Project Summary
Intracellular communications **rely on the support of** cell cytoskeleton**s** and cellular junctions. Spermatogenesis is **constituted by a series of cellular events which also depends on precise** communication**s** between the nursing cells**, i.e.** Sertoli cells and **the developing** germ cells **in the seminiferous epithelium. In short, the Sertoli cells support germ cell development at a Sertoli:germ cell ratio of ~1:30-1:50 to maintain the daily production of germ cells in adult animals and humans. This is made possible through intriguing and precise coordination between Sertoli and germ cells via cell junctions (e.g.,** communicat**ing** gap junctions**). Since the entire events of post-meiotic spermatid development takes place behind the** blood-testis barrier (BTB), **and the observation that meiosis initiates following the establishment of a functional BTB, the BTB** integrity and its maintenance are important **contributing** factor**s to spermatogenesis**. Blood-testis barrier, which is an important structure between adjacent Sertoli cells near the basement membrane and it contains **a testis-specific anchoring junction called basal** ectoplasmic specialization **(basal ES)**. **In short, basal ES, t**ight junctions, **gap junctions together with the supporting** actin filament bundles generate a**n** important **ultrastructure known as the BTB to support germ cell development.** Actin is the most abundant protein in the cytoplasm of mammalian cells, accounting for 10–20% of the total cytoplasmic protein content. Actin exists either as a globular monomer (G-actin), the globular monomers polymerize into polar two-stranded helical polymers, called filamentous actin (F-actin). During spermatogenesis, for the **transport** of **preleptotene** spermatocytes across the BTB to continue **their** development, junctions between Sertoli cells have **to undergo continuous restructuring of the actin-based cytoskeletons to support cell junction** “deadhesion” and readhesion” which is regulated by cargo vesic**le** dynamics in Sertoli cells. During spermatogenesis, **while** the morphological details are known that germ cell **transport** is **supported** by actin-based cell cytoskeleton, the mechanism **that supports** reorganization in the cell cytoskeleton is not yet clear. **Since in order to better** understand the **biology of** spermatogenesis, we **seek to examine the biology of** cell-cell communications between Sertoli cells and germ cells. Cell-cell communication **involves** the transport of cargo**es** through filopodes, which **serve as** bridges between cells. These filopodial structures are defined as “tube” **or** “nanotunnels” because of the inter**-**change**s** of cargo material**s within** the tunnel**s** between cells. These tunneling nanotubes between cells provide **rapid** exchange**s** of both cell-surface molecules and cytoplasmic substance**s**. In cancer cells, it is **known** that some proteins **such as** ezrin and fascin 1 play a role in cell-cell communication **by constituting** the tunneling nanotubes. In our previous studies, we showed that ezrin and fascin 1 proteins play a role with focal adhesion kinases (FAK) and actin filaments in the **basal/**BTB reorganization which regulate the spermatogenesis **using an in vitro** Sertoli cell culture **system that mimics the Sertoli cell BTB in vivo. A**fter silencing of ezrin and fascin 1, formation of tunneling nanotubes and F-actin together between Sertoli cells were disturbed. We know from the literature that the actin filaments and actin binding proteins can be changed under the effects of electromagnetic field (EMF). In this study, we will evaluate the **effects of** electromagnetic field and electrical current effect **on** cytoskeleton dynamics **during** spermatogenesis between Sertoli and germ celss. **At** this point, the important question **is:** in which physical conditions the signaling pathways of cells **during** spermatogenesis **could be affected to perturb spermatogenesis**? In this project, to evaluate the intracellular actin organization and the cargo proteins, which helps to communicate between cells, firstly, we aim to follow **the nanotunnels’s speed and direction between Sertoli-germ cell co-culture. Secondly we will observe the changes of actin organization after silencing FAK by siRNA, the visualization of the transition of ezrin, fascin 1 and Arp3/NWASP proteins which are related to actin organization in the cells. Finally, we will visualize the orientation of cargo vesicles under the electromagnetic field without FAK to see the changes in the cell-cell communication by live records.** We planned the study groups as **Control group (C), Testosteron treated groups (T),**

FAK small interpherase RNA (siRNA) treated groups (FAK RNAi), electromagnetic field treated groups (EMF), electromagnetic field treated groups together with FAK RNAi treated groups (EMF+FAK RNAi). Thus we can evaluate the transition of cargo vesicles, which includes actin binding proteins, and essential organelles as mitochondria in different culture conditions between Sertoli-Sertoli and Sertoli-germ cells in spermatogenesis process. To visualize the vesicles if they transit to right place in right time by using electromagnetic field with RNAi technology combination in the Sertoli germ cell co-culture, we may create an *in vitro* spermatogenesis model with this project via the evaluation of actin electric charges and the magnetic field between the cells. Additionally, the control of the cell-cell communication and their growing times can be also understood by visualizing the intracellular cargo transition. **For clinical application**, we **will also examine if we can** support the maintenance of spermatogenesis in infertile patients who have sperm production failure by using this cell culture model to develop germ cells *in vitro.* In short, our obtained data can give some aid**s** to understand the **role of cytoskeleton and cargo trafficking on** cell-cell communication by using siRNA and electromagnetic field**. Results of these studies should also be applicable** to study in other cells with othe**r** proteins in further investigat**e the biology of** intracellular cell **trafficking and** dynamics.

Keywords: Spermatogenesis, tunneling nanotubes, electromagnetic fields, F-actin, ezrin, fascin 1