Sialyl-Lewis(x) interaction for sperm selection in assisted reproductive treatment: abridged secondary publication

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KEY MESSAGES

1. Capacitated human spermatozoa initiate fertilisation via binding to the zona pellucida (ZP). The sialyl-Lewis(x) (sLeX) sequence is the most abundant terminal sequence on the glycans of human ZP glycoproteins involved in spermatozoa-ZP binding. Compared with unbound spermatozoa, ZP- or sLeX-bound spermatozoa have better fertilisation potential and quality in terms of morphology, DNA integrity, chromatin integrity, protamination, and global methylation.
2. Four sLeX-binding proteins of capacitated spermatozoa were identified: chromosome 1 open reading frame 56 (C1orf56), ZP-binding protein 1, heat shock-related 70 kDa protein 2, and sperm acrosome membrane–associated protein 1.
3. Clorf56 translocated to the cell surface of the spermatozoa acrosomal region during capacitation. Treatment with anti-C1orf56 antibody inhibited spermatozoa-ZP binding and ZP-induced acrosomal reactions. Purified C1orf56 from capacitated spermatozoa were able to bind human ZP.
4. The in vitro fertilisation rate was not associated with the percentage of capacitated spermatozoa expressing C1orf56. However, the percentage of C1orf56-positive spermatozoa in the acrosome-reacted population was significantly lower in cycles with a fertilisation rate <60% than in cycles with a fertilisation rate ≥60%. These results suggest that C1orf56 has important roles after ZP-binding and acrosomal reactions.
5. Sperm quality can be significantly enhanced by selection methods involving ZP, sLeX, and annexin V microbeads.

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Introduction

Infertility affects approximately 15% of reproductive-aged couples worldwide. The live birth rate after use of assisted reproductive technology (ART) is approximately 30%. Sperm quality is a major factor influencing fertilisation success. Only fertilisation-competent spermatozoa survive natural selection mechanisms involving anatomical, biochemical, and physiological barriers in a highly specialised microenvironment.[1] In ART, motile and morphologically normal spermatozoa are routinely isolated by either swim-up or density gradient centrifugation. However, these two markers are unable to select spermatozoa with high fertilisation potential and genetic quality.

Human fertilisation begins when a capacitated spermatozoon binds to the zona pellucida (ZP) of an oocyte. The spermatozoa-ZP interaction serves as a natural sperm selection mechanism in vivo and can potentially be used for sperm evaluation and selection in clinical settings.[2] The sialyl-Lewis(x) (sLeX) sequence is the most abundant terminal sequence on the glycans of human ZP glycoproteins involved in spermatozoa-ZP binding.[3] This phenomenon suggests that the human ZP selectively interacts with spermatozoa exhibiting high fertilisation capacity and genetic integrity. This study aimed to determine the roles and clinical applications of human ZP and sLeX in selecting fertilisation-competent spermatozoa.

Methods

Semen samples were collected from normal men. Oocytes were collected from infertile women. The direct swim-up method was used to isolate motile and viable spermatozoa. Spermatozoa-ZP coincubation assays were performed. Ionophore- or ZP-induced acrosomal reactions were performed to prepare acrosome-reacted sperm. ZP- or sLeX-bound spermatozoa were collected. The fertilisation competence and quality of ZP- or sLeX-bound spermatozoa were determined in terms of

morphology, acrosome status, motility, viability, ZP-binding capability, DNA fragmentation rate, DNA damage, chromatin integrity, protamine deficiency, and methylation level.

Sperm plasma membrane proteins were extracted. Surface sLeX-binding protein expression patterns were investigated on uncapacitated, capacitated, and acrosome-reacted spermatozoa. The associations between surface sLeX-binding protein expression and spermatozoa fertilisation competence/quality were determined. Capacitated spermatozoa were pre-incubated in medium supplemented with functional blocking anti–chromosome 1 open reading frame 56 (C1orf56) antibody. Isotype-matched antibody was used as a control. Spermatozoa were washed before evaluation of their fertilisation competence. The effects of 1 mg/mL anti-C1orf56 antibody for 120 minutes on the sLeX binding capabilities of spermatozoa were determined by flow cytometry. C1orf56 was purified from human spermatozoa using immuno-affinity chromatography. Purified C1orf56 was labelled with Alexa Fluor-594. Matched hemizona were incubated with 1 mg/mL labelled C1orf56 with or without anti-C1orf56 neutralising antibody for 3 hours; binding was observed using a fluorescence microscope.

To investigate clinical applications of the sperm-sLeX interaction, couples attending the infertility clinic were recruited. The standard gonadotrophin-releasing hormone agonist long protocol was used. Conventional insemination was performed 4 hours after oocyte retrieval, and fertilisation was checked 16 to 18 hours later. Normal fertilisation was regarded as the appearance of two pronuclei. The fertilisation rate (FR) was defined as the number of two pronuclei zygotes observed divided by the total number of inseminated oocytes × 100. Men with in vitro FR ≥60% or <60% were compared. The surface expression of C1orf56 in spermatozoa was determined by flow cytometry. The potential application of sLeX for sperm selection during intracytoplasmic sperm injection (ICSI) was determined.

Results

Sperm-ZP binding is associated with two protein markers: heat shock 70 kDa protein 2 (HSPA 2) and sperm acrosome membrane–associated protein (SPACA) 3. Compared with unbound spermatozoa, ZP- or sLeX-bound spermatozoa had significantly higher expression levels of HSPA2 and SPACA 3, as well as significantly higher rates of normal morphology, DNA integrity, chromatin integrity, protamination, and global methylation.[4] Additionally, altered H3K9Me1 histone methylation and DNA methylation of small nuclear ribonucleoprotein polypeptide N were demonstrated in ZP- or sLeX-bound spermatozoa.[4]

We identified four sLeX-binding proteins in capacitated spermatozoa: C1orf56, ZP-binding protein 1, HSPA 2, and SPACA 1.[5] The acrosomal region of spermatozoa exhibited C1orf56 immunoreactive signals with intensities that increased after capacitation; this phenomenon indicated C1orf56 translocation to the cell surface during capacitation. Treatment with anti-C1orf56 antibody decreased the number of capacitated spermatozoa bound to the ZP, spermatozoa-sLeX binding, and ZP-induced acrosomal reactions of capacitated spermatozoa. Fluorescence-labelled C1orf56 also specifically bound to the ZP of human oocytes.[5] However, spermatozoa fertilisation competence/quality was not associated with surface C1orf56, clusterin, HSPA 2, SPACA 4, or ZP-binding protein 1 (data not shown).

The FR rate was not associated with the percentage of capacitated spermatozoa expressing C1orf56. However, the percentage of C1orf56-positive spermatozoa in the acrosome-reacted population was significantly lower in cycles with an FR <60% than in cycles with an FR ≥60%; these results suggest that C1orf56 has important roles after ZP-binding and acrosomal reactions.[5] The high- and low-motility groups did not significantly differ in terms of C1orf56 expression. Similarly, samples with normal morphology of >4% and ≤4% did not significantly differ in the capacitated and acrosome-reacted subpopulations.[5]

Compared with unselected controls, ZP-bound spermatozoa had a significantly higher rates of normal morphology, DNA integrity, protamination, and global methylation (Table). Similar enhancing effects were observed in sLeX-bound and annexin V microbead-selected sperm.

Discussion

In the present study, ZP-bound spermatozoa had significantly higher expression levels of HSPA 2 and SPACA 3, compared with unbound spermatozoa. Moreover, ZP-bound spermatozoa had significantly higher rates of normal morphology, DNA integrity, chromatin integrity, protamination, and global methylation, compared with unbound spermatozoa.[4] These findings confirmed the utility of the spermatozoa-ZP interaction in the selection of fertilisation-competent spermatozoa for ART and in provision of diagnostic information about the fertilisation potential and genetic qualities of spermatozoa. Methods involving ZP, sLeX, and annexin V microbeads are viable approaches for sperm selection.

The contribution of C1orf56 to the spermatozoa-ZP interaction was demonstrated by the binding of purified C1orf56 to the ZP, as well as the inhibitory effect of anti-C1orf56 antibody on the spermatozoa-ZP interaction.[5] There was

TABLE. Comparisons of fertilisation competence and quality among sperm samples (n=10) isolated by various selection methods

Sperm selection method	DNA fragmentation rate, %	Protamination deficiency, fluorescence intensity/sperm	Methylation, fluorescence intensity/sperm	Normal morphology, %
Unselected control	16.2±5.0	119189.4±33450.8	346.7±115.5	4.6±2.3
Zona pellucida	4.4±2.7*	31426.6±14122*	1212.6±428.1*	15.2±7.0*
Sialyl-Lewis(x) sequence	10.5±5.3*	98729.6±27056.3	691.0±383.4*	8.2±3.1*
Physiological intracytoplasmic sperm injection dish	11.9±4.6	90530.6±47736.8	540.6±399.3	7.1±3.3
Annexin V microbeads	8.7±4.1*	108768.8±63358.l	833.3±367.2*	8.7±3.8*

* P<0.05, compared with unselected controls

no significant difference in C1orf56 expression on capacitated spermatozoa between the high FR (≥60%) and low FR (<60%) groups. This lack of difference is likely due to the presence of multiple ZP receptors; a decrease in one such receptor can be compensated by others.[2] The C1orf56 level in the acrosome-reacted spermatozoa was positively associated with FR. This observation suggests that C1orf56 has important roles after ZP-binding and acrosomal reactions.

Conclusion

Our results support the notion that the development of a robust and reproducible selection method incorporating the ZP binding ability of spermatozoa can improve the overall workflow and pregnancy outcomes of ART.

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Disclosure

The results of this research have been previously published in:

1. Leung ETY, Lee BKM, Lee CL, et al. The role of spermatozoa-zona pellucida interaction in selecting fertilization-competent spermatozoa in humans. Front Endocrinol (Lausanne) 2023;14:1135973.
2. Leung ETY, Lee CL, Tian X, et al. Simulating nature in sperm selection for assisted reproduction. Nat Rev Urol 2022;19:16-36.
3. Wang Y, Zhao W, Mei S, et al. Identification of sialyl-Lewis(x)-interacting protein on human spermatozoa. Front Cell Dev Biol 2021;9:700396.

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5. Wang Y, Zhao W, Mei S, et al. Identification of sialyl-Lewis(x)-interacting protein on human spermatozoa. Front Cell Dev Biol 2021;9:700396.