# Evaluation of human papilloma virus in semen as a risk factor for low sperm quality and poor in vitro fertilization outcomes: a systematic review and meta-analysis

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**Objective:** To analyze the effect of human papilloma virus (HPV) sperm infection on sperm parameters and in vitro fertilization (IVF) outcomes.
**Design:** Systematic review and meta-analysis.
**Setting:** Not applicable.
**Patients:** Men with HPV sperm infection and couples undergoing IVF.
**Interventions:** Searches were conducted in the following databases: Medline(R), PubMed, Embase, Web of Science, Scopos, and the Cochrane Library. We included studies examining sperm parameters and IVF results in patients with and without HPV sperm infection.
**Main Outcome Measures:** Sperm analysis (concertation, count, volume, motility, morphology), according to the World Health Organization manual, pregnancy rate (PR), and miscarriage rate (MR).
**Results:** Sixteen studies were included in this meta-analysis. The presence of HPV had a significant association with impaired sperm parameters in terms of concentration (mean difference [MD] −4.48, 95% confidence interval [CI] − 6.12 to −2.83), motility (MD −11.71, 95% CI − 16.15 to −7.26), and morphology (MD −2.44, 95% CI − 4.08 to −0.79. A review of the literature regarding ART outcomes showed an association between HPV infection and decreased PR, and an even stronger association between HPV infection and increased MR.
**Conclusion:** Our meta-analysis shows a negative effect of HPV on sperm concentration, motility, and morphology. Further subgroup and categorical analysis confirmed the clinical significance of impaired sperm motility in HPV-infected sperm, although the sperm count and morphology must be carefully analyzed. The studies reviewed reported lower PR and increased MR in couples with HPV-infected sperm. As most studies had a moderate risk of bias, these observations warrant further large, well-designed studies before introducing clinical management recommendations.
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**El resumen está disponible en Español al final del artículo.**

**Key Words:** Human papilloma virus, sperm analysis, in vitro fertilization outcomes, male infertility



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M.W. has nothing to disclose. C.S-S.N. has nothing to disclose. I.F. has nothing to disclose. J.B. is an investigator in Merck's nonavalent HPV vaccine studies.
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Human papilloma virus (HPV) is one of the most common sexually transmitted pathogens found in both men and women (1, 2). More than 200 different types of HPV have been identified. The genital types are classified as high-risk (HR) and low-risk (LR) subtypes (3–5). HPV infection is a subject of concern mostly due to its oncogenic features. The HR HPV types (mainly 16 and 18) were reported as the main etiological factor of cervical cancer (6–8), the fourth most common cancer worldwide in women (6).

In men, HR HPV types are associated with anal cancer, penile cancer, and a fraction of head and neck cancers (9). Moreover, HPV may also be present in the semen of asymptomatic men (1, 10–13). However, most epidemiological studies and natural history investigations of HPV infections relate to women, whereas limited amount of data is available about men (4, 14). Unlike routine Papanicolaou (Pap) test or the liquid-based cytology testing in conjunction with HPV DNA testing, as done in women, no such screening tests are currently available or authorized for men as a routine screening. Therefore, gathering data on asymptomatic HPV infection in men is more challenging (15).

Although it is well established that HPV is primarily transmitted through direct epithelial contact, until recently little attention has been paid to other consequences that the presence of HPV in semen might have (3, 16). The influence of HPV in semen on sperm parameters and sperm quality has become an additional matter of concern. Recent data have shown that HPV semen infection is a possible risk factor for male infertility (17, 18) by interfering with sperm parameters such as count, vitality, motility, and morphology; by altering the composition of seminal fluid such as pH, semen viscosity, or leukocyte number; or by increasing DNA fragmentation (1, 12, 13, 19, 20). Other recent studies have not confirmed these findings (10, 21, 22), and additional research is needed to determine whether HPV infection contributes to male infertility.

Apart from the possible effect of HPV on male fertility, its potential effect on fertility treatments and assisted reproductive technology (ART) results is also a matter of interest. Sperm infection may alter sperm quality and thus may have a negative influence on ART outcomes. Following in vitro fertilization (IVF) treatment, implantation and pregnancy rates (PR) were similar in infected and noninfected males, but a lower number of good quality embryos and increased miscarriage rates (MR) were found in the presence of HPV-positive sperm (23). Similarly negative outcomes were also observed in intrauterine insemination (IUI) procedures (24).

The prevalence of couples seeking fertility treatment and undergoing IVF is rising (25). In 2016, 1.8% of infants born in the United States and 4% of infants born in Israel were conceived using ART (26).

In view of emerging data on the possible effect of HPV on sperm quality and ART outcomes, a meta-analysis was undertaken.

## MATERIALS AND METHODS

### Searches

The systematic review of the literature was conducted accordingly to the Meta-analysis of Observational Studies in Epidemiology (MOOSE) guidelines (27). Study protocol can be assessed at PROSPERO International prospective register of systematic reviews (http://www.crd.york.ac.uk/PROSPERO, registration number CRD42019127419). Searches were conducted by an experienced research librarian in the following databases: MEDLINE using the OvidSP interface and PubMed, Embase, Web of Science, Scopos, and the Cochrane Library. The search strategies are detailed in Supplemental Appendices 1 and 2. Reference lists of all the related reviews and guidelines as well as electronically retrieved articles selected for inclusion in the present review were hand searched to ensure comprehensive coverage of all relevant literature. The research questions were as follows: Research question 1 [RQ1]: Is there an association between HPV in sperm and sperm quality? Research question 2 [RQ2]: Does the presence of HPV in sperm have an impact on ART outcomes?

In our protocol published in PROSPERO, we mentioned our intention to study the different effects of HR and LR types of HPV on sperm parameters. However, as described later in the text, only a few studies published their data regarding this issue, and therefore we were not able to conduct a subgroup analysis according to HPV types.

### Types of Study Included

This meta-analysis considered for inclusion experimental and nonexperimental studies, including randomized controlled and observational studies (case-controlled, cohort, and cross-sectional studies), examining the sperm parameters and in vitro fertilization (IVF) results in patients with and without sperm HPV infection. Sperm parameters were evaluated according to the World Health Organization manual (28). Studies that were selected were adjusted according to major confounding factors.

### Outcomes Measured

The primary outcome was sperm analysis (concertation, count, volume, motility, morphology). Secondary outcomes were pregnancy rate (PR), defined as positive $\beta$-hCG or clinical pregnancy (a sac with or without fetal heartbeat); and miscarriage rate (MR), defined as a pregnancy that did not proceed beyond week 20.

### Data Extraction

The search described above was used to select abstracts for screening. Titles and abstracts were independently screened by two researchers to select papers for full-text assessment based on title, keywords, and abstract. Case reports and case series, reviews, editorials. and nonhuman studies were excluded. Abstracts of studies were excluded if the full article was not published. Nonmatching literature sources such as reports, dissertations, theses, and databases were excluded. Studies that were published in a non-English language were excluded as well. Primary data collection was performed by two reviewers using a standardized data extraction procedure, with disagreements being settled by in-depth discussion. If two publications seemed to be duplicate publications based

on authors, institution, and description of the study population, only one of the studies was included. In articles representing an overlapping population (e.g., a large study including patients from a previously reported sample), only the larger study was included.

## Risk of Bias (Quality) Assessment

Methodological quality assessment of observational studies was conducted using the Newcastle–Ottawa Quality Assessment Scale (low, ≤5 points; medium, 6–7 points; high, 8–9 points). The Risk of Bias in Non-randomized Studies of Interventions tool was used to evaluate the quality of included studies (29). According to the guidelines of this tool, an intervention is referred to either "treatment" or "exposure" even in studies with no actual intervention implemented by the investigators.

Two independent reviewers assessed trial quality, and any disagreements were resolved through consensus adjudication. In addition, the overall quality of the evidence was assessed using criteria recommended by the Grading of Recommendations Assessment, Development, and Evaluation Working Group (GRADE).

## Strategy for Data Synthesis

A quantitative synthesis was conducted using RevMan 5.3 (Cochrane Collaboration). A two-tailed $P$ value of $<.05$ was considered statistically significant. Heterogeneity across studies was assessed using the $\chi^2$ test (significance set at $P<.1$) and the $I^2$ statistic. Pooling of the results was performed using the Mantel–Haenszel fixed effects model. The results were measured either by risk ratio (RR) or by mean difference (MD), presenting the confidence interval (CI), and $P$ value.

Publication bias was assessed by the Begg and Mazumdar test and the Egger regression asymmetry test, as well as contour-enhanced funnel plots. Sensitivity analyses was conducted by omitting from the analyses studies with highest weight, by removing outliers, and by omitting one study at a time to evaluate whether the results could have been affected markedly by a single study.

# RESULTS

According to the two research questions (RQ), two systematic searches were conducted. For the first RQ (RQ1), 468 references were identified through database searching, and for the second RQ (RQ2), 1,708 references were identified. Supplemental Tables 1 and 2 provide the reasons for excluding full-text articles.

Finally, we selected 21 studies (1, 4, 5, 7, 10–13, 16, 20, 21, 23, 30–38) for RQ1, comprising 4,679 patients. Four studies (23, 34, 37, 39) were selected for RQ2, comprising 641 couples (Supplemental Fig. 1). Given the fact that only four studies were found to be answering the inclusion criteria for RQ2, a meta-analysis was not further conducted; instead a review of the literature was done. Eight studies (1, 4, 7, 10, 23, 30, 33, 38) reported median values in their results (compared to most studies that reported the mean value) and/or did not present the mean or standard deviation (SD). Thus, these studies were not entered to the comparisons of absolute sperm parameters in our meta-analysis. After removing these studies, 2750 patients remained in the study group for RQ1. Two studies reported their results as mean values and 95% CI (16, 21). Based on the Cochrane Handbook, the CI was converted to the SD and presented in our meta-analysis. Some studies reported their results as the number of patients with oligospermia, asthenospermia, and/or teratospermia. Three of the studies excluded because of data presentation (30, 33, 38) were included these comparisons. Finally, 16 studies were included in all our meta-analysis comparisons. Table 1 and Supplemental Table 3 summarize the included studies' characteristics. It is worth mentioning that the inclusion and exclusion criteria are summarized in Table 1 to avoid results that are affected by different methodological issues. Most studies included in their analysis only patients with infertility; however, five papers included fertile patients as part of the study group (31–33, 35, 38). Accordingly, a subgroup analysis of infertile patients was conducted. In papers presenting a case group analysis of infertile patients, only this case group was analyzed in our meta-analysis (5, 12, 13).

Three studies (16, 21, 30) compared sperm parameters between HR and LR HPV types, four other studies (20, 34, 36, 38) investigated the effect of HR HPV types exclusively. In our study protocol, we stated our intention to investigate the different effect of HR and LR HPV sperm infection on sperm parameters. That said, the data on this issue are insufficient for the meta-analysis comparison. The only possible comparisons regarding HR HPV sperm infection are of sperm concentration and motility. The data are insufficient regarding sperm volume, morphology, and count in sperm with HR HPV infection. The number of studies reporting the results on sperm with LR HPV infection is also small; thus, a comparison between HR and LR HPV sperm infections could not have been conducted. We will review these relevant data in the Discussion section.

In light of only two studies (16, 30) reporting the number of leukocytes in sperm, a comparison on this outcome was not conducted. We note that six papers from the same group were included in our meta-analysis (11–13, 32, 35, 37). Considering this, we contacted the authors, who confirmed that each study group was based on a different cohort of patients. Thus, all six studies were included.

As all included studies were observational, they presented moderate to severe risk of bias, mostly due to selection bias, elimination of confounding factors, or bias in selection of the reported results.The risk of bias summary is presented in Figure 1, and quality assessment is presented in Table 1 and Supplemental Table 4.

According to Cochrane Handbook for Systematic Reviews of Interventions, testing for publication bias by funnel plot asymmetry should not be conducted when less than 10 studies are included in the meta-analysis in order to avoid a false result. In our meta-analysis, the comparisons of sperm concentration and sperm motility included 12 studies and were assessed for publication bias by a funnel plot (Supplemental Fig. 2). No asymmetry was detected, and the risk of publication bias is low.

## TABLE 1

**Characteristics of the included studies (research question 1 [RQ1]).**

| Study (first author) | Country | Study design | n | Inclusion criteria | Exclusion criteria | Outcomes measured | Comments | Quality (NOS) |
|---|---|---|---|---|---|---|---|---|
| Boeri (2019) | Italy | Cross-sectional study | 729 | Male factor infertility | Cryptorchidism, abnormal karyotyping, skin lesion compatible with HPV infection, or symptoms of genitourinary infections; a history of vasectomy; infertility treatment in the preceding year and a positive semen culture | Concentration, colume, morphology, leukocytes, motility | Comparison between high- and low-risk HPV genotypes; values presented as median and range. | High |
| Damke (2017) | Brazil | Prospective cohort study | 229 | Age: 18 y or older. Semen analysis requested as part of a fertility evaluation | Symptoms of genitourinary infections, antibiotic treatment within the previous 3 months, reproductive system abnormalities, a history of vasectomy; infertility therapy in the preceding year; positive sexually transmitted diseases | Volume, morphology, motility, leukocytes, concentration | Comparison between high- and low-risk HPV genotypes; values presented as mean and range | Medium |
| Fedder (2019) | Denmark | Prospective cohort study | 43 | Unselected, nonvasectomized, azoospermic men; 43 proven fertile healthy men as control | Not mentioned | Sperm count, concentration, volume | None | Medium |
| Foresta (2010) | Italy | Cross-sectional clinical study | 100 | Men attending a project of andrological prevention | Previous history of cryptorchidism, prostate infections, testicular trauma, or post-mumps orchitis, presence of sperm antibodies, varicocele, and seminal infections | Volume, concentration, count, motility, morphology | Results presented per HPV type | High |

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## TABLE 1

**Continued.**

| Study (first author) | Country | Study design | n | Inclusion criteria | Exclusion criteria | Outcomes measured | Comments | Quality (NOS) |
|---|---|---|---|---|---|---|---|---|
| Foresta (2010a) | Italy | Cross-sectional clinical study | 108 (group c) | Patients either with risk factors for HPV semen infection or with male factor infertility | History of cryptorchidism, testicular trauma, or post-mumps orchitis, varicocele, seminal infection | Motility | 1. The main results of this paper include sperm HPV infection and exfoliated cells. 2. Only group c (infertile patients) results were entered in our meta-analysis | High |
| Foresta (2015) | Italy | Cohort study | 619 | Male infertility patients with female partners without main genital diseases | History of cryptorchidism, testicular trauma, post-mumps orchitis, prior knowledge of HPV infection, previous or ongoing vaccination at the time of enrolment, varicocele, and bacterial seminal infections | Concentration, count, motility, morphology | None | High |
| Garolla (2012) | Italy | Case-control study | 35 | Semen samples from 22 HPV-infected patients and from 13 normozoospermic noninfected volunteers | Not mentioned | Volume, concentration, count, motility, morphology | None | Medium |
| Garolla (2013) | Italy | Cross-sectional clinical study | 165 | Infertile male patients with normal female partners | History of cryptorchidism, testicular trauma, or post-mumps orchitis, varicocele, and seminal infections | Concentration, count, motility, morphology | In our meta-analysis, we included the results of the comparison between infertile HPV-infected patients and infertile noninfected patients (an additional control group was not entered) | Medium |
| Garolla (2016) | Italy | Cross-sectional clinical study | 226 | Males aged 25–40 y with normal or altered sperm parameters | Azoospermic patients; current infection of sexually transmitted diseases; patients with genetic alterations; additional exclusion criteria for women | Volume, concentration, count, motility, morphology | HPV present in sperm and exfoliated cells | High |

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## TABLE 1

**Continued.**

| Study (first author) | Country | Study design | n | Inclusion criteria | Exclusion criteria | Outcomes measured | Comments | Quality (NOS) |
|---|---|---|---|---|---|---|---|---|
| Kim (2017) | Korea | Cross-sectional study | 381 (HPV group) | Age: 19–40 years; had sexual intercourse with a woman within the past year | Sex gene abnormality/ serious structural disorder of the pelvic organs/inability to ejaculate due to physical or mental disease; male sterilization operation; history of an STI within the previous year, genital warts, penile or anal cancer, testicular trauma, cryptorchidism, post-mumps orchitis, varicocele, or seminal infection; or penile discharge or unusual penile symptoms such as severe pain within the previous 6 months | Volume, concentration, count, motility, morphology | None | High |
| Lai (1997) | China | Cross- sectional clinical study | 24 | Randomly selected patients who attended fertility clinic | Not mentioned | Morphology, motility | High-risk HPV types only | Low |
| Luttmer (2016) | Netherlands | Cross-sectional study | 430 | Male partners in couples seeking fertility evaluation | Men with a history of vasectomy or testicular cancer | Volume, concentration, count, motility | Comparison between high- and low-risk HPV genotypes; values presented as mean and range | High |
| Moghimi (2019) | Iran | Case-control study | 70 | Male infertility patients<br>Control group included fertile men who had at least one child | Chromosome abnormalities, azoospermia, undescended testis, history of orchitis or varicocele, men whose spouses had histories of uterine and ovarian disorders<br>Exclusion criterion for the control group was the presence of genital warts | Concentration, morphology, motility | High-risk HPV types only<br>Results of the infertile group were included in our meta-analysis | High |

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## TABLE 1

**Continued.**

| Study (first author) | Country | Study design | n | Inclusion criteria | Exclusion criteria | Outcomes measured | Comments | Quality (NOS) |
|---|---|---|---|---|---|---|---|---|
| Rintala (2004) | Finland | Cross-sectional study | 65 | Voluntary fathers-to-be | Not mentioned | Volume, concentration, count, motility | High-risk HPV types only; standard deviation not mentioned in text | Low |
| Tanaka (2000) | Japan | Case-control study | 86 couples (male HPV) | Not mentioned | Not mentioned | Concentration, motility | High-risk only | High |
| Yang (2013) | China | Case-control study | 615 (case group) | Male infertility patients | Presence of antisperm antibodies, azoospermia, undescended testis, chromosome abnormalities, mumps, orchitis, or hypergonadotropic/hypogonadotropic hypogonadism. In addition, subjects with a spouse who had tubal, uterine, or cervical abnormalities or bilateral fallopian tube obstruction were excluded | Volume, concentration, motility, morphology | Only the case group was included in our meta-analysis (control group comprised fertile patients) | Medium |

*Note*: HPV = human papilloma virus; NOS = Newcastle–Ottawa Quality Assessment Scale; STI = sexually transmitted infection.

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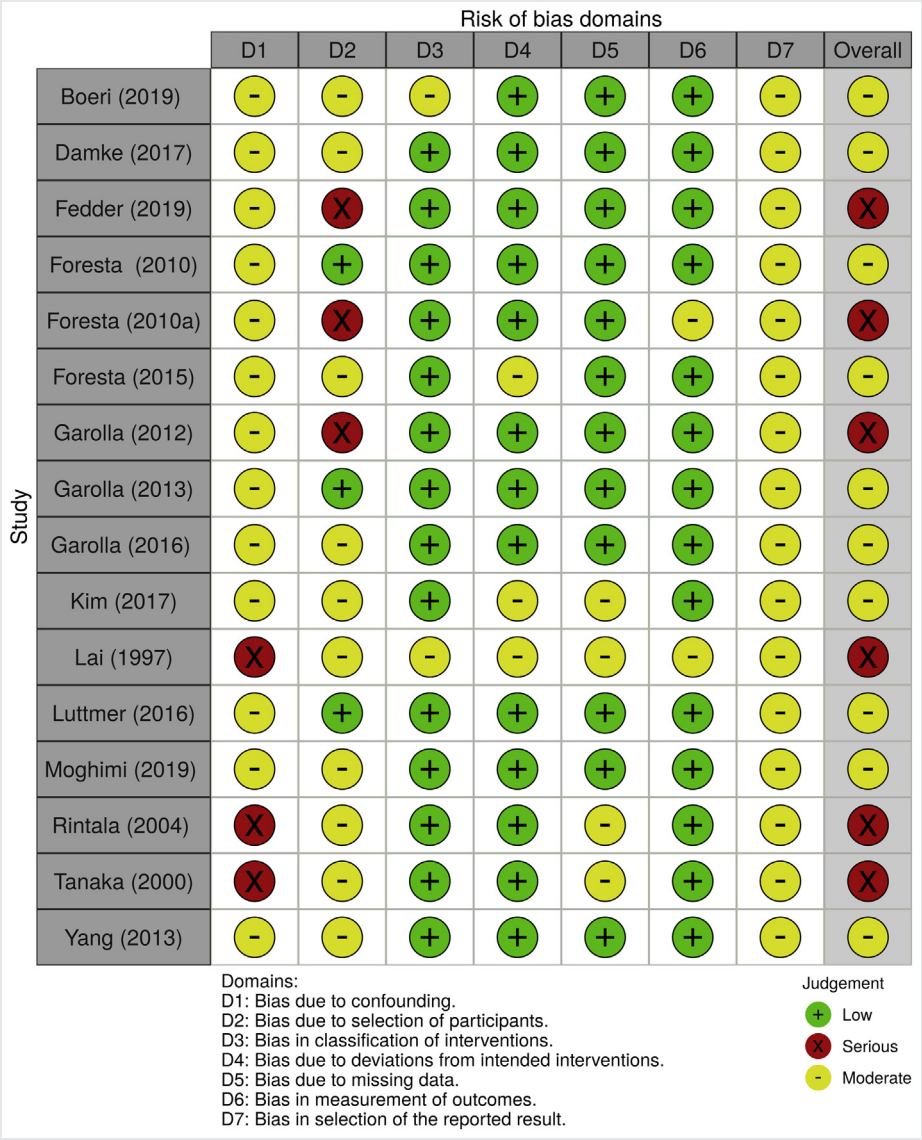
**FIGURE 1**




Risk of bias summary.

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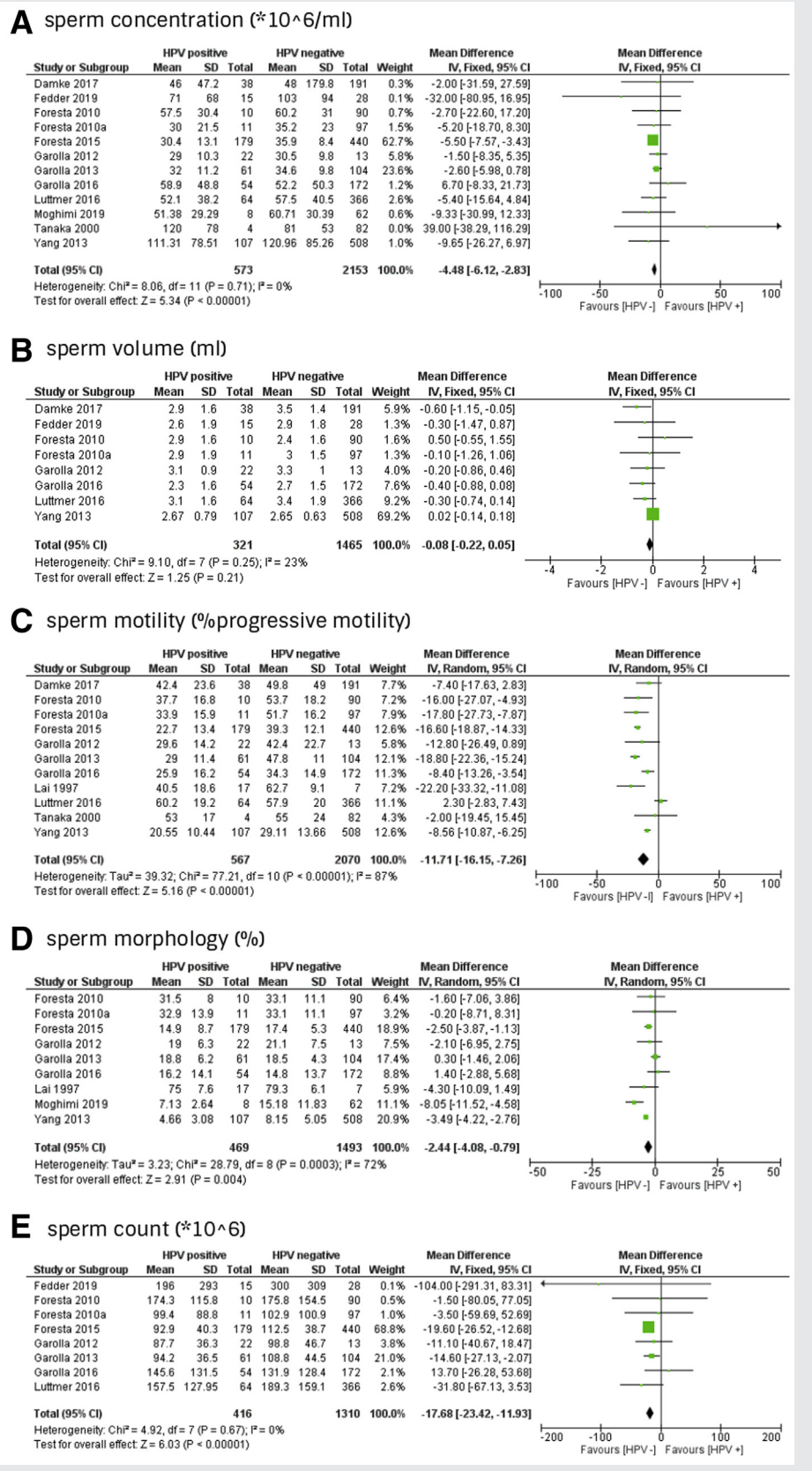
## Meta-analysis

**Review question 1.** The forest plots for sperm parameters are presented in Figure 2. Taking for comparison all studies that measured sperm concentration, the plot shows that the outcome was significantly lower in the HPV-positive group (MD −4.48, 95% CI − 6.12 to −2.83), $P < .00001$). In the subgroup analysis of high-quality studies, the plot shows that the concentration is still significantly lower in the HPV-positive group (MD −5.25, 95% CI − 7.23 to − 3.28), $P < .00001$).

Taking for comparison all studies that measured sperm volume, the plot shows that the outcome was not significantly different between the HPV-positive and HPV-negative groups (MD −0.08, 95% CI − 0.22 to 0.05, $P = .21$). In the subgroup analysis of high-quality studies, the plot shows that the difference in sperm volume is still nonsignificant (MD −0.26, 95% CI − 0.56 to 0.04, $P = .09$).

Taking for comparison all studies that measured sperm motility, the plot shows that the outcome was significantly lower in the HPV-positive group (MD −11.71, 95% CI − 16.15 to −7.26, $P < .00001$). In the subgroup analysis of high-quality studies, the plot shows that the percentage of motile sperm is still significantly lower in the HPV-positive group (MD −11.51, 95% CI − 17.74 to −5.29, $P = .0003$). It is worth mentioning that one study reported very low motility in both HPV-positive and HPV-negative subjects (0.63% and 6%, respectively) (20), implying a significant difference in population characteristics. For this reason, and to achieve

## FIGURE 2




Sperm parameters. (**A**) Sperm concentration (*10^6/mL); (**B**) sperm volume (mL); (**C**) sperm motility (% progressive motility); (**D**) normal morphology (%); and (**E**) sperm count (*10^6).

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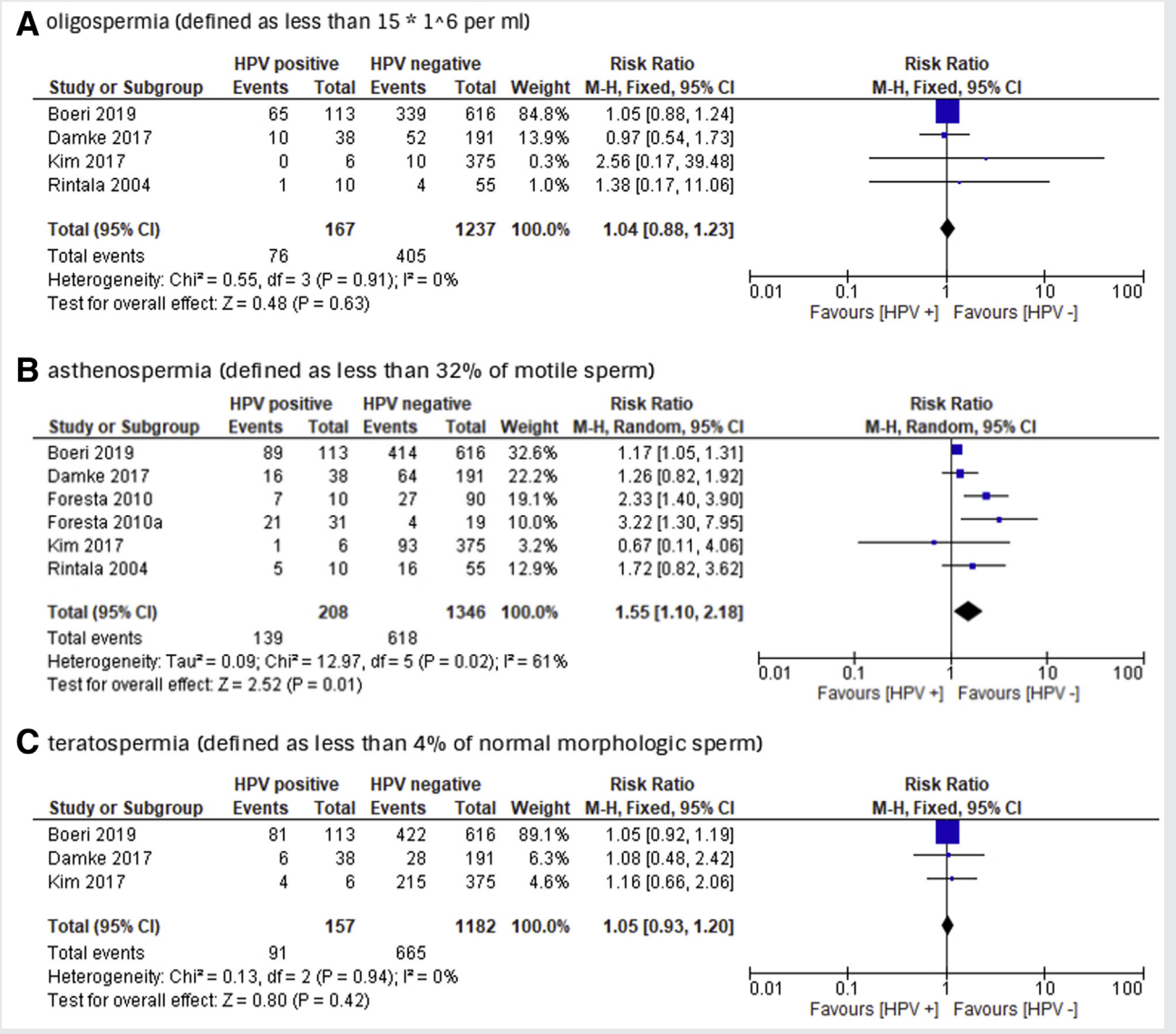
## FIGURE 3




Abnormal sperm parameters. (**A**) Oligospermia (defined as <15 * 1^6 per mL); (**B**) asthenospermia (defined as <32% of motile sperm); and (**C**) teratospermia (defined as <4% of normal morphologic sperm).

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accurate estimations, the study was excluded from the motility analysis.

Taking for comparison all studies that measured sperm morphology, the plot shows that the outcome was significantly lower in the HPV-positive group (MD −2.44, 95% CI − 4.08 to −0.79, $P = .004$). In the subgroup analysis of high-quality studies, the plot shows that the percentage of sperm with normal morphology was not significantly lower in the HPV-positive group (MD −2.64, 95% CI − 5.76 to 0.49, $P = .1$).

Taking for comparison all studies that measured sperm count, the plot shows that the outcome was significantly lower in the HPV-positive group (MD −17.68, 95% CI − 23.42 to −11.93, $P < .00001$). In the subgroup analysis of high-quality studies, the plot shows that sperm count was still significantly lower in the HPV-positive group (MD −18.76, 95% CI − 25.39 to −12.13, $P < .00001$).

Some studies reported the results as the number of patients with oligospermia, asthenospermia, and/or teratospermia. A few of these studies were excluded from the comparisons of absolute sperm parameters because of presentation of their results as median values or omission of the mean or SD (30, 33, 38). Therefore, in addition to the mean concentration, motility, and morphology rates, we analyzed the data according to these results (Fig. 3).

Taking into comparison all studies that measured the number of patients with: oligospermia, the plot shows that the outcome was not significantly different between the

HPV-positive and HPV-negative groups (RR 1.04, 95% CI 0.88 to 1.23, $P = .63$); asthenospermia, the plot shows that the outcome was significantly higher in the HPV-positive group (RR 1.55, 95% CI 1.10 to 2.18, $P = .01$); and teratospermia, the plot shows that the outcome was not significantly different between the HPV-positive and HPV-negative groups (RR 1.05, 95% CI 0.93 to 1.20, $P = .42$).

When considering only the subgroup of high-quality studies, the results remained nonsignificant for oligospermia and teratospermia and remained significant for asthenospermia. Hypospermia and leukospermia were not analyzed as an outcome because of insufficient data.

As previously described, five papers included fertile patients as part of the study group. Because this may be a confounding factor, we added a subgroup analysis of the studies that included infertile patients only (Supplemental Fig. 3).

For sperm concentration, motility, morphology, and sperm count, the significant decrease in the HPV-positive group remained significant when analyzing the results of infertility patients only. The nonsignificant effect on sperm volume remained nonsignificant. Oligospermia and teratospermia also remained nonsignificantly different between the HPV-positive and HPV-negative groups when including the results of infertility patients only. Asthenospermia remained higher in the HPV-positive group; however, the effect was nonsignificant (RR 1.38, 95% CI 0.94 to 2.04, $P = .1$).

Studies regarding HPV in semen differ with regard to the methods used to analyze for the presence of HPV (fluorescence in situ hybridization [FISH], polymerase chain reaction [PCR]). As this heterogeneity may be a potential confounder, a subgroup analysis was conducted according to the detection technique. When removing the studies that detected HPV by FISH or serology only (and not by PCR) (11, 12, 37), differences in sperm concentration and sperm count were found to be of borderline significance ($P = .1$ and $P = .09$, respectively). Yet, the effect on sperm motility and normal morphology remained significant (Supplemental Fig. 4).

Using the GRADE criteria, the overall quality of existing evidence was initially described as "low" in light of observational studies regarding data acquisition. This impression may be problematic, as most of the CIs were moderate, and yet, considering the high consistency, the grade was not reduced to "very low."

**Review question 2.** Only four papers were found to be relevant to RQ2: Perino et al. (39) found a significantly increased risk of MR at the presence of HPV sperm infection, and even noted 100% MR when both men and women were infected; Garolla et al. (37) found a significant decrease in PR following IUI and intracytoplasmic sperm injection (ICSI) and a firm association between fetal infection, blastocyst deformation, and a higher MR; Tangal et al. (23) found HPV infection to be a causative factor in decreased quality of embryos during ART, but because of the limited number of cases, the effect of HPV infection on implantation rates PR or MR remained an unresolved issue; and Tanaka et al. (34) also reported their limited number of cases to be a restrictive factor for any conclusion to be drawn. Performing a meta-analysis was unfortunately not within reach because of a lack of data. Nevertheless, these studies were analyzed because of their important data on PR and MR.

In an attempt to integrate the data from these four studies, it was noticed that the definition of pregnancy was not clearly stated in two studies (34, 37). The other two papers (23, 39) defined the pregnancy rate as clinical pregnancy rate (fetal heartbeat seen). Given the limited number of studies on this issue, a conclusion cannot be drawn regarding the effect of HPV-infected sperm on PR. However, a trend toward lower PR is observed in each study.

The MR was also defined differently in each study. Garolla et al. (37) defined the MR as pregnancy loss from all pregnancies (spontaneous and ART pregnancies); Perino et al. (39) defined it as pregnancy loss of clinical pregnancies only; Tangal et al. (23) included also chemical pregnancies; and Tanaka et al. (34) did not define the MR clearly in the text. Even so, all studies reported elevated MRs in couples with HPV-infected sperm.

## DISCUSSION

Our meta-analysis showed that the presence of HPV in sperm had a significant association with decreased sperm concentration, motility, and morphology, but not with semen volume. These findings did not change for most outcomes when only high-quality studies were included or when including studies analyzing male infertility patients only.

Although HPV infects and replicates in epithelial cells, semen is considered a medium of HPV transport during intercourse (15, 40, 41), and a reservoir for HPV infection is found in the testes, seminal vesicles, and ductus deferens (42).The prevalence of HPV sperm infection was estimated at 16% in the population of infertile patients (41). HPV may bind to sperm heads (35) and/or may be present in exfoliated epithelial cells in semen samples (13, 42).

The mechanisms by which HPV could impair sperm quality or embryo development is unclear. It is tempting to speculate that persistent HPV infection in men reflects reduced immune competence, as this may be associated with the development of HPV-related cancers; however the correlation between this assumption and the negative effect on sperm parameters is unclear. When discussing infertility due to HPV infection, many studies found a change in sperm parameters such as decreased motility of HPV infected semen (35); some asserted that HPV induces sperm DNA fragmentation (19, 43); others suggested that HPV interferes with the ability of spermatozoa to bind and to penetrate the oocyte (44); and others associated HPV infection with impaired embryo development, blastocyst implantation, and placental dysfunction (45, 46).

A number of studies included in our meta-analysis reported the results of HPV influence on sperm quality using the parameters oligospermia, teratospermia, and asthenospermia (13, 16, 30, 33, 35, 38). When addressing sperm parameters and correlation with fertility, this categorical classification may be more clinically significant. When these were considered exclusively, only motility was significantly reduced.

In these comparisons, HPV presence in sperm was found to have a negative effect on sperm motility. This effect remained significant when considering only the subgroup of high-quality studies addressing the general male population; however, the effect was not found to be significant when analyzing the results of infertility patients only. Only four and three studies were included in the analysis regarding oligospermia and teratospermia, respectively, with most of the studies comprising a small HPV-positive samples. Thus, it is possible that the availability of insufficient data was the reason for not reaching significance. In the comparison of asthenospermia, six studies were included, comprising a greater study group with the ability to achieve statistical significance. In addition, categorical classification, reporting the number of patients with oligospermia, teratospermia, or asthenospermia (instead of reporting the absolute results of sperm parameters) may decrease the ability to achieve significant results per se. It is worth mentioning that within the same category (oligospermia for example), there is importance to the subclassification according to severity. It is possible that the effect seen across the whole population may tilt the balance from oligospermia to severe oligospermia; this, too, is of clinical significance, yet remains to be proved.

Three studies compared HR HPV to LR HPV regarding their influence on male fertility (16, 21, 30), all of these studies found the HR HPV 16 to be the most common infective agent. Although HR HPV was shown to have a more negative effect of sperm motility (30) and seminal viscosity (16) compared to LR HPV, there were not enough studies conducted to draw a definite conclusion. Furthermore, one study showed that no significant association was found between either HR HPV or LR HPV on sperm parameters (21). Similarly, four studies evaluated the influence on seminal parameters and quality of HR HPV exclusively (20, 34, 36, 38), but the data were insufficient to conclude that a specific subtype has a different implication from another.

The higher rates of HR-related fertility impairment may be due to infection severity. An increased apoptotic phenomena in sperm exposed to E6/E7 (found in HR types) was found (19, 43). Moreover, the transfer of E6/E7 genes from infected sperm to the oocyte and further on to the blastocyst was shown (23). A recent study on HR cervical HPV tested in women, showed no association between HPV infection and female factor infertility (47).

As for PR, this outcome was found to be decreased in the HPV-positive group in studies detailed in our review. This may show a trend toward a negative effect of HPV on ART outcome, but further studies must be conducted. A very interesting finding was the higher MR in the HPV-positive group in the studies reviewed. It is important to emphasize that the terms of PR and MR were defined differently in each study, as described earlier in text. Nevertheless, studies showed the negative influence of HPV sperm infection on MR.

Because of the limitations described above, it is important to emphasize that although significant results were found, it is still premature to recommend a change in clinical management of IVF patients. Moreover, moderate to severe risk of bias was observed in the included studies. Thus, we must interpret the results with caution, and more studies must be conducted. However, it seems that HPV infection may certainly be another factor in understanding infertility and MR in the future.

It is worth mentioning that studies regarding HPV in semen differ with regard to the methods used to analyze for the presence of HPV(FISH, PCR) and with regard to the location of HPV present (existing in exfoliated epithelial cells, attached to sperm cells, seminal fluid, etc.). After conducting a subgroup analysis and comparing the different viral detection methods, a significant effect on motility and morphology were still present using either method. Thus, the potential HPV effect on sperm quality appears to be mostly achieved by impairing sperm motility and normal morphology. The results for sperm concentration and count should be carefully analyzed, as the laboratory techniques may be a possible confounder. Moreover, there are insufficient data regarding the method of insemination (IVF or ICSI). Although one can assume that ICSI may minimize the transfer of HPV to the embryo, the negative effect of HPV infection was observed regardless of the insemination technique. Future studies should address this issue as well. In addition, the different abstinence periods before semen sampling reported in the included studies, ranging from 2 to 5 days, may have affected the sperm motility analysis. Finally, most studies lack information regarding possible confounders such as simultaneous female partner genital HPV infection, other genital infections, and lifestyle factors—all possibly affecting fertility and serving as treatable causes.

In conclusion, our meta-analysis investigated the influence of HPV sperm infection on sperm parameters and ART results. The results show a negative association between HPV in sperm and sperm count, motility, and morphology. When considering only categorical analyses, only motility was significantly affected. As such, the clinical significance of sperm morphology and sperm count results is still not clear. In a review of literature, four studies reported a trend toward lower ART PR and an increase in MR.

As HPV vaccination was reported to be effective in reducing the mean clearance time in men with HPV semen infection (11, 48), in the future, after accumulating more evidence, it may be considered as a recommendation for men's health in general and specifically for its potential effect on reproductive outcomes. Meanwhile, in men infected with HPV, the direct swim-up procedure may reduce the number of HPV-infected sperm cells, and this may be an optional treatment for men with HPV-infected sperm (32, 37, 49), but it demands further study.

The association found between HPV in sperm and MR should be further studied, as a trend toward higher MR among males with HPV-infected sperm was noted. Finally, more studies are required in order to confirm the association between the presence of HPV in semen and male infertility.

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**Evaluación del virus del papiloma humano en semen como factor de riesgo para baja calidad seminal y pobre pronóstico en fecundación in vitro: revisión sistemática y meta-análisis**

**Objetivo:** Analizar el efecto de la infección en semen por el virus del papiloma humano (VPH) sobre los parámetros seminales y en el pronóstico de la fecundación in vitro (FIV).

**Diseño:** Revisión sistemática y meta-análisis.

**Pacientes:** Hombres con infección seminal por VPH y parejas sometidas a FIV.

**Intervenciones:** Se realizaron búsquedas en las siguientes bases de datos: Medline (R), PubMed, Embase, Web of Science, Scopos y Librería Cochrane. Se incluyeron estudios que examinaban los parámetros seminales y los resultados de la FIV en pacientes con y sin infección seminal por VPH. El protocolo de revisión está disponible en PROSPERO (CRD42019127419).

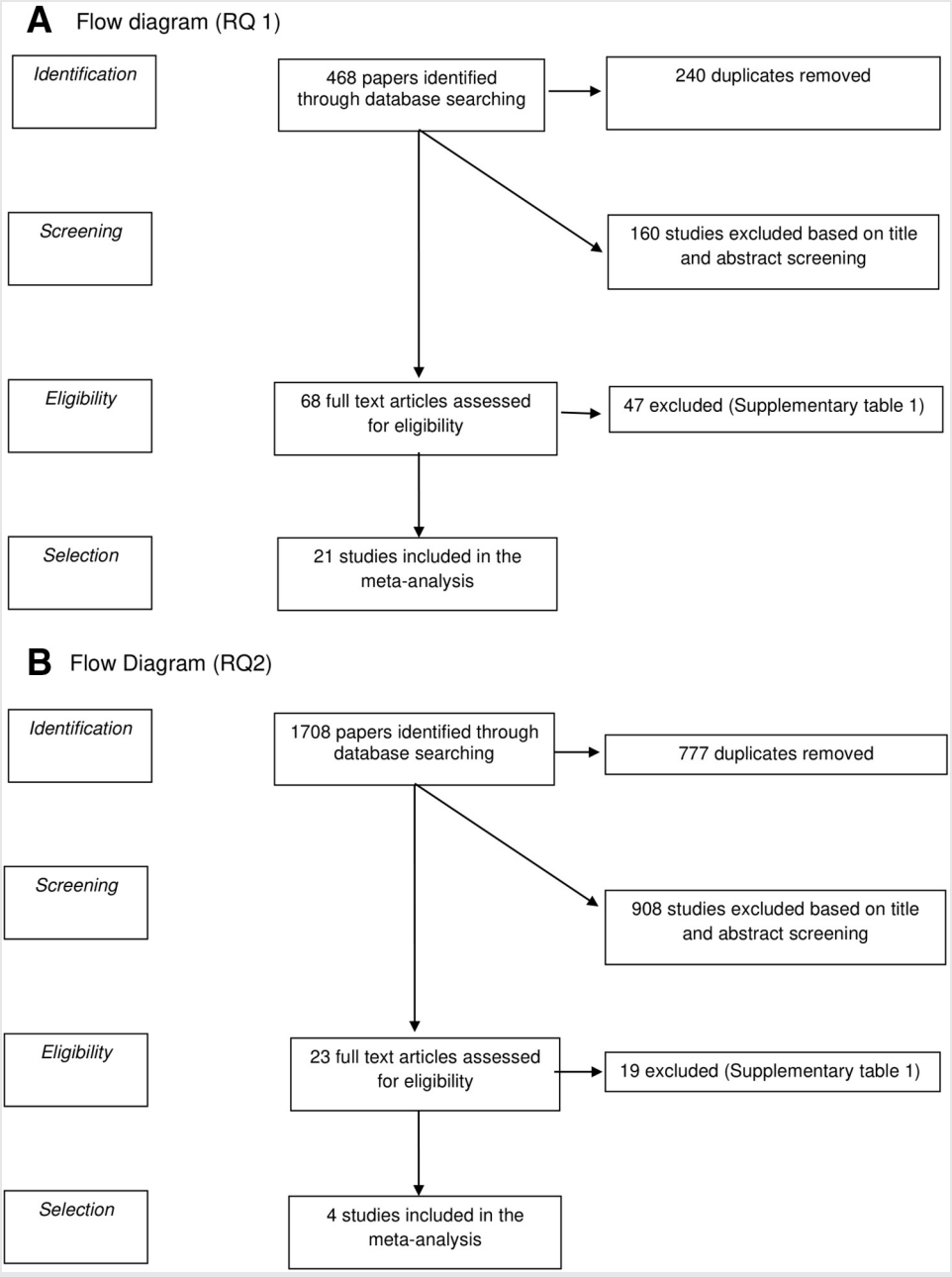
**Principales Medidas del Resultado:** Análisis seminal (concentración, recuento, volumen, motilidad, morfología) de acuerdo al manual de la Organización Mundial de la Salud, tasa de embarazo (TE) y tasa de abortos (TA).

**Resultados:** El meta-análisis incluyó dieciséis estudios. La presencia de VPHse asoció de manera significativa con parámetros seminales alterados en términos de concentración (MD -4.48, 95% CI -6.12 to -2.83), motilidad (MD -11.71, 95% CI -16.15 to -7.26) y morfología (MD -2.44, 95% CI -4.08 to -0 .79). Una revisión de la literatura en lo referente a pronóstico en ART mostró asociación entre infección por VPH y reducción en TE e incluso una asociación más potente entre infección por VPH y aumento de TA.

**Conclusión:** Nuestro meta-análisis demuestra un efecto negativo del VPH sobre el recuento, motilidad y morfología espermáticas. El posterior análisis categórico y de subgrupos confirmó el significado clínico de la alteración de la motilidad espermática en el semen infectado por VPH, aunque el recuento y la morfología espermáticas deben ser analizados cuidadosamente. Los estudios revisados informaron de menores TE y TA aumentadas en parejas con infección espermática por VPH. Puesto que la mayoría de estudios tenían un moderado riesgo de sesgo, estas observaciones justifican más estudios de mayor tamaño y bien diseñados antes de introducir recomendaciones de manejo clínico.

**Palabras clave:** Virus del Papiloma Humano, análisis seminal, pronóstico en Fecundación in Vitro, infertilidad masculina.

## SUPPLEMENTAL FIGURE 1




Study flow diagram. (**A**) Review question 1 (RQ1); (**B**) review question 2 (RQ2).

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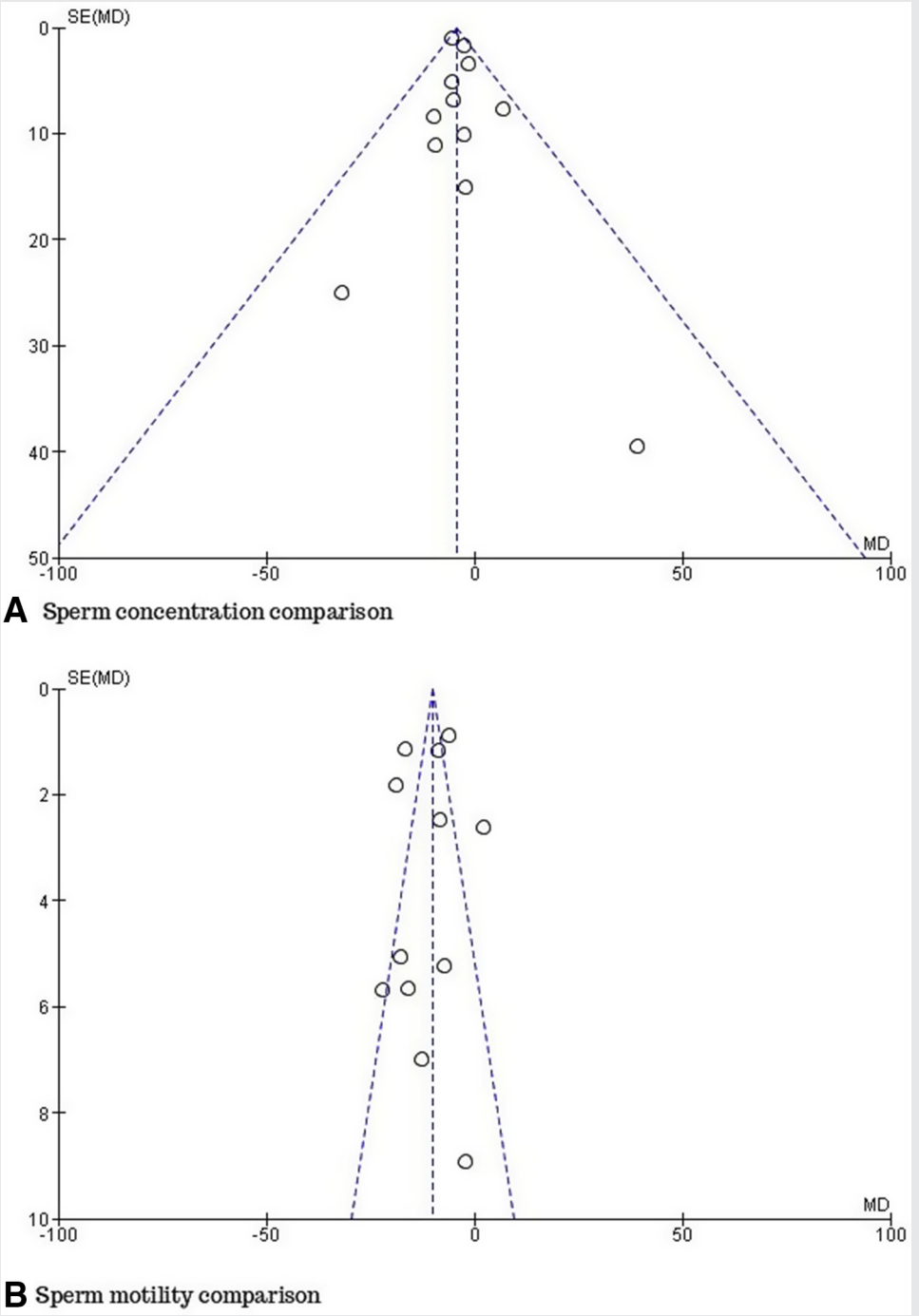
## SUPPLEMENTAL FIGURE 2




Funnel plots. (**A**) Sperm concentration comparison; (**B**) sperm motility comparison.

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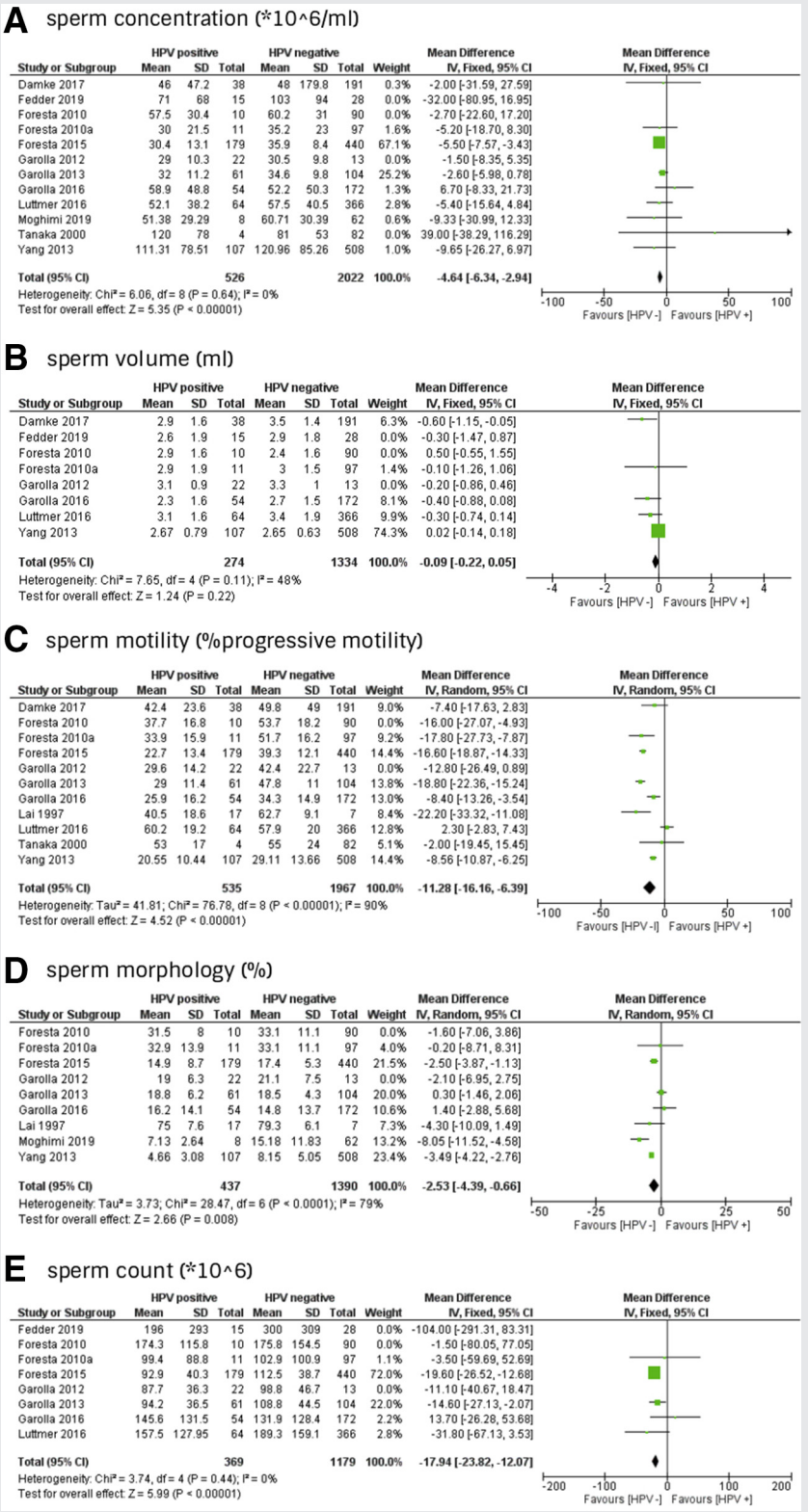
## SUPPLEMENTAL FIGURE 3




Forest plot of sperm parameters: subgroup analysis of infertility patients only. (**A**) Sperm concentration (*10^6/mL); (**B**) sperm volume (mL); (**C**) sperm motility (% progressive motility); (**D**) normal morphology (%); (**E**) sperm count (*10^6).

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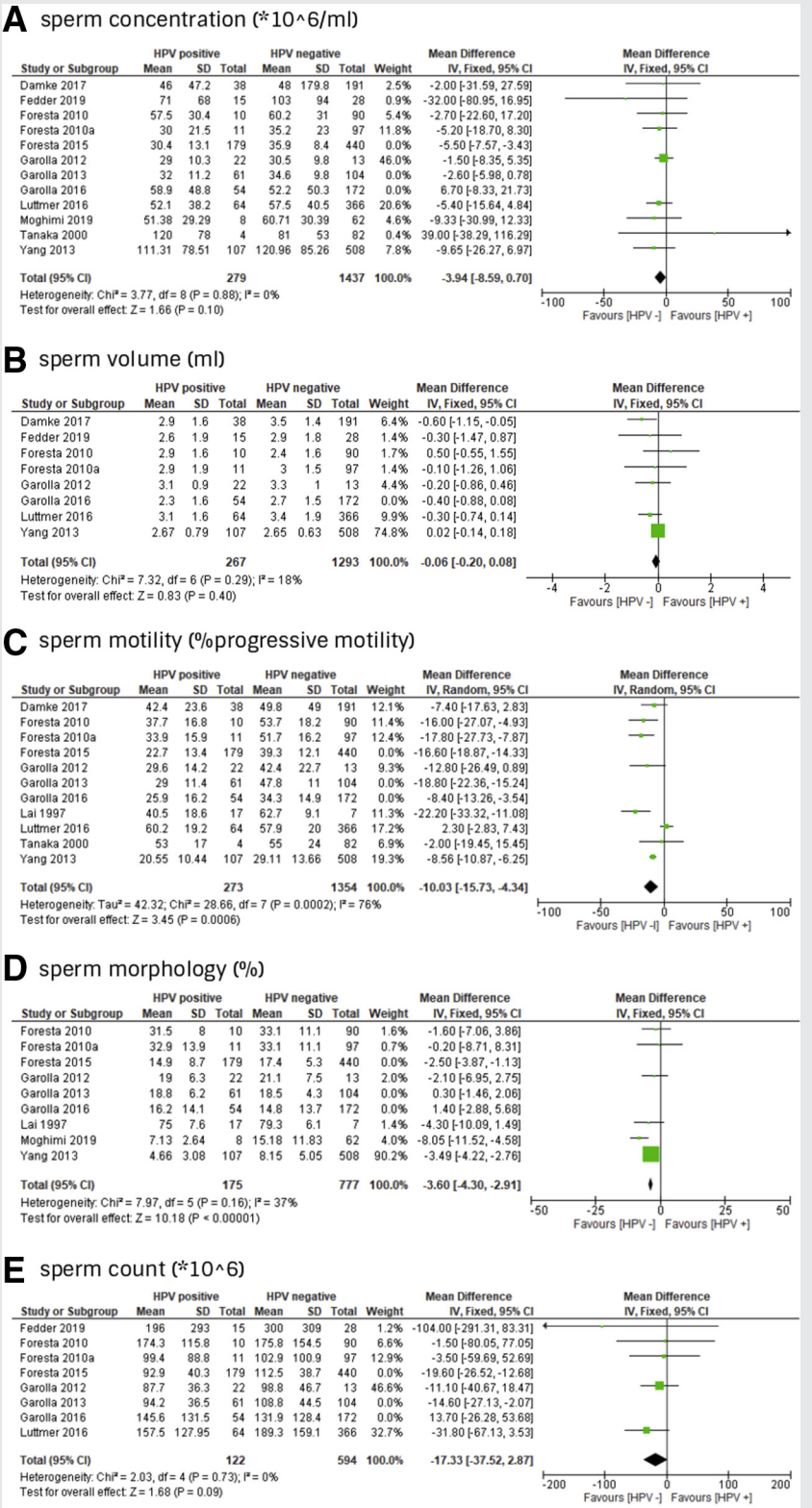
## SUPPLEMENTAL FIGURE 4




Forest plot of sperm parameters: subgroup analysis according to detection technique. (**A**) Sperm concentration (*10^6/mL); (**B**) sperm volume (mL); (**C**) sperm motility (% progressive motility); (**D**) normal morphology (%); (**E**) sperm count (*10^6).

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