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CASE REPORT

Unexpected viral presence: detection of oncogenic HPV-56 in human follicular fluid - a case report

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Abstract

Background

Human papillomavirus type 56 (HPV-56) is a high-risk oncogenic genotype not included in the nonavalent vaccine and has been associated with cervical, anal, and head and neck cancers. Because FF plays a key role in oocyte maturation, the detection of a high-risk virus within this microenvironment may have implications for fertility and early embryonic development.

Case presentation

We describe the detection of HPV-56 in the follicular fluid of a woman undergoing assisted reproductive technology (ART). Molecular analysis revealed HPV-56 exclusively in the FF aspirated from the second ovary according to the predefined retrieval order. The first ovary, aspirated immediately before, tested negative, reducing the likelihood of procedural contamination.

Discussion

Identifying a high-risk oncogenic HPV genotype in a compartment traditionally considered relatively protected challenges current assumptions about viral localization and ovarian vulnerability. Given the established oncogenic potential of HPV-56, its presence in FF warrants further investigation, as it may potentially affect oocyte competence, embryonic development, and ART outcomes.

Conclusion

This novel finding underscores the need for further research into HPV localization beyond the cervix and its possible impact on fertility and ART outcomes.

Keywords: HPV-56, Follicular Fluid, ART, oocyte, Embryo-development.

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Introduction

Human papillomavirus type 56 (HPV-56) is a high-risk oncogenic genotype associated with cervical cancer and has also been detected in anal, head and neck, and other anogenital malignancies [1-3]. Unlike other high-risk genotypes, HPV-56 is not included in the current nonavalent HPV vaccine [4-5], making its identification particularly relevant for prevention and reproductive health [6-7]. Although detection of HPV-56 at the cervical level is clinically significant, its identification in additional anatomical compartments, such as follicular fluid, may provide new insights into viral dissemination and its potential impact on fertility [8,9].

A growing body of evidence indicates that HPV infection may adversely affect both male and female reproductive function [10]. In men, HPV has been shown to bind to the sperm head, impair sperm motility, and alter sperm morphology, potentially reducing fertilization capacity [11-13]. In women, accumulating evidence suggests that HPV may interfere with early embryonic development and compromise reproductive outcomes. Collectively, these findings have raised concern about the potential role of HPV in subfertility and in the success of assisted reproductive technology (ART) [10].

Only recently has HPV DNA been detected in the follicular fluid (FF) of women undergoing ART, challenging the long-standing assumption that the ovarian environment is sterile [10,14-16]. The follicular microenvironment plays a critical role in oocyte maturation, protection, and developmental competence. The presence of viral DNA within this niche may disrupt the delicate biochemical balance required for optimal oocyte quality and subsequent embryo development [10,17].

To date, no published reports have specifically identified HPV-56 in follicular fluid. The detection of this high-risk genotype in FF therefore represents a novel and clinically relevant finding with potential implications for reproductive medicine. In this case report, we describe the first identification of HPV-56 in the follicular fluid of a woman undergoing ART, highlighting the need for further investigation into the reproductive consequences of this viral localization.

Case presentation

A 44-year-old woman underwent oocyte retrieval as part of an assisted reproductive technology (ART) cycle. Before oocyte retrieval, she underwent routine cervical cytology (Pap smear), which was reported as negative for intraepithelial lesions or malignancy (NILM). The cytology report described keratosis and a pyknotic index of 30%. No molecular HPV testing was recommended at that time, and no HPV DNA testing was performed before the ART cycle.

On the day of oocyte retrieval, a vaginal swab was collected as part of the center's standard protocol. Molecular analysis detected high-risk HPV-56. Transvaginal ultrasound-guided follicular aspiration yielded five oocytes, of which four were mature (metaphase II [MII]). All four mature oocytes were successfully fertilized, and two embryos were transferred. The patient subsequently experienced a spontaneous miscarriage.

To investigate the presence of HPV within the ovarian microenvironment, molecular testing for HPV DNA was performed on follicular fluid (FF) collected separately from each ovary. The right ovary was aspirated first according to the predefined retrieval sequence. HPV DNA testing of the right-sided FF was negative. The left ovary was aspirated immediately thereafter, and molecular analysis of its FF detected HPV-56. The detection of HPV-56 exclusively in the FF from the left ovary, together with the negative result from the right ovary, makes procedural contamination during oocyte retrieval or laboratory processing less likely. If contamination had occurred during aspiration or sample handling, both FF specimens would be expected to test positive. Although contamination cannot be completely excluded, the compartment-specific detection supports a true biological localization of HPV-56 within the left ovarian follicular environment.

No cervical HPV-56 infection had been documented before the ART cycle, and the patient had no clinical history of HPV-related disease. The unexpected identification of HPV-56 in follicular fluid represents a novel finding and raises important questions regarding viral dissemination, ovarian susceptibility, and the potential implications for oocyte competence and reproductive outcomes.

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Discussion

The detection of HPV-56 in the FF of the left ovary, but not in the right ovary aspirated immediately beforehand, suggests that the virus was unlikely to have been introduced during the ART procedure [18]. Instead, this compartment-specific positivity supports the hypothesis that HPV-56 was present within the ovarian microenvironment. If contamination had occurred during aspiration or laboratory handling, both FF samples would be expected to test positive. The unilateral detection therefore raises the possibility of true biological localization of the virus within the left ovarian follicular niche [10].

The presence of HPV-56 in FF raises important questions regarding the virus's ability to cross anatomical and immunological barriers. The ovarian environment has traditionally been considered relatively protected by multiple structural and immune defense mechanisms. The identification of a high-risk oncogenic HPV genotype within this compartment challenges this assumption and suggests that HPV may have mechanisms that allow it to be detected beyond the cervical epithelium. Whether this occurs through ascending infection, hematogenous spread, or cellular trafficking remains unknown [1,19].

A particularly relevant aspect concerns the potential interaction between HPV and granulosa cells. These cells play a central role in nurturing the oocyte, regulating follicular maturation, and maintaining the biochemical integrity of the FF. If HPV-56 exhibits affinity for granulosa cells, it may gain proximity to the oocyte itself. In men, HPV is known to bind to the sperm head, impairing motility and morphology [12-13,20]. If a similar mechanism were possible in oocytes, the implications for fertilization, embryo development, and ART outcomes could be significant. Although direct evidence of HPV binding to human oocytes is currently lacking, the detection of viral DNA in FF warrants further investigation into this possibility [10,19,21].

The identification of HPV-56 in FF also prompts reflection on the limitations of current preventive strategies. HPV-56 is not included in the nonavalent vaccine, underscoring that vaccination does not provide protection against all oncogenic genotypes [1,4,22]. As ART increasingly involves women of advanced reproductive age — many of whom may not have been vaccinated or may be exposed to non-vaccine HPV types — the potential reproductive impact of high-risk genotypes becomes an important area of inquiry [1,19,23].

This case also highlights the need to reconsider pre-ART screening protocols. A negative Pap smear, as in this patient, does not exclude the presence of HPV at the cervical level, nor does it provide information regarding HPV infection status beyond the cervix [24,25]. Molecular HPV testing may offer a more accurate assessment of viral status prior to ART [26]. Furthermore, given emerging evidence of HPV detection in FF, targeted viral DNA testing of follicular samples could provide additional insights into the ovarian microenvironment, particularly in cases of unexplained infertility or repeated ART failure.

Overall, the detection of HPV-56 in follicular fluid represents a novel and clinically relevant finding that challenges current assumptions about HPV localization and raises important questions regarding its potential impact on oocyte competence and reproductive outcomes.

Conflict of Interest

The authors report no conflict of interest.

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