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Local transfer of testosterone and aromatase activity in the spermatic cord in wild boar/pig hybrids in short-daylight and long-daylight periods

Anna Tabęcka-Łonczyńska [a,c,*], *Przemysław Gilun* [b], *Stanisława Stefańczyk-Krzymowska* [b], *Marek Koziorowski* [a,c]

[a] *Department of Animal Physiology and Reproduction, University of Rzeszów, ul. Rejtana 16C, 35-959 Rzeszów, Poland*

[b] *Department of Local Physiological Regulation, Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Tuwima 10, 10-747 Olsztyn, Poland*

[c] *Centre of Applied Biotechnology and Basic Sciences, University of Rzeszow, Werynia, 36-100 Kolbuszowa, Poland*

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ABSTRACT

The local transfer of testosterone (T) and immunolocalization of cytochrome P450 aromatase (P450arom) in the spermatic cord vessels of ten male wild boar × domestic pig hybrids were examined in December (short-daylight period) and June (long-daylight period). Total T concentration was determined in the jugular vein (JV) and free T concentration was estimated in the common carotid artery (CA), branches of the testicular artery supplying the testis (TA) and epididymis (EA), as well as in testicular veins draining blood from the testis (TV) and spermatic cord (SV). P450arom was immunolocalized in the arterial and venous vessels of the spermatic cord. The concentrations of total T in the JV and free T in the CA did not differ between the examined periods. However, in December, free T concentrations in the TA and EA were higher ($p < 0.01$–0.001) than in the CA. In June, free T concentration was higher ($p < 0.01$) in EA than in CA and TA. The concentrations of free T in the TV and SV were higher ($p < 0.001$) than in the JV regardless of the period. Also, free T concentration in the SV was higher ($p < 0.05$) in June than in December. P450arom was expressed in all layers of the arterial and venous vessels of the spermatic cord. In June, the intensity of the P450arom staining was higher than in December. The results suggest that the local supply of the male reproductive organs with steroid hormones operate in the hybrids of wild boar × domestic pig. This supply includes the local transfer of testosterone and the P450arom action.

* *Corresponding author at*: Department of Animal Physiology and Reproduction, University of Rzeszów, ul. Rejtana 16C, 35-959 Rzeszów, Poland. Tel.: +48 17 872 32 57; fax: +48 17 872 32 61.
E-mail address: annaurz@wp.pl (A. Tabęcka-Łonczyńska).

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1. Introduction

The permeation of endogenous or tritium-labeled exogenous testosterone (T) from the testicular vein into the testicular artery has been reported in several species [1,2]. High T levels were found in the lymph leaving the boar testis and flowing through the vessels which run along the spermatic cord [3]. However, there is lack of detailed information about the local transfer of hormones in the male reproductive organs.

The structure of the spermatic cord and morphological adaptation of its vasculature have been demonstrated in males of several species including the boar [1,2] and the morphological relationships between the testicular arteries and testicular veins in the boar have been presented in detail [4]. The well-developed, multi-layer venous network on the wall of the testicular artery branches has also been described [4]. A detailed examination of the vessel morphology has identified four types of testicular veins according to their perivascular elements and location in relation to the testicular artery. The deepest layer of the venous network, located in the tunica adventitia and occasionally penetrating the tunica media of the testicular artery, was considered to be an important element for decreasing the barrier between the blood of the testicular artery and the testicular veins. The authors believe that the vascular adaptation is the morphological basis for the permeation of hormones from outflowing testicular venous blood and lymph to the arterial blood [4].

Androgens are crucial for reproductive organ functions in male mammals. The T concentration in the boar gonadal fluids is high [5]. The relationship between the supply of the male reproductive organs with androgens and the course of spermatogenesis has been previously demonstrated [6]. Testosterone exerts its biological effects on spermatogenesis *via* androgen receptors (AR) localized in Sertoli cells [7], as well as in spermatozoa [8]. There is also evidence for a non-genomic action of androgens, in which T elicits responses through secondary messengers such as cAMP and signaling pathways different from classical AR-mediated transcription [9]. Although T is recognized as the primary male steroid hormone, the cytochrome P450 aromatase (P450arom) which transforms androgens to estrogens is present in almost all cell types of the mammalian testis [10].

The dependence of boar testis function on the light and photoperiodicity [5] and significant differences in boar sperm quality between the winter and summer seasons [11,12] have been reported. It is also known that the secretory activity of the testis in the male domestic pig and the wild boar is influenced by the season [5,13–15]. We decided to use hybrids resulting from the mating of domestic sows (Duroc) with a wild boar that strongly maintain seasonal reproduction. We hypothesized that the local transfer of testicular hormones from the venous effluent into the arterial blood might modulate the supply of the testis with these hormones. The main object of interests was T which may be subjected to the local transfer as well as may be aromatized. Since it has been established that only free steroid molecules (not bound to proteins) may be locally transferred [16], the aim of the present study was to determine differences between free T concentration in systemic arterial blood (common carotid artery; CA) and arterial blood supplying the testis and epididymis (branches of the testicular artery; TA and EA, respectively). In addition, the presence of P450arom in the arterial and venous vessels of the spermatic cord was estimated to evaluate whether the conversion of androgens to estrogens is possible in these vessels.

2. Materials and methods

2.1. Animals and photoperiod management

Ten mature wild boar × domestic pig hybrid males (aged 12 months, body mass 100–120 kg), housed at Experimental Farm Branch Campus of the Faculty of Biotechnology, University of Rzeszów, Kolbuszowa, Poland (50° N), were used in the study. The experiments were performed during the short-daylight period (December), when the activity of reproductive processes in the animals is high and the long-daylight period (June), when the reproductive processes are reduced. In December, the animals were housed in a room with windows and exposed to a range of 40–50 lux of natural illumination during the day. The mean daily temperature was 12 °C. In June, the animals were maintained in an open-sided shed and exposed to natural daytime illumination with an intensity of approximately 30,000 lux. The light intensity was measured repeatedly (9 times) at animal eye level (about 20 cm from the eyes). Each measurement consisted of five readings made at one minute intervals, and the values were averaged. The measurement was performed using lux meter (Voltcraft, DT-8820; Hirschau, Germany), which measured the level of intensity in the range of 20–20,000 lux (±5%). The mean ambient temperature was 24 °C during the day and 12 °C during the night. All procedures were approved by the Local Ethics Committee on Animal Experimentation in Lublin No. 8/2007. For experimental purposes, the animals were premedicated with atropine (Atropinum, 0.05 mg/kg bm, i.m., Biowet, Gorzów Wielkopolski, Poland) and azaperone (Stresnil, 2 mg/kg bm, i.m., Janssen Pharmaceutica, Beerse, Belgium). General anesthesia was induced and maintained with sodium thiopental (Thiopental, Thiopental Sandoz GmbH, Austria; 10 mg/kg bm, i.v. per animal every 20 min). Separate silastic catheters were inserted into the jugular vein (JV) and common carotid artery (CA) (od 2.4 mm, id 1.8 mm).

2.2. Surgical procedures, sample collection and plasma T determination

Under general anesthesia, blood samples were collected from the cannulated: 1/common carotid artery (CA), 2/external jugular vein (JV), 3/from the branches of the testicular artery supplying the testis (TA) and 4/epididymis (EA), as well as 5/ from the branches of the testicular vein draining blood from the testis (TV) and 6/spermatic cord (SV), using injection needles (od 0.7 mm, id 0.6 mm; Fig. 1) every 10 min for 2 h. When the blood collection was completed, the animals were castrated, and tissue samples from the testes (control tissue) and the middle part of spermatic cord were removed for immunolocalization of P450arom in the blood vessels. The heparinized blood samples were centrifuged, and the plasma free T and total T (JV only) concentrations were determined