Testicular Sperm Extraction

Subjects: Andrology

Contributor: Kaan Aydos

Retrieving spermatozoa from the testicles has been a great hope for patients with non-obstructive azoospermia (NOA), but relevant methods have not yet been developed to the level necessary to provide resolutions for all cases of NOA. Although performing testicular sperm extraction under microscopic magnification has increased sperm retrieval rates, in vitro selection and processing of quality sperm plays an essential role in the success of in vitro fertilization. Moreover, sperm cryopreservation is widely used in assisted reproductive technologies, whether for therapeutic purposes or for future fertility preservation. In recent years, there have been new developments using advanced technologies to freeze and preserve even very small numbers of sperm for which conventional techniques are inadequate.

Keywords: testicular azoospermia ; non-obstructive azoospermia ; sperm selection ; sperm ; cryopreservation ; in vitro maturation

1. Introduction

To date, although testicular spermatozoa from patients with non-obstructive azoospermia (NOA) have been used widely for intracytoplasmic sperm injection (ICSI), this method's effectiveness still has potential for further improvement. NOA is characterized by the absence of any spermatozoa, whether dead or alive, in the ejaculate due to reduced or nonexistent sperm production in the testicle [1]. Testicular fine needle aspiration (FNA or testicular sperm aspiration—TESA) is an effective and non-invasive method used to obtain sperm, especially from patients with obstructive azoospermia [2]. Although its simpler and less traumatic features have made FNA the preferred method, testicular sperm extraction (TESE) is the treatment of choice for patients with NOA, with a satisfactory number of successful spermatozoa retrieved in approximately half of the patients. In the conventional TESE procedure, the testis is exposed through a small incision in the tunica albuginea, and multiple biopsies are taken randomly [3]. However, microTESE carried out at high magnification under an operating microscope allows visualization of whitish, larger, and more opaque seminiferous tubules likely to contain mature germ cells [4]. Although not randomized, most studies have reported that the sperm recovery rate from microTESE is superior to that from conventional TESE [5][6][7]. In fact, a recent controlled, randomized study verified the efficacy of microTESE compared with that of TESE in retrieving spermatozoa from patients with NOA [8]. In addition to the surgical technique, however, in vitro extraction of sperm from surgically excised testicular tissue or tubules is also important for obtaining spermatozoa of sufficient quality and quantity for use in ICSI.

2. Processing Immature Germ Cells

Despite extensive searching, mature spermatozoa can be obtained with TESE in only approximately 40% to 60% of cases of NOA for use in ICSI [9][10]. In the remaining cases, round spermatids were attempted as a last resort, although until recently, the results were not satisfactory enough to encourage routine practice [11]. However, in 2015, Tanaka et al. reported 14 healthy babies born with round spermatid injection (ROSI) in oocytes previously activated by electric current; 3 years later, from the 2-year follow-up results of 90 ROSI babies, it was determined that round spermatids enabled patients with NOA to have their own genetic offspring [12][13]. Subsequently, Papuccu et al. reported their results of 472 couples who underwent 904 cycles using elongating (Sb2) spermatids for the ROSI technique and achieved a 9.6% ongoing pregnancy rate [14]. Since transformation of immature germ cells into spermatozoa with fully developed flagella has had limited success in in vitro experiments, culturing samples to achieve at least haploid round spermatids may have wider clinical application [15].

If testicular tissue samples from TESE do not contain spermatozoa, various approaches have been described for in vitro maturation of early stage germ cells. For in vitro maturation, either (1) testicular tissue is used in whole pieces while preserving its three-dimensional (3D) structure or (2) after isolation and purification, different cell types are exposed to culture conditions in which the spermatogenic process is recreated. In TESE-negative cases, it makes sense to try culturing small fragments of testicular tissue or intact pieces of tubules first. However, since the limited diffusion rates of

the tissue do not allow tissue viability to be maintained over a long period, it is challenging to culture the tissue as a whole. After long-standing efforts, in 2011, Sato et al. reported healthy offspring from haploid cells developed from testicular fragments cultured on agarose gel in modified Minimum Essential Medium (α-MEM) supplemented with knockout serum replacement [16]. Others have also shown culturing of frozen/thawed testicular tissue on agarose gel to restore spermatogenesis up to haploid spermatids, leading to offspring [17]. Furthermore, from seminiferous tubule segments cultured in chitosan hydrogel bioreactors, the development of spermatids and spermatozoa were achieved on days 34 and 55, respectively [18]. In another organotypic culture system described for human immature testicular tissue, germ cells were shown to differentiate up to round spermatids within 16 days [19]. However, the inability of organ culture systems to restore spermatogenesis in cryopreserved human testicular specimens has also been reported [20]. These contradictions in results may be due to the fact that culture methods are not yet fully optimized or that the nature of subcellular defects is different [21]. Later, developed microfluidic technology further improved organotypic culture systems, allowing ex vivo sustainability of the structure and viability of germ cells in testicular tissue for producing mature sperm [22]. As an option for in vitro maturation of isolated germ cells, 2D culture systems have been developed to support enzymatically digested testicular cell suspensions. In 2D cultures, isolated SSCs are maintained either on feeder cells or on mixed cell populations co-cultured with somatic cells [23]. Different feeders, including SIM mouse embryo-derived thioguanine and ouabain resistant (STO), mouse embryonic fibroblast, bovine Sertoli cells and laminin-coated plate were used to support spermatogenesis [24]. Apart from feeders, different culture systems, such as human amnion mesenchymal stem cells [25], in vitro reprogramming of fibroblasts to human induced Sertoli-like cells [26], and isolated cell culture with growth factor supplementation [27], have been defined to support in vitro spermatogenesis. Nevertheless, 2D culture systems provided in vitro restoration of spermatogenesis and supported the development of haploid spermatids with fertilization potential [28]. Experiences gained from previous studies ultimately led to the development of artificially constructed 3D structures [29]. By creating structures that mimic the composition of the main testicular components, 3D cultures allow immature germ cells to be reconstructed similarly to their original tissue architecture, thereby allowing further maturation [30][31][32][33]. Although this system has the ability to direct the differentiation of germ cells, microenvironmental conditions favorable for complete maturation must also be achieved.

In addition to attempting complex and intricate methods for in vitro maturation of immature germ cells from testicular tissue, there is a need for simple methods that can be used more easily in clinical practice. When Aslam et al. compared suspensions of mixed cell populations and isolated homogeneous populations of spermatogenic cells prepared from testicular tissue, they showed that most of the isolated round spermatids developed tails and remained intact and viable for 72 h in modified Eagle's minimum essential medium with no hormonal supplementation [34]. However, since they used a mixture of obstructive and non-obstructive tissue samples, the contribution of in vitro culturing to the development of flagella in immature germ cells in cases of NOA is not clear. Similarly, it has been shown that human round spermatids can mature up to spermatozoa when cocultured on Vero cell monolayers [35]. Other researchers have also verified the maturation of primary spermatocytes into haploid spermatids through in vitro coculture with Vero cells [36]. Subsequently, round spermatids generated from human SSCs were shown to fertilize mouse oocytes [27]. However, even without a co-culture, in vitro hormonal supplementation has been demonstrated to be capable of providing sufficient support to mature premeiotic germ cells [37]. Thus, culturing testicular samples from patients with NOA in medium containing recombinant FSH and testosterone for 48 h transformed FISH-proven primary spermatocytes into mature round spermatids, after which injection into the oocyte resulted in healthy offspring [38]. It has been shown that hormones added to in vitro culture medium in cases of NOA not only accelerate spermiogenesis but also improve apoptosis-related cell damage in enhancing the reproductive performance of germ cells [39]. Contrary to most studies indicating that FSH and testosterone added to testicular culture media play a role in the development of different stages of in vitro spermatogenesis, it has also been suggested that their supplementation does not induce meiotic and post-meiotic cells and therefore cannot differentiate premeiotic germ cells [40]. Differences in germ cell development in in vitro maturation studies may be due to insufficient support of established culture conditions. The maintenance of healthy spermatogenesis from SSCs can only be achieved with the support of a complicated and precise "niche" microenvironment [41]. Sertoli cells establish the most important component of the "niche," and by producing growth factors and cytokines, they regulate proper self-renewing and differentiation of SSCs, transition to meiosis, and, finally, differentiation of round spermatids into spermatozoa [42].

Under proper culture conditions, reaggregation of Sertoli cells forms organized monolayer structures. Therefore, in most of the in vitro maturation studies performed on testicular tissue samples, a co-culture with Sertoli cells has been used effectively to provide structural and nutritional support for differentiation of germ cells [43][44][45]. In the co-culture of round spermatids and Sertoli cells, it has been shown that supplementation with recombinant FSH and testosterone contributes significantly to the differentiation of round spermatids into elongating spermatids [46]. However, other studies have also reported that no matter how much FSH stimulation in organotypic cultures of immature testicular tissue increases the percentage of premeiotic cells, it does not allow for further maturation [21]. Some similar studies have also confirmed that

FSH supplementation in cultures does not support post-meiotic maturation [47]. Actually, the underlying mechanism for the contradictions in the reports may be the impaired ability of testes to respond to the endogenous hormonal milieu due to compromised androgen receptor and FSH receptor (FSHR) signaling pathways [48]. In fact, when compared to obstructive azoospermia, the FSHR expression level in isolated and purified Sertoli cell cultures was found to be 2.7 times lower in the NOA group; hence, it has been claimed that there may be an altered Sertoli cell response to in vitro FSH stimulation [49]. Alternatively, without the need for hormone supplementation, spermatogenesis from testicular SSCs to fertility-competent sperm formation could be induced in organ cultures using different techniques [50][51]. Considering this fact, before choosing a method to be used in co-culture studies with Sertoli cells, it is important to investigate the hormone/receptor interaction along with the response to FSH and testosterone.

To date, a number of studies have been conducted on the development of many different culture systems, with varying levels of success. Furthermore, with their innovations in in vitro germ cell maturation, advanced technology products created in the field of regenerative medicine using cell/tissue culture, biomaterials, and bioactive products have become promising treatment alternatives for patients with NOA [52][53]. However, before suggesting potential clinical uses of haploid male gametes exposed to in vitro manipulations, further analyses of fecundity, epigenetic consequences, and safety are essential.

3. Conclusions

The primary outcome associated with the efficiency of ARTs is successful, healthy live births. In addition to allowing only a small amount of sperm retrieval, cases of NOA require a more demanding process in the treatment of infertility due to the fact that the available sperm are also products of impaired spermatogenesis. The whole process begins with collection of the highest-quality surgical specimens possible. Following microTESE, with its verified efficacy, researchers have attempted sophisticated techniques such as Raman spectroscopy, multiphoton microscopy, and full field optical coherence tomography to identify testicular tubules with spermatogenesis. As further developments, laser-assisted sperm selection and microfluidic systems appear to be promising for extracting viable spermatozoa from surgically removed testicular samples. Moreover, there is an ongoing effort to develop optimized freezing protocols and effective technologies that will allow patients with NOA to retain their fertility by implementing cryopreservation of even a single sperm. However, the initial promising results of all of these developments must be confirmed by large studies in the context of clinical practice. The use of nanoparticles for in vitro maturation of germ cells is also another promising innovation, as it will allow previously unsuccessful patients with NOA to have children using their own biological material. Undoubtedly, the clinical consequences of all of these manipulations that could result from potential changes in the offspring's genomes must be followed very carefully.

References

1. Deruyver, Y.; Vanderschueren, D.; Van der Aa, F. Outcome of microdissection TESE compared with conventional TESE in non-obstructive azoospermia: A systematic review. Andrology 2014, 2, 20–24.
2. Meng, M.V.; Cha, I.; Ljung, B.M.; Turek, P.J. Testicular fine-needle aspiration in infertile men: Correlation of cytologic pa ttern with biopsy histology. Am. J. Surg. Pathol. 2001, 25, 71–79.
3. Ubaldi, F.; Nagy, Z.; Liu, J.; Tournaye, H.; Camus, M.; Smitz, J.; Liebaers, I.; Devroey, P.; Van Steirteghem, A. A survey of four years of experience with intracytoplasmic sperm injection. Acta Eur. Fertil. 1995, 26, 7–11.
4. Schlegel, P.N. Testicular sperm extraction: Microdissection improves sperm yield with minimal tissue excision. Hum. Re prod. 1999, 14, 131–135.
5. Amer, M.; Ateyah, A.; Hany, R.; Zohdy, W. Prospective comparative study between microsurgical and conventional testi cular sperm extraction in non-obstructive azoospermia: Follow-up by serial ultrasound examinations. Hum. Reprod. 200 0, 15, 653–656.
6. Okada, H.; Dobashi, M.; Yamazaki, T.; Hara, I.; Fujisawa, M.; Arakawa, S.; Kamidono, S. Conventional versus microdis section testicular sperm extraction for nonobstructive azoospermia. J. Urol. 2002, 168, 1063–1067.
7. Westlander, G. Utility of micro-TESE in the most severe cases of non-obstructive azoospermia. Ups J. Med. Sci. 2020, 125, 99–103.
8. Colpi, G.M.; Colpi, E.M.; Piediferro, G.; Giacchetta, D.; Gazzano, G.; Castiglioni, F.M.; Magli, M.C.; Gianaroli, L. Micros urgical TESE versus conventional TESE for ICSI in non-obstructive azoospermia: A randomized controlled study. Repro d. Biomed. Online 2009, 18, 315–319.

9. Schlegel, P.N. Nonobstructive azoospermia: A revolutionary surgical approach and results. Semin. Reprod. Med. 2009, 27, 165–170.
10. Mehmood, S.; Aldaweesh, S.; Junejo, N.N.; Altaweel, W.M.; Kattan, S.A.; Alhathal, N. Microdissection testicular sperm extraction: Overall results and impact of preoperative testosterone level on sperm retrieval rate in patients with nonobst ructive azoospermia. Urol. Ann. 2019, 11, 287–293.
11. Gross, K.X.; Hanson, B.M.; Hotaling, J.M. Round Spermatid Injection. Urol. Clin. N. Am. 2020, 47, 175–183.
12. Tanaka, A.; Nagayoshi, M.; Takemoto, Y.; Tanaka, I.; Kusunoki, H.; Watanabe, S.; Kuroda, K.; Takeda, S.; Ito, M.; Yana gimachi, R. Fourteen babies born after round spermatid injection into human oocytes. Proc. Natl. Acad. Sci. USA 2015, 112, 14629–14634.
13. Tanaka, A.; Suzuki, K.; Nagayoshi, M.; Takemoto, Y.; Watanabe, S.; Takeda, S.; Irahara, M.; Kuji, N.; Yamagata, Z.; Yan agimachi, R. Ninety babies born after round spermatid injection into oocytes: Survey of their development from fertilizati on to 2 years of age. Fertil. Steril. 2018, 110, 443–451.
14. Pabuccu, R.; Sertyel, S.; Pabuccu, E.; Aydos, K.; Haliloglu, A.H.; Demirkıran, D.; Keles, G.; Tanaka, A. Live Births with a Novel ROSI Technique Using Elongating Spermatids for Non Obstructive Azoospermia Patients: A European Cohort Study. In Proceedings of the ESHRE 36th Annual Meeting, Copenhagen, Denmark, 5–8 July 2020; p. O-125.
15. Sousa, M.; Cremades, N.; Alves, C.; Silva, J.; Barros, A. Developmental potential of human spermatogenic cells co-cult ured with Sertoli cells. Hum. Reprod. 2002, 17, 161–172.
16. Sato, T.; Katagiri, K.; Gohbara, A.; Inoue, K.; Ogonuki, N.; Ogura, A.; Kubota, Y.; Ogawa, T. In vitro production of functio nal sperm in cultured neonatal mouse testes. Nature 2011, 471, 504–507.
17. Yokonishi, T.; Sato, T.; Komeya, M.; Katagiri, K.; Kubota, Y.; Nakabayashi, K.; Hata, K.; Inoue, K.; Ogonuki, N.; Ogura, A.; et al. Offspring production with sperm grown in vitro from cryopreserved testis tissues. Nat. Commun 2014, 5, 4320.
18. Perrard, M.H.; Sereni, N.; Schluth-Bolard, C.; Blondet, A.; D Estaing, S.G.; Plotton, I.; Morel-Journel, N.; Lejeune, H.; D avid, L.; Durand, P. Complete Human and Rat Ex Vivo Spermatogenesis from Fresh or Frozen Testicular Tissue. Biol. Reprod. 2016, 95, 89.
19. de Michele, F.; Poels, J.; Vermeulen, M.; Ambroise, J.; Gruson, D.; Guiot, Y.; Wyns, C. Haploid Germ Cells Generated i n Organotypic Culture of Testicular Tissue from Prepubertal Boys. Front. Physiol 2018, 9, 1413.
20. Portela, J.M.D.; de Winter-Korver, C.M.; van Daalen, S.K.M.; Meißner, A.; de Melker, A.A.; Repping, S.; van Pelt, A.M. M. Assessment of fresh and cryopreserved testicular tissues from (pre)pubertal boys during organ culture as a strategy for in vitro spermatogenesis. Hum. Reprod. 2019, 34, 2443–2455.
21. Medrano, J.V.; Vilanova-Pérez, T.; Fornés-Ferrer, V.; Navarro-Gomezlechon, A.; Martínez-Triguero, M.L.; García, S.; G ómez-Chacón, J.; Povo, I.; Pellicer, A.; Andrés, M.M.; et al. Influence of temperature, serum, and gonadotropin supplem entation in short- and long-term organotypic culture of human immature testicular tissue. Fertil. Steril. 2018, 110, 1045–1057.e1043.
22. Komeya, M.; Hayashi, K.; Nakamura, H.; Yamanaka, H.; Sanjo, H.; Kojima, K.; Sato, T.; Yao, M.; Kimura, H.; Fujii, T.; et al. Pumpless microfluidic system driven by hydrostatic pressure induces and maintains mouse spermatogenesis in vitr o. Sci. Rep. 2017, 7, 15459.
23. Ibtisham, F.; Honaramooz, A. Spermatogonial Stem Cells for In Vitro Spermatogenesis and In Vivo Restoration of Fertili ty. Cells 2020, 9, 745.
24. Nasiri, Z.; Hosseini, S.M.; Hajian, M.; Abedi, P.; Bahadorani, M.; Baharvand, H.; Nasr-Esfahani, M.H. Effects of different feeder layers on short-term culture of prepubertal bovine testicular germ cells in-vitro. Theriogenology 2012, 77, 1519–1528.
25. Qian, C.; Meng, Q.; Lu, J.; Zhang, L.; Li, H.; Huang, B. Human amnion mesenchymal stem cells restore spermatogene sis in mice with busulfan-induced testis toxicity by inhibiting apoptosis and oxidative stress. Stem Cell Res. Ther. 2020, 11, 290.
26. Liang, J.; Wang, N.; He, J.; Du, J.; Guo, Y.; Li, L.; Wu, W.; Yao, C.; Li, Z.; Kee, K. Induction of Sertoli-like cells from hum an fibroblasts by NR5A1 and GATA4. Elife 2019, 8.
27. Yang, S.; Ping, P.; Ma, M.; Li, P.; Tian, R.; Yang, H.; Liu, Y.; Gong, Y.; Zhang, Z.; Li, Z.; et al. Generation of haploid sper matids with fertilization and development capacity from human spermatogonial stem cells of cryptorchid patients. Stem Cell Rep. 2014, 3, 663–675.
28. Marh, J.; Tres, L.L.; Yamazaki, Y.; Yanagimachi, R.; Kierszenbaum, A.L. Mouse round spermatids developed in vitro fro m preexisting spermatocytes can produce normal offspring by nuclear injection into in vivo-developed mature oocytes. Biol. Reprod. 2003, 69, 169–176.

29. Lee, J.H.; Kim, H.J.; Kim, H.; Lee, S.J.; Gye, M.C. In vitro spermatogenesis by three-dimensional culture of rat testicular cells in collagen gel matrix. Biomaterials 2006, 27, 2845–2853.

30. Abu Elhija, M.; Lunenfeld, E.; Schlatt, S.; Huleihel, M. Differentiation of murine male germ cells to spermatozoa in a soft agar culture system. Asian J. Androl. 2012, 14, 285–293.

31. Lee, J.H.; Gye, M.C.; Choi, K.W.; Hong, J.Y.; Lee, Y.B.; Park, D.W.; Lee, S.J.; Min, C.K. In vitro differentiation of germ cells from nonobstructive azoospermic patients using three-dimensional culture in a collagen gel matrix. Fertil. Steril. 2007, 87, 824–833.

32. Gholami, K.; Pourmand, G.; Koruji, M.; Sadighigilani, M.; Navid, S.; Izadyar, F.; Abbasi, M. Efficiency of colony formation and differentiation of human spermatogenic cells in two different culture systems. Reprod. Biol. 2018, 18, 397–403.

33. Yi, H.; Xiao, S.; Zhang, Y. Stage-specific approaches promote in vitro induction for spermatogenesis in vitro. Cell. Dev. Biol. Anim. 2018, 54, 217–230.

34. Aslam, I.; Fishel, S. Short-term in-vitro culture and cryopreservation of spermatogenic cells used for human in-vitro conception. Hum. Reprod. 1998, 13, 634–638.

35. Cremades, N.; Bernabeu, R.; Barros, A.; Sousa, M. In-vitro maturation of round spermatids using co-culture on Vero cells. Hum. Reprod. 1999, 14, 1287–1293.

36. Tanaka, A.; Nagayoshi, M.; Awata, S.; Mawatari, Y.; Tanaka, I.; Kusunoki, H. Completion of meiosis in human primary spermatocytes through in vitro coculture with Vero cells. Fertil. Steril. 2003, 79 (Suppl. 1), 795–801.

37. Movahedin, M.; Ajeen, A.; Ghorbanzadeh, N.; Tiraihi, T.; Valojerdi, M.R.; Kazemnejad, A. In vitro maturation of fresh and frozen-thawed mouse round spermatids. Andrologia 2004, 36, 269–276.

38. Tesarik, J.; Bahceci, M.; Ozcan, C.; Greco, E.; Mendoza, C. Restoration of fertility by in-vitro spermatogenesis. Lancet 1999, 353, 555–556.

39. Tesarik, J.; Greco, E.; Mendoza, C. Assisted reproduction with in-vitro-cultured testicular spermatozoa in cases of severe germ cell apoptosis: A pilot study. Hum. Reprod. 2001, 16, 2640–2645.

40. Solomon, R.; AbuMadighem, A.; Kapelushnik, J.; Amano, B.C.; Lunenfeld, E.; Huleihel, M. Involvement of Cytokines and Hormones in the Development of Spermatogenesis In Vitro from Spermatogonial Cells of Cyclophosphamide-Treated Immature Mice. Int. J. Mol. Sci. 2021, 22, 1672.

41. Liu, S.; Tang, Z.; Xiong, T.; Tang, W. Isolation and characterization of human spermatogonial stem cells. Reprod. Biol. Endocrinol. 2011, 9, 141.

42. Mohammadzadeh, E.; Mirzapour, T.; Nowroozi, M.R.; Nazarian, H.; Piryaei, A.; Alipour, F.; Modarres Mousavi, S.M.; Ghaffari Novin, M. Differentiation of spermatogonial stem cells by soft agar three-dimensional culture system. Artif. Cells Nanomed. Biotechnol. 2019, 47, 1772–1781.

43. Pramod, R.K.; Mitra, A. In vitro culture and characterization of spermatogonial stem cells on Sertoli cell feeder layer in goat (Capra hircus). J. Assist. Reprod. Genet. 2014, 31, 993–1001.

44. Nowroozi, M.R.; Ahmadi, H.; Rafiian, S.; Mirzapour, T.; Movahedin, M. In vitro colonization of human spermatogonia stem cells: Effect of patient's clinical characteristics and testicular histologic findings. Urology 2011, 78, 1075–1081.

45. Jabari, A.; Sadighi Gilani, M.A.; Koruji, M.; Gholami, K.; Mohsenzadeh, M.; Rastegar, T.; Khadivi, F.; Ghanami Gashti, N.; Nikmahzar, A.; Mojaverrostami, S.; et al. Three-dimensional co-culture of human spermatogonial stem cells with Sertoli cells in soft agar culture system supplemented by growth factors and Laminin. Acta Histochem. 2020, 122, 151572.

46. Hasegawa, H.; Terada, Y.; Ugajin, T.; Yaegashi, N.; Sato, K. A novel culture system for mouse spermatid maturation which produces elongating spermatids capable of inducing calcium oscillation during fertilization and embryonic development. J. Assist. Reprod. Genet. 2010, 27, 565–570.

47. Roulet, V.; Denis, H.; Staub, C.; Le Tortorec, A.; Delaleu, B.; Satie, A.P.; Patard, J.J.; Jégou, B.; Dejucq-Rainsford, N. Human testis in organotypic culture: Application for basic or clinical research. Hum. Reprod. 2006, 21, 1564–1575.

48. Majumdar, S.S.; Sarda, K.; Bhattacharya, I.; Plant, T.M. Insufficient androgen and FSH signaling may be responsible for the azoospermia of the infantile primate testes despite exposure to an adult-like hormonal milieu. Hum. Reprod. 2012, 27, 2515–2525.

49. Heidargholizadeh, S.; Aydos, S.E.; Yukselten, Y.; Ozkavukcu, S.; Sunguroglu, A.; Aydos, K. A differential cytokine expression profile before and after rFSH treatment in Sertoli cell cultures of men with nonobstructive azoospermia. Andrologia 2017, 49, e12647.

50. Komeya, M.; Kimura, H.; Nakamura, H.; Yokonishi, T.; Sato, T.; Kojima, K.; Hayashi, K.; Katagiri, K.; Yamanaka, H.; Sanjo, H.; et al. Long-term ex vivo maintenance of testis tissues producing fertile sperm in a microfluidic device. Sci. Rep. 2016, 6, 21472.

51. Sato, T.; Katagiri, K.; Kojima, K.; Komeya, M.; Yao, M.; Ogawa, T. In Vitro Spermatogenesis in Explanted Adult Mouse T estis Tissues. PLoS ONE 2015, 10, e0130171.
52. Reuter, K.; Schlatt, S.; Ehmcke, J.; Wistuba, J. Fact or fiction: In vitro spermatogenesis. Spermatogenesis 2012, 2, 245 –252.
53. Galdon, G.; Atala, A.; Sadri-Ardekani, H. In Vitro Spermatogenesis: How Far from Clinical Application? Curr. Urol. Rep. 2016, 17, 49.