

DEBATE

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Is it time to shift the attention on early stages embryo development to avoid inconclusive evidence on HPV-related infertility: debate and proposal

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Abstract

Background: Current evidence about *in-vivo* effects of HPV cannot definitively clarify the possible negative role of this worldwide common infection in early embryo development. However *in-vitro* evidence, seems to underline a possible negative effect of HPV in increasing blastocyst apoptosis and in reducing the endometrial implantation of trophoblastic cells. On these bases we believe that a new scientific approach is necessary to better understand the real role of male and female HPV infection in infertility and early pregnancy development.

Methods: English literature review of manuscripts focused on HPV infection and human reproduction was conducted. We performed a critical analysis of evidence and possible bias affecting both *in-vivo* and *in-vitro* studies regarding this topic.

Results: The biggest limitation of the *in-vivo* studies is due to the inappropriate timing of HPV effects evaluation since evidence about *in-vitro* studies strongly suggests that a large part of HPV negative effects occurs during a very early stage of embryo development. All the efforts of the scientific community to investigate the real role of HPV in human reproduction disorders cannot underestimate the severe BIAS of actual evidence in postulating new hypothesis and research projects which are fundamental to clarify if HPV may be associated with unexplained couples infertility and early miscarriages.

Conclusions: If the relationship between HPV gametes infection and early human reproduction step impairment will be confirmed, the HPV male and couple vaccination may represent a reliable option to improve fertility in some couples affected by infertility actually classified as “idiopathic” but maybe linked to HPV infection.

Keywords: HPV infection, Human reproduction, Sperm infection, Couple infertility, Early miscarriage, Study BIAS, Embryo development, Blastocyst, Trophoblastic cells

Background

Starting from our recent systematic literature review about “Male and Couple Fertility Impairment due to HPV-DNA Sperm Infection” we focused our attention on the controversies concerning HPV infection in human reproduction impairment [1]. Current evidence about *in-vivo* effects of HPV cannot definitively clarify the possible negative role of this worldwide common infection in early embryo development [2-6]. However, *in-vitro* evidence

seems to underline a possible negative effect of HPV in increasing blastocyst apoptosis and in reducing the endometrial implantation of trophoblastic cells [7-15]. Despite Matovina et al. detected the HPV-DNA in miscarriages specimens without any significant association [16], in our knowledge only two clinical studies demonstrated a possible relationship between HPV and early pregnancy loss. Hermonat et al. comparing the prevalence of HPV in samples from spontaneous miscarriages and elective abortions found that 15 of the 25 spontaneous samples (60%) were positive for HPV-DNA sequences compared to only 3 of the 15 elective samples (20%) [2]. The higher percentage

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of HPV-DNA detection, if compared to voluntary abortions, lead the Authors to consider HPV as one of the possible etiologic agents responsible for early pregnancy loss. Perino et al., on a total of 199 couples undergoing to IVF cycles (*in-vitro fertilization*), found an increased risk of pregnancy loss both in case of male sperm infection (66.7% *versus* 15%) and of female cervical infection (40% *versus* 13.7%) [3].

Anyway the interesting results of Perino et al. should be interpreted with caution since several Authors reported approximately 24% of cervical HPV-DNA detection in women with ongoing pregnancy [17,18].

Other 3 available studies (other than Matovina et al. [16]) failed to find a possible correlation between HPV infection and early miscarriages [4-6]. Skoczynski et al., on a total of 129 women, found the HPV-DNA in 17.7% of spontaneous miscarriages samples and in 24.4% of placenta from term deliveries [4]. Also Conde-Ferraz et al., comparing 127 women with spontaneous abortion attending for curettage and 127 pregnant women at-term, found an HPV cervical prevalence of 24.4% and 15.2% respectively without significant correlation between HPV infection and pregnancy loss [5]. Ticconi et al., analyzing retrospectively 49 cases of women with unexplained recurrent miscarriages and 475 healthy controls, found a cervical HPV prevalence of 26.53% in cases (13 of 49) and 61.89% in controls (294 of 475) confirming the absence of a causal association between HPV and miscarriages [6].

Discussion

We believe that all the *in-vivo* studies conducted till now (except for the perspective one of Perino et al.) are affected by different BIAS which probably led to confusion in considering the final results. The first possible BIAS is related to the HPV types detected: all the cited studies focused in particular on high-risk (Hr) genotype 16 and 18 extending the search only to few other Hr-genotypes or low risk HPV 6/11. The second BIAS is related to the sample detection methods of HPV presence which did not permit to rule out the possible contaminations derived by the passage through the genital tract. Adjunctively the study by Conde-Ferraz et al. and Ticconi et al., focusing only on cervical HPV detection, did not consider the possible BIAS related to the viral clearance (at time of cervical sample the patients may have already cleared the virus) which can lead to an underestimation of the problem [19]. The third BIAS is related to the fact that all these studies are built without considering the new inputs provided by recent *in-vitro* evidence which demonstrated that HPV sperm infection seems to have a possible role in adverse blastocyst development [7,14]. In particular it has been demonstrated that the spermatozoa is a vector for HPV transmission into fertilized oocytes and that the infected zygote (if the

oocyte is fertilized) is able to perpetuate the viral genome expression at blastocyst stage and subsequently in trophoblastic cells [7,13,20].

On these bases we believe that a new scientific approach is necessary to better understand the real role of male and female HPV infection in early pregnancy development and infertility. We think that the recent *in-vitro* evidence on this field represents a good starting point to plan new perspective studies and to avoid the above mentioned BIAS.

Yet in 1996 Cabrera et al. in a murine experimental study demonstrated that spermatozoa carrying HPV-16 and -18 viral genome can be transfected into a mouse blastocyst with viral DNA localization both in the inner cells mass and trophoblastic cells [14]. The consequences of this possible "colonization" were subsequently evaluated by different Authors who confirmed the possible adverse effects of HPV at different steps of human embryo development. Calinisan et al. found that mouse blastocysts incubated with HPV-DNA-16 fragments showed an increased rate of DNA fragmentation and apoptosis [15]. This interesting result was subsequently confirmed and deepened by Henneberg et al. who defined the exact timing of HPV embryo impairment. In particular the Author showed how HPV-16 and -18 are able to inhibit the embryo development only at 2 cells stage (but not at 4-8 cells stage) with a peak percentage of about 30% after HPV-16 exposure and a major inhibition of blastocyst hatching process after HPV-18 exposure [10]. Different Authors demonstrated also the negative effect of HPV presence in trophoblastic cells which could result in abnormal placentation and maybe in early pregnancy loss. You et al. demonstrated that trophoblastic cells, like squamous epithelium, are broadly permissive for HPV showing some similarities in the gene expression repertoire between these two cells. She found an active viral genome expression (both early and late genes) in 3A trophoblastic cells previously cultured with HPV 16,18,11,31. Moreover she found, in different *in-vitro* studies, that HPV-31 infection and HPV-16 E6 and E7 oncogenes caused both a decrease in 3A trophoblastic cells number and a low trophoblast-endometrial cells adhesion in the first week after the exposure [12,13]. The evidence that HPV-16 is able to complete its life cycle in trophoblastic cells was previously reported by Liu et al. [21]. These findings provided to expand HPV biology, to support the hypothesis of a possible link between HPV and some spontaneous abortions, and to search a new technology for studying HPV.

These effects were also confirmed by Gomez et al. who found a 3- to 6- fold greater rate of apoptosis in trophoblastic cells transfected with a plasmid containing the entire HPV-16 genome and a progressive decrease of trophoblast invasion ability (25.2-57.6%) from

day 3 until day 15 after transfection compared to negative controls [9]. Also Hong et al. showed a similar trend with a reduction both of implantation rate (less 37.2%) and of migratory/invasive activity in embryos exposed to HPV-16 [7].

The reasons which could explain the reduction of invasiveness of trophoblastic cells were suggested by Boulenouar et al. who found a down-regulation of E-cadherin (a fundamental protein for an adequate cell-to-cell adhesion) in trophoblastic cells expressing HPV-16 viral genome [8].

Although all the considered studies have important limitations (which should not be underestimated) linked to the *in-vitro* artificial and experimental conditions, most of the analyzed data suggested a possible adverse effect of HPV infection in early pregnancy development and maybe in idiopathic couple infertility. The demonstration that also the spermatozoa can act as a vector of HPV viral genome into oocyte [20] force the scientific community to understand what may be the clinical implication of this discovery. In fact, in 2010 Syrjanen reported that peri-conceptual transmission could theoretically occur via infected oocyte or spermatozoa [22]. So, although currently no studies exist on HPV detection in oocytes, theoretically, subsequent virus transmission might originate from the embryos soon after fertilization (such as via spermatozoa carrier) [20,22].

This fact acquires an even greater importance since the recent estimated prevalence of HPV sperm infection is about 10% (95% CI:7–14%) in general male population and 16% (95% CI:10–23%) in men affected by unexplained infertility [23].

So, focusing the investigations on HPV implications in human reproduction, it is mandatory to shift the older concept of infection with female gender target (i.e. oncologic risk in infected women respect to men) and to start in considering the HPV infection as a couple or, even better, a men problem.

In agreement with this new conception, it is intuitive that all the available *in-vivo* studies are not useful to completely understand the HPV exact role in human infertility because study methods, patients selection and idea conception seem to be inadequate.

The biggest limitation of the *in-vivo* studies is due to the inappropriate timing of HPV effects evaluation since evidence about *in-vitro* studies strongly suggests that a large part of HPV negative effects are carried out during a very early stage of embryo development (within the first two weeks after conception). These effects are very difficult to be demonstrated by clinical studies since it is possible to detect a causal-effects ratio on human reproduction only starting from the third week after conception (five week of amenorrhea). In this way all clinical studies lose a large part of negative effects demonstrated by *in-vitro* experimental studies.

Anyway clinical evidence can elucidate the consequences of HPV in late embryos development and pregnancy evolution. The detection of HPV in the placental tissue [24] lead to speculate about its possible role in adverse pregnancy outcomes since only few recent studies find an association between viral detection, placental abnormalities and preterm delivery [9,25,26]. Despite the increasing evidence of HPV vertical transmission [27,28], this route is regarded as less clinically important because of the detections of transient HPV-DNA. However, recent studies have provided clear evidence of papillomavirus productive infection in lymphocytes and placenta [29]. Furthermore, a model of papillomavirus latency has been recently proposed that could explain the failure or transience in HPV detection observed in some infected infants. This new evidence of hematogeneous and vertical spread of HPV suggests that these modes of transmission should be investigated in greater detail to obtain a better understanding of the infection and a fuller awareness of the preventive measures that can be taken against HPV-related diseases [30].

Conclusions

In conclusion all the efforts of the scientific community to investigate the real role of HPV in human reproduction disorders cannot underestimate the severe BIAS of previous evidence in postulating new hypothesis and research projects. Even if the choice of more appropriate laboratory techniques (ISH rather than PCR) could partially solve the BIAS linked to potential contamination of specimen, shifting the attention on men and couples rather than only on women represent a first step to solve the dilemma. As suggestion to Clinicians interested in the topic we invite to focus the attention on idiopathic infertile couples undergoing ART cycles, theoretically the most appropriate cohort to plan both *in-vitro* and *in-vivo* further studies.

We must definitively understand if the hypothesized adverse effects of HPV in human reproduction is only a supposition derived from a “inconclusive” scientific speculation or if we are faced to a new important discovery which can potentially clarify the exact mechanism of some cases of unexplained couples infertility and of early miscarriages linked to HPV infection.

So If the suspected HPV role in early human reproduction impairment will be confirmed by well conducted further studies, the couple vaccination before attempting both spontaneous and assisted pregnancy could be a reliable and effective tool [31].

The rationale for proposing the use of HPV vaccination (Gardasil® or Cervarix®) in couple attempting ART cycle is based on the evidence that in man the HPV sperm infection seems associated with some spermatic parameters impairment [1] and in women HPV vaccination strength the immunological response to the HPV

genotype already detected preventing de novo coinfection and superinfection by a different hr-HPV and lr-HPV genotypes [31]. As theoretical assumption, couple vaccination may potentially reduce some “undetected early miscarriage” actually erroneously considered as ART failure (lack of embryos implantation) increasing the pregnancy rate (first aim of ART procedures).

If in infertile couple the fertility rate will result increased after vaccination, this can be considered an indirect confirmation of our hypothesis and a suggestion to start more appropriate *in-vitro* and epidemiological studies on this field.

Improvement in the knowledge of HPV-DNA sperm infection mechanisms, timing, and link to fertility impairment may explain some of the actual “idiopathic” male and couple infertility.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

MN & SG carried out literature search, critical analysis of data and written the manuscript. AA carried out language revision and critical comment. GBN and GA participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

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