A subovarian exchange mechanism for the countercurrent transfer of ovarian steroid hormones in the pig

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Summary. Sow ovaries with their ovarian pedicle were isolated and supplied with blood from the middle uterine artery. During the 30 min of infusion with $[^3]$H)testosterone into the ovarian vein 3 cm below the ovary and for the 30 min after the infusion, radioactivity was detected in tissue fluid 3 cm laterally from the ovarian vein and artery. When $[^3]$H)testosterone was infused into the muscles of the ovarian pedicle radioactivity was detected in the ovarian artery branches near the ovary. Of the total amount of blood entering the ovarian artery $63.7\pm4.2\%$ reached the ovary and $36.3\pm3.1\%$ travelled to the muscles and connective tissue of the ovarian pedicle. It was demonstrated that the ovarian arterial branches supplying the ovarian pedicle muscles after capillarization form the veins which descend within the ovarian artery network, redivide and create the venous mesh covering the spiralling ovarian artery branches.

It is suggested that a special subovarian exchanging mechanism exists in the ovarian pedicle for countercurrent transfer of ovarian steroid hormones and that ovarian function can thereby be regulated.

Introduction

The countercurrent transfer of testosterone from the spermatic vein to the artery has been demonstrated in rats (Free, Jaffe, Jain & Gomes, 1973; Free & Jaffe, 1975, 1978), rams (Ginther, Mapletoft, Zimmermann, Meckley & Nuti, 1974), monkeys (Dirschke, Walsh, Mapletoft, Robinson & Ginther, 1975), man (Bayard, Boulard, Huc & Pantonniere, 1975), bulls (Amann & Ganjam, 1976) and rabbits and guinea-pigs (Free, 1977).

Barrett et al. (1971), Goding, Baird, Cumming & McCracken (1972) and McCracken et al. (1972) showed how prostaglandin F-2α was transferred from the uterine vein of the ewe to the associated ovarian artery and thence to the ovary. The luteolytic effect of prostaglandin when infused into the anterior uterine vein of the ewe or sow has been confirmed by several workers (Gleeson, 1974; Goding, 1974; Land, Baird & Scaramuzzi, 1976; Lindloff, Holtz, Elsaesser, Kreikenbaum & Smidt, 1976; Krzymowski, Kotwica, Okrasa, Doboszyńska & Zięcik, 1978; Kotwica, 1980). Inert radioactive gases, xenon-133 or krypton-85, infused into the uterine horn of the mouse, hamster and guinea-pig or into the utero-ovarian vein of sheep or women reach higher concentrations in the adjacent rather than in the opposite ovary (Einer-Jensen, 1974; Einer-Jensen & McCracken, 1977; Bendz, Einer-Jensen, Lundgren & Janson, 1979).

Countercurrent transfer of progesterone in the sheep ovarian pedicle was first reported briefly by McCracken & Einer-Jensen (1976) and confirmed by Walsh, Yutrzenka & Davis
(1979). Oestradiol-17β and testosterone have been shown to pass from the ovarian vein into the ovarian artery in cows (Krzymowski et al., 1982a; Kotwica, Williams & Marchello, 1981a). In the pig testosterone, progesterone and oestradiol are transferred from the uterine vein into the ovarian artery in the ovarian pedicle (Krzymowski, Kotwica & Stęfańczyk, 1979, 1981a; Kotwica et al., 1981b; Krzymowski, Kotwica, Stęfańczyk, Dębek & Czarnocki, 1981b, 1982b); We have now attempted to explain the mechanism of these processes.

Materials and Methods

The pigs were of the Polish Large White breed and weighed 120–150 kg. Oestrus was observed 6–7 days after weaning and was designated Day 0. On various days throughout the oestrous cycle laparotomies were performed and ovaries with their associated ovarian pedicle were isolated by the technique of Krzymowski et al. (1981a), modified as described by Krzymowski et al. (1981b, 1982b). All experiments on the isolated ovary and ovary pedicle were performed under the control of a stereomicroscope at × 12.5 magnification. Autologous arterial blood from the uterine artery was supplied to the ovary by means of a peristaltic miniflow pump (type 304 ELMED, Unipan, Poland). The flow rate was 7.36 ml/min. The pressure in the ovarian artery approximately 10 cm below the convoluted area and measured by a direct method was 80–100 mmHg (10.64–13.3 kPa).

Anatomy of the ovarian pedicle vasculature

An ovary with its pedicle continuously supplied with arterial blood was placed under the stereomicroscope and the coils of the ovarian artery branches were exposed by means of an electrocoagulator and photographed. After some time blood flow was stopped and physiological saline (9 g NaCl/l) with heparin (7 i.u./ml) at approximately 39°C was introduced into the ovarian artery under the same pressure. After 3 min of circulation of the fluid, when the blood vessels were completely discoloured as checked under the stereomicroscope, a ligature was placed round the pedicle at the hilum of the ovary and the latter was then cut off. Two different experiments were done. (1) Arterial blood flow was again connected to the ovarian artery and the consequent filling of the blood vessels and their course were followed under the microscope and some chosen fields of vision were photographed. (2) Arterial blood was introduced by syringe into the ovarian vein and the sequence of filling up of blood vessels in the ovarian pedicle was observed under the stereomicroscope. After the experiments the ovarian vein was incised lengthwise and its wall was photographed to demonstrate the number and size of the veins draining into it.

Distribution of blood flow to the ovary and its pedicle

For determination of the blood volume flowing through the ovarian artery and that supplying the muscles and connective tissue of the ovary pedicle and the ovarian tissue, a clamp regulating blood flow by means of a screw was placed below the ovary and the ovary was then cut off. The clamp regulated the blood flow to maintain blood pressure in the ovarian artery at the level recorded in earlier experiments (80–100 mmHg); the pressure was checked with a mercury manometer. Blood flowing from the utero–ovarian vein or from incised ovarian artery branches was collected separately over 30 min and its volume was measured.

Determination of [3H]testosterone penetration from the ovarian vein to the tissues of the ovarian pedicle

In 5 experiments the ovary and its pedicle were isolated and supplied with arterial blood. Cannulae for ovarian venous blood collection or for steroid hormone infusion were implanted
into the utero-ovarian vein as previously described (Krzymowski et al., 1982b). Using an electrocoagulator the ovarian pedicle muscles were incised 1 cm along the pedicle below the ovary and at a lateral distance of 3 cm from the ovarian vein. Pushing aside the muscles a small hollow was prepared. In the field of vision under the stereomicroscope, venous, arterial and lymphatic vessels were seen as well as the interstitial fluid accumulating in small quantities. The small hollow formed was completely free of bleeding as checked under the microscope. 

\[1,2,6,7^3H\]Testosterone (sp. act. 81 Ci/mmol; Radiochemical Centre, Amersham, Bucks, U.K.), purified on thin-layer chromatographic plates (Krzymowski et al., 1981a) and dissolved in 3 ml saline, was infused into the ovarian vein through the cannula for 30 min at a rate of 0-1 ml/min. During the infusion (5 \times 10^7 c.p.m. corresponded of 166 ng testosterone) and for 30 min after the end of the infusion, autologous arterial blood with added heparin was dropped by syringe into the hollow prepared earlier in the ovarian pedicle tissue. Blood was administered in 0-2 ml portions and was in contact with the surrounding cells for 15 sec before being collected into test tubes; the samples were pooled for every 10-min period. Estimation of radioactivity in the blood samples was determined by the method described previously (Krzymowski et al., 1981a).

**Determination of \[^1\text{H}\]testosterone penetration from the tissue surrounding the ovarian pedicle vessels to the ovarian artery**

In 5 experiments the ovaries were isolated and supplied with arterial blood. The ovarian artery branches were exposed by means of the electrocoagulator 3 cm below the ovary. At 3 cm below the ovary and 3 cm lateral from the ovarian vein and convoluted ovarian artery branches, an injection needle was inserted into the musculature of the uterine broad ligament. Through this needle \[^3\text{H}\]testosterone (~5 \times 10^7 c.p.m. corresponding to 166 ng), purified before experiment and dissolved in 1.5 ml saline, was infused for 30 min at a rate of 0.05 ml/min. During and 30 min after the infusion, blood samples from the incised ovarian artery branch were collected as described previously (Krzymowski et al., 1982b).

**Results**

**Anatomy of the ovarian pedicle vasculature**

There were several ovarian artery branches on the dorsal side of the ovarian vein or next to it which formed a multilayered convolution resembling in arrangement the small intestine in the abdominal cavity (Pl. 1, Fig. 1). From the muscle of the ovarian pedicle, single venous vessels, or several in a bundle, descended directly onto the ovarian artery branches and after ramification a fine venous network extensively enmeshed the surface of the coiled artery branches (Pl. 1, Figs 1 and 2). The fine network of venous vessels drained into vessels with a larger diameter and these in turn flowed into the ovarian vein. A fragment of the ovarian vein wall with the outlets of the minute venules is shown in Pl. 1, Fig. 4.

During contraction of the branches of the ovarian artery walls after electric or thermal stimulation, the lumen of part or all of the venous vessels forming a network opposite to the arterioles closed. Plate 1, Fig. 3 shows the network of minute venous vessels covering the loops of the ovarian artery branches when blood flow restricted by the contraction of the artery branch wall. Between the coils of the ovarian artery branch covered with fine venous vessels there were numerous minute and larger lymphatic vessels (Pl. 1, Figs 1 and 3). Many other fine lymphatic vessels made a superficial net covering the ovarian pedicle.

The main vessels of the ovarian pedicle are covered on the abdominal and lateral sides with highly developed muscles. The muscles and connective tissue are supplied with blood from branches of the ovarian artery. When the ligature was placed below the ovary and the latter was then cut off, blood introduced into the ovarian artery filled the venous vessels of the vascular...
pedicle and flowed out of the ovarian vein. When blood was washed out with saline (Pl. 2, Figs 5 and 7) and filled with blood from the ovarian artery, with flow to the ovary closed off (Pl. 2, Figs 6 and 8), small venules covering the walls of the coiled arterioles were filled and became visible as well as the larger vessels into which they drained. When the ovarian pedicle was washed out with saline and the ovarian vein was filled with blood from a syringe, the contrary direction of blood flow was obtained, small venules covering the walls of the arterioles were filled and visible, while the arterioles were empty (Pl. 2, Fig. 9). In this experiment vessels filled up in the following order: (i) venous vessels lying between the branches of the ovarian artery; (ii) fine venous vessels enmeshing the branches of the ovarian artery; (iii) larger venous vessels lying between the branches of the ovarian artery and ovarian pedicle muscles; (iv) capillaries of muscles shown by change of colour of the muscles; and (v) the ovarian artery with blood flowing out.

These experiments indicate that the fine venous vessels enmeshing the branches of the ovarian artery are the secondary network of venous vessels from which the larger venous vessels are originated that pass to the ovarian vein. It demonstrates the existence of a rete mirabile pattern of venous vessels, i.e. a portal subovarian vascular system.

**Distribution of blood flow to the ovary and its pedicle**

In 5 experiments an average of 63.7 ± 4.2 (s.e.m.)% of the blood entering the ovarian artery travelled to the ovary and 36.3 ± 3.1% to the muscular and connective tissue of the pedicle.

**Determination of [³H]testosterone penetration from the tissue surrounding the ovarian pedicle vessels to the ovarian artery**

[³H]Testosterone infused into the muscles of the ovarian pedicle permeated to the blood of the coiled branches of the ovarian artery and the mean ± s.e.m. values at 10, 20 and 30 min were 1128 ± 422, 2204 ± 985 and 2990 ± 1464 c.p.m./ml during the infusion and 1994 ± 770, 1496 ± 433 and 1050 ± 324 c.p.m./ml after the infusion respectively.

**Determination of [³H]testosterone penetration from the ovarian vein to the tissue of the ovarian pedicle**

At 10, 20 and 30 min during and after the end of the infusion the mean ± s.e.m. radioactivity values were 453 ± 176, 240 ± 65, 1617 ± 1347 and 907 ± 693, 711 ± 516 and 650 ± 443 c.p.m./ml respectively, indicating that the hormone had diffused from the ovarian vein and penetrated the tissue surrounding the ovarian vein and artery.

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**PLATE 1**

Fig. 1. Ovarian artery branches which form multilayered convolutions. The fine thin-walled mesh of venous vessels covers the surface of the artery network extensively. a, A lymph vessel crossing the artery branches.

Fig. 2. One helix of an ovarian artery branch and the fine venous mesh covering its surface.

Fig. 3. The influence of contraction of the wall of the artery branch and the consequent restriction of blood flow within the venous mesh covering the loops of the ovarian artery. a, A lymph vessel crossing the artery branches.

Fig. 4. An internal surface of a fragment of the ovarian vein wall. Many outlets of the minute venules are visible.
PLATE 1

(Facing p. 460)
Discussion

The physiological role of the intimate association of the extensive convolutions of the ovarian artery branches with the ovarian veins is not known. Reynolds (1948) investigated spiral ovarian arteries in rabbits and reported that the spirals reduced blood flow and equalized distribution of blood to the ovarian stroma. Del Campo & Ginther (1973) studied the morphology of the uterine vasculature in horses, sheep and pigs by latex injections and concluded that the contact area between the vein and artery was increased by the tortuous path of the artery over the surface of the vein in sheep and by the location of the artery between 2 or 3 channels of the vein in pigs. The ovarian artery in horses did not contact the ovarian vein.

In our previous (Krzymowski et al., 1979, 1981a, 1982b) and present experiments high radioactivity was demonstrated in ovarian arterial blood as late as 30 min after the end of steroid hormone infusion into the ovarian vein. The demonstration that testosterone infused into the ovarian vein penetrates not only to the ovarian artery blood but also into the surrounding smooth muscles and connective tissues and that labelled hormone infused into the muscles of the ovarian pedicle diffuses into the blood of the ovarian artery suggests that the radioactivity present in the blood of the ovarian artery may be the result of the direct penetration of steroid hormone through the apposed walls of the ovarian artery and ovarian vein or its indirect passage via the interstitial fluid and the system of fine venous vessels enmeshing the microvascular branches of the ovarian artery. Direct permeation is supported by histological observations of the local thinning of the walls of veins and arteries at the site of their immediate contact (Del Campo & Ginther, 1974; Lee & O'Shea, 1975).

The existence of a venous network covering the spiralling ovarian artery branches may be of particular importance in the mechanism of permeation of steroid hormones into the blood of the ovarian artery. It consists of fine venous vessels which originate from venous vessels descended from the muscle layer and connective tissue of the ovarian pedicle. The minute veins of the mesh join together to form larger vessels adhering to the arterioles which flow into the ovarian vein (Text-fig. 1).

The androgen concentrations in the ovarian artery were significantly higher than those in the uterine blood supplying the ovarian artery and in the ovarian vein (Kotwica et al., 1981b). However, the high steroid values in the lymph and tissue fluid collected from the pedicle close to the ovary (Kotwica et al., 1981b) suggest that ovarian steroid hormones from the tissue fluid and lymph may be the main source for the increased concentrations in the ovarian artery. This view is supported by the results of Lindner, Sass & Morris (1964) who found far greater concentrations of oestrogen and progesterone in the ovarian lymph than in the systemic circulation of the ewe. Significantly higher values of all steroid hormones in lymph taken at the ovary base than in blood flowing out or reaching the ovary (Kotwica et al., 1981b) confirmed that the ovary releases a great amount of steroid hormones particularly to the intercellular fluid. The mechanism of this process has been explained by many experiments. Short (1962, 1964)

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PLATE 2

Figs 5 and 7. The loops of an ovarian artery after a 3-min infusion of physiological saline instead of blood into the ovarian artery. There is no blood in the venous mesh covering the arterial network.

Figs 6 and 8. The same loops of the ovarian artery shown in Figs 5 and 7. After ligation and removal of the ovary, arterial blood flow was reconnected to the ovarian artery. The blood-filled fine venous mesh on the ovarian artery branches has become visible.

Fig. 9. The ovarian artery shown in Fig. 7. After ligation and removal of the ovary, arterial blood flow was reconnected to the ovarian vein. There is no blood in the ovarian artery but the fine venous mesh on the ovarian artery branch is filled.
and YoungLai & Short (1970) have pointed out that secretions of the well vascularized theca interna cells pass directly into the tissue fluid and capillaries surrounding them. Baird (1977) demonstrated that secretion of oestradiol was depressed during the infusion of antibodies to testosterone and suggested that androgens synthesized by the theca interna leave the cell and traverse the extracellular space and basement membrane before being converted to oestrogen by the avascular granulosa cells. Tissue fluid from the ovary is drained by a net of fine lymphatic vessels which, in the sow, run close to the vein and the ovarian artery and also make a fine net draining the whole ovarian pedicle.

Our present results show that the tissues of ovarian pedicle are supplied with arterial blood by branches of the ovarian artery and 36.3% of this blood passes to the muscles and connective tissue of the ovarian pedicle. We also show that introduction of labelled testosterone into the ovarian pedicle muscles led to its appearance in the ovarian artery.
All the foregoing information indicates that within the ovarian pedicle there may be two pathways of steroid hormone penetration: (1) a direct pathway from the ovarian vein to the arterial blood supported by anatomical observations of connections (Del Campo & Ginther, 1974; Lee & O'Shea, 1975); and (2) an indirect pathway into the arterial blood through the system of capillary and lymphatic and venous vessels of the pedicle. With our in-vitro system we have been unable to show any differences according to the stage of the oestrous cycle but this is probably due to the extreme contractility of the arterial vessels.

Ford, Weber & Stormshak (1976) demonstrated that a uterine artery adjacent to an ovary bearing the corpus luteum in heifers responds to in-vitro periarterial nerve stimulation with greater increases in constriction than the contralateral uterine artery. Kalsner (1969), McKercher, Van Order, Bhatnagar & Burke (1973), Resnik, Killam, Battaglia, Makowski & Meschia (1974), Caton, Abrams, Clapp & Barron (1974), Ford et al. (1976, 1977a, b), Krall, Tuck & Korenman (1977) and Ford, Chenault & Echternak (1979) have demonstrated the role of progesterone and oestrogen in uterine vasoconstriction and described the mechanism of this regulation. Lee & Novy (1978) and Varga & Greenwald (1979) demonstrated that LH and ACTH may directly stimulate ovarian blood flow and, depending on the stage of the cycle, may participate in ovarian steroid secretion. The control of ovarian secretory function by an extrahypophysial neural mechanism (Kawakami, Kubo, Uemura & Nagase, 1979) may also be via the autonomic innervation of the ovarian pedicle vasculature. Many experiments have demonstrated that PGF-2α reduces blood flow to the ovary and suggested that PGF-2α affects the vascular component of the corpus luteum (Pharriss, Cornette & Gutknecht, 1970; Nett, McClellan & Niswender, 1976; Wehrenberg, Dierschke & Wolf, 1979).

The ability of the ovarian artery to absorb ovarian steroid hormones may be considered as a local means of regulation of the function of the ovary. Leung, Goff, Kennedy & Armstrong (1978), Leung, Henderson & Armstrong (1979) and Leung & Armstrong (1979a, b) showed that excess oestrogen production in the membrana granulosa cells may inhibit formation of androgens. Moreover, Caffrey, Nett, Abel & Niswender (1979) demonstrated that the 3β-hydroxy-Δ5-steroid dehydrogenase-Δ5-Δ4-isomerase complex involved the conversion of pregnenolone to progesterone and was inhibited by feedback of its own product, that is progesterone. Thus, if the steroid hormone levels can locally regulate the formation of their precursors (progesterone and testosterone) and in this way limit the final production of these hormones, then the ability to reabsorb steroid hormones in the ovarian pedicle demonstrated in this study must also affect ovarian secretion and is an essential regulatory mechanism.

The fact that the mechanism of subovarian reabsorption of steroid hormones is not a simple diffusion through the walls of the ovarian vein and artery, but is associated with an anatomical modification and adaptation of the whole circulatory system in the ovarian pedicle, is evidence of the significant and special role of this mechanism in regulatory processes.

Based on our results and those of other authors we suggest that there may be the following control processes:

1. Local regulation
   (a) regulation of hormonal secretion at the cellular level based on negative feedback between the secretory ovarian cells (the shortest regulatory loop);
   (b) regulation by organs via a subovarian exchange mechanism of reabsorption and secondary utilization of hormones produced in the ovary, based on the regulatory interactions of the ovary and the uterus (short regulatory loop);

2. Central regulation
   (a) hormonal regulation through the hypothalamus and hypophysis based on negative feedback (long regulatory loop).
   (b) regulation through the cortical centres and the limbic system and further via the nervous pathways which reach indirectly to the reproductive organs and their vascularization (longest regulatory loop).
Many of the papers cited above indicate that a subvarian regulatory system may be universal, occurring with particular modifications in the various species, and such a system is probably analogous to that in the pampiniform plexus of males.

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References


Steroid exchange mechanism in sow ovarian pedicle


Krzymowski, T., Kotwica, J., Stefańczyk, S., Dębek, J. & Czarnocki, J. (1982b) Steroid transfer from the ovarian vein to the ovarian artery in the sow. J. Reprod. Fert. 65, 000–000.


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