The action of trenbolone acetate, a synthetic anabolic steroid, on ovarian function in the guineapig

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Summary

The action of trenbolone acetate, a synthetic anabolic steroid, on ovarian function was investigated in the guineapig. Certain comparisons were made with testosterone, the naturally occurring androgen, administered as the phenylpropionate ester. Two milligrammes trenbolone acetate per kg given subcutaneously on alternate days for 20 days blocked oestrous cyclicity and ovulation in 9 of 10 animals. A similar effect was shown by 2·2 mg of testosterone phenylpropionate. Treatment of trenbolone acetate-treated animals with exogenous gonadotrophins suggested that the production of follicle-stimulating hormone had been suppressed. Signs of abnormality were seen in the livers of animals receiving 2 mg trenbolone acetate and 2·2 mg testosterone phenylpropionate.

Keywords: Guineapig, Trenbolone acetate; Anabolic steroid; Androgens; Oestrous cycle; Anovulation

Trenbolone acetate (TBA, androst-1,9(10), 11-trien-3-one, 17 acetate) is a synthetic anabolic steroid with androgenic activity (Neumann, 1976). It has been used in a number of countries as a growth promoter in ruminants. Reynolds *et al.* (1981) and Zarkawi *et al.* (1990) have reported that oestrous cyclicity was disrupted when TBA was used on cows and heifers. The site of action of this effect has not been identified.

The guineapig, unlike the rat, mouse and rabbit, but like the cow, the ewe and the human, is both a spontaneous ovulator and has a spontaneous luteal phase (Hilliard, 1973). The guineapig is, therefore, an appropriate model for studying factors affecting ovarian function in ruminants and man. The aim of the present study was to investigate the effect of TBA and a natural androgen, testosterone, on oestrous cyclicity and ovarian function in the guineapig, and to identify the site of its inhibitory action.

Materials and methods

Animals

Adult female albino Dunkin-Hartley strain guineapigs (A. Tuck and Son Ltd, Essex, UK) were housed under controlled conditions (temperature 21–22 °C, light : dark, 14 : 10 h, with lights on 0700–2100 h). Guineapigs were fed SGP pellets (K & K Greets Ltd, UK) *ad libitum*, supplemented with carrots and cabbage. Vitamin C was dissolved in the drinking water (0·7 g/l). The oestrous cycles were followed by twice daily examination of the vaginal membrane and a smear was taken whenever the vagina was open. The day on which vaginal cornification preceded an influx of leucocytes in the vaginal smear was considered the day of oestrus and designated day 1 of the oestrous cycle. Animals were used on experiment after exhibiting 2 normal (15–18 day) oestrous cycles.

Chemicals

Trenbolone acetate (Finajet 30) was obtained from Hoechst, UK made up in arachis oil. Grade II

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bovine serum albumin (BSA), human chorionic gonadotrophin (hCG) and porcine follicle-stimulating hormone (FSH) were obtained from Sigma Chemical Company Ltd (Dorset, UK). One unit (u) of FSH, equivalent to 1 mg Armour standard 264-151-X, has a potency of 9·8 i.u. 2nd IRP HMG in the Steelman & Pohley (1953) bioassay. BSA/saline was prepared as a 1% (w/v) solution in 0·9% (w/v) NaCl. Both hCG and FSH were prepared in BSA/saline and stored at −20 °C. Testosterone phenylpropionate (TPP, Androjet) was obtained from Intervet Ltd, UK and made up in arachis oil.

Treatments

Experiment 1 Fifteen animals were allocated to 3 treatments comprising 5 animals each as follows: (*a*) control, given 0·33 ml arachis oil per injection; (*b*) TBA 0·4, given 0·4 mg TBA per kg body weight per injection; (*c*) TBA2, given 2 mg TBA per kg body weight per injection. Ten injections were given subcutaneously on alternate days starting on day 8 of the oestrous cycle. The animals were killed by stunning and exsanguination one day after the last injection.

Experiment 2 Twenty-five animals were allocated to 5 treatments comprising 5 animals each as follows: (*a*) control, given 0·33 ml arachis oil per injection; (*b*) TBA 0·4, given 0·4 mg TBA per kg body weight per injection; (*c*) TBA2, given 2 mg TBA per kg body weight per injection; (*d*) TPP 0·44, given 0·44 mg TPP per kg body weight per injection; (*e*) TPP 2·2, given 2·2 mg TPP per kg body weight per injection. Ten injections were given subcutaneously on alternate days starting on day 8 of the oestrous cycle. The animals were killed by stunning and exsanguination one day after the last injection.

Experiment 3 Twenty animals were allocated to 4 treatments comprising 5 animals each as follows: (*a*) TBA alone, given 10 injections of 2 mg TBA per kg body weight per injection subcutaneously on alternate days starting on day 8 of the oestrous cycle; (*b*) TBA plus FSH, given the same as group (*a*) with 250 mu FSH being given subcutaneously twice daily on days 11–15 inclusive; (*c*) TBA plus hCG, given the same treatment as group (*a*) with 50 i.u. hCG, being given i.p. on day 15; (*d*) TBA plus FSH and hCG given the same as treatment (*b*) with 50 i.u. hCG being given i.p. on day 15. All animals were killed by stunning and exsanguination one day after the last TBA injection (day 17).

Analytical procedures

Vaginal opening and smears During treatment animals were examined twice daily to check whether the vagina was open or closed. If open, a vaginal smear was taken. An influx of leucocytes following a cornified smear was indicative of ovulation having occurred.

Progesterone assay and ovarian observations At death, blood samples were collected by cutting the vessels in the neck, serum was prepared and stored at −20 °C until assayed. Progesterone was assayed by radioimmunoassay (Zarkawi *et al.*, 1990). The intra- and inter-assay coefficients of variation are 9% and 18%, respectively. Ovaries, removed at death, were serially sectioned at 10 μm and stained with haematoxylin and eosin. The number of recent corpora lutea (indicating the presence or absence of recent ovulations) and the number of luteinized follicles, with trapped oocytes (experiment 3 only), were recorded. In some cases (see results) the maximum size of non-atretic (Byskov, 1978) follicles was measured.

Liver abnormalities Livers were examined for gross abnormalities. Tissue samples were sectioned at 10 μm, stained with haematoxylin and eosin, for microscopic examination.

Statistical comparisons The data showed heterogeneity of variance and skewed distributions, and hence are presented as geometric means and ranges. Comparisons have been made using the non-parametric Mann–Whitney (2 sample) and Kruskal–Wallis (more than 2 samples) tests (Campbell, 1974).

Table 1. The effect of TBA and TPP given on day 8 of the cycle on oestrous cyclicity and ovulation in guineapigs

Treatment group	No. of animals	Pretreatment cycle length (days)*	Treated cycle length (days)*	No. of animals cycling on treatment	No. of animals ovulating on treatment	No. of corpora lutea*	Progesterone concentration: Ovulating animals* (p mol/l)	Progesterone concentration: Non-ovulating animals* (p mol/l)
Experiment 1:								
Control	5	16·5 (16–17)	16·1 (16–17)	5	5	3·9 (3–5)	18·0 (12·9–26·5)	—
TBA 0·4	5	16·7 (16–18)	>19·2 (16–>27)	4	4	4·2 (3–5)	11·7 (9·8–13·1)	1·7
TBA 2	5	16·7 (16–17)	—	0	1	3	6·5	1·7 (1·0–3·0)
Experiment 2:								
Control	5	16·3 (16–17)	16·3 (16–17)	5	5	3·5 (3–5)	17·5 (7·3–27·5)	— —
TBA 0·4	5	16·3 (16–17)	18·8 (17–21)	5	5	3·4 (3–4)	9·6 (7·8–10·5)	—
TBA 2	5	16·3 (16–17)	—	0	0	0	— —	1·8 (1·1–3·1)
TPP 0·44	5	16·3 (16–17)	20·8 (18–27)	5	4	4·2 (4–5)	16·8 (15·9–19·3)	2·1
TPP 2·2	5	16·9 (16–17)	—	0	0		—	2·1 (1·3–4·3)

*Values are geometric means and ranges.

Results

The effect of TBA and TPP given on day 8 of the cycle on oestrous cyclicity and ovulation is shown in Table 1. In experiment 1, TBA 0·4 treatment slightly delayed vaginal opening in one animal (vaginal opening occurring in 4 animals on days 16, 17, 18, 20) and prevented vaginal opening (>27 day cycle) and ovulation in another animal. TBA2 treatment blocked vaginal opening and prevented ovulation in 4 of 5 animals. In experiment 2, the effects of TBA were confirmed. Vaginal opening occurred on days 17, 18, 19, 19 and 21 in the TBA 0·4 group with all animals ovulating; whereas vaginal opening and ovulation were blocked in all animals in the TBA2 group. The effects of equimolar amounts of testosterone phenylpropionate were similar to those of TBA, with TPP 0·44 delaying vaginal opening (opening occurring on days 18, 19, 19, 22, 27) and preventing ovulation in one animal over the injection period; whereas TPP 2·2 prevented both vaginal opening and ovulation in all cases. When ovulation occurred in TBA- or TPP-treated animals ovulation rate was unaltered ($P>0·05$), though there was an almost significant ($P=0·05$) reduction in progesterone production by corpora lutea of TBA-treated animals. No follicles greater than 700 μm were present in non-ovulating androgen-treated animals.

The effects of exogenous gonadotrophins on ovarian functions in TBA-treated animals is

Table 2. The number of corpora lutea, luteinized follicles and concentration of progesterone in TBA-treated guineapigs and TBA-treated guineapigs given exogenous gonadotrophins

Treatment group	No. of animals	No. of corpora lutea*	No. of luteinized follicles*	Progesterone concentration (p mol/l)*
TBA alone	5	0	0	1·4 (1·0–1·9)
TBA + FSH	5	9·0 (6–13)	0	6·5 (4·0–12·2)
TBA + HCG	4†	0‡ (0–1)	9·4 (4–18)	2·8 (2·1–3·7)
TBA + FSH + HCG	5	11·2 (4–17)	0	6·6 (3·4–24·2)

*Values are geometric means and ranges.
†One animal was excluded as vagina opened during pregonadotrophin treatment period.
‡One of 4 animals had a single ovulation.

shown in Table 2. As expected from experiments 1 and 2, animals treated with TBA2 failed to ovulate. Animals treated with FSH on days 11–15 had many active corpora lutea as did the group treated with FSH followed by hCG. The group treated with hCG, apart from one animal with a single ovulation, all had large numbers of luteinized follicles.

Signs of abnormality were seen on the livers of animals receiving 2 mg TBA and 2·2 mg TPP. On gross inspection small brown or yellowish lesions or nodules were present on otherwise normal livers. Histologically these areas tended to be haemorrhagic with areas of disorganized tissue with pycnotic nuclei.

Discussion

The results of the present experiments show the TBA treatment in the guineapig, started in the mid-luteal phase of the oestrous cycle, blocks or delays vaginal opening and ovulation depending on dose, in a similar fashion to equimolar concentrations of testosterone ester. There was evidence that failure, of both oestrogen production to induce vaginal opening, and ovulation, was due to an inhibition of follicular recruitment and normal follicular development. The effects on the disruption of ovarian function and oestrous cyclicity agree well with studies on both laboratory and domestic species using natural and synthetic androgens (Laqueur & Fluhmann, 1942; Labhsetwar & Diamond, 1970; Dekker & Hutchinson, 1973; Neumann, 1976; Heitzman, Harwood & Mallison, 1977; Ward *et al.*, 1978; Reynolds *et al.*, 1981; Galbraith & Lawrie, 1984; Zarkawi *et al.*, 1990). Depending on the dose of steroid and species employed, oestrous cycle disruption, lack of ovulation with reduced follicular growth or in some cases induction of follicular cysts, are seen. In the present study in the guineapig rather higher doses of TBA (ten 2 mg per kg injections given subcutaneously over 20 days) were required to block ovarian cyclicity than in similar studies in beef heifers (Zarkawi *et al.*, 1990) in which implants of approximately 1 mg per kg were effective. These dose levels may be considered in the context of TBA used in growth studies where subcutaneous implants of 0·4 to 1·3 and 0·6 mg per kg were given to mature sheep (Sulieman *et al.*, 1988) and cattle (Galbraith, 1980), respectively; and 4·2 to 10 mg per kg injected subcutaneously thrice weekly for up to 6 weeks in female rabbits (Lobley *et al.*, 1983). In the present experiments serial injection of FSH to TBA-treated animals led to ovulation induction. This suggests that TBA has blocked the FSH release from the pituitary that is involved in follicular recruitment. Such a mechanism of action of androgens was suggested in previous experiments in rats and hamsters (Dekker & Hutchinson, 1973). The similarity of response of the FSH plus hCG group with the FSH alone group suggests that the mechanisms involved with the induction of the ovulatory gonadotrophin surge are not impaired by TBA. The induction of luteinized follicles rather than ovulation in animals treated with hCG alone would tend to confirm that TBA-treated animals contain only immature or abnormal follicles that are not capable of being ovulated but which, on stimulation with hCG, undergo luteinization with trapped oocytes. An additional finding in the present study was the presence of small lesions on the livers of guineapigs receiving the higher doses of steroid. The possibility of liver damage including liver tumours (Evans, 1978) from androgens, particularly excess anabolic steroids, is recognized.

In conclusion, the guineapig has been used to investigate the mode of action of TBA on oestrous cycle disruption and the induction of anovulation. The data suggest that a primary locus of action is to prevent the FSH release that is normally involved in preovulatory follicle recruitment.

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References

Byskov AG (1978) Follicular atresia. In *The Vertebrate Ovary* (ed. RE Jones), pp. 533–562. New York: Plenum Publishing

Campbell RC (1974) *Statistics for Biologists*, Second edition. Cambridge: Cambridge University Press

Dekker A & Hutchinson JSM (1973) Effect of testosterone propionate on fine structure and gonadotrophin content of the female rat and hamster anterior pituitary. *Zeitschrift für Zellfforschung und Microskopische Anatomie* **143**, 291–300

Evans DJ (1978) Liver tumors elicited by specific factors: synthetic androgens and anabolic steroids. In *Primary Liver Tumours* (ed. H Remmer, HM Bolt, P Bannasch & H Poppek), pp. 213–216. Lancaster: MTP Press

Galbraith H (1980) Effect of trenbolone acetate on growth, blood metabolites and hormones of cull beef cows. *Veterinary Record* **107**, 559–560

Galbraith H & Lawrie P (1984) Response of the female rat to trenbolone acetate and oestradiol-17β given alone or combined. *Proceedings of the Nutrition Society* **43**, 42A

Heitzman RJ, Harwood DJ & Mallinson CB (1977) The effect of an anabolic steroid, trenbolone acetate, on oestrous cycling in dairy cows. *Acta Endocrinologica* **85** (suppl 2), 462

Hilliard J (1973) Corpus luteum in guinea-pigs, hamsters, rats, mice and rabbits. *Biology of Reproduction* **8**, 203–221

Labhsetwar AP & Diamond M (1970) Ovarian changes in the guinea-pig during various reproductive stages and steroid treatments. *Biology of Reproduction* **2**, 53–57

Laqueur GL & Fluhmann CF (1942) Effects of testosterone propionate in immature and adult female rats. *Endocrinology* **30**, 93–101.

Lobley GE, Walker A, Connell A & Galbraith H (1983) The effect of trenbolone acetate on growth rate and carcass composition of young female rabbits. *Animal Production* **36**, 111–115

Neumann F (1976) Pharmacological and endocrinological studies on anabolic agents. In *Anabolic Agents in Animal Production*, Vol. V (eds F Coulston & F Korte), pp. 253–264. Stuttgart: George Thieme

Reynolds IP, Harrison LP, Mallison CB, Harwood DJ & Heitzman RJ (1981) The effect of trenbolone acetate on the bovine oestrous cycle. *Animal Production Science* **4**, 107–116

Steelman SL & Pohley FM (1953) Assay of the follicle-stimulating hormones based on the augmentation with human chorionic gonadotrophin. *Endocrinology* **53**, 604–616

Sulieman AH, Galbraith H & Topps JH (1988) Growth performance and body composition of wether lambs implanted at two different initial live weights with trenbolone acetate combined with oestradiol 17β. *Animal Production* **47**, 65–74

Ward RC, Costoff A & Mahesh VB (1978) The induction of polycystic ovaries in mature cycling rats by the administration of dehydroepiandrosterone. *Biology of Reproduction* **18**, 614–623

Zarkawi M, Galbraith H & Hutchinson JSM (1990) Influence of trenbolone acetate, zeranol and oestradiol-17β implantation on growth performance and reproductive function in beef heifers. *Animal Production* (in press)