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CHANGES IN PRE AND POST PUBERTY LEVELS OF PROGESTERONE, ESTRADIOL AND GHRELIN IN GILTS

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Abstract

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The onset of puberty in pigs is a physiological process influenced by many factors. Some of the most important among them are age, breed, nutrition, technology of breeding, seasons and more. The reproductive system is particularly sensitive to energy reserve and metabolic status of the organism. In recent years it was discovered the role of so-called "metabolic" hormones (leptin and ghrelin) performing interaction between energy reserves and fertility. The purpose of this study was to characterize the changes in pre- and post-puberty levels of progesterone, estradiol and ghrelin in blood sera of gilts from different cross-breeds. An evaluation of the physiological maturity in gilts from LW x L (Large White x Landrace) crossbreed (n=10) and newly developed breed group (developed in the Agricultural Institute – Shumen, Bulgaria) (NBG) (Danube White x Landrace) (n=10), by comparison of the blood serum levels of progesterone, estradiol and ghrelin was carried out. It was established a significantly higher progesterone level ($p < 0.001$) in the blood sera of LWxL gilts at the age of 210 days (3 ng/ml), compared with that of the age of 165 days (0.98 ng/ml). Similar data were obtained in the NBG – 2.68 ng/ml at the age of 220 days and 0.66 ng/ml at the 160 days old gilts. In the LWxL gilts estradiol levels significantly ($p < 0.001$) increase from 113 pg/ml at 165 days of age to 130 pg/ml at 210 day, in NBG gilts the increase was respectively from 77.4 pg/ml at 160 day to 156.8 pg/ml at 220 day. There was a weak negative correlation between ghrelin and progesterone levels in pre pubertal period in both breeds (r_p = -0.31 for LW x L and r_p = -0.28 for NBG). However, at the post puberty there was a weak to moderate positive correlation between the two hormones in both breeds (r_p = 0.34 for LWxL and r_p = 0.42 for NBG). There was no correlation between the levels of estradiol and ghrelin before and after puberty onset in each breed.

Key words: gilts; breed; puberty; progesterone; estradiol; ghrelin
Abbreviations: NBG: newly developed breed group; FSH*:* follicle stimulating hormone; LH*:* luteinizing hormone; GnRH*:* gonadotropin-releasing hormone; ELISA*:* enzyme linked immunosorbent assay; KNDy*:* kisspeptin/neurokinin B/dynorphin

Introduction

The age of puberty onset in pigs varies from 120-130 days to 200-250 days (Brade, 1988). There are many factors which can influence the puberty onset in gilts. One of them is the breed (Germanova, 1990; Benkov and Shostak, 1991; Marchev and Shostak, 2004). Kirov and Marchev (2004) found that the average age of the first estrus is at 186.2 days for the Danube white and 193.3 days for Landrace breed in Bulgaria. Marchev and Petrova (2012) established that the sexual maturity in LWxL gilts, expressed by manifestation of estrus is at the age of 178.6 days with deviations from 159 days to 202 days.

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The development of the reproductive system of the domestic pig is separated into several periods - a period before puberty (first month after birth); period of infantilism (second month), inactive (third - fourth month) during which begins secretion of estrogens.

In the last (prepubertal) period, the releasing of GnRH by hypothalamus stimulates the incretion of FSH and LH from the anterior pituitary gland, which provokes the production of estrogen and progesterone by the ovaries. Studies of Coffey et al. (1997) showed that the main part of hormonal changes occurred before the age in which gilts show estrus.

In the post pubertal period steroid secretion of ovaries is activated, which is expressed by increase of the progesterone and estrogens concentration (Anderson, 2009).

The reproductive system is particularly sensitive to energy reserve and metabolic status of the organism. In the recent years it was discovered the role of so-called "metabolic" hormones (leptin and ghrelin) performing interaction between energy reserves and fertility (Prunier and Quesnel, 2000; Tena-Sempere et al., 2013). Tena-Sempere (2007) showed that the ghrelin is secreted not only in the stomach, but also from other peripheral tissue such as the pancreas, ovaries, adrenal cortex. The concentration of ghrelin in the blood plasma depends on changes in energy balance of the body and is associated with the physiological condition of the reproductive system (Scrimgeour et al., 2008; Torres-Rovira et al., 2011).

The purpose of this study was to characterize the changes in pre- and post puberty levels of progesterone, estradiol and ghrelin in blood sera of gilts from LWxL crossbreed and newly developed breed group (NBG) (Danube White x Landrace).

Materials and Methods

Experimental animals

For the purposes of the study were used clinically healthy gilts, equalized by age from LWxL crossbreed (n=10) and from newly developed breed group (NBG) (n=10).The trails were conducted in the experimental base of the Agricultural Institute Shumen.

Collection of samples

Blood sera were collected before the onset of puberty and after registration of second estrus - 165 and 210 days for LWxL and 160 and 220 days for NBG.

The animals were raised in groups in secured floor area 1.2 m² per capita and fed "ad libitum" with equal rations contents in 1 kg 161 g of crude protein and 3008 kcal metabolizable energy at 150-180 days and 147 g of crude protein and 3031 kcal metabolizable energy at 180-230 days of age. They received water from nipple drinkers. Live weight of animals was measured. Blood samples were obtained by puncture of *sinus venous* in the medial eye angle.

Detection of sex hormones and ghrelin levels in blood sera

The levels of progesterone, estradiol and ghrelin in the blood sera were tested by applying the enzyme linked immunosorbent assay /ELISA/. Commercial kits of Cusabio (China) (cat.CSB-E12869; CSB-E09437; CSB-E09436) were used and the analysis was performed according to the manufacturer's protocol.

Statistical analysis

All data were statistically analyzed by the software package Statistica (StatSoft 10). Differences were considered significant at $P < 0.05$.

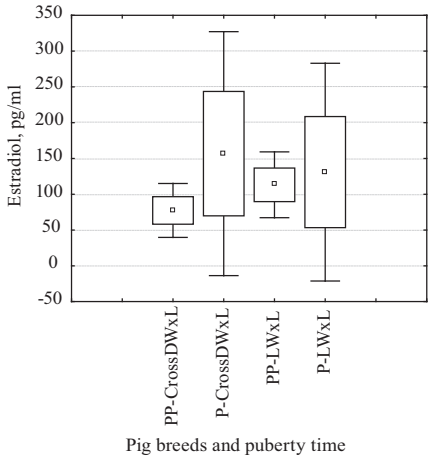
Results

The results of the evaluation of the physiological changes in sex hormone levels and ghrelin, before and after sexual maturity are shown on Figure 1.

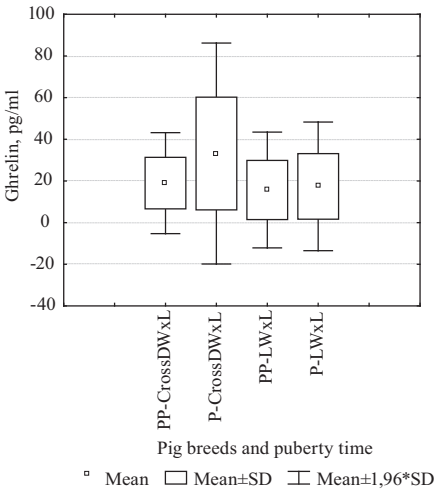
It was established a significantly higher progesterone level ($p < 0.001$) in the blood sera of LWxL gilts at the age of 210 days (3 ng/ml), compared to the age of 165 days (0.98 ng/ml). Similar data were obtained in the NBG – 2.68 ng/ml at the age of 220 days and 0.66 ng/ml at the 160 days old gilts. The serum levels of estradiol significantly ($p < 0.01$) increased after puberty, in LWxL gilts from 113 pg/ml at 165 days to 130 pg/ml at 210 days of age. In NBG gilts the estradiol levels rise from 77.4 pg/ml on 160 day to 156.8 pg/ml on day 220. Ghrelin levels in the blood sera from LWxL gilts were with insignificant difference in the comparison between the two ages (18.9 pg/ml and 17.4 pg/ml). At age 210-220 days the animals from both groups reached the typical for the pubertal gilts live weight (Table 1.).

In the postpubertal period the levels of ghrelin in the blood sera from NBG gilts was found at a higher level (33.2 pg/ml) vs. prepubertal one (18.9 pg/ml), but the difference is insignificant, probably due to the smaller number of animals. Weak negative correlations (r_p = -0.35 for LWxL and r_p = -0.28 for NBG) between ghrelin and progesterone in prepubertal period were established in both breeds (Table 2).

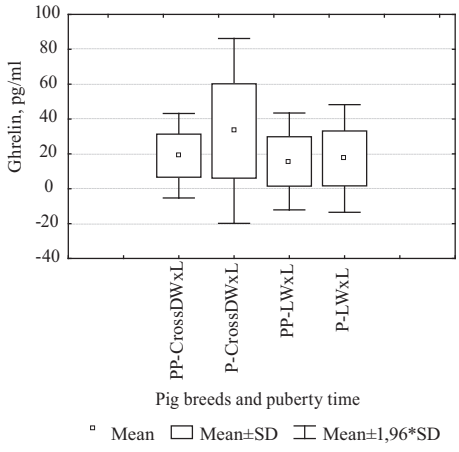
However, in the postpubertal period there is a trend of positive correlation between these hormones (r_p = 0.34 and r_p = 0.42). There was no correlation between the levels of estradiol and ghrelin before and after puberty onset in each breed.



a) Progesterone



b) Estradiol



c) Ghrelin

Fig. 1. Comparison of the sex steroids and ghrelin levels in blood plasma of LWxL and NBG (DW x L) in prepubertal (PP) and pubertal (P) period

Table 1
Live weight of pigs before and after the onset of puberty

Traits	LW x L		NBG	
	x±Sx	x±Sx	x±Sx	x±Sx
Age, days	165	210	160	220
Live weight, kg	78.8±2.4	105.5±6.8	69.4±3.9	116.4±7.2

Table 2
Correlations between levels of sex steroids and ghrelin (Pearson) in blood serum of LW x L and NBG during the puberty onset

Origin	Hormones	Sextual maturity	Relationships with Ghrelin
LW x L	Progesterone, ng/ml	prepuberty	-0.35
		puberty	0.34
	Estradiol, pg/ml	prepuberty	-0.04
		puberty	0.1
NBG	Progesterone, ng/ml	prepuberty	-0.28
		puberty	0.42
	Estradiol, pg/ml	prepuberty	-0.19
		puberty	0.15

Discussion

The new knowledge of this work is characterization of the pre and post pubertal changes in the blood levels of the sex hormones progesterone and estradiol and the metabolic hormone ghrelin in gilts from the newly created crossbreed NBG. These results are compared with the hormonal characteristic of the puberty onset in the LWxL crossbreed. Steroid hormones produced from developing gonads during sexual maturation are key factors in KNDy (kisspeptin/neurokinin B/dynorphin) neuron activation and regulation (Merkley et al., 2012). Prior to puberty, estrogen and progesterone from the ovary are produced in low levels and are not yet capable of exerting positive and negative feedback on the hypothalamus. The results of our investigation confirm the opinion of Christenson et al (1985) that serum concentrations of progesterone is lower than 1 ng/ml in pre-pubertal gilts. As the female matures she is able to produce sufficient levels of steroid hormones to activate the feedback loop of the KNDy neuropeptides and reproduction cycle (Goodman et al., 2013). This evidence of physiological and physical determinants provides clues of an animal's impending puberty onset. The serum concentration of estrogens after puberty onset is greater than at age before puberty (Goodman et al., 2013).

Onset of puberty in the gilts was preceded by a significant increase in serum concentrations of estrogens (Lutz et al., 1984). The results of our study support these finding and the

concentration of estradiol in our observation was significantly ($P<0.01$) greater in postpubertal gilts. Our data supports the hypothesis that a reduction in metabolism of gonadal steroids, regardless of the existence of different gonadal steroid profiles in female pigs during pubertal development, may allow gonadal steroids to advance sexual development. This coupled with reduced metabolism of gonadal steroids are other physiological components also associated with sexual maturation.

Reproductive function is highly sensitive to energy reserves of the organism and the metabolite status. It is known that the fertility is determined by a multi-hormonal effect, included not only sex and gonadotropic hormones, but also "metabolic" hormones – leptin and ghrelin. These hormones "include" signals for puberty onset and the subsequent maintenance of reproductive ability (Tena-Sempere, 2007). Our data also show that at the onset of the puberty there is a correlation between the level of progesterone and ghrelin in the blood serum of both groups of animals. Prunier and Quesnel (2000) considers that female pigs need a sufficient reserve of energy and an optimal state of metabolism for reaching the good fertility conditions.

Conclusion

In the present study was found that the sexual maturation of the newly developed breed group does not deviate a lot from the parental breeds. In both controlled breeds at 160-165 days of age, the levels of progesterone and estradiol were low, which is typical for prepuberty. On the 220 days (after the second estrus) the blood serum levels of the tested hormones were typical for sexually mature gilts. In postpubertal period it was established a significantly ($P <0.01$) higher level of progesterone and estradiol in the blood serum of LWxL and NBG gilts compared to the prepubertal period. These values do not distinguish reliably than other studied breeds. The correlation between the progesterone and ghrelin levels in the blood serum of the animals was established. The relationships between progesterone and ghrelin show that animals with an optimal diet actively entering puberty, but in the postpubertal period the high food intake does not stimulate sexual activity.

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