Effects of Human Chorionic Gonadotrophin and Castration on Plasma Gonadal Steroid Hormones of the Dog

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SUMMARY

An intramuscular injection of 500 I.U. of human chorionic gonadotrophin resulted in an increase of plasma testosterone and progesterone concentrations in the intact male dog, but had no effect on plasma 17B-estradiol concentration. Castration caused a rapid decrease in concentration of testosterone, progesterone, and 17B-estradiol, indicating that the tests were the major organs producing these hormones in the male dog.

RÉSUMÉ

Effets de la gonadotrophine chorionique humaine et de la castration sur la teneur du plasma en hormones stéroïdes gonadiques, chez le chien

L'injection intramusculaire de 500 U.I. de gonadotrophine chorionique humaine à des chiens mâles, se traduisit par une élévation de la teneur du plasma en testostérone et en progestérone; elle ne produisit cependant aucun effet sur celle de l'oestradiol-17B. La castration entraîna par ailleurs une baisse rapide de ces trois hormones, dans le plasma, indice que les testicules sont les organes qui en secrètent la majeure partie, chez le chien.

INTRODUCTION

It is generally believed that the gonadal steroidal hormones play an important role in the proper functioning of the skin (6,7). Androgens increase the production of sebum by favoring formation of new sebaceous cells and hypertrophy of mature cells, and therefore, augmenting the synthetic potential of each cell (2). Progesterone may also have a direct stimulating effect on sebaceous glands. Estrogens have been shown to reduce both the sebaceous gland size and sebum production (2) and to promote keratinization (5).

In general, relatively little is known about the effect of gonadal hormones on apocrine glands. Androgens seem to have a positive effect and estrogen a negative effect on these glands (2). This is based on the fact that larger apocrine glands were found in male than in female rabbits and this difference is more evident at sexual maturity. Ovariohysterectomized rabbits have increased apocrine gland size. In addition, estrogen but not androgen reduces the rate of hair growth in rabbits (2).

In the dog, two testicular disorders, the Sertoli cell tumor and the male feminizing syndrome have been cited most frequently to be associated with pathological dermatological manifestations (6). Signs associated with these syndromes are gynecomastia, decreased libido, alopecia, and hyperpigmentation (7). Seborrhea has been observed with the male feminizing syndrome but was absent in those with the Sertoli cell tumor (3,7). The male feminizing syndrome is a confusing entity of unknown etiology although it has been attributed to hypoandrogenism as well as hyperestrogenism (7). However, no difference has been found in the concentration of estradiol and testosterone in peripheral blood nor in spermatic venous blood of dogs with the male feminizing syndrome (6). This has also been shown in man (9). The Sertoli cell tumor has been associated with hyperestrogenism (5,7). Although the hormonal causes are not fully understood, possible explanations include: a) hormone excesses, b) hormone deficiencies, c) imbalances in ratio of hormones, d) abnormal responses to pituitary gonadotropins, e) others. It is not known whether: a) gonadal hormone deficiencies or excesses are real entities or, b) an imbalance in the relative concentrations of gonadal hormones are important. Assays of plasma estrogen, testosterone and progesterone were used in the present study to document values in male dogs, and to study their responses to luteinizing hormone stimulation and castration.

MATERIALS AND METHODS

Animals

Mature, one to two years old, healthy, cross bred male dogs weighing 15 to 25 kg, housed individually in an artificially illuminated (14 h light:10 h dark) area, were used. Feed[1] and water were available *ad lib*, except before surgery when feed was withheld for 24 h.

Experimental Design

To determine levels in normal male dogs, blood samples were taken from a jugular vein catheter at 36, 24, 18, 12, six and zero hours before an intramuscular injection of 500 I.U. human chorionic gonadotrophin (HCG).[2] To determine the responses to HCG, the same dogs were sampled at one, two, four, six and eight hours after the injection. To determine the response to castration, dogs were divided randomly into two groups eight hours after the HCG injection. Dogs in group 1 were anesthetized with sodium thiamylal[3] (10 mg/kg), intubated,

[1]Dog Chow, Ralston Purina Co., Mississauga, Ontario.
[2]Chorionic Gonadotropin, E.L. Stickley and Co. Ltd., Brantford, Ontario.
[3]Bio-Tal, MTC Pharmaceuticals, Hamilton, Ontario.

maintained with halothane[4] for one half hour and allowed to recover. Dogs in group 2 were treated the same as those of group 1 except that they were castrated under anesthesia. Blood samples were taken from both groups at four, 12, 24, and 36 h after halothane was discontinued. Removed testicles were submitted for histological examination.

Blood Sampling

Ten mL blood samples were collected and placed in heparinized tubes. The catheters were flushed with 2.5 mL of heparinized saline. Blood samples were centrifuged immediately and plasma was stored at -20° C.

Assays

Progesterone and 17B-estradiol were measured by radioimmunoassay using specific antisera, validation of these assays has been published previously (8). Testosterone was extracted from plasma with diethyl ether and the extracts were assayed using an antiserum raised in rabbit specific for testosterone (NCR-98-18-4-78).[5] Cross reactions of this antiserum (1) with major steroids are listed in Table I. Duplicate determinations were made for all samples using 200 μL plasma and all determinations were corrected for procedural losses. The assay standard curve ranged from 5 pg to 1 ng to 1 ng per assay tube and assay sensitivity, defined as the lowest concentration of testosterone that significantly displaced labelled testosterone, was 25 pg/mL. The average extraction efficiency was 77 ± 2.4% (n = 11). The assay blank was less than assay sensitivity and the interassay coefficient of variation was 15% (n = 17).

TABLE I
PERCENTAGE CROSS REACTIONS OF STEROIDS WITH TESTOSTERONE ANTISERUM (NCR-98-18-4-78)[a]

Steroid	% Cross Reaction
Testosterone	100
Progesterone	<0.2
Estradiol-17B	<0.2
Cortisol	<0.02
Cholesterol	<0.02
Dehydroepiandrosterone	8.0
Pregnenolone	0.48
Dihydrotestosterone	100
Androstenedione	4.4

[a]Calculated as described by Abraham *et al* 1970.

Statistical Analysis

For each of the hormones the mean basal level (± standard deviation) was determined for the 36 h before administration of HCG by pooling all results. Response to HCG injection was expressed as a mean (± s.d.) for all dogs at each time interval and a one way analysis of variance was calculated using time intervals as the independent variable. When a significant "F value" was obtained, each sample time was compared to the basal (0 h) level by the least significant difference multiple range test (10).

The data for hormone concentrations were analysed statistically by a two by five analysis of variance, using treatment (castrated and noncastrated animals) and time (0, 4, 12, 24, 36 h) as independent variables. When a significant "F value" was calculated, mean hormone levels in castrated dogs were compared to the mean basal (0 h) level and to the level at the same time in noncastrated dogs.

RESULTS

The mean plasma testosterone, progesterone, and estrogen concentrations before HCG injection were: 622 ± 353 pg/mL, 1151 ± 729 pg/mL, and 38 ± 21 pg/mL respectively (Figures 1, 2 and 3). A single intramuscular injection of 500 I.U. of HCG resulted in a significant ($P \leq 0.05$) increase in plasma testosterone and in plasma progesterone by 6 h (Figures 1 and 2). Concentration of testosterone and progesterone peaked at four to eight hours and six to eight hours respectively, but there was no significant effect on 17B-estradiol (Figure 3), after injection of HCG. Plasma testosterone concentrations in intact dogs remained elevated but progesterone concentration decreased to basal values within 36 h after HCG administration. Plasma estrogen concentra-

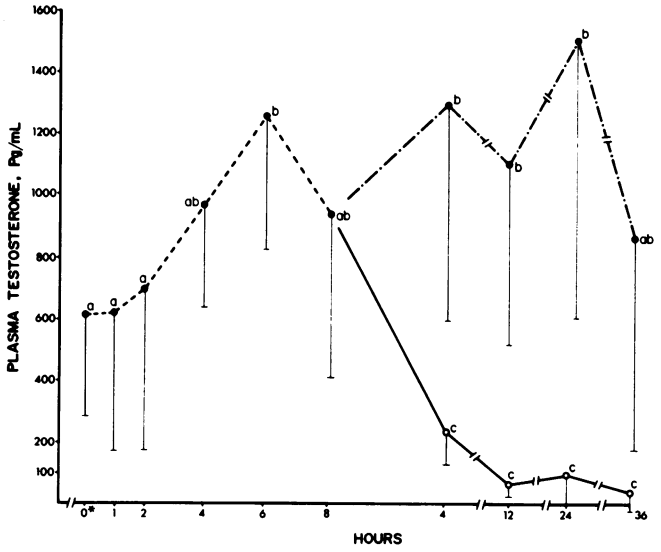

FIGURE 1. Influence of HCG and castration on plasma testosterone level (mean ± s.d.). 500 I.U. of HCG was injected intramuscularly at 0 h. Eight hours after injection of HCG, dogs were randomized into two groups. Group 1 served as controls (n = 9) and Group 2 were castrated (n = 8). a.b.c. Mean testosterone levels over time or between samples at the same time, with superscripts in common were not significantly different ($P \leq 0.05$). *Pooled results of six collections at variable time intervals of 17 dogs prior to treatment.

• — · — · — • intact animals ○———○ castrated animals

[4]Fluothane, Ayerst Laboratories, Montreal, Quebec.

[5]N.C. Rawlings, Reproductive Endocrine Lab., Department of Veterinary Physiological Science, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Saskatchewan.

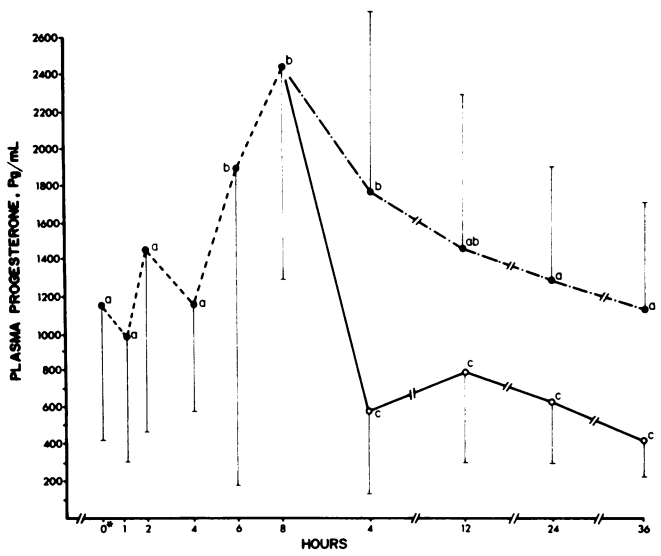

FIGURE 2. Influence of HCG and castration on plasma progesterone level (mean ± s.d.). 500 I.U. of HCG was injected intramuscularly at 0 h. Eight hours after injection of HCG, dogs were randomized into two groups. Group 1 served as controls (n = 9) and Group 2 were castrated (n = 8). a.b.c. Mean progesterone levels over time or between samples at the same time, with superscripts in common were not significantly different ($P \leqslant 0.05$). *Pooled results of six collections at variable time intervals of 17 dogs prior to treatment.

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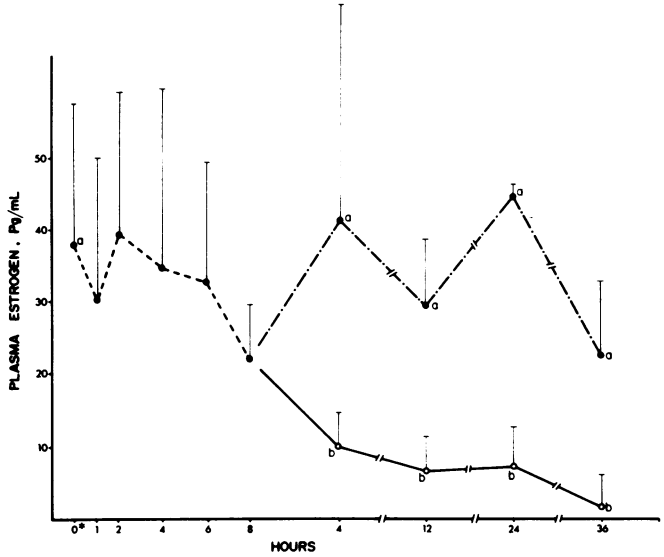

FIGURE 3. Influence of HCG and castration on plasma estrogen level (mean ± s.d.). 500 I.U. of HCG was injected intramuscularly at 0 h. Eight hours after injection of HCG, dogs were randomized into two groups. Group 1 served as controls (n = 9) and Group 2 were castrated (n = 8). a.b. Mean estrogen levels over time or between samples at the same time, with superscripts in common were not significantly different ($P \leqslant 0.05$). *Pooled results of six collections at variable time intervals of 17 dogs prior to treatment.

• ·—·—·—· • intact animals ○———○ castrated animals

tion remained within the pre-HCG injection range of values throughout the study. The plasma testosterone, progesterone and estrogen concentrations decreased significantly ($P \leqslant 0.05$) in dogs within four hours after castration (Figures 1, 2 and 3), at which time the values were significantly less than prior to HCG injection. Removed testicles were histologically normal.

DISCUSSION

The plasma testosterone concentration found in this study is in agreement with a previous report for the dog (4). After an intramuscular injection of 500 I.U. HCG to intact dogs the concentration increased, peaked at six hours and remained elevated for the duration of the study. This has also been observed in the bull (11). The peripheral plasma testosterone concentration decreased rapidly within hours following castration. It was observed that before the HCG injection testosterone levels fluctuated within individuals which is in agreement with a previous report for the dog (4). Further studies are necessary to determine if a daily rhythm of testosterone occurs in the dog.

Plasma progesterone concentration increased markedly after HCG injection and declined rapidly following castration, indicating that the testicles are a major source of progesterone production. This increase in blood progesterone concentration presumably results from the effect of HCG on the testicular Leydig cells. Since progesterone is an intermediate product in testosterone biosynthesis (12), the major effect of HCG must be at a level prior to pregnenolone, therefore HCG can be expected to increase testicular progesterone and testosterone production.

In this study, peripheral plasma 17B-estradiol concentration did not increase after HCG injection, but declined rapidly after castration indicating that the testicle was a major source of estrogen production. The Sertoli cells have been shown to be the primary source of testicular estrogen (12), therefore HCG injection appears to affect Leydig but not Sertoli cells. This work has also demonstrated that the testicles are a major source not only of testosterone, but also of progesterone and estrogen.

ACKNOWLEDGMENTS

The author greatly acknowledges the financial support of the Canadian Veterinary Research Trust Fund.

REFERENCES

1. ABRAHAM, G.E., W.D. O'DELL, R. EDWARDS and J.M. PURDY. Solid phase radioimmuno-assay of estrogens in biological fluids. Acta endocr. Copenh. Suppl. 147: 332. 1970.
2. AMOROSO, E.C. and F.J. EBLING. Allergic and endocrine dermatosis in the dog and cat. II. Hormones and skin. J. small Anim. Pract. 7: 755-775. 1966.
3. DOERING, G.D. and M.E. JENSEN. Clinical Dermatology of Small Animals. 2nd Ed. p. 91. St. Louis: C.V. Mosby. 1973.
4. DePALATIS, L., J. MOORE and R.E. FALVO. Plasma concentration of testosterone and LH in the male dog. J. Reprod. Fert. 52: 201-207. 1978.
5. HALLIWELL, R.E.W. Steroid therapy in skin disease. *In* Current Veterinary Therapy VI. R.W. Kirk, Ed. pp. 541-547. Philadelphia: W.B. Saunders Co. 1976.
6. MATTHEEUWS, D. and F. COMHAIRE. Oestradiol and testosterone in male dogs with alopecia and feminization without testicular neoplasm. Br. vet. J. 131: 65-69. 1975.
7. MULLER, G.H. and R.W. KIRK. Textbook of Small Animal Dermatology. 2nd Ed. p. 17. Philadelphia: W.B. Saunders Co. 1976.
8. RAWLINGS, N.C., L. WEIR, B. TODD, J. MANNS and J. HYLAND. Some endocrine changes associated with the post partum period of the suckling beef cow. J. Reprod. Fert. 60:

301-308. 1980.
9. SOUTHREN, A.L., D. ROSS, D.C. SHARMA, G. GORDON, B.W. ALLEN and R.J. DORFMAN. Plasma concentration and biosynthesis of testosterone in the syndrome of feminizing testis. J. clin. Endocr. 25: 518-525. 1965.
10. STEEL, G.D. and J.H. TORRIE. Principles and Procedures of Statistics. pp. 106-107. New York: McGraw-Hill Book Co.
11. THIBIER, M. and O. ROLLAND. Levels of testosterone and luteinizing hormone in the plasma of young post-pubertal bulls after injection of human chorionic gonadotrophin. J. Endocr. 75: 451-452. 1977.
12. VANDERMOLEN, H.J. and K.B. EIK-NES. Biosynthesis and secretion of steroids by the canine testis. Biochim. biophys. Acta 248: 343-362. 1971.

BOOK REVIEW/ANALYSE DE VOLUME

Reproduction in the Pig. P.E Hughes and M.A. Varley. Published by Butterworth Publishers, Inc., Massachusetts. 1980. 241 pages. Price US$39.95, paperback US$23.95.

The main objective of this book is to present the biological relations which control the important facts of the annual productivity in the sow: the number of piglets born and the number of litters produced each year by the sow.

The book is divided into three parts. The first one gives the basics on reproductive physiology. This very short chapter is meant for people without any knowledge on the general reproductive physiology. The second part deals with the sow and the third one with the boar. Chronologically, the authors present puberty, estral cycle, ovulation, fertilization and conception, lactation and weaning. The last part deals with boar puberty, the assessment of fertility potential, coupling (behavior) and artificial insemination. A conversion table and a list of important words with definitions are given at the end of the book.

Each chapter begins with a flash back on scientific facts of the pig reproductive physiology then goes on with the practical implication of these facts. The reader can complete his information by referring to the bibliography listed at the end of each chapter.

After reading this book, one main conclusion can be drawn: reproductive control in the pig is under the direct dependence of good management.

This book is meant for everyone (including pig-breeder). However, veterinarians should have it as a referring book, especially those involved in the supervision of breeding farms.

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L'objectif principal de ce livre est de présenter les relations biologiques qui contrôlent les principaux facteurs de la productivité annuelle de la truie: le nombre de porcelets qui naissent ainsi que le nombre de portées produites par année par la truie. Pour ce faire, le livre est divisé en trois parties. La première donne les bases de la physiologie de la reproduction. Ce chapitre, très court, s'adresse à des personnes qui n'ont aucune connaissance sur la physiologie générale de la reproduction. La deuxième partie est consacrée à la truie et la troisième, au verrat. Chronologiquement, les auteurs présentent la puberté, le cycle oestral, l'ovulation, la fertilisation et la conception, la gestation, la lactation et le sevrage. Pour le verrat, les auteurs donnent aussi la puberté, l'évaluation de la fertilité, l'accouplement (le comportement) et l'insémination artificielle. Le livre se termine avec des tables de conversion ainsi que d'une liste de termes utilisés dans le texte avec leur définition.

Chaque chapitre rappelle d'abord les données scientifiques sur la physiologie de la reproduction chez le porc pour ensuite donner les implications pratiques de ces connaissances toujours basées sur des résultats d'analyses scientifiques. Le lecteur peut compléter sa documentation en consultant les références appropriées présentées à la fin de chaque chapitre. Une conclusion générale peut être soulignée après avoir lu ce livre. Le contrôle de la reproduction chez le porc est sous la dépendance directe de la régie. Les résultats d'analyses démontrent que les techniques naturelles sont, jusqu'à présent, plus efficaces que toute thérapeutique hormonale.

Ce livre s'adresse donc à toute personne intéressée par la reproduction chez le porc. Même un éleveur de porc peut en retirer beaucoup grâce à la simplicité et la clarté des textes et figures. Toutefois, il sera dans de meilleures mains si les médecins vétérinaires l'ont pour bien expliquer et utiliser les bienfaits de la régie dans le domaine de la reproduction.

D. Bousquet.

ABSTRACT

Little, T.W.A. and Harding, J.D.J. The interaction of *Haemophilus parahaemolyticus* and *Pasteurella multocida* in the respiratory tract of the pig. British Veterinary Journal (1980) 136: 371-383. (Central Vet. Lab., New Haw, Weybridge, Surrey, UK).

More than 20% of deaths in fattening pigs are caused by pneumonia associated with *P. multocida* but this organism does not cause pneumonia when it is given experimentally to pigs. In an outbreak of pneumonia in store pigs, only *P. multocida* could be isolated by conventional means, but selective media and a dilution method (to dilute out the inhibitory effect of *P. multocida*) yielded also *H. parahaemolyticus*. Ten day old pigs from a hysterectomy-derived herd kept in isolation were infected with either or both of the two organisms. *P. multocida* alone caused no disease but together with *H. parahaemolyticus* caused severe pneumonia which was fatal in pigs infected through the trachea. *H. parahaemolyticus,* also, caused fatal pneumonia when given alone.

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