Megestrol and Chlormadinone Acetates in the Hamster: Fertilization-Capacitation Effects[1] (35891)

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It has been 20 years since Austin and Chang first demonstrated that sperm require a period of uterine or oviductal incubation (capacitation) before they are capable of penetrating the outer investments of the ovum to cause fertilization. The role of the endocrines in this process was first shown by Chang (1), when he reported that uterine capacitation was inhibited in pseudopregnant or progesterone-treated rabbits.

The work of Yanagimachi (2) on the hamster, has verified the requirement for capacitation in hamsters and he has successfully capacitated hamster sperm *in vitro*.

Bedford (3) and Bedford and Shalkovsky (4) have demonstrated the possibility of two or more distinct phases of rabbit sperm capacitation and that the first phase can be accomplished in a foreign uterine environment, *viz.*, in the uterus of the estrous rat. This principle was utilized in our work (5), where the first phase of rhesus and human sperm capacitation was demonstrated, by indirect techniques, to occur in the uterus of the rat, mouse, hamster, and rabbit.

Martinez-Manautou *et al.* (6) studied the effect of levels of chlormadinone acetate from 50 to 500 μg/day on contraceptive effectiveness in humans and found a dose–response effect on contraceptive effectiveness. The compounds allowed ovulation but prevented pregnancy. This suggested an effect of the low dose oral contraceptive compounds on sperm capacitation on the fertilization process. Harrison and Dukelow (7) have demonstrated a dose-related effect of megestrol acetate at levels from 50 to 500 μg/day on ovulation in the squirrel monkey (*Saimiri sciureus*).

The present work was conducted to examine the effects of megestrol and chlormadinone acetate on hamster reproduction and the effect of the hamster uterine environment on rabbit sperm capacitation.

Material and Methods. Solutions of megestrol and chlormadinone acetates were administered to adult (12 week old) hamsters subcutaneously in 0.1 ml of cottonseed oil at doses ranging from 10 to 500 μg/day. To separate anti-implantation effects from ovulation-fertilization effects, these injections were given for 4 days either: (a) for one estrous cycle before mating; or (b) from days 2 to 5 (day 1 represents the day of mating and ovulation). In a third series of experiments, vaginal smears were examined for sperm the morning after mating to determine if the compounds exerted an effect on mating behavior.

To determine if the hamster uterus was capable of completing the first phase of rabbit sperm capacitation, an assay system described by Dukelow (8) was used. Twice washed rabbit sperm were placed in the ligated, estrous hamster uterus. Twelve hours later the sperm were recovered, concentrated by centrifugation at 862*g* for 5 min and inseminated (2.5×10^4 sperm in 0.05 ml of saline) into the oviducts of rabbits either 12 or 14 hr after the does received 2.5 mg of LH. Fertilization of ova in the 12-hr but not the 14-hr rabbits was interpreted to mean that only the first phase of capacitation has been accomplished; whereas fertilization of ova from both types of rabbits would indicate complete capacitation.

Results. Unlike the rabbit, fertilization in

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TABLE I. Percentage of Animals Becoming Pregnant After Natural Mating, with Varying Doses of Chlormadinone and Megestrol Acetates Administered Subcutaneously for One Estrous Cycle (4 days) Before Mating.

Treatment (μg)	No. of hamsters	% Preg.	Total corpora lutea	Total embryos	Implantation index[a]
Cottonseed oil (control)	17	82.3	186	176	94.5
Chlormadinone acetate					
10	9	77.8	104	86	82.6
50	14	85.8	134	131	97.8
100	15	60.0	112	108	100.0
250	14	21.4	34	37	100.0
500	5	0	—	—	—
Megestrol acetate					
50	16	75.0	143	153	100.0
100	15	60.0	120	100	83.4
250	24	25.0	86	77	89.6

[a] Implantation index = (no. of embryos implanted on day 8/no. of corpora lutea present on day 8) × 100.

the hamster is an "all or none" situation resembling the phenomena observed in the pig. In recoveries of ova from over 500 hamsters, we have observed only one case where both fertilized and unfertilized ova were recovered from the same animal. Because of this, one can conveniently measure hormonal effects in terms of the percentage of animals becoming pregnant, a pregnant animal being defined as having one or more fertile ova (in instances of ova recovery) or implantation sites (postimplantation).

When chlormadinone and megestrol acetates were administered at levels above 50 μg/day to hamsters for one estrous cycle prior to mating (Table I), there was a decreasing fertility level. There were no observed differences in the implantation indices among treatments. At a level of 500 μg of chlormadinone/day there were noticeable gross effects on the reproductive tract including increased inflammation and failure of implantation. Ovulation occurred in virtually all animals including those receiving compounds at the 500-μg level.

Table II, indicates that neither chlormadinone nor megestrol acetate (with the exception of the highest level of the latter) exerted an adverse effect on the percentage of animals becoming pregnant when administered from days 2 through 5, *i.e.*, during the time when implantation occurred.

TABLE II. Percentage of Animals Becoming Pregnant After Natural Mating, with Varying Doses of Chlormadinone and Megestrol Acetates Administered Subcutaneously for 4 Days During the Time of Implantation (days 2 through 5 after mating).

Treatment (μg)	No. of hamsters	% Preg.	Total corpora lutea	Total embryos	Implantation index
Cottonseed oil (control)	16	62.5	109	106	97.3
Chlormadinone acetate					
50	15	66.7	123	118	96.0
100	14	57.2	110	109	99.1
250	9	66.7	87	71	81.6
Megestrol acetate					
50	15	66.7	137	124	90.6
100	16	81.4	162	163	100.0
250	14	28.6	51	48	94.2

TABLE III. Percentage of Animals Mating and Becoming Pregnant, with Varying Doses of Chlormadinone and Megestrol Acetates Administered Subcutaneously for One Estrous Cycle (4 days) Before Mating.

Treatment (μg)	No. of hamsters	No. with sperm	No. pregnant	% Mating	% of those mating that are pregnant
Cottonseed oil	11	9	9	81.7	100.0
Chlormadinone acetate					
50	10	9	6	90.0	66.7
100	17	8	4	47.1	50.0
250	13	11	6	84.6	54.5
Megestrol acetate					
50	16	10	7	62.5	70.0
100	8	6	5	75.0	83.2
250	5	4	1	80.0	25.0

Since chlormadinone acetate has been implicated as interfering with the mating behavior of rats (G. Bialy, personal communication), a third series of hamsters received the test compounds and vaginal smears were examined on the day after mating for the presence of sperm. The results (Table III) showed no consistent effect of the compounds on the percentage of animals mating. Hamsters receiving the highest levels of chlormadinone and megestrol acetates had 84.6 and 80.0% mating, respectively, compared to 81.7% in control animals.

Comparison of the effect of varying doses of the compounds on only those animals that mated shows that 100% of the controls became pregnant, while treated hamsters had fertility levels from 17 to 75 percentage units lower. These values represent the effect of the compounds on the events occurring during capacitation and fertilization and differentiate the effects from implantation and mating behavior.

When rabbit sperm were incubated in the hamster uterus and inseminated into test rabbits 2 hr after ovulation, 53% of the ova were fertilized (Table IV). Inseminaton 4 hr after ovulation resulted in very low fertility (4%).

TABLE IV. First Stage Capacitation of Rabbit Sperm in the Uterus of the Hamster.

Time of insemination (hr postov.)	No. of oviducts	Total ova	No. fertilized	% Fertilized
2	11	34	18	53.0
4	13	54	2	3.7

Discussion. The data reported here demonstrate the usefulness of the hamster in the screening of pharmacologic agents as antifertility compounds. The two compounds tested, megestrol acetate and chlormadinone acetate, exerted their antifertility effect during the time of capacitation-fertilization. At the levels tested, no effect of either compound was noted on ovulation or implantation in the hamster.

Mating behavior, as measured by the presence or absence of sperm in the vaginal smear on day 2, was not affected by the compounds, unlike the effect observed in the rat with chlormadinone acetate.

The high level of fertility noted with hamster-incubated rabbit sperm inseminated into rabbit oviducts 2 hr after ovulation with an accompanying low fertility level with 4-hr-old ova indicates that only the first stage of capacitation had been accomplished in the hamster uterus. Chang (9) reported a fertilization level of 88% with 12-hr uterine-incubated sperm and 4-hr-old ova. These data confirm the species-specificity requirement for the second stage of capacitation as stated by Bedford, and at the same time, demonstrate that the hamster, as well as the rat [Bedford (3)], and dog [Hammer and Sojka (10)] can be used to accomplish the first stage of rabbit sperm capacitation.

Summary. A differential assay is described for testing compounds with hamsters which

allows one to separate anti-implantation and antimating behavior effects from influences exerted at the time of capacitation and fertilization. Such tests with chlormadinone and megestrol acetate emphasize the effects of these compounds on the later phenomenon. Demonstration that the first phase of rabbit sperm capacitation occurs readily in 12 hr in the hamster uterus indicates the usefulness of this animal for cross-species studies of capacitation.

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