Impact of Ultrasound Gels on Sperm Function Safety Assessment of Transvaginal Ultrasound for Trying-To-Conceive Couples

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Dedicatória

“Há quem diga que amor de pais

É o maior do mundo

Esquecendo que o de avós

É eterno e profundo.”

Para a minha querida avó, cujos humildes sonhos jamais contemplariam o rumo que minha vida tomou e por quem carrego a responsabilidade de tudo o que deixou.

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Aos que tornaram a Covilhã casa, pelo conforto de saber que nos temos uns aos outros quer nas alegrias, quer nos momentos em que o coração aperta.

Resumo

Introdução: A ultrassonografia desempenha um papel central no diagnóstico e tratamento da infertilidade, afetando casais que estão a tentar engravidar. Esta técnica requer o uso de géis de ultrassom para os quais não existe atualmente regulamentação ou diretrizes claras, comprometendo a sua segurança.

Objetivo: O presente estudo visa caracterizar físicoquimicamente quatro géis de ultrassom quanto ao pH, osmolalidade e viscosidade, bem como avaliar o seu potencial impacto negativo na motilidade espermática, utilizando um modelo bovino *in vitro*.

Materiais e métodos: Quatro géis de ultrassom, comercializados na Europa, foram testados quanto ao pH (por imersão), osmolalidade (via depressão crioscópica) e viscosidade (usando um viscosímetro rotativo). Foram depois incubados a 10% (v/v)/37ºC, com espermatozoides bovinos previamente capacitados, durante 60 minutos. A motilidade espermática foi avaliada aos 30 e 60 minutos para cada amostra/controlo, em triplicado, para determinar a percentagem de motilidade espermática. O ensaio foi realizado em duplicado. Foi aplicado o teste ANOVA *one*-way, com o *post hoc* de *Dunnett* ($p<0{,}05$), através do GraphPad©, para averiguar a significância, comparando a motilidade entre as amostras e o Controlo.

Resultados e Discussão: Todos os géis de ultrassom apresentaram pH ácido, sendo um hipoosmolar e os restantes hiperosmolares. O Quick-Eco Gel® apresentou a maior viscosidade, enquanto o Clear Eco Supergel® a menor. Os espermatozoides progressivos para o Controlo Negativo foram 86% (30') e 83% (60'), e menos de 5% para os restantes géis de ultrassom (Sterile Aquasonic® 4%/2%, Quick-Eco Gel® 3%/1%, Clear Eco Supergel® 1%/2% e "Gel Ultrassom Azul" 1% aos 30 e 60'). Todos os géis demonstraram ser significativamente diferentes do controlo aos 30 e 60 minutos.

Conclusão: Este estudo destaca o potencial impacto negativo dos géis de ultrassom na motilidade espermática, enfatizando a necessidade de regulamentação e mais pesquisas para garantir sua segurança, especialmente para casais que estão a tentar engravidar.

Palavras-chave:

Géis-de-ultrassom;Motilidade;pH;Osmolalidade;Viscosidade

Abstract

Introduction: Ultrasonography plays a central role in the diagnosis and treatment of infertility, affecting trying-to-conceive couples. This technique requires the use of ultrasound gels which, lacking regulation and clear guidelines, can compromise safety standards.

Objective: This study aims to characterize physicochemically four ultrasound gels for their pH, osmolality and viscosity, and evaluate their potential negative impact on sperm motility, using a bovine *in vitro* model.

Materials and Methods: Four ultrasound gels marketed in Europe were tested for pH (by immersion), osmolality (via cryoscopic depression) and viscosity (using a viscometer). After, they were incubated at 10%(v/v)/37ºC, for 60', with bovine spermatozoa previously capacitated. Sperm motility was evaluated at 30' and 60' for each sample/control, in triplicate, to assess the percentage of sperm motility. Two independent runs were performed. GraphPad© was used to perform one-way-ANOVA with Dunnett's *post hoc* test ($p<0.05$) for significance, comparing %Motility between samples and Negative Control (sample without ultrasound gel).

Results and Discussion: All the ultrasound gels have an acidic pH one is hypoosmolal and the other three hyperosmolar. Quick-Eco Gel® exhibited the highest viscosity while Clear Eco Supergel® had the lowest. The percentage of progressive spermatozoa for Negative Control was 86% (30') and 83% (60') and less than 5% for all ultrasound gels (Sterile Aquasonic® 4%/2%, Quick-Eco Gel® 3%/1%, Clear Eco Gel® 1%/2% and "Gel Ultrassom Azul" 1% at 30' and 60'). Statistical analysis was significant for all ultrasound gels at both 30 and 60 minutes.

Conclusion: This study demonstrates that tested gels could have a detrimental impact on sperm motility which underscores the importance of further research to contribute to the regulation of these gels, especially for trying-to-conceive couples use.

Keywords:

Ultrasound-gels;Motility;pH;Osmolality.Viscosity

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Acronym List

ART	Assisted Reproductive Techniques
WHO	World Health Organization
TTC	Trying-To-Conceive
UG	Ultrasound Gel
FDA	Food and Drug Administration
SA	Sterile Aquasonic®
QEG	Quick-Eco Gel®
CES	Clear Eco Supergel®
GA	“Gel Ultrassom Azul”
Spmtz	Spermatozoa
NC	Negative Control
PR	Progressive Spermatozoa
NP	Non-progressive Spermatozoa
IM	Immotile Spermatozoa

1. Introduction

Ultrasonography is an emerging imaging technique that has assumed an increasing central role in diagnosis and treatment, particularly in the fields of gynecology and obstetrics. Since its inception in this area, in the 1950s, this technique has undergone significant evolution. In general, ultrasound allows for the investigation of the anatomy and physiology of the female reproductive system throughout its various stages (1).

During pregnancy, ultrasound provides a primary diagnostic for assessing placental and umbilical cord characteristics, as well as monitoring real-time fetal development across all three trimesters. Additionally, this technique enables a comprehensive gynecological evaluation, assessing the anatomy and physiology of the female reproductive system. It aids in identifying potential adnexal masses and causes of infertility, such as endometriosis, adnexal adhesions, anatomical anomalies, among others (1,2).

The accuracy of ultrasound diagnoses is closely related to the mastery of the technique and the clinician's experience. Hence, training in this technique has been actively encouraged, and the scientific community is continuously working to develop guidelines that appropriately define its application to harness its multiple potentials. The ongoing efforts aim to ensure that clinicians are well-equipped to leverage the capabilities of ultrasound for precise and effective diagnostics in the realm of gynecology and obstetrics. This technique allows the visualization of internal structures of the body, in real-time, by emitting ultrasound waves. The passage of waves between the transducer and the skin is ensured by a ultrasound gel, which is a colloid suspension that prevents ultrasound dissipation and allows the formation of a clear image (1,2).

In the field of infertility, ultrasound plays a particularly important role, offering both diagnostic insights and opportunities for treatment. When it comes to assessing the etiology of infertility, ultrasonography proves valuable by allowing the visualization of endometrial abnormalities and ruling out conditions such as endometriosis, adhesions, or masses that could hinder oocyte implantation (2).

Furthermore, it is essential in evaluating the patency of the fallopian tubes, which can impact the migration of the oocyte towards the uterus. Ultrasound also facilitates the assessment of ovarian follicle reserve and identifies abnormalities like polycystic disease that may affect oocyte production (2).

In the realm of treatment, once etiology is established, ultrasound can be employed to monitor follicular development, assess ovulation timelines, and facilitate the collection of oocytes for *in vitro* fertilization. This technique proves instrumental in the subsequent implantation of fertilized oocytes into the uterus. Overall, ultrasound emerges as a versatile tool in both understanding and addressing infertility concerns, offering a comprehensive approach from diagnosis to treatment in the field. It is routinely used in clinical practice both in infertility studies associated with female factors and in the monitoring of follicular development during Assisted Reproductive Techniques (ART) (2,3).

Infertility is a pathology whose prevalence has been increasing over the last decades, affecting more than 186 million people worldwide. In fact, based on a 2021 study by the World Health Organization (WHO), approximately 17.5% of the global population, or 1 in 6 people, is estimated to grapple with infertility. This issue transcends borders, affecting various nations and sparking worry over the increasing prevalence of countries facing inadequate fertility rates to sustain future generations (4–7).

Clinically, infertility is defined as the absence of uterine pregnancy after either a year of regular unprotected sexual intercourse or donor insemination, in women younger than thirty-five years. After this diagnosis in trying-to-conceive (TTC) couples, it becomes pressing to dissect various factors that could be accountable for it, including the anatomy of the female reproductive system, namely through the usage of ultrasonography as it has been previously discussed (5,8).

Although there are clear guidelines defining procedures for determining the etiology of infertility when it resides in the female reproductive system, these guidelines are silent regarding the specifications for gels required for performing ultrasound. In fact, despite being classified as medical devices, there are no clear guidelines defining their ingredients, concentrations, physicochemical properties, or even their mode of application. Some literature actually reports a deleterious effects of vaginal lubricants for personal use, as well as ultrasound gels (UG), on sperm function, mainly referring to a decrease in sperm motility after *in vitro* tests (3,7,9,10).

The observed impact on sperm function could be related to the dose of gel and the duration of exposure, as well as its composition and physicochemical characteristics, such as pH, osmolality and viscosity, and this impact must be analyzed in depth, since sperm motility is one of the most important parameters for the success of the fertilization process (3,7).

Despite this, studies about the influence of UG on sperm function are still scarce. In a recent study, Soriano *et al*. examined the effects of two market available UG, purportedly free of toxic ingredients known for their deleterious impact on motility, such as glycerin. The research highlighted detrimental effects observed after thirty minutes of sperm incubation in Aquasonic® gel and this effects were proportional to the duration and consistent with prior studies (3,7).

Literature states that the thick consistency of lubricating gels can pose challenges for spermatozoa (spmtz) motility due to the increased resistance encountered as they attempt to move through it. Essentially, the high viscosity of these gels requires spmtz to expend greater energy and exert more force to make progress, potentially impeding their movement and overall viability (11). However, this impact has never been documented regarding UG.

The Food and Drug Administration (FDA) identifies the potential toxicity of these UG based on very low pH values (pH <3), very high osmolality (exceeding 3,000 mOsm/kg), and/or the presence of certain membrane-penetrating chemicals, like glycerol, known to be toxic at or above room temperature. To ensure safety, the FDA categorizes lubricating gels into three classes, designating class 2 personal lubricants as compatible with gametes, fertilization, and embryos. In order for lubricants to obtain this safety code (PEB) and be considered a class 2 gel compatible with gametes, fertilization, and embryos, they have to undergo a more in-depth battery of tests that includes tests which aim to replicate the environment of semen and cervical mucus upon ovulation period as well as safety fertilization embryo development environment. For FDA, gels with PEB code are considered safe for use in couples attempting conception, encompassing sexual intercourse, diagnostic procedures, and clinical interventions. Among the numerous properties that should be met, these UG should have a neutral pH (ranging around 7) to be safe for sperm viability and have an ideal osmolality around 300 mOsm/kg. Brands should also test their class 2 UG to make sure that theirs products don't produce endotoxins and/or have a negative impact on sperm survival, motility, chromosomal integrity and capacity to permeate through the gel and cervical mucus (12,13).

The reality is that the majority of commercially available UG in Europe do not specify their physicochemical properties, components and concentrations on their labels. Furthermore, there is a notable absence of specific guidelines concerning their usage, with no recommendations regarding these essential characteristics. This lack of transparency raises concerns about the potential risks associated with their use, particularly in medical applications such as transvaginal ultrasound procedures. Establishing clear guidelines and standards for UG composition and usage is crucial to ensuring the safety and well-being of individuals undergoing ultrasound examinations, especially in the context of fertility-related procedures for TTC couples (7,9,12).

Hence, the goals of the current study are:

1. To characterize four UG currently employed in transvaginal ultrasound by examining their pH, osmolality and viscosity;

2. To assess the potential toxic effects of UG in sperm motility, following *in vitro* tests, using a bovine model;

3. To compare the obtained results with a negative control (incubation of capacitated sperm without addition of UG) and relate these results to the physicochemical characteristics of ultrasound gels and the results described in the relevant literature.

The importance of understanding the physicochemical characteristics of commercially available UG and their potential deleterious effect on sperm motility is thus linked to the urgency of developing guidelines that protect the population that can benefit from the application of ultrasound, especially TTC couples.

2. Materials and Methods

The fieldwork was divided into two phases. Initially pre-tests were performed for methodological validation. Afterwards, the animal model tests were performed in triplicates in two independent runs.

Four UG marketed in Europe were used, namely:

- Sterile Aquasonic® (SA) – REF 01-01, Parker Laboratories, Inc. Hannover, Germany.

- Quick-Eco Gel® (QEG) "Cristal Version" – REF 99.800.25, AB Medica Group, SA. Barcelona, Spain.

- Clear Eco Supergel® (CES) – REF 730.25, Ceracarta, SPA. Italy.

- "Gel Ultrassom Azul" (GA)- REF 25.GU, Naturdermo, Lda. Valongo, Portugal

2.1. Ultrasound Gel Preparation and Characterization

Testing for pH and osmolality were performed as described in Cunha *et al.* (10).

Concerning pH testing of UG, this was determined as described in previous studies, at room temperature through the direct immersion of a pH electrode (Testo, 206-pH2, Digital pH meter) into the samples (10,14). This measurement was preformed in duplicate and the mean value was then assessed.

The osmolality of the tested products was assessed using a Osmometer (Micro-Osmometer Model 3300, Advanced Instruments, Massachusetts, United States), employing the freezing point depression method, as detailed by previous studies (10,15). Whole samples were utilized, except in instances where the osmolality values exceeded the maximum limit of the apparatus (2,000 mOsm/kg). In such cases, products underwent a dilution, and the osmolality values were calculated by multiplying the experimental value by the dilution factor. Preliminary experiments demonstrated consistent agreement between osmolality values obtained before and after the dilution of gels.

Viscosity measurements were conducted using an absolute rotational viscometer, specifically a Cone/Plate Rheometer CP-52Z, where a defined sample quantity (0.5 mL) was placed onto a supporting plate. The shear rate of 2,000 dyne/cm^2 was used for all samples and the rotational speed of the cone was carefully selected to maintain torque within the range of 10-100%, ensuring the accuracy of the readings. Each measurement lasted enough time for the cone to complete at least four full rotations. The Cone/Plate Rheometer utilized in this study adheres to the standards outlined in the Portuguese Pharmacopoeia 10.0.

The pH, osmolality and viscosity values were subsequently analyzed together for the purpose of identifying eventual correlations with sperm motility changes.

2.2. Bovine Sample Preparation

Straws of bovine semen, previously stored in a liquid nitrogen chamber were properly removed, allowed to reach room temperature for forty-five seconds, and then thawed completely by heating in a 37°C water bath. Subsequently, 5 µL of semen sample were pipetted into the Makler® Counting Chamber (Sefi-Medical Instruments, 0.01 sq.mm 10µm deep) and observed under an optical microscope (Olympus, model CX41) with an amplification of 200x (20x objective and 10x ocular) to estimate the initial sperm concentration in the sample used. The Makler® Counting Chamber has a grid of 10x10 squares, which allows great precision in sperm count. This count was performed in triplicate (the number of spmtz was counted in each one of 3 rows) and the final concentration obtained corresponds to the mean of these three counts.

Next, a capacitation of the bovine semen was carried out using the swim-up method, through which it is expected that, after 45 minutes at 37ºC, incubated in a commercial medium (Bo-SemenPrep®, from IVFBioscience, United Kingdom), with the tube at a 45º angle, spmtz with better viability will reach the top. This upper portion of the medium is then collected to obtain a sample with the highest possible concentration of viable spmtz (16).

After this period, the initial portion of the sample was collected, and a new count was performed under an optical microscope using a Makler® Counting Chamber with a 20x objective. Following this, the concentration was adjusted to 20 million spmtz using the commercial medium.

2.3. Motility Analysis

Capacitated sperm was incubated at 10% (v/v) of each one of the four previously characterized UG. All UG were handled using a Positive Displacement Micropipette P100 (Gilson®, Microman E M100E). The ratio of 10% (v/v) is well described in literature and aims to replicate as accurately as possible the amount of gel remaining in the vagina after transvaginal US (7,9,11,17). A negative control (NC) was prepared, consisting only of capacitated sperm without any addition of UG. All the said samples, as well as the NC, were then incubated at 37°C, and a timer was set for 30 and 60 minutes. The selected incubation time ranges aligns with prior published studies, which propose that, physiologically, it is expected that within the first thirty minutes, spmtz detach from the ejaculate and advance within the female cervix (9,18).

After 30 minutes, 5 µL from each sample was collected, placed in the Makler® Counting Chamber, and observed under an optical microscope sequentially. At this point, spmtz motility was evaluated in triplicates for each sample and classified as progressive (PR, the spmtz which advanced in the chamber), non-progressive (NP, the spmtz that remained in the same place, performing a continuous circular move), and immotile (IM, the spmtz without movement). The same procedure was repeated after 60 minutes of incubation.

The obtained spmtz motility results for each UG were then compared with the ones referring to the NC and related with the physicochemical characteristics of ultrasound gels.

2.4. Statistical Analysis

For the presentation of the data, tables and graphs were employed, with statistical data preceded by analysis. To assess differences between samples, a One-way ANOVA test and Dunnett's *post hoc* were performed, using $p<0.05$ for statistical significance. In order to do so GraphPad© Prism 10.1.2 was used (Dotmatics, Boston, Massachusetts, United States of America). Results of % motility of each UG were compared with NC.

3. Results

3.1. Ultrasound Gel Characterization

The obtained pH, osmolality and viscosity values are presented in Table 1.

Table 1: Mean pH, osmolality and viscosity (cone CP-52Z, shear rate 2,000 dyne/cm²) values for each UG.

	pH (mean)	Osmolality (mOsm/kg)	Viscosity (cP)
Sterile Aquasonic®	6.78	1,437.1	62310
Quick-Eco Gel®	6.78	194.5	81160
Clear Eco Supergel®	5.65	427.5	42370
“Gel Ultrassom Azul”	5.76	590.5	66180

3.2. Motility Assessment

Motility assessment are presented in Table 2 as well as in Figures 1 and 2. Table 2 displays the mean percentage of PR spmtz at 30 and 60 minutes of incubation with 10% (v/v) of each UG and the negative control. Figure 1 shows the results for the percentage of motility (percentage PR) after 30 minutes of incubation, while Figure 2 shows the percentage of motility after 60 minutes. Statistical analysis was performed, comparing each UG results with results obtained for NC and asterisks represent statistical significance ($p<0.05$).

Table 2: Mean percentage of motile spmtz after 30 and 60 minutes incubation with 10%(v/v) of each gel (NC- negative control; SA -Sterile Aquasonic®; QEG - Quick-Eco Gel®; CES - Clear Eco Supergel®; GA – “Gel Ultrassom Azul”).

	NC	SA	QEG	CES	GA
Motility at 30minutes	86%	4%	3%	1%	1%
Motility at 60 minutes	83%	2%	1%	2%	1%

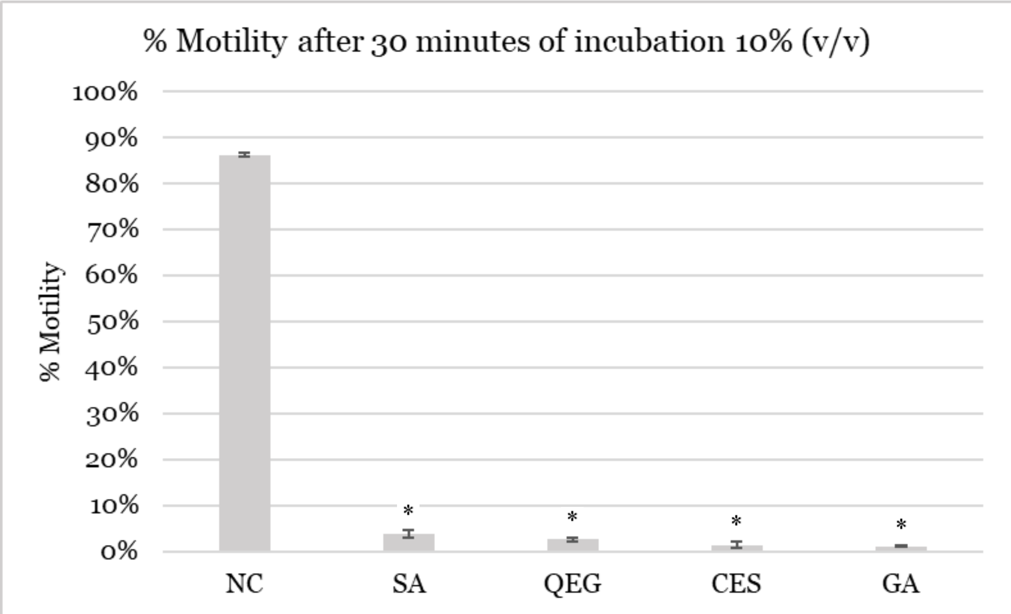

Figure 1: Percentage of motile spmtz after 30 minutes incubation with 10%(v/v) of each gel (NC- negative control; SA -Sterile Aquasonic®; QEG - Quick-Eco Gel®; CES - Clear Eco Supergel®; GA – "Gel Ultrassom Azul"). Asterisks (*) denote statistical significance ($p<0.05$) compared to NC.

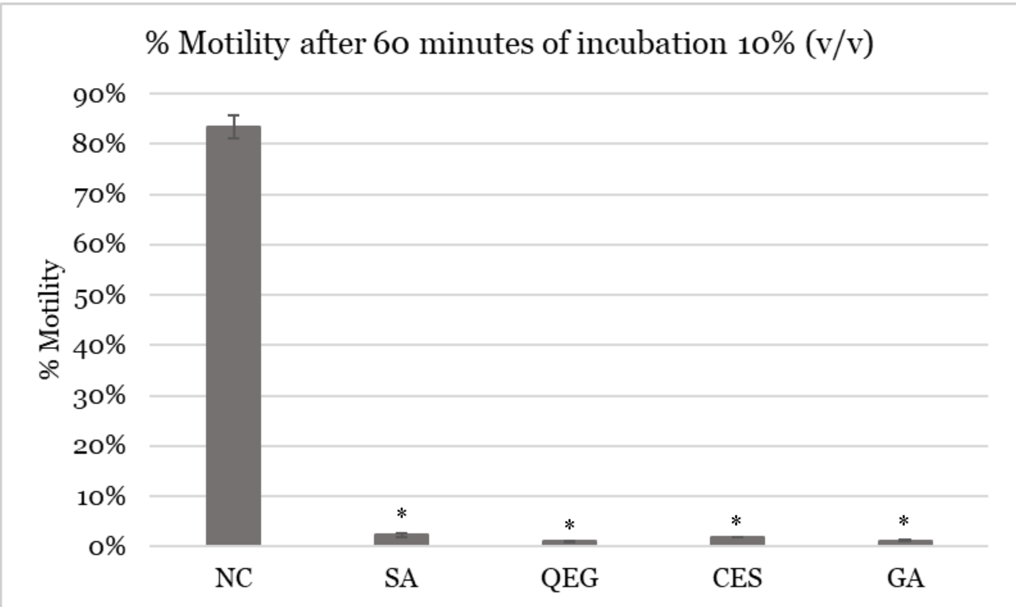

Figure 2: Percentage of motile spmtz after 60 minutes incubation with 10%(v/v) of each gel (NC- negative control; SA -Sterile Aquasonic®; QEG - Quick-Eco Gel®; CES - Clear Eco Supergel®; GA – "Gel Ultrassom Azul"). Asterisks (*) denote statistical significance ($p<0.05$) compared to NC.

4. Discussion

Regarding mean pH values, both Sterile Aquasonic® (SA) and Quick-Eco Gel® (QEG) showed a pH slightly lower than the FDA's recommended level of 7 (Table 1). This contrasts with Clear Eco Supergel® (CES) and "Gel Ultrassom Azul" (GA), which both had a pH below 6. Normal pH of vaginal fluids is reported to vary between 3.8 and 5, and the optimal pH for human semen is between 7.2 and 7.8 (7,19,20). However, values below 7 have been reported to result in spermatozoa immobilization (7,12,21). Therefore, the mean pH of all four tested gels falls outside the previously mentioned intervals. A pH between the optimal ranges for vaginal fluid could pose a threat to sperm motility, as it is more acidic than the optimal pH of semen. Indeed, vaginal pH is known to vary with the presence of semen due to semen's high buffering capacity. In any case, all four tested ultrasound gels failed to meet the optimal pH neutrality recommended for gamete and fertilization compatibility and are lower than the optimal pH for human semen which could lead to potential deleterious effects on sperm motility (12,22).

Concerning osmolality, SA UG was proven to be hyperosmotic, with a mean osmolality value of 1,437.1 mOsm/kg (Table 1). Literature suggests that spermatozoa motility is ideal within means with osmolalities of approximately 300-320 mOsm/kg, while hyperosmolarities above 400 mOsm/kg result in a nearly 50% decrease in motility (7,12,23). Therefore, the osmolality value of SA is markedly higher than the recommended level for gamete and fertilization-compatible ultrasound gels. In contrast, Quick-Eco Gel® was proven to be hypoosmotic, with a mean osmolality of 194.5 (Table 1). While this value is not optimal, it was the closest to the FDA recommendations for gels that are gamete and fertilization compatible (12). CES and GA were proven to be hyperosmotic, and therefore suboptimal for spermatozoa, with values higher than the recommended levels but not as elevated as SA (427.5 and 590.5, respectively) (Table 1).

Pertaining to viscosity, QEG presented the highest viscosity of all tested UG with value of 81160 cP (Table 1). In direct opposition, CES was proven to have the lowest viscosity value of the group of 42370 cP. SA and GA had similar intermediate values of 62310 and 66180 cP respectively. While there are no recommendations concerning UG viscosity this value may be analyzed in light of natural physiology. It is known that, upon ejaculation, human semen undergoes rapid transformation into a solid, gel-like coagulum, which then undergoes spontaneous and complete liquefaction within 30 minutes. Following this liquefaction process, the ejaculate exhibits a fluid consistency with viscosity levels at 37ºC that can vary around 3 to 4 cP (low viscosity values, typical from solutions). Elevated viscosity levels are associated with decreased sperm motility, primarily due to mechanical factors that involve sperm entrapment and hinderance of their typical movement patterns (21,24).

In relation to spmtz motility, the present results demonstrate that all four UG decreased sperm motility significantly ($p<0.05$) at both 30 and 60 minutes (Table 2 and Figures 1 and 2). Specifically, the percentage of PR spmtz for NC was 86% at 30 minutes and 83% at 60 minutes, whereas it was less than 5% for all UG (Sterile Aquasonic® 4%/2%, Quick-Eco Gel® 3%/1%, Clear Eco Gel® 1%/2% and “Gel Ultrassom Azul” 1%/1%) at 30 and 60 minutes. These results for SA UG are consistent with previous ones that relate a decrease in spmtz motility upon incubation at 1 hour and 24 hours, thus reenforcing the fact that it should not be used to perform transvaginal ultrasound in women that are TTC (7).

Literature states that the toxic effect seems to be related to the concentration and duration of exposure (7). While one might argue that the 10% concentration of gel used might be higher than the real concentration of UG found in the vagina after ejaculation, this value seems to be consistent all through previous literature (7,9,11,14,17). It would be interesting for subsequent studies to dissect the variables around UG concentrations in the vagina, such as concentrations of UG below 10% (v/v) (for instants at 5% or 1%), in order to better understand whether the potential deleterious effect persists or if any of these gels have a dose-dependent effect. Additionally, variations in volume and composition of cervical mucus accordingly to the age and phase of the reproductive cycle of women, as well as to the volume of ejaculated semen could also play a central role in this phenomenon and should, therefore, be tested. The relevance of these results and its translation to clinical practice also rely on the persistence of these gels in the vagina over time, which largely depends on its bioadhesive behaviour (22).

While the results show that the pH, osmolality and viscosity seem to be related to the negative impact on sperm motility, it is not possible to establish a direct correlation to the potential toxicity in sperm function, as both hypo- and hyperosmolar conditions appear to cause the same degree of decrease in spmtz motility. The same holds true for pH, as two of the UG tested presented values close to neutrality as recommended by the FDA but seem to produce the same effect on sperm motility as UG with pH values more acidic. The exact same can be said regarding viscosity as the more viscous UG, QEG, seems to be as harmful for spmtz progressiveness as the other UG which have lower viscosities. Therefore, other factors, such as qualitative and quantitative composition of the gels, could also play a role in this drastic reduction in sperm motility.

To this matter, future studies should focus on assessing other UG variables, such as DNA and chromatin integrity, as well as the ability of spmtz to maintain acrosomal reaction. These findings should be cross-referenced with additional studies regarding the potential toxicity of each UG, as the FDA suggests that certain molecules commonly used in these gels (for example, glycerol and propylene glycol) are osmotically active and thus capable of penetrating cell membranes. This could have a potential toxic effect, not only by impairing spermatozoa viability but also triggering cytokine production and, therefore, inflammation. The same goes for endotoxin production, which could impair spermatozoa motility, as suggested by the FDA and remains unclear (12).

Additionally, the reversibility of this effect should also be studied, and it would be particularly interesting if future works could focus on the development of an ultrasound gel that could have a beneficial effect on spermatozoa viability, in contrast to the effect assessed. In a way, these new ultrasound gels could not only be helpful for diagnosis in women trying to conceive but also potentiate spermatozoa motility and, therefore, fertilization.

5. Conclusion

All of the four tested UG presented pH and osmolality values which deviate from the ranges recommended by the FDA for UG compatible with gametes and fertilization. Additionally, all referred UG presented very high viscosity values. These observations raise concerns about the potential impact on reproductive processes when using these medical devices in fertility treatments.

The experimental results also revealed a statistically significant decrease in spmtz motility after incubation with all four UG, both at 30 and 60 minutes, when compared to the negative control ($p<0.05$). This decline in motility raises concerns about a potential toxic effect on sperm function, emphasizing the need for careful consideration and assessment of ultrasound gel formulations in the context of reproductive health.

Notably, the deviation in pH and osmolality appears to be associated with a negative effect on sperm motility. While these factors are significant contributors, the current study suggests that a comprehensive understanding of the physicochemical properties, including specific molecule analysis, is crucial for a more thorough evaluation. Examining these additional parameters can provide a more nuanced perspective on the potential implications of UG use in the context of TTC couples.

While acknowledging the preliminary nature of our findings, it is imperative to highlight the immediate necessity for the regulation and monitoring of UG, especially those used by couples undergoing fertility treatments. The presented research underscores the urgency of further studies to delve into the complexities of UG formulations and their potential impact on gametes and fertilization. By establishing clear guidelines and regulations, we can enhance the safety and efficacy of UG in the realm of assisted reproductive technologies, contributing to the well-being of TTC couples.

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