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'Advanced Puberty' in Female Guinea Pigs Treated with Human Chorionic Gonadotrophin (HCG) or Testosterone Enantate (TE) at Birth

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Abstract. Newborn female guinea pigs treated with HCG or testosterone enantate at birth display a 'precocious puberty' i.e., advanced vaginal opening and normal estrous cycles.

Animals treated with a high dose of HCG (1,500 RU) had smaller ovaries than the controls or those treated with 500 RU. All groups had corpora lutea in their ovaries.

Hemispaying reaction was 89.8% in 1,500-RU animals, 67.2% in the 500-RU group, and 37.2% in the controls.

It is concluded that the 'ovarian refractory period' in the newborn guinea pig is not present under our experimental conditions. The influence of the ovarian steroid secretion on these results is discussed.

Key Words
HCG
Testosterone enantate
Newborn guinea pig
Hemispaying
Precocious puberty differentiation
Tonic-fasic

Precocious vaginal opening in the rat has been used as evidence of advanced puberty, in response to early treatments with gonadotrophins, estrogens or androgens. All of these treatments are ineffective during the first 3 weeks called 'refractory period' [CRITCHLOW and BAR SELA, 1966].

A similar refractory period has also been described in guinea pig, based on histological studies [FREED and COPPOCK, 1936]. However, FALK and PAPANICOLAOU [1936] and BUÑO [1959] have shown that HCG injected in newborn guinea pigs induces 'masculinization' of external genitalia, appointing to an 'abnormal' response of these ovaries.

Previous results from this laboratory have shown that testosterone propionate (TP) injected in newborn guinea pigs modified neither puberty (first vaginal opening) nor the following estrous cycles [CARLEVARO *et al.*, 1969].

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The present study was conducted to know if HCG or a long-acting androgen testosterone enantate (TE) given during the perinatal period are able to modify the time of puberty and/or the following estrous cycles in guinea pigs.

Material and Methods

Newborn female guinea pigs from our stock were divided into the following groups:

Group I. (A) Fourteen animals received a subcutaneous (s.c.) pellet of 10 mg of TE, under ether anesthesia. (B) Ten animals were anesthetized, recovered, and used as controls.

Group II. (A) Fourteen animals were injected with 100 RU (rat unit) of HCG/day s.c., during days 1–5 post-delivery (day 1 = day of delivery). (B) Eleven animals received 500 RU of HCG/day s.c. during days 1–3. (C) Thirteen animals received saline s.c. and were used as control group.

All animals were weaned at 10 days of age. They were housed in small cages (4 per cage) in a light-controlled room, with a 14-hour ligth + 10-hour darkness schedule. Barley, alfalfa and tap water were supplied *ad libitum*.

Puberty and estrous cycles were determined as in previous papers [CARLEVARO *et al.*, 1969].

At 160–170 days of age, animals of group II (HCG-treated) were hemispayed under ether anesthesia. Left ovary was always removed, weighed, fixed and prepared for histological study. Serial sections of 10 μm were stained with PAS-H. Hemispaying was performed between the 9th and 11th days of the estrous cycle (day 1 = vaginal opening day). After 45–50 days post-hemispaying, the animals were autopsied between the 9th and 11th days of estrous cycle.

Animals from group I (TE-treated) were autopsied at 145–155 days of age, also between the 9th and 11th days of the estrous cycle.

Autopsy procedure. The animals were killed by bleeding under ether anesthesia. The endocrine organs were dissected, weighed, fixed and prepared for histological study. Serial sections of the ovaries of all animals were performed as described above. Uteri, adrenals, pituitaries and thyroids were also sectioned and stained with PAS-H.

Statistical results were analyzed using Student's t test, analysis of variance, and Duncan's test [STEEL and TORRIE, 1960].

Results

All the animals treated with HCG or implanted with TE showed a clitoridean hypertrophy which disappeared at about 25 days of age.

Animals implanted with TE had the first vaginal opening *(puberty)* at 34 ± 1.9 days of age, while the control group had it at 69 ± 7.9 days. This difference is highly significant ($p<0.001$). The following estrous cycles in

Table I. Body and organ weights of female guinea pigs implanted with TE (10 mg s.c.) at birth. Values are mean ± standard error

Group	Body weight		Ovaries	Uterus	Adrenals	Thyroid	Pituitary
	initial	final					
Control	75 ± 2.4	517 ± 17.8	72 ± 6.4	932 ± 43	350 ± 11	70 ± 5.8	14 ± 0.6
TE	75 ± 2.7	533 ± 15.0	67 ± 5.9	896 ± 31	347 ± 8.4	65 ± 2.3	15 ± 0.5

Table II. Body and organ weights of female guinea pigs treated with HCG at birth and hemispayed between 160 and 170 days of life

Group	Body weight		Ovaries		Percentage of hypertrophy[3]	Uterus	Adrenal	Thyroid	Pituitary
	initial	final	left	right					
Control	80±3.3[1]	575±61	34±1.5	45±2.7	37.2	976± 55	380±17	66±3.8	16±0.6
500 RU of HCG	80±4.9	598±25	33±2.1	53±3.7	67.2	1,079±123	389±21	67±3.4	17±0.4
1,500 RU of HCG	84±6.3	602±21	25±1.4[2]	48±0.9	89.8	1,039± 62	424±17	69±4.1	16±0.8

[1] Mean ± standard error.

[2] $p<0.01$ (Duncan's test).

[3] Percentage of hypertrophy $= \frac{OD - OI}{OI} \times 100$.

OD = Right ovarian weight.

OI = Left ovarian weight.

both treated and control animals had a normal length (19 days). (In our colony the average estrous cycle length is 19 ± 2.5 days.)

We observed no significant differences in weight of organs between treated and control animals (table I). Ovaries of both groups showed corpora lutea and growing follicles suitable to the day of estrous cycle at autopsy.

Animals treated with HCG demonstrated a clear advance in vaginal opening, which was earlier in animals injected with 500 RU than in those receiving 1,500 RU. The following cycles of both groups had normal length (fig. 1).

The left ovaries of 1,500-RU treated guinea pigs were smaller than both those of the 500-RU treated ones and the control group. Histological study evidenced normal corpora lutea and growing follicles, as a consequence of previous ovulations.

After 45–50 days post-hemispaying the 1,500-RU treated guinea pigs had the higher compensatory hypertrophy (89.9%), larger than 500-RU treated animals (67.2%) and control animals (37.2%; table II). Right ovaries of all animals, treated and control, had normal corpora lutea and follicles.

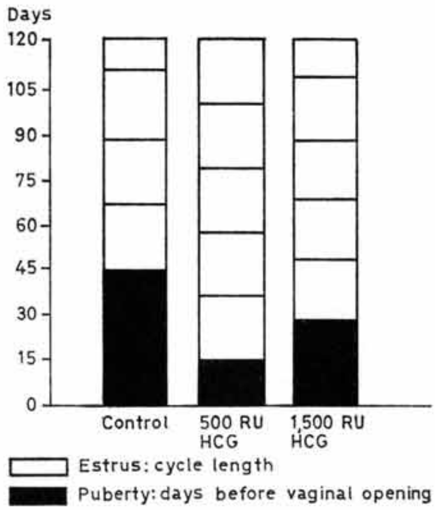

Fig. 1. Puberty and estruos cycles of female guinea pigs treated with two different doses of HCG at birth.

Discussion

In our experiments we found that it is possible to induce precocious puberty (advanced vaginal opening followed by normal estrous cycles) through hormonal treatment during the immediate postnatal life employing two different models.

The HCG results suggest that the ovarian 'refractory period' in the guinea pig is not as absolute as can be suspected from FREED and COPPOCK'S [1936] experiments, since we induced precocious puberty which evidences an ovarian response to exogenous, and possibly, to endogenous gonadotrophins. BUÑO [1959] also showed a histological response of guinea pig ovaries treated with HCG at birth.

Precocious puberty induced by HCG treatment could imply an endogenous gonadotrophin participation through a positive feedback action of the initial steroid ovarian secretion. Since HCG induced a more advanced precocious puberty than TE implants, it is suggested that also in prepuberal ovaries the follicle sensibility to gonadotrophin is increased by estrogens as was shown in adult animals by ARON *et al.* [1968]. TP [a shorter acting androgen than TE; JUNKMANN, 1957] injected to newborn female guinea pigs did not modify puberty [CARLEVARO *et al.*, 1969] as TE did. The difference could be related directly to the different acting time of the two androgens and the maturation the animals have during this period.

The TE-induced precocious puberty could be explained by a testosterone transformation to estrogens and their posterior action. It is well known that androgens can be converted to estrogens through an aromatization process [MCKERNS, 1969]. In rats, precocious puberty has been induced by estrogen injected in the prepuberal period or by hypothalamic implants [SMITH and DAVIDSON, 1968]. Another possibility to explain our results can be a direct positive feedback action of androgen on gonadotrophin secretion. JOHNSON and NAQVI [1969] show a positive feedback action of androgen on prepuberal rats. We do not have yet evidences to support any of these hypotheses; nevertheless, both can be accepted and are probably complementing each other.

Normal ovulation and estrous cycles in newborn TE-implanted guinea pigs agree with previous results from this laboratory, which proved that sexual gonadotrophin control is differentiated in guinea pigs prenatally [CARLEVARO *et al.*, 1969; BUÑO *et al.*, 1967; DOMÍNGUEZ *et al.*, 1968].

Different doses administered in HCG treatment clearly affected the first vaginal opening as well as the weight of the left ovary. However, response to hemispaying was unequal between control and HCG-treated animals. The highest hemispaying response was observed in the HCG-treated guinea pigs. It seems that the initial steroid secretion by the ovary (estrogen, androgen, or both) induced by HCG treatment, altered the reactivity of the gonadotrophin secretion system to estrogen levels or the ovarian response to gonadotrophin elevations. Similar alteration in the hemispaying response has been shown in bewborn guinea pigs treated with TP [CARLEVARO *et al.*, 1969].

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