Journal Pre-proof

Human sperm chaperone HSPA2 distribution during *in vitro* capacitation

Natalia Huerta-Retamal, Paula Sáez-Espinosa, Laura Robles-Gómez, Manuel Avilés, Alejandro Romero, Jon Aizpurua, María José Gómez-Torres

PII: S0165-0378(20)30167-4

DOI: https://doi.org/10.1016/j.jri.2020.103246

Reference: JRI 103246

To appear in: *Journal of Reproductive Immunology*

Received Date: 28 July 2020

Revised Date: 28 September 2020

Accepted Date: 5 November 2020

Please cite this article as: Huerta-Retamal N, Sáez-Espinosa P, Robles-Gómez L, Avilés M, Romero A, Aizpurua J, Gómez-Torres MJ, Human sperm chaperone HSPA2 distribution during *in vitro* capacitation, *Journal of Reproductive Immunology* (2020), doi: https://doi.org/10.1016/j.jri.2020.103246

Human sperm chaperone HSPA2 distribution during *in vitro* capacitation

Authors:

Natalia Huerta-Retamal[1], Paula Sáez-Espinosa[1], Laura Robles-Gómez[1], Manuel Avilés[2], Alejandro Romero[1], Jon Aizpurua[3,4] and María José Gómez-Torres[1,4]

Affiliations:

[1]Departamento de Biotecnología, Universidad de Alicante, Alicante, 03690, Spain

[2]Departamento de Biología celular e Histología, Universidad de Murcia, Instituto Murciano de Investigación Sociosanitaria (IMIB-Arrixaca), Murcia, 30003, Spain

[3]IVF Spain, Medicina Reproductiva, Alicante, 03540, Spain

[4]Cátedra Human Fertility, Universidad de Alicante, Alicante, Spain

*Correspondence: Dr. MªJ Gómez Torres (mjose.gomez@ua.es).

Highlights

- Sperm capacitation changes need longer time than the suggested by WHO guidelines.
- Capacitation-associated plasma membrane remodelling includes HSPA2 modifications.
- HSPA2 must be at the equatorial band and acrosomal region during gamete interaction.
- HSPA2 may be a good biomarker of capacitation and sperm maturity.

Abstract

Human fertilization success depends on the ability of the spermatozoa to undergo capacitation. Even though this process can be conducted *in vitro*, the optimal time for a sperm cell to complete capacitation *in vitro* is still under discussion due to the lack of proper capacitation biomarkers. Here, we evaluated the influence of *in vitro* capacitation time on HSPA2 distribution over human sperm head testing this chaperone as a potential capacitation biomarker. The chaperone was assessed in human spermatozoa from 16 normozoospermic donors using indirect immunofluorescence in uncapacitated, one and four-hour capacitated spermatozoa. The percentage of HSPA2 immunofluorescent cells before and after one hour of capacitation did not differ significantly. However, after four hours of capacitation, we observed a significantly higher percentage of HSPA2 labelled cells. In fluorescent cells analysed before capacitation, we could not identify a predominant distribution pattern. Meanwhile, after capacitation, most sperm showed a highly labelled equatorial band accompanied by a homogeneous fluorescence throughout the acrosomal region. Our findings suggest that HSPA2 needs more than one hour of *in vitro* capacitation for being correctly distributed in the anterior region of the sperm head. In conclusion, the present study provides solid evidences for the utility of HSPA2 as a biomarker of human sperm *in vitro* capacitation. Due to its importance during egg-sperm recognition, the use of HSPA2 as a biomarker before an artificial reproduction technique may be suggested, in addition to a longer capacitation time during sperm preparation.

Keywords: Capacitation, Heat shock protein A2, HSP70, Immunofluorescence, Spermatozoa.

1. Introduction

The spermatozoa fertilizing capacity is acquired after a period of time in the female tract. This time-dependent process, known as "capacitation", is necessary for the sperm cell to become fully able to interact with the oocyte (Austin, 1952, 1951; Chang, 1951; De Jonge, 2017; Florman and Fissore, 2014). At this point, it is the female tract environment which induces a sequence of physiological sperm changes including cholesterol loss (De Jonge, 2017), reorganization of glycoconjugates (Sáez-Espinosa et al., 2018), protein phosphorylation (Matamoros-Volante et al., 2018) and hyperactivation (Quill et al., 2003; Shur et al., 2004; Stival et al., 2016). Specifically, the tyrosine phosphorylation of flagellar proteins is an important and common capacitation biomarker (Nassar et al., 1999; Naz and Rajesh, 2004; Quill et al., 2003; Sakkas et al., 2003; Visconti et al., 1995).

This knowledge about the molecular mechanisms occurring during capacitation has been enabled by the possibility of carrying out *in vitro* capacitation (Baker, 2016; De Jonge, 2017; Gervasi and Visconti, 2016; Jin and Yang, 2017). However, there is still controversy regarding the optimal time to perform this process *in vitro* (De Jonge, 2017; Ostermeier et al., 2018). To date, the World Health Organization (WHO) guidelines have been considered for standard procedures, suggesting a time of one hour for swim-up selection under capacitating conditions (World Health Organization, 2010). Nevertheless, these current protocols do not consider the presence of multiple subpopulations in one sperm sample, which have been demonstrated to capacitate at different incubation times (Ostermeier et al., 2018). Furthermore, previous studies suggest that sperm cells capacitated for less than two hours fail to bind the egg zona pellucida (ZP) (Rankin et al., 2003). However, after three to four hours of *in vitro* capacitation the spermatozoa are able to bind the ZP (Baibakov et al., 2012). This process is achieved by the interaction of a group of several candidate sperm molecular

receptors (Aitken and Nixon, 2013) with the ZP during fertilization (Bernabò et al., 2014; Dun et al., 2012). In addition, the correct location of these sperm-ZP receptors is intimately regulated by chaperones (Dun et al., 2012; Redgrove et al., 2012, 2011).

The Heat Shock Protein A2 (HSPA2), one of the chaperones involved in ZP recognition during fertilization, is considered a key target of study due to its participation in the dynamics of the hyaluronidase sperm adhesion molecule 1 (SPAM1) and the zona-receptor molecule Arylsulfatase A (ARSA) (Redgrove et al., 2013). In fact, the reduced expression of HSPA2 on sperm cells is associated with a low number of ZP binding sites on the sperm and failure to bind the oocyte ZP (Dix et al., 1996; Huszar et al., 2000; Motiei et al., 2013; Redgrove et al., 2013) and reduced fertility potential (Huszar et al., 2003, 1992).

Relevant reports on HSPA2 (Huszar et al., 2000; Motiei et al., 2013; Redgrove et al., 2013) are based on the supposition that this protein is colocalized with ARSA and SPAM1 in the periacrosomal region of sperm head. This postulation, however, is controversial since other authors (Motiei et al., 2013) found this chaperone distributed over the different sperm head domains (acrosome, postacrosome and equatorial band) and associated these different distributions with fertility. Moreover, these studies were developed without taking into account the capacitation time.

Consequently, and considering the current mismatches about HSPA2 distribution and the lack of studies on optimal *in vitro* capacitation time, the goal of this study was to know the presence and spatial location of HSPA2 on human sperm before and during one and four hours of *in vitro* capacitation.

2. Materials and methods

2.1 Seminal sample analysis

Semen samples were obtained from donors (*n*=15) after 3 to 4 days of abstinence. A basic semen analysis was performed prior to one hour and morphology was assessed before and after one and four hours of *in vitro* capacitation using strict criteria (World Health Organization, 2010). Only samples classified as normozoospermic according to the WHO (World Health Organization, 2010) were included and considered for further analyses. The samples were then divided into three aliquots in order to be studied before (uncapacitated sperm) and after one- and four-hour *in vitro* capacitation. This study was approved by the Bioethics Committee of the University of Alicante (Spain) in accordance with the Declaration of Helsinki principles and informed written consent was obtained from each donor.

2.2 In vitro capacitation

The seminal plasma was removed by centrifugation for 10 minutes at 250 x g at room temperature. Sperm cells were then washed in human tubal fluid medium (HTF, Origio, Denmark) for 5 minutes at 250 x g. The samples were capacitated by the swim-up technique for one (World Health Organization, 2010) and four hours (Baibakov et al., 2012) using HTF medium supplemented with 5 mg/ml of bovine serum albumin (BSA, Sigma-Aldrich, Inc.) at 37ºC and 5% CO_2. The supernatant fraction was then collected and washed in phosphate buffered saline (PBS, Life Technologies, Thermo Fisher Scientific Inc.).

2.3 Spontaneous acrosome reaction assessment

After capacitation, the acrosomal integrity was verified fixing 5µl of sample on coverslips with methanol for 30 minutes. After washing, the samples were incubated in dark with *Pisum sativum* agglutinin conjugated with fluorescein-5-isothiocyanate

(PSA-FITC, Sigma-Aldrich, Inc.) at a final concentration of 50 µg/mL for 30 minutes as previously reported (Cross et al., 1986). After three washes in PBS, samples were mounted using Vectashield® H-100 mounting medium containing 4', 6-diamidine-2'-phenylindole dihydrochloride (DAPI, Vector Laboratories, Inc.). Appropriate negative control experiments were performed.

2.4 Tyrosine phosphorylation

Before and after capacitation, samples were fixed in 2% paraformaldehyde for 1 hour at 4ºC. Finally, they were re-suspended in PBS to reach a final concentration of 1 x 10^6 sperm cells/ml and preserved at 4ºC.

In order to quantify one of the molecular changes (Sakkas et al., 2003) taking place during capacitation, flagellum tyrosine phosphorylation was analysed. The assays were based on standard procedures, as described previously (Sati et al., 2014). Fixed cells were permeabilized in 0.1% Triton X-100 (Sigma-Aldrich, Inc.) for 10 minutes. After PBS washing, unspecific binding was blocked by incubating with 2% BSA-PBS for 30 minutes. Phosphorylated tyrosine was labelled by incubating with a mouse monoclonal anti-phosphotyrosine antibody (PY20, Sigma-Aldrich, Inc.) at 1:500 dilution in PBS for 1 hour, followed by a polyclonal donkey anti-mouse cyanine-3 antibody (©Jackson ImmunoResearch, UK) at 1:300 dilution in PBS for 1 hour in darkness. After a final 15 minutes wash in PBS, the coverslips were mounted with Vectashield® H-100 mounting medium containing DAPI (Vector Laboratories, Inc.). Negative control experiments were performed omitting the anti-phosphotyrosine antibody.

2.5 Heat Shock Protein A2 (HSPA2) immunolocalization

To evaluate the HSPA2 localization on the sperm, we modified the method previously described by Motiei *et al.* (2013). Briefly, previously fixed samples were placed on coverslips and, once dried, they were rehydrated in PBS for 15 minutes at 5 minutes intervals and permeabilized with Triton X-100 at 0.2% in PBS for 10 minutes at room temperature. After permeabilization, the coverslips were incubated with the primary rabbit polyclonal anti-HSPA2 antibody (Sigma-Aldrich, Inc.) at a final concentration of 1:100 in blocking solution overnight. After being washed with PBS three times for 5 minutes, samples were incubated with a polyclonal donkey anti-rabbit IgG-FITC (Vector Laboratories, Inc.) antibody at 1:100 dilution in blocking solution for 1 hour in the dark. Finally, three washes were carried out at 5 minutes intervals. Once dry, the coverslips were mounted with Vectashield® H-100 mounting medium containing DAPI (Vector Laboratories, Inc.). Negative control experiments were performed omitting the primary antibody.

2.6 Statistical analysis

The staining patterns in the sperm head and the presence of phosphorylated tyrosine at the flagellum level were quantified as percentages (%). A total of 400 cells were evaluated from each seminal sample and physiological condition (uncapacitated, one-hour capacitated and four-hour capacitated) using a Leica TCS SP2 Laser Scanning Confocal Microscope (Germany). Data were analysed using two-way analysis of variance (ANOVA) followed by univariate analysis and Bonferroni post hoc tests. When analysing HSPA2 distribution, only the patterns present in more than 5% of sperm in a sample, either before or after capacitation, were taken into account. Descriptive and statistical results were obtained using IBM SPSS Statistics 22.0. Two-sided *P*-values <0.05 were considered to be statistically significant.

3. Results

3.1 Seminal sample analysis

All semen samples were classified as normozoospermic according to WHO reference values (World Health Organization, 2010). The descriptive statistics (mean ± SD) of sperm concentration, motility percentage, normal sperm morphology, spontaneous acrosome reaction and tyrosine phosphorylation are shown in **Table 1.**

3.2 Spontaneous acrosome reaction assessment

Spermatozoa showing fluorescence in the acrosomal region were considered acrosome-intact, instead of those presenting equatorial band labelling or no labelling were perceived as acrosome reacted (Sáez-Espinosa et al., 2019). There were no significant differences between the percentage of cells reacted before and after one-hour capacitation (7.7% vs 11.9% respectively). However, after four-hours capacitation, the percentage of reacted cells increased significantly (18.5%, Bonferroni difference, BD; $P<0.001$) (**Table 1**).

3.3 Tyrosine phosphorylation

Spermatozoa were classified as phosphorylated when they had partial or complete fluorescence in their flagellum (Sáez-Espinosa et al., 2019). We found a significant capacitation-dependent increase in sperm with phosphorylated tyrosine. In uncapacitated sperm, 8.9% of the cells showed phosphorylated tyrosine in their flagellum. After capacitation, this percentage significantly increased (BD; $P<0.001$) to 31.5% and 42.6% after one- and four- hour respectively (**Table 1**), with significant differences between capacitation times (BD; $P<0.05$).

3.4 Distribution of the Heat Shock Protein A2 (HSPA2)

The assessment of the presence of HSPA2 in sperm head showed no statistical differences between the percentage of cells labelled before and after one-hour capacitation (29.52% vs 23.12% respectively) (**Figure 1B**). However, we observed a significant increase of labelled cells for HSPA2 after four hours of capacitation (70.46%, BD; $P<0.001$). On the other hand, when we analysed in detail the distribution of HSPA2 in labelled cells, we identified three head staining patterns (**Figure 1A**): Pattern 1 (P1) showed high fluorescence in the post-acrosomal region, Pattern 2 (P2) was expressed in the equatorial band, and Pattern 3 (P3) displayed fluorescence in the equatorial region with less intense but homogeneous labelling throughout the acrosomal region.

Before capacitation, the chaperone was expressed heterogeneously in labelled cells, displaying a P1 distribution over 12.05%. This pattern was not expressed in one-hour capacitated sperm. However, after four-hours *in vitro* capacitation, this distribution pattern was observed in 17.61% of cells (**Figure 1B**).

In contrast, once the spermatozoa were *in vitro* capacitated, most of the labelled cells for HSPA2 (13.34% and 31.65% after one and four hours respectively) showed intense fluorescence in the equatorial region accompanied with less intense but homogeneous labelling throughout the acrosomal region (P3). This pattern was significantly less representative in uncapacitated cells (10.48%) than after four-hours capacitation (BD; $P<0.001$) (**Figure 1B**).

Furthermore, an intense fluorescence at the equatorial band level (P2) was observed in 6.99% and 9.77% of spermatozoa before and after one-hour capacitation respectively, without significant differences. However, this immunolabelling was

significantly higher (21.20%) when the cells were capacitated during four hours (BD; $p < 0.001$) (**Figure 1B**). Control samples were devoid of fluorescence, confirming the specificity of the method (data not shown).

4. Discussion

The failure of the male gamete to recognize and adhere to the ZP is one of the most frequent problems during human fertilization (E. Bromfield et al., 2015; Liu and Baker, 2000; Nixon et al., 2015; Redgrove et al., 2012; World Health Organization, 2010). In this context, the molecular complex consisting on SPAM1, HSPA2 and ARSA has been implicated in the gamete recognition and binding process (Redgrove et al., 2013, 2012). Due the role of HSPA2 modulating the action of ARSA and SPAM1, the study of the dynamics of HSPA2 during capacitation, may lighten about the correct localization of this protein complex implicated in fertilization. To our knowledge, no study has ever mapped the temporal and spatial behaviour of HSPA2 in human spermatozoa during *in vitro* capacitation.

Because of the long time of capacitation (four hours), a capacitation-required event such flagellar tyrosine phosphorylation was tested as a reference (Florman and Fissore, 2014; Nassar et al., 1999; Sati et al., 2014). Our results showed a capacitation time-dependent increase on phosphorylation. These data are consistent with previous studies, which confirm the presence of tyrosine phosphorylation in sperm at the flagellum level and its capacitation-dependent increase (Nassar et al., 1999; Naz and Rajesh, 2004; Sati et al., 2014) and demonstrates that the capacitation time used in this study is adequate for tyrosine phosphorylation.

The study of HSPA2 by fluorescence microscopy showed a low percentage of unacapacitated and one-hour capacitated spermatozoa labelled for this chaperone. On

the contrary, we observed that in most of the cells capacitated during four hours HSPA2 was detected. These results are consistent with other studies where it was observed by flow cytometry an increase in HSPA2 surface labelling after three-hour capacitation in spermatozoa (Motiei et al., 2013; Nixon et al., 2015).

Our immunofluorescence study showed three different distribution patterns of this receptor. Previous reports, however, characterized a higher number of HSPA2 patterns (i.e. total head, anterior head, posterior head, equatorial band, mid piece, tail, cytoplasm and membrane) (Motiei et al., 2013). This discrepancy was probably due to different experimental conditions used in both studies since Motiei recorded HSPA2 distribution only over uncapacitated cells and included patterns present in less than 5% of sperm. In this context, we did not consider the HSPA2 labelling of the sperm flagellum as a labelling pattern because only a total of 2.84% of cells showed this distribution.

Interestingly, in contrast to uncapacitated labelled sperm, in which HSPA2 did not evidence a majoritarian pattern, during *in vitro* capacitation, the majority of cells presented an equatorial band accompanied by slight fluorescence in the acrosome, independently of the capacitation time. Other authors have illustrated a similar HSPA2 distribution on the sperm (Bromfield, Aitken, Anderson *et al.*, 2015; Huszar *et al.*, 1992, 2003; Liu and Baker, 2000; Motiei *et al.*, 2013; Redgrove *et al.*, 2013), suggesting a possible relationship between this HSPA2 location and high fertility capacity. This HSPA2 pattern (P3) showed a time-dependent increase that was significant after four-hour capacitation, indicating that the chaperone requires a longer capacitation time to be located on the equatorial band and acrosomal region.

Other pattern observed after capacitation was at equatorial band level (P2). This distribution was observed in around 10% and 20% of capacitated cells (one and four

hours respectively), being these rates similar to the percentage of reacted cells found at these capacitation times. These spermatozoa could be acrosome reacted, according to other authors who reported that several of the proteins involved in gamete fusion are relocated to the equatorial band during the acrosomal reaction of the spermatozoa, in order to perform their function after external acrosome membrane removal (Flesch and Gadella, 2000; Gadella, 2008; Satouh *et al.*, 2012; Okabe, 2014; Sáez-Espinosa *et al.*, 2018). Even though studies in mouse show that spermatozoon is already reacted before reaching the egg (Gahlay *et al.*, 2010; Inoue *et al.*, 2011; Jin *et al.*, 2011; Okabe, 2014), in human fertilization, the interaction with the ZP would be a key step to trigger this crucial reaction.

On the basis of the results reported in this study, we could deduce that when sperm are allowed to capacitate for a longer time, the number of cells with labelling for HSPA2 increased. In the same way, a greater sperm subpopulation that have been able to localize this protein to the oocyte-interaction region was recovered. The distribution of HSPA2 at the equatorial band and the acrosomal domain in capacitated cells in contrast to the labelling in the post-acrosomal region of uncapacitated sperm head could allow the protein complex ARSA, HSPA2 and SPAM1 to be temporarily and spatially located to play its role on sperm-oocyte interaction (E. G. Bromfield et al., 2015a, 2015b; Nixon et al., 2015; Redgrove et al., 2013, 2011).

These results lead us to suggest that the chaperone HSPA2 may be a good biomarker of capacitation and sperm maturity, since the spermatozoa that, after capacitation, fail to localize this protein correctly, will not be able to present ARSA or SPAM 1, both involved in the interaction of the spermatozoa with the oocyte (Bromfield, Aitken, Anderson *et al.*, 2015; Bromfield, McLaughlin, *et al.*, 2015; Nixon *et al.*, 2015; Redgrove *et al.*, 2013). These conclusions are supported by studies

indicating that a high percentage of sperm expressing this chaperone in the anterior and equatorial regions of the head is related to high fertility (Motiei et al., 2013). Moreover, our results highlight the need for a longer time than the hour suggested by WHO guidelines for swim-up capacitation. At least, four hours are needed for the optimal capacitation-associated remodelling of the sperm plasma membrane, which could include post-translational modifications of HSPA2 that may be uncovering the epitopes of HSPA2, resulting in the different chaperone locations observed in this study.

Nevertheless, further research is needed on the location of this protein complex, replicating *in vitro* the different conditions to which spermatozoa are exposed until fertilization takes place.

On the basis of our results, it could be concluded that capacitation-associated plasma membrane remodelling might include post-translational modifications of HSPA2. Furthermore, at least four hours of incubation in capacitating media would be required for an optimal HSPA2 localization on the sperm plasma membrane. This leads us to propose that some of the sperm physiological changes associated with capacitation, such plasma membrane remodelling and tyrosine phosphorylation, need a longer time than the hour suggested by WHO guidelines to reach ideal values.

On the other hand, we could suggest that the presence of HSPA2 at the equatorial band and the acrosomal region after capacitation might be essential to play its role on sperm-oocyte interaction. So, by increasing the incubation time to four hours under capacitating conditions, we should be able to recover a greater sperm subpopulation that have been able to show and localize this protein into the oocyte-interaction region.

In view of these factors, the chaperone HSPA2 might be proposed as good capacitation and maturity biomarker.

5. Declaration of interest

All authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Conflict of interest

All authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

6. Aknowledgements

This work was supported by the Human Fertility Cathedra of the University of Alicante and R&D&I projects financed by competitive public entities (ViGrob-186, UAIND17-03 and AGL2015).

References

Aitken, R.J., Nixon, B., 2013. Sperm capacitation: a distant landscape glimpsed but unexplored. Mol. Hum. Reprod. 19, 785–793. https://doi.org/10.1093/molehr/gat067

Austin, C.R., 1952. The capacitation of the mammalian sperm. Nature 170, 326. https://doi.org/10.1038/170326a0

Austin, C.R., 1951. Observations on the penetration of the sperm in the mammalian egg. Aust. J. Sci. Res. B. 4, 581–96.

Baibakov, B., Boggs, N.A., Yauger, B., Baibakov, G., Dean, J., 2012. Human sperm bind to the N-terminal domain of ZP2 in humanized zonae pellucidae in transgenic mice. J. Cell Biol. 197, 897–905. https://doi.org/10.1083/jcb.201203062

Baker, M.A., 2016. Proteomics of post-translational modifications of mammalian spermatozoa. Cell Tissue Res. 363, 279–287. https://doi.org/10.1007/s00441-015-2249-x

Bernabò, N., Ordinelli, A., Di Agostino, R., Mattioli, M., Barboni, B., 2014. Network analyses of sperm-egg recognition and binding: Ready to rethink fertility mechanisms? Omi. A J. Integr. Biol. 18, 740–753. https://doi.org/10.1089/omi.2014.0128

Bromfield, E., Aitken, R.J., Nixon, B., 2015. Novel characterization of the HSPA2-stabilizing protein BAG6 in human spermatozoa. Mol. Hum. Reprod. 21, 755–769. https://doi.org/10.1093/molehr/gav041

Bromfield, E.G., Aitken, R.J., Anderson, A.L., McLaughlin, E.A., Nixon, B., 2015a. The impact of oxidative stress on chaperone-mediated human sperm-egg

interaction. Hum. Reprod. 30, 2597–2613. https://doi.org/10.1093/humrep/dev214

Bromfield, E.G., McLaughlin, E.A., Aitken, R.J., Nixon, B., 2015b. Heat shock protein member A2 forms a stable complex with angiotensin convertingenzymeand protein disulfide isomerase A6 in human spermatozoa. Mol. Hum. Reprod. 22, 93–109. https://doi.org/10.1093/molehr/gav073

Chang, M.C., 1951. Fertilizing capacity of spermatozoa deposited into the fallopian tubes. Nature 168, 697–8. https://doi.org/10.1038/168697b0

Cross, N.L., Morales, P., Overstreet, J.W., Hanson, F.W., 1986. Two simple methods for detecting acrosome- reacted human sperm. Gamete Res. 15, 213–226. https://doi.org/10.1002/mrd.1120150303

De Jonge, C., 2017. Biological basis for human capacitation-revisited. Hum. Reprod. Update 23, 289–299. https://doi.org/10.1093/humupd/dmw048

Dix, D.J., Allen, J.W., Collins, B.W., Mori, C., Nakamura, N., Poorman-Allen, P., Goulding, E.H., Eddy, E.M., 1996. Targeted gene disruption of Hsp70-2 results in failed meiosis, germ cell apoptosis, and male infertility. Proc. Natl. Acad. Sci. U. S. A. 93, 3264–8. https://doi.org/10.1073/pnas.93.8.3264

Dun, M.D., Aitken, R.J., Nixon, B., 2012. The role of molecular chaperones in spermatogenesis and the post-testicular maturation of mammalian spermatozoa. Hum. Reprod. Update 18, 420–35. https://doi.org/10.1093/humupd/dms009

Flesch, F.M., Gadella, B.M., 2000. Dynamics of the mammalian sperm plasma membrane in the process of fertilization. Biochim. Biophys. Acta 1469, 197–235.

Florman, H.M., Fissore, R.A., 2014. Fertilization in Mammals, Fourth Edi. ed, Knobil and Neill's Physiology of Reproduction: Two-Volume Set. Elsevier. https://doi.org/10.1016/B978-0-12-397175-3.00004-1

Gadella, B.M., 2008. Sperm membrane physiology and relevance for fertilization.

Anim. Reprod. Sci. 107, 229–236. https://doi.org/10.1016/j.anireprosci.2008.05.006

Gahlay, G., Gauthier, L., Baibakov, B., Epifano, O., Dean, J., 2010. Gamete recognition in mice depends on the cleavage status of an egg's zona pellucida protein. Science (80-.). 329, 216–219. https://doi.org/10.1126/science.1188178

Gervasi, M.G., Visconti, P.E., 2016. Chang's meaning of capacitation: A molecular perspective. Mol. Reprod. Dev. 83, 860–874. https://doi.org/10.1002/mrd.22663

Huszar, G., Ozenci, C.C., Cayli, S., Zavaczki, Z., Hansch, E., Vigue, L., 2003. Hyaluronic acid binding by human sperm indicates cellular maturity, viability, and unreacted acrosomal status. Fertil. Steril. 79 Suppl 3, 1616–24. https://doi.org/10.1016/s0015-0282(03)00402-3

Huszar, G., Stone, K., Dix, D., Vigue, L., 2000. Putative Creatine Kinase M-Isoform in Human Sperm Is Identifiedas the 70-Kilodalton Heat Shock Protein HspA21. Biol. Reprod. 63, 925–932. https://doi.org/10.1095/biolreprod63.3.925

Huszar, G., Vigue, L., Morshedi, M., 1992. Sperm creatine phosphokinase M-isoform ratios and fertilizing potential of men: a blinded study of 84 couples treated with in vitro fertilization. Fertil. Steril. 57, 882–8.

Inoue, N., Satouh, Y., Ikawa, M., Okabe, M., Yanagimachi, R., 2011. Acrosome-reacted mouse spermatozoa recovered from the perivitelline space can fertilize other eggs. Proc. Natl. Acad. Sci. U. S. A. 108, 20008–20011. https://doi.org/10.1073/pnas.1116965108

Jin, M., Fujiwara, E., Kakiuchi, Y., Okabe, M., Satouh, Y., Baba, S.A., Chiba, K., Hirohashi, N., 2011. Most fertilizing mouse spermatozoa begin their acrosome reaction before contact with the zona pellucida during in vitro fertilization. Proc. Natl. Acad. Sci. U. S. A. 108, 4892–4896.

https://doi.org/10.1073/pnas.1018202108

Jin, S.-K., Yang, W.-X., 2017. Factors and pathways involved in capacitation: how are they regulated? Oncotarget 8, 3600–3627. https://doi.org/10.18632/oncotarget.12274

Liu, D.Y., Baker, H.W.G., 2000. Defective sperm-zona pellucida interaction: A major cause of failure of fertilization in clinical in-vitro fertilization. Hum. Reprod. 15, 702–708. https://doi.org/10.1093/humrep/15.3.702

Matamoros-Volante, A., Moreno-Irusta, A., Torres-Rodriguez, P., Giojalas, L., Gervasi, M.G., Visconti, P.E., Treviño, C.L., 2018. Semi-automatized segmentation method using image-based flow cytometry to study sperm physiology: the case of capacitation-induced tyrosine phosphorylation. Mol. Hum. Reprod. 24, 64–73. https://doi.org/10.1093/molehr/gax062

Motiei, M., Tavalaee, M., Rabiei, F., Hajihosseini, R., Nasr-Esfahani, M.H., 2013. Evaluation of HSPA2 in fertile and infertile individuals. Andrologia 45, 66–72. https://doi.org/10.1111/j.1439-0272.2012.01315.x

Nassar, A., Mahony, M., Morshedi, M., Lin, M.H., Srisombut, C., Oehninger, S., 1999. Modulation of sperm tail protein tyrosine phosphorylation by pentoxifylline and its correlation with hyperactivated motility. Fertil. Steril. 71, 919–23. https://doi.org/10.1016/s0015-0282(99)00013-8

Naz, R.K., Rajesh, P.B., 2004. Role of tyrosine phosphorylation in sperm capacitation / acrosome reaction. Reprod. Biol. Endocrinol. 2, 75. https://doi.org/10.1186/1477-7827-2-75

Nixon, B., Bromfield, E., Dun, M., Redgrove, K., McLaughlin, E., Aitken, Rj., 2015. The role of the molecular chaperone heat shock protein A2 (HSPA2) in regulating human sperm-egg recognition. Asian J. Androl. 17, 568.

https://doi.org/10.4103/1008-682X.151395

Okabe, M., 2014a. Mechanism of fertilization: a modern view. Exp. Anim. 63, 357–65. https://doi.org/10.1538/expanim.63.357

Okabe, M., 2014b. Mechanisms of fertilization elucidated by gene-manipulated animals. Asian J. Androl. 17, 646–52. https://doi.org/10.4103/1008-682X.153299

Ostermeier, G.C., Cardona, C., Moody, M.A., Simpson, A.J., Mendoza, R., Seaman, E., Travis, A.J., 2018. Timing of sperm capacitation varies reproducibly among men. Mol. Reprod. Dev. 85, 387–396. https://doi.org/10.1002/mrd.22972

Quill, T.A., Sugden, S.A., Rossi, K.L., Doolittle, L.K., Hammer, R.E., Garbers, D.L., 2003. Hyperactivated sperm motility driven by CatSper2 is required for fertilization. Proc. Natl. Acad. Sci. U. S. A. 100, 14869–14874. https://doi.org/10.1073/pnas.2136654100

Rankin, T.L., Coleman, J.S., Epifano, O., Hoodbhoy, T., Turner, S.G., Castle, P.E., Lee, E., Gore-Langton, R., Dean, J., 2003. Fertility and Taxon-Specific Sperm Binding Persist after Replacement of Mouse Sperm Receptors with Human Homologs. Dev. Cell 5, 33–43. https://doi.org/10.1016/S1534-5807(03)00195-3

Redgrove, K.A., Anderson, A.L., Dun, M.D., McLaughlin, E.A., O'Bryan, M.K., Aitken, R.J., Nixon, B., 2011. Involvement of multimeric protein complexes in mediating the capacitation-dependent binding of human spermatozoa to homologous zonae pellucidae. Dev. Biol. 356, 460–474. https://doi.org/10.1016/j.ydbio.2011.05.674

Redgrove, K.A., Anderson, A.L., McLaughlin, E.A., O'Bryan, M.K., Aitken, R.J., Nixon, B., 2013. Investigation of the mechanisms by which the molecular chaperone HSPA2 regulates the expression of sperm surface receptors involved in human sperm-oocyte recognition. Mol. Hum. Reprod. 19, 120–135.

https://doi.org/10.1093/molehr/gas064

Redgrove, K.A., Nixon, B., Baker, M.A., Hetherington, L., Baker, G., Liu, D.Y., Aitken, R.J., 2012. The Molecular Chaperone HSPA2 Plays a Key Role in Regulating the Expression of Sperm Surface Receptors That Mediate Sperm-Egg Recognition. PLoS One 7. https://doi.org/10.1371/journal.pone.0050851

Sáez-Espinosa, P., Huerta-Retamal, N., Robles-Gómez, L., Avilés, M., Aizpurua, J., Velasco, I., Romero, A., Gómez-Torres, M.J., 2019. Influence of in vitro capacitation time on structural and functional human sperm parameters. Asian J. Androl. https://doi.org/10.4103/aja.aja_104_19

Sáez-Espinosa, P., Torrijo-Boix, S., Huerta-Retamal, N., Avilés, M., Aizpurua, J., Romero, A., Gómez-Torres, M.J., 2018. La capacitación y la reacción acrosómica se asocian con cambios en la localización del ácido siálico y en la morfometría de la región cefálica del espermatozoide humano. Rev. Int. Androl. 16, 20–27. https://doi.org/10.1016/j.androl.2017.03.002

Sakkas, D., Leppens-Luisier, G., Lucas, H., Chardonnens, D., Campana, A., Franken, D.R., Urner, F., 2003. Localization of tyrosine phosphorylated proteins in human sperm and relation to capacitation and zona pellucida binding. Biol. Reprod. 68, 1463–9. https://doi.org/10.1095/biolreprod.102.011023

Sati, L., Cayli, S., Delpiano, E., Sakkas, D., Huszar, G., 2014. The pattern of tyrosine phosphorylation in human sperm in response to binding to zona pellucida or hyaluronic acid. Reprod. Sci. 21, 573–581. https://doi.org/10.1177/1933719113504467

Satouh, Y., Inoue, N., Ikawa, M., Okabe, M., 2012. Visualization of the moment of mouse sperm-egg fusion and dynamic localization of IZUMO1. J. Cell Sci. 125, 4985–4990. https://doi.org/10.1242/jcs.100867

Shur, B.D., Rodeheffer, C., Ensslin, M. a, 2004. Mammalian fertilization. Curr. Biol. 14, R691–R692. https://doi.org/10.1038/270084a0

Stival, C., Puga Molina, L.D.C., Paudel, B., Buffone, M.G., Visconti, P.E., Krapf, D., 2016. Sperm capacitation and acrosome reaction in mammalian sperm, in: Advances in Anatomy Embryology and Cell Biology. Springer Verlag, pp. 93–106. https://doi.org/10.1007/978-3-319-30567-7_5

Visconti, P.E., Bailey, J.L., Moore, G.D., Pan, D., Olds-Clarke, P., Kopf, G.S., 1995. Capacitation of mouse spermatozoa. 1. Correlation between the capacitation state and protein tyrosine phosphorylation. Development 121, 1129–1137.

World Health Organization, 2010. WHO laboratory manual for the examination and processing of human semen. World Heal. Organ. Edition, V, 286. https://doi.org/10.1038/aja.2008.57

Figure 1. Expression and frequency (mean % ± σ) of HSPA2 patterns in human spermatozoa in different physiological conditions. Fluorescence patterns observed: post-acrosomal region (P1), equatorial band (P2), equatorial band and acrosomal region (P3). Significant differences at $P<0.05$ (*); no significant differences (NS). Scale bar: 10 µm common to all images.

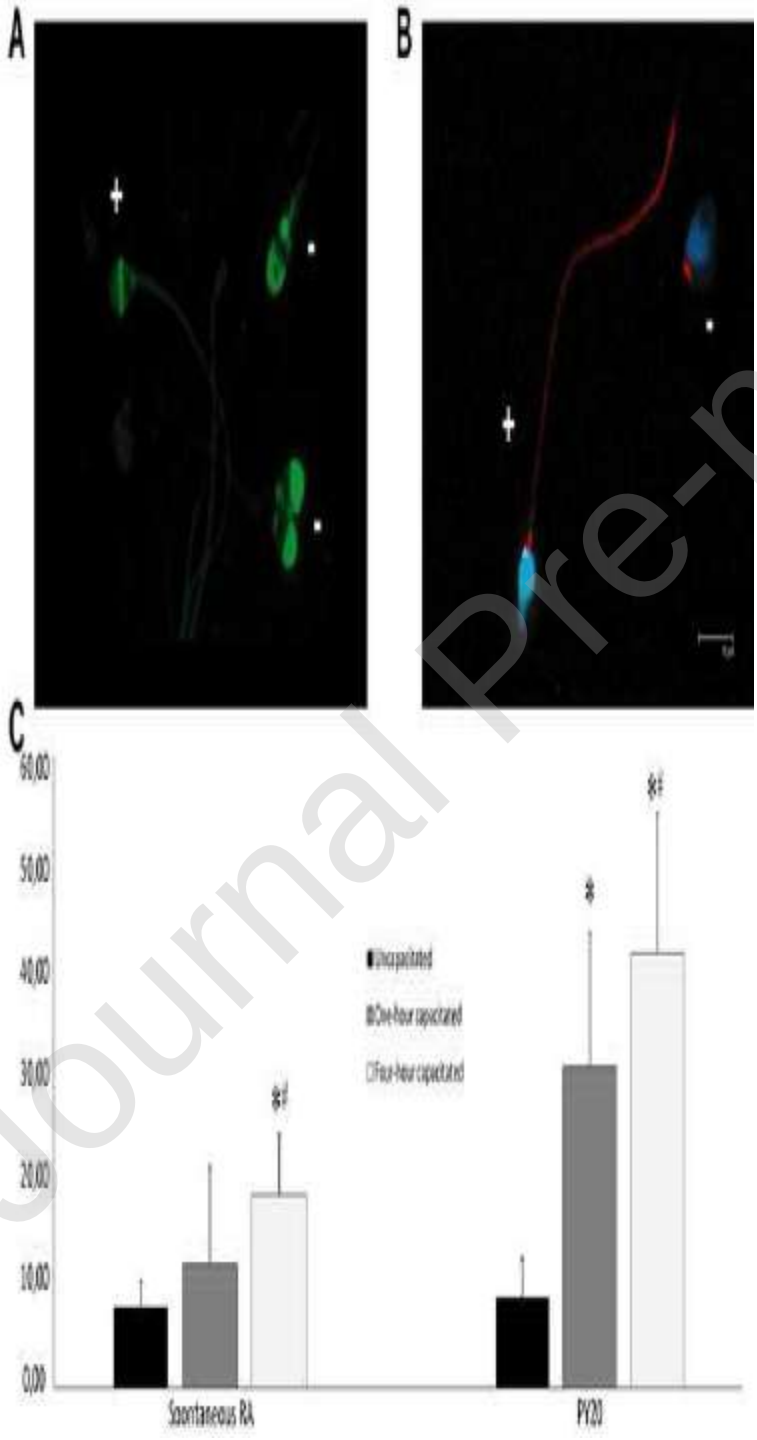

Figure 2

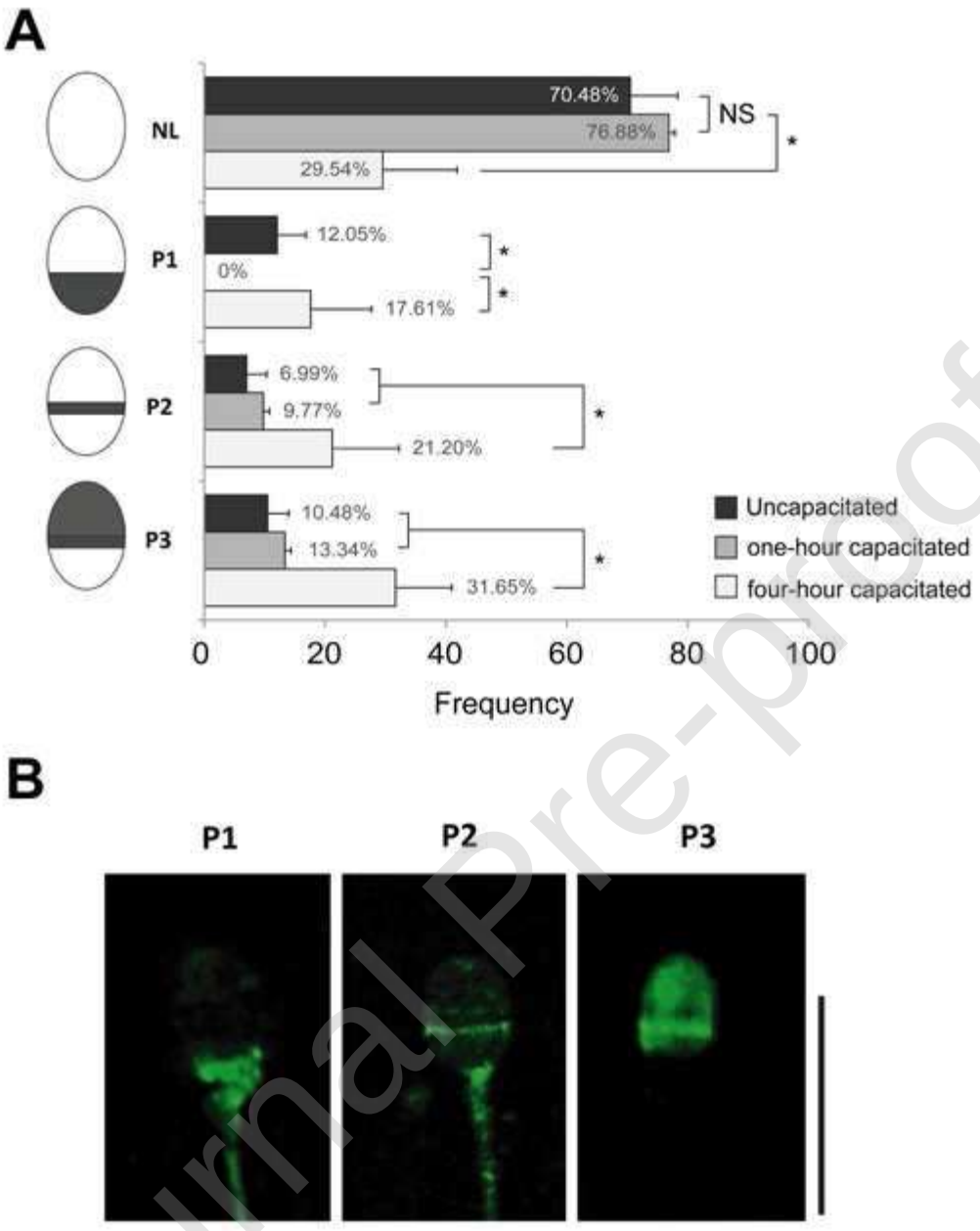

Table 1. Seminal sample analysis result (*n*=15) before and after *in vitro* capacitation.

Parameter	Mean ± σ		
	Uncapacitated	One-hour capacitated	Four-hour capacitated

Concentration (10^6 cells/ml)	70.7 ± 20.5	5.8 ± 2.8*	4.0 ± 2.4*
Motility (%)	70.4 ± 14.5	96.0 ± 4.7*	97.8 ± 1.9*
Normal Morphology (%)	13.6 ± 6.3	18.8 ± 6.1*	23.3 ± 6.6*
Spontaneous acrosome reaction (%)	7.7 ± 2.4	11.8 ± 9.2	18.5 ± 5.7*#
Flagellar tyrosine phosphorylation (%)	8.8 ± 3.8	31.5 ± 13.0*	42.6 ± 13.6*#

Significant differences against Uncapacitated cells at $P<0.05$ (*); Significant differences against One-hour capacitated cells at $P<0.05$ (#)