一、 英文摘要：

The gonadotropin FSH binds to its membrane receptor and initiates a cascade of cellular responses in target Sertoli cells. FSH-receptor couples to Gs protein and stimulates adenyl cyclase, elevates c-AMP, then activates a series of kinases and finally alters target Sertoli cell functions. Previous reports suggested that tTG covalently cross-linked FSH and receptors, and stabilized the FSH-receptor complexes. However, the existence of cross-linked FSH-receptor complexes have not yet been demonstrated.

tTG is found in various cell types and tissues. Recent data have shown that tTG has multiple functions. It also acts like a G protein when it binds with GTP and was designated as $G\alpha_h$, a 50 kilodalton protein. The inhibitory effect of the tTG destabilizes/dissociates FSH-receptor binding is due to it's transglutaminase function or its $G\alpha_h$ function remains to be elucidated. Recent findings show that TSH couples with more than 10 different kinds of G proteins. Similarly, in addition to coupling with Gs, whether or not FSH-receptor also couples with other G proteins, such as $G\alpha_h$, is not known. It is, therefore, intended to demonstrate the existence of cross-linked FSH-receptor complexes. On the other hand, efforts will also be made to trace the possible function of tTG in FSH target cells; *i. e.*, tTG may act like a G protein such as $G\alpha_h$. Or, other G proteins may couple with FSH-receptor. In other words, experiments will be performed to test if FSH would activate phospholipase C-δ_1(PLC-δ_1), elevate IP_3 and DAG, or mobilize intracellular calcium etc. in the target cells.

Our recent data have demonstrated the presence of tTG in cultured Sertoli cells using western blot analysis. In this study, the dose effect and time course of FSH on the elevation of intracellular cAMP in Sertoli cells were determined. The ED50 of FSH was observed between 100-300 IU/L. Thus 300 IU/L FSH was administrated to Sertoli cells in subsequent studies. The levels of intracellular cAMP level were maximal after administration of FSH for 7.5-minutes MIX, a phosphodiesterase inhibitor, pretreated Sertoli cells. On the other hand, phospholipase C-δ1 was also when the light membrane fraction of Sertoli cell after 60-min treatment of FSH was analyzed by Western blot. Furthermore, three different molecular weight protein bands of 85, 74 and 50($G\alpha_h$) kilodalton were detected by anti-tTG monoclonal Ab. Further effets be made to determine the elevation/activation sequences of cAMP, PLC-δ_1, IP_3 and DAG.

Keywords: gonadotropin, FSH, receptor, transglutaminase, G proteins, Gs, $G\alpha_h$, phospholipase C-δ_1, IP_3, DAG, c-AMP, Sertoli cells.

二、 中文摘要：

性線激素 FSH 與其膜上的受器結合後會開啟標的細胞-Sertoli cell-內的一連串生化反應。FSH-受器與 Gs 蛋白結合後會刺激 Adenyl cyclase，提昇 c-AMP 然後活化一連串激媒的反應最終改變標的細胞的功能。從前的報告推斷 tTG 促使 FSH 與受器之間的共價交錯連結(cross-linked)穩定 FSH-受器之複和物而起動一連串標的細胞之生化反應。然而，FSH-受器複和物的交錯連結目前為止尚未被發現。

tTG 廣泛地存於各種細胞及組織中，在最近的研究報告中指出 tTG 具有多樣性的功能。當 tTG 與 GTP 結合時，則具有 G 蛋白(G-protein)的功能，其名為 Gα_h，為一 50KD 的蛋白質。抑制 tTG 的去穩定性和 FSH 受器結合及分離的能力是因其執行轉麩胺媒的功能或者進一步執行 Gα_h的作用至今仍未被闡明。近來的研究發現顯示 TSH 會與超過十種以上不同的 G 蛋白結合。同樣的，除了與 G 蛋白結合外，FSH-受器是否也會與 G 蛋白如 Gα_h結合，目前並不瞭解。因此我們想要證實 FSH-受器複和物的交錯連結是否存在。另外，我們亦會致力於探索 tTG 在 FSH 標的細胞是否具有其他功能；即 tTG 是否具有 G 蛋白如 Gα_h的功能，或是其他的 G 蛋白是否會與 FSH-受器結合。換句話說，將測試 FSH 是否會活化標的細胞內 phospholipas C-δ_1的活性、升高 IP_3及 DAG 的量，或使細胞內鈣的流通等。

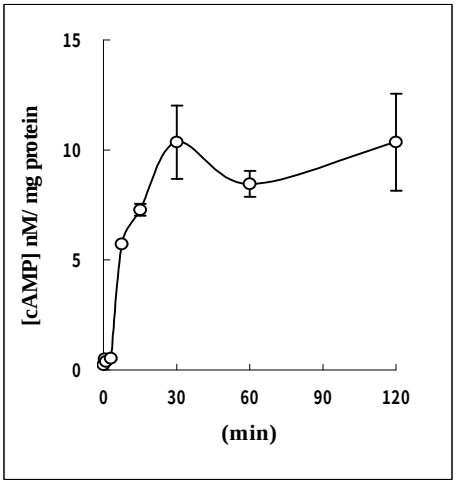
利用 Western blot 分析法，我們最新的研究數據證明了 Sertoli cells 內確實有 tTG 的表現。在這次的研究中，我們以偵測細胞內 cAMP 的增加程度來觀察不同濃度及時間點時 FSH 對細胞作用的影響。我們發現 FSH 之 50%有效作用劑量在 100-300 IU/ L 之間，往後的實驗就以 300 IU/L 的劑量做。在以 phosphodiesterase 的抑制劑 MIX，對 Sertoli cells 做一前處理後，加入 100 IU/ L 的 FSH，發現在 FSH 處理 7.5 分鐘時細胞內的 cAMP 開始累積到最大量。另外，我們也發現在以 FSH 處理細胞一小時後，phospholipas C-δ_1在細胞膜上的含量以西方墨點法檢測有增加的情形。進一步的實驗也觀察到以 tTG 之抗體在西方墨點法的結果中，我們也發現有三條分子量分別約為 87、74 及 50kD（Gα_h）的蛋白被顯現出來。日後將觀研 FSH 在 Sertoli cells 活化 phospholipas C-δ_1的路徑是經由 cAMP 導致內鈣濃度增加而引起或是 FSH 之直接作用。

三、研究結果：

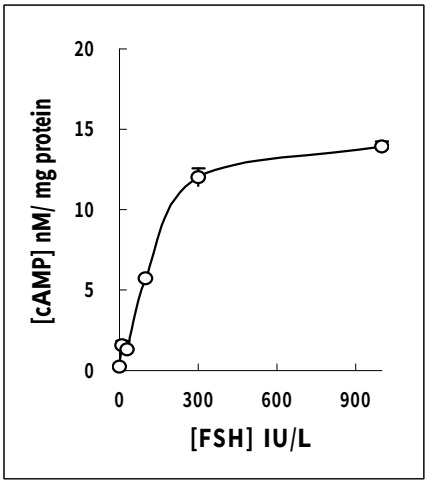
1. **<u>FSH induced intracellular cAMP accumulation in a time- and dose-dependent manner in Sertoli cells</u>**

FSH (100IU/ ml) was administered to 6 days-cultured Sertoli cells which were pretreated with 1mM of methyl-isobutyl xanthine (MIX), a phosphodiesterase inhibitor. After incubation, Sertoli cells were scrapped from culture plate in the extraction buffer and centrifuged at 20,000 x g at 4℃ for 30 minutes. The supernatants were used to measure the concentration of intracellular cAMP with an WLISA kit. The data from three independent experiments were calculated with SigmaPlot software as shown in Fig. 1a and b. The levels of intracellular cAMP were maximal 7.5-minutes after administration of FSH. The ED50 of FSH was observed between 100-300 IU/ L.

a.

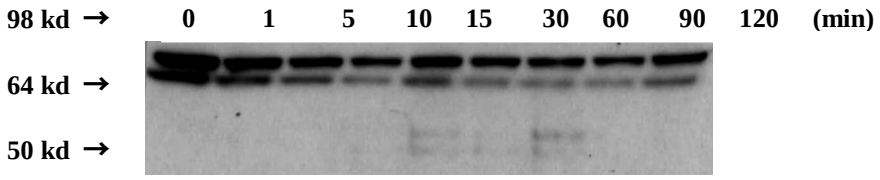


b



2. **<u>Anti-tTG antibodies recognized three different fragments of tTG in light membrane of Sertoli cells</u>**

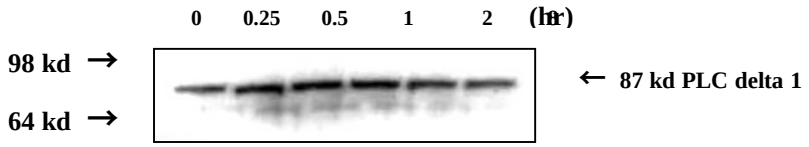
After 6 days of culture Sertoli cells were treated with 300 IU/ L of FSH was at defined time intervals. After incubation, Sertoli cells were scrapped from culture plates in extraction buffer and the cell lysates were centrifuged 300 at xg at 4℃ for 10 minutes. Furthermore, the supernatants were transferred to the new eppendorf tube and then centrifuged at 50,000 xg at 4 ℃ for 60 minutes. The pellets (light membrane) were resolved by western blotting analysis. The data shown that three bands of 87, 74 and 50 kD proteins were recognized by anti-tTG antibody.



3. <u>FSH stimulated the PLC ¢1 activation and translocation from cytosol to membrane</u>

After 6 days of culture, Sertoli cells were treated with 300 IU/ L of FSH at the defined time intervals. FSH treated-Sertoli cells were scrapped from culture dish in the extraction buffer and the lysates were centrifuged 300 xg at 4℃ for 10 minutes. Subsequently, the supernatants were transferred to new eppendorf tube and then centrifuged at 50,000 xg at 4 ℃ for 60 minutes. The pellets (light membrane) and supernatants (cytosol) were analyzed by western blotting . The data shown that PLC δ1 was increased after 60-min FSH treatment in the light membrane of Sertoli cells. G3PDH was used as an internal control of loading protein concentration.

membrane



cytosol