Extracellular CD147 as a diagnostic marker for defective acrosome reaction in asthenozoospermia and idiopathic infertility: abridged secondary publication

E Fok*

KEY MESSAGES

1. Sperm from patients with asthenozoospermia exhibits reduced motility and decreased induction of the acrosome reaction (AR), which may be associated with CD147 deficiency.
2. CD147 plays an important role in fertilisation by regulating sperm motility and the AR.
3. Recombinant CD147 treatment could improve sperm function and fertilisation outcomes in sperm from patients with asthenozoospermia.
4. The soluble CD147 levels in seminal plasma and follicular fluid can serve as predictive markers for fertilisation rates and in vitro fertilisation outcomes.

Hong Kong Med J 2023;29(Suppl 4):S31-5
HMRF project number: 06170476

E Fok

School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong SAR, China

* Principal applicant and corresponding author: ellisfok@cuhk.edu.hk

Introduction

Successful fertilisation requires the maintenance of sperm motility and induction of the acrosome reaction (AR), a process of enzyme release from the sperm head that allows penetration of the egg. Asthenozoospermia is a condition in which sperm display insufficient motility. Such sperm also exhibits a significantly lower rate of AR induction. However, the molecular mechanisms underlying defects in asthenozoospermia that lead to infertility remain unclear.

CD147 is a member of the immunoglobulin superfamily that is commonly detected in the reproductive tract and other glandular epithelial cells of various organs. CD147 is also highly expressed in cancers; it has been associated with tumour progression and invasion. CD147 is expressed in both membrane-bound and soluble forms; these two forms interact and form dimers that promote tumour invasion.[1] CD147 has essential roles in reproduction. CD147 knockout mice display both male and female infertility.[2] Loss of CD147 in oocytes and surrounding cumulus cells leads to a significant decrease in the rate of fertilisation with wild-type sperm during in vitro fertilisation (IVF),[3] suggesting an essential role for cumulus cell-derived CD147 in fertilisation. CD147 is also expressed in sperm. However, the functions of CD147 in sperm and during fertilisation remain elusive.

This study aimed to characterise the expression patterns of CD147 in sperm, seminal plasma, and follicular fluid (FF), as well as the associations of CD147 with sperm parameters and pregnancy outcomes, and to evaluate the effects of CD147 on the AR and the fertilisation potential of sperm from patients with infertility.

Methods

Semen and FF samples were collected from patients with asthenozoospermia and couples with unexplained infertility. To evaluate the expression of CD147 in extracellular compartments, extracellular vesicles (ie, membranous vesicles secreted by cells into the extracellular space) were isolated. All samples were used for expression profiling to evaluate the level of CD147 protein on extracellular vesicles, on sperm, and in FF. Selected samples were used as in vitro models to study the effects of extracellular/soluble CD147 on sperm function, along with the underlying molecular mechanisms.

The levels of CD147 on sperm, in seminal plasma, and in FF were assessed by enzyme-linked immunosorbent assays (ELISAs), immunofluorescence staining, and semi-quantitative Western blotting. The effects of CD147 on sperm function were examined by treating sperm samples with CD147-neutralising antibody or soluble CD147 (recombinant CD147 [rCD147]). Sperm motility and hyperactivated motility were measured by computer-assisted sperm analysis (CASA); AR was evaluated by fluorescein-*Pisum sativum* agglutinin (FITC-PSA) assays. Fertilisation capacity was measured by hyaluronan-binding and sperm penetration assays. Detailed experimental protocols are available in previous publications.[4,5]

Results

Under immunofluorescence staining, CD147

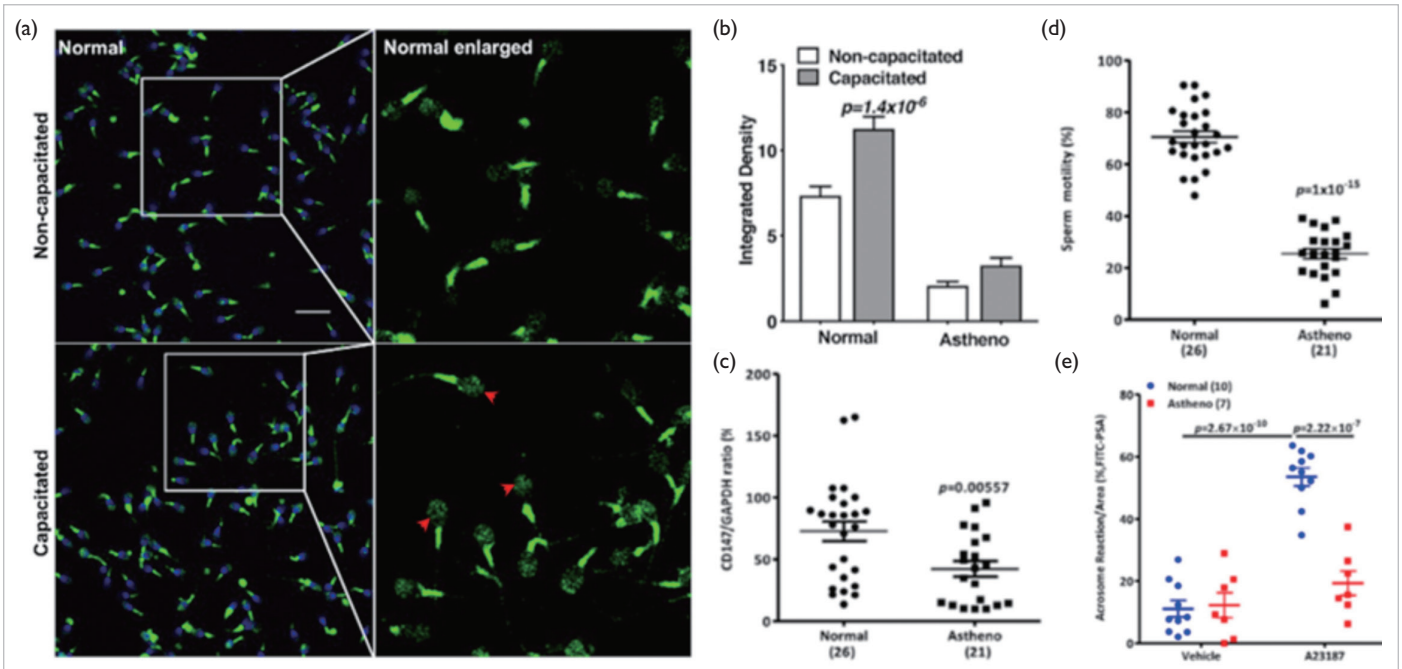

FIG 1. CD147 deficiency and acrosome reaction impairment in sperm from patients with asthenozoospermia. (a) Representative confocal images showing the expression and localisation of CD147 (green) in the head and midpiece before capacitation in normal sperm. The intensity of CD147 on the sperm head is increased after capacitation (red arrowheads). (b) Quantification of fluorescent signals showing a significant increase in the integrated density of CD147 in the normal sperm head region after capacitation. (c) Quantification of Western blots showing decreased CD147 expression in patients with asthenozoospermia, compared with healthy men. (d) Computer-assisted sperm analysis of motility in sperm from healthy men and patients with asthenozoospermia. (e) Quantification of ionophore (A23187)-induced initiation of the acrosome reaction, as measured by fluorescein-*Pisum sativum* agglutinin staining. Initiation of the acrosome reaction is significantly reduced in sperm from patients with asthenozoospermia than in sperm from healthy men.

protein levels were lower in asthenozoospermic sperm samples than in normal sperm samples (Fig 1). A stronger CD147 signal was observed in the head region upon capacitation, a process induced by the female reproductive tract essential for sperm to fertilise. The reduced expression of CD147 in patients with asthenozoospermia was confirmed by Western blotting. In addition, ionophore-induced AR was also reduced in asthenozoospermic sperm. These results suggest that sperm from patients with asthenozoospermia exhibits reduced motility and decreased induction of the AR, which may be associated with CD147 deficiency.

CD147 was expressed in membrane-bound and soluble forms; thus, the female reproductive tract was assumed to secrete soluble CD147 and regulate sperm function. Indeed, ELISAs showed that soluble CD147 was present in human FF (Fig 2). CD147 was identified in a subpopulation of extracellular vesicles isolated from FF.

To investigate the role of CD147 in sperm function, sperm-bound CD147 was blocked by a neutralising antibody. The effect of a rCD147 protein that resembled soluble CD147 was tested. Although rCD147 treatment did not increase sperm motility, anti-CD147 antibody treatment significantly reduced sperm motility (Fig 2), suggesting that sperm-bound CD147 contributes to the maintenance of sperm motility. Hyperactivated motility and the AR, both associated with capacitation, were assessed by CASA and *Pisum sativum* agglutinin (PSA) staining, respectively. Although progesterone, a physiological inducer of hyperactivation and the AR, induced a twofold increase in the percentage of sperm with hyperactivated motility, rCD147 treatment did not affect this process. These findings suggested that soluble CD147 is not essential for sperm hyperactivation. Notably, rCD147 treatment induced a threefold increase in AR, comparable with the increase triggered by progesterone, indicating that rCD147 is a potent AR inducer. Effects of CD147 on fertilisation outcomes were examined using hyaluronan-binding and human sperm-hamster oocyte penetration assays. Anti-CD147 antibody treatment significantly decreased the percentage of sperm bound to hyaluronan and the number of oocyte-penetrating sperm. rCD147 treatment significantly increased the number of oocyte-penetrating sperm but showed only a modest increase in the percentage of sperm bound to

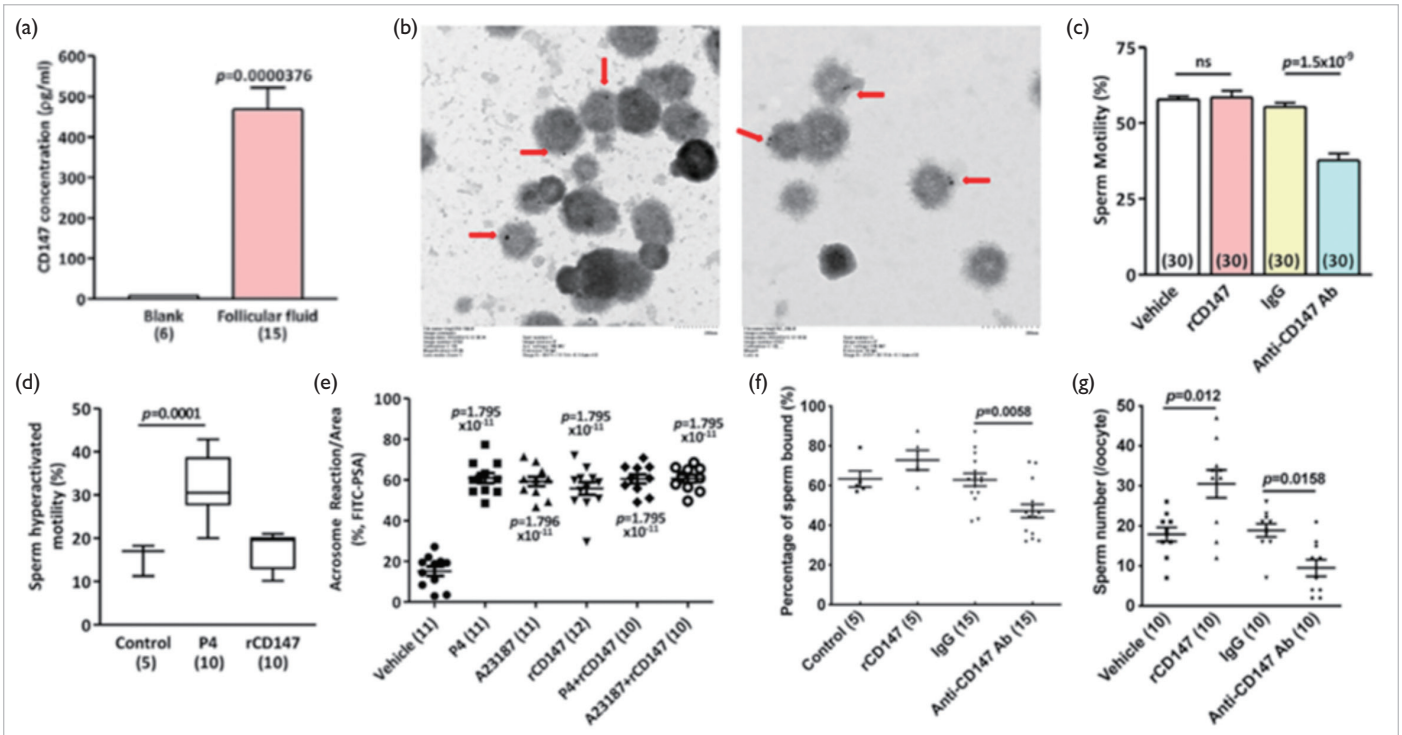

FIG 2. Soluble CD147 promotes sperm motility and induces the acrosome reaction. (a) Enzyme-linked immunosorbent assay results showing the presence of soluble CD147 in human follicular fluid. (b) Representative electronic microscopy images showing the presence of CD147-labelled gold particles (red arrow) on extracellular vesicles isolated from follicular fluid. (c) Computer-assisted sperm analysis (CASA) results showing the effect of recombinant CD147 (rCD147) or CD147-neutralising antibody (anti-CD147 Ab) treatment on sperm motility. (d) CASA results showing the effect of rCD147 and progesterone on hyperactivated motility in capacitated sperm. (e) Fluorescein-*Pisum sativum* agglutinin staining results showing the effect of rCD147, progesterone, the ionophore A23187, or the indicated combined treatments on the acrosome reaction in sperm. (f) Hyaluronan-binding assay (g) and human sperm-hamster oocyte penetration assay results showing the effect of rCD147 or anti-CD147 Ab treatment on hyaluronan-binding and oocyte-penetrating abilities in sperm.

hyaluronan. These results suggest that CD147 plays an important role in fertilisation by regulating sperm motility and the AR.

Sperm function was tested after augmenting the dimerisation of CD147 through increasing the amount of soluble CD147. Results showed that rCD147 significantly enhanced motility in sperm and that this effect could be abolished by treatment with the CD147-neutralising antibody (Fig 3). Despite a decrease in the level of AR induction, rCD147 treatment was able to trigger a 3.7-fold increase in AR induction, which could be inhibited by anti-CD147 antibody treatment. Importantly, rCD147 treatment significantly increased the percentage of bound sperm in samples from patients with asthenozoospermia. These results demonstrated that rCD147 treatment could improve sperm function and fertilisation outcomes in sperm from patients with asthenozoospermia.

The level of CD147 could serve as a predictive marker for fertilisation outcomes. Couples with unexplained (idiopathic) infertility who were undergoing IVF treatment were recruited. The soluble CD147 level in FF from women with idiopathic infertility significantly decreased, compared with women whose partners were diagnosed with male infertility (Fig 3), suggesting that soluble CD147 in FF can be a diagnostic marker for AR defects in couples with idiopathic infertility. Furthermore, the level of CD147 in seminal plasma was positively correlated with the fertilisation rate. The level of CD147 in seminal plasma was significantly lower in sperm from men whose partners did not become pregnant after IVF treatment. This suggested an association between seminal CD147 levels and IVF outcomes. Thus, the soluble CD147 levels in seminal plasma and FF can serve as predictive markers for fertilisation rates and IVF outcomes.

Discussion

Sperm-bound CD147 is involved in sperm motility, the AR, and sperm-egg interactions. Deficiency in sperm-bound CD147 may result in poor motility and AR in patients with asthenozoospermia; therefore, CD147 is a potential diagnostic marker for male infertility.

The ability of rCD147 to trigger AR induction

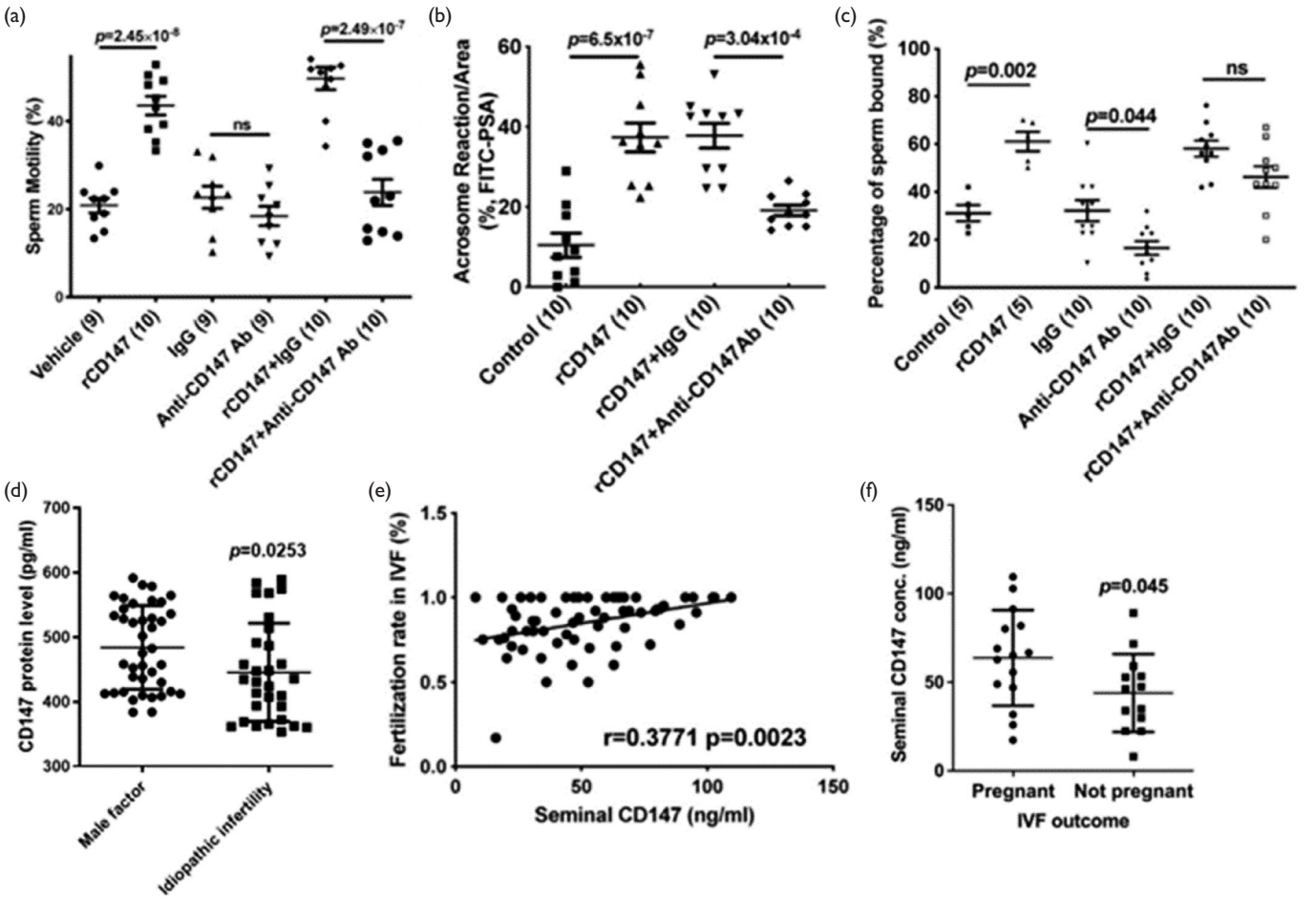

FIG 3. Potential application of soluble CD147 level in assisted reproduction. (a) Computer-assisted sperm analysis results showing the effect of CD147-neutralising antibody (anti-CD147 Ab) and/or recombinant CD147 (rCD147) treatment on motility in sperm from patients with asthenozoospermia. (b) Fluorescein-*Pisum sativum* agglutinin (FITC-PSA) staining results showing the effect of rCD147 on the acrosome reaction in sperm from patients with asthenozoospermia, in the presence or absence of anti-CD147 Ab. (c) Hyaluronan-binding assay results showing the effect of anti-CD147 Ab and/or rCD147 on hyaluronan-binding ability in sperm from patients with asthenozoospermia. (d) Enzyme-linked immunosorbent assay (ELISA) results showing reduced level of CD147 in follicular fluid from women with idiopathic infertility. Female partners with normal profiles and their male partners diagnosed with male infertility were recruited as controls. (e) Correlation analysis of seminal plasma CD147 level with the fertilisation rate after in vitro fertilisation (IVF). (f) ELISA results showing the level of CD147 in seminal plasma from men with infertility, according to IVF outcome.

and augment CD147 signalling, thereby improving sperm motility and fertilising capacity, has high translational value. Successful IVF and intrauterine insemination outcomes rely on sperm motility. The use of rCD147, which mimics the physiological source of soluble CD147 in semen and the female reproductive tract, may improve the success rate by restoring sperm motility and enhancing fertilising capacity.

Intracytoplasmic sperm injection (ICSI) is usually conducted when patients lack motile or morphologically normal sperm. Assessment of AR capability has been proposed to guide the selection of IVF or ICSI. The level of soluble CD147 in seminal plasma was correlated with the rates of fertilisation and pregnancy, suggesting that CD147 could be an indicator to guide the assisted reproductive technology regimens. Additionally, introduction of large amounts of acrosomal enzymes into the oocyte cytoplasm disrupts the cytoskeleton and damages oocytes and decreases the success rate of ICSI. The removal of acrosomes before ICSI improves oocyte survival and embryonic development. The AR can be triggered by rCD147 treatment, which efficiently removes acrosomal enzymes and increases the success rate of ICSI.

Conclusion

CD147 has a key role in sperm function. CD147 deficiency results in poor sperm motility and AR, contributing to infertility outcomes associated with asthenozoospermia. The levels of soluble CD147 in FF and seminal plasma may serve as indicators

to guide the personalised assisted reproductive technology regimens.

Funding

This study was supported by the Health and Medical Research Fund, Health Bureau, Hong Kong SAR Government (#06170476). The full report is available from the Health and Medical Research Fund website (https://rfs2.healthbureau.gov.hk).

Disclosure

The results of this research have been previously published in:

1. Chen H, Shi X, Li X, et al. CD147 deficiency is associated with impaired sperm motility/acrosome reaction and offers a therapeutic target for asthenozoospermia. Mol Ther Nucleic Acids 2021;26:1374-86.

References

1. Wu J, Hao ZW, Zhao YX, et al. Full-length soluble CD147 promotes MMP-2 expression and is a potential serological marker in detection of hepatocellular carcinoma. J Transl Med 2014;12:190.
2. Bi J, Li Y, Sun F, et al. Basigin null mutant male mice are sterile and exhibit impaired interactions between germ cells and Sertoli cells. Dev Biol 2013;380:145-56.
3. Kuno N, Kadomatsu K, Fan QW, et al. Female sterility in mice lacking the basigin gene, which encodes a transmembrane glycoprotein belonging to the immunoglobulin superfamily. FEBS Lett 1998;425:191-4.
4. Choy KHK, Chan SY, Lam W, et al. The repertoire of testicular extracellular vesicle cargoes and their involvement in inter-compartmental communication associated with spermatogenesis. BMC Biol 2022;20:78.
5. Chen H, Shi X, Li X, et al. CD147 deficiency is associated with impaired sperm motility/acrosome reaction and offers a therapeutic target for asthenozoospermia. Mol Ther Nucleic Acids 2021;26:1374-86.