Ann. Anim. Sci., Vol. 10, No. 1 (2010) 3–8

LIPOSOMES IN GAMETE AND EMBRYO BIOTECHNOLOGY

I z a b e l a G r a d

Department of Biotechnology of Animal Reproduction,
National Research Institute of Animal Production, 32-083 Balice n. Kraków, Poland

Abstract

Liposomes are highly flexible vesicular structures that already found practical application in pharmaceutics and cosmetology in the 1980s. Current research on liposomes concentrates on their possible use in medicine and animal reproduction biotechnology, including the use of liposomes as a model of sperm cell membrane and as carriers of unstable or easily soluble compounds, and the determination of their usefulness in cryoconservation of gametes and embryos, and in animal transgenesis. This review discusses the possibility of using liposomes in animal reproduction biotechnology.

Key words: biotechnology, liposomes, gametes, embryos, mammals

Liposomes are vesicular structures composed of phospholipids that enclose aqueous solutions. They are built from concentrically arranged lipid bilayers, similar to the lipid arrangement of biological membranes. Liposomes are formed because of the amphiphilic properties of lipids, which can self-organize into bilayer structures in aqueous solutions. According to the principle of lowest energy, lipid bilayers tend to form vesicles. Liposomes were first observed in 1911 and named as “artificial cells”. The types and characteristics of liposomes are shown in Table 1.

Table 1. Classification and characteristics of liposomes

Types of liposome	Liposome diameter	Number of lipid bilayers
Small liposomes – SUV	20–80 nm	1
Large liposomes – LUV	80–1000 nm	1
Giant liposomes – GUV	1–2 μm	1
Multilamellar liposomes – MLV	300 nm–20 μm	several

Liposomes are the subject of many experiments. Research on the use of liposomes in gamete and embryo biotechnology is mainly focused on:

– the possibility of using liposomes as biological membrane models,

– the possibility of increasing the efficiency of biotechnology methods through compounds delivered by liposomes.

Most studies did not verify a toxic influence of liposomes on cell viability, but Ziętkiewicz and Słomski (1984) suggested that excess of liposomes per cell and overly long incubation period may have a toxic effect.

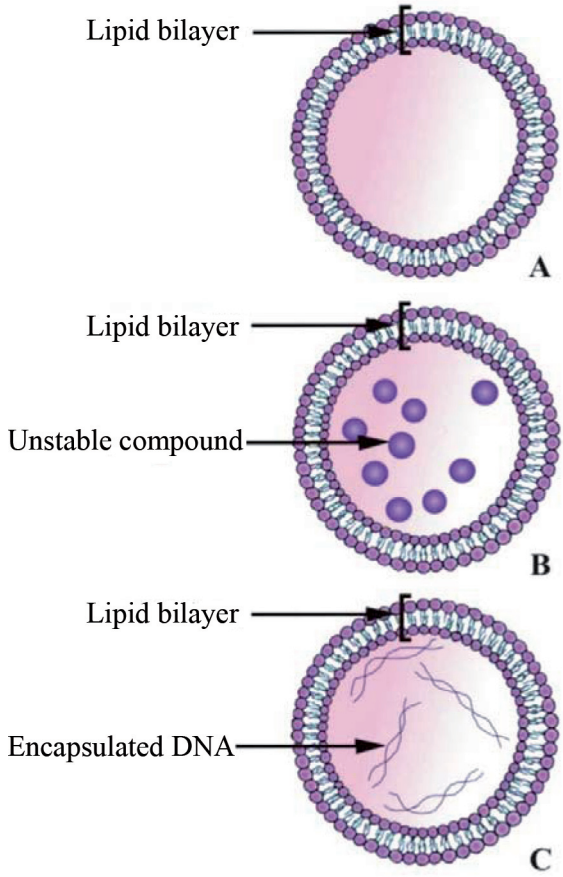

Figure 1. Schematic diagram of liposomes
A. Monolayer liposome as plasma membrane model
B. Liposome – unstable compound carrier
C. DNA – liposome complex

Liposomes as cell membrane models

As noted above, the structure of liposomes is analogous to the structure of cell membranes, as a result of which they can be used as simple biological membrane models to study principal functions of cell membranes such as permeability, stability and integrity (Figure 1 A).

Because during *in vitro* fertilization process in mammals, the plasma membrane of spermatozoa is the site of molecular changes that lead to reduced stability, it is important to identify the mechanism of these changes. LUV liposomes as a model were used by Cheetham et al. (1990) to study the interaction between membrane stabilizer (cholesterol) and calcium ions.

A very important role in cell electrophysiology is played by transport of ions such as Na^+, K^+, Ca^{2+} and Cl^-, whereas transport of Ca^{2+} in sperm cells is of crucial importance in capacitation and acrosomal reaction. Ions are transported across cell membranes due to integral proteins that include ion channels. Bai and Shi (2001) used giant liposomes as models to study electrophysiology and activity of Cl- ion channels in human spermatozoa plasma membrane. The authors demonstrated the presence of three types of Cl- ion carriers by means of patch-clamp technique, which allows the study of single ion channels in liposomes. Moreover, Cl- ions were found to be active in different time intervals of the fertilization process.

Liposomes in in vitro fertilization

Liposomes and fertilizing capacity of sperm

In addition to the appropriate concentration of morphologically normal spermatozoa per dose in every species, the fertilizing capacity of spermatozoa is affected by motility, course of capacitation, acrosomal reaction, and penetration of zona pellucida. One of the biotechnology methods associated with male reproduction is sperm cryoconservation. Cryoconserved spermatozoa show a much lower fertilizing capacity compared to spermatozoa in fresh ejaculate. Quite often, spermatozoa in fresh semen are also characterized by lower fertilizing capacity, which has prompted researchers to find possible ways of improvement. Research on fertilizing capacity of sperm has most often used dilauroylphosphatidylcholine (PC12) liposomes. It was demonstrated that incubation of sperm with PC12 liposomes before fertilization had an influence on percentage of spermatozoa which undergo acrosomal reaction in bull, ram and stallion (Graham et al., 1987), whereas addition of liposomes to thawed bovine sperm induced acrosomal reaction and increased fertilizing capacity (Graham and Foote, 1987). PC12 liposomes also induce the acrosomal reaction of stallion sperm stored for 24 hours at 4°C (Padilla et al., 1991). Further research showed that treatment of human sperm with PC12 activates spermatozoa motility (Holden and Trounson, 1992).

Liposomes as carriers of unstable compounds

It is possible to increase the efficiency of experimental methods through compounds delivered by a given liposome (Figure 1 B). Liposomes are used as carriers of hydrophilic or unstable compounds, which are easily degraded without protection. This is also the case with the bioenergy compound ATP (adenosine triphosphate)

that is particularly sensitive to enzymatic hydrolysis, which makes it a very unstable compound in biological fluids. Puisieux et al. (1994) attempted to incorporate ATP encapsulated within MLV liposomes into human spermatozoa. They demonstrated that encapsulated liposome ATP, incubated with spermatozoa, induces *in vitro* capacitation and may be helpful in *in vitro* fertilization procedure. Skiba-Lahiani et al. (1995) indicate that ATP encapsulated in PC12 liposomes has a great potential for changing the structure of spermatozoa membrane necessary for fertilization.

Liposomes in cryoconservation of gametes and embryos

It is known that sensitivity of mammalian gametes and embryos to low temperature considerably reduces their susceptibility to cryoconservation. In order to protect cells against detrimental effects of low temperature and thus to increase the efficiency of cryoconservation, protective agents known as cryoprotectants are used.

Our preliminary experimental work (unpublished data) demonstrated that supplementation of a standard bull semen freezing medium with liposomes improves the medium's performance. Both semen viability after thawing and spermatozoa membrane's integrity have improved. One of possible problems for more extensive application of liposomes may be lack of standardization. The same study demonstrated significant performance differences depending on which batch of tested liposomes was used.

Experiments with phosphatidylcholine (EPC) and dipalmitoylphosphatidylcholine (DPPC) liposomes used to supplement cryoprotectants for chilling bovine spermatozoa and oocytes (Zeron et al., 2002) showed their favourable effect on susceptibility of spermatozoa to low temperature and proportion of embryos undergoing first divisions. The beneficial effect of liposome supplementation was also demonstrated for cryoconserved semen of the boar (He et al., 2001) and stallion (Wilhelm et al., 1996). Meanwhile, experiments with cryoconservation of bovine embryos in medium supplemented with liposomes (Pugh et al., 1998) containing lecithin, sphingomyelin and cholesterol did not produce the expected results. It was only affirmed that liposome supplementation had no negative effect on development of embryos to the blastocyst stage and on their survival after thawing. Recent research (Tamargo-Miguel et al., 2008) suggests that liposomes made from soybean lipids could effectively replace hen egg yolk in a diluent for freezing bovine semen.

Liposomes in transgenesis of animals

The methods which allow introducing genes or synthetic oligonucleotides into cells are divided into physical methods like microinjection or electroporation, and chemical methods such as DEAE-dextran transfection of synthetic carriers (e.g. liposomes) and transfection using viruses. The method that enables exogenous DNA to integrate into spermatozoa with the aid of liposomes is known as sperm lipofection. Spermatozoa with internalized foreign DNA are often used as vectors when introducing a gene or gene fragment into the oocyte during transgenesis. The findings of Kim et al. (2007) indicate that sperm can be transfected during incubation with DNA–liposome complex (Figure 1 C) and this method can be used to transfer exogenous DNA into the oocyte. The experiments with stallion semen (Ball et al.,

2008) showed the possibility of transfecting stallion spermatozoa with exogenous DNA provided by liposomes. Lai et al. (2001) used the ICSI method for fertilization, in which spermatozoa were previously incubated with exogenous DNA–liposome complex (Figure 1 C), but after transplantation of the embryos obtained, none of the recipients was pregnant.

Conclusions

Because of simple structure and the possibility of regulating their properties, liposomes are a flexible tool for research in many fields of science including biophysics, biochemistry, molecular biology and medicine. Research has shown that liposomes can also be effectively used in animal reproduction biotechnology. The application of liposomes as a model of sperm plasma membrane gives better insight into biochemical and structural changes occurring on membrane surface during acrosomal reaction and capacitation, while enabling the molecular mechanisms of transport across cell membranes to be identified. Furthermore, the use of liposomes in *in vitro* fertilization increases the fertilizing capacity of sperm while providing high-energy but biologically unstable compounds such as ATP. It was also demonstrated that liposomes reduce the sensitivity of gametes and embryos to chilling and make them more susceptible to cryoconservation. In addition, the fact that sperm can be transfected using liposomes with exogenous DNA enables their use in animal transgenesis.

The growing interest in liposomes and the discovery of new applications indicate that liposome structures have a high potential to be used in animal reproduction biotechnology in the future.

References

Bai J.P., Shi Y.L. (2001). A patch-clamp study on human sperm Cl^- channel reassembled into giant liposome. Asian J. Androl., 3 (3): 185–191.

Ball B.A., Sabeur K., Allen W.R. (2008). Liposome-mediated uptake of exogenous DNA by equine spermatozoa and applications in sperm-mediated gene transfer. Equine Vet. J., 40 (1): 76–82.

Cheetham J.J., Chen R.J., Epand R.M. (1990). Interaction of calcium and cholesterol sulphate induces membrane destabilization and fusion: implications for the acrosome reaction. Biochim. Biophys. Acta, 1024 (2): 367–372.

Graham J.K., Foote R.H. (1987). Dilauroylphosphatidylcholine liposome effects on the acrosome reaction and *in vitro* penetration of zona-free hamster eggs by bull sperm: II. A fertility assay for frozen-thawed semen. Gamete Res., 16 (2): 147–158.

Graham J.K., Foote R.H., Hough S.R. (1987). Penetration of zona-free hamster eggs by liposome-treated sperm from the bull, ram, stallion and boar. Biol. Reprod., 37 (1): 181–188.

He L., Bailey J.L., Buhr M.M. (2001). Incorporating lipids into boar sperm decreases chilling sensitivity but not capacitation potential. Biol. Reprod., 64 (1): 69–79.

Holden C.A., Trounson A.O. (1992). Effect of dilauroylphosphatidylcholine on human spermatozoa. J. Androl., 13 (3): 260–265.

Kim T.S., Cao Y., Cheong H.T., Yang B.K., Park C.K. (2007). DNA take-up by sperm and *in vitro* embryo development by sperm-mediated gene transfer in the pig. Reprod. Fertil. Dev., 19 (1): 317–318.

Lai L., Sun Q., Wu G., Murphy C.N., Kuhholzer B., Park K.W., Bonk A.J., Day B.N., Prather R.S. (2001). Development of porcine embryos and offspring after intracytoplasmic sperm injection with liposome transfected or non-transfected sperm into *in vitro* matured oocytes. Zygote, 9 (4): 339–346.

Padilla A.W., Tobback C., Foote R.H. (1991). Penetration of frozen-thawed, zona-free hamster oocytes by fresh and slow-cooled stallion spermatozoa. J. Reprod. Fertil., Suppl., 44: 207–212.

Pugh P.A., Ankersmit A.E., McGowan L.T., Tervit H.R. (1998). Cryopreservation of *in vitro*-produced bovine embryos: effects of protein type and concentration during freezing or of liposomes during culture on post-thaw survival. Theriogenology, 50: 495–506.

Puisieux F., Fattal E., Lahiani M., Auger J., Jouannet P., Couvreur P., Delattre J. (1994). Liposomes, an interesting tool to deliver a bioenergetic substrate (ATP), *in vitro* and *in vivo* studies. J. Drug Target., 2 (5): 443–448.

Skiba-Lahiani M., Auger J., Terribile J., Fattal E., Delattre J., Puisieux F., Jouannet P. (1995). Stimulation of movement and acrosome reaction of human spermatozoa by PC12 liposomes encapsulating ATP. Int. J. Androl., 18 (6): 287–294.

Tamargo-Miguel C., Perez-Garnelo S.S., Beltran Brena P., Pałasz A.T., De La Fuente J., Rodriguez A., Hildalgo C.O. (2008). Freezing of semen from endangered Asturiana de la Montana Bulls in zwitteronic lipids-based extenders. Reprod. Fertil. Dev., 20 (1): p. 161.

Wilhelm K.M., Graham J.K., Squires E.L. (1996). Effects of phosphatidylserine and cholesterol liposomes on the viability, motility and acrosomal integrity of stallion spermatozoa prior to and after cryopreservation. Cryobiology, 33 (3): 320–329.

Zeron Y., Tomczak M., Crowe J., Arav A. (2002). The effect of liposomes on thermotropic membrane phase transitions of bovine spermatozoa and oocytes: implications for reducing chilling sensitivity. Cryobiology, 45 (2): 143–152.

Ziętkiewicz E., Słomski R. (1984). Liposomes as carriers in the transfer of substances of biological importance into cell. Post. Biochem., 30: 149–172.

Accepted for printing 24 II 2010

IZABELA GRAD

Liposomy w biotechnologii gamet i zarodków

STRESZCZENIE

Liposomy – niezwykle plastyczne struktury pęcherzykowe – znalazły już w latach 80. XX wieku praktyczne zastosowanie w farmacji i kosmetologii. Obecne badania nad liposomami koncentrują się głównie na możliwości wykorzystania ich w medycynie oraz biotechnologii rozrodu zwierząt. Badania te dotyczą m.in.: użycia liposomów jako modelu błony komórkowej plemników oraz nośników niestabilnych lub łatwo rozpuszczalnych związków, a także ustalenia ich przydatności w kriokonserwacji gamet i zarodków oraz w transgenezie zwierząt. Artykuł omawia możliwości zastosowania liposomów w biotechnologii rozrodu zwierząt.