Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.
STUDIES OF PUBERTAL DEVELOPMENT IN BOARS:
RELATIONSHIPS BETWEEN REPRODUCTIVE ORGAN DEVELOPMENT AND
PERIPHERAL PLASMA LEVELS OF LUTEIMIZING HORMONE AND TESTOSTERONE

A thesis presented in partial fulfilment of
the requirements for the
Degree of Master of Philosophy
at Massey University

SIMPLICIA VALENZUELA FLORCRUZ
1977
Studies of Pubertal Development in Boars: Relationships between Reproductive Organ Development and Peripheral Plasma Levels of Luteinizing Hormone and Testosterone

by Simplicia Valenzuela FlorCruz

Two studies were conducted to obtain fundamental data on the process of puberty in Large White x Landrace boars. The first was a longitudinal study designed to evaluate the course of gonadal and epididymal development and the concurrent endocrine changes during the period of sexual maturation.

Twenty-four animals were castrated sequentially at ages ranging from 43 to 241 days. Increases in testicular weight were more highly correlated with body weight ($r = 0.953, P < 0.001$) and age ($r = 0.919, P < 0.001$) than were similar increases in epididymal weight ($r = 0.558$ and $0.593, P < 0.01$, respectively). The age at onset of spermatogenesis varied between boars in the range 90 to 127 days. First observations of spermatozoa in seminiferous and epididymal tubules were made at 127 and 146 days of age, respectively.

Longitudinal profiles of LH and testosterone secretion were investigated by assaying plasma samples collected at fortnightly intervals from each of ten boars aged 41 to 236 days. Until 82 days of age mean LH levels were low (0.20 - 1.25 ng/ml), then rose to a peak of 2.19 ng/ml at 110 days. Later LH levels declined gradually and after 166 days fluctuated between 0.5 and
1.0 ng/ml. Mean testosterone levels also were low in the prepubertal period (0.10 - 0.27 ng/ml), then between 110 and 138 days increased from 0.60 to 8.00 ng/ml. Subsequently testosterone concentrations fell slowly, then except for an isolated peak of 7.73 ng/ml at 194 days, fluctuated between 1.40 and 3.80 ng/ml. These results indicated that the major changes in LH and testosterone secretion during puberty in boars were similar to those which have been reported to occur in males of other species.

Two short term studies of LH and testosterone secretory profiles were carried out to evaluate the effects of stage of sexual maturity on the patterns of secretion of these hormones. Four pubertal and three post-pubertal boars were subjected to plasma sampling every twenty minutes for 24 hours. During puberty, plasma profiles of LH varied in a manner indicative of a highly pulsatile mode of secretion. Likewise, large fluctuations in plasma testosterone levels were noted at this age but they were not as frequent as those of LH. In contrast, plasma LH and testosterone profiles of post-pubertal boars showed fewer and smaller fluctuations in hormone concentrations. The respective overall mean levels of LH and testosterone were 0.82 and 1.04 ng/ml in pubertal boars, and 0.39 and 0.81 ng/ml in post-pubertal boars. These values were consistent with those obtained in the longitudinal study and confirmed the greater output of both hormones during puberty and their decline thereafter. At both pubertal and post-pubertal ages, associations between LH and testosterone pulses were not always consistent, nor was there any evidence of diurnal variations in plasma hormone concentrations.

The results reported in this thesis indicated that puberty in boars occurred between the ages of 90 and 146 days, at which
ages, mean body weights were 35.3 and 58.6 kg, respectively. This period was characterized by a rapid growth of the testes and epididymides and was accompanied by concurrent elevations of plasma LH and testosterone concentrations. The less pulsatile nature of LH and testosterone secretion recorded from the post-pubertal boars probably reflected the maturation (increased sensitivity) of hypothalamic negative feedback mechanisms which normally regulate hormone secretion. The existence of such a mechanism was confirmed by an elevation of plasma LH levels following castration of 5 boars at 215 days of age.
ACKNOWLEDGMENTS

My sincere gratitude goes to the many people without whose help and assistance this thesis would not have been possible: firstly, to my supervisor Dr. K.R. Lapwood for his patient guidance, encouragement, and valuable help in all phases of this project; to the Dept. of Physiology and Anatomy for providing the facilities; to Prof. R.E. Munford and Mr. P.R. Wilson for their kind help in the statistical analyses; to Mr. R.H. Telfer for assisting me in the collection of blood samples; to Mrs. H. Carter, Mr. B. Vautier, and Mr. N. Ward for their technical assistance in the laboratory; to the Massey Pig Research Center staff, Mr. J. Carr, Mr. T. Rogers, and their personnel for their cooperation and help in the management of experimental animals; to Mr. M. Copland and Mr. M. Kurosawa for doing the castrations, and to Mr. M. Birtles for his expertise in the histological processing and photography of testicular and epididymal sections.

Grateful acknowledgments also are due to Dr. L.E. Reichert Jr., Emory University, U.S.A. for supplying the porcine LH for iodination and for assay standards, and to Dr. G.D. Niswender, Colorado State University, U.S.A. for providing the antisera to testosterone and porcine LH.

I am indebted to the Colombo Plan Scholarship Committee for the personal financial support, to the University of the Philippines for granting me the privilege to undertake this study under special detail, and to the Foreign Aid Division, Ministry of Foreign Affairs, New Zealand for providing research maintenance funding.

Finally, I appreciate warmly the moral and spiritual support of my families in the Philippines especially the perseverance and patience of my husband Jorge, who allowed and encouraged me to pursue this 18 month study in New Zealand.
CHAPTER I: INTRODUCTION

A. Physiology of puberty in males
   1. General considerations
   2. Physiological changes associated with puberty
      a. Testicular development
      b. Development of other organs of reproduction
         (1) The epididymides
         (2) The accessory sex glands
         (3) The external genitalia
      c. Development of secondary sex characters
      d. Hormonal changes associated with pubertal development
         (1) Gonadotrophins
         (2) Gonadal steroids
         (3) Other hormones
            (a) Adrenal sex steroids
            (b) Prolactin
            (c) Growth hormone

B. Physiology and endocrinology of reproductive development in boars
   1. Reproductive development
   2. Endocrinology of reproductive development
a. Gonadotrophins 22
b. Testicular steroids 23
c. Hypothalamic-anterior pituitary-testicular relationships during puberty 26
C. The purpose of the present study 27

CHAPTER II: MATERIALS AND METHODS
A. Animals 28
B. Animal management 28
C. Surgical techniques 29
1. Anaesthesia 29
2. Jugular vein catheterization 29
   a. Insertion of the cannula into the jugular vein 29
   b. Exteriorization of the cannula 30
3. Castration 30
4. Post-surgical care 31
D. Blood collection 31
1. Fortnightly sampling 31
2. Twenty-four hr sampling 32
E. Hormone radioimmunoassay procedures 32
1. LH Assay 32
   a. Materials and procedure 32
   b. Assay validation 33
2. Testosterone assay 35
   a. Materials and procedure 35
   b. Assay validation 38
F. Experimental design and analysis 39

CHAPTER III: A LONGITUDINAL STUDY OF PUBERTAL DEVELOPMENT IN BOARS: INVESTIGATION OF THE RELATIONSHIPS BETWEEN GONADAL AND EPIDIDYMAL DEVELOPMENT AND PLASMA LH AND TESTOSTERONE PROFILES
A. Introduction 40
B. Materials and methods 41
   1. Animals and experimental procedure 41
   2. Statistical analysis 42
CHAPTER IV: TWENTY-FOUR HOUR PLASMA PROFILES OF LUTEINIZING HORMONE AND TESTOSTERONE IN PUBERTAL AND POST-PUBERTAL BOARS

A. Introduction
B. Materials and methods
C. Results
   1. LH
   2. Testosterone
   3. LH-testosterone relationships
D. Discussion
   1. Hormone profiles in pubertal boars
   2. Hormone profiles in post-pubertal boars
   3. LH-testosterone relationships

CHAPTER V: GENERAL DISCUSSION AND CONCLUSIONS

A. Longitudinal study
B. Twenty-four hour hormone profile study
C. Possible applications of the present findings

REFERENCES
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>FIGURE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>34</td>
</tr>
<tr>
<td>2.2</td>
<td>37</td>
</tr>
<tr>
<td>3.1</td>
<td>45</td>
</tr>
<tr>
<td>3.2</td>
<td>46</td>
</tr>
<tr>
<td>3.3</td>
<td>47</td>
</tr>
<tr>
<td>3.4</td>
<td>48</td>
</tr>
<tr>
<td>3.5</td>
<td>49</td>
</tr>
<tr>
<td>3.6</td>
<td>51</td>
</tr>
<tr>
<td>3.7</td>
<td>52</td>
</tr>
<tr>
<td>3.8</td>
<td>54</td>
</tr>
<tr>
<td>3.9</td>
<td>54</td>
</tr>
<tr>
<td>3.10</td>
<td>55</td>
</tr>
<tr>
<td>3.11</td>
<td>55</td>
</tr>
<tr>
<td>3.12</td>
<td>57</td>
</tr>
<tr>
<td>3.13</td>
<td>57</td>
</tr>
<tr>
<td>3.14</td>
<td>59</td>
</tr>
<tr>
<td>3.15</td>
<td>59</td>
</tr>
<tr>
<td>3.16</td>
<td>61</td>
</tr>
<tr>
<td>FIGURE</td>
<td>PAGE</td>
</tr>
<tr>
<td>--------</td>
<td>------</td>
</tr>
<tr>
<td>3.17</td>
<td>Photomicrograph of a testicular section from a 241 day old boar</td>
</tr>
<tr>
<td>3.18</td>
<td>Profile of plasma LH levels in boars during sexual maturation</td>
</tr>
<tr>
<td>3.19</td>
<td>Comparison of the profiles of plasma LH levels between two litters of boars</td>
</tr>
<tr>
<td>3.20</td>
<td>Profile of plasma testosterone levels in boars during sexual maturation</td>
</tr>
<tr>
<td>3.21</td>
<td>Comparison of the profiles of plasma testosterone levels between two litters of boars</td>
</tr>
<tr>
<td>4.1</td>
<td>LH secretory profiles recorded from plasma samples collected from four pubertal boars sampled at 20 min intervals for 24 hours</td>
</tr>
<tr>
<td>4.2</td>
<td>LH secretory profiles recorded from plasma samples collected from three post-pubertal boars sampled at 20 min intervals for 24 hours</td>
</tr>
<tr>
<td>4.3</td>
<td>Twenty-four hr cumulative LH levels showing pooled regression lines</td>
</tr>
<tr>
<td>4.4</td>
<td>Testosterone secretory profiles recorded from plasma samples collected from four pubertal boars sampled at 20 min intervals for 24 hours</td>
</tr>
<tr>
<td>4.5</td>
<td>Testosterone secretory profiles recorded from plasma samples collected from three post-pubertal boars sampled at 20 min intervals for 24 hours</td>
</tr>
<tr>
<td>4.6</td>
<td>Twenty-four hr cumulative testosterone levels showing pooled regression lines</td>
</tr>
</tbody>
</table>