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Effects of dietary vitamin supplementation and semen collection frequency on hormonal profile during ejaculation in the boar

I. Audet [a], N. Bérubé [b], J.L. Bailey [b], J.-P. Laforest [b], H. Quesnel [c], J.J. Matte [a,*]

[a] *Dairy and Swine Research and Development Centre, Agriculture and Agri-Food Canada, 2000 College Street, P.O. Box 90, Lennoxville STN, Sherbrooke, Que., Canada J1M 1Z3*

[b] *Department of Animal Science, Centre de Recherche en Biologie de la Reproduction (CRBR), Laval University, Ste-Foy, Que., Canada G1K 7P4*

[c] *INRA, UMR1079, Systèmes d'élevage, Nutrition Animale et Humaine, F-35590 Saint-Gilles, France*

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Abstract

To evaluate the effect of dietary and management factors on boar hormonal status during ejaculation, 39 boars were canulated to determine the profiles of luteinizing hormone (LH), follicle-stimulating hormone (FSH), 17β-estradiol (E_2), and testosterone (T) in blood plasma and seminal fluid. Prior to canulation, 18 boars were fed a basal diet (control), whereas the remainder ($n = 21$) were fed a basal diet supplemented with extra vitamins (supplemented). Within each dietary treatment, two regimens of semen collection were used over the 3 mo preceding the hormonal evaluation: three times per 2 wk (3/2) or three times per wk (3/1). Plasma E_2 was lower ($P < 0.01$) before ejaculation (232.5 ± 22.6 pg/mL) than at the onset of ejaculation (255.2 ± 27.1 ng/mL). Plasma T increased from 5.14 ± 0.72, before ejaculation to 5.87 ± 0.86 ng/mL at the onset of ejaculation in supplemented boars, whereas it decreased from 5.15 ± 0.65 to 4.87 ± 0.70 ng/mL in controls (diet by time, $P < 0.05$). At the onset of ejaculation, plasma FSH was higher in 3/2 boars (0.436 ± 0.06 ng/mL) than in 3/1 boars (0.266 ± 0.04 ng/mL; $P < 0.05$). During ejaculation, plasma LH increased linearly ($P < 0.01$) from 0.59 ± 0.07 to 0.97 ± 0.10 ng/mL, and plasma E_2 and T concentrations were correlated ($r = 0.62$, $P < 0.01$). Plasma FSH before and during ejaculation was negatively correlated with sperm production ($r = -0.60$, $P < 0.01$) and testicular weight ($r = -0.50$, $P < 0.01$). In conclusion, dietary and management factors had few impacts on hormonal profiles during ejaculation, but homeostasis of some hormones was related to some criteria of reproductive performance in boars.

Keywords: Vitamin; Sperm collection frequency; Ejaculation; Hormone profile; Boar

1. Introduction

Maximizing the production of high-quality sperm is of paramount importance for swine AI. However, little is known about the impact of external factors, such as nutrition and management, on semen production in boar and on the physiology and homeostasis during the process of semen collection for AI.

Boars are known to produce large amounts and a wide spectrum of sex steroids [1], including androgens such as testosterone (T), androstenedione and dehydroepiandrosterone-sulphate [2–4]. Testosterone is recognized as the primary hormone required to maintain spermatogenesis in adult rats [5] and is a regulator of gonadotropin secretion in boars [6]. In the male, testosterone is transformed into estradiol in the brain by an aromatase; this enzyme is also present in the Leydig

* Corresponding author. Tel.: +1 819 565 9171; fax: +1 819 564 5507.
E-mail address: mattej@agr.gc.ca (J.J. Matte).

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cells of the boar, but apparently not in other mammals [7]. The boar produces considerable amounts of estrogens in the Leydig cells [8] and has a much higher concentration of circulating blood estrogens than males of most other species [9]. In boars, as well as bulls, estrogen concentrations are higher in semen than in blood plasma, whereas the opposite is true for androgens [1,8]. Estrone and 17β-estradiol (E_2) represent the main estrogens in boar blood plasma [1], and E_2 is important in different aspects of male reproduction such as testicular maturation [10], germ cell survival [11] and sperm motility [12]. Gonadotropins are essential for testicular growth and development and for the support of testicular factors necessary for spermatogenesis initiation and maintenance [13,14]. Luteinizing hormone (LH) stimulates testosterone secretion by Leydig cells; both of these hormones play critical roles in boar reproduction [15]. Also, testicular size and total daily sperm production are negatively correlated with concentrations of plasma follicle-stimulating hormone (FSH) [16,17]. Steroid production in the domestic boar, in contrast to the wild pig, is not considered to be strongly affected by season [1]. Other factors, such as nutrition, might be more important. For example, protein intake might affect sperm production through an alteration of steroid production and/or secretion [9]. A recent study showed that vitamin supplements may influence the reproductive performance of boars, particularly during a period of stress induced by intensive semen collection [18].

The present experiment was therefore undertaken to better characterize hormonal profiles during mounting and ejaculation in boars and to verify the hypothesis that vitamin supplements and different regimens of semen collection influence the hormonal profile.

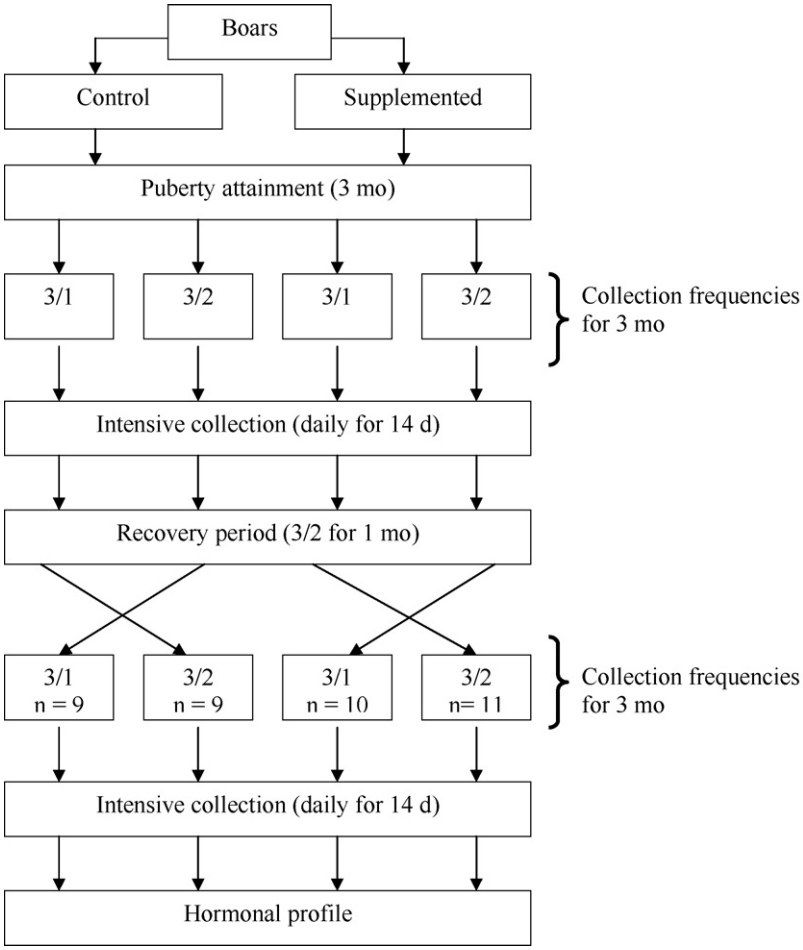

Fig. 1. Experimental design used prior to hormone profiles measured before and during ejaculation of boars.

2. Materials and methods

2.1. Animals and treatments

Fifty Duroc boars were selected at 209.2 ± 2.79 d of age (mean ± S.E.M.) for this study and were housed individually in pens on partial-slatted floors. They were used for the purpose of another experiment (unpublished data, manuscript in preparation; Fig. 1). The average body weight was 136.4 ± 1.70 kg (mean ± S.E.M.) at initiation of treatments and 270.3 ± 2.51 kg at the end of the experiment. Boars were randomly allocated, according to body weight and age, to one of two dietary treatments: (1) basal diet (Table 1), with a vitamin premix providing concentrations corresponding to the industry average which exceeded the recommended NRC [19] levels (Table 2; control, $n = 25$) and (2) basal diet supplemented with the control premix plus extra fat-soluble and water-soluble vitamins (Table 2; supplemented, $n = 25$). Premixes were kept at 4 °C until they were given to the animals. The daily feed allowance for the whole experimental period was 3.0 kg and the premixes were given as a top-dressing of 50 g. After puberty, the animals were trained to mount an artificial sow for semen collection; those not trained satisfactorily by the end of this period were culled. Thirty-nine boars were ultimately selected for the experiment and allocated to different collection frequencies within each dietary treatment: three times per wk (3/1, high frequency) or three times per 2 wk (3/2, regular frequency) for 3 mo (Fig. 1). Afterwards, the animals were subjected to an intensive collection regimen (daily collection for 2 wk), followed by a ''recovery'' period of 1 mo, during which the collection frequency was twice weekly. Subsequently, there was a 3-mo period during which the collection frequencies high (3/1) and regular (3/2) were reversed in relation to the first 3-mo period and then, again, an intensive collection regimen (daily collection for 2 wk) followed. Sperm production was estimated for each ejaculate throughout the experiment. Ten days after the end of the second intensive collection frequency, all boars were canulated for blood sampling during ejaculation, in order to monitor hormonal concentrations. According to previous results [18], this interval was sufficient for a complete recovery to the previous semen production. Two days after blood collection for the hormonal profile, boars were euthanized by an intravenous overdose of ketamine hydrochloride (Vetrepharm Canada Inc., Belleville, ON, Canada) and then exsanguinated. Testes were measured, weighed, and volumes were noted by water displacement. In the present report, the collection frequency refers to the

Table 1
Composition of the basal diet fed to boars[a]

Ingredient[b]	Proportion (%)
Corn	31.4
Wheat bran	30
Barley	15
Soybean meal (48% crude protein)	12.2
Gluten	7.5
Limestone	1.47
Dicalcium phosphate	0.83
Fat	0.5
Salt	0.49
L-Lysine-HCl	0.3
DL-Methionine	0.185
Anti-mould	0.1
Delquin	0.015

[a] The analytical value for riboflavin was 4.98 μg/g; for pyridoxine, 2.47 μg/g; for folates, 1.27 μg/g; and vitamin E and vitamin B12 were undetectable.

[b] The calculated composition for ME, CP, lysine, Ca and P of the basal diet were (as-fed basis) 2810 kcal/kg, 15.9%, 0.96%, 0.81% and 0.79%, respectively.

Table 2
Daily vitamins for boars provided by the premix in each treatment[a,b]

Vitamin	Control[c]	Supplemented
Vitamin A (KIU)	20	100
Vitamin D (KIU)	3	10
Vitamin E (IU)	120	600
Menadione (mg)	2	10
Choline (mg)	400	4000
Pantothenic acid (mg)	40	40
Riboflavin (mg)	10	100
Folic acid (mg)	4	40
Niacin (mg)	50	500
Thiamine (mg)	2	20
Pyridoxine (mg)	6	60
Vitamin B_{12} (mg)	0.04	0.4
Biotin (mg)	0.5	5

[a] 50 g of premix were given as top-dressing. Analytical value (means ± S.E.M.) for vitamin E was 687.7 ± 8.5 IU for supplemented vs. 116.6 ± 3.6 IU for control; for riboflavin, 96.6 ± 3.5 mg for supplemented vs. 10.4 ± 0.25 mg for control; for pyridoxine, 66.2 ± 1.8 mg for supplemented vs. 6.4 ± 0.22 mg for control; for folic acid, 35.9 ± 0.94 mg for supplemented vs. 4.9 ± 0.29 mg for control and for Vitamin B_{12}, 0.39 ± 0.01 mg for supplemented vs. 0.04 ± 0.004 mg for Control.

[b] Each premix provided the following daily: Mn as manganous oxide, 60 mg; Zn as zinc oxide, 200 mg, Fe as ferrous sulfate, 200 mg; Cu as copper sulfate, 30 mg; I as calcium iodate, 0.54 mg; Se as selenite, 0.54 mg.

[c] Vitamin premix provided concentrations corresponding to a survey carried out among the feed industry by BASF Corp., Vitamin and Supplementation Rates for U.S. Commercial Poultry, Swine and Dairy Cattle, Mount Olive, NJ, 1993.

residual effect of the last semen collection frequency used for the boars during the last 3-mo period of the overall trial. The boars remained on their dietary treatment up to slaughter. All animals were used and cared for in accordance with Canadian Council on Animal Care guidelines [20].

2.2. Sampling and laboratory analysis

Jugular catheters were inserted into the boars using a nonsurgical technique [21] 2 d before the onset of repeated blood sampling. Blood was collected from the catheters between 0700 and 1200 h on fasting boars (>15 h). Four samples were taken at 15-min intervals in the boar's pen and then the animal was moved to the collection room and presented to the artificial sow for semen collection. Semen was collected using the gloved hand technique. During ejaculation, blood was collected through the catheter at a rate of 10 mL per min until the end of ejaculation. Tubes were centrifuged at $1800 \times g$ for 12 min at 4 °C, and the plasma was separated into aliquots and frozen at −20 °C for hormonal analysis. The collected semen was strained through sterile gauze in a prewarmed insulated container kept at 37 °C, in order to remove gelatinous material. Semen volume was weighed (±1 mg) and the sperm concentration was estimated using a spectrophotometer (Novaspec II, Pharmacia Biotech, Cambridge, England) precalibrated with a haemocytometer. The semen was centrifuged at $1800 \times g$ for 20 min at 4 °C. The seminal plasma was aliquoted and frozen at −20 °C for hormonal analysis.

Blood and seminal plasma E_2 were measured in duplicate by radioimmunoassay (RIA) using commercial kits containing ^{125}I-estradiol (ImmuChem™ Double Antibody 17β-estradiol; ICN Biomedicals Inc., Costa Mesa, CA, USA), according to the method of Audet et al. [18]. Intra- and inter-assay CVs were 4.2% and 7.6%, respectively. Blood plasma testosterone (T) was measured in duplicate by RIA using commercial kits containing ^{125}I-testosterone (ImmuChem™ Double Antibody Testosterone, ICN Biomedicals Inc.). Blood samples were diluted with 30 μL of sample added to 20 μL of diluent buffer. Validation tests for the measurement of T in blood plasma had intra- and inter-assay coefficients of variation (CV) of 7.6% and 6.0%, respectively, and the recovery rate was 107.5%. Validation tests for the measurement of T in seminal plasma had intra- and inter-assay CVs of 6.1% and 6.2%, respectively, and the recovery rate was 103.1%. Blood and seminal plasma LH and FSH were measured by the Prairie Diagnostic Service Laboratory (University of Saskatoon, Saskatoon, SK, Canada), using the method of Kingsbury and Rawlings [22]. Intra-assay CVs were 3.2% and 4.7%, respectively for reference plasma FSH with means of 0.69 and 2.43 ng/mL. Intra-assay CVs were 8.5% and 7.8% for reference sera LH with means of 0.64 and 4.14 ng/mL. Sensitivity of the LH and FSH assays were 0.06 and 0.13 ng/mL.

2.3. Statistical analysis

The data were analyzed using the Mixed Procedure of SAS (SAS Institute Inc., Cary, NC, USA) [23] according to a 2×2 factorial arrangement of treatments, with dietary supplements (control and supplemented boars) and semen collection frequencies (3/1 and 3/2) as main effects. For repeated measurements (blood samples), polynomial contrasts were used to evaluate the time effect. Differences were considered significant at $P < 0.05$. Because each animal had different ejaculation durations, the ejaculation periods were standardized by dividing the total duration into quarters to facilitate analysis of the hormonal profiles during ejaculation. Means from 1, 2, or 3 blood samples were calculated for each quarter, in cases where the quarter boundaries were smaller or equal on each side. The analysis was done on time response for the period of ejaculation and also between the basal level and the beginning of the ejaculation. The boar was considered as the experimental unit. The following model was used:

$$Y_{ij} = \mu + B_i + F_j + (B_i \times F_j) + e_{ij}$$

where Y_{ij} = dependent variable, B_i = vitamin supplements, F_j = collection frequency, $B_i \times F_j$ = interaction between vitamin supplements and collection frequency, and e_{ij} = residual error.

To normalize for experimental error, a logarithmic transformation was done for E_2 and a square root transformation was done for FSH, LH and T. Pearson correlation coefficients were used to compare seminal and plasma hormones before and during ejaculation with testicular weight, testicular volume, and cumulative sperm production (excluding the intensive collection and recovery periods) over the 3-mo periods.

3. Results

Two types of hormonal response are presented. The first one, in pre-ejaculation, corresponds to the mean value of four samples taken at 15-min intervals before the onset of ejaculation compared to value at the onset

of ejaculation (Quarter 1). The second type of hormonal response corresponds to the hormonal variations during ejaculation divided in four quarters of equal time, according to the individual length of ejaculation for each individual boar (i.e. (time at end of ejaculation − time at beginning of ejaculation)/4).

3.1. Plasma hormonal concentrations before and at the onset of ejaculation

At the onset of ejaculation, the concentration of blood plasma E_2 was higher ($P < 0.01$) than before the onset of ejaculation (255.2 ± 27.1 and 232.5 ± 22.6 pg/mL, respectively). For blood plasma LH, there were no effects of diet, collection frequency, or time ($P > 0.35$) on concentrations before (0.54 ± 0.07 ng/mL) or at the onset of ejaculation (0.59 ± 0.07 ng/mL). Concentrations of blood plasma T in supplemented boars was lower before than at the onset of ejaculation (5.14 ± 0.72 and 5.87 ± 0.86 ng/mL, respectively), whereas it was the opposite for control boars (5.15 ± 0.65, and 4.87 ± 0.70 ng/mL, respectively; diet by time interaction, $P < 0.05$). For the concentrations of blood plasma FSH, no diet, collection frequency or time effects were detected ($P > 0.06$) on the concentrations before (0.33 ± 0.03 ng/mL) or at the onset of ejaculation (0.35 ± 0.04 ng/mL).

3.2. Hormonal time response during ejaculation

Neither diet nor collection frequency affected the concentrations or total amounts of seminal hormones (LH, FSH, T and E_2; $P > 0.24$; Table 3). There was a linear increase for the concentrations of blood plasma LH over the duration of ejaculation (0.59 ± 0.07, 0.73 ± 0.08, 0.90 ± 0.08, and 0.97 ± 0.10 ng/mL for Quarters 1–4, respectively; $P < 0.01$). Concentrations of blood plasma T decreased between Quarter 1 and Quarter 2 (5.41 ± 0.57 and 4.67 ± 0.42 ng/mL, respectively) and increased thereafter (4.88 ± 0.42 and 5.42 ± 0.55 ng/mL for Quarters 3 and 4; $P < 0.01$).

Table 3
Concentrations (means ± S.E.M. of 39 boars) and total amount of hormones in seminal plasma of ejaculates collected during hormonal profiles

Hormone	Concentration (ng/mL)	Total amount per ejaculate (ng)
Estradiol	0.02 ± 0.005	4.41 ± 1.08
Testosterone	0.25 ± 0.03	45.31 ± 7.26
LH	0.10 ± 0.03	20.46 ± 6.53
FSH	0.13 ± 0.04	25.21 ± 8.11

The concentrations of blood plasma E_2 was not influenced either by treatments or quarter of ejaculation ($P > 0.31$). At the onset of ejaculation (Quarter 1), concentrations of plasma FSH was higher in 3/2 boars than in 3/1 boars ($P < 0.05$), but this effect disappeared during ejaculation (collection frequency by time interaction, $P > 0.08$; Fig. 2).

In terms of correlations among variables, there was a correlation between blood plasma E_2 and blood plasma T concentrations ($r = 0.62$, $P < 0.01$). Blood plasma T was correlated with seminal plasma T ($r > 0.74$; $P < 0.01$), but no other correlation was obtained between seminal and blood plasma hormones. Blood plasma FSH before and during ejaculation was negatively correlated with the anterior cumulative (3 mo) sperm production ($r = -0.60$, $P < 0.01$) and with testicular weight ($r = -0.50$, $P < 0.01$). Testicular weight was positively correlated with testicular volume ($r = 0.87$, $P < 0.01$) and with cumulative sperm production ($r = 0.63$, $P < 0.01$). There were no treatment (semen collection frequency or dietary supplements) effects on cumulative sperm production, testicular weight or testicular volume with overall

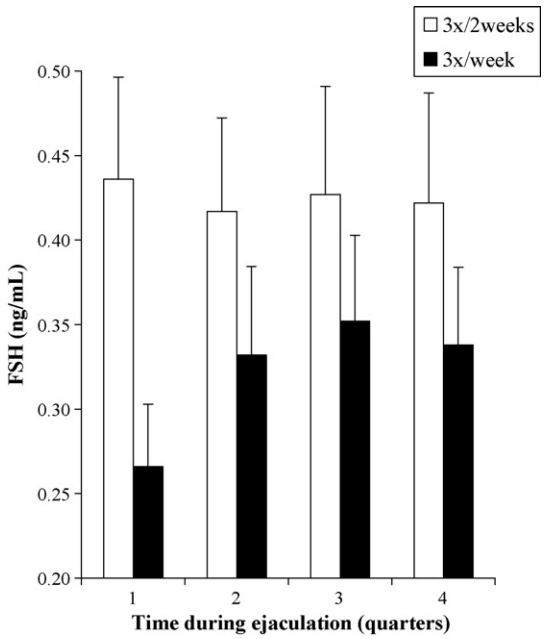

Fig. 2. Plasma FSH concentrations of boars during ejaculation. Ejaculation length was divided into four quarters to standardize for all animals (Quarters 1, 2, 3, and 4). Boars were subjected to two regimens of semen collection frequency during the 3 mo preceding hormonal challenge: 3 times per 2 wk or 3 times per wk. Values are means ± S.E.M. The effect of semen collection frequency at the first quarter ($P = 0.04$) disappeared thereafter (collection frequency by time interaction, $P > 0.08$).

means of 802.2 ± 29.9 doses (3×10^9 sperm cells), 1024.2 ± 27.5 g, and 948.7 ± 28.9 mL, respectively.

4. Discussion

This study provides novel information regarding hormonal profiles in blood and seminal plasma of boars just before and during ejaculation. All hormones measured in both blood plasma and semen had high coefficients of variation. Nevertheless, taking into account the number of animals involved and the sampling procedure, the present study can be regarded as one of the most reliable evaluations to date of the relationship between hormonal homeostasis during ejaculation and reproductive performance in AI boars.

In the present experiment, hormonal concentrations in seminal plasma appeared to be independent of blood plasma concentrations, except for testosterone. Such a correlation was also reported previously for testosterone in boars and was attributed to a direct transfer of T from Leydig cells to peripheral blood plasma in pigs [1]. However, in contrast with findings of Claus et al. [1], no correlation was detected in the present experiment between seminal and blood plasma 17β-estradiol. In terms of the total content of unconjugated estrogens, 17β-estradiol represented 77% and estrone, 23% [24]. Moreover, it has been shown that approximately half of the estrogen in the semen of boar is bound to the sperm membrane [24]. Factors of animal origin, such as genetics and age, which differed between Claus et al. and the present experiment, might have interfered with the partitioning of those types of estrogens.

Nutritional factors such as protein intake affected sperm production in rats [25], bulls [26] and boars [9] by altering hormonal production or secretion. To the best of our knowledge, the present experiment is the first one to investigate the effects of vitamin supplementation on hormonal profiles before and during ejaculation. Globally, there were few treatment effects on hormonal profiles either during or before ejaculation, except for an interaction diet by time for T before ejaculation. In rats, increased testicular steroid dehydrogenase activity and plasma testosterone concentrations were reported after a dietary supplement of ascorbic acid [27]. In the present experiment, however, the vitamin premix did not contain a supplement of ascorbic acid. During ejaculation, the vitamin supplementation effect disappeared, but there was a global decrease in blood plasma testosterone concentrations at the beginning of ejaculation and then an increase. It is likely that this late increase in testosterone during ejaculation originated from the testes, as 95% of blood testosterone is synthesized from the testes, with the remainder coming from the adrenal glands [6]. The constant rise in LH during ejaculation might contribute to the increase in testosterone at the end of ejaculation, because LH is known to stimulate testosterone secretion by Leydig cells [28]. Conversely, an increase in cardiac rhythm during the mount could have caused a change in blood flow that might explain the initial decrease in testosterone. Accelerated blood circulation will increase the amount of testosterone going through the liver and being metabolized [29], thereby decreasing blood testosterone concentrations. Such a mechanism has been proposed for progesterone in sows [30].

The collection frequency treatment applied before the hormonal challenge affected blood plasma FSH concentrations, but only during the first quarter of ejaculation. In fact, in 3/2 boars, blood plasma FSH was higher than for 3/1 boars. This transient effect of semen collection regimen is difficult to explain, taking into account the limited information available in the literature on that matter. Nevertheless, those variations of FSH were more likely attributable to the release of this gonadotropin than to its synthesis, taking into account the relatively short interval involved (ejaculation). Under normal conditions, FSH promotes spermatogenesis and testosterone secretion; in the male, testosterone, in turn, is the principal inhibiting factor for FSH secretion, whereas inhibin is considered a secondary factor [31]. In the present study, testosterone values were not related to FSH measurements. In fact, the coefficient of correlation (not shown in results) was low ($r = -0.056$) and not significant. As far as inhibin is concerned, no measurements were made in the present experiment. Therefore, the transient effect of collection regimen on plasma concentrations of FSH during the first quarter of ejaculation was apparently not sufficient to affect either testosterone secretion or semen production.

In the present experiment, several time responses were observed before and/or during ejaculation. Blood plasma LH concentrations increased linearly throughout ejaculation, but were stable prior to ejaculation. Similarly, Ellendorff et al. [32] also reported a significant elevation following copulation, in spite of considerable fluctuations. Sexual stimulation and mating has been reported to increase the secretion of LH in males of various species [33–36], but not in all studies [37–39]. It can be hypothesized that this transient release of LH during ejaculation is induced by GnRH, given the peak of plasma gonadotropin observed 15–30 min following injection of GnRH [40]. Copulatory behaviour has also been associated with increased plasma cortisol values in boars [41], which

may enhance the release of LH in response to endogenous GnRH stimulation [42]. This endogenous GnRH stimulation might be linked to the increase in blood pressure caused by sexual excitation and the physical effort of the mount and ejaculation. In our study, there was no significant correlation between blood plasma LH and blood plasma testosterone or FSH before or during ejaculation. This lack of correlation between FSH and LH was in line with differential effects of semen collection frequency on these two hormones. Low correlations between FSH and LH were reported in repeated blood plasma of adult intact boars at rest over a period of 12 h [43].

The present experimental design and the large number of animals allowed a reliable assessment of correlations among different hormonal and reproduction criteria. The correlation between blood E_2 and T concentrations during ejaculation ($r = 0.62$ and $P < 0.01$) was likely due to the fact that T is transformed in E_2 in the brain by an aromatase and this aromatase is also present in the Leydig cells of boars [6]. In a previous experiment, E_2 has been associated to some aspects of boar semen production [9]. Although a time response was found for E_2 in the present experiment, there was no relationship with any criteria of semen production. Those differences between experiments may be related in part to different experimental conditions in particular a GnRH challenge used by Louis et al. [9]. The negative correlation between plasma FSH and testis size found in this experiment was similar to results previously reported for boars [16,17]. Total daily sperm production being correlated positively with testicular size in boars [44,45], testicular size is of great economic importance in selecting animals for AI. Based on the present results, we inferred that measurements of reproductive hormone concentrations might become another valuable selection tool, in combination with testis size, for predicting reproductive performance in AI.

In summary, the present dietary and management factors are unlikely to be major modulators of hormonal homeostasis during the process of ejaculation under AI conditions. However, the predictive value of both FSH and testis size for reproduction performance deserves to be further validated and could be promising as additional criteria for the selection of the most appropriate boars for AI purposes.

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