GLP-1 Follicular Extracellular Vesicles: β-Cell Regenerative Therapy in Type 2 Diabetes Mellitus and PMOS Dysfunction

GLP-1 Vésicules folliculaires extracellulaires : thérapie régénérative à cellules β dans le diabète sucréel de type 2 et les dysfonctionnements du PMOS

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Abstract | Résumé

Type 2 Diabetes Mellitus (T2DM) is characterized by β-cell dysfunction and loss of insulin-producing mass in the pancreas (1). Current glucose-lowering therapies slow disease progression but fail to reverse β-cell decline (2). Polyendocrine Metabolic Ovarian Syndrome (PMOS) and T2DM share underlying metabolic dysfunction, including insulin resistance and chronic inflammatory signaling. Restoring β-cell health may represent a promising strategy to improve metabolic regulation, improve glycemic management, and potentially alleviate PMOS-related infertility (3).

This study proposes engineering ovarian follicular-fluid-derived extracellular vesicles (FF-EVs) to support pancreatic β-cell regeneration. FF-EVs are intended to enhance β-cell mass, improve glucose-stimulated insulin secretion, and reduce T2DM-linked reproductive dysfunction like PMOS (4).

Follicular fluid from hormonally stimulated female C57BL/6 mice will be used to isolate EVs enriched in reproductive microRNAs and insulin-like growth factors (5). These are engineered to display glucagon-like-peptide-1 (GLP-1) on their membrane surface through fusion with EV-anchoring Lysosome-Associated Membrane Protein 2B (LAMP2B), enabling selective pancreatic β-cell uptake through GLP-1 receptor binding. The engineered EVs will be loaded with pro-regenerative cargo proteins (harmine and mRNAs encoding PDX1, NGN3, and MAFA), promoting β-cell proliferation and β-cell mass restoration (6).

Optimal EV formulations will be tested in C57BL/6 mice (no-STZ, STZ-induced). β-cell viability, diabetic markers (Glut2, Glut4, INSR, IRS1/2) were assessed *in vitro* through glucose-stimulated insulin secretion, then *in vivo* evaluation of pancreatic β-cell proliferation (7).

Translational evaluation will use human β-cell lines and *in vitro* human organoids (pancreas-ovarian follicles) to validate β-cell recovery and improvements of metabolic-reproductive parameters.

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Le diabète sucré de type 2 (DT2) se caractérise par un dysfonctionnement des cellules β et une perte de masse productrice d'insuline dans le pancréas (1). Les thérapies actuelles de réduction de la glycémie ralentissent la progression de la maladie mais ne parviennent pas à inverser le déclin des cellules β (2). Le syndrome ovarien métabolique polyendocrine (PMOS) et le DT2DM partagent des dysfonctionnements métaboliques sous-jacents, notamment la résistance à l'insuline et la signalisation inflammatoire chronique. Restaurer la santé des cellules β pourrait représenter une stratégie prometteuse pour améliorer la régulation métabolique, améliorer la gestion glycémique et potentiellement atténuer l'infertilité liée au PMOS (3).

Cette étude propose d'ingénier des vésicules extracellulaires dérivées du liquide folliculaire ovarien (FF-EV) pour soutenir la régénération pancréatique β-cellulaire. Les FF-EV visent à augmenter la masse cellulaire β, à améliorer la sécrétion d'insuline stimulée par le glucose et à réduire les dysfonctionnements reproductifs liés au DT2DM comme le PMOS (4).

Le liquide folliculaire de souris C57BL/6 stimulées hormonalement sera utilisé pour isoler les EV enrichies en microARN reproducteurs et en facteurs de croissance de type insuline (5). Ces appareils sont conçus pour afficher du peptide similaire-glucagon-1 (GLP-1) à leur surface membranaire par fusion avec la protéine membranaire lysosome-associée 2B (LAMP2B) ancrant les EV, permettant une captation sélective des cellules pancréatiques β via la liaison aux récepteurs GLP-1. Les VE conçus seront chargés de protéines cargo pro-régénératives (harmine et ARNm codant pour PDX1, NGN3 et MAFA), favorisant la prolifération des cellules β et la restauration de masse des cellules β (6).

Les formulations optimales de la cellule électrique seront testées chez des souris C57BL/6 (sans STZ, induite par STZ). La viabilité des β-cellules et les marqueurs diabétiques (Glut2, Glut4, INSR, IRS1/2) ont été évalués *in vitro* via la sécrétion d'insuline stimulée par le glucose, puis une évaluation *in vivo* de la prolifération des β pancréas (7). L'évaluation translationnelle utilisera des lignées β humaines et des organoïdes humains *in vitro* (follicules pancréas-ovaires) pour valider la récupération des cellules β et l'amélioration des paramètres métabolo-reproducteurs.

Keywords: Follicular fluid, pancreatic β-cell regeneration, EV-based therapeutics, LAMP2B fusion proteins, EV cargo loading (siRNA, mRNA, miRNA), vesicle-mediated RNA delivery, ovarian microenvironment, Type 2 Diabetes Mellitus

Background Information

T2DM is a prevalent metabolic disorder, and while current therapies control hyperglycemia, they do not address underlying β-cell dysfunction, making β-cell preservation and regeneration critical therapeutic goals. T2DM intersects with reproductive disorders (PMOS), where affected women have a 4-8.8-fold higher risk of developing T2DM (8). This shared metabolic-hormonal dysfunction highlights the need to improve pancreatic β-cell functions. EVs are biocompatible with therapeutic carriers capable of transporting proteins and bioactive molecules. FF-EVs are appealing because they contain developmental and endocrine signals that support cell survival. For β-cell targeting, FF-EVs can be engineered to display GLP-1 fused to the EV membrane protein LAMP2B, enabling receptor-specific delivery.
FF-EVs is expected to reverse β-cell loss, addressing T2DM and its intersection with reproductive metabolic disorders.

Research Idea

Two therapeutic benefits are offered by FF-EV-GLP-1-LAMP2B construct. First, the GLP-1 cargo helps maintain β-cell identity while promoting β-cell regeneration and enhancing insulin secretion. Second, the inherent bioactivity of FF-EVs, including growth signaling factors and developmental microRNAs, further supports metabolic regulation and glycemic control (9).
When combined, this produces an EV platform that can improve β-cell function in diabetic conditions and resolve T2DM-mediated reproductive issues in females.

Rationale

Conventional T2DM therapies are limited by poor β-cell targeting and low durability *in vivo* (10). FF-EVs transport developmental miRNAs, metabolic regulators, and proteins directly to endocrine targets. This minimizes off-target exposure and improves β-cell-specific therapeutic action (11). FF-EVs attach to pancreatic β-cells when designed with GLP-1R-targeting LAMP2B fusion peptide, enabling selective docking and transmission of regeneration signals (6). This study will also examine reproductive markers owing to correlations between female reproductive disorders and T2DM (3). The MIN6 mouse cell line is utilized as it transports glucose and secretes insulin similarly to pancreatic β-cells (12)

Hypothesis

FF-EVs engineered with a GLP-1R β-cell–homing peptide fused to LAMP2B are hypothesized to enhance β-cell targeting and improve glycemic regulation in T2DM. Furthermore, the resulting improvements in metabolic function may contribute to alleviating reproductive imbalances associated with PMOS.

Methodology

For β-cell specificity, engineered FF-EV-GLP-1-LAMP2B fusion protein is used (13). C-terminus GLP-1 will be fused to the N-terminus of LAMP2B through glycine–serine flexible linker (G_4S), preventing steric hindrance, allowing proper folding and surface presentation (13, 14). Granulosa cells will be isolated from hormonally stimulated adult female mice, cultured *in vitro*. Cells are transfected with fusion plasmid GLP-1-LAMP2B construct (CMV promoter and SV40 polyA terminator), enabling secretion of FF-EVs displaying GLP-1 on the surface for GLP-1R–mediated β-cell homing (15, 16). Purified FF-EVs will be loaded with β-cell cargo proteins such as harmine, a selective DYRK1A inhibitor, to promote β-cell proliferation (17). Furthermore, mRNAs encoding PDX1, NGN3, and MAFA will be included to induce pancreatic endocrine fate through NGN3, establish β-cell lineage identity through PDX1, and enhance glucose responsiveness through MAFA (18). miR-132 will also be incorporated to enhance β-cell survival and proliferation through PTEN/AKT/FOXO3 signaling pathways (19). Additionally, betacellulin, a growth factor involved in β-cell differentiation, survival, and insulin secretion, will be included within the FF-EVs. The mRNA and miRNA will be incorporated using electroporation; harmine, and Betacellulin through sonication (20).

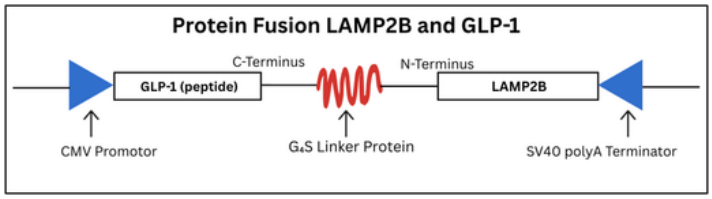

Figure 1. Structure of GLP-1-LAMP2B fusion protein. Figure 1 shows a schematic representation of the modified fusion protein utilized for β-cell selectivity in extracellular vesicles formed from follicular fluid (FF-EVs). A flexible glycine–serine linker (G_4S) fuses the C-terminus of glucagon-like peptide-1 (GLP-1) to the N-terminus of lysosome-associated membrane protein 2B (LAMP2B), reducing steric hindrance and facilitating appropriate protein folding and surface display. A cytomegalovirus (CMV) promoter drives the construct, while an SV40 polyadenylation signal ends. GLP-1's surface presentation makes it easier for EVs to target and home to pancreatic β-cells via the GLP-1 receptor (GLP-1R).

Experimental Design

In the proposed study, the independent variable is the type of treatment administered to MIN6 cells, mice, human cells, and organoids. Dependent variables include β-cell functional outcomes and expression levels of diabetic markers (*D) and reproductive markers (*R). The control variables include cell line (MIN6), standardized EV isolation procedures, uniform culture conditions (DMEM composition, temperature, CO_2 levels, and confluence at treatment), EV dosing (1×10^8–1×10^9 particles/mL), treatment duration (24-hour incubation), controlled 0.5-1mM STZ exposure conditions.

Table 1. Diabetic Molecular Markers

Experimental Model	Diabetic Markers (*D)
In-Vitro MIN6 Cell Line	Ins1/Ins2
In-Vivo C57BL/6 Mice Model	Serum markers: insulin, glucose, C-peptide, leptin, adiponectin, TGs Tissue markers: Pdx1, MAFA, Ppary
Human β-Cell Line	INS, C-peptide
Pancreatic Organoids	Glut1, Glut2, Glut4, INSR, IRS1/2

Table 1. shows a summary of key metabolic markers used to evaluate β-cell function and glucose homeostasis *in vitro* and *in vivo* models. MIN6 cells will be analyzed for insulin gene expression through Ins1/Ins2, while the C57BL/6 mouse model includes both serum markers (insulin, glucose, C-peptide, leptin, adiponectin, triglycerides) and pancreatic tissue markers (Pdx1, MAFA, Ppary). Human β-cell lines were assessed for insulin (INS) and C-peptide levels, and pancreatic organoids were evaluated for glucose transport and insulin signaling markers (GLUT1, GLUT2, GLUT4, INSR, IRS1/2).

Table 2. Reproductive Endocrine Markers

Experimental Model	Reproductive Markers (*R)
In-Vitro MIN6 Cell Line	ESR1, ESR2, FSHR
In-Vivo C57BL/6 Mice Model	Esr1/2, Fshr Serum: Estradiol, Progesterone, LH, FSH,
Human β-Cell Line	ESR1, ESR2, FSHR
Ovarian Follicle Organoids	ESR1/ESR2, FSHR

Table 2. shows a summary of reproductive and hormonal markers used to assess endocrine function across experimental systems. MIN6 and human β-cell lines will be evaluated for estrogen receptor expression (ESR1, ESR2) and follicle-stimulating hormone receptor (FSHR). *In vivo* C57BL/6 mice will be analyzed for both gene expression (Esr1/2, Fshr) and circulating reproductive hormones (estradiol, progesterone, luteinizing hormone [LH], and follicle-stimulating hormone [FSH]. Ovarian follicle organoids will be assessed for ESR1/ESR2 and FSHR expression.

In Vitro

Cell culture: MIN6 cells will be grown in DMEM with 10% heat-inactivated FBS, 100 U/mL penicillin, 100 µg/mL streptomycin, and 2.0 mM glutamine in CO_2 incubator at 37°C with 5% CO_2. Cells seeded into 12-or 24-well plates to reach 70-80% confluence over 24h before treatment (21).

Induction of β-cell injury (STZ): The normal β-cell will receive no STZ. MIN6 cells will be treated to STZ (0.5-1mM) for 24h to elicit T2D-like/β-cell damage. The wells will be cleansed and reintroduced to fresh medium before treatment. Insertion of EVs into MIN6 cells will be accomplished by directly adding EV-containing media to wells at 1×10^8, 5×10^8, 1×10^9 particles/mL dosage. EVs will be incubated with MIN6 cells for 24h and absorbed by cells through endocytosis.

Experimental groups: Three treatment groups will be created for STZ and No STZ. The control group contains MIN6 cells with no EV. FF-EV-GLP1 group will contain MIN6 cells in medium containing FF-EVs with GLP-1. FF-EV-GLP1-LAMP2B group will contain MIN6 in medium containing FF-EVs with GLP-1-LAMP2B.

Detection: Following EV exposure, qPCR/Western blot for β-cell markers (PDX1, MAFA, INS, GLUT2) will be conducted. Following those studies, best EV conditions for restoring/enhancing β-cell activity in STZ-injured MIN6 cells will be chosen for mouse studies. *D markers will be validated using ELISAs (22).

In Vivo

Selection process; n=10: The in-vivo mice profile includes: C57BL/6 female mice aged 8-10 weeks, 20-25g, kept in standardized settings (12h light/dark cycle, controlled temperature/humidity, consistent food/water) (23). Before treatment, baseline fasting blood glucose (FBG) levels, body weight, and health condition recorded. Mice randomly assigned to no-STZ or STZ-induced groups (24). Diabetes in STZ group produced using low-dose STZ (50 mg/kg intraperitoneally, 5 days), hyperglycemia confirmed before EV therapy (25).

Experimental groups: Experimental groups will consist of both no-STZ and STZ-induced conditions, each further divided into three treatment groups. The control group will contain normal β-cells receiving vehicle treatment only. The FF-EV-GLP1 group will receive GLP-1-enriched FF-EVs administered through subcutaneous injections twice per week for four weeks (26). Similarly, the FF-EV-GLP1-LAMP2B group will receive LAMP2B-engineered GLP-1 EVs through subcutaneous injections twice per week for four weeks.

Monitoring/Detection: Weekly measurements of body weight and fasting blood glucose (FBG) will be collected throughout the study. Intraperitoneal glucose tolerance tests will be conducted at designated intervals, while ELISA assays will be used to quantify plasma insulin levels (27). At the conclusion of the study, pancreatic tissues will be collected for gene expression analysis and histological assessment of β-cell mass and apoptotic indicators. Ovarian tissues will also be analyzed to assess follicle morphology, with *D and *R levels detected through ELISA assays.

Translational study with human β-cell and organoids: Both diabetic and non-diabetic human β-cell lines will contain identical experimental groups, including a control group receiving vehicle only, an FF-EV-GLP1 group treated with GLP-1 enriched FF-EVs, and an FF-EV-GLP1-LAMP2B group treated with LAMP2B-engineered GLP-1 EVs. *D screened conditions will also be included. Similarly, human pancreatic and follicular organoid models will undergo the same treatment conditions. Under non-diabetic conditions, the study will assess EV penetration into three-dimensional tissue structures. Under diabetic conditions, the study will evaluate whether EV mediated GLP-1 delivery improves β-cell function within 3D tissue models mimicking Type 2 Diabetes Mellitus (28). Both *D and *R screened conditions will be included

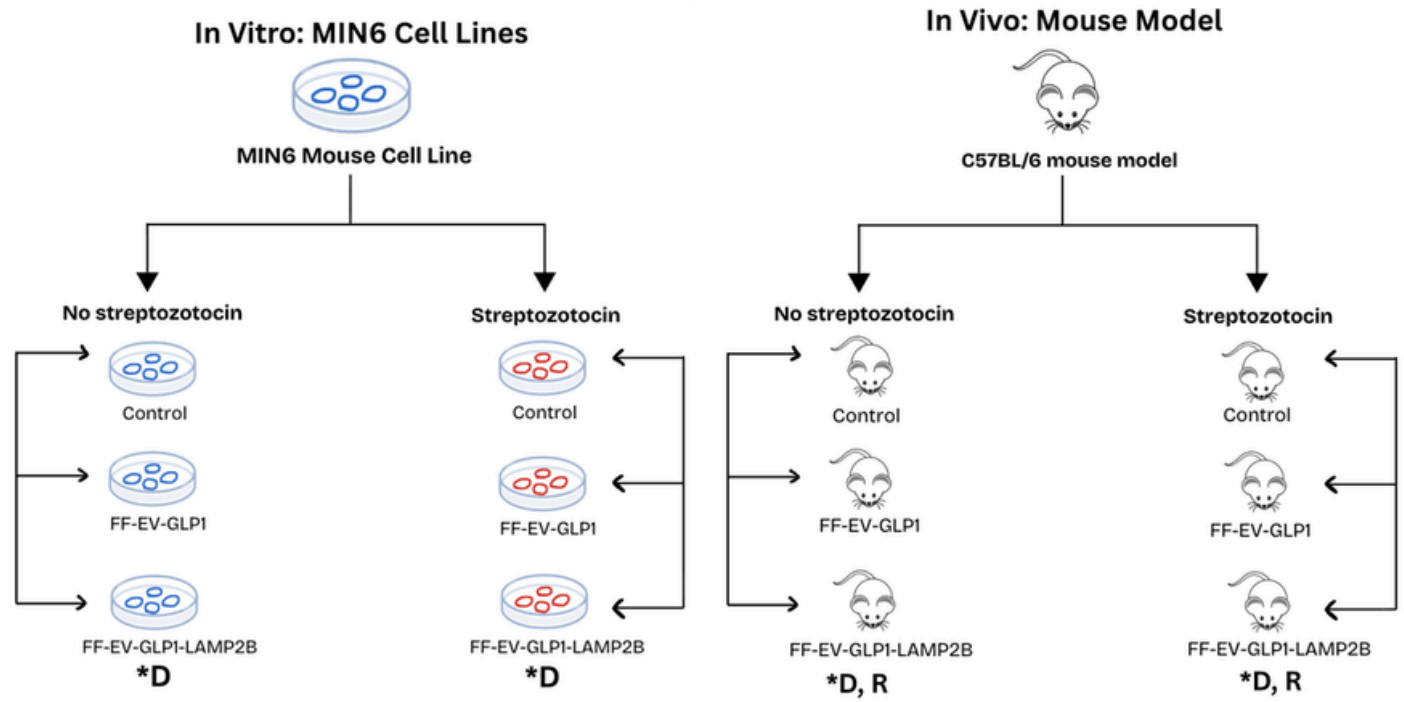

Figure 2. FF-EV based β-cell targeting β MIN6 cells and C57BL/6 mouse models. Figure 2. shows a schematic overview of experimental design in MIN6 β-cell lines and C57BL/6 mouse models. Cells and animals were divided into control, FF-EV-GLP1, and FF-EV-GLP1-LAMP2B treatment groups under both basal (no streptozotocin) and diabetic (streptozotocin-induced) conditions. Outcomes were assessed using diabetic metabolic markers (*D) *in vitro* and both metabolic and reproductive markers (*D, R) *in vivo*.

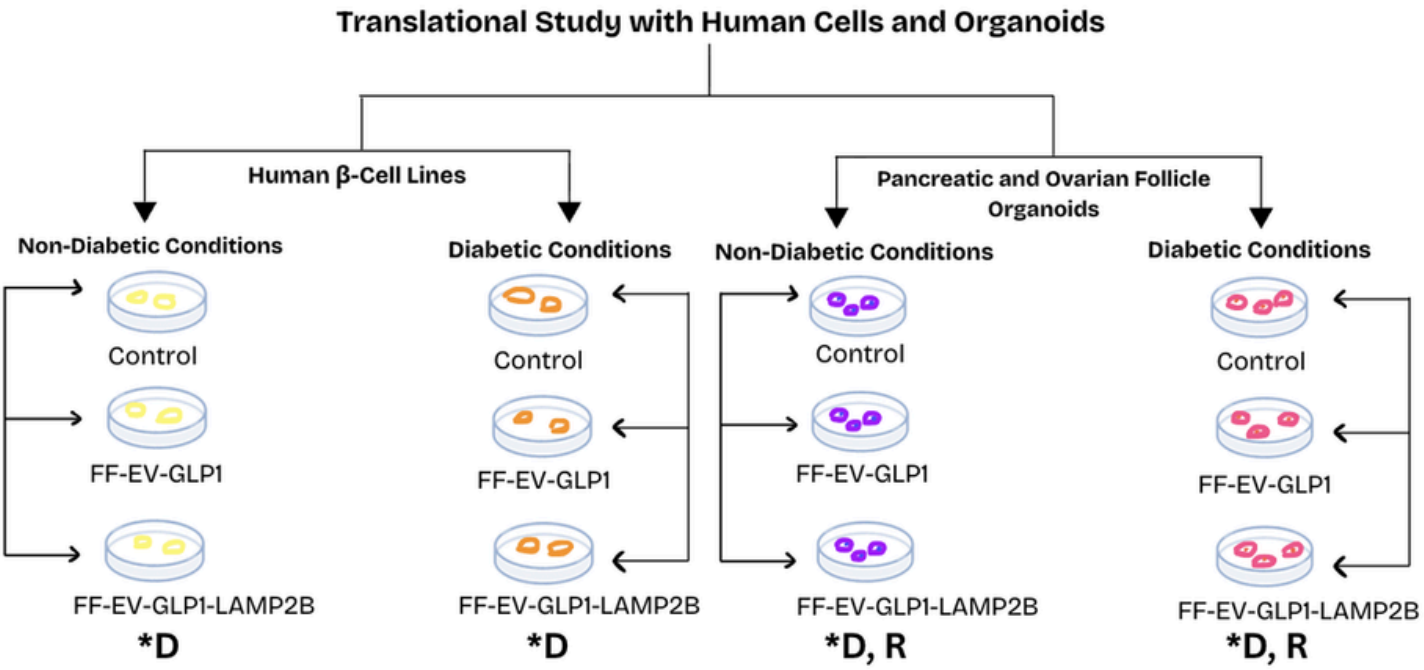

Figure 3. Translational validation of FF-EV–based therapy in human β-cell lines and pancreatic and ovarian follicle organoid systems. Figure 3 shows a translational validation assessing therapeutic efficacy under non-diabetic and diabetic conditions in human β-cell lines and pancreatic and ovarian follicle organoids. Treatment groups included control, FF-EV-GLP1, and FF-EV-GLP1-LAMP2B. Outcomes were evaluated using diabetic metabolic markers (*D) and reproductive endocrine markers (*R) to assess both metabolic and endocrine function

within the organoid studies.

Expected Outcomes & Impact

Engineered follicular extracellular vesicles (FF-EV-GLP-1-LAMP2B) are expected to improve pancreatic β-cell survival, proliferation, and glucose-stimulated insulin secretion in both *in vitro* and *in vivo* models, along with improved glycemic control and metabolic marker normalization. Additionally, because T2DM and reproductive dysfunction share metabolic pathways, EV treatment may improve endocrine signaling and reproductive markers. If effective, this method has the potential to extend regenerative therapies beyond symptom management and into disease-modifying medicines for T2DM and associated disorders.

Limitations & Risk Assessment

There may be some restrictions and risks associated with this proposed study. Reproducibility and therapeutic dependability may be impacted by variations in EV isolation, cargo loading efficiency, and batch consistency. While the goal of GLP-1-LAMP2B surface modification is to improve β-cell targeting, variations in EV uptake among cell types may decrease targeting specificity and result in off-target consequences. Furthermore, the intricacy of human T2DM may not be fully captured by widely used diabetes models, such as STZ-induced systems, which limit translational applicability.

Conclusion

If the alternative hypothesis is accepted, FF-EV-GLP1-LAMP2B will target pancreatic β-cells, deliver regenerative cargo, enhance β-cell proliferation, and reduce metabolic dysfunction in diabetes and PMOS. This would support EV-based therapeutics as a biocompatible strategy for reversing β-cell loss in T2DM and improving reproductive health.

Future studies will use letrozole-induced C57BL/6 mice to model PMOS-associated dysfunction, as letrozole is a potent aromatase inhibitor that interferes with the conversion of androgens to estrogens, resulting in hyperandrogenemia and key metabolic and reproductive characteristics of PMOS (32). This model makes it possible to look into metabolic and endocrine issues related to the relationship between T2DM and PMOS. Future research in this area will test the therapeutic efficiency of modified follicular extracellular vesicles (FF-EV-GLP-1-LAMP2B) *in vivo*, particularly their capacity to improve PMOS-related and reproductive dysfunction while simultaneously promoting β-cell regeneration. By confirming treatment effects across interrelated physiological systems, this strategy will increase the study's translational value.

Definitions

1. Betacellulin: A growth factor from the epithelial growth factor family that promotes pancreatic β-cell proliferation, survival, and maturation; often used to enhance insulin-producing cell development (29).
2. DSPE-PEG–Peptide Insertion: A membrane-engineering method where a lipid–PEG–peptide conjugate (DSPE-PEG–peptide) is spontaneously inserted into the extracellular vesicle (EV) membrane. DSPE is a hydrophobic phospholipid that anchors into the EV lipid bilayer, while PEG provides a flexible spacer that displays the peptide on the EV surface. This allows targeted ligands (GLP-1) to be added to EVs without genetic modification (30).
3. Electroporation: A laboratory method that uses short electrical pulses to temporarily open pores in cell or EV membranes, allowing DNA, RNA, or drugs to enter (31).
4. ELISA: A biochemical assay used to quantify proteins by using antigen–antibody binding coupled with a colorimetric or fluorescent readout (32).
5. GLP1: An incretin hormone that enhances glucose-stimulated insulin secretion and slows gastric emptying; β-cells express its receptor (GLP-1R), enabling targeted therapies (33).
6. Harmine: A small-molecule inhibitor of DYRK1A that stimulates robust proliferation of human pancreatic β-cells by releasing cell-cycle suppression (34).
7. Hyperglycemia: A condition characterized by abnormally high blood glucose levels, typically resulting from impaired insulin secretion, insulin resistance, or both (35).
8. Incretin: A class of gut-derived hormones (such as GLP-1 and GIP) released after eating that enhance glucose-dependent insulin secretion and help regulate blood sugar levels (36).
9. LAMP2B: An EV-associated membrane protein commonly used as a scaffold for displaying targeting peptides on extracellular vesicles through fusion-protein engineering (6).
10. PMOS: Polyendocrine Metabolic Ovarian Syndrome, a prevalent reproductive endocrine disorder in women characterized by hormonal imbalance, irregular ovulation, metabolic dysfunction, and an increased risk of insulin resistance and Type 2 diabetes (8).
11. Pancreatic β-cell: A specialized endocrine cell located in the islets of Langerhans responsible for producing, storing, and secreting insulin in response to glucose (37).
12. Follicular Extracellular vesicles: Nanoparticles naturally present in ovarian follicular fluid that carry proteins, RNAs, and signaling molecules involved in reproductive and metabolic regulation (38).
13. qPCR: A laboratory technique used to quantify gene expression by measuring mRNA levels (39).
14. Sonification: A technique that uses high-frequency sound waves to break apart membranes or mix biological samples; in EV work, can be used to load cargo into vesicles (40).
15. Streptozotocin: A chemical β-cell toxin that induces diabetes *in vitro* and *in vivo* by causing DNA alkylation and selective β-cell death; used to create diabetic models (7).
16. Western blot: A protein-based technique used to detect and quantify specific proteins via antibody binding (41).

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