發育生物學與再生醫學中心研究快報

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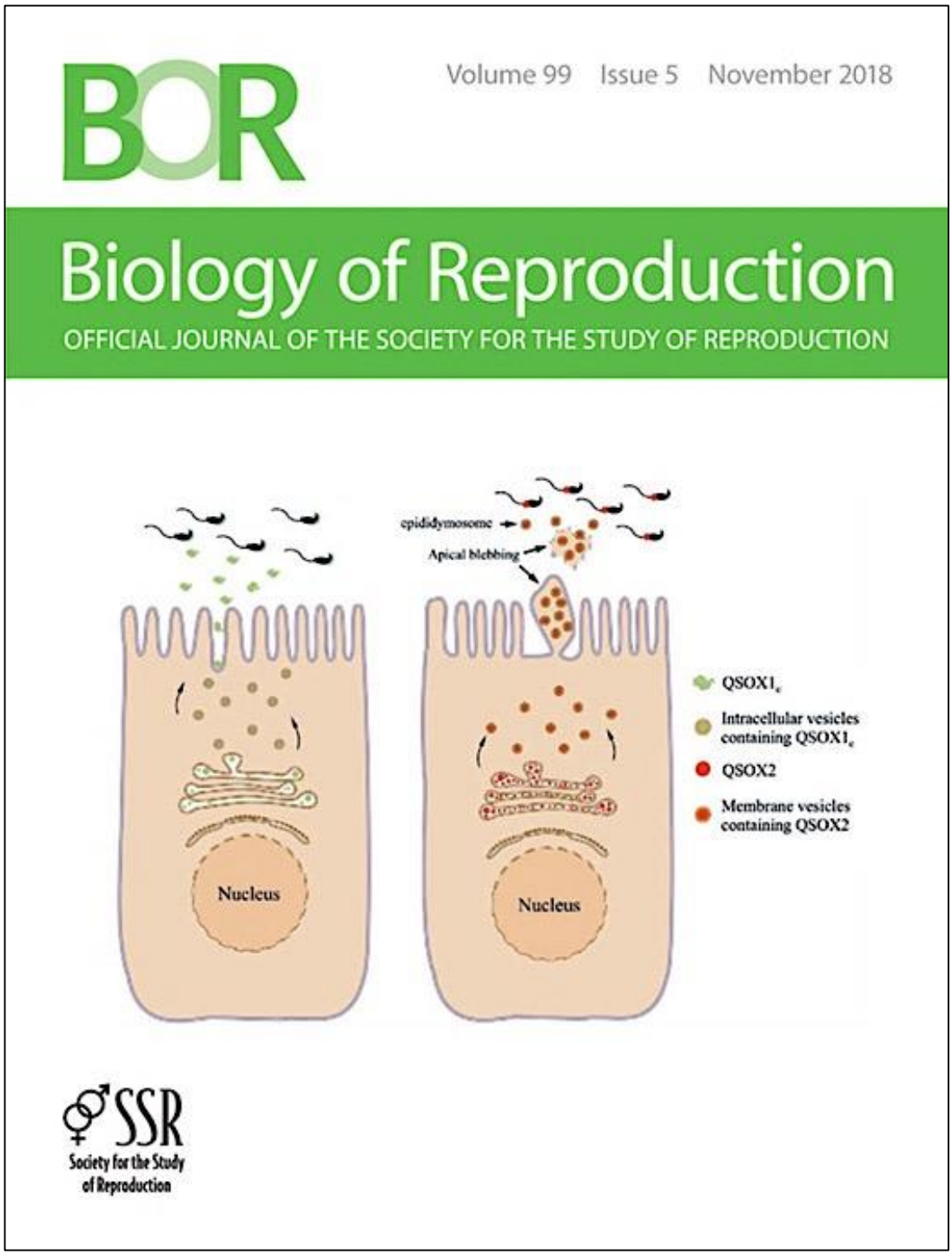
附睪蛋白質協助精子成熟與結構穩定提高受孕成功機率

不孕症一直以來為全球關心的重要議題之一，台灣每年花費在治療不孕症相關的生物技術總值超過台幣 200 億，然而，在缺乏全面性了解不孕症病因與機制的情形下，並無法完全解釋或改善不孕症，亦無法有效提高人工生殖技術的成功率。台大獸醫專業學院暨獸醫學研究所蔡沛學助理教授主持的繁殖生理與細胞生物學實驗室其中一個研究主題為了解生殖道於精、卵結合與精子成熟過程中所扮演的角色與功能。本次刊登於國際期刊 Biology of Reproduction 的文章（Mouse Quiescin Sulfhydryl Oxidases Exhibit Distinct Epididymal Luminal Distribution With Segment-Specific Sperm Surface Associations）出自於獸醫學研究所基礎組博士班二年級汪澤恩的研究主題 ”The Involvement of Epididymal Proteins in the Maturation Processes of Sperm Cells”。在此研究中，蔡老師實驗室藉由科技部三年期的“傑出學者養成計劃”及台灣大學國際處（與東京大學）“重點姐妹校經費”，與國內（馬偕醫學院醫學研究部李勝祥研究員）及國外（東京大學繁殖生理研究室 Kei-ichiro Maeda 教授, Fuko Matsuda 副教授, Dr. Shiori MINABE; University of Newcastle Brett Nixon 教授, Matt Dunn 教授）實驗室共同合作，結合大規模 proteomic screening, whole tissue imaging, epididymosome isolation 與 glycan analyses，針對附睪內分泌型蛋白質 Quiescin Q6 Sulfhydryl Oxidase (QSOX)的調控與功能進行深入研究。在此研究中，我們首次證實男性生殖道蛋白硫氫氧化酶 QSOX (quiescin Q6/sulfhydryl oxidases protein) 的兩種亞型 QSOX1、 QSOX2，大量分佈於雄性生殖道中，並於精子成熟過程的重要器官-附睪中呈現互補性的分佈 (complementary distribution)。我們更透過附睪分泌小體 (epididymosome) 的純化與蛋白質定性、定量測量，證實 QSOX2 受到附睪上皮細胞特殊的 apical blebbing 分泌機制調控，於分泌後附著於精子頭、頸交接處，對於穩定精子構造與尾部擺動扮演重要的角色。然而另一亞型，QSOX1 則分布於管腔中，並特異性的緊密黏附在精子頭帽處，調控附睪精子，提供附睪內精子去活化，抑制精、卵結合前非特異性頭帽反應的重要保護機制。我們藉由與東京大學獸醫系 Prof.

MAEDA, Prof. MATSUDA 實驗室的合作，以 Kisspeptin 基因轉殖鼠結合 *in vitro* cell-based assays 了解到 QSOX2 的基因與蛋白質表現受到睪固酮 (testosterone) 的調控，並可能藉由附睪上皮細胞 principal cells 上的非典型雄性激素受體 (atypical androgen receptor) 來調控 QSOX2 的表現與分泌。我們也透過此計畫中所建立的 QSOX1-eGFP 與 QSOX2-eGFP 系統，證實另一亞型 QSOX1 的表現與分泌並非如 QSOX2 單純受到生殖荷爾蒙的調控，而是與管腔中是否有精子的存在相關（即精子為調控 QSOX1 分泌的主要因素）。此一重大發現徹底顛覆了教科書上傳統生殖道上皮細胞以單方向傳遞精、卵結合所需物質給精子的想法，進一步證實精子可能透過分泌細胞訊息傳遞所需物質給生殖道上皮細胞，建立雙向(bi-directional) 溝通與互動的機制。此次研究計畫與成果，不僅發表於繁殖生理知名國際期刊 Biology of Reproduction，更登上 11 月份期刊封面（Tse-En Wang; Sheng-Hsiang Li; Shiori Minabe; Amanda Anderson; Matt Dun; Kei-Ichiro Maeda; Fuko Matsuda; Hui-Wen Chang; Brett Nixon; Pei-Shiue Jason Tsai*. Mouse Quiescin Sulfhydryl Oxidases Exhibit Distinct Epididymal Luminal Distribution With Segment-Specific Sperm Surface Associations. Biology of Reproduction 2018 Nov. 99(5);1022-1033, (https://www.ncbi.nlm.nih.gov/pubmed/2980009) ）。

圖一：蔡沛學助理教授繁殖生理與細胞生物學實驗室成員（右一，博班學生汪澤恩）。



圖二：發表之論文登上繁殖生理知名國際期刊 Biology of Reproduction 2018 年 11 月份 cover story。Biology of Reproduction 2018 Nov. 99(5);1022-1033, (https://www.ncbi.nlm.nih.gov/pubmed/2980009)

Epididymal Proteins Facilitate Sperm Maturation and Structural Stabilization

輪播引言：NTU team's breakthrough study on the regulation and function of epididymal secretory proteins, which facilitate successful fertilization, was published and selected as cover story in *Biology of Reproduction* in Nov, 2018.

Fertilization is a decisive moment that enables the combination of two gametes to form a new organism; however, the decreasing fertilization rate has become a global issue and concern in the past decades. One of the research focuses of the Laboratory of Reproductive Physiology and Cell Biology, chaired by Assistant Prof. Pei-Shiue Tsai (蔡沛學) from the NTU School of Veterinary Medicine, is to understand the roles and functions of the reproductive tract in the process of sperm-egg binding and sperm maturation.

The research article published in the world-famous international journal, *Biology of Reproduction*, was conducted by second-year doctoral student Tse-En Wang (汪澤恩) on the topic of "The Involvement of Epididymal Proteins in the Maturation Processes of Sperm Cells." The study was supported by the Young Investigator Merit Award of the Ministry of Science and Technology (MOST) and the Strategic Partnership Grants of the NTU Office of International Affairs (with the University of Tokyo). With these funds, Dr. Tsai's lab was able to connect and integrate resources from different laboratories around the world (e.g., Dr. S.H. Li from the Mackay Memorial Hospital, Taiwan; Prof. K.-I. Meada, Associate Prof. F. Matsuda, and Dr. S. Minabe from the University of Tokyo, Japan; and Profs. B. Nixon and M. Dunn from the University of Newcastle, Australia) to perform large-scale proteomic screening, whole tissue imaging, epididymosome isolation, and glycan analyses for an in-depth study on the regulation and function of the epididymal secretory protein Quiescin Q6 Sulfhydryl Oxidase (QSOX).

In this study, we demonstrated for the first time that the two subtypes QSOX1 and QSOX2 are abundantly distributed in the male reproductive tract and display a complementary distribution throughout the epididymis. We further confirmed by the qualitative and quantitative measurement of the isolated epididymosome that QSOX2 is regulated by the apical blebbing secretion mechanism of the epididymal epithelial cells, and thereafter attached to the sperm head-neck junction after secretion to stabilize

the structure of the spermatozoa. By contrast, the other subtype, QSOX1, is distributed in the lumen. The protein specifically adheres to the sperm cap region (acrosome) and inhibits the non-specific and immature acrosome reaction before the sperm encounters the egg. By using the Kisspeptin gene-knockout mice in combination with *in vitro* cell-based assays, we revealed that QSOX2 gene and protein expression is regulated by testosterone, likely via the atypical androgen receptors on the principal cells of epithelial cells. We also confirmed through the QSOX1-eGFP and QSOX2-eGFP systems established in this study that, unlike QSOX2 protein, the secretion of QSOX1 is not regulated by the reproductive hormones, but rather by the presence of sperm cells in the lumen. This major discovery overturns the traditional concept of unidirectional communication from the epithelial cells to the sperm in current textbooks, and shows the potential mechanism of bidirectional communication and interactions between the male reproductive tract and gametes. These research findings were not only published in the prestigious international journal, *Biology of Reproduction*, but also selected as the cover story in the November 2018 issue.

Reference:

Tse-En Wang; Sheng-Hsiang Li; Shiori Minabe; Amanda Anderson; Matt Dun; Kei-Ichiro Maeda; Fuko Matsuda; Hui-Wen Chang; Brett Nixon; Pei-Shiue Jason Tsai. "Mouse Quiescin Sulfhydryl Oxidases Exhibit Distinct Epididymal Luminal Distribution with Segment-Specific Sperm Surface Associations." *Biology of Reproduction*, 99 (5): 1022-1033, Nov. 2018.

(Source: Dr. Pei-Shiue Tsai, NTU School of Veterinary Medicine)

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