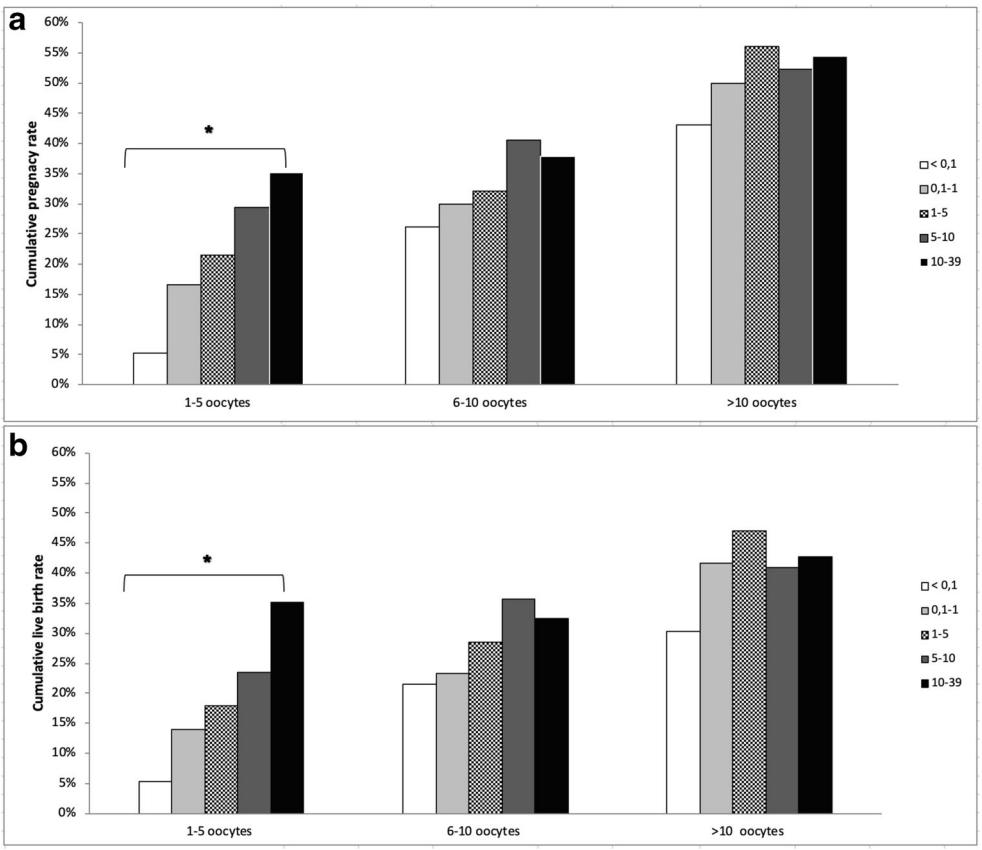


**Fig. 3** Cumulative outcome rates comparatively assessed in cycles classified according to number of retrieved oocytes and subanalysed based on TSC. **a** $*P = 0.022$; **b** $*P = 0.017$

the formation of a viable embryo and, after implantation, establishment of a viable pregnancy goes well beyond fertilization. In fact, while sperm are marginally affected by chromosomal aneuploidies, they are not at all immune from other genetic, epigenetic and cellular factors that intervene at postfertilization stages and can compromise the genomic and developmental integrity of the embryo [23]. For example, several studies [24–27], including a previous investigation reported by our group [28, 29], suggest that damage of paternal DNA may not emerge with detectable effects in developmental parameters of the preimplantation embryo or in implantation rates, but can determine an increase in miscarriage rates. Very recently, we have also shown that decreased sperm quality is positively associated with the rate of mosaic blastocysts in cases of preimplantation genetic testing for aneuploidy (PGT-A) [15]. There are, therefore, well-founded reasons to extend our knowledge on the implication of semen quality on reproduction and, in particular, fertility treatments outcome.

To measure the effect of TSC on treatment outcome, we adopted the rates of cumulative pregnancy and live birth as endpoints. Cumulative outcome derived from the use of all viable embryos generated in a single ovarian stimulation cycles has gained progressive importance as a more comprehensive and reliable measure of efficacy in assisted reproduction. In our study, analysis on the overall data set highlighted the importance of TSC in the determination of cumulative outcome, with progressively higher CPR and CLBR associated with higher TSC values. Other parameters were excluded from our analysis and call for an extension of the present study. Further subanalyses revealed other, more intriguing, relationships. Breaking down data according to oocyte yield, the above association was confirmed in cycles with few collected oocytes (1–5), but not in those with a higher number of retrieved oocytes (6–10 and > 10). Of particular note, while the average number of embryos as a function of TSC did not change within each oocyte yield group (1–5, 6–10 and > 10), in cycles with 1–5 retrieved oocytes the CLBR increased five-folds across progressively higher TSC values. At first sight, this inconsistency is puzzling considering the notion that, in the general population, higher rates of cumulative success derive also form a downstream effect of a higher number of embryos generated in a single stimulation cycle. However, taken together, our data may lend credit to an alternative, but not mutually exclusive, hypothesis that in young women a low ovarian response is associated with reduced oocyte quality that in turn may reveal the impact of reduced TSC on clinical outcome. In other words, It may be plausible that in such low response cases compromised sperm function, as suggested in this study by low TSC, might not be compensated for by reparative capacities of the oocyte and therefore might have an impact on development, not necessarily at fertilization or during preimplantation development, but at peri- or postimplantation stages. Three lines of evidence are relevant to this hypothesis; (i) sperm DNA damage, which is believed to affect assisted reproduction outcome as also reported above [28], was observed to be strongly associated with poor semen quality [30, 31]; (ii) human oocytes express numerous genes involved in DNA repair whose transcripts, abundantly present at the mature stage and during preimplantation development, can intervene to rescue sperm-derived damaged DNA sequences [32, 33]; (iii) the human oocyte transcriptome is affected by a condition of poor ovarian reserve [34]. On the contrary, in cases characterised by normal or high ovarian response, and therefore unaltered oocyte quality according to the above hypothesis, possible sperm defects could be repaired, or compensated for, during early development, preventing significant implications for postimplantation embryo viability. A similar scenario is not merely theoretical. Many studies are in fact consistent with the emerging notion that the detrimental consequences of DNA damage on embryo development depend not only on the magnitude and type of DNA modifications carried over by gametes but also on the reparative abilities of the oocyte [35]. Collectively we are very intrigued by the study outcome. However, we recognise significant limitations of our analysis that should be addressed in future investigations. In fact, while TSC has emerged as an important factor determining clinical outcome in association with poor ovarian response, in a similar fashion other sperm parameters should be assessed, such as morphology and DNA fragmentation.

## Conclusions

In final analysis, the present study highlights the general importance of sperm TSC for the efficacy of assisted reproduction treatments. At the same time, it suggests that the consequences of low sperm count on clinical outcome may remain relatively cryptic in association with normal or high ovarian response, but can emerge decisively in case of reduced ovarian response. Moreover, the study is suggestive of new themes for future research. For example, it would be very interesting also from a biological standpoint to assess whether specific oocyte competences, e.g. DNA damage repair that may impact also sperm function at postfertilisation stage are compromised in subject characterised by a reduced ovarian function, irrespective of age.

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## Compliance with ethical standards

Approval for the study was obtained from the local Institutional Review Board. Informed consent was obtained from selected patients.

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