Artificial microcontainers for cryopreservation of solitary spermatozoa

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1. Purpose

Some cases of male infertility caused by severe oligozoospermia (cryptozoospermia) or teratozoospermia only leave a few spermatozoa suitable for ICSI fertilization. Isolation of functional gametes from testicular or epididymal aspirates requires considerable workload in azoospermia, while multiple surgical invasions may entail negative consequences. These problems can be solved by sperm cryopreservation, but there is a high risk of losing unique genetic material in the course of freezing, storing, and thawing. Placing small groups of spermatozoa in microcontainers eliminates these risks and makes conventional cryopreservation protocols applicable.

Use of the cryopreservation technique utilizing empty *zonae pellucidae* (ZP's) of human or animal oocytes, which was proposed by J. Cohen in 1997, is limited in common clinical practice due to its potential infection and contamination implications. Furthermore, preparing ZP's requires individual microsurgical evacuation of oocyte cytoplasm; spermatozoa can get stuck on the internal ZP surface; while some substances found in ZP's might induce an acrosomal reaction.

2. Methods and Materials

Presented is a technique allowing to obtain an artificial ZP analogue, empty microspheres made of agarose gel and used for cryopreservation of solitary spermatozoa. Such agarose microspheres are inactive and sterile biologically. We used microspheres made of 2% agarose gel and measuring approximately 100 μm in diameter.

[The hollow microspheres made of agarose gel] see: [The hollow microspheres made of agarose gel]

Motile spermatozoa left after ICSI procedures performed in 18 patients with severe oligoteratozoospermia, were placed in described microspheres (1 to 10 in each), in the same manner as in ICSI and using the same microinstruments, in 10% PVP solution.

Microspheres loaded with spermatozoa were then placed in a 1:1 solution, Sperm Preparation Medium (MediCult, 1069/1070) and Sperm Freezing Medium (MediCult, 10670005/10670010), for 5 min. Afterwards, 1 to 5 injected microspheres were put into 250 μL plastic straws, and these straws were frozen in liquid nitrogen evaporation for 10 min and then placed in liquid nitrogen.

The straws were thawed at room temperature. Microspheres were washed in five Sperm Preparation Medium drops and incubated at +37 ^{0}C for an hour. Spermatozoon viability was assessed by motility recovery, as well as in an eosin supravital test. There was no medical fertilization of human oocytes in these sperm studies.

3. Results

In total, 318 motile spermatozoa were frozen in 67 microspheres and 19 straws. Two out of 67 microspheres (3%) containing 7 spermatozoa (2% of the total amount) were lost following thawing. Out of 243 remaining spermatozoa, 311 (78%) recovered motility after incubation. The eosin supravital test demonstrated that 81% of these spermatozoa (251/311) had preserved membrane integrity.
[Human spermatozoon inside of agarose microsphere] see: [Human spermatozoon inside of agarose microsphere]

4. Conclusion

A new technique is presented (The EAPO Pat.No007992) allowing to obtain and utilize sterile and biologically safe empty microspheres for efficient cryopreservation of solitary spermatozoa in IVF-ICSI programmes.
[Eurasian Patent â„–007992 (Russian)] see: [Eurasian Patent â„–007992 (Russian)]

5. References

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6. Mediafiles:

Eurasian Patent №007992 (Russian)

ЕВРАЗИЙСКАЯ ПАТЕНТНАЯ ОРГАНИЗАЦИЯ
ЕВРАЗИЙСКОЕ ПАТЕНТНОЕ ВЕДОМСТВО

ЕВРАЗИЙСКИЙ ПАТЕНТ

№ 007992

Название изобретения:
«СПОСОБ ПОЛУЧЕНИЯ ПОЛЫХ МИКРОСФЕР И ПОЛАЯ МИКРОСФЕРА, ПОЛУЧЕННАЯ ДАННЫМ СПОСОБОМ»

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Настоящим удостоверяется, что евразийский патент выдан на изобретение, изложенное в прилагаемом описании и формуле изобретения.

При уплате установленных годовых пошлин патент действует на территории государств участников Евразийской патентной конвенции – Азербайджанской Республики, Кыргызской Республики, Республики Армения, Республики Беларусь, Республики Казахстан, Республики Молдова, Республики Таджикистан, Российской Федерации, Туркменистана

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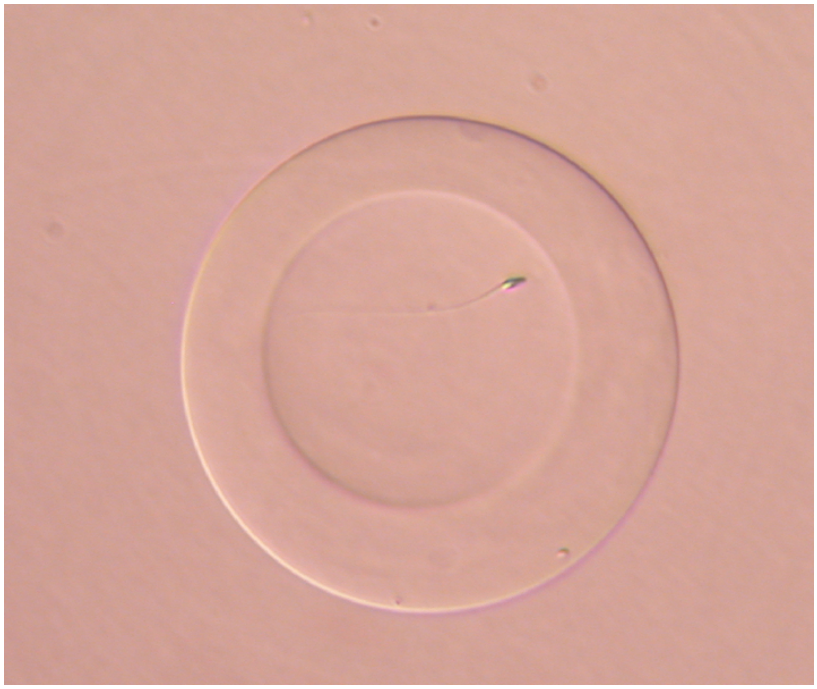
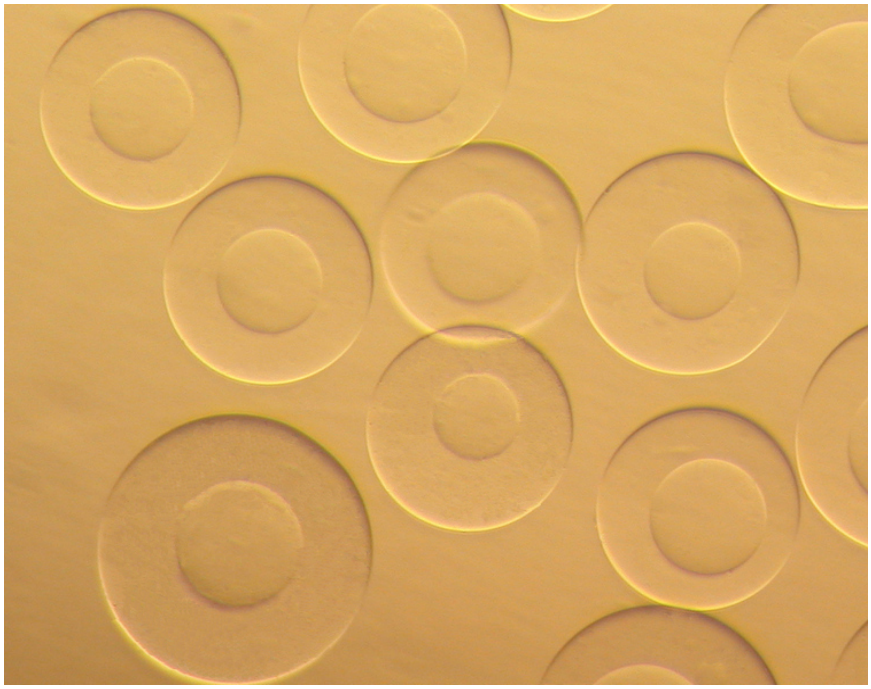
Eurasian Patent №007992 for producing agarose microspheres. Document in Russian.

Human spermatozoon inside of agarose microsphere



Solitary spermatozoa placed into agarose microsphere by similar to ICSI technique and with the same microtools can be frozen by conventional methods. (DIC. 40x).

The hollow microspheres made of agarose gel



These agarose microspheres that can be used as vehicles for single sperm cryopreservation are biologically inert, sterile, prevent sperm losses during manipulation and provide good post-thaw survival rate. (DIC. 40x).