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# Synergistic cooperation between the β-catenin and SF1 regulates progesterone synthesis in laying hen ovarian granulosa cells

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**ABSTRACT**

The development of ovarian follicles in poultry is a key factor affecting the performance of egg production. Ovarian follicle development is regulated via the Wnt/β-catenin signaling pathway, and β-catenin, encoded by *CTNNB1*, is a core component of this pathway. In this study, using ovary GCs from laying hens, we investigated the regulatory role of *CTNNB1* in steroid synthesis. We found that *CTNNB1* significantly regulates the expression of *StAR* and *CYP11A1* (key genes related to progesterone synthesis) and the secretion of progesterone (P4). Furthermore, simultaneous overexpression of CTNNB1 and SF1 resulted in significantly higher levels of *CYP11A1* and secretion of P4 than in cells overexpressing CTNNB1 or SF1 alone. We also found that in GCs overexpressing *SF1*, levels of *CYP11A1* and secreted P4 were significantly greater than in controls. Silencing of *CYP11A1* resulted in the inhibition of P4 secretion while overexpression of *SF1* in *CYP11A1*-silenced cells restored P4 secretion to normal levels. Together, these results indicate that synergistic cooperation between the β-catenin and SF1 regulates progesterone synthesis in laying hen ovarian hierarchical granulosa cells to promote *CYP11A1* expression.

**KEYWORDS**

Laying hens; β-catenin; granulosa cells; SF1; *CYP11A1*; progesterone

## Introduction

Laying performance in hens, a very important economic trait, is affected by myriad factors, but depends mainly on ovarian function and follicle development.[1] A follicle, the basic functional unit of the ovary, is composed of thecal cells, follicular granulosa cells (GCs), and oocytes.[2] The differentiation and proliferation of GCs and their synthesis of follicle steroid hormones, play an important role in follicle recruitment, selection, growth, and maturation.[3] Earlier studies have reported several factors regulating GC proliferation and differentiation, most of which are related to the Wnt/β-catenin signaling pathway. For example, *SMAD4* inhibits granulosa cell apoptosis;[4] bone morphogenetic protein-15 promotes follicular selection in hen granulosa cells;[5] PITX2 regulates granulosa cell proliferation and steroid hormone production in dairy goats.[6] β-catenin, encoded by *CTNNB1*, is a protein in the classical Wnt signaling pathway; it can activate the transcription of target genes related to cell proliferation and hormone secretion, and its abnormal activation may lead to the formation of tumors.[7–9] Early studies in mice showed that β-catenin binds to the transcription factor TCF/LEF activating the expression of corresponding target genes and promoting the production of steroid hormones, thereby regulating the development of ovarian follicles.[10] Castanon et al. report, that in bovines, FSH regulates *CTNNB1* expression and induces secretion of steroid hormones from granulosa cells thus promoting ovarian follicle development,[11] and Bae et al. report that *CTNNB1* can be used as an estrogen-inducing gene to influence the development of fallopian tubes in chicks.[12] Thus, β-catenin plays a crucial role in follicular development by participating in the synthesis of steroid hormones.

The hypothalamic-pituitary-ovarian axis plays a critical part in animal reproduction by regulating the synthesis of steroid hormones.[13] During ovarian

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growth and maturation, follicular granulosa cells undergo a series of physiological and biochemical changes, including the expression of gonadotropin receptors and the synthesis of steroids.[14] The production of steroid hormones is regulated by several factors, including genes related to steroid production, hormones, and various other regulatory factors.[14,15] Progesterone receptor is located in the nucleus of GCs and binds specifically to hormones to form hormone receptor complexes, which are involved in follicle growth and development, maturation, and ovulation, as well as steroid synthesis.[16] Steroidogenic acute regulatory protein (StAR), cholesterol side chain cleavage cytochrome P450 (P450scc), and 3β-hydroxysteroid dehydrogenase (3β-HSD) are directly involved in the synthesis of progesterone.[6,16] In addition, several regulatory factors (IGF1, EGF, TGF-β, and SF1) and transcription factors (FOXL2, WT1, AP1, and SP1) regulate the expression of ovarian steroid synthesis genes, as well as hormone secretion, by activating multiple molecular signaling pathways.[16] Among them, steroidogenic factor 1 (SF1, officially designated NR5A1) is an important transcription factor in hormone synthesis; it regulates the transcription of *CYP11A1*, *StAR,* and *3β-HSD*.[17] SF1 regulates the activity of the *CYP11A1* promoter in bezoar somatic cells;[18] PITX2 interacts with SF1 to participate in the WNT pathway and enhance the production of E2 and P4 in GCs of dairy goats;[6] In rat granulosa cells, SF1 interacts with β-catenin to regulate the expression of *CYP19A1*.[10]

Many of the studies on the regulation of reproduction focus mainly on mammals, there are many fewer reports on the regulation of reproduction in laying hens. To determine the regulatory factors and related signaling pathways that can improve egg production, it is necessary to elucidate the molecular mechanisms of laying performance. We previously reported that CTNNB1, as the target gene of miR-458b-5p, is highly expressed in hen hierarchical follicular granulosa cells,[19] so we speculated that CTNNB1 may regulate the development of hierarchical follicular granulosa cells in laying hens by participating in progesterone synthesis. In this study, we investigated the role of β-catenin and SF1 as regulatory factors of progesterone secretion from follicular hierarchical granulosa cells in laying hens.

## Materials and methods

### Ethics approval

All animal experiments were approved by the Institutional Animal Protection and Use Ethics Committee of Shandong Agricultural University (SDAUA-2018-018) and conducted in accordance with the "Guidelines for Experimental Animals" of the Ministry of Science and Technology of China.

### Sample collection

Three healthy Hyline-brown hens of about 30 weeks of age were randomly selected from a local research farm affiliated with Shandong Agricultural University. Hens were slaughtered via carotid artery bleeding, the abdominal organs were incised with surgical scissors, and all hierarchical follicles (diameters > 12mm, F6-F1) were placed in sterile beakers containing 5% penicillin/streptomycin PBS (HyClone, Logan, UT) to remove residual connective tissue and attached blood filaments. Using forceps, the outer membrane on the follicles was peeled away, follicles were then punctured and the granulosa cell layer was separated by gentle shaking.

### Cell culture and transfection

The granulosa cell layer was placed in PBS, washed three times, and then digested by adding 0.25% trypsin (Gibco, Grand Island, NY) and incubating 8-10 min at 37 °C in a 5% $CO_2$ humidified atmosphere. After digestion, single cells were obtained by passing the suspension through a 200 μm filter. After centrifugation, cells were suspended in M199 complete medium (Gibco) containing 10% fetal bovine serum (Gibco) and 1% penicillin/streptomycin (Solarbio, Beijing, China), aliquoted into 6-well culture plates at a density of $5 \times 10^5$ cells/well, and incubated for about 12 hours at 37 °C and 5% $CO_2$. When the cell density reached more than 70%, cells were transfected (the plasmid and siRNA used for transfection were 2 ng and 8 μL per well); Lipofectamine 2000 (Invitrogen, Carlsbad, CA) was used for the transfection of recombinant expression plasmids and Lipofectamine RNAiMAX (Invitrogen) was used for the transfection of siRNA. Transfections were performed according to the manufacturer's protocol.

### RNA extraction, cDNA synthesis, and quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from GCs using TRIzol reagent (Novizan, Nanjing, China). OD was measured using a spectrophotometer (Thermo, Carlsbad, USA), and RNA purity and concentration were determined by the 260/280 ratio. All samples had

an acceptable purity (absorbance ratio from 1.9 to 2.1). cDNA was synthesized by reverse transcription using a HiScrip®II First Strand cDNA Synthesis Kit (Novizan) containing gDNA wiper. The samples were stored frozen at −20 °C until use. ChamQ Universal SYBR qPCR Master Mix (Novizan) was used for qRT-PCR. Primer sequence information is listed in Table 1. The quantitative real-time PCR protocol was as follows: 2xChamQ SYBR qPCR Master Mix 10.0 µL, Forward primer (10 µmol/L) 0.4 µL, Reverse primer (10 µmol/L) 0.4 µL, template (cDNA) 1.0 µL, and RNase Free ddH2O 8.2 µL. The reaction conditions were performed as follows: 95 °C for 30 s; 95 °C for 10 s and 60 °C for 30 s for 40 cycles; 95 °C for 15 s, 60 °C for 60 s, and 95 °C for 15 s. The relative expression levels of genes were determined by the $2^{-\Delta\Delta C}{}_{T}$ method.

### Plasmids and siRNA

The siRNA and negative control (NC) used in the experiment were designed and synthesized by GenePharma (Shanghai, China). pcDNA3.1 is kept by the laboratory. The recombinant expression plasmids pcDNA3.1-CTNNB1 and pcDNA3.1-SF1 were constructed by Tsingke (Beijing, China). siRNA sequences are listed in Table 2.

### ELISA

GCs were transfected into a 6-well plate for 24 h, and cell supernatant was collected. Concentrations of progesterone (P4) was determined using a chicken P4 ELISA kit (Enzyme-linked Biotechnology, Shanghai, China; Meimian Biotechnology, Jiangsu, China) according to the manufacturer's instructions (The sensitivity of ELISA Kits of P4 was tolerance within batch and tolerance between batches of CV < 10% and there was no cross-reactivity with ELISA kit). Absorbance (OD) was measured at 450 nm using the Molecular Devices SpectraMax i3x Multi-Mode Microplate Detection System. The concentration of P4 in the samples is then determined by comparing the OD of the samples to the standard curve.

### Statistical analysis

All data were statistically analyzed using SPSS version 22.0 and are presented as the means ± standard deviation. The two groups were compared by t-test, and the multiple groups were compared by one-way analysis of variance (ANOVA). Multiple comparisons were made using the Duncan's test. Statistical significance is defined as $P \leq 0.05$. GraphPad Prism 8.0 software was used for the visualization of all data.

## Results

### β-catenin regulates progesterone secretion through hierarchical follicular granulosa cells

Figure 1a illustrates, as determined by qRT-PCR, the increased expression of *CTNNB1* in hierarchical follicular granulosa cells transfected with pcDNA3.1-CTNNB1 ($P < 0.01$), and Figure 1b illustrates the silencing efficiency of *CTNNB1* in cells transfected with siRNA-CTNNB1 ($P < 0.01$). In cells over-expressing *CTNNB1*, levels of *StAR* and *CYP11A1* (genes related to progesterone) were significantly increased over empty vector control, and in cells under-expressing *CTNNB1*, levels of *StAR* and *CYP11A1* were significantly decreased over siRNA-NC control ($P < 0.01$ and $P < 0.05$ respectively; Figure 1c–f). ELISA results showed that levels of P4 was significantly increased after transfection with pcDNA3.1-CTNNB1 ($P < 0.05$; Figure 1g), and significantly decreased after transfection with siRNA-CTNNB1 ($P < 0.01$; Figure 1h). These results suggest that *CTNNB1* regulates the synthesis and secretion of progesterone in GCs.

**Table 1.** Primer sequences.

| Primer name | Primer sequence (5′–3′) | Melting temperature (°C) | Product length (bp) | GenBank number |
|---|---|---|---|---|
| CTNNB1 | F:TCATTGGCAGCAGCAGTCATAT | 56.6 | 253 | NM_205081.3 |
| | R: CATTCCTTCATCCAGTGTTTCG | 53.7 | | |
| SF1 | F: GCGGGAGGAATAAGTT | 47.9 | 169 | NM_205077.2 |
| | R: ATGGCGTGGATGCTGT | 54.5 | | |
| StAR | F:GACAACATGGAGCAGATGGGCGACTGG | 66.3 | 160 | NM_204686.3 |
| | R: GCCGGGAGCACCGAACACTCACAA | 67.1 | | |
| CYP11A1 | F: ACCGCAACAAGCCCTACG | 58.9 | 238 | NM_001001756.2 |
| | R: CACACAGACTCCAAGGCAAAG | 56.5 | | |
| β-actin | F: CACGATGGAGGGGCCGGACTCATC | 67.1 | 241 | NM_001101.5 |
| | R: TAAAGACCTCTATGCCAACACAGT | 55.4 | | |

CTNNB1, catenin beta-1; SF1, nuclear receptor subfamily 5 group A member 1; StAR, steroidogenic acute regulatory; CYP11A1, cytochrome P450 family 11 subfamily A member 1; β-actin, actin-beta.

### β-catenin and SF1 regulate progesterone secretion in hierarchical follicular granulosa cells

Figure 2a illustrates the transfection efficiency of pcDNA3.1-SF1 into hierarchical follicular granulosa cells ($P<0.01$), the level of *SF1* was significantly increased over cells transfected with empty vector; and Figure 2b illustrates the silencing efficiency of *SF1* after transfection of with three different siRNAs-SF1 ($P<0.05$). We next co-overexpressed *CTNNB1* and *SF1* and quantified the levels of *CYP11A1* and the secretion of P4. By qPCR, simultaneous overexpression of *CTNNB1* and *SF1* resulted in significantly higher levels of *CYP11A1* over controls ($P<0.05$; Figure 2c) and, by ELISA, significantly higher levels of secreted P4 ($P<0.05$; Figure 2d). Cells were then cotransfected with pcDNA3.1-CTNNB1 and siRNA3-SF1, and Figure 2e shows the transfection efficiency ($P<0.01$). In SF1-silenced cells overexpressing *CTNNB1,* levels of *CYP11A1* were significantly decreased over controls ($P<0.01$; Figure 2f), and the levels of secreted P4 returned to normal ($P>0.05$; Figure 2g).

We therefore speculated that β-catenin promotes the secretion of P4 in hierarchical follicular granulosa cells by synergizing with SF1.

**Table 2.** siRNA sequences.

| Name | Sequence (5′–3′) |
|---|---|
| siRNA-CTNNB1 | 5′-CCCUAUGAUGGAACAUGAATT-3′<br>5′-UUCAUGUUCCAUCAUAGGGTT-3′ |
| siRNA-SF1 | 5′-GCUCCACUUUCAUAGCACUTT-3′<br>5′-AGUGCUAUGAAAGUGGAGCTT-3′ |
| siRNA-CYP11A1 | 5′-GGCUGACAAAUGUAUCCAATT-3′<br>5′-UUGGAUACAUUUGUCAGCCTT-3′ |
| siRNA-NC | 5′-UUCUCCGAACGUGUCACGUTT-3′<br>5′-ACGUGACACGUUCGGAGAATT-3′ |

### SF1 and CYP11A1 regulate progesterone secretion in hierarchical follicular granulosa cells

Song et al. discussed the regulatory effect of SF1 on *CYP11A1* in goats[20], so we investigated its potential

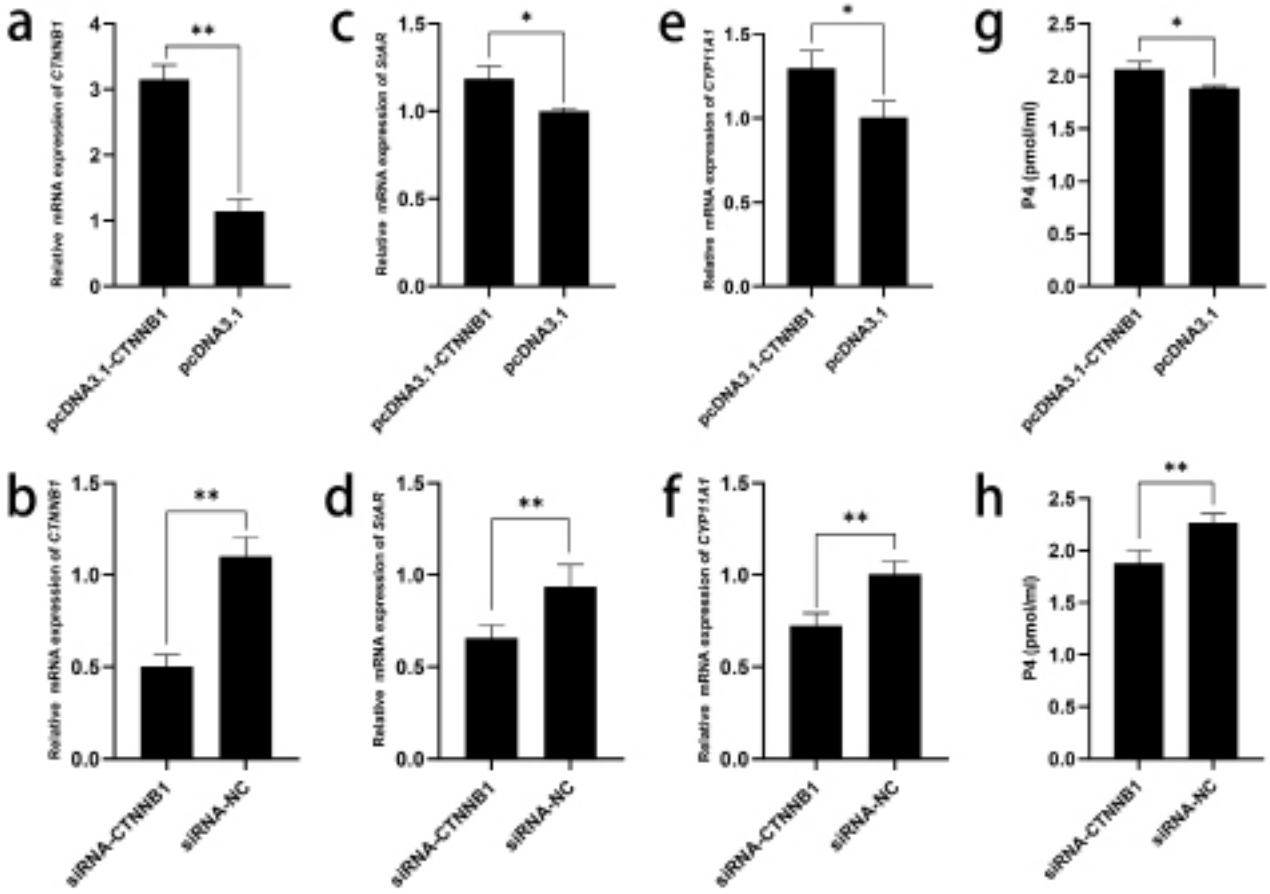


**Figure 1.** β-catenin promotes the secretion of progesterone in GCs. (a) Relative level of *CTNNB1* after transfection with pcDNA3.1-CTNNB1. (b) Relative expression of *CTNNB1* mRNA after transfection with siRNA-CTNNB1. (c) Relative expression of *StAR* after transfection with pcDNA3.1-CTNNB1. (d) Relative expression of *StAR* after transfection with siRNA-CTNNB1. (e) Relative expression of *CYP11A1* after transfection with pcDNA3.1-CTNNB1. (f) Relative expression of *CYP11A1* after transfection with siRNA-CTNNB1. (g) Levels of P4 after transfection with pcDNA3.1-CTNNB1. (h) Levels of P4 after transfection with siRNA-CTNNB1. Results are presented as means ± SE; n = 3; * $P<0.05$; ** $P<0.01$.

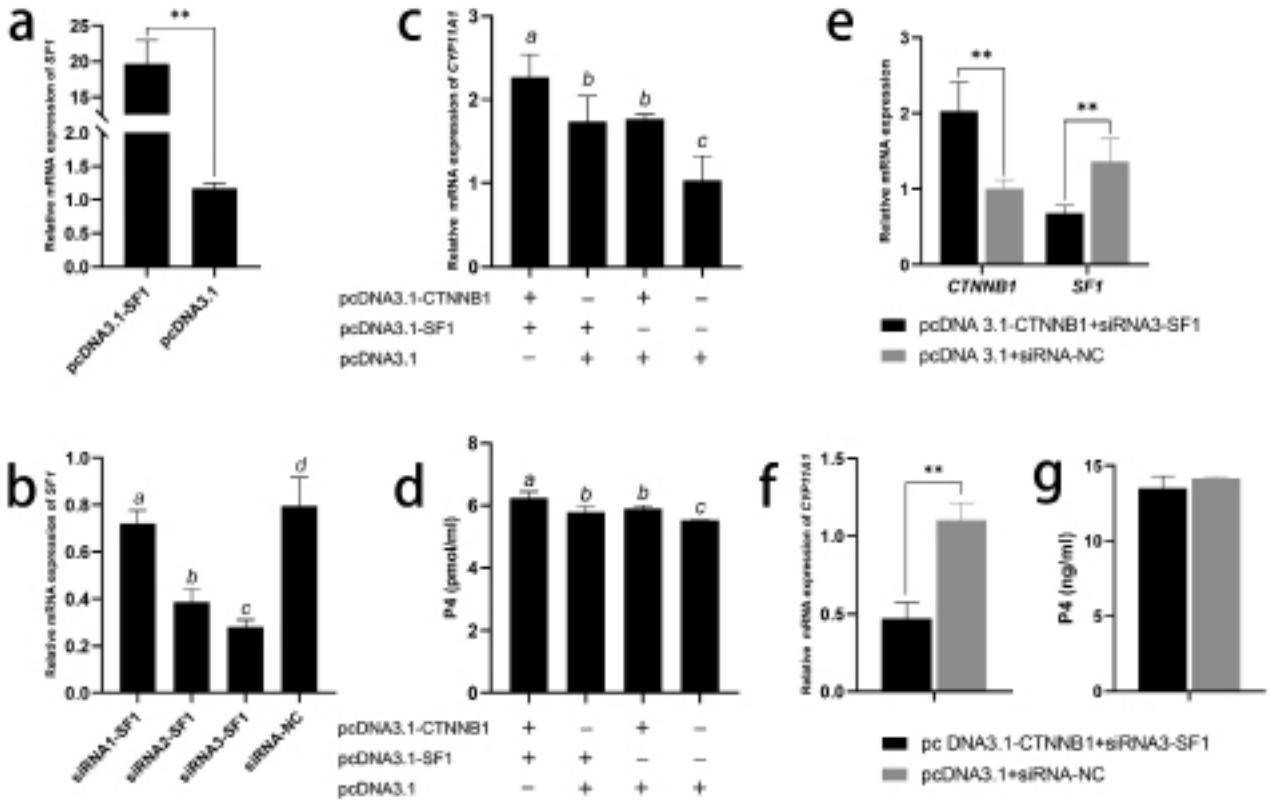


**Figure 2.** β-catenin and SF1 co-promote the secretion of progesterone in GCs. (a) Relative expression of *SF1* after transfection with pcDNA3.1-SF1 cells. (b) Relative expression of *SF1* after transfection of with three different siRNAs-SF1. (c) Relative expression of *CYP11A1* after cotransfection with pcDNA3.1-CTNNB1 and pcDNA3.1-SF1. (d) Levels of secreted P4, measured by ELISA, after cotransfection with pcDNA3.1-CTNNB1 and pcDNA3.1-SF1. (e) Relative expression of *CTNNB1* and *SF1* after cotransfection with pcDNA3.1-CTNNB1 and siRNA3-SF1. (f) Relative expression of *CYP11A1* after cotransfection with pcDNA3.1-CTNNB1 and siRNA3-SF1. (g) Level of secreted P4 after cotransfection with pcDNA3.1-CTNNB1 and siRNA3-SF1. Results are presented as means ± SE; n = 3; * $P < 0.05$; ** $P < 0.01$; *a, b,* and *c* indicate significant different values ($P < 0.05$).

effect on *CYP11A1* in chickens. We first determined the silencing efficiency of three siRNAs-CYP11A1 ($P < 0.05$; Figure 3a). In cells cotransfected with siRNA2-CYP11A1 and pcDNA3.1-SF1 we found that the level of *SF1* was significantly increased, and that of *CYP11A1* was significantly decreased ($P < 0.01$; Figure 3b) over controls. In cells overexpressing pcDNA3.1-SF1 the level of *CYP11A1* was significantly increased over control and in SF1 silenced cells, the level of *CYP11A1* was significantly decreased compared to control ($P < 0.01$, Figure 3c, d). By ELISA, cells overexpressing *SF1* had significantly higher levels of secreted P4 than control, cells with *CYP11A1* silenced had significantly lower levels of secreted P4 than control, and cells that simultaneously overexpressed SF1 and under-expressed *CYP11A1* were restored to the normal levels of secreted of P4 ($P < 0.01$ and $P > 0.05$; Figure 3e). These results further suggest that SF1 plays a role in regulating progesterone secretion by targeting *CYP11A1*.

## Discussion

Follicle development is the basis of reproduction in female animals.[16] Follicular development is regulated by hormones and growth factors secreted or paracrine by granulosa cells, thecal cells, and oocytes. Granulosa cells not only provide nutrition for oocyte growth but are also the main site for the synthesis of steroid hormones.[21]

β-catenin, encoded by *CTNNB1*, is a crucial regulator in the Wnt/β-catenin signaling pathway. Numerous studies have demonstrated that the Wnt/β-catenin signaling pathway is involved in the regulation of myriad cell functions, some of which are cell proliferation, differentiation, apoptosis, and steroid synthesis.[19] Bu et al. reported that in goat GCs, steroidogenesis-related gene expression was decreased by siRNA-CTNNB1.[6] Guo et al. reported that by up-regulating the Wnt/β-catenin pathway in mouse cells, cell proliferation increased as did steroidogenic enzyme expression.[22] Abedini et al. found that WNT5A

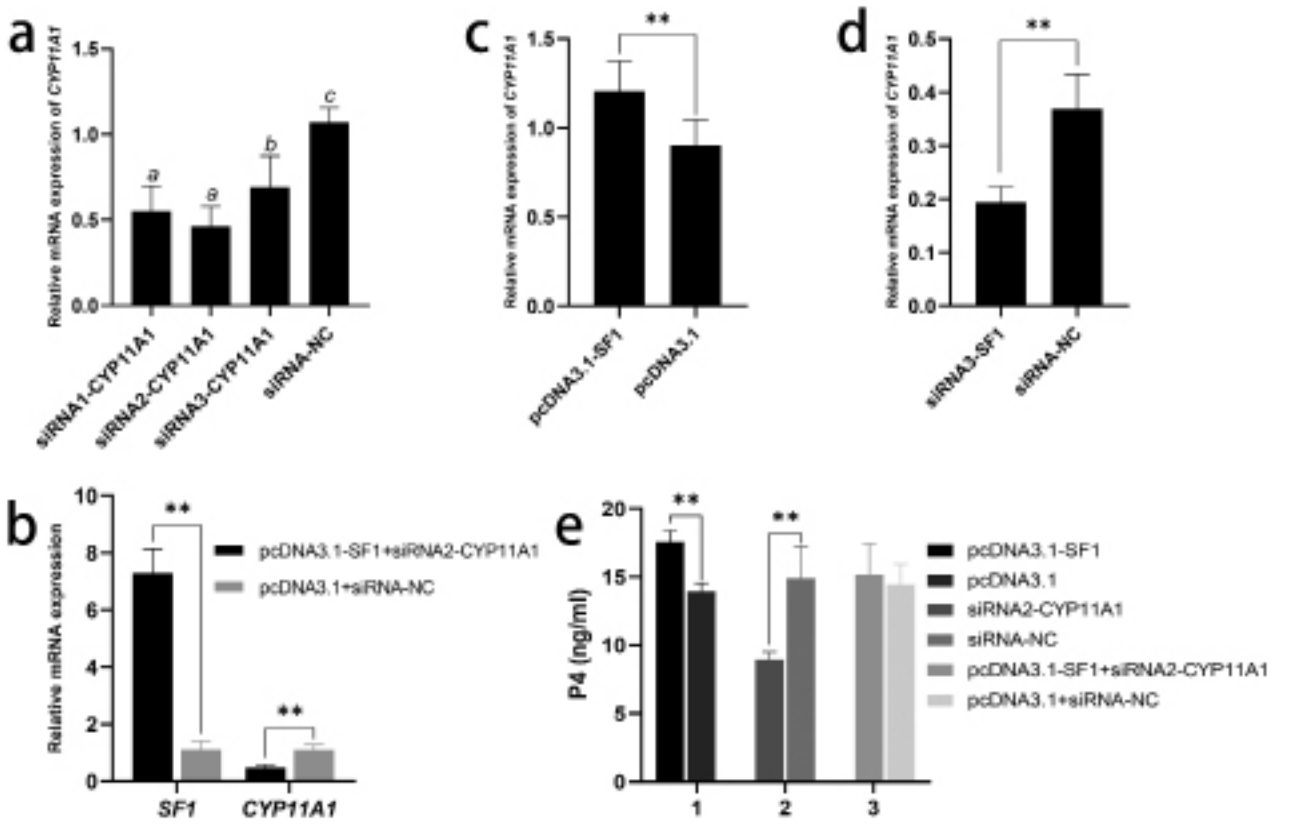


**Figure 3.** SF1 and *CYP11A1* co-promote the secretion of progesterone in GCs. (a) Relative expression of *CYP11A1* after transfection of with three different siRNAs-CYP11A1. (b) Relative expression of *SF1* and *CYP11A1* after cotransfection with pcDNA3.1-SF1 and siRNA2-CYP11A1. (c) Relative expression of *CYP11A1* after transfection with pcDNA3.1-SF1. (d) Relative expression of *CYP11A1* after transfection with siRNA3-SF1. (e) Level of secreted P4 in cells cotransfected with pcDNA3.1-SF1 and siRNA2-CYP11A1. Results are presented as means ± SE; n = 3; * $P < 0.05$; ** $P < 0.01$; *a, b,* and *c* indicate significant different values ($P < 0.05$).

acts as a negative regulator of FSH-stimulated steroid production in bovine granulosa cells.[23] These results demonstrate that the Wnt/β-catenin signaling pathway has many important effects on the regulation of steroids in mammalian follicular granulosa cells, but as its role in laying hen ovarian granulosa cells is not well known, we speculate that *CTNNB1* plays a similar role in the GCs of laying hens.

E2, P4, and androgen are the main steroids involved in the regulation of female fertility.[20] The synthesis of E2 and P4 is mainly controlled by StAR, CYP11A1, and CYP19A1.[24] Steroidogenic acute regulatory protein (StAR) carries free cholesterol in the cytoplasm and transports it to the inner mitochondrial membrane where it is converted into pregnenolone (P5) by cytochrome P450 (CYP11A1). P5 is catalyzed by 3β-HSD to form progesterone, which is finally converted to androstenedione by the steroid 17α-hydroxylase; this synthetic pathway is known as the Δ4 pathway. Androstenedione is catalyzed by 17β-hydroxysteroid dehydrogenases to form testosterone, which is finally catalyzed by aromatase to produce estrogen (E2).[25–27] In mammals, E2 and P4 can be synthesized by GCs, but the main site of P4 synthesis is in the corpus luteum cells after ovulation, in birds, E2 and P4 can also be synthesized by granulosa cells, and the main site of E2 synthesis is TCs, so there are some significant differences in the regulation of steroid synthesis between the two.[28]

Here, we determined that *CTNNB1* promotes the secretion of P4 in hierarchical follicular granulosa cells. Jordan et al. showed that there is a synergistic effect between β-catenin and SF1 in mouse cells, and the two can promote the secretion of P4 in transfected cells.[29] We cotransfected hierarchical follicular granulosa cells with pcDNA3.1-CTNNB1 and pcDNA3.1-SF1, and pcDNA3.1-CTNNB1 and siRNA-SF1, and found that *CTNNB1* and *SF1* together significantly promoted the secretion of P4. GCs overexpressing *CTNNB1* and *SF1* also had significantly higher levels of secreted P4 than cells overexpressing *CTNNB1* and *SF1* alone. In *SF1* silenced cells overexpression of *CTNNB1* still affects the expression of *CYP11A1* and the secretion of P4. Therefore, we concluded that *CTNNB1* promotes the secretion of P4 in hierarchical follicular granulosa cells in concert with *SF1*.

The steroidogenic factor SF1 belongs to the nuclear receptor family of transcription factors and has the typical structure of the nuclear receptor family, including a C-terminal ligand-binding domain, an intermediate hinge domain, and an N-terminal DNA-binding domain. SF1 plays a key role in the development and function of steroid organs, it transcribes a range of factors required to regulate steroid hormone biosynthesis and is required for gene expression in the pituitary and reproductive tracts.[30–32] SF1 regulates the transcription of *CYP11A1*,[17] and a large number of studies have shown that in mammals, the highly conserved sequence (SF1RE: TAGCCTTGA) in the promoter region of *CYP11A1* is the binding site of SF1.[33–35] By comparing this sequence in chickens and mammals, we found that there was a difference of one base. As we thought, overexpression of *SF1* results in increased secretion of P4, and silencing of *CYP11A1* in *SF1* overexpressing cells resulted in decreased secretion of P4. Therefore, we speculate that SF1 and *CYP11A1* jointly promote the secretion of P4. However, the binding of SF1 to the core promoter region of *CYP11A1* needs to be verified in laying hens. Although this study verified the effects of β-catenin, SF1, and *CYP11A1* on P4 synthesis, they are by no means the only three variables regulating P4 synthesis, so the specific mechanism of steroid hormone synthesis in laying hens needs to be studied further.

## Disclosure statement

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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## References

1. Du Y, Liu L, He Y, Dou T, Jia J, Ge C. Endocrine and genetic factors affecting egg laying performance in chickens: a review. *Br Poult Sci*. 2020;61(5):1–9.
2. McGee EA, Hsueh AJ. Initial and cyclic recruitment of ovarian follicles. *Endocr Rev*. 2000;21(2):200–214.
3. Eppig JJ. Reproduction: oocytes call, granulosa cells connect. *Curr Biol*. 2018;28(8):R354–R356.
4. Du X, Li Q, Yang L, Liu L, Cao Q, Li Q. SMAD4 activates the Wnt signaling pathway to inhibit granulosa cell apoptosis. *Cell Death Dis*. 2020;11(5):373.
5. Stephens CS, Johnson PA. Bone morphogenetic protein 15 may promote follicle selection in the hen. *Gen Comp Endocrinol*. 2016;235:170–176.
6. Bu Q, Liu S, Wang Z, et al. PITX2 regulates steroidogenesis in granulosa cells of dairy goat by the WNT/β-catenin pathway. *Gen Comp Endocrinol*. 2022;321–322:114027.
7. Boerboom D, White LD, Dalle S, Courty J, Richards JS. Dominant-stable β-catenin expression causes cell fate alterations and Wnt signaling antagonist expression in a murine granulosa cell tumor model. *Cancer Res*. 2006;66(4):1964–1973.
8. Boyer A, Paquet M, Laguë M-N, Hermo L, Boerboom D. Dysregulation of WNT/CTNNB1 and PI3K/AKT signaling in testicular stromal cells causes granulosa cell tumor of the testis. *Carcinogenesis*. 2009;30(5):869–878.
9. MacDonald BT, Tamai K, He X. Wnt/β-catenin signaling: components, mechanisms, and diseases. *Dev Cell*. 2009;17(1):9–26.
10. Parakh TN, Hernandez JA, Grammer JC, et al. Follicle-stimulating hormone/cAMP regulation of aromatase gene expression requires beta-catenin. *Proc Natl Acad Sci U S A*. 2006;103(33):12435–12440.
11. Castañon BI, Stapp AD, Gifford CA, Spicer LJ, Hallford DM, Hernandez Gifford JA. Follicle-stimulating hormone regulation of estradiol production: possible involvement of WNT2 and beta-catenin in bovine granulosa cells. *J Anim Sci*. 2012;90(11):3789–3797.
12. Bae S-M, Lim W, Jeong W, et al. Hormonal regulation of beta-catenin during development of the avian oviduct and its expression in epithelial cell-derived ovarian carcinogenesis. *Mol Cell Endocrinol*. 2014;382(1):46–54.
13. Matsuda F, Inoue N, Manabe N, Ohkura S. Follicular growth and atresia in mammalian ovaries: regulation by survival and death of granulosa cells. *J Reprod Dev*. 2012;58(1):44–50.
14. Zhang C, Shimada K, Saito N, Kansaku N. Expression of messenger ribonucleic acids of luteinizing hormone and follicle-stimulating hormone receptors in granulosa and theca layers of chicken preovulatory follicles. *Gen Comp Endocrinol*. 1997;105(3):402–409.
15. Yamamura N, Takeishi M, Goto H, et al. Expression of messenger RNA for gonadotropin receptor in the granulosa layer during the ovulatory cycle of hens. *Comp Biochem Physiol A Mol Integr Physiol*. 2001;129(2–3):327–337.
16. Wu X, Zhang N, Li J, et al. gga-miR-449b-5p regulates steroid hormone synthesis in laying hen ovarian granulosa cells by targeting the IGF2BP3 gene. *Animals*. 2022;12(19):2710.
17. Juengel JL, Larrick TL, Meberg BM, Niswender GD. Luteal expression of steroidogenic factor-1 mRNA during the estrous cycle and in response to luteotropic and luteolytic stimuli in ewes. *Endocrine*. 1998;9(3):227–232.
18. Liu Z, Simpson ER. Steroidogenic factor 1 (SF-1) and SP1 are required for regulation of bovine CYP11A gene expression in bovine luteal cells and adrenal Y1 cells. *Mol Endocrinol*. 1997;11(2):127–137.
19. Wang W, Teng J, Han X, Zhang S, Zhang Q, Tang H. miR-458b-5p regulates ovarian granulosa cells proliferation through Wnt/β-catenin signaling pathway by targeting catenin beta-1. *Anim Biosci*. 2021;34(6):957–966.

20. Wen X, Li D, Tozer AJ, Docherty SM, Iles RK. Estradiol, progesterone, testosterone profiles in human follicular fluid, and cultured granulosa cells from luteinized pre-ovulatory follicles. *Reprod Biol Endocrinol*. 2010; 8(1):117.
21. Song S, Ding W, Yao H, et al. BMP6 promotes the secretion of 17 beta-estradiol and progesterone in goat ovarian granulosa cells. *Animals*. 2022;12(16):2132.
22. Guo X, Chen Y, Hong T, et al. Induced pluripotent stem cell-derived conditional medium promotes Leydig cell anti-apoptosis and proliferation via autophagy and Wnt/β-catenin pathway. *J Cell Mol Med*. 2018;22(7): 3614–3626.
23. Abedini A, Zamberlam G, Boerboom D, Price CA. Non-canonical WNT5A is a potential regulator of granulosa cell function in cattle. *Mol Cell Endocrinol*. 2015;403:39–45.
24. Manna PR, Dyson MT, Stocco DM. Role of basic leucine zipper proteins in transcriptional regulation of the steroidogenic acute regulatory protein gene. *Mol Cell Endocrinol*. 2009;302(1):1–11.
25. Sechman A, Pawlowska K, Hrabia A. Effect of 3,3',5-triiodothyronine and 3,5-diiodothyronine on progesterone production, cAMP synthesis, and mRNA expression of STAR, CYP11A1, and HSD3B genes in granulosa layer of chicken preovulatory follicles. *Domest Anim Endocrinol*. 2011;41(3):137–149.
26. Hu MC, Hsu HJ, Guo IC, Chung BC. Function of Cyp11a1 in animal models. *Mol Cell Endocrinol*. 2004;215(1–2):95–100.
27. Taraborrelli S. Physiology, production and action of progesterone. *Acta Obstet Gynecol Scand*. 2015;94 (S161):8–16.
28. Bahr JM, Wang SC, Huang MY, Calvo FO. Steroid concentrations in isolated theca and granulosa layers of preovulatory follicles during the ovulatory cycle of the domestic hen. *Biol Reprod*. 1983;29(2):326–334.
29. Jordan BK, Shen JH, Olaso R, Ingraham HA, Vilain E. Wnt4 overexpression disrupts normal testicular vasculature and inhibits testosterone synthesis by repressing steroidogenic factor 1/beta-catenin synergy. *Proc Natl Acad Sci U S A*. 2003;100(19):10866–10871.
30. Borud B, Mellgren G, Lund J, Bakke M. Cloning and characterization of a novel zinc finger protein that modulates the transcriptional activity of nuclear receptors. *Mol Endocrinol*. 2003;17(11):2303–2319.
31. Hanley NA, Ball SG, Clement-Jones M, et al. Expression of steroidogenic factor 1 and Wilms' tumour 1 during early human gonadal development and sex determination. *Mech Dev*. 1999;87(1–2):175–180.
32. Morohashi KI, Omura T. Ad4BP/SF-1, a transcription factor essential for the transcription of steroidogenic cytochrome P450 genes and for the establishment of the reproductive function. *Faseb J*. 1996;10(14):1569–1577.
33. Guo IC, Shih MC, Lan HC, Hsu NC, Hu MC, Chung BC. Transcriptional regulation of human CYP11A1 in gonads and adrenals. *J Biomed Sci*. 2007;14(4):509–515.
34. Shih MC, Hsu NC, Huang CC, Wu TS, Lai PY, Chung BC. Mutation of mouse Cyp11a1 promoter caused tissue-specific reduction of gene expression and blunted stress response without affecting reproduction. *Mol Endocrinol*. 2008;22(4):915–923.
35. Shih MM, Chiu Y, Hu M, Guo I, Chung B. Regulation of steroid production: analysis of Cyp11a1 promoter. *Mol Cell Endocrinol*. 2011;336(1–2):80–84.