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Relation between basal sperm morphology and sperm
recovered by swim up

*Relación entre la morfología basal de los espermatozoides y los espermatozoides
recuperados al nadar*

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ABSTRACT

Objective: The purpose of this study is to investigate a possible correlation between basal sperm morphology and sperm recovered by swim up technique, in patients with fertility problems.

Methods: Semen analysis was performed in 112 patients following the laboratory manual for the examination and processing of human semen of the World Health Organization (WHO), fifth edition. Sperm were separated by swim up technique and sperm parameters were reevaluated. Relation between basal sperm morphology and sperm parameters after swim up were evaluated by correlation coefficient analysis.

Results: Means of basal parameters were above lower limits established by the WHO. After swim up, the average of sperm recovery, total motility and progressive motility were 8.89 ± 0.86 , 91.74 ± 0.97 , 97.41 ± 0.66 respectively. Sperm parameters after swim up showed an apparently lower concentration of sperm recovered when normal sperm morphology is lower in basal samples; however, these results were not statistically significant.

Conclusion: There is no correlation between basal sperm morphology and quality sperm recovered after swim up, despite an apparently lower concentration of sperm after sperm separation is observed in patients with a decreased normal sperm morphology.

Keywords: Sperm morphology, sperm preparation, semen analysis, swim up technique.

RESUMEN

Objetivo: El objetivo de este estudio es investigar una posible correlación entre la morfología basal de los espermatozoides y los espermatozoides recuperados mediante la técnica de natación, en pacientes con problemas de fertilidad. **Métodos:** Se realizó análisis de semen en 112 pacientes siguiendo el manual de laboratorio para el examen y procesamiento del semen humano de la Organización Mundial de la Salud (OMS), quinta edición. Los espermatozoides fueron separados por la técnica de nadar y los parámetros de los espermatozoides fueron reevaluados. La relación entre la morfología del esperma basal y los parámetros espermáticos después de la inmersión se evaluó mediante el análisis del coeficiente de correlación. **Resultados:** los promedios de parámetros basales estuvieron por encima de los límites inferiores establecidos por la OMS. Después de nadar, el promedio de recuperación de espermatozoides, motilidad total y motilidad progresiva fueron 8.89 ± 0.86 , 91.74 ± 0.97 , 97.41 ± 0.66 respectivamente. Los parámetros espermáticos después de nadar mostraron una concentración aparentemente menor de espermatozoides recuperados cuando la morfología espermática normal es más baja en las muestras basales; sin embargo, estos resultados no fueron estadísticamente significativos. **Conclusión:** No existe una correlación entre la morfología basal de los espermatozoides y la calidad de los espermatozoides recuperados después de nadar, a pesar de una concentración aparentemente menor de espermatozoides después de la separación espermática en pacientes con una morfología espermática normal disminuida.

Palabras claves: Morfología espermática, preparación de esperma, análisis de semen, técnica de natación.

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Introducción.

Infertility affects around 60-80 million couples worldwide and the causes can be given by female, male, combined or unknown factors (Avellino, Theva, & Oates, 2017; Mascarenhas, Flaxman, Boerma, Vanderpoel, & Stevens, 2012). Male factor affects around 40% of infertility cases (Avellino et al., 2017), for this reason the evaluation of infertility also involves the male.

Semen analysis implicate the study of macroscopic and microscopic characteristics, where normal and abnormal sperm morphology evaluation are included (World Health Organization, 2010). Different kinds of malformations can be presented in spermatozoa (Chang, Garcia, Hitschfeld, & Härtel, 2017; Chang, Heutte, Petitjean, Härtel, & Hitschfeld, 2017; Gatimel, Moreau, Parinaud, & Léandri, 2017; World Health Organization, 2010), so its evaluation represent an important part for clinical decisions in infertile couple (Badawy, Elnashar, & Eltotongy, 2009).

In assisted reproduction treatments, sperm separation from seminal fluid is a necessary step to sperm selection; consequently, several techniques for this step have been described (Lampiao & du Plessis, 2006; Morshedi, Duran, Taylor, & Oehninger, 2003; Rappa et al., 2016; Urbano, Masson, VerMilyea, & Kam, 2017; Yamanaka et al., 2016).

Density gradient centrifugation and swim up are the most used sperm separation technique (Lampiao & du Plessis, 2006; Morshedi, Duran, Taylor, & Oehninger, 2003; Rappa et al., 2016; Urbano, Masson, VerMilyea, & Kam, 2017; Yamanaka et al., 2016). Sperm selection by swim-up is based on motility and consequently dependent on sufficient motile sperm in the

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sample (Thijssen et al., 2014), so its use is recommended in normozoospermic and moderate oligoasthenoteratozoospermic cases (Murcia-Robayo, Jouanisson, Beauchamp, & Diaw, 2018; Palini et al., 2017). Swim up allows a significant improvement in sperm morphology and motility compared with basal parameters (Elberger et al., 2006; Erdem et al., 2009; Kim, Jee, & Kim, 2015); nevertheless, in some asthenozoospermic cases, sperm recovery is less than expected.

The purpose of this study is to investigate a possible correlation between sperm morphology and sperm recovered by swim up technique in male patients who evaluate their fertility.

Materials and methods

Study population and semen collection

Men over 18 years old, attended in Innafest, were included. All volunteers had the conditions for semen collection established by the WHO manual, Fifth edition. The Ethics Human Committee of the Kennedy Hospital Group - Ecuador, approved this study.

Semen analysis

Semen analysis was done one hour after sample collection, following the WHO criteria (fifth edition) for examination and processing of human semen. The microscopic parameters included were sperm concentration (10^6 per ml), total motility (progressive motility + non-progressive motility, %), progressive sperm motility (PR, %) and sperm morphology (%). For the staining of sperm was used diff - Quik kit. Normal sperm morphology was evaluated

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considering WHO criteria and morphological defects were classified as head defects, neck-midpiece defects and tail defects.

Sperm Preparation

After semen analysis, sperm were separated by swim up technique, overlaying 1.5 ml of culture medium (global® w/ HEPES with 10% albumin) above 1 ml of sample. After 60 ~90 minutes of incubation at 37°C, sperm were taken from the top and centrifuged 10 minutes at 300 g. Samples were reevaluated after swim up.

Statistical Analysis

The variables were expressed as mean \pm error standard of mean (SEM). Relation between variables were evaluated by correlation coefficient and a P value of < 0.05 was considered statistically significant.

Results.

One hundred twelve patients with age range 20 ~57 years (34.61 ± 0.63) were included. Semen samples were collected with a minimum of two and a maximum of seven days of sexual abstinence (3.61 ± 0.10).

Sperm concentration, total motility and progressive motility were evaluated before and after swim up. Sperm morphology was analyzed only in basal samples. The means of the results are described in table 1.

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Table 1. Semen parameters before and after swim up

		Pre – swim up	Post - Swim
		Mean ± SEM	Mean ± SEM
Sperm concentration (10⁶ per ml)		47.64 ± 2.89	8.89 ± 0.86
Total motility (%)		52.83 ± 1.56	91.74 ± 0.97
Progressive sperm motility (%)		90.45 ± 0.92	97.41 ± 0.66
Normal sperm morphology		13.51 ± 0.78	-
Abnormal sperm morphology	Head	46.02 ± 1.16	-
	Neck-midpiece	22.62 ± 0.62	-
	Tail	17.30 ± 0.72	-

Regarding sperm morphology, table two describes the cases in three groups based on normal sperm morphology and semen parameters after swim up.

Table 2. Normal sperm reference and sperm quality after swim up.

Normal sperm morphology	Number of patients	Sperm concentration (10⁶ per ml)	Total motility (%)	Progressive motility (%)
≤ 4%	20	6.13 + 1.74	89.75 + 3.40	96.95 + 1.49
5 – 14 %	40	7.31 + 1.05	89.75 + 1.54	96.57 + 1.47
≥ 14 %	50	11.61 + 1.50	93.34 + 1.08	98.26 + 0.63

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Correlation analysis of normal and abnormal sperm morphology with semen parameters after swim up are described in table 3.

Table 3. Correlation coefficient analysis between variables

	Sperm morphology			
	Normal	Abnormal sperm		
		Head	Neck-midpiece	Tail
Sperm Concentration (10^6 per ml)	0.199	-0.176	0.124	-0.035
Total motility (%)	0.160	-0.080	0.026	-0.054
Progressive motility (%)	-0.060	0.076	-0.100	0.020

Discussion.

The aim of this study was to evaluate the relation between basal sperm morphology and sperm parameters after swim up. The age range of patients included was 20~57 years. A specific age was not considered as inclusion or exclusion criteria because many patients with fertility problems decide to evaluate their fertility in an intermediate adult age.

Means of semen basal parameters evaluated are over the lower limits established by WHO laboratory manual for the examination and processing of human semen, fifth edition.

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Several studies such as Eisenberg (2017) reveals the influence of different factors on semen quality as age, finding a decline in semen volume, sperm motility and normal sperm (%) between men with 30 and 50 years (Eisenberg & Meldrum, 2017). Other factors such as alcohol consumption as well as diseases like prostate cancer can also negatively affect spermatogenesis and semen quality; (E. Ricci et al., 2017; Sigman, 2017). On the other hand, it has been suggested that racial differences exist in semen quality at the time of infertility evaluation (Khandwala et al., 2017).

Sperm morphology allows to clarify the potential fertility of semen samples (Avellino et al., 2017). the most common classification system used is the WHO criteria (Badawy et al., 2009); however, we still find a wide variety of morphology evaluation technique (Yamanaka et al., 2016). Further, morphology evaluation depends on the experience of the evaluator (Murcia-Robayo et al., 2018); therefore, the subjective nature of this evaluation makes it difficult to compare the results with the parameters established by the WHO (Yamanaka et al., 2016).

There are several techniques of sperm separation in assisted reproduction, being gradient and swim up the most used. In the present work, direct swim up with final centrifugation of supernatant was used. One hundred and twelve samples were processed, with not sperm recovery in two samples.

Swim up technique is based on motility and consequently dependent on a sufficient number of motile spermatozoa in the sample (Ramos et al., 2015), sperm recovery is less than other techniques of sperm separation but sperm isolated have better motility (Morshedi et al., 2003; G. Ricci et al., 2009; Yamanaka et al., 2016). This is consistent with our results, where

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average of sperm recovery, total motility and progressive motility after swim up were 8.89 ± 0.86 , 91.74 ± 0.97 , 97.41 ± 0.66 respectively.

Concerning the two samples in which sperm were not recovered after swim up, we are not clear about the cause of it. Samples were processed in the same way as the other samples because they had the required criteria. In both cases, basal parameters of sperm concentration, total motility and progressive sperm motility were found over the lower limits established by the WHO; nevertheless, one case had normal sperm morphology below this limit (3%) and the other case was in borderline (6%). It is described that swim up as well as the gradient technique is indicate in normospermia or moderate oligoasthenoteratozoospermia cases (Palini et al., 2017); nevertheless, in samples with minor and moderate teratozoospermia can be obtained an acceptable number of functional sperm recovery (count 10^6 x motility % x normal morphology %) after swim up (Correa-Pérez, Fernández-Pelegrina, Córdova-Claudio, Kaskar, & Panayiotis, 2003).

Patients were divided in three groups, considering normal sperm morphology: ≤ 4 %, $5 \sim 14$ % and >14 %. An apparently lower concentration of sperm recovery after swim up in patients with normal sperm ≤ 4 % and between $5 \sim 14$ % was observed; however, these results were not statistically significant. In the same way, no relationship was found between specific sperm abnormalities (head, neck-midpiece and tail) and parameters of the samples after swim up. Our results are consistent with G. Ricci (2009), who reported that any basal semen parameters are an independent predictor to determine the recovery rate of progressive motility sperm.

Conclusion

There is no correlation between basal sperm morphology and quality sperm recovered after swim up, despite an apparently lower concentration of sperm after sperm separation is observed in patients with a decreased normal sperm morphology.

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