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EFFECTS OF TIOCONAZOLE ON PARTURITION AND SERUM LEVELS OF 17β-OESTRADIOL, PROGESTERONE, LH AND PRL IN THE RAT

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Abstract—Tioconazole, an imidazole antifungal agent, was administered orally at 100 mg/kg/day to pregnant rats according to two regimens; in one, treatment started on day 15 post-insemination (p.i.) and in the other it started on day 18 p.i. The first regimen caused a delay in onset of parturition and a prolongation of labour. Serum progesterone was decreased from days 17 to 21 p.i., 17β-oestradiol decreased on day 21 p.i., LH increased on day 17 p.i., and the normal surge of prolactin on day 21 p.i. abolished. The parturition disorders disappeared when 17β-oestradiol (0.125 μg/animal/day s.c.) was given with tioconazole from day 15 p.i. In the second regimen, tioconazole treatment advanced by about 24 hours the onset of parturition and the normal fall in serum progesterone and the surge in prolactin. Serum 17β-oestradiol was unaffected, but LH was raised on days 19 and 20 p.i. In animals receiving progesterone (2.5 mg/animal/day, s.c.) and tioconazole from day 18 p.i. parturition was no longer advanced. In conclusion, the parturition disorders observed in rats during tioconazole treatment are associated with a modification of progesterone and 17β-oestradiol serum levels. These findings have questionable relevance for the human situation as the roles of these steroid hormones in parturition in women are different from those in rats.

Tioconazole, an imidazole antifungal agent, has been shown to inhibit 14-demethylation of lanosterol, a microsomal cytochrome P-450 dependent enzyme system [1, 2] and this is considered to be the basis of its antifungal action. Other imidazole derived antifungal agents inhibit the biosynthesis of various other steroids [3, 4]. Peri-natal toxicology studies carried out in the Pfizer Laboratory (Amboise, France) showed that tioconazole (100 mg/kg/day) administered orally to pregnant rats induced some disorders in parturition [5]. The protocol recommended for evaluation of potential adverse effects of pharmaceutical substances on parturition requires drug administration to cover the last third of pregnancy [6, 7]. When tioconazole was administered to pregnant rats from day 15 p.i., it caused a prolongation of pregnancy and a difficult labour. Litter size was unaffected. Suspecting a hormonal mechanism and being cognisant of how rapidly the hormonal status of rats changes in late pregnancy [8], we subsequently investigated the effect of starting tioconazole administration on day 18 p.i., instead of day 15 p.i. In contrast to the earlier findings, this protocol led to an advance in the onset of parturition, the process of parturition itself being normal. Disturbances of parturition have been reported for other imidazole antifungal drugs [9–11]. In the case of tioconazole no adverse effect was observed at oral dose levels up to 20 mg/kg.§ The present study was undertaken to investigate whether these littering disorders were related to variations induced by tioconazole of the serum levels of key hormones involved with the end of gestation in the rat (progesterone, 17β-oestradiol, prolactin and luteinizing hormone).

Tioconazole was administered orally according to two protocols: in one group from day 15 p.i., in the other group from day 18 p.i. until parturition. First results revealed treatment-related reductions in the serum concentrations of 17β-oestradiol and progesterone on certain days. Consequently, in a second phase pregnant rats were subjected to the same two tioconazole protocols, but this time with concomitant administration of either 17β-oestradiol or progesterone, according to which hormone was lacking. The doses of the hormones were chosen on the basis of literature data [12] or preliminary studies (unpublished) such as to provide serum levels similar to those which occur in normal pregnancy.

MATERIALS AND METHODS

Animals and observations. Inseminated female rats Crl:COBS-CD(SD)BR (Charles River, France), aged 8–10 weeks were used. Naïve females were caged overnight with males and checked on the following morning for the presence of vaginal plugs as an indication of copulation. The day of the finding was called day 0 p.i. Females were observed daily throughout the study. Towards the end of the gestation period, they were inspected every hour (between 09.00 and 24.00) and the duration of the labour (when it occurred during the observation period) was noted. The onset of parturition was assigned either to the "day" period (0900–2100) or the night period (2100–0900).

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§ Tioconazole (Trosyd®) is only marketed for application by topical routes. This results in a maximum systemic exposure to man at least 25 times lower than that which occurs in rats after an oral dose of 20 mg/kg (Pfizer, unpublished data).

Compound preparation. Tioconazole (Pfizer Central Research, Sandwich, Kent, U.K.) was suspended at room temperature in a 25% aqueous solution of Cremophor EL (BASF) at the appropriate concentration to provide a dose of 100 mg/10 ml/kg body weight. 17β-Oestradiol (Sigma Chemical Co, St Louis, MO) was solubilized in a 2.5% methyl alcoholic olive oil solution; progesterone (Roussel-Uclaf, Paris, France): 100 mg were dissolved in 2 ml absolute alcohol and then suspended in 2 ml olive oil.

Treatment schedule. In treatment I, tioconazole was administered every morning at 0900 by oesophageal intubation with metal catheters from day 15 p.i. till the day before sacrifice or till delivery. In treatment II, tioconazole was given from day 18 p.i. till parturition. Control females of the two groups received 10 ml vehicle/kg body weight under the same conditions.

17β-Oestradiol was administered daily by the subcutaneous route at 1300 from day 15 to day 20 p.i. and at 0700 on day 21 p.i. (0.125 μg/0.1 ml/animal/day). Progesterone was administered daily by the subcutaneous route at 1300 on days 18 p.i. and 19 p.i.; and at 0900 and 1700 on day 20 p.i. (2.5 mg progesterone/0.1 ml/animal/injection). Control females received 0.1 ml of olive oil solution under the same conditions.

Blood sampling. Females were sacrificed by decapitation for blood collection. Serum, separated by centrifugation, was distributed into several tubes for the radioimmunoassays and frozen at −20°.

Steroid hormone radioimmunoassays. Serum concentrations of 17β-oestradiol and of progesterone were measured by radioimmunoassay using tritium based kits from Biomérieux, France, according to the methods of Abraham [13, 14]. Serum samples of 1 ml were used for each assay (in duplicate) and results were calculated by reference to standard curves, established from triplicate assays.

Polypeptide hormone assays. Serum concentrations of LH and prolactin were measured by radioimmunoassay, using iodine-125 kits from Nat. Inst. Arthritis, Diabetes, and Digestive and Kidney Diseases; Nat. Hormone and Pituitary Program, Baltimore, MD 21201, U.S.A; following the method supplied by this Institute. Serum samples of 0.15 and 0.05 ml were used for assays (in duplicate) of LH and prolactin respectively. Results were calculated by reference to standard curves, established from triplicate assays.

Statistical analysis. Serum levels of 17β-oestradiol, progesterone and PRL were analysed by Student's *t*-test. For LH values for untreated animals were often below the limit of detection, so that Student's *t*-test was inapplicable. Instead, a chi-squared test was used, grouping where necessary control values into a <300 pg/ml category.

The duration of labour was analysed by a chi-squared test.

Pregnancy length was ranked in ascending order. A Kruskal–Wallis test was applied to test the hypothesis of no difference in mean rank for the groups. When the class number k is 2, this test is similar to the U-squared Mann–Whitney variable. Under the hypothesis of no difference among the groups, this variable is distributed as χ^2 with one degree of freedom.

RESULTS

Progesterone serum levels (Fig. 1)

Tioconazole treatment from day 15 p.i. decreased progesterone serum levels on days 17 p.i. and 20 p.i. ($P < 0.05$). Tioconazole treatment from day 18 p.i. induced a sharp fall of progesterone serum levels on day 20 p.i. ($P < 0.001$), i.e. 24 hr before that in control females.

17β-Oestradiol serum levels (Fig. 2)

Tioconazole treatment from day 15 p.i. caused a marked decrease in 17β-oestradiol serum levels on day 21 p.i. ($P < 0.001$), i.e. a few hours before littering. Tioconazole treatment from day 18 p.i. did not seem to modify 17β-oestradiol serum levels.

Luteinizing hormone serum levels (Fig. 3)

In control females LH was below the limit of detection (300 pg/ml) on days 17, 18 and 19 of pregnancy; an increase of LH levels was observed on day 21 p.i. In females treated with tioconazole from day 15 p.i. serum LH levels were high on day 17 p.i. ($P < 0.01$). In females treated with tioconazole from day 18 p.i. a marked release of LH occurred on days 19 and 20 p.i. ($P < 0.001$ and $P < 0.05$ respectively).

Prolactin serum levels (Fig. 4)

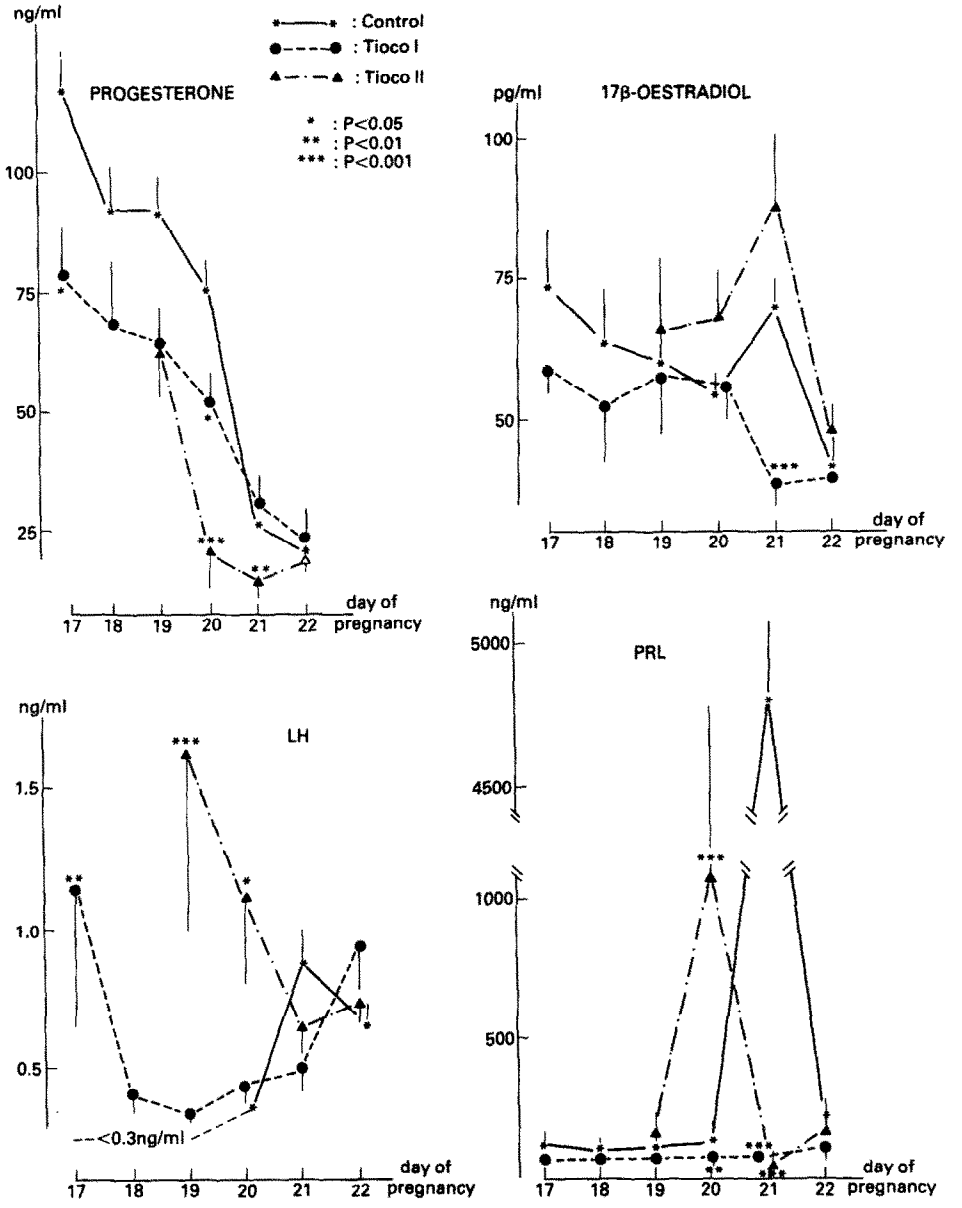
In control females an enormous release of prolactin occurred on day 21 p.i. This peak did not occur in females treated with tioconazole from day 15 p.i. ($P < 0.001$), but was advanced ($P < 0.001$) by 24 hr in females given tioconazole from day 18 p.i.

Pregnancy length and duration of labour (Figs 5 and 6)

Most controls delivered on day 21 p.i. Parturition was observed in 23/28 and, with one exception, lasted less than 2 hr. 17β-Oestradiol treatment affected neither pregnancy length nor duration of labour.

Of the 21 females treated with tioconazole from day 15 p.i., most littered later than control females. Although pregnancy was significantly longer in this group than in the control group ($P < 0.05$), dispersion of parturition over days 20 p.i. to 23 p.i. is apparent. The duration of labour was longer in females treated with tioconazole from day 15 p.i. than in controls ($P < 0.001$). When 17β-oestradiol was added to the tioconazole treatment from day 15 p.i. pregnancy length was not significantly different from controls and labour was normal.

In females treated with tioconazole from day 18 p.i. pregnancy was significantly shorter (on average, 24 hr) than in the control group ($P < 0.001$). The duration of labour was normal. Progesterone treatment alone from day 18 p.i. induced a prolongation of pregnancy. When progesterone was added to the tioconazole treatment from day 18 p.i., the advanced parturition observed after tioconazole alone no longer occurred.



Figs 1–4. Serum hormone levels in pregnant rats treated with tioconazole. Control values were similar in each experiment and have been pooled; Tioco I: administration of tioconazole from day 15 p.i.; Tioco II: administration of tioconazole from day 18 p.i.; Numbers of animals at each sampling point:

Day	17	18	19	20	21	22
Controls	8–9	10–11	17–18	16–18	13–19	13–25
Tioco I	3–4	3	5–6	8–9	7–13	4–10
Tioco II	—	—	6	6	6	5–6

DISCUSSION

Control animals used in these studies had a normal length of pregnancy and duration of labour [15]. The serum level patterns of progesterone, 17β-oestradiol, PRL and LH in our control females at the end of pregnancy were similar to those described in the literature [16–18]. Vehicle treatment of control females did not disturb the pregnancy length, the duration of labour or hormone levels. Progesterone treatment of control rats at the end of gestation prolonged pregnancy as has already been demonstrated [12]. The 17β-oestradiol treatment used in this study had, as expected, no effect on pregnancy length or the duration of labour of control females.

When the tioconazole treatment began on day 15 p.i., parturition was dispersed over three days. Pregnancy length was, on average, longer than in

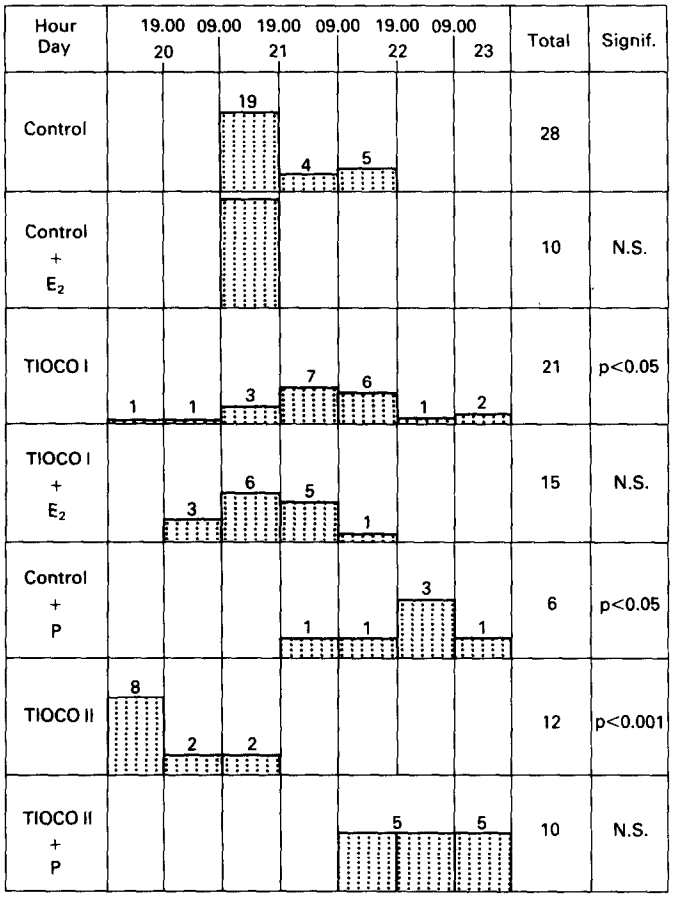


Fig. 5. Time to onset of parturition in pregnant rats treated with tioconazole. As there was no difference between the control groups the data have been combined. Tioco I: administration of tioconazole from day 15 p.i. Tioco II: administration of tioconazole from day 18 p.i. E_2: administration of 17β-oestradiol from day 15 p.i. P: administration of progesterone from day 18 p.i.
N: number of females starting to litter during this period.
▨: column heights represent.

the control group and the duration of labour was prolonged. The significant deficiency of 17β-oestradiol, noted on day 21 p.i., seems to underlie these effects, inasmuch as when 17β-oestradiol was added to such a tioconazole treatment the delays in parturition and difficulties of labour disappeared. 17β-Oestradiol is known to have an important role in the activation of the uterus during premature labour [16].

Females treated with tioconazole from day 18 p.i. littered about 24 hr before controls and the well known parturition-associated fall in serum progesterone level also occurred 24 hr sooner than in the control group. When progesterone treatment was added to this tioconazole treatment this premature parturition no longer occurred. These results suggest that a tioconazole induced premature fall in serum progesterone is responsible for the advance in parturition.

Tioconazole thus seems to induce parturition disorders in the rat by modifying steroid serum levels at the end of pregnancy. Tioconazole has been shown to inhibit sterol biosynthesis by inhibiting 14-demethylation of lanosterol, a microsomal cytochrome P-450 dependent system [1, 2]. Furthermore, several other imidazole-containing antifungal drugs have been shown to inhibit steroid aromatase activity of human placental microsomes [4] and other imidazole-containing antifungal drugs, such as miconazole and ketoconazole, have been shown to inhibit other cytochrome P-450 dependent steroidogenic enzyme activities [3, 19–23]. The conversion of androstenedione to estrogens is catalyzed by a cytochrome P-450 dependent mono-oxygenase, aromatase enzyme [24]. As the biosynthesis of ovarian steroids requires many cytochrome P-450 dependent systems, it is possible that tioconazole inhibits ovarian steroid biosynthesis by inhibiting one or more of these cytochrome P-450 systems. The possible direct action of tioconazole on ovarian steroid biosynthesis is presently under study in our laboratory.

It should be noted that it may not be justifiable to

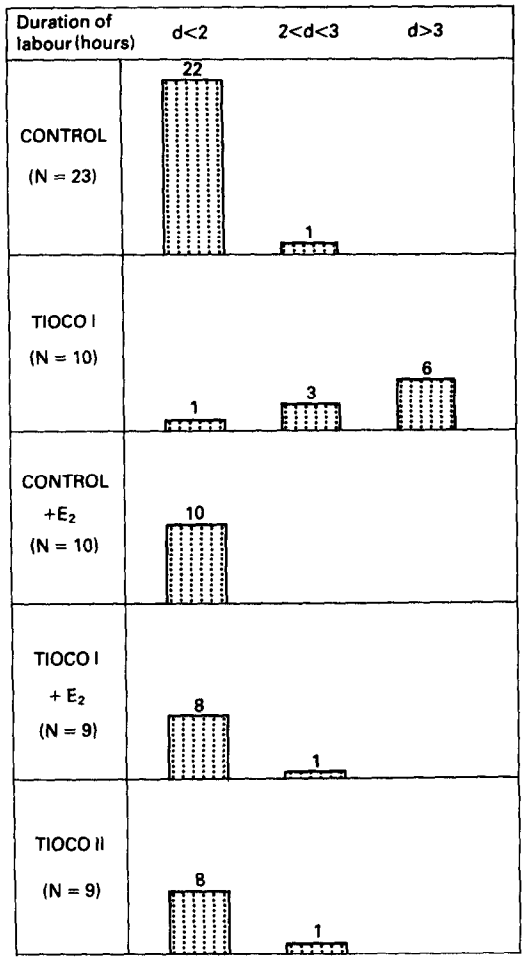


Fig. 6. Duration of labour in pregnant rats treated with tioconazole. Tioco I: administration of tioconazole from day 15 p.i. Tioco II: administration of tioconazole from day 18 p.i. E_2: administration of 17β-oestradiol from day 15 p.i.

extrapolate from the explanation offered here for the effects of tioconazole on parturition in rats to the human situation. The mechanisms controlling the timing of parturition are still not well understood, but a number of inter-species differences are clear [8, 25]. Although the levels of progesterone and oestrogen in the rat are important, these hormones may only have inhibitory or permissive roles for the decisive later actions of other hormones such as relaxin, oxytocin or prostaglandin-$F_{2\alpha}$ [8, 26, 27]. In the present context, inter-species differences in the role and consequences of the pre-partum changes in steroid hormones are relevant. Thus, the late rise in serum 17β-oestradiol and the ability of progesterone to block the onset of parturition in the rat do not have counterparts in the human and other primates. In women parturition can occur without changes in circulating levels of 17β-oestradiol or progesterone [28, 29].

The modification of the polypeptide hormone serum levels observed in this study may be related to the serum steroid modifications. High LH levels may be a consequence of decreases in serum progesterone or oestrogen. It is possible that the known luteolytic properties of LH [30] may have an influence if they occur at the moment when the ovarian–uterine axis is in a receptive state. The variations in the prolactin surge at parturition in rats may just reflect the advance or retardation of the onset of parturition/lactation [31]. The present view seems to be that prolactin does not play a significant part in control of the timing of parturition in the rat [32].

In conclusion, a working hypothesis to explain the contrasting results of this investigation can be envisaged. The surge of LH soon after commencing tioconazole treatment on day 18 p.i. could be significantly luteolytic to trigger the onset of parturition. Gordon and Sherwood have reported that administration of LH to rats on day 19 p.i. advances the onset of parturition [33]. On the other hand, when tioconazole treatment starts on day 15 p.i. the short-lived LH surge which follows is without effect as the uterus is not yet "prepared" for parturition. Now the crucial event appears to be the suppression of the normal pre-partum rise in 17β-oestradiol, resulting in a delayed onset in parturition. The rapidity with which several hormones fluctuate in a tightly synchronised manner over the last few days of pregnancy in rats [8, 32], means that the consequences of administering an agent which can modulate any of the key steps will depend in a quite sensitive manner on the timing of such treatments. For example, Downing *et al.* [34] showed that administration of single doses of 17β-oestradiol to pregnant rats on days 17, 18 or 19 p.i. resulted in either an advancement or a retardation of the onset of parturition, according to the day of administration.

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REFERENCES

1. M. S. Marriott, *J. gen. Microbiol.* **117**, 253 (1980).
2. G. W. Pye and M. S. Marriott, *Sabouraudia* **20**, 325 (1982).
3. A. Pont, P. Williams, D. Loose, D. Feldman, R. Reitz, C. Bochra and D. Stevens, *Ann. Int. Med.* **97**, 370 (1982).
4. J. Mason, B. Murry, M. Olcott and J. J. Sheets, *Biochem. Pharmac.* **34**, 1087 (1985).
5. M. S. Marriott, K. W. Brammer, J. Faccini, J. K. Faulkner, S. Jevons, A. M. Monro, J. Nachbaur, J. Perraud and M. H. Tarbit, *Gynäk. Rdsch.* **23 S1**, 1 (1983).
6. U.S. Food and Drug Administration (1966).
7. *Official Journal of the Europ. Commun.* L332, Directive 83/571/EEC (1983).
8. G. D. Thorburn and J. R. G. Challis, *Physiol. Rev.* **59**, 863 (1979).
9. D. Thienpont, J. Van Cutsem, J. M. Van Nueten, C. J. E. Niemegeers and R. Marsboom, *Arzneimittel-Forsch.* **25**, 224 (1975).
10. A. J. Janssen and W. F. M. Van Bever, in *Pharmacological and Biochemical Properties of Drug Sub-*

stances, Vol. 2 (Ed. M. E. Goldberg), p. 333. American Pharmaceutical Association, New York (1979).
11. C. Hito, S. Tsukuda, K. Inoue, S. Kasuya, I. Fukuzawa, K. Nakano, Y. Shibutani and H. Ohnishi, *Iyakuhin Kenkyu* **7**, 535 (1976).
12. A. I. Csapo and W. G. Wiest, *Endocrinology* **85**, 735 (1969).
13. G. E. Abraham and W. D. Odell, in *Immunologic Methods in Steroid Determination*, p. 87. F. G. Peron and B. V. Caldwell, New York (1970).
14. G. E. Abraham, R. S. Swerdloff, D. Tulchinsky and W. D. Odell, *J. clin. Endocr. Metab.* **32**, 619 (1971).
15. P. W. Nathanielsz, *Semin. Perinatol.* **2**, 223 (1978).
16. D. M. Linkie and G. D. Niswender, *Endocrinology* **90**, 362 (1972).
17. W. K. Morishige, G. J. Pepe and I. Rothchild, *Endocrinology* **92**, 1927 (1973).
18. A. A. Shaikh, *Biol. Reprod.* **5**, 297 (1971).
19. D. S. Loose, P. B. Kan, M. A. Hirst, R. A. Marcus and D. A. Feldman, *J. clin. Invest.* **71**, 1495 (1983).
20. R. J. Santen, H. Van Den Bossche, J. Symoens, J. Brugmans and R. de Costen, *J. Clin. Endocr. Metab.* **57**, 732 (1983).
21. T. Schurmeyer and E. Nieschlag, *Acta Endocr. Copenh.* **105**, 275 (1984).
22. H. Watanabe, J. A. Menzies, *Res. Comm. Chem. Pathol. Pharmac.* **48**, 141 (1985).
23. A. Pont, P. L. Williams, S. Azhar, R. Reitz, C. Bochra, E. S. Smith and D. Stevens, *Archs Interm. Med.* **142**, 2137 (1982).
24. E. Thompson, Jr. and P. K. Siiteri, *J. biol. Chem.* **149**, 5373 (1974).
25. P. W. Nathanielsz, *Ann. Rev. Physiol.* **40**, 411 (1978).
26. D. G. Porter, S. J. Downing and J. M. C. Bradshaw, *J. Endocr.* **89**, 399 (1981).
27. G. Weiss, *Ann. Rev. Physiol.* **46**, 43 (1984).
28. A. L. Hartikainen-Sorri, A. Kauppila, R. Tuimaka, L. Viinikka and D. Ylikorkala, *Acta Obstet. Gynaecol. Scand.* **60**, 497 (1981).
29. W. L. Willcox, J. L. Yovich, S. C. McColm and J. M. Phillips, *Br. J. Obstet. Gynaecol.* **92**, 66 (1985).
30. I. Rothchild, *Recent Prog. Horm. Res.* **37**, 183 (1981).
31. D. Lincoln and J. C. Doyle, *I.R.C.S.* **6**, 436 (1976).
32. D. W. Lincoln and D. G. Porter, *Anim. Repro. Sci.* **2**, 97 (1979).
33. W. L. Gordon and O. D. Sherwood, *Endocrinology* **111**, 1299 (1982).
34. S. J. Downing, D. G. Porter and D. W. Lincoln, *J. Reprod. Fert.* **62**, 519 (1981).