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What is the fertility-enhancing effect of tubal flushing? A hypothesis article

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ABSTRACT

Hysterosalpingographies (HSGs) have formed an essential part of the fertility workup for more than a century. More recently, tubal flushing, especially with oil-based contrast, has been shown to significantly improve the natural conception rates. Critically, the mechanism of this fertility-enhancing effect during tubal flushing is still unclear. This article postulates hypotheses, based on published and own research, on the potential mechanisms and root cause of tubal flushing fertility enhancement. Possible explanations for the increased fertility rates, especially with oil-based contrast, are divided into the biochemical and interfacial effects derived from the contrast properties. The biochemical effects may include the immunological response of the endometrium or peritoneum, the impact on the endometrial opioid receptors or the iodine content. The interfacial effects may include improvement of interfacial factors due to the lubricant effect or dislodgement of mucus debris within the Fallopian tubes.

IMPACT STATEMENT

- **What is already known on this subject?** Tubal flushing during hysterosalpingographies (HSGs) increases natural conception rates, and using oil-based over water-based contrast increases that effect even further. However, the underlying mechanism of the observed fertility-enhancing effect is still poorly understood.
- **What do the results of this study add?** This article postulates different hypotheses on the potential mechanisms and root cause of the fertility enhancement from tubal flushing.
- **What are the implications of these findings for clinical practice and/or further research?** We suggest additional research on the different hypotheses, intending to determine which subfertile women will benefit most from tubal flushing using oil-based contrast and at which stage of their subfertility. Furthermore, we suggest research on administering tubal flushing with oil-based contrast, besides in HSG.

KEYWORDS

Hysterosalpingography; subfertility; tubal flushing; oil-based contrast; tubal patency testing





Introduction

Rindfleisch introduced hysterosalpingography (HSG) in 1910 but only evaluated the uterine cavity by injecting a bismuth solution (Rindfleisch 1910). In 1914, Cary was the first to describe tubal patency assessment using HSG with collergol, a silver solution, as the contrast medium (Cary 1914). It is, however, HSG's therapeutic potential, first suggested by Weir and Weir that continues to gather interest (Weir and Weir 1951).

HSGs still have an essential role in daily practice and are advised for tubal patency testing in many countries (NICE Clinical guidelines [CG156]) (NICE 2017). Different contrasts are available for this diagnostic procedure, based on either water or oil-based medium.

Tubal flushing with oil-based contrast compared to no flushing significantly increases the live-birth rate, with an odds ratio of 3.3 (95% CI 1.6–6.9). Tubal flushing with water-based contrast may also increase the live birth rate compared to no flushing, with an odds ratio of 1.1 (95% CI 0.67–1.9) (Wang et al. 2020). Additionally, a long-term follow-up study of the largest RCT on the comparison of water-based versus oil-based contrast, showed that there were significantly more naturally conceived pregnancies in women who underwent an HSG with oil-based contrast compared to water-based contrast (van Rijswijk et al. 2020).

Over the years, scientific reports have focussed on differences in pregnancy outcomes between HSG contrasts, but the observed fertility-enhancing effect's underlying mechanism is not well understood. Most studies focus on women with unexplained

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subfertility and a smaller number on subfertile women with endometriosis or repeat implantation failure undergoing IVF (Johnson et al. 2004; Brent et al. 2006; Reilly et al. 2019).

Knowledge of the mechanism of action of tubal flushing is essential, as it can help us understand which women, and at which stage of their subfertility, can benefit from tubal flushing. This paper aims to summarise the different hypotheses on the mechanisms of the fertility-enhancing effect of tubal flushing. In doing so, we also aim to clarify the knowledge gaps and to suggest topics for further research.

Methods

Electronic databases including PubMed®, Scopus and Web of Science were searched for relevant publications. The key search items included 'hysterosalpingography', 'oil contrast', 'ethiodized oil', 'ethiodol', 'lipiodol', 'therapeutic', 'peritoneum', 'endometrium', 'immune cells', 'phagocytosis', 'sperm', 'iodine', 'endometriosis', 'ciliary motion', 'sperm transport', 'sperm capacitation', 'isthmic plugs', 'tubal obstruction', 'cilia', 'tubal flushing', 'shear stress' and 'vascular mechanobiology'.

Hypotheses

The different hypotheses focus on the possible fertility-enhancing effect of tubal flushing with relation to; the biochemical effects of the contrast and the interfacial effects of the contrast properties (Figure 1). We will discuss each hypothesis, and its available evidence, separately.

Hypothesis 1. Immunological effect on the endometrium and peritoneum

The first hypothesis is that oil-based contrast positively affects fertilisation and implantation due to altering the immune response to spermatozoa and the conceptus.

Uterine bathing, the effect of oil-based contrast on the endometrium

A 1987 rodent study showed the induction of decidua formation after intrauterine oil injection in appropriately sensitised uteri (Milligan 1987). Later research in a mouse model, showed that CD205+ dendritic cells decrease after the instillation of oil-based contrast, compared with sham and saline infusion treatments. Dendritic cells present antigens and thereby stimulate the T-cells of the immune system. The decrease in these cells reduces antigen sampling and the immune system's presenting capability in the uterus. The fertility-enhancing mechanism of oil-based contrast may be via damping the immune response to antigens, including a conceptus. Two hypotheses for the decrease in dendritic cells after oil-based contrast injection are; a toxic effect on the dendritic cells or stimulation of dendritic cells's migration to lymphoid organs (Johnson et al. 2005).

However, in a randomised trial in women with endometriosis or repeated implantation failure, no evidence was found that additional flushing with oil-based contrast increases the success of IVF compared to no additional flushing. A limitation of this study is that it was underpowered (including only 70 of the planned 350 women) (Reilly et al. 2019).

Peritoneal bathing, the effect of oil-based contrast on the peritoneum

Clinical studies have shown that the positive effect of oil-based contrast, on pregnancy rate, is higher in women with endometriosis-related subfertility than unexplained subfertility. The relative risk to become pregnant after flushing with oil-based contrast compared to no flushing was 4.4 (1.61–12.21) in women with endometriosis compared to 1.6 (0.81–3.16) in women with unexplained subfertility (Johnson et al. 2004). The following possible mechanisms may explain these results.

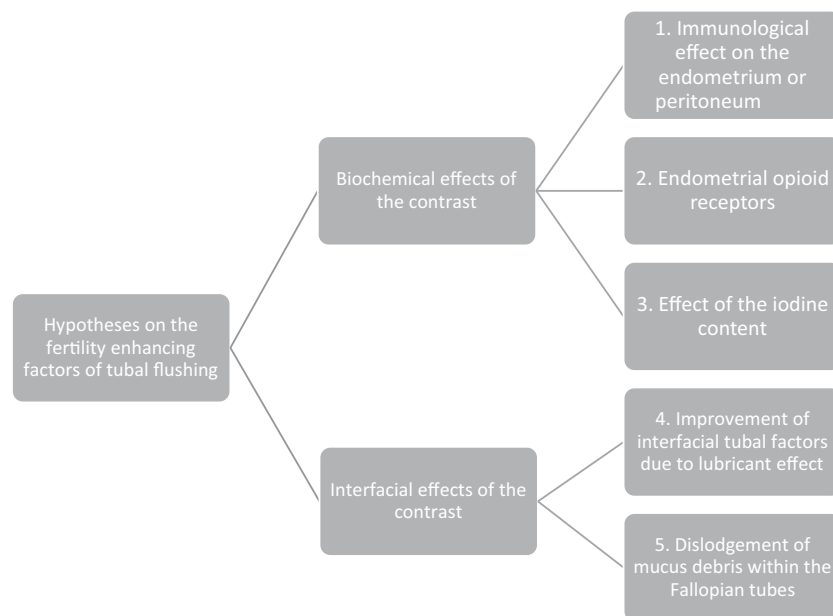


Figure 1. The hypotheses on the fertility-enhancing factors of tubal flushing.

Inhibiting effect of spermatozoa phagocytosis. Peritoneal macrophages from women with endometriosis exhibit higher levels of phagocytosis capacity of spermatozoa *in vitro*. This effect suggests that the peritoneal macrophages might negatively influence fertilisation by phagocytising spermatozoa (Muscato, Haney and Weinberg 1982). In an *in vitro* study, exposure of oil-based contrast (Ethiodol) to the peritoneal fluid of six women with unexplained subfertility or endometriosis inhibited phagocytosis of the pelvic macrophages. The proposed mechanism is that the lipids form a layer on the macrophages' cytoplasmic membrane, which hinders the contact between the macrophages and the spermatozoa (Mikulska et al. 1994). By inhibiting macrophages' activity, oil-based contrast may positively affect fertilisation (Boyer et al. 1986).

Stimulating effect on dendritic cells in peritoneal fluid. An *in vitro* study shows that human dendritic cells that incorporate oil-based contrast show greater cellular complexity, indicating maturity compared to cells cultured without oil-based contrast. The same phenotype of dendritic cells is present in the peritoneal fluid of women who previously underwent an HSG with oil-based contrast. It may be that the dendritic cells phagocytose oil-based contrast, consequently initiating an immune response and then become mature. Mature dendritic cells may create a more favourable peritoneal environment for conceiving, presumably because dendritic cells' maturation reduces their phagocytosis activity. This process may be critical in women with immunological abnormalities in the peritoneal cavity, such as endometriosis (Izumi et al. 2017).

Hypothesis 2. Endometrial opioid receptors

The second hypothesis is that oil-based contrast increases endometrial receptivity due to acting on endometrial opioid receptors.

The commercial available oil-based contrast, Lipiodol Ultra Fluid[®], is derived from the seeds from the *Papaver somniferum*, which contain natural opium (Shenoy and Lui 2019). Research has demonstrated that opioid receptors express in human endometrial cells and that their expression changes during the menstrual cycle. mRNA expression increases during the proliferative phase and decreases during the menstrual cycles secretory phase with maximum values around ovulation time. This cyclic upregulation of opioid receptors, especially during the mid-proliferative to the mid-secretory phase, suggests a role in endometrial receptivity and consequently implantation. Therefore, we hypothesize that oil-based contrast may act through endometrial opioid receptors increasing endometrial receptivity, forming part of explaining the aforementioned increased pregnancy rates after using oil-based contrast (Totorikaguena et al. 2017).

Hypothesis 3. Effect of the iodine content

The third hypothesis is that the iodine content of the contrast positively affect ovulation and implantation.

The commercially available oil-based contrast media (Lipiodol Ultra Fluid[®]) contains more iodine than the most common water-based contrast media, 480 mg I/mL vs. 250–300 mg I/mL, respectively (depending on the type of water-based contrast media selected). Additionally, water-based contrast, and its iodine content, is more rapidly excreted from the body than oil-based contrast (Miyamoto et al. 1995).

Worldwide, there has been a drastic decrease in urinary iodine-concentration (UIC) in pregnant women. In 1971, the National Health and Nutrition Examination Surveys (NHANES) began measuring the UIC levels within the United States. The median UIC was 327 µg/L for pregnant women in 1971–1974. The second survey (1988–1994) showed a median UIC of 141 µg/L for pregnant women (Hollowell et al. 1998).

Further studies from the United States published have shown a median UIC of 100.5 µg/L, in women wishing to conceive (Stagnaro-Green et al. 2015). The reference values for an adequate mean UIC are 100–199 µg/L for adults and 150–249 µg/L for pregnant women, according to the World Health Organisation (WHO) (WHO 2013). An inadequate iodine intake may lead to a decrease in the production of thyroid hormones. Eleven percent of women in Washington, DC, in 2015, displayed subclinical hypothyroidism at pre-conceptual consultations (Stagnaro-Green et al. 2015).

An inadequate iodine intake can lead to subclinical hypothyroidism with a further negative effect on ovulation (Fairley and Taylor 2003). Furthermore, in ovulating women with low UIC, the fecundability is significantly decreased (Mills et al. 2018). This reduction may be because thyroxine increases progesterone secretion, and, in a lesser amount, the oestradiol secretion of granulosa cells (Wakim et al. 1995). Additionally, there may be an effect on the endometrium/myometrium or both, containing thyroid hormone receptors (Kirkland et al. 1983). Interestingly, a study in ewes and rams with a low UIC showed a significant increase in pregnancy rate after a subcutaneous injection of iodised oils (Lipiodol[®]), from 37% to 100% ($p = .007$) (Ferri et al. 2003).

We hypothesise that the fertility-enhancing effect of oil-based contrast during HSGs may be partially due to the iodine content of the contrast influencing ovulation and implantation.

Hypothesis 4. Improvement of interfacial factors due to the lubricant effect of the contrast medium

This hypothesis states that the lubricating properties of the oil-based contrast have a fertility-enhancing effect, due to improvement in cilia's action and gametes transport within the oviduct.

Cilia factors

Ciliary activity within the Fallopian tubes influences tubal secretions flow and the gamete transport (Jansen 1984). The cilia structure consists of a central pack containing two single microtubules-axoneme, surrounded by nine other microtubules arranged in doublets (see Figure 2). The doublet microtubules sliding drives the rhythmical beating movement of

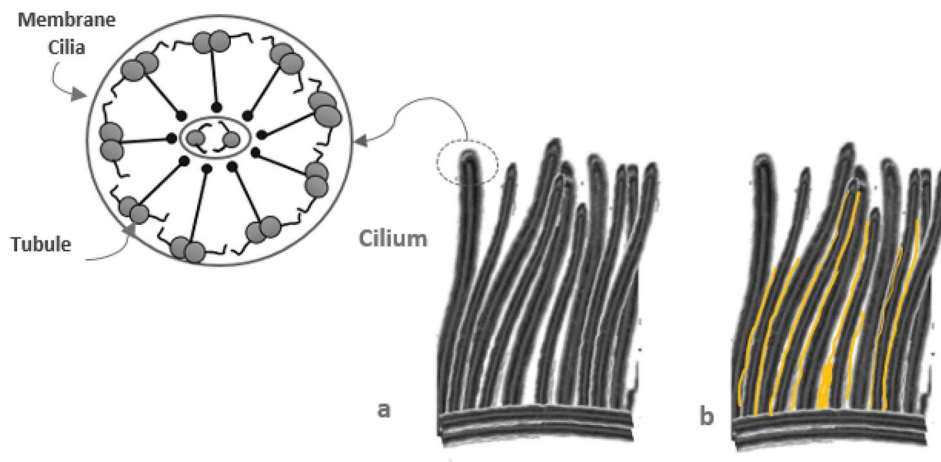


Figure 2. The cilia and a schematic image of the axoneme structure and its microtubules: (a) cilia before flushing and (b) cilia after lubrication with oil-based contrast.

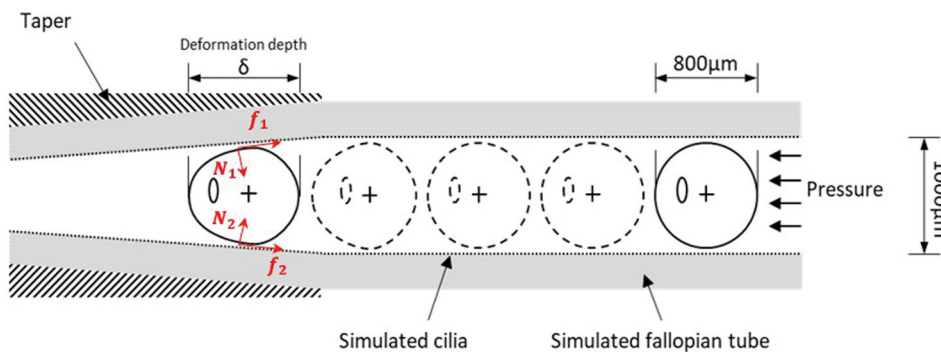


Figure 3. A schematic of oocyte transit within microchannel and exerted forces on it. The tangential force (f_1 and f_2) acts as resistance to cells' movement with the flow, and the normal force (N_1 and N_2) is the support force exerted on the cell from the channel's wall.

the cilia, which generates intratubal flow (Satir and Matsuoka 1989; Shi et al. 2011).

Oil-based contrast media reduces the friction between the cilia themselves, due to its lubricating effect and by filling the cilium's irregular surface and the inner surface of the Fallopian tube. By reducing the friction, the cilia's beating movement may be enhanced, which may positively affect gametes and embryo transportation through the Fallopian tube.

Additionally, the cilia movement's improvement increases mixing of tubal secretions and gametes (Muglia and Motta 2001).

Oocyte transit. Oil-based contrast may function as a lubricant to enhance the cilia movement, reducing friction between individual cilia and between cilia and gametes, easing the transition through the Fallopian tube. Well-lubricated, low friction conditions will improve spermatozoa and oocyte transportation by reducing tangential resistance.

A mathematical model can describe friction between the oocyte and the Fallopian tube walls, where the friction coefficient depends on the physical and geometric properties of the oocyte and the physical and chemical properties of the contrast (see Appendix 1). High friction restricts the movement of the oocyte, and a low friction coefficient facilitates movement. Figure 3 illustrates the oocyte transit within

a microchannel, which provides insight into the influence of the tribological properties of the HSG contrasts. We hypothesize that an oil-based contrast reduces friction offering less resistance to ciliary movement and oocyte transport than water-based contrast media.

Motility effect of spermatozoa. The female genital tract contains oviductal fluid, produced by the transudate fluid from the systematic circulation and secretory ciliated epithelial cells within the oviduct (Li and Winuthayanon 2017). When the spermatozoa come into contact with the oviductal fluid, they undergo essential changes, starting with the capacitation process. As a result of this, the motility of the spermatozoa increases through hyperactivation. This process provides a vital force to overcome the attraction between the spermatozoa and the tubal-epithelium (see Figure 4). The epithelium itself also plays a crucial role in the spermatozoa's detachment; however, this process's mechanisms are unknown (Suarez and Pacey 2006). Oil-based contrast may reduce interfacial bonding during capacitation and hyperactivation, and therefore enhance the fertility outcomes. There are no studies available that tested this hypothesis.

An *in vitro* study demonstrated that tubal fluid conditioned by cultured endosalpingeal cells, heparin or both improve bull sperm capacitation, reducing binding to

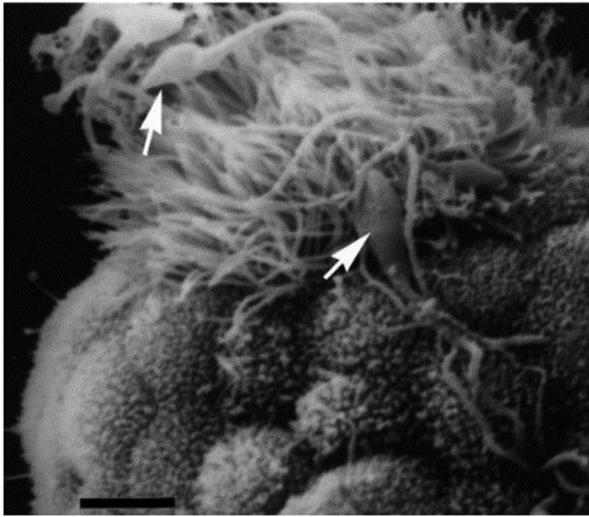


Figure 4. A sample of scanning electron micrograph illustrating the human spermatozoa attached to the cilia (Suarez and Pacey 2006).

endosalpingeal epithelium (Chian et al. 1995; Mahmoud and Parrish 1996). An oil-based contrast may also reduce interfacial bonding to improve fertility outcomes.

In summary, the fertility-enhancing effect of oil-based contrast may be partially due to its lubricating properties, either through reducing resistance during gamete transport or reducing interfacial bonding providing free movement of the cilia or during capacitation and hyperactivation of spermatozoa.

Hypothesis 5. Dislodgement of mucus debris within the Fallopian tubes

The fifth and final hypothesis states that oil-based contrast leads to more effective dislodgement of tubal debris, which improves tubal transport.

Amorphous casts of unknown aetiology can form within the Fallopian tube's proximal region (Sulak et al. 1987). These 'debris plugs' consist of mucus and aggregates of histiocyte-like cells, originating, potentially from endometrial stromal or mesothelial cells (Gillespie 1965; Kerin et al. 1991).

Such debris may cause partial or total tubal occlusions in otherwise anatomically normal Fallopian tubes (Sulak et al. 1987). This debris's existence may inhibit the complete operation of the cilia within the Fallopian tube by negatively influencing the cilia's beating pattern (Jackson-Bey et al. 2020), and therefore prevent natural transportation of the oocyte and spermatozoa. Tubal flushing during HSG could provide a mechanical means of removing debris (Gillespie 1965), and may increase natural pregnancy and live birth rates (Wang et al. 2019). From a technical perspective, the contrast's flow creates intratubular pressure and generates shear forces acting against the debris that may contribute to the dislodgement, similar to that found in occluded blood vessels (Lu and Kassab 2011).

An *in vitro* fluid dynamics model incorporating both shear stress and contrast resistance (see Appendix 2) shows that higher viscosity fluids cause higher resistance within an artificial tube during the flow. This mechanical loading may assist

in detaching the mucus plugs within the Fallopian tube. The contrast's viscosity will be essential and is directly proportional to the shear stresses and tubal resistance. However, this is not the only physical property contributing to the pressure build-up and mucus dislodgement within the Fallopian tube. Other contrasts' characteristics, such as density and surface tension, may also play a role in the dislodgement.

Perceived pain levels may also help support the pressure build-up hypothesis. Van Welie et al. report that women who experience moderate to severe pain (visual analogue score ≥ 6) during oil-based contrast HSG benefit from higher ongoing pregnancy rates than the procedure conducted with water-based media (30 to 50%, RR 1.7; 95% CI 1.1–2.5). Below this pain threshold, there is no significant effect on pregnancy outcomes between oil-based and water-based contrast (van Welie et al. 2019). Some women's pain levels during HSG may be indicative of tubal pressure build-up followed by dislodgement of the intra-tubal debris.

It may be that tubal flushing with oil-based contrasts dislodge the debris plugs and clear occlusions more efficiently and leave less debris residue, resulting in improved fertility outcomes by restoring the cilia operation. Contrast specific properties may determine the effectiveness of the debris dislodgement.

Discussion

The different hypotheses for the fertility-enhancing effect of tubal flushing, especially with oil-based contrast presented here, are: the immunological effect on the endometrium or peritoneum, the effect on the endometrial opioid receptors, the impact of the iodine content on ovulation and implantation, the improvement of interfacial factors due to the lubricant effect or the dislodgement of mucus debris within the Fallopian tubes.

Strengths and limitations

This article is the first to provide an overview of the published data on the potential mechanisms, the hypotheses, of the fertility-enhancing effect from tubal flushing. However, the amount of evidence for each hypothesis is limited and the current knowledge behind the hypotheses is partly based on *in vitro* studies and/or animal studies. Nevertheless, summarising the insights from animal studies is an important first step in determining subsequent human studies. Our hypotheses are drawn from critical analysis of medical and clinical engineering research sources.

Clinical implications

The ultimate goal is to understand which women, and at which stage of their subfertility, can benefit from tubal flushing, especially with the use of oil-based contrast. However, with the published studies until now this is not yet possible to determine. We suggest further research to obtain more data to test each hypothesis. Additionally, we advise research on the route of administration of tubal flushing, other than

the traditional HSG, for example, hysterosalpingo-foam/contrast sonography (HyCoSy/HyFoSy), transvaginal hydrolaparoscopy (THL) or laparoscopy. These techniques are being used more frequently in daily practice because they do not require radiation and may, in the future, even replace traditional HSG. However, there is no knowledge on tubal flushing's effect and safety with oil-based contrast in these alternative tubal patency testing methods.

Conclusions

We summarised the different hypotheses on the fertility-enhancing mechanism of tubal flushing, especially with oil-based contrast, during the fertility workup. More research is needed to determine which subfertile women will benefit from tubal flushing using oil-based contrast, at which stage of their subfertility and after which route of administration.

Authors contributions

IR, AMH, MB, VM, BWM, CK and KDD contributed to the study conception and design. IR and AMH prepared the manuscript. MB, VM, BWM, CK and KDD critically revised the paper. All authors approved the final version of the article.

Disclosure statement

I.R. reports receiving a travel fee for presenting at the ASRM 2019 from Guerbet. A.M.H. has nothing to disclose. K.D.D. has nothing to disclose. V.M. reports receiving travel- and speakers fee as well as research grants from Guerbet. M.B. has nothing to disclose. B.W.M. is supported by an NHMRC Practitioner Fellowship (GNT1082548). B.W.M. reports consultancy for ObsEva, Merck KGaA and Guerbet and travel and research grants from Merck KGaA and Guerbet. C.K. has nothing to disclose.

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Appendix 1

Equation (1) is used to calculate the coefficient of friction:

$$\mu_k = \frac{3[F_D - \rho_{cell} V_{cell} a]}{(\rho_{oocyte} - \rho_{contrast}) \times \pi d^3 g \cdot \cos \theta} \quad (1)$$

where ρ represents the densities of the oocyte and the contrast, d and V indicate the oocyte diameter and volume, respectively. The friction coefficient μ_k confirms the sole contribution of the contrasts on the tribology properties between the cell and surface as the results will be comparative and not absolute.

Appendix 2

In a way similar to blood vessels, Fallopian tubes are under mechanical loading from the pressure of the flow which causes internal shear and circumferential wall stresses (Lu and Kassab 2011). Based on shear stress and contrast resistance equations (Equations (2) and (3)), it is demonstrated that the higher viscosity fluids cause higher resistance to the wall during the flow. Based on this modelling, the viscosity has a direct proportional relationship with shear stresses and resistance within the tube.

$$\tau_s = \frac{4\mu Q}{\pi R^3} \quad (2)$$

and

$$R = \frac{8\mu L}{\pi R^4} \quad (3)$$

where μ indicates the viscosity of the contrast, L and R are the length and radius of the Fallopian tube, respectively, and Q is the flow rate of the contrasts.