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REVIEW ARTICLE

# A Concise Review of Spermatogenic Cells Culture: Assessing the Apoptotic Pathway

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## Abstract

In the absence of sperm retrieved from azoospermic male, thus considered in utilizing developed spermatogenic cells including spermatogonia to spermatid in restoring male fertility. Spermatogenic cells obtained from testicular tissue culture need to be expanded prior its utilization, yet the excessive apoptosis of resulting cell culture is a challenge. This review was aimed to discuss article dealing with the factors and the underlying mechanism inducing apoptosis of spermatogenic cells culture. A computerized search of PubMed and google scholar database was conducted for general terms such as "male infertility", "azoospermia", "human spermatogenesis, "spermatogenic cells" and "apoptosis" and followed by screening and evaluation of collected paper accordingly. Various agents induced the apoptosis in spermatogenic cells such as high temperature, hypothermic storage, metabolite of cell culture derived and gonadotropin suppression driving to apoptosis. Thus all of the agents were paving the specific pathways of inducing apoptosis, through extrinsic and intrinsic, leading to the activation of caspase initiators followed by the activation of caspase executor in various types of spermatogenic cells.

**Keywords:** *Human spermatogenic cells, Apoptosis, Culture.*

## Background

Defined as an absence of sperm retrieval through epididymis or directly through the testis, azoospermia is a major problem of male infertility. Biopsy generally performed in achieving the sperm, once it could not be obtained, thus it is considered to use the existing spermatogenic cells, of which spermatogonia, spermatocytes or spermatid. The constructed biology therapy using spermatogenic cells is a promising approach in restoring fertility. Spermatogenic cells were the potential candidate of restoring male fertility. Of which by conducting spermatogonial stem cells (SSCs) culture in order to expand in achieving appropriate amount prior the utilization.

SSCs could be propagated in vitro and programmed to differentiate forming functional sperm. The established culture system should support the long term cell stability, comprising the culture environment, the supplementation of serum or growth factors. Throughout the culture the existing obstacle is hampering the successful of the propagation of spermatogenic cells in vitro. Over the challenge is the exaggerated apoptosis induced by the environment of the culture. Apoptosis is the integral part of spermatogenesis to reduce the excessive of the developing spermatogenic cells in vivo. Nevertheless the rate of apoptosis is tending to increase in the culture in vitro. An assessment of the number of cells undergoing apoptosis could be measured and worthwhile as a parameter determining the optimal. This review was aimed to discuss article dealing with the affecting factors and the underlying mechanism inducing apoptosis of spermatogenic cells culture.

## Methodology

A computerized search of PubMed and Google scholar database was conducted for general terms such as "male infertility", "azoospermia", "human spermatogenesis, "human spermatogenic cells", "spermatogonial stem cells" and "apoptosis". Followed by screening and evaluation of

included criteria, the collected paper was analyzed accordingly.

## Result & Discussion

In adults, apoptosis plays an important role in the stage of spermatogenesis by preventing excessive production of germ cells from seminiferous tubules. Testicular homeostasis is maintained by the prosurvival system and the proapoptotic system by working together to regulate the apoptotic level of spermatogenic cells in an effort to produce quality spermatozoa [1, 3].The maturation process in spermatogenesis, spermatogenic cell apoptosis is used to selectively maintain the number and type of each spermatogenic cell. Mature sperm is a product of the developmental sequence of the results of carefully controlled proliferation, differentiation, self-renewal and apoptosis [4].

**Table 1: Factors inducing apoptosis of spermatogenic cells**

| Factors | Mechanism | Reference |
|---|---|---|
| High temperature (37C) | Accelerating tubular morphology loss and intratubular cell death in vitro | Medrano 2018 |
| Hypothermic storage | Inducing stress factor leading to transmembran ionic imbalances and intracellular acidosis | Faes, 2016 |
| Gonadotropin suppression | Reducing number of Spermatogonia type B through apoptosis via intrinsic pathway | Saleela, 2008 |
| Metabolite cells culture | lactate, alanine, and ammonia attribute to the growth reduction and cell death | Cotter, 1995 |

Apoptosis is a strictly regulated process which initially attempt by the presence of a signal death including physiological (hormones and cytokines), biological (viruses, bacteria, parasites), chemical (drugs), or physical (radiation and toxins) [5, 6].Followed by the integration or regulation stage consist of the signal transduction and the activation of related apoptotic genes. Subsequently the implementation of apoptosis is observed by morphological change, DNA degradation, cell disassembly, and apoptotic body formation [6]. The execution phase is marked by an increase of catabolic enzymes and the production of reactive oxygen species (ROS) which will simultaneously reduce mitochondrial permeability. The final stage is phagocytosis or elimination by macrophages, dendritic or cells adjacent to apoptotic cells. The microscopically of apoptotic cells such as cells shrink and become larger, cytoplasm appears denser, membran blebbing, condensed chromatin and solid fragmentation in the core membrane, chromatin clustered in peripheral parts, DNA fragmentation and resulting to the fragment of apoptotic cells [7].

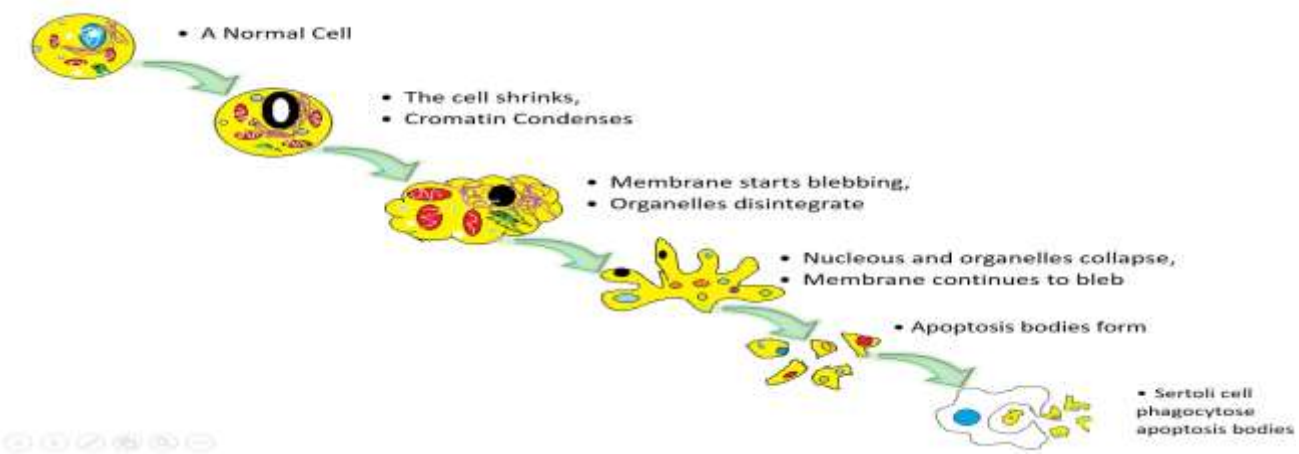


**Figure 1: Cell processes undergo apoptosis**

Two underlying mechanisms of spermatogenic cells apoptosis are the intrinsic and extrinsic pathways [7]. Paving the extrinsic pathway is begin in stimulation of death receptors from Fas, apol which member of tumor necrosis factor family, nerve growth factor receptor and TNF receptor-related apoptosis inducing ligand

 

with each specific ligand. The specific binding of the ligand to its receptor induce the recruitment of Fas Activated Death Domain (FADD), followed by the recruitment and the activation of ProPase 8 and leading to the activation of executor Caspase 3 [7, 10].The intrinsic pathway contains translocation of bax in to the mitochondria result in the release of cytochrome C to the cytosol [11].Cytochrome C binds to Apaf-1 forming a complex as apoptosome complex along with pro-caspase 9[12, 13].

Apoptosome complex will activate the executor caspase, 3, 6, and 7 which will degrade the intracellular protein and affect the apoptosis of the cell [14].Inappropriate apoptosis due to the altered gene expression or environment stimuli contribute to the various testicular pathologic conditions of infertile male [15]. Meanwhile the regulated of apoptosis, sustained by the pro-survival system and the pro-apoptotic system,[1] is essential for maintaining the equilibrium of testicular homeostasis in an effort to produce the functional sperm throughout life [1, 16].

The germ cell apoptosis in testis occurs when the excessive of damaged germ cells presence. FasR/FasL system will be activated inducing the apoptosis through extrinsic pathway [17].FasL is expressed by Sertoli cells which having a role in nurturing and controlling the germ cell production [17, 19].During establishing the spermatogenic cells culture, there are many obstacles heading, such as the inappropriate temperature, gonadotropin reduction and the metabolite secreted by the culture cell [20].Levels of apoptosis are quantified by staining and visualizing cells or tissues for TUNEL, annexin-V, nuclear condensation and activated caspases.

The conducted study by Medrano 2018 presented the high temperature contributed to the acceleration of tubular morphology loss and intratubular cell death in vitro. Previous study (Van Demark and Free, (1970) [21] stated the proper spermatogenesis require the less temperature 2-3C than body temperature [21]. Meanwhile the study by Faes 2016 demonstrated the effect of hypothermic storage towards the spermatogenic cells affecting the inducement of of stress factor, trans-membrane ionic imbalances and intra cellular acidosis [22].Study by Saleela using hormonal contraceptive regiment figured out the decrease significant number of spermatogonia type B, indicating an increase of apoptosis via the intrinsic pathway. Gonadotropin regulates spermatogonia type B survival, not the proliferation [23].

The metabolite secreted by the resulting cell culture such as lactate, alanine, and ammonia influence the cell behavior driving to the growth reduction and cell death through apoptosis [24].The problem with high density culture production is the enhanced environmental perturbation, thus stressing cells due to nutrient and oxygen transport limitation, accumulation of metabolic by-products[24] and elevated osmolarity [25, 26].

To best of our knowledge, the inappropriate culture technique, insufficient the proper medium and supplementation used in culture were contributed to cell culture loss. The limitation of the study was there were a few of conducting study in investigating the particular factors inducing apoptosis in spermatogenic cells therefore the limit agents attributed to germ cell apoptosis could be reported.

## Conclusion

Various agents induced the apoptosis in spermatogenic cells culture such as high temperature, hypothermic storage and gonadotropin suppression driving to apoptosis. Thus all of the agents were paving the specific pathways of inducing apoptosis, through extrinsic and intrinsic, leading to the activation of caspase initiators followed by the activation of caspase executor in various types of spermatogenic cells.

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