

Retrograde transfer of testosterone to the porcine ovary in follicular and luteal phase of the estrous cycle *in vivo*

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SUMMARY

This study was designed to determine the efficiency and rate of testosterone (T) retrograde transfer during the follicular and luteal phase of the estrous cycle in gilts (n=27). The efficiency and the rate of the retrograde transfer of T from the ovarian effluent into blood supplying the ovary were determined for the first time under *in vivo* conditions. Ovarian arterial blood concentration of T was higher than that in systemic blood during both, the follicular phase (p<0.01) and luteal phase (p<0.0001). The efficiency of the retrograde transfer of T into ovarian arterial blood was not dependent on concentration of testosterone in the ovarian venous blood. However, the efficiency and the rate of the retrograde transfer of T differed between phases of the estrous cycle. The presented results suggest that local retrograde transfer enriched the porcine ovary with an additional amount of T which may affect the ovarian secretory function. *Reproductive Biology* 2006 6:149–159.

Key words: retrograde transfer, testosterone, ovary, gilts

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INTRODUCTION

Steroid hormones were found to affect follicular [2, 3, 15, 16] and luteal [6] steroidogenesis in several species. The retrograde transfer of steroids from the uterine vein into the ovarian artery appears to be one of the factors influencing ovarian function. Indeed, in experiments in which radiolabeled (^3H) progesterone (P_4), testosterone (T) or estradiol (E_2) were infused into the ovarian vein, we demonstrated the retrograde transfer of steroid hormones into ovarian arterial blood [10, 12, 13]. Next, we detected ^3H -T in the ovarian artery branches near the ovary after its infusion into the muscles of the ovarian pedicle [11]. At the same time we studied the anatomy of the vasculature and the distribution of blood flow in the mesovarium [11]. We also showed the accumulation of steroids in mesovarian tissues [8] and the steroid binding by tissue proteins [18]. The local transfer of ovarian steroid hormones from the ovarian vein to ovarian artery in the pig during the periovulatory period was also demonstrated by Hunter et al. [5]. In a view of these results, we presented a concept concerning the mechanism of the steroid hormone permeation into ovarian arterial blood. The mechanism involved the contribution of mesovarian vasculature, interstitial fluid and lymph in the retrograde transfer of steroid hormones [11, 13, 19].

This concept was further confirmed by the results of the *ex vivo* [20] and *in vivo* [21] experiments in which the efficiency and rate of P_4 and E_2 retrograde transfer were estimated. Moreover, differences were found in the retrograde transfer efficiency and rate between the follicular and luteal phase. It is of interest that these coefficients were not dependent on the hormone concentration in ovarian venous blood. There is no information, however, on the intensity and efficiency of the androgen retrograde transfer in cycling gilts. The aim of this study was to examine: 1/ whether a native T is back transferred in periovarian vascular complex during the follicular and luteal phase of the estrous cycle, 2/ what the efficiency and the rate of this transfer are, and 3/ whether these coefficients differ between follicular and luteal phases of the porcine estrous cycle.

MATERIALS AND METHODS

Animals and experimental procedures

The study was conducted in accordance with protocol No 37/N approved by the local Ethical Commission for Animal Experiments. Crossbreed gilts (n=27; bw: 100 kg) after two controlled estrous cycles (21 days) bred in a commercial farm were used in this study. Seventeen gilts (the follicular phase: n=5; the luteal phase: n=12) were used for blood sample collection and 10 gilts (the follicular phase: n=5; the luteal phase: n=5) for the measurement of blood flow in the ovarian artery.

On days 17-19 (the follicular phase) or days 10-12 (the luteal phase) of the estrous cycle, the gilts were premedicated with atropine (0.05 mg/kg, im, Biowet Gorzów Wielkopolski, Poland), azaperone (Stresnil, 2 mg/kg, im, Jansen Pharmaceutica, Beerse, Belgium) and ketamine hydrochloride (Ketamidol, 15 mg/kg, im, Richter Pharma, Austria). A silastic catheter was inserted through the brancho-cephalic vein into the jugular vein [7]. General anaesthesia was induced with thiopental sodium (Thiopental, iv, Biochemie GmbH, Kundl, Austria) and maintained with supplementary doses according to the symptoms observed. The reproductive tract was exposed *via* mid-ventral laparotomy under aseptic condition. Cannulation of vessels (fig. 1) was conducted according to the method described earlier [21]. Briefly, a catheter (o.d.: 1.0 mm; i.d.: 0.8 mm) was inserted into the ovarian vein 2 cm below its connection of ovarian vein with the uterine vein. Next, to enable the collection of ovarian venous blood, the tip of the catheter was situated 4 cm below the ovary where a few ovarian venous branches join the common vessel – the ovarian vein, and 3-4 cm above the connection of the ovarian vein and uterine vein. Another catheter (o.d.: 0.8 mm; i.d.: 0.6 mm) was inserted into a branch of the uterine artery connected by anastomoses with the ovarian artery. The cannulated uterine artery branch was ligatured below anastomoses. This way of cannulating the uterine artery branch enables the collection of arterial blood supplying the ovary. Simultaneous blood samples were collected every 5 min during a 30-min period from the jugular vein and ovarian vein, and arterial blood

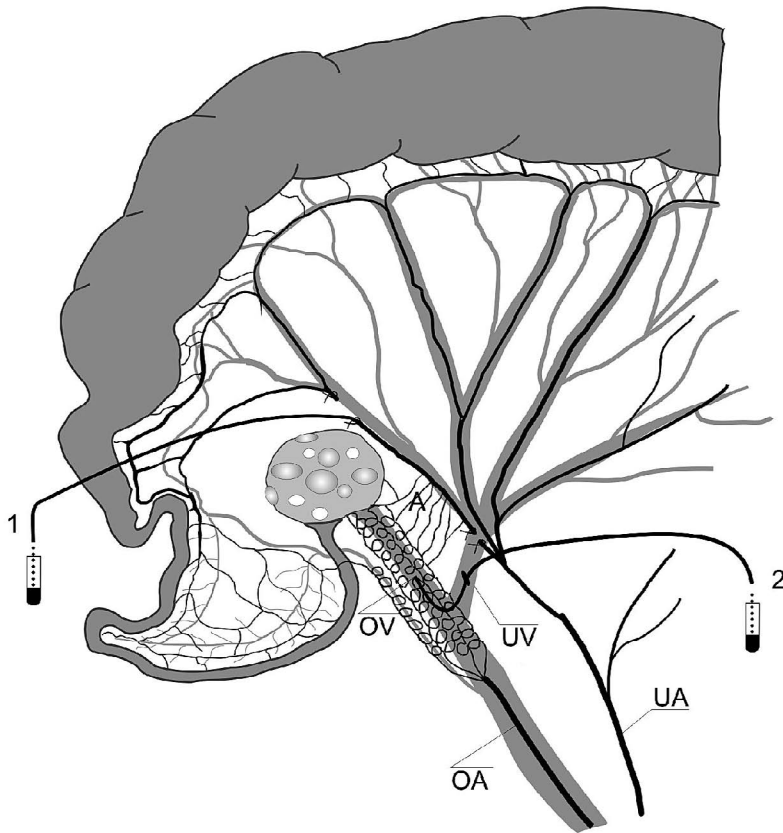


Figure 1. Experimental design to study the efficiency and rate of the retrograde transfer of testosterone in anaesthetized gilts. Catheters were inserted: 1/ into a branch of uterine artery connected by anastomoses with the ovarian artery to collect arterial blood supplying the ovary; 2/ into the ovarian vein to collect ovarian venous blood. OA: ovarian artery; UA: uterine artery; A: anastomoses connecting the ovarian artery and branch of the uterine artery; OV: ovarian vein; UV: uterine vein.

supplying the ovary from the branch of the uterine artery. The heparinized blood was centrifuged and the blood plasma was stored at -70°C until hormonal assays were completed.

Radioimmunoassay of testosterone

The plasma concentration of samples for T was determined by radioimmunoassay [9]. The antibodies were obtained from the Department

of Animal Physiology, University of Warmia and Mazury, Olsztyn, Poland. Antibodies against testosterone (code BS/88/312) were characterized earlier [22]. The extraction efficiency for T was 68.7%. The sensitivity of the assay was: 2.3 pg/ml. The intra- and inter-assay coefficients of variations were: 4.1% and 7.0%, respectively.

Blood flow measurement

After laparotomy, an electric transducer (2 mm, Narcomatic, USA) was implanted bilaterally on the ovarian artery, 3-5 cm below its branching. The reproductive tract was replaced in the abdominal cavity in its physiological position. The blood flow in the ovarian artery was recorded by an electromagnetic flowmeter (RT 500 Narcomatic) for 60 min after the control period for stabilization (30-60 min).

Calculation of the results and statistical analysis

The efficiency and rate of the retrograde transfer of T were calculating according to the following formula as described earlier [20]:

$$E = (a-b) \times 100/c$$

E is the efficiency of the retrograde transfer of the hormone to the ovary expressed as a percentage of the hormone concentration in the ovarian venous effluent. Next, **a** is the hormone concentration in blood supplying the ovary (collected from the ovarian artery 1-2 cm below the ovary, from cannulated anastomoses). Then, **b** is the hormone concentration in blood collected from the jugular vein (representing systemic blood reaching the ovarian artery). Finally, **c** is the hormone concentration in the ovarian venous effluent.

The rate of the retrograde transfer of T from the ovarian venous effluent to the ovary was calculated according to the following formula:

$$R = (a-b) \times V$$

R is the rate of the retrograde transfer of hormone expressed in pg per 1 min, **V** is the volume of blood plasma flowing into the ovarian artery in millilitres per 1 min, and **a** and **b** are as defined above. All hormone concentrations are measured in pg per 1 ml of blood plasma.

All the data are presented as means±SEM. The concentrations of T in blood samples collected from the ovarian artery were compared with T concentration in blood collected from the jugular vein (systemic blood reaching the initial part of the ovarian artery) by paired *t* test. The mean blood flow and also the T retrograde transfer efficiency and rate were compared between the phases of the estrous cycle by unpaired *t* test. Analyses were performed using Prism (Prism 4.0 GraphPad Software, San Diego, USA).

RESULTS

The mean concentration of T in ovarian venous blood was higher ($p<0.0001$) in the luteal phase (169.5 ± 18.7 pg/ml) than in the follicular phase (42.4 ± 3.3 pg/ml). During the follicular phase ($p<0.01$) and luteal phase ($p<0.0001$), the concentration of T was higher in ovarian arterial blood (see M&M) than in jugular vein (peripheral blood; tab. 1, fig. 2).

The efficiency of the retrograde transfer (E) of T into ovarian arterial blood was not dependent on the concentration of testosterone in the ovarian venous blood. However, the efficiency and the rate (R) of testosterone

Table 1. Testosterone (T) concentrations (means±SEM) in jugular and ovarian veins, and ovarian artery as well as efficiency and rate of the T retrograde transfer

	Testosterone	
	Follicular phase	Luteal phase
Jugular vein (pg/ml)	12.2±0.5	13.3±0.1
Ovarian artery (pg/ml)	14.2±0.6*	16.3±0.4*
Ovarian vein (pg/ml)	42.4±3.3	169.5±18.7 ^a
Mean efficiency (E) of the retrograde transfer of T (%)	3.72±0.7	1.85±0.3 ^b
Mean rate (R) of the retrograde transfer of T (pg/min)	20.38±4.1	47.4±5.9 ^c

*depicts the differences between ovarian artery and jugular vein in T level during the follicular phase ($p<0.01$) and luteal phase ($p<0.0001$); ^{a,b,c} depicts differences in examined parameters between the follicular and luteal phase; a: $p<0.0001$; b: $p<0.05$; c: $p<0.01$

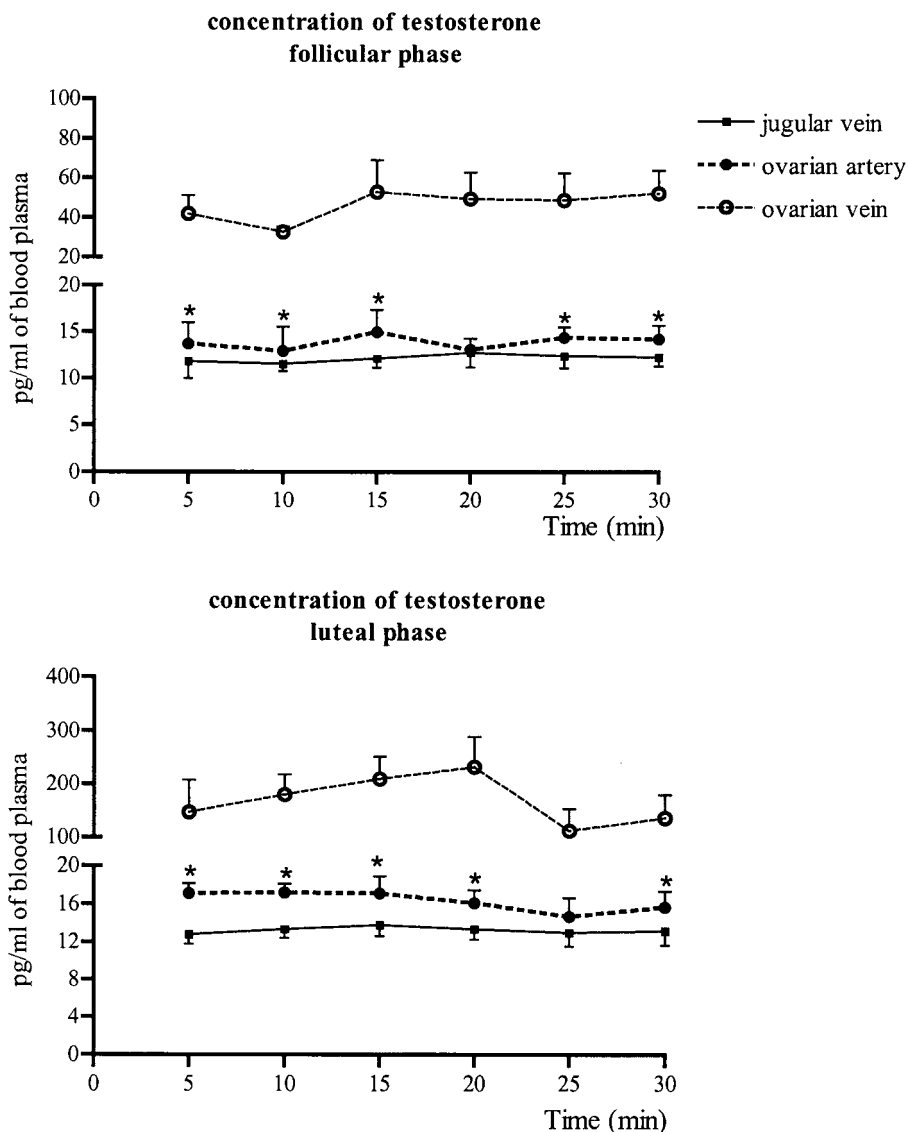


Figure 2. Plasma testosterone concentration (means±SEM) during the follicular and luteal phase of the porcine estrous cycle. Simultaneous samples were taken from a branch of the uterine artery (above anastomoses between branches of the ovarian and uterine artery), from the ovarian and the jugular vein during the follicular and luteal phase of the estrous cycle in the pig. *depicts differences in T concentration between ovarian artery and jugular vein during the follicular phase ($p < 0.01$) and luteal phase ($p < 0.0001$). The obvious differences in T concentration between ovarian vein and other vessels are not depicted.

retrograde transfer differ between the follicular and luteal phase of the estrous cycle (tab. 1). The mean blood flow in the ovarian artery was 19.9 ± 0.3 ml/min in the follicular phase and 25.9 ± 0.6 ml/min in the luteal phase of the estrous cycle ($p < 0.0001$).

DISCUSSION

In many earlier studies the retrograde transfer of steroid hormones was characterized by the percentage of radioactive steroids permeating into ovarian arterial blood following steroid infusion into the ovarian vein [1, 10, 12]. In ewes, such retrograde transfer of radioactive P_4 expressed as the percentage was 0.5-1% [1]. In pigs, the transfer of radioactive T differed among particular days of the estrous cycle [10], however, exact characteristics of the T retrograde transfer were not included. In these reports only a direct transfer of hormone from ovarian venous blood into ovarian arterial blood was analyzed. This classic counter current exchange, however, is only a part of a very complex process, which also involves an indirect steroid transfer from blood and lymph flowing through the periovarian vascular complex [20]. Our recent study [23] revealed that the efficiency of the retrograde transfer of P_4 and E_2 into arterial blood supplying the ovary was many times higher than that estimated for radiolabeled hormones during the both phases of the porcine estrous cycle [10, 12].

In the current study, the efficiency of retrograde transfer of T, during both phases of the estrous cycle, was lower than those of P_4 and E_2 [21]. This may be caused by differences in the steroid molecule structure. McCracken et al. [17] demonstrated a different efficiency of retrograde transfer for particular pairs of steroids (P_4 and 20α -dihydroprogesterone, androstenedione and T, estrone and E_2). These authors showed a more effective transfer of less polar forms of steroids and postulated that there is a relationship between the steroid structure and its permeation into arterial blood.

Additional hormonal supply to the ovary depends on the efficiency of hormone retrograde transfer in the periovarian vascular complex and ovarian blood flow i.e. the rate of the retrograde transfer [19, 20, 21]. The rate of the

T retrograde transfer found in this study demonstrated that the amount of hormone additionally transferred into the ovary was not negligible.

The influence of ovarian steroid hormones on steroidogenesis has been reported to be stimulatory or inhibitory [14, 15]. Retrograde transfer of steroid hormones elevates their concentration in ovarian arterial blood and, in consequence, in the ovary. For that reason, it was postulated that the secondary utilization of the T transferred to the ovary contributed to the regulation of ovarian steroidogenesis by local regulatory short loop [11, 19]. Testosterone retrograde transferred to the ovary may be involved in the regulation of both P_4 and E_2 synthesis by granulosa cells. The stimulatory effect of T on E_2 secretion in bovine follicles was dose-dependent² and could result from T being a substrate for E_2 synthesis. Moreover, a long lasting exposure of bovine granulosa cells to T, increased the activity of aromatase¹. On the other hand, low physiological concentration of T inhibited P_4 secretion by granulosa cells of small bovine follicles [3]. Since the T effects on E_2 and P_4 secretion were more pronounced in small follicles, elevated concentrations of T were suggested to participate in the selection of developing follicles¹.

The inhibitory effect of T on P_4 synthesis was also found in bovine luteal cells. This effect may be caused by several mechanisms including a decrease in P450_{scc} expression and a suppression of 3 β -HSD activity [4]. The described T effects on estrogens and progestagens synthesis/secretion may reflect the processes present under *in vivo* conditions. Therefore, the permanent retrograde transfer of T to the ovary appears to play an important role in the regulation of ovarian function.

In the current paper, we demonstrated that T is back transferred in the periovarian vascular complex during the estrous cycle. Moreover, the efficiency and rate of the T retrograde transfer differed between the follicular and luteal phase of the porcine cycle. These results suggest that local retrograde transfer enriched the porcine ovary with an additional amount of T which may affect the secretory function of the ovary.

¹Bassini G, Grasselli F, Baratta M, Tamanini C 1994 Testosterone modulates estradiol-17 β production in bovine granulosa cells cultured in vitro. *Journal of Reproduction and Fertility, Abstract Series*, abs. 14.

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REFERENCES

1. Einer-Jensen N, McCracken JA **1981** The transfer of progesterone in the ovarian vascular pedicle of the sheep. *Endocrinology* **109** 685-690.
2. Fortune JE, Vincent SE **1983** Progesterone inhibits the induction of aromatase activity in rat granulosa cells in vitro. *Biology of Reproduction* **28** 1078-1089.
3. Grasselli F, Bassini G, Bussolati S, Tamanini C **1995** Follicle-stimulating hormone – testosterone interaction in modulating steroidogenesis in bovine granulosa cells. I. Effect on progesterone. *European Journal Endocrinology* **132** 759-764.
4. Guo IC, Wu LS, Lin JH, Chung BCh **2001** Differential inhibition of progesterone synthesis in bovine luteal cells by estrogens and androgens. *Life Sciences* **68** 1851-1865.
5. Hunter RHF, Cook B, Poyser NL **1983** Regulation of oviduct function in pigs by local transfer of steroids and prostaglandins: a mechanism of influence sperm transport. *European Journal of Obstetrics and Gynecology: Reproductive Biology* **14** 225-232.
6. Jarry H, Einspanier A, Kanngieser L, Dietrich M, Pitzel L, Holtz W, Wuttke W **1990** Release and effects of oxytocin on estradiol and progesterone secretion in porcine corpora lutea as measured by an *in vivo* microdialysis system. *Endocrinology* **126** 2350-2357.
7. Kotwica J, Krzymowski T, Dębek J **1978** Cannulation of venous vessels in swine for endocrine studies (in Polish). *Medycyna Weterynaryjna* **34** 118-120.
8. Kotwica J, Krzymowski T, Stefańczyk S, Nowicka R, Dębek J, Czarnocki J, Kuźnia S **1981** Steroid concentrating mechanism in the sow's ovarian vascular pedicle. In *Advances of Physiological Sciences, Advances in Animal and Comparative Physiology* **20** 149-152. Eds Pethes G and Frenio VL Akadémiai Kiadó, Budapest, Hungary.
9. Kotwica J, Williams GL, Marchello MJ **1982** Countercurrent transfer of testosterone by the ovarian vascular pedicle of the cow: evidence for relationship to follicular steroidogenesis. *Biology of Reproduction* **27** 778-789.
10. Krzymowski T, Kotwica J, Stefańczyk S **1981** Venous-arterial counter current transfer of ³H-testosterone in the vascular pedicle of the sow ovary. *Journal of Reproduction and Fertility* **61** 317-323.
11. Krzymowski T, Kotwica J, Stefańczyk J, Czarnocki J, Dębek S **1982** A subovarian exchange mechanism for the countercurrent transfer of ovarian steroid hormones in the pig. *Journal of Reproduction and Fertility* **55** 457-465.
12. Krzymowski T, Kotwica J, Stefańczyk S, Dębek J, Czarnocki J **1982** Steroid transfer from the ovarian vein to the ovarian artery in sow. *Journal of Reproduction and Fertility* suppl. **40** 179-191.
13. Krzymowski T, Kotwica J, Stefańczyk-Krzybowska S **1990** Uterine and ovarian counter current pathways in the control of ovarian function in the pig. *Journal of Reproduction and Fertility* **40** 179-191.

14. Leung PCK, Armstrong DT **1979** A mechanism for the intraovarian inhibitory action of estrogen on androgen production. *Biology of Reproduction* **21** 1035-1042.
15. Leung PCK, Goff AK, Kennedy TG, Armstrong DT **1978** An intraovarian inhibitory action of estrogen and androgen production *in vivo*. *Biology of Reproduction* **19** 641-647.
16. Maggofin DA, Ericson GF **1981** Mechanism by which oestradiol-17 β inhibits ovarian androgen production in rat. *Endocrinology* **108** 962-969.
17. McCracken JA, Schramm W, Einer-Jensen N **1984** The structure of steroids and their diffusion through blood vessel walls in a counter-current system. *Steroids* **43** 293-303.
18. Stefańczyk S **1984** Mechanism of binding of testosterone by ovarian vascular pedicle and uterus. (in Polish) *Zeszyty Problemowe Postępów Nauk Rolniczych* **309** 181-189.
19. Stefańczyk-Krzymowska S, Krzymowski T **2002** Local adjustment of blood and lymph circulation in the hormonal regulation of reproduction in female pigs – facts, conclusions and suggestions for future research. *Reproductive Biology* **2** 115-132.
20. Stefańczyk-Krzymowska S, Krzymowski T, Wąsowska B, Chłopek J **2002** Retrograde transfer of ovarian steroid hormones to the ovary in the porcine periovarian vascular complex. *Experimental Physiology* **87** 361-371.
21. Stefańczyk-Krzymowska S, Wąsowska B, Chłopek J, Grzegorzewski W **2004** Retrograde transfer of steroid hormones to the ovary in luteal and follicular phases of porcine oestrous cycle *in vivo*. *Experimental Physiology* **89** 140-144.
22. Szafrńska B, Zięcik A, Okrasa S **2002** Primary antisera against selected steroids or proteins and secondary antisera against γ -globulins – an available tool for studies of reproductive process. *Reproductive Biology* **2** 187-203.