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COMPARISONS OF THE POTENTIAL UTILITY OF LHRH AGONISTS AND ANTAGONISTS FOR FERTILITY CONTROL

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Summary—Prospects for the use of LHRH analogs for human fertility control have been reviewed with particular reference to two highly potent representatives. Nafarelin acetate, the LHRH agonist, has a potency about 200 × that of LHRH and is consistently effective in suppressing gonadal function in females through a desensitization of LHRH receptors in the pituitary. Such agents show promise as ovulation inhibitors for women although concern has been expressed over the dangers of unopposed estrogen or alternatively hypoestrogenemia. Although early studies indicated luteolysis in women and interceptive action in baboons it is now clear that the LHRH agonists will not be useful clinically to terminate pregnancy. Wide species differences in the male response to LHRH agonists exist. Unfortunately azoospermia has not been achieved in men. The LHRH antagonists, typified by [*N*-Ac-D-Nal(2)^1, D-pCl-Phe^2, D-Trp^3, D-hArg(Et$_2$)^6, D-Ala^{10}]LHRH, require high doses to competitively inhibit responses to endogenous LHRH. Their advantages include a rapid induction of the hypogonadal state with apparently little species or sexual variation in response. Based on animal studies, preferable utility of the antagonists would lie in male contraception and pregnancy interception.

INTRODUCTION

Luteinizing hormone releasing hormone (LHRH) must be secreted and act at its pituitary receptors in a pulsatile manner to exert its physiological effect of activating and maintaining the reproductive cascade from gonadotropin synthesis and release to gametogenesis and gonadal steroidogenesis [1–3]. Continuous presence of LHRH results in a down regulation of its receptors on the pituitary gonadotropes and a shutdown of gonadotropin release.

Very potent agonistic analogs of LHRH are now available and several are undergoing clinical evaluation. Their increased potency over LHRH itself results from increased receptor binding affinity and/or resistance to metabolism. This is typified by nafarelin acetate {[6-D-(2-naphthyl)alanine]LHRH} which has approx 200 × the potency of LHRH in rats [4], has a K_a of $2.1 \times 10^{10}\ M^{-1}$ for rat gonadotrope receptors (2 log orders greater than LHRH) and a circulating half life of 2 h in humans (approx 4 × LHRH). These attributes combine to make it unlikely that such highly potent agonistic analogs will ever be useful to treat infertility. A glance at the literature will show however that they are under intensive investigation for treatment of gonadal hormone-dependent syndromes and for antifertility effects.

More recently very potent antagonistic analogs of LHRH have been synthesized. These analogs compete with endogenous LHRH for its receptors and, having binding affinities equivalent to those of the most potent agonistic analogs, require only low doses *in vivo* to interfere with the gonadotropin surge necessary for ovulation. Somewhat higher doses will shutdown tonic or basal secretion of gonadotropins and again terminate gametogenesis and gonadal steroidogenesis. A particularly potent and long lived example of this class of analogs is [*N*-Ac-D-Nal(2)^1, D-pCl-Phe^2, D-Trp^3, D-hArg(Et$_2$)^6, D-Ala^{10}]LHRH [5], RS-68439, which from a single injection is capable of suppressing gonadotropin levels for 48–72 h [6]. The antagonistic analogs cause a precipitous fall in gonadotropins and steroids in contrast to the effects of even the most potent agonists, where a stimulatory phase precedes shutdown. This difference, together with a suggestion that it may be possible to achieve a greater degree of gonadal inhibition with antagonists than agonists, may well have an influence on their relative utility, particularly in the area of fertility control.

The range of uses of LHRH analogs, with particular reference to the agonists, has recently been reviewed [7] and studies toward male contraceptive potential have been summarized [8]. Rapid progress in the study of LHRH antagonists makes it appropriate to compare the effects of the two classes of analog and perhaps forecast their relative potential.

EFFECTS IN MALES

Rats

Daily, or even less frequent, administration of LHRH agonists to mature rats was early shown to have suppressive effects on both steroidogenesis and spermatogenesis [9–12]. These observations were forecast as leading to an eventual contraceptive use for men [13]. More detailed study has revealed however that, whether judged by histology or by mating experiments, complete suppression of fertility cannot be achieved in rats by use of the agonists [14, 15]. This is in spite of the findings of multiple mechanisms of action of LHRH agonists in the rat but not in other

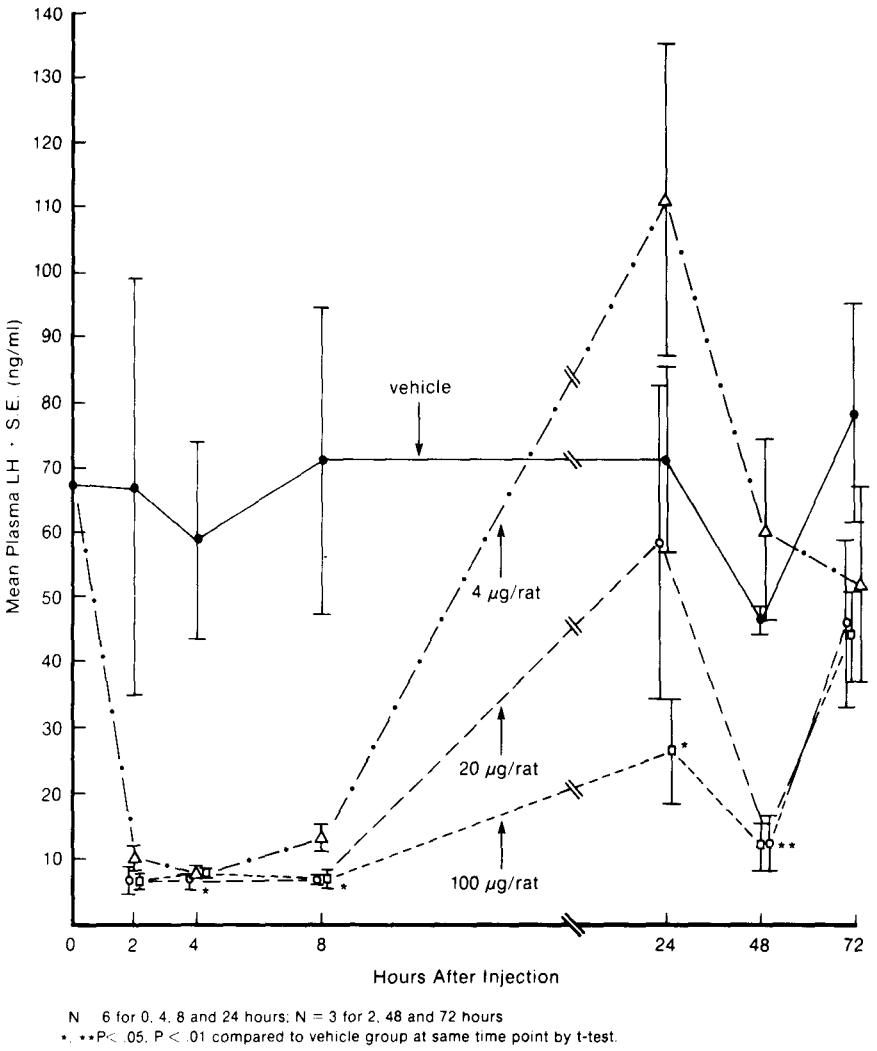

Fig. 1. Plasma LH in the intact male rat following a single subcutaneous injection of RS-68439.

species. In addition to pituitary receptor down regulation [16], LHRH can interact directly with homologous, high affinity and specificity receptors on the Leydig cell [17] and, by a mechanism poorly understood, suppress testicular 17α-hydrolase and 17,20 desmolase activities [18–20]. Also the LHRH-stimulated high levels of LH (or exogenously administered HCG) can down regulate its homologous receptors at the gonad, again leading to inhibitory effects on testicular function [21, 22]. In addition, supplemental testosterone is reported to further decrease spermatogenesis [23] but the combined effect on fertility does not appear to have been studied.

Historically, LHRH antagonist analogs were of low potency and sensitive *in vivo* models such as the castrate male rat were required to assess their action. The more potent analogs, such as RS-68439, have striking activity in this system in that as little as 8 μg per rat (25 μg/kg) as a single injection results in a precipitous decline in LH levels which then are depressed for 48 h or more [6]. FSH levels in the same system are less affected. As one would expect, in the intact animal the effect of these agents is to depress circulating gonadotropins and testosterone [24, 25]. However, for unknown reasons considerably more analog is required in the intact than castrate rat, even 100 μg/rat (300 μg/kg) of RS-68439 giving only partial suppression at 24 h following single injection (Fig. 1). With daily treatment, there is a cumulative effect on gonadotropins and testosterone (Fig. 2) and spermatogenesis is totally, rather than focally as with the agonists, inhibited (Fig. 3). An enhancement of the potency results in rats if the antagonist is given continuously via a subcutaneously implanted osmotic pump (Fig. 4). There is a suggestion that a greater degree of inhibition can result from treatment with antagonist than agonist (Fig. 5) however longer term studies are required to confirm this. While the LHRH antagonists will bind to the LHRH receptors on the Leydig cells, no direct effect of the antagonists, other than an ability to block effects of the agonists, has been documented [19].

Dogs

The male beagle is particularly sensitive to the suppressive effects of LHRH agonists on testicular func-

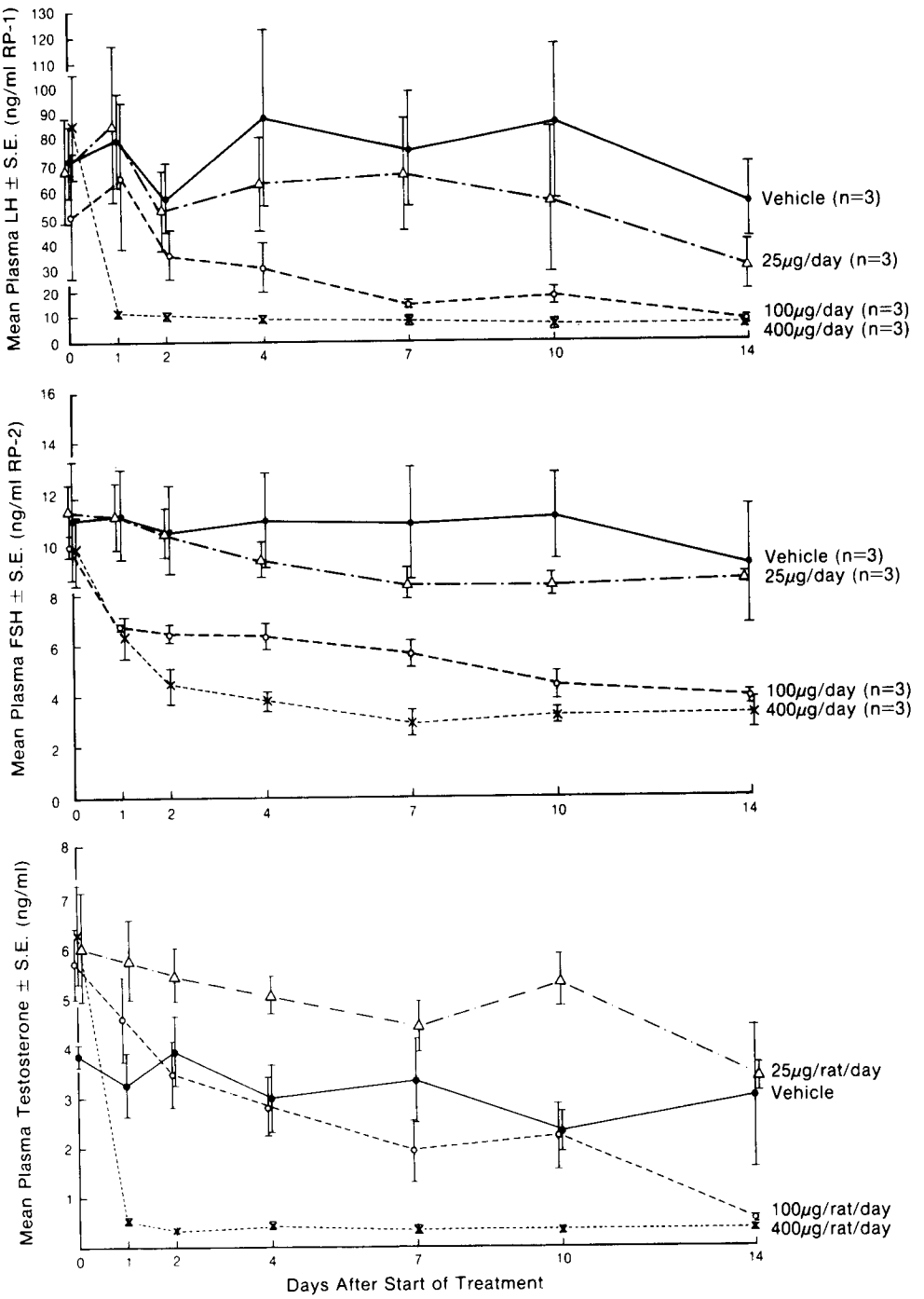

Fig. 2. Plasma LH, FSH and testosterone levels in the rat over 14 days of daily subcutaneous injection of various doses of RS-68439.

tion, which however appear to be mediated solely through pituitary receptor down regulation. Exogenous gonadotropin is still capable of stimulating steroidogenesis [8]. Inhibition of spermatogenesis is rapid at higher dose levels, already noticeable histologically by 10 days of treatment at 2 or 10 μg/kg/day but slower at 0.5 μg/kg/day, probably a reflection of the time to down regulation at the pituitary [27]. Eventually however at all of those dose levels, total inhibition of spermatogenesis to presence of spermatogonia and Sertoli cells only, is achieved. After cessation of longer term treatment, up to at least 1 year, recovery of spermatogenesis is rapid and spermatozoa begin to appear in the ejaculate after 7–8 weeks [28]. Supplementation with testosterone is required to maintain ejaculatory function but is without effect on the antispermatogenic effect.

The dog is somewhat more sensitive than the rat to LHRH antagonist effects. A single injection of 100 μg/kg of RS-68439 in the intact beagle keeps testosterone levels in the castrate range for more than 24 h (Fig. 6). Repetitive daily injection results in dry

ejaculates after 2 weeks. Ejaculatory function is maintained by supplemental testosterone but azoospermia is still achieved (Fig. 7).

Primates

Early reports were that male macaques were poorly sensitive to the LH-releasing effects of LHRH agonists [29, 30]. It is perhaps not too surprising then that poor sensitivity to the down regulating effects of these analogs has also been a routine finding [31–33]. Long term treatment of rhesus monkeys by twice daily administration was reported to suppress testicular function in a percentage of animals [34], although this should be viewed with caution as male rhesus are known to have seasonal changes in testicular function [35]. Greater suppression has been obtained in rhesus monkeys by continuous administration of agonist [36, 37]. It has been claimed that supplementation with testosterone, if delayed some weeks, is compatible with azoospermia but that simultaneous treatment with testosterone and agonist is not [38, 39]. However, in other studies with controlled release formulations, pronounced inter animal variation in response in both rhesus [40] and cynomolgus has been seen, even in the face of comparable blood levels of compound between animals (Fig. 8).

Early studies in men used low daily doses of agonist and gave inconsistent suppression of testosterone levels and no effect upon sperm count [41]. Higher dose studies with another analog did result in consistent suppression of circulating levels of testosterone and a fall in sperm count in all subjects, although 5 out of the 7 treated men discontinued

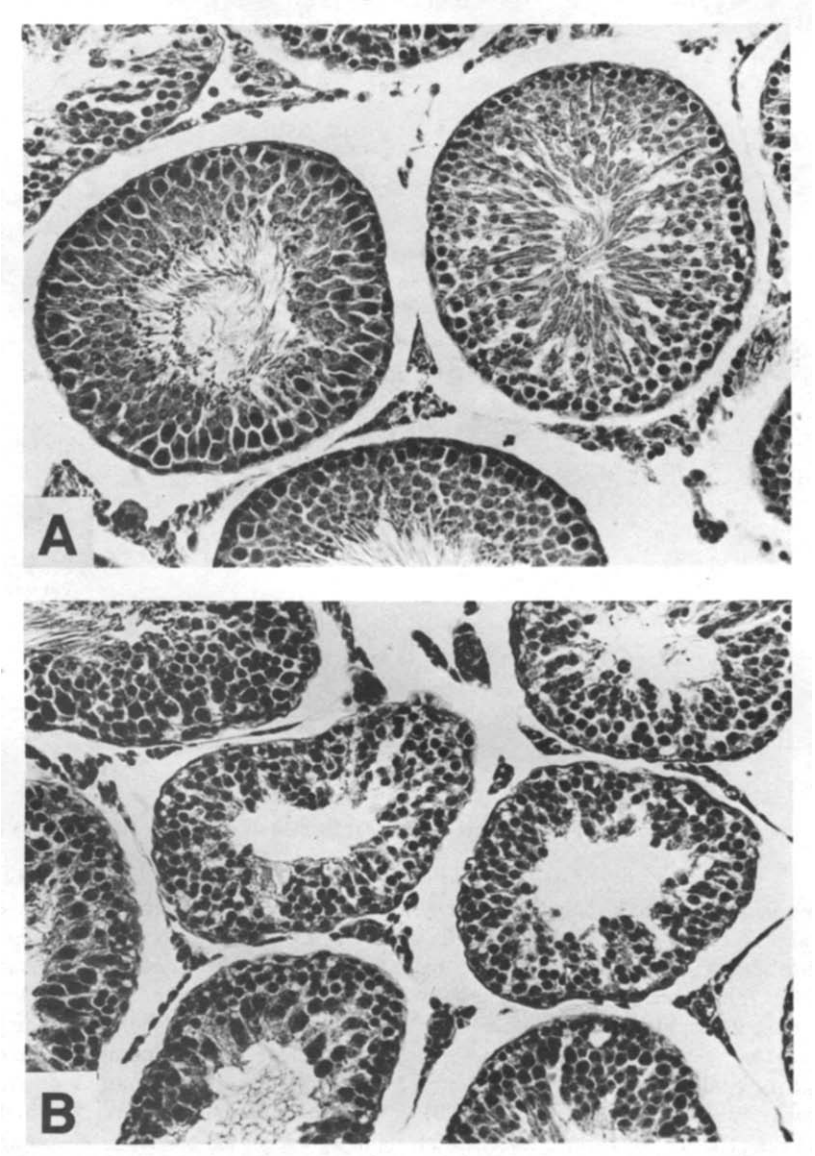

Fig. 3. Histological appearance of the testis of rats following 14 days of daily subcutaneous injection of 400 μg/rat of RS-68439. (A: vehicle; B: RS-68439)

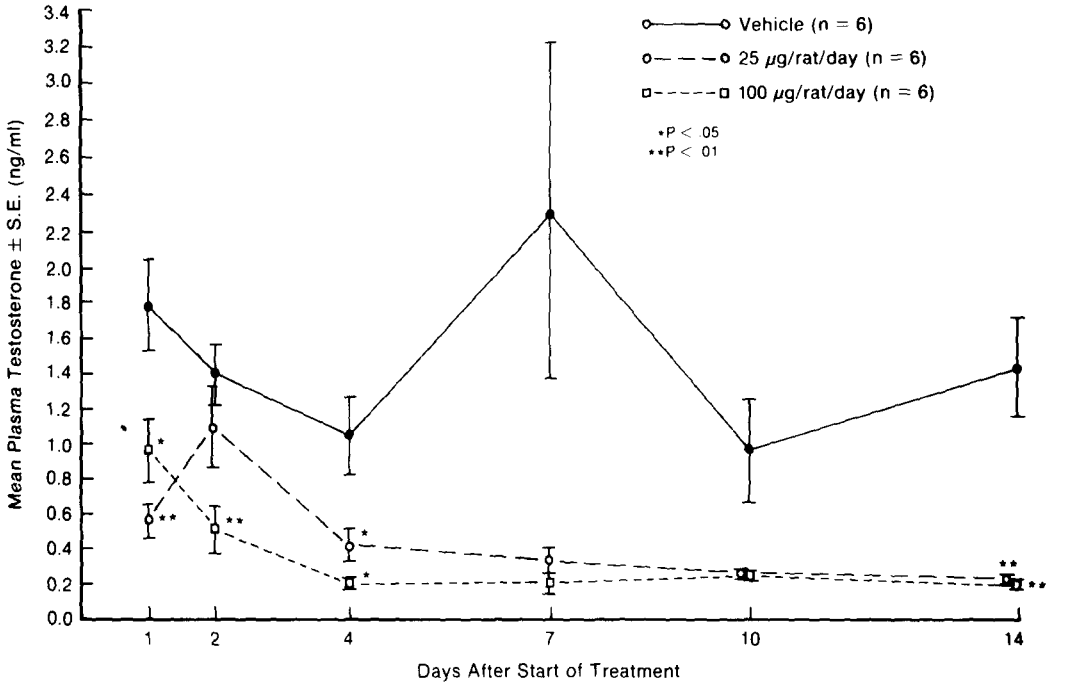

Fig. 4. Plasma testosterone levels in male rats receiving continuous administration of RS-68439 via subcutaneously implanted osmotic minipumps.

treatment after only 6–7 weeks because of impotence and/or hot flashes [42]. Longer term [43] or higher dosage treatment [44] by daily injection has induced only oligospermia although there is a suggestion that these results may have been compromised by the concomitant and early onset testosterone supplementation [38]. The long term, high dose, unsupplemented LHRH agonist treatment used in prostatic cancer patients appears to result in aspermatogenic testes [45] although there is no indication of the level of testicular function prior to treatment in these cases. Continuous infusion studies are underway in men with LHRH agonists [46, 47] Azoospermia has not been achieved although a dose related degree of oligospermia has been noted.

The castrate male monkey is more sensitive to the inhibitory effects of LHRH antagonists than is the intact animal, to the extent that in early studies suppression could only be detected in the castrate, even with doses as high as 2 mg/kg [48]. However, as in other species, the highly potent RS-68439 will rapidly and effectively suppress both pituitary and testicular function in intact male monkeys either by injection (Fig. 9) or by infusion [49] in the range of 400–500 μg/kg per day. Azoospermic ejaculates are consistently noted after 9 weeks of treatment. No

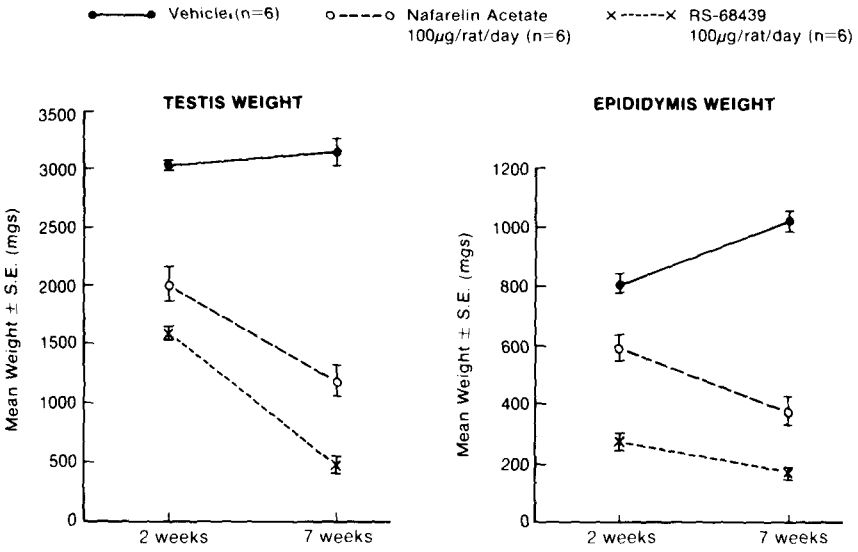

Fig. 5. Comparative effects of nafarelin and RS-68439 on organ weights in rats.

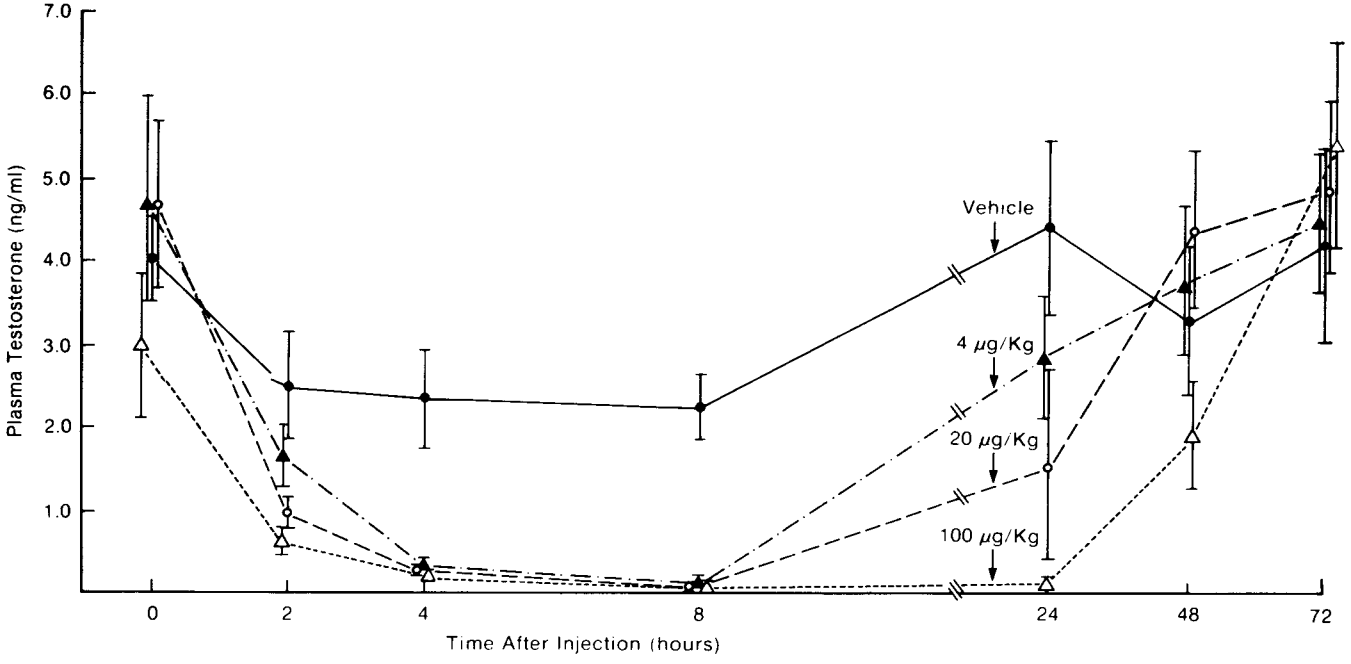

Fig. 6. Effect of a single subcutaneous injection of 4, 20 or 100 μg/kg of RS-68439 on plasma levels of testosterone in male beagles.

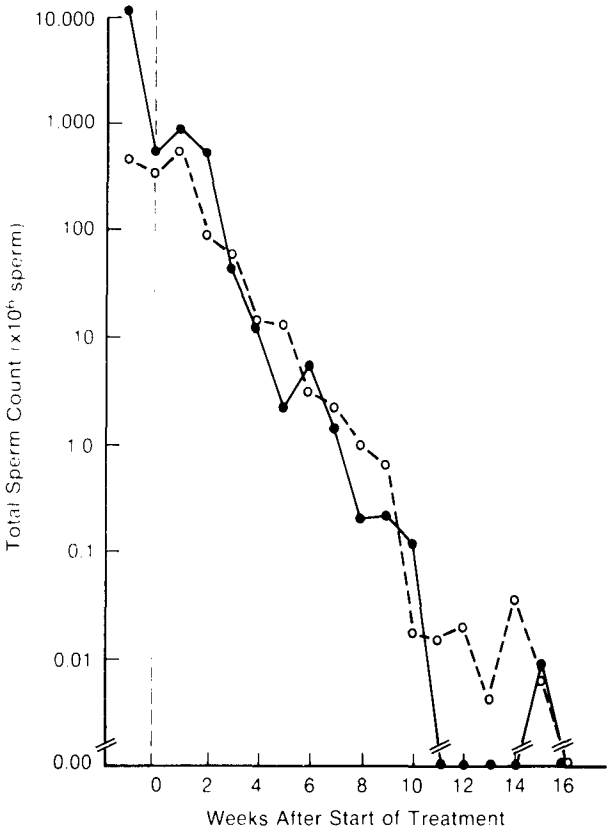

Fig. 7. Effect of daily injection of 100 μg/kg of RS-68439, in two male beagles bearing subcutaneous silastic capsules of testosterone, on total sperm count in weekly ejaculates.

reports of evaluation of LHRH antagonists in men have appeared.

EFFECTS IN FEMALES

Rats

Although the LHRH agonists are acutely stimulatory and will induce ovulation at all stages of the cycle in rats [50], persistent treatment will block ovulation and stop estrous cyclicity [4]. As in male rats the mechanisms involved in female rats are complicated and multifactorial. In addition to effects exerted at the pituitary receptors, specific LHRH receptors do exist at both the follicular and luteal level [51, 52]. Direct gonadal actions and interference with the steroidogenic effects of the gonadotropins by the agonists is now well documented [20]. Pregnancy may be terminated in rats by a single injection of LHRH agonist and this effect would be attributable to the inappropriately high levels of gonadotropins down regulating their homologous receptors at the corpus luteum [53]. However, specific ipsilateral pregnancy termination has been noted following direct injection of LHRH agonist into one uterine horn in the pregnant rat. There have even been claims of interaction between LHRH agonists and uterine steroid receptors, although the evidence is somewhat flimsy [54].

Blockade of ovulation in rats with LHRH antagonists is easy to demonstrate and has long been the basis for potency screening [55]. However, a single injection of antagonist may delay ovulation for several days [56] or alternatively block ovulation even when given once several days before ovulation [25]. It appears that there is a different circulating blood level/receptor occupancy requirement for LHRH antagonists to inhibit ovulation and to suppress estrous cyclicity, for the latter requires dosages an order of magnitude greater than the former. This probably reflects a difference between the requirement for blockade of the pre-ovulatory surge in gonadotropins and that for suppression of basal levels of gonadotropins. LHRH antagonists will terminate pregnancy in rats [57]. Efficacy varies with stage of gestation, the optimum time for dosing is day 10. Either progesterone or hCG administration will reverse the effect.

Dogs

It has now been shown that long term administration of nafarelin in female dogs is capable of suppressing both cyclicity (as judged by proestrous discharge and by vaginal or behavioral estrus) and ovulation [58]. Prevention of ovulation for periods of up to 18 months is consistent with a return of ovulation in a few weeks. Interestingly, as already reported for the heifer [59] an already entrained

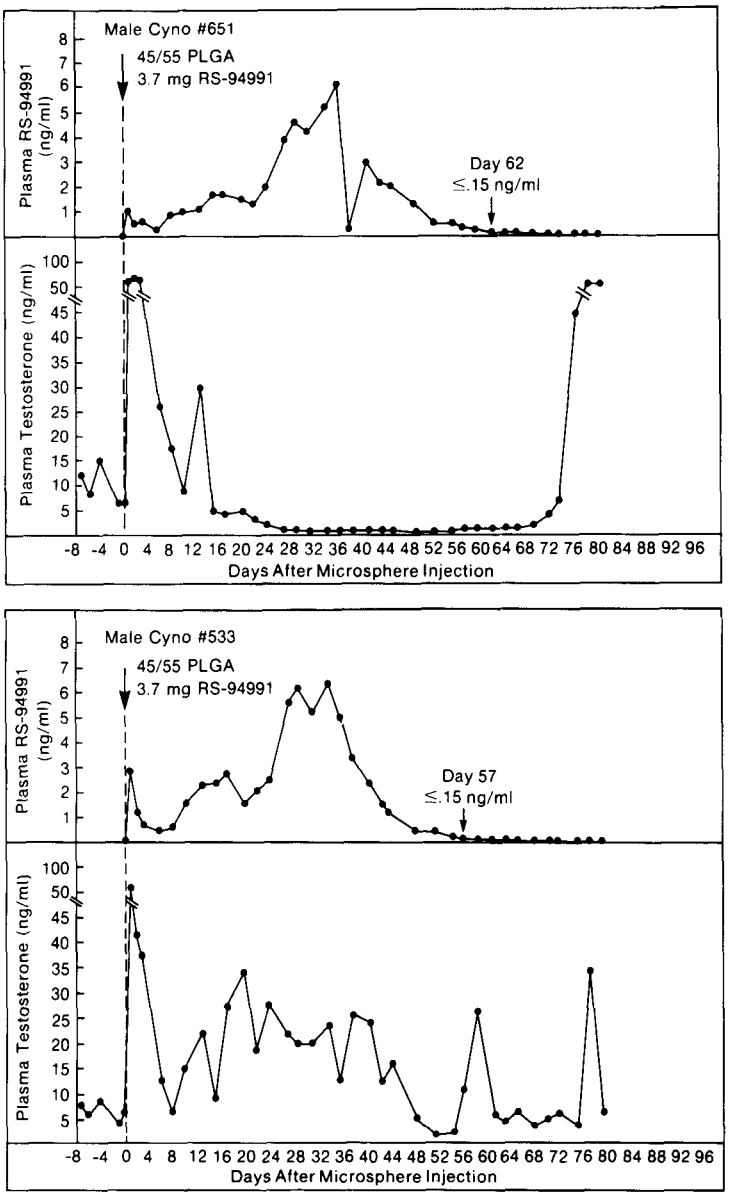

Fig. 8. Blood levels of nafarelin acetate and testosterone in 2 cynomolgus monkeys after subcutaneous injection of nafarelin acetate incorporated into PLGA microspheres.

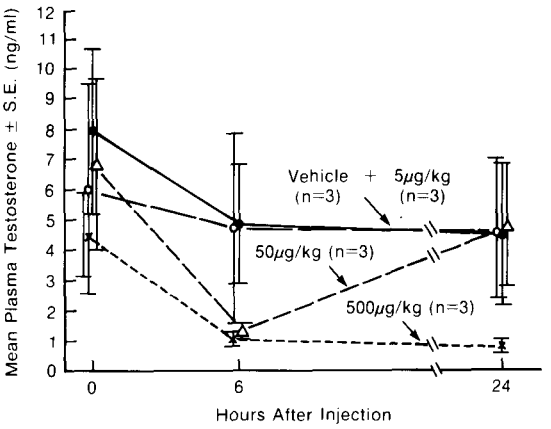

Fig. 9. Effect of a single subcutaneous injection of RS-68439 on circulating levels of testosterone—male cynomolgus monkeys.

ovulation cannot be prevented with an LHRH agonist. Dosage during the luteal phase of the nonpregnant bitch results in a significant reduction in integrated levels of progesterone [58]. However, the same doses administered throughout gestation had no effect on the outcome of pregnancy. Whether this indicates the presence of, and interaction with, a luteotrophic factor in the pregnant bitch, as in higher species, is not known.

Preliminary results indicate the ability to suppress luteal function and terminate pregnancy in the bitch with an LHRH antagonist (B. H. Vickery, G. I. McRae and J. C. Goodpasture, unpublished observations). It is not yet clear whether luteolysis can be achieved, or merely that progesterone secretion is prevented for the duration of the period in which gonadotropin secretion is blocked, followed by return

of luteal function when gonadotropin levels rise again.

Primates

It is now well established that in monkeys as well as in women ovulation may be suppressed by comparatively low daily doses of LHRH agonists [14, 60–62]. There does seem some dispute as to whether the dose can be titrated so that the dangers of, on the one hand, unopposed estrogen and endometrial hyperplasia and on the other vasomotor and bone density symptoms of estrogen withdrawal can be avoided [63, 64]. One group goes so far as to propose regular induction of withdrawal bleeding using a course of progestogen each month [65]. It appears that, as in male primates, more profound suppression of pituitary and ovarian function can be achieved in female primates by continuous administration of the LHRH agonist from controlled release formulations [65, 66].

Luteolysis, or better luteal suppression, has been a routine observation in response to LHRH agonists in nonpregnant monkeys and women [67–69]. However, the ability of exogenous chorionic gonadotropin to antagonize this effect [70, 71] no doubt accounts for the inability of these agents to terminate pregnancy in primates [71–73], with the notable exception of the baboon [74, 75]. The demonstrations of LHRH receptors in human placental tissue [76, 77], a suppressive effect on chorionic gonadotropin production *in vitro* with LHRH agonists on human placental tissue (T. Siler-Khodr and B. Vickery, unpublished) and the observation of suppression of chorionic gonadotropin production *in vivo* in pregnant baboons [74] suggests that a difference in placental–blood barrier may account for the differences.

Not unexpectedly, reports are now appearing which document the ability of LHRH antagonists to inhibit ovulation in primates [78, Fig. 10]. As with the agonists, however, whether it will be possible to avoid the problems associated with either unopposed estrogen or estrogen deprivation is unknown but considered unlikely. Although early reports concluded that the nonpregnant primate corpus luteum was independent of pituitary support and that LHRH antagonists were without effect [78], it is now clear that luteal progesterone output can be precipitously decreased with these agents [79] resulting in withdrawal bleeding within 1–2 days (Fig. 11). This effect is not luteolysis because discontinuation of antagonist treatment after 2–3 days may allow progesterone levels to rise again and, as in the case with agonists, chorionic gonadotropin supplementation will prevent the fall and/or cause a rise in progesterone levels. *In vitro* studies with human placental explants incubated with an LHRH antagonist have shown remarkable inhibition of both steroids and chorionic gonadotropin into the medium [80]. Studies in pregnant baboons, with a low potency LHRH antagonist, at a time when chorionic gonadotropin levels were low did show transitory falls in progesterone [81]. Of course, even if pregnancy should be terminated in baboons, prior experience with the agonists would reserve judgement until studies in women had been completed.

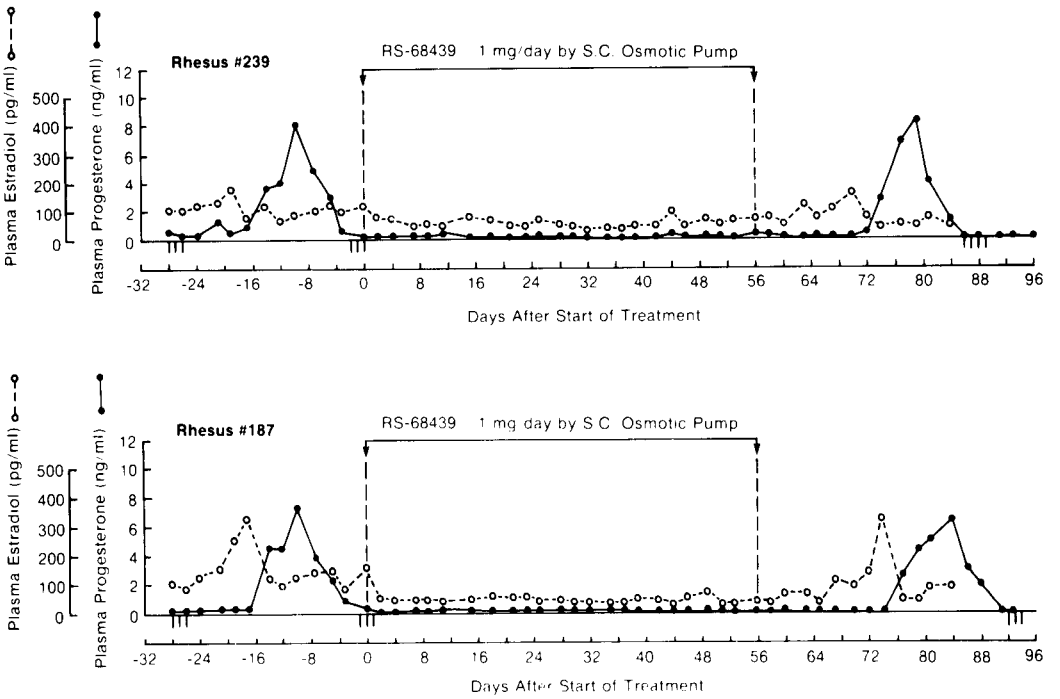

Fig. 10. Effect of RS-68439 on ovulation in rhesus monkeys.

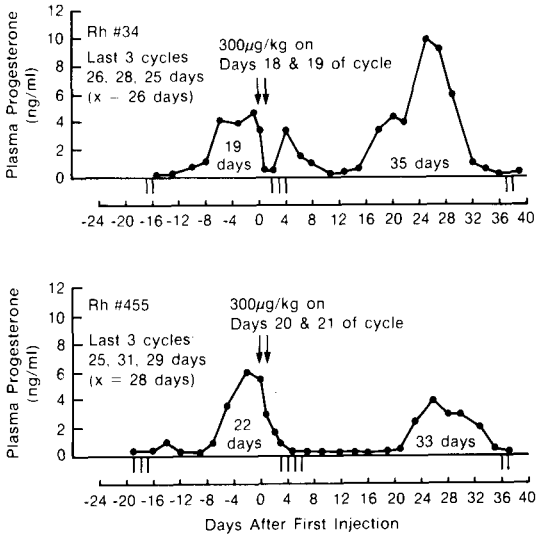

Fig. 11. Effect of RS-68439 injected on two consecutive days in the midluteal phase on circulating levels of progesterone in female rhesus monkeys.

CONCLUSIONS

LHRH agonist analogs are now available of such potency that very low doses may be used to control fertility in certain species and gender. However, particularly in males, the interspecies variability in response is large, varying from the mouse in which down regulation cannot be achieved, to the rat where only focal disruption of spermatogenesis is observed, to the rabbit where interanimal variation is noted, to the dog where total azoospermia is easily achieved [7, 36, 82]. In men testosterone levels may be lowered to the extent that hot flashes are experienced and yet azoospermia has not been achieved [41–44]. Some hope is held out by the recent results in the very resistant macaques by using continuous administration [37, 39] and this may be likened to the "megadose" therapy used in men with prostatic carcinoma [83]. However even though autopsy material shows aspermatogenic histology [45], it must be realized that these are patients with a terminal disease rather than young healthy men. Responses in the former will not necessarily be applicable to the latter. Even further, should continuous administration therapy result in consistent induction of azoospermia, it will await availability of controlled release formulations for both the LHRH agonist and possibly also for the testosterone supplementation.

For control of fertility in females, the outlook is somewhat brighter. Suppression of ovulation is a consistent effect over a broad range of species and long term contraceptive efficacy has been reported with one of these agents [61]. While there are concerns about the potential risks of unopposed estrogen and about the effects of estrogen deprivation, perhaps these would be minimized by short courses of therapy of 3–4 months at a time. Such a regimen might be of particular use for postpartum contraception. It seems unlikely to the present author, however, that the combination of LHRH agonist and progestins would gain wide acceptance. It is now clear that neither menstrual induction or pregnancy termination will be achieved with LHRH agonists in women.

Evaluation of LHRH antagonists is in the early stages. However, interspecies variation in response has not been reported. In male rats, dogs and macaque monkeys azoospermia and aspermatogenesis has been consistently obtained. Although high level testosterone supplementation in rats has been shown to reverse the antifertility effect, this is not expected in other species including man.

Although it is logical to assume that ovulation suppression can be achieved in women as in other animal species with the LHRH antagonists, it is unlikely that they would offer any advantages over the agonists. The hope for an interceptive or menstrual inducing utility for the antagonists, is still with us from the results of the animal studies. However, with the recent experience with the agonists in mind, judgement will be reserved until the human studies have been completed.

At first sight it appeared that the same end results would be achieved by the two distinct mechanisms by which the LHRH agonists and antagonists interfere with reproduction. The present review suggests that there may be a place for both classes of analog in future control of fertility.

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