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*Original article*

# Concentrations of oestrogens in blood plasma and seminal plasma of boars during the postpuberal period

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## Abstract

Concentrations of oestrogens in the blood plasma and seminal plasma of mature boars are high. However, little is known about their concentrations after reaching sexual maturity. The aim of this study was to determine the concentration of oestrogens in blood plasma and seminal plasma of boars during the postpuberal period. Free and conjugated oestrone and oestradiol-17β were determined by radioimmunoassay in blood from the testicular vein and artery, and peripheral circulation as well as in seminal plasma collected from 18 Polish Landrace boars. The animals were divided into three groups (n = 6) according to age (8, 12 and 16 months, respectively). Oestrone was predominant free and conjugated oestrogen. The highest values of oestrogens were measured in the testicular vein ($p \leq 0.05$). The concentrations of oestrogens in seminal plasma did not differ from those found in the peripheral circulation. An age-dependent increase in levels of all four oestrogens ($p \leq 0.05$) was observed. This can be associated with biochemical maturation of the reproductive system during the postpuberal period.

**Key words**: boar, oestrogens, blood plasma, seminal plasma, age effect

## Introduction

The sexual maturity starts in the boar between 5 and 9 months of age (Cameron 1987). The achievement of sexual maturity in the boar is followed by "biochemical maturation" of the reproductive system. Biochemical properties of semen are changing. Protein content, inhibition of ascorbate and $Fe^{2+}$-induced lipid peroxidation, activity of superoxide dismutase, concentration of L-glutathione (GSH) and activity of GSH-related enzymes as well as a total antioxidant status in seminal plasma increase after reaching sexual maturity to the age of 18 months (Strzeżek 2000, 2002, Kowalowka et al. 2008, Koziorowska-Gilun 2009). The "biochemical maturation" of the reproductive system seems to be essential for production of semen with high biological properties.

The boar is characterized by the secretion of large quantities of testicular oestrogens. They occur also in seminal plasma. In the boar, oestrogens play an important role in the regulation of the testicular development and function (for review, see Hess and Carnes 2004, Zduńczyk and Janowski 2009).

Concentrations of oestrogens in the blood plasma of mature boars are high and exceed those found in sows during oestrus (Claus and Hoffmann 1980,

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Claus et al. 1983, 1985). However, little is known about their concentrations in blood plasma and seminal plasma during the early postpuberal period in the boar. There are no studies on oestrogen secretion in the Polish Landrace boar at this period. Therefore, the aim of this study was to determine the concentration of free and conjugated oestrone and oestradiol-17β in blood plasma and seminal plasma of Polish Landrace boars between 8 and 16 month of age.

## Materials and Methods

The investigations were carried out on 18 Polish Landrace boars from October to December 2009 in a pig insemination station in the north-east of Poland. The animals were kept in individual pens and fed the standard diet. The animals were divided into three groups (n = 6) according to age (8, 12 and 16 months, respectively). Ejaculates were collected by the "gloved-hand" technique and the gel portion was removed by double gauze. Seminal plasma was obtained by centrifugation (3000 x g for 10 min) of gel-free semen. The boars were anesthetized with azaperone (20 mg/kg i.m.) and ketamine (20 mg/kg i.v.) and castrated. The protocol was approved by the Local Ethics Commission for Animal Experiments. During surgery, blood samples were collected by puncture from the auricular vein, and the testicular artery and vein into heparinized vials and centrifuged (3000 x g for 20 min). The plasma was stored until -20°C until assayed.

Concentrations of oestrone (E1), oestrone sulphate (E1S), oestradiol-17β (E2) and oestradiol-17β sulphate (E2S) were measured by RIA. Oestrone sulphate and oestradiol-17β sulphate were determined after removal of free oestrogens with toluene and hydrolysis with β-glucuronidase/arylsulphatase from *Helix pomatia* (Hoffmann et al. 1997).

The antiserum against oestradiol-17β was provided by Dr. B. Szafrańska and characterized elsewhere (Szafrańska et al. 2002). Intra-assay and inter-assay coefficients of variation of the assay for estradiol-17β were 6.0% and 9.2%, respectively.

The antiserum against oestrone (a gift of Prof. Hoffmann) was previously described (Hoffmann et al. 1996). Intra-asay and inter-asay coefficients of variation of the assay for estrone were 8.2% and 15.8%, respectively.

The data were shown as the mean ± SEM. The statistical significance of differences in hormone concentrations were assessed by on-way ANOVA followed by Bonferroni;s multiple comparison test (GraphPad PRISM, GraphPad Software Inc., Sand Diego, Ca, USA). A value of $p \leq 0.05$ was set as the limit of statistical significance.

## Results

The predominant free and conjugated oestrogen in blood plasma and seminal plasma of boars was oestrone. Maximal concentrations of E1 in the peripheral circulation, testicular vein, testicular artery and seminal plasma of boars aged 12 months were 0.54, 1.37, 0.61 and 0.40 ng/ml, respectively. The respective concentrations of E1S were 10.37, 34.42, 12.20 and 1.17 ng/ml. Free and conjugated oestradiol-17β were also found in blood plasma and seminal plasma, but their concentrations were low. They were 251.30, 391.20, 304.60 and 248.90 pg/ml for E2S, and 49.10, 241.10, 123.50 and 37.50 pg/ml for E2, respectively. The highest values of estrogens were measured in the testicular vein ($p \leq 0.05$). The concentrations of oestrogens in seminal plasma did not differ from those in the peripheral circulation. An age-dependent increase in levels of all four oestrogens ($p \leq 0.05$) was observed (Figs. 1-4).

## Discussion

Oestrone was the predominant free and conjugated oestrogen in blood plasma and seminal plasma of the boars. This finding agrees with the results of Claus and Hoffmann (1980). The range in concentrations of oestrogens is within that reported for adult boars in other studies (Rostalski 2005, Hoffmann et al. 2010).

Concentrations of oestrogens in the testicular artery were partially higher than those found in the auricular vein. Higher concentrations of E2 in the testicular artery compared to those determined in the peripheral circulation were found by Tabędzka-Łonczyńska (2010). This indicates the possibility of the counter-current transfer of oestrogens across the pampiniform plexus.

All four oestrogens were present in the seminal plasma, but their concentrations did not differ from those found in the peripheral circulation, which indicates that they were not actively enriched into the seminal plasma of the boar. Similar results were obtained by Rostalski (2005) and Hoffmann et al. (2010). In contrary, Claus et al. (1983, 1985) found higher concentrations of unconjugated oestrogens in seminal plasma compared to blood plasma. Oestrogens from boar semen increase the myometrial contraction frequency in the sows by stimulation of PGF2α release (Claus et al. 1987, Claus 1990). They influence also LH release and contribute to the timing of ovulation in response to mating or insemination (Claus 1990, Weiler and Claus 1991, Waberski et al. 1996).

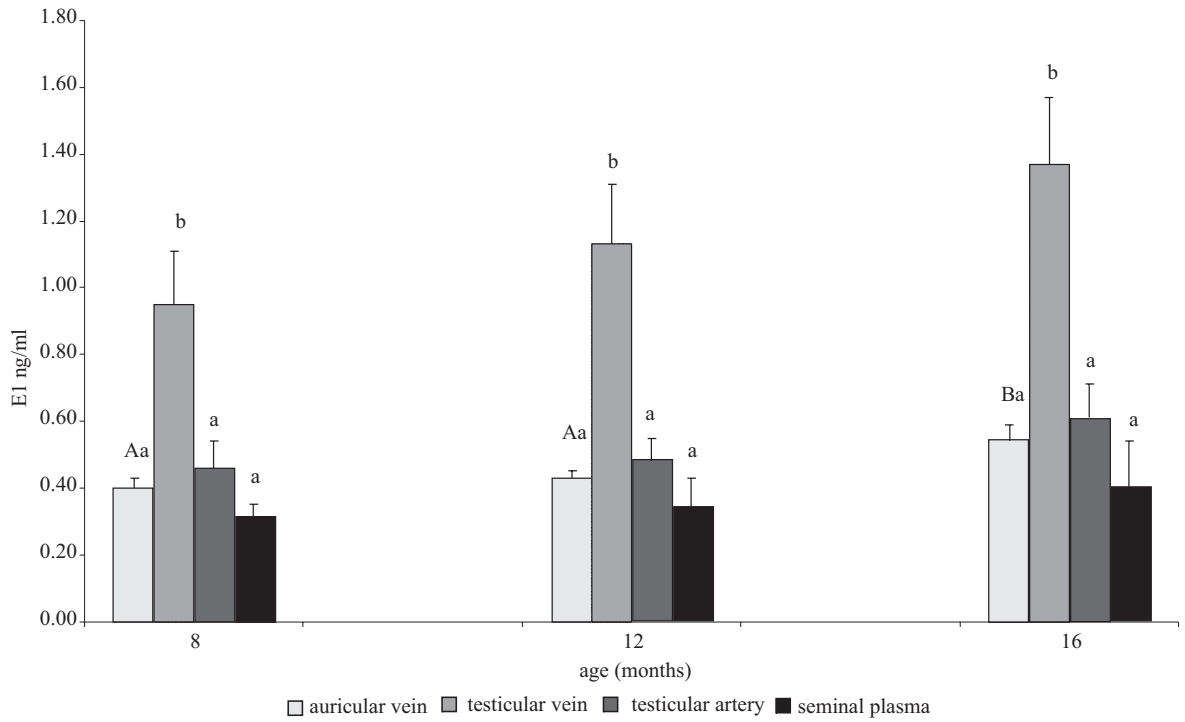


Fig. 1. Concentrations of oestrone (ng/ml, x ± SEM) in the auricular and testicular vein, testicular artery and in seminal plasma. a-b – differences between sampling sites statistically significant at $p \leq 0.05$. A-B – differences between age groups statistically significant at $p \leq 0.05$.

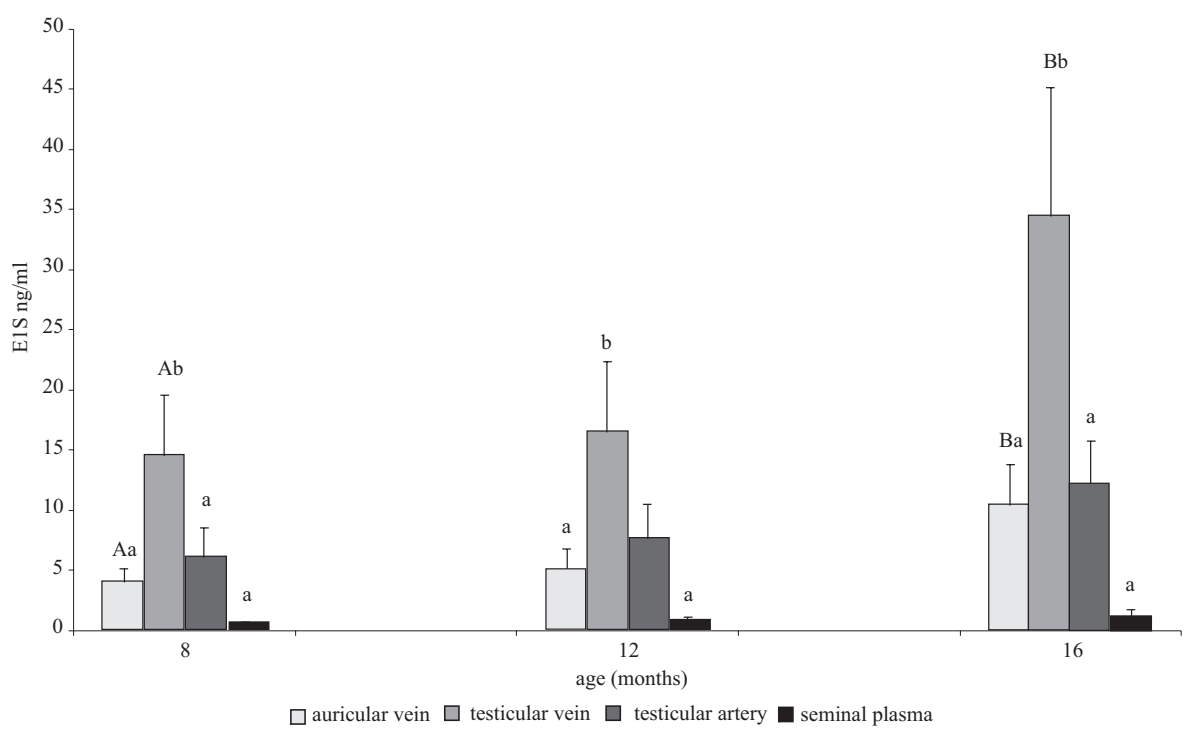


Fig. 2. Concentrations of oestrone sulphate (ng/ml, x ± SEM) in the auricular and testicular vein, testicular artery and in seminal plasma. a-b – differences between sampling sites statistically significant at $p \leq 0.05$. A-B – differences between age groups statistically significant at $p \leq 0.05$.

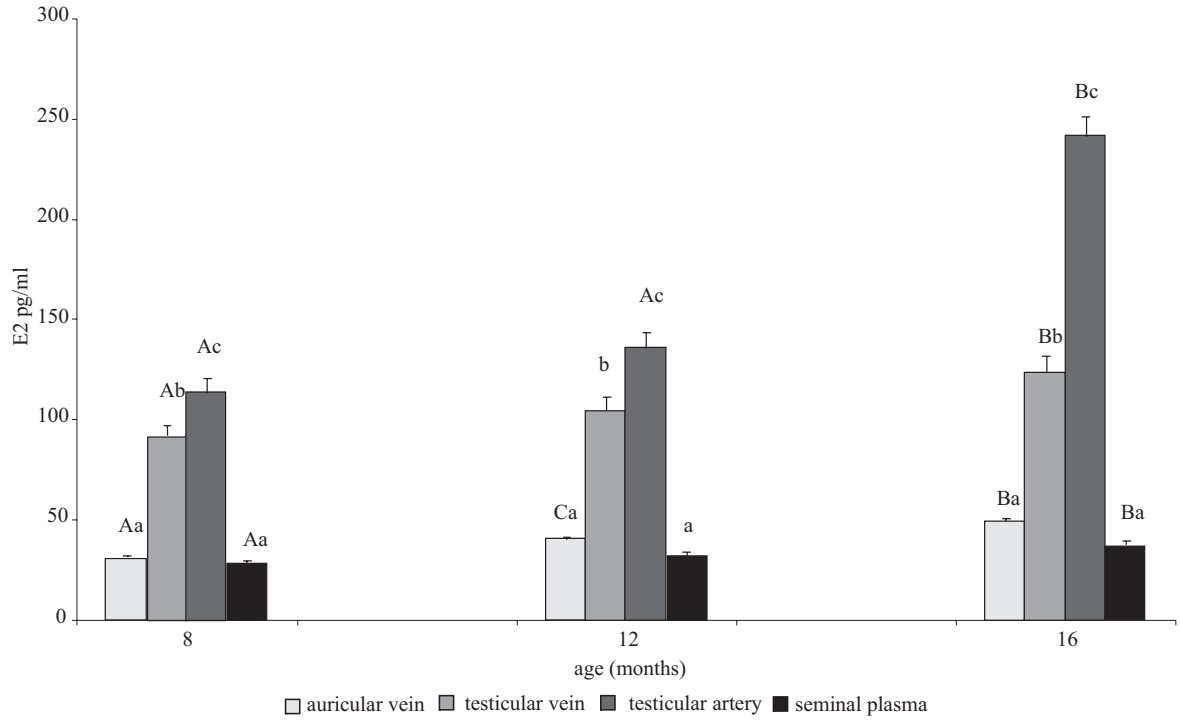


Fig. 3. Concentrations of oestradiol-17β (pg/ml, x ± SEM) in the auricular and testicular vein, testicular artery and in seminal plasma. a-c – differences between sampling sites statistically significant at $p \leq 0.05$. A-C – differences between age groups statistically significant at $p \leq 0.05$.

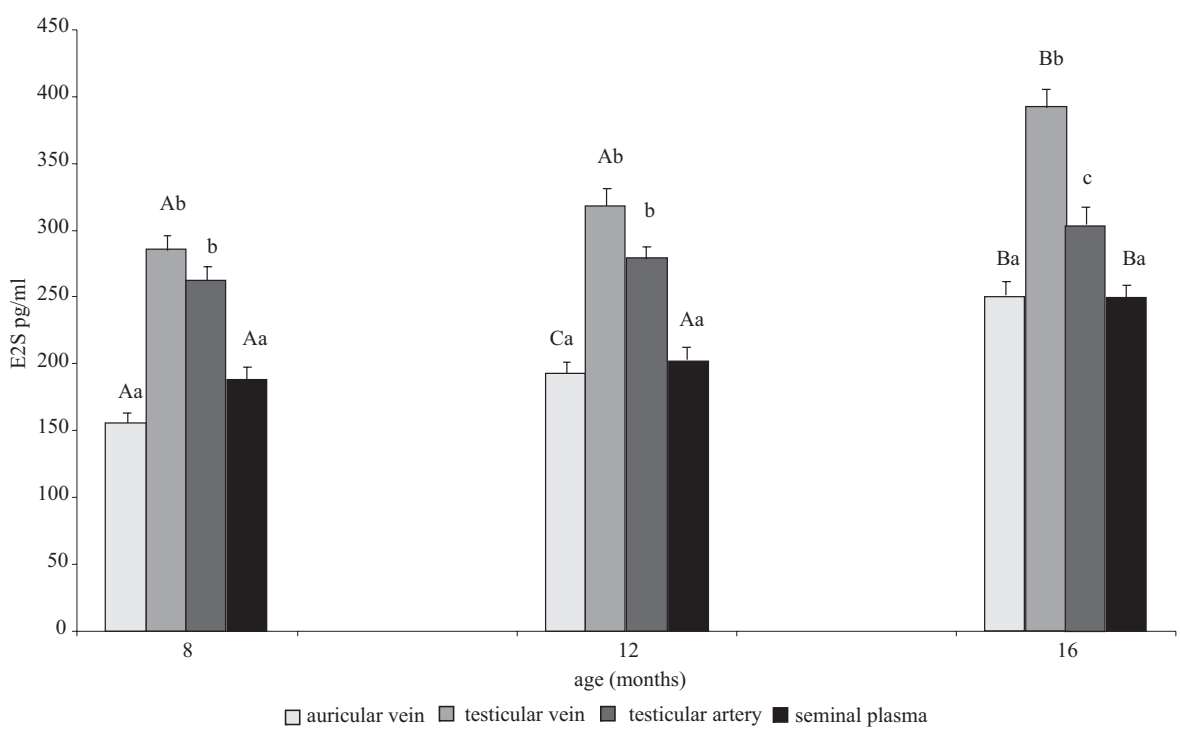


Fig. 4. Concentrations of oestradiol-17β sulphate (pg/ml, x ± SEM) in the auricular and testicular vein, testicular artery and in seminal plasma. a-c – differences between sampling sites statistically significant at $p \leq 0.05$. A-C – differences between age groups statistically significant at $p \leq 0.05$.

Concentrations of oestrogens increased significantly from 8 to 16 months of age. A significant increase in E1S and E1 concentrations in the peripheral circulation from day 100 to day > 365 was also observed by Hoffmann et al. (2010). This indicates that oestrogens might be involved in the process of "biochemical maturation" of the reproductive system after reaching sexual maturity in the boar. They influence the development and secretory activity of accessory sex glands. Injections of oestrone in combination with androgen in prepubertally castrated boars resulted in an increase in the weight of the accessory sex glands, and in zinc level in the seminal vesicles (Booth 1980). Treatment with the aromatase inhibitor, Letrozole, during neonatal period reduced accessory sex gland weights in postpuberal boars (Berger et al. 2008). However, further studies are necessary to clarify the effect of oestrogen concentration on the composition of seminal plasma.

In conclusion, our data showed that oestrone was predominant free and conjugated oestrogen in blood plasma and seminal plasma of the boars. The highest values of oestrogens were measured in the testicular vein. The concentrations of oestrogens in seminal plasma did not differ from those found in the peripheral circulation, which suggest that they were not actively secreted into the seminal plasma. The rise of oestrogens concentration from 8 to 16 months of age can be associated with the process of "biochemical maturation" of the reproductive system during the postpuberal period.

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