FERTILITY AND STERILITY
Vol. 28, No. 3, March 1977
Printed in U.S.A.

EFFECT OF INTRAUTERINE ESTRIOL ON REPRODUCTIVE FUNCTION IN THE RABBIT*†

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The effect of intrauterine estriol on spontaneous ovulation, ovum fertilization, blastocyst development, and blastocyst implantation in rabbits has been investigated. Estriol-releasing intrauterine capsules constructed of biocompatible polymer were implanted in one uterine horn of adult New Zealand White female rabbits, while placebo-containing capsules were implanted into the contralateral horn. The animals were artificially inseminated and ovulated or mated to fertile bucks. The does were killed and their reproductive tracts were examined 54 hours, 6 days, and 10 days after ovulation-inducing injection or after coitus. The results indicate that intrauterine estriol released at a steady state rate of 1.25 μg/day effectively inhibits blastocyst development and implantation. This contraceptive effect was clearly local, since implantations in the contralateral, placebo-bearing horn were not inhibited. Furthermore, the same dose of estriol, when released systemically from a subcutaneously placed capsule, had no effect on implantation. Intrauterine estriol appeared also to have no effect on spontaneous ovulation or ovum fertilization.

Uterine receptivity to implantation is dependent on precisely timed endocrine events where both estrogen and progesterone secretion play a key role. Estrogen-dependent uterine RNA synthesis is necessary for implantation in several species of laboratory rodents.[1, 2]

In immature rats, estriol, like estradiol, binds to uterine cytoplasm receptor and, like estradiol, causes equal accumulation of the receptors in the nucleus.[3] Estriol also shares with estradiol the capability of causing early uterotropic events, i.e., histamine release, decrease in number of mast cells, increase in eosinophilic leukocytes, vasodilation, increased blood flow, and edema.[2]

Accepted October 15, 1976.
*Supported by National Institutes of Health Contract NO1-HD-3-2739.
†Presented in part at the Thirty-Second Annual Meeting of The American Fertility Society, April 5 to 9, 1976, Las Vegas, Nev.

Estradiol, however (but not estriol), is capable of inducing in rats true uterine growth, as shown by increased dry weight 24 hours after a single estradiol injection.[3] Estriol, therefore, while unable to cause true uterine growth, may interfere with estradiol-induced RNA synthesis by competing with this steroid for uterine receptors.[4] Since late estrogen-induced RNA synthesis is necessary for implantation, an anti-implantation effect of estriol can be postulated. In fact, estriol has reportedly been shown to interfere with conception in rats, rabbits, and hamsters when administered systemically in small amounts.[5, 6] However, systemic administration of a steroid may involve endocrine events outside the uterus.

We attempted to test the strictly-uterine contraceptive effectiveness of estriol by local chronic administration of estriol in very minute amounts to the uterine horn via a small intrauterine capsule.

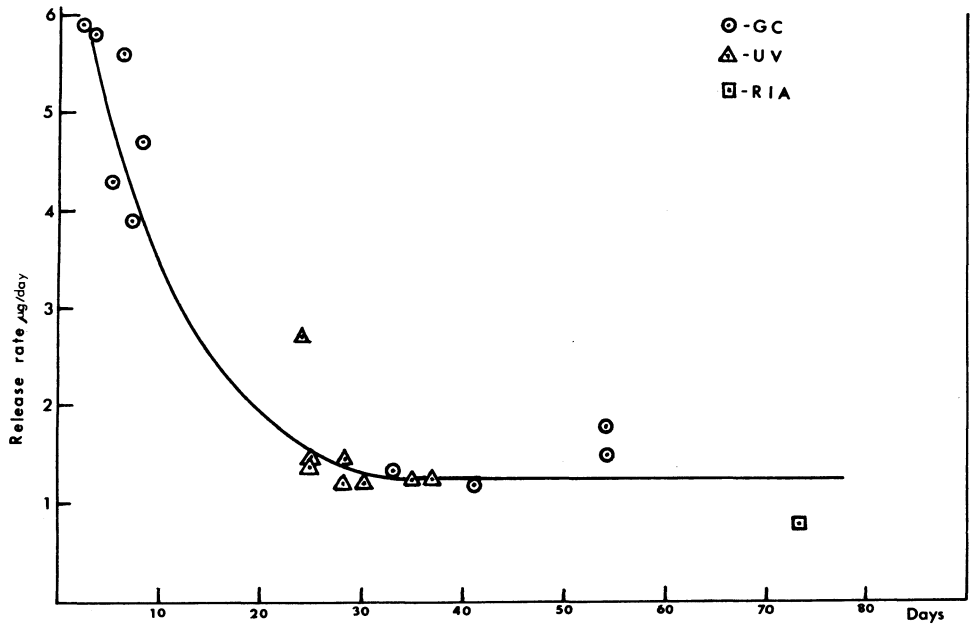

FIG. 1. Release rate of 1.25-μg estriol devices as determined in vitro over an 80-day period by Alza Research, using gas chromatography (*GC*), radioimmunoassay (*RIA*), and ultraviolet (*UV*) techniques.

MATERIALS AND METHODS

Estriol-releasing capsules measuring 1.5 × 10 mm were manufactured by Alza Research, Palo Alto, Calif. The capsules were made of a biocompatible polymer and released estriol at a steady state rate of 1.25 or 0.04 μg/day. Initial release rates were significantly higher for all capsules but decreased exponentially with time, so that within 3 to 4 weeks a predetermined steady state release rate was achieved (Fig. 1). All release rates were determined in vitro by Alza Research, using gas chromatography, radioimmunoassay, and ultraviolet techniques. For each estriol-releasing capsule an identical placebo capsule was prepared which contained only the carrying vehicle without estriol.

Estriol release rates from randomly selected capsules were also determined in our laboratory before insertion and after removal from rabbit uteri. For this purpose each device was prewashed in a bath of running tap water at the controlled temperature of 37° ± 1° C with continuous stirring. After washing, the capsules were suspended from a vertically reciprocating shaker and incubated in 20 to 40 ml of 0.9% isotonic saline. The capsules were agitated in the saline solution for 6 to 24 hours. Aliquots of the incubation media were then extracted with anhydrous ether. Tritium-labeled estriol was added for determination of procedural losses. Chromatographic purification was performed with Sephadex LH-20 columns, using isooctane-benzene-methanol (62:20:18, v/v) as eluent. The amount of estriol was determined by radioimmunoassay technique using sheep antiserum against estriol-6-keto-oxime-human serum albumin,[7] purchased from Dr. G. E. Abraham, Torrance, Calif. Release rates so determined corresponded to those indicated by Alza Research. Specifically, 1.25-μg devices were releasing 1.8 ± 0.1 μg/24 hours (mean ± standard deviation of 15 determinations) before insertion and 1.3 ± 0.3 μg/24 hours (mean ± standard deviation of 11 determinations) after removal from the uterine horns.

Adult New Zealand White female rabbits and adult males of proven fertility were used. Laparotomies were performed under pentobarbital or nitrous oxide and halothane inhalation anesthesia, through a midline incision using sterile technique. The capsules were introduced into the uterine horn through a small incision near the uterocervical junction and secured to the uterine wall with a silk suture, so that they remained free in the lumen.

Estriol-releasing capsules were placed in one horn, while placebo-containing capsules were placed in the contralateral horn, to serve as control. When specifically stated, the capsules were implanted subcutaneously in the neck region. About 4 weeks after insertion of the capsules, the

does were artificially inseminated with semen collected from adult bucks with the aid of an artificial vagina. Prior to insemination, the semen was diluted with 0.9% saline solution to an average concentration of 50 million sperm/ml. When specifically stated, the does were mated with fertile bucks. Immediately following insemination the does were ovulated with 150 IU of human chorionic gonadotropin (HCG [Follutein, E. R. Squibb & Sons, Princeton, N. J.]) administered intravenously.

To study the effect of intrauterine estriol on fertilization, development of blastocysts, and implantation, several experimental modifications were designed.

Experimental Design

Effect of Intrauterine Estriol on Blastocyst Implantation. This effect was evaluated for capsules releasing 1.25 and 0.04 μg of estriol/24 hours. The does were killed 10 days after insemination and HCG injection. At autopsy, the entire reproductive systems were removed and examined. Implantation sites in each horn were examined and their number was recorded. The number of corpora lutea in each ovary was counted and recorded.

Effect of Intrauterine Estriol on Ovum Fertilization. This effect was evaluated for capsules releasing 1.25 μg of estriol/24 hours. The does were killed 54 hours after insemination and ovulation-inducing injection. At autopsy the ovaries were removed and the corpora lutea on each side were counted. The oviducts were dissected and flushed separately with 0.05% methylene blue in normal saline. The washings were collected in a watch glass and examined immediately for the presence of eggs under a dissecting microscope. The eggs recovered were separated and counted. Uterine horns were also flushed and the washings examined for presence of cleaved ova.

Effect of Intrauterine Estriol on Egg Development. This effect was evaluated for capsules releasing 1.25 μg of estriol/24 hours. The does were killed 6 days after insemination and ovulation-inducing injection. At autopsy the number of corpora lutea in each ovary was recorded and the uterine horns were flushed as described above. The blastocysts recovered from uterine washings were counted and examined under a microscope. The number of blastocysts measuring 3 mm or more was counted and recorded for each horn. Similarly, the number of smaller blastocysts was counted and recorded separately. The oviducts were also flushed and the washings examined.

Effect of Intrauterine Estriol on Coitus-Induced Ovulation. This effect was evaluated for capsules releasing estriol at a steady state rate of 1.25 μg/24 hours. The does were not inseminated artificially, and HCG injections were not given. Instead, they were mated with fertile bucks. The does were killed 10 days after mating, and their reproductive systems were examined for the number of implantation sites and the number of corpora lutea.

Systemic Effect. This effect was evaluated for capsules releasing 1.25 μg of estriol/24 hours. Estriol or placebo capsules were implanted subcutaneously in the neck region. Four weeks later the does were inseminated and ovulated. The does were killed 10 days after HCG injection. At autopsy the reproductive systems were examined for the number of implantation sites and the number of corpora lutea.

In all experiments the percentage of ova (or blastocysts) recovered was calculated by the formula: No. of ova recovered/no. of corpora lutea × 100. The statistical significance of the results was analyzed by means of the *t*-test for percentages.[8]

RESULTS

Effect on Blastocyst Implantation. Examination of reproductive systems 10 days after insemination and HCG injection revealed that an average of 2.4 implantations occurred in each uterine horn bearing a placebo device (Table 1). By comparison, only 1 implantation was observed in as many as 13 horns fitted with capsules releasing

TABLE 1. *Effect of Intrauterine Estriol on Blastocyst Implantation: Does Were Killed 10 Days after Insemination*

	No. of horns	No. of corpora lutea	No. of implanted blastocysts	% Blastocysts recovered	
Release rate: 1.25 μg/24 hr					
Active device side	13	98	1	1	$P < 0.001^a$
Placebo side	13	84	31	37	
Release rate: 0.04 μg/24 hr					
Active device side	9	66	4	6	$P < 0.001^a$
Placebo side	9	63	16	25	

[a]The *t*-test for significance of the difference between percentages was used.

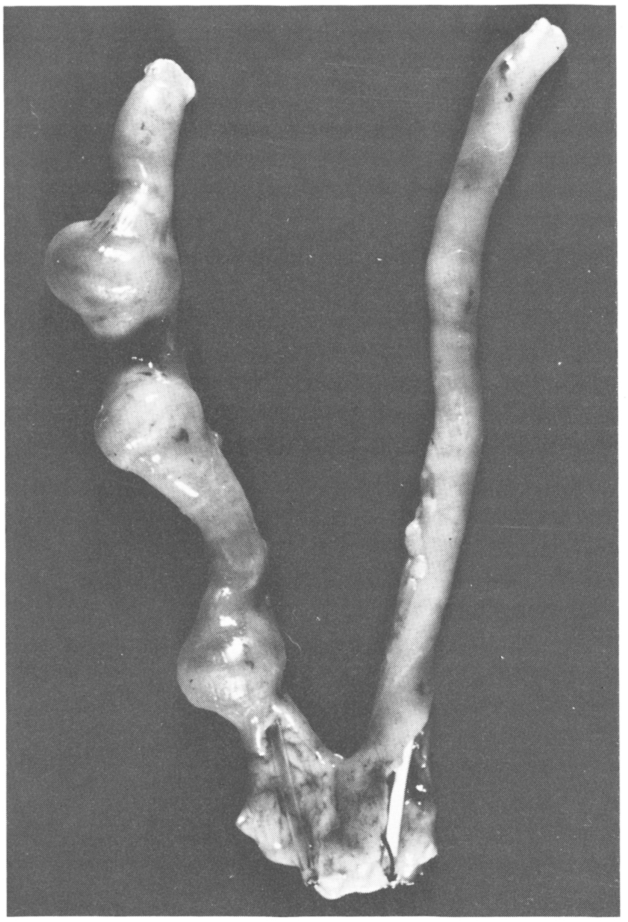

FIG. 2. Anti-implantation effect of intrauterine estriol: three implantations in a control, placebo-bearing horn and no implantations in the contralateral horn bearing an estriol-releasing device.

1.25 μg of estriol/24 hours. Figure 2 shows a photograph of the rabbit uterus with three implantation sites in a control horn bearing a placebo device and no implantations in a contralateral horn fitted with an estriol-releasing device.

The percentages of implanted ova were 37 for uterine horns fitted with a placebo device and 1 for horns fitted with an active device. The difference when evaluated statistically was highly significant ($P < 0.001$).

The number of implantation sites and the percentage of implanted ova in horns bearing capsules releasing estriol at a lower rate (0.04 μg/24 hours) were higher than the corresponding numbers for 1.25-μg releasing capsules. However, when evaluated statistically by comparison with placebo-bearing horns, the contraceptive effect of a 0.04-μg estriol device was still significant at the 0.001 level.

Effect on Ovum Fertilization. An average of two cleaved ova were recovered from oviducts on the side of the active device 54 hours after insemination and HCG injection, and an average of 4 cleaved ova on the contralateral, control side (Table 2). The percentages of ova recovered on active and placebo sides were 61 and 74, respectively. The difference was not significant statistically.

Effect on Egg Development. Table 3 shows the number and the size of 6-day-old blastocysts recovered from uterine horns bearing active and placebo devices. The over-all percentage of blastocysts recovered from the horns bearing active devices was significantly smaller than the corresponding percentage for placebo-bearing horns. There was no significant difference in the percentage of blastocysts smaller than 3 mm in diameter recovered from treated or control horns. However, significantly smaller numbers and percentages of blastocysts 3 mm or larger were recovered from horns bearing estriol-releasing devices as compared with control horns.

Effect on Coitus-Induced Ovulation. Reproductive systems were examined 10 days after mating. HCG was not administered to this group of animals. The results are shown in Table 4. Mating induced ovulation in all animals. No blastocysts were implanted in horns bearing an active device, while a significant percentage of implanted ova was recovered in horns bearing placebo devices.

Lack of Systemic Effect. Reproductive systems were examined 10 days after insemination and ovulation. The animals had no intrauterine devices. Estriol-releasing or placebo capsules were implanted subcutaneously. Of 13 animals bearing estriol-releasing capsules, 12 became pregnant, while of 5 bearing placebo capsules, 4 conceived (Table 5). There was no difference in the number of implantation sites between right and left horns, and there was no difference between the percent-

TABLE 2. *Absence of Effect of Intrauterine Estriol (1.25 μg/24 Hours) on Ovum Fertilization: Does Were Killed 54 Hours after Insemination*

	No. of horns	No. of corpora lutea	No. of cleaved ova recovered	% Cleaved ova recovered	
Active device side	7	23	14	61	NS[a]
Placebo side	7	39	29	74	

[a]The t-test for significance of the difference between percentages was used. NS, Not significant.

TABLE 3. *Effect of Intrauterine Estriol (1.25 μg/24 Hours) on Blastocyst Development and Recovery: Does Were Killed 6 Days after Insemination*

	No. of horns	No. of corpora lutea	Blastocysts ≥ 3 mm		Blastocysts < 3 mm		Total blastocysts recovered		
			No. recovered	% Recovery	No. recovered	% Recovery	No.	%	
Active device side	10	52	2	4	12	23	14	27	$P < 0.001$[a]
Placebo side	10	53	24	45	14	26	38	72	

[a]The *t*-test for significance of the difference between percentages was used.

age of ova implanted in animals fitted subcutaneously with estriol or placebo capsules.

DISCUSSION

The results of this study indicate that estriol released from intrauterine capsules at the in vitro estimated rate of 1.25 μg/24 hours effectively inhibits blastocyst implantation in rabbits (Tables 1 and 4). The anti-implantation effect at this release rate is close to 100%. However, even at a lower release rate (0.04 μg/24 hours) the anti-implantation effect, although less pronounced, is still significant.

This contraceptive effect is quite clearly local, since implantations in the contralateral placebo-bearing horn were not inhibited. Furthermore, the same dose of estriol when released systemically from a subcutaneously placed capsule had no effect on blastocyst implantation (Table 5).

Lack of implantation sites in horns bearing estriol-releasing devices may indicate an anti-implantation effect of this steroid or may indicate that other events preceding implantation and necessary for normal reproduction were inhibited. Several experiments were performed to evaluate whether intrauterine estriol interferes with fertilization, development of the ovum, or spontaneous ovulation.

No effect of intrauterine estriol on coitus-induced ovulation was observed. An average of five ovulations occurred in the ovary on the treated side and the same number on the opposite, control side (Table 4).

There was no statistically significant effect of intrauterine estriol on ovum fertilization, as judged by the number of cleaved ova recovered from the oviducts (Table 2). However, ovum development in the early blastocyst phase appeared to be affected. A significant decrease in normally developed 6-day-old blastocysts was observed in treated horns (Table 3).

Thus, the anti-implantation effect of intrauterine estriol appears to be exerted through an inhibitory effect of this steroid on development of the blastocysts and on their subsequent implantation. It is of interest to note that a very similar effect was observed for intrauterine progesterone.[9] The mechanism of this effect is not entirely clear. Seshadri et al.[9] postulated that accelerated endometrial maturation was a possible cause of the anti-implantation effect of intrauterine progesterone. However, this does not seem likely, since a very similar defect in the development of early blastocysts can be observed for both intrauterine progesterone and estriol.

Wotiz et al.[10] postulated that the anti-implantation effect of estriol, an impeded estrogen, is mediated through inhibition of the synthesis of a specific estradiol-dependent protein necessary for successful implantation. Estriol is known to bind competitively to the cytoplasmic estradiol receptors, making them unavailable to estradiol. Unlike estradiol, the estriol-receptor complex is not retained by the nucleus and does not induce late protein synthesis.[3]

However, recent data seem to make this hypothesis less tenable. Anderson et al.[3] have shown that repetitive injections of estriol at intervals not less frequent than 3 hours result in an increase in uterine dry weight (true long-term uterine growth) equivalent to that produced by repetitive injections of estradiol.

The presence of intrauterine capsules continuously releasing estriol would be expected to be equivalent to repeated estriol injections and therefore also capable of inducing late protein synthesis. In this light, the prevention of implan-

TABLE 4. *Effect of Intrauterine Estriol (1.25 μg/24 Hours) on Spontaneous Ovulation and Blastocyst Implantation: Does Were Killed 10 Days after Mating*

	No. of horns	No. of corpora lutea	No. of implanted blastocysts	% Blastocysts recovered	
Active device side	4	21	0	0	$P < 0.01$[a]
Placebo side	4	20	15	75.0	

[a]The *t*-test for significance of the difference between percentages was used.

TABLE 5. *Absence of Effect of Systemic Estriol on Blastocyst Implantation in Rabbits*

	No. of animals	No. of horns	No. of corpora lutea	No. of implanted blastocysts	% Ova implanted	
Estriol capsules[a]						
Pregnant does	12	24	141	92	65	
Nonpregnant does	1	2	1	0		NS[b]
Placebo capsules						
Pregnant does	4	8	39	20	51	
Nonpregnant does	1	2	2	0		

[a]Capsules releasing estriol at a rate of 1.25 μg/24 hours or placebo were implanted subcutaneously in the neck region. The does were inseminated and ovulated with HCG. Reproductive systems were examined and implantation sites counted 10 days after insemination.

[b]The *t*-test for significance of the difference between percentages was used. NS, Not significant.

tation by the intrauterine estriol capsule may indicate that its mechanism of action does not involve interference with late protein synthesis by the uterus. However, additional studies are needed to determine the effect of the estriol capsule on uterine protein synthesis.

The process of implantation in many species involves a complex interaction between embryo and uterus. In mice and rats, the blastocysts become activated in a uterus which has been stimulated by estrogens. In such an environment, the blastocyst's rate of RNA and protein synthesis increases, and enlargement and outgrowth of trophoblastic cells take place. If the uterus has not been sensitized by estrogens, the blastocysts enter a state of dormancy and implantation is delayed. Available evidence suggests that estrogens do not affect the blastocysts directly, but may cause the removal from the uterine secretions of a factor(s) which inhibits the metabolic activity of the blastocyst.[2]

It is also known that a specific uterine protein, blastokinin, is required for the successful growth and development of the blastocyst.[11] The synthesis of this protein appears to be under the control of progesterone and not estradiol.[12] However, the effect of estriol, if any, on the synthesis of this protein is unknown.

Another mechanism for the anti-implantation effect of intrauterine estriol may also be postulated. The over-all blastocyst recovery from treated horns 6 days after insemination was 27% as opposed to 72% for the control horns (Table 3). These data may suggest either inhibition of the development of the blastocysts and their subsequent degeneration (as was already postulated) or it may indicate an accelerated transport of normally developed blastocyts through the uterine horn fitted with the estriol capsule.

It may be of interest to point out that the percentage of blastocysts implanted in placebo-bearing horns was significantly higher following mating (75%; Table 4) than following artificial insemination and ovulation induction (25% and 37%; Table 1). This difference may be due, in part, to luteinization of the immature follicles caused by the rather large ovulatory dose of HCG. Such follicles, containing entrapped ova, would not contribute to the number of implantations, while they would be indistinguishable from normal corpora lutea. The number of implantations per each control horn, however, also seems to be higher in mated animals as opposed to those artificially inseminated (3.75 versus 1.8 and 2.4, respectively). This may be explained by the fact that artificial insemination was performed with diluted semen and that there was a time lapse between semen collection and insemination.

If the contraceptive effectiveness of estriol is demonstrated in species other than laboratory rodents and rabbits, this steroid may prove to be useful for contraception via intrauterine release in women. In view of the very small amount of hormone released, no systemic effects can be reasonably anticipated.

Acknowledgment. We are grateful to Alza Research, Palo Alto, Calif., for its generous assistance in providing the estriol-releasing capsules.

REFERENCES

1. Segal SJ, Wada K, Schuchner E: Role of RNA in estrogen-induced nidation of blastocysts. In Proceedings of the 47th Meeting of the Endocrine Society, New York, 1965, p 26
2. Psychoyos A: Endocrine control of egg implantation. In Handbook of Physiology, Sect 7: Endocrinology, Vol 2: Female Reproductive System, Part 1, Edited by RO Greep, EB Astwood. Washington DC, American Physiological Society, 1973, p 187
3. Anderson JN, Peck EJ Jr, Clark JH: Estrogen-induced uterine responses and growth: relationship to receptor estrogen binding by uterine nuclei. Endocrinology 96: 160, 1975
4. Brecher PI, Wotiz HH: Competition between estradiol and estriol for end organ receptor proteins. Steroids 9:431, 1967

5. Scublinksy A, Wotiz HH: The contraceptive action of impeding oestrogens. I. Activity of oestriol in the rat. J Reprod Fertil 26:139, 1971
6. Scublinsky A, Wotiz HH: The contraceptive action of impeding oestrogens. VII. Effectiveness of oestriol in the rabbit and the hamster. J Reprod Fertil 26:365, 1971
7. Tulchinsky D, Abraham GE: Radioimmunoassay of plasma estriol. J Clin Endocrinol Metab 33:775, 1971
8. Hill B: Principles of Medical Statistics, Seventh Edition. New York, Oxford University Press, 1961, p 308
9. Seshadri B, Gibor Y, Scommegna A: Antifertility effects of intrauterine progesterone in the rabbit. Am J Obstet Gynecol 109:536, 1971
10. Wotiz HH, Smith S, Shapiro B, Scublinsky A: The contraceptive action of impeding oestrogens. IV. Effect of oestradiol on the anti-implantation action of oestriol in the rat. J Reprod Fertil 26:237, 1971
11. El-Banna AA, Daniel JC Jr: Stimulation of rabbit blastocysts in vitro by progesterone and uterine proteins in combination. Fertil Steril 23:101, 1972
12. Arthur AT, Daniel JC Jr: Progesterone regulation of blastokinin production and maintenance of rabbit blastocysts transferred into uteri of castrate recipients. Fertil Steril 23:115, 1972