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Assessment of a tablet drug delivery system incorporating nonoxynol-9 coprecipitated with polyvinylpyrrolidone in preventing the onset of pregnancy in rabbits

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Objective: To assess the in vivo efficacy of the tablet drug delivery system containing nonoxynol-9 coprecipitated with polyvinylpyrrolidone by delivering the spermicidal agents vaginally and evaluating their ability to prevent the onset of pregnancy in rabbits.

Design: Controlled clinical study.

Setting: Division of Laboratory and Animal Resources, College of Pharmacy, University of Kentucky.

Animal(s): Forty-two New Zealand White female rabbits.

Intervention(s): The rabbits were artificially inseminated at various intervals after vaginal insertion of the tablet drug delivery system containing either polyvinylpyrrolidone only (0 minutes) or nonoxynol-9 coprecipitated with polyvinylpyrrolidone (polyvinylpyrrolidone/nonoxynol-9; 0, 3, 30, 180, and 360 minutes). The rabbits were induced to ovulate 6 hours before insemination by IM injection of hCG (200 IU).

Main Outcome Measure(s): The onset of pregnancy in the rabbits was evaluated after insertion of the tablet drug delivery system containing polyvinylpyrrolidone only or polyvinylpyrrolidone/nonoxynol-9 at various intervals, followed by artificial insemination.

Result(s): The onset of pregnancy was not reduced significantly when the tablet drug delivery system containing polyvinylpyrrolidone or polyvinylpyrrolidone/nonoxynol-9 was used and insemination was performed immediately after tablet insertion (time 0). However, pregnancy rates (PRs) were reduced significantly in the rabbits that received the tablet drug delivery system containing polyvinylpyrrolidone/nonoxynol-9 and were inseminated at 3, 30, 180, and 360 minutes after tablet insertion. The highest PR reduction occurred between 30 and 180 minutes after insertion of the tablet drug delivery system containing polyvinylpyrrolidone/nonoxynol-9.

Conclusion(s): The tablet drug delivery system is an efficient method of delivering the tested spermicidal agents vaginally. The design and dosage used in preparing the tablet drug delivery system provide short- and long-term release of the spermicidal agents, which results in almost immediate and extended enhancement of their contraceptive properties. (Fertil Steril® 1998;69:768–73. ©1998 by American Society for Reproductive Medicine.)

Key Words: Tablet, drug delivery, nonoxynol-9, polyvinylpyrrolidone, vaginal contraceptives

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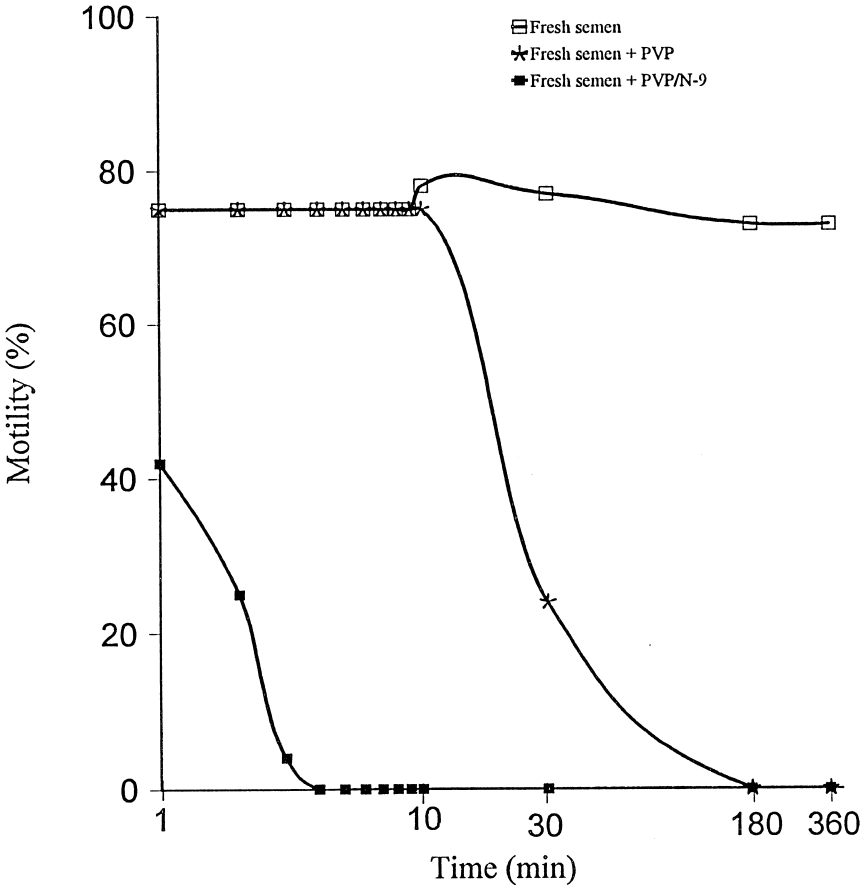
Nonoxynol-9 (nonylphenol [polyethoxy] ethanol) is a nonionic surfactant used as the active ingredient in most of the commercially available spermicides. The action of nonoxynol-9 on sperm was evaluated previously with the use of electron microscopy techniques (1, 2). These studies showed that nonoxynol-9 lysed sperm membrane regions, which resulted in complete immobilization or death of the sperm. Polyvinylpyrrolidone is one of the most commonly used polymers in medicine because of its safety for human use and its hydrophilic properties (3). The ability of polyvinylpyrrolidone to function as a carrier of compounds such as nonoxynol-9 is attributed to its membrane-seeking and membrane-coating properties (4, 5).

We previously showed that spermicidal formulations of nonoxynol-9 coprecipitated with polyvinylpyrrolidone, which yields polyvi-

FIGURE 1

Percentage of motile sperm in rabbit seminal specimens exposed to the tablet drug delivery systems containing polyvinylpyrrolidone or polyvinylpyrrolidone/nonoxynol-9 during a 6-hour incubation period (logarithmic scale). PVP = polyvinylpyrrolidone; N-9 = nonoxynol-9.



nylpyrrolidone/nonoxynol-9, could act as potent spermicides as assessed by in vitro studies using human and rabbit sperm (6–8). We also demonstrated that the coprecipitation of nonoxynol-9 and polyvinylpyrrolidone is a necessary step to alter the chemical state (liquid–powder) of nonoxynol-9 (6–8). The in vitro spermicidal abilities of various formulations containing polyvinylpyrrolidone/nonoxynol-9 were tested, and minimum lethal doses were calculated using the parameters established by the Sander-Cramer and cervical mucus penetration tests (7, 8). The results obtained in those studies clearly delineated the efficacy of the various formulations under consideration.

Studies also were performed to assess the delivery and efficacy of the developed spermicidal agents in vivo (9). The spermicidal agents were prepared in the form of a capsule and a tablet for the purpose of delivering them vaginally (9). The capsule and tablet drug delivery systems were designed with two compartments, an inner core and an outer core, to provide spermicidal release over short and long periods (9). The outer core released its contents first (short-term release), whereas the inner core released its contents after dissolution of the outer core (long-term release). The tablet proved to be a more efficient delivery system than the capsule in dissolving and releasing its contents and in preventing the onset of pregnancy in rabbits at various intervals after tablet insertion and artificial insemination (9).

The objective of this study was to improve further the efficacy of the tablet drug delivery system as a vaginal contraceptive in rabbits and for possible future use in humans.

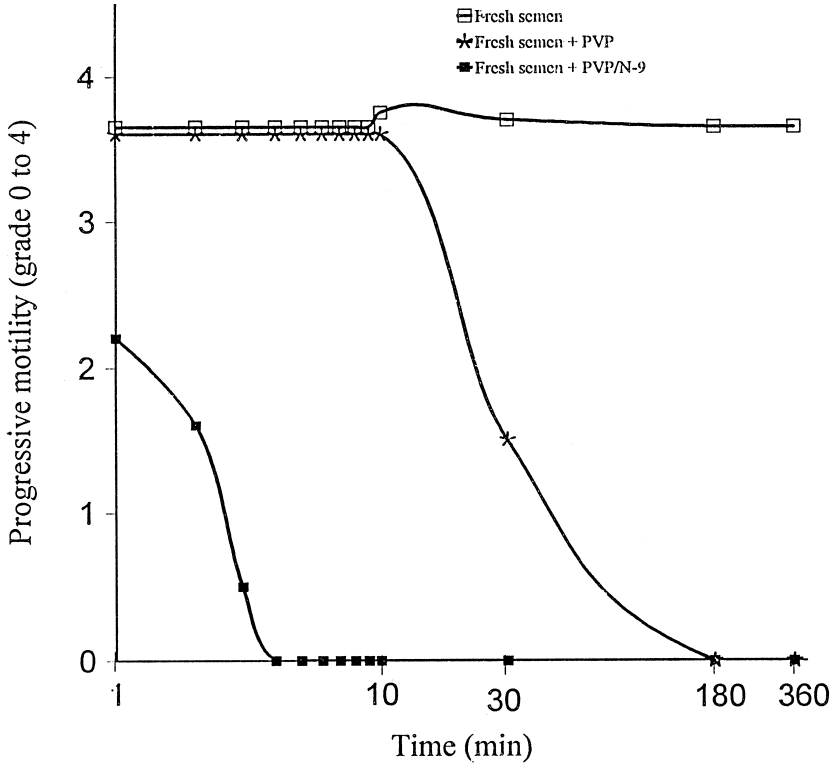
MATERIALS AND METHODS

Animal Care and Handling

Fifty New Zealand White rabbits (42 females and 8 males) were used in this study. The rabbits were housed in individual metal cages and fed high-fiber rabbit formula (6–8 oz/d, ProLab Animal Diet; Agway, Inc., Syracuse,

FIGURE 2

Progressive motility (grade 0–4) in rabbit seminal specimens exposed to the tablet drug delivery system containing polyvinylpyrrolidone or polyvinylpyrrolidone/nonoxynol-9 during a 6-hour incubation period (logarithmic scale). PVP = polyvinylpyrrolidone; N-9 = nonoxynol-9.



NY). A constant room temperature of 21°C was maintained, and the rabbits were exposed to a lighting regimen of 14 hours of light and 10 hours of dark. The rabbits were monitored for general health and well-being according to the guidelines approved by the University of Kentucky Institutional Animal Care and Use Committee.

Semen Collection

Artificial vaginas for the collection of rabbit semen were constructed and used as previously described (10–12). A doe (female rabbit) was introduced to each buck (male rabbit) at the time of semen collection. Each buck was teased 3–4 times without allowing it to copulate with the doe (false mount). The prewarmed (45°C) artificial vagina was placed close to the male's penis, and penile insertion (thrust) into the artificial vagina and ejaculation occurred.

The test tube containing the semen was maintained at 37°C before in vitro testing was performed. Semen specimens were assessed for volume (milliliters), concentration (1×10^6 sperm per milliliter), and motility characteristics. Semen specimens were pooled and split into three (1-mL) aliquots. Aliquot 1 was used to assess sperm viability during the incubation period, and the specimen was not exposed to the tablet drug delivery system. Aliquots 2 and 3 were used to test the tablet drug delivery systems containing polyvinylpyrrolidone or polyvinylpyrrolidone/nonoxynol-9.

Assessment of Spermicidal Activity In Vitro

Spermicidal formulations were prepared as tablets in a manner similar to a previously described technique (13). The tablet drug delivery system consisted of an inner core and an outer core (coating) containing polyvinylpyrrolidone or polyvinylpyrrolidone/nonoxynol-9. The outer core was applied as a coating layer over the inner core. The outer core contained a lesser amount of polyvinylpyrrolidone only or polyvinylpyrrolidone/nonoxynol-9 than did the inner core. The outer core was designed to release its contents first (short-term release), whereas the inner core released its contents after dissolution of the outer core (long-term release).

The nonoxynol-9 content in the tablet drug delivery system was calculated using the results of previous studies in which the minimum dose necessary to kill sperm in vitro (within 20 seconds of exposure) and the characteristics of spermicide release were assessed (6–8). The percentage of polyvinylpyrrolidone and nonoxynol-9 in the tablet drug

delivery system was calculated using the weight of the inner and outer cores (coating). The tablet drug delivery system consisted of 10–11 mg of nonoxynol-9.

Two tablet drug delivery systems containing either polyvinylpyrrolidone or polyvinylpyrrolidone/nonoxynol-9 formulations were placed in vials containing 1 mL of semen (aliquots 2 and 3). The vials containing the semen and the spermicidal formulations were incubated at 37°C, and samples were taken at various intervals and analyzed for percentage and grade of motility. Measurements were taken at 20 seconds, at 1-minute intervals up to 15 minutes, at 5-minute intervals up to 30 minutes, or at hourly intervals up to 6 hours of incubation. Specimens were assessed until complete sperm killing was established. Killing of sperm was confirmed by supravital staining (eosin-nigrosin) (7, 8).

Vaginal Delivery of Spermicidal Formulations

Forty-two does were allocated into seven groups (6 does per group) according to the insemination protocol used. Does in groups 1 and 2 received one- and two-tablet drug delivery systems containing polyvinylpyrrolidone, respectively. Does in groups 3–7 received a two-tablet drug delivery system containing polyvinylpyrrolidone/nonoxynol-9 (4 mg/kg). At each insemination, 1 doe in each group received the corresponding number of tablets and was inseminated at distinct intervals after tablet insertion.

Does that received the tablet drug delivery system containing polyvinylpyrrolidone were inseminated immediately after vaginal insertion of the tablet. Does that received the tablet drug delivery system containing polyvinylpyrrolidone/nonoxynol-9 were inseminated at 0, 3, 30, 180, and 360 minutes after vaginal insertion of the tablet. The does were held in a supine position, and the tablet drug delivery systems were placed vaginally (10–12). A properly lubricated and sterilized Pasteur pipette (blunted end) was used to introduce and place the drug delivery system into the vagina at a consistent depth (10–12 cm).

Artificial inseminations were performed with the use of the same technique used for the vaginal insertion of the tablet drug delivery system. Semen specimens were collected and pooled as previously described. The insemination dose consisted of 0.5 mL of pooled semen containing $70-80 \times 10^6$ sperm with 70%–80% motility. The insemination dose was aspirated into the insemination rod, which then was introduced through the vulva into the vagina and guided to a depth of 8–10 cm where the semen was deposited vaginally (10–12).

Does that were scheduled to be inseminated were induced to ovulate 6 hours in advance of the expected time of insemination. Ovulation was induced by IM injection of hCG (200 IU) (10–12). Pregnancy rates (PRs) were calculated as the proportion of does that became pregnant and delivered newborn rabbits. Newborn rabbits were maintained through lactation to weaning and monitored accordingly for general health and survival. The experiment was replicated six times.

RESULTS

Fresh specimens (aliquot 1) remained viable during the incubation period and in vitro assessment of the tablet drug delivery system. Sperm specimens exposed to the tablet drug delivery system containing polyvinylpyrrolidone/nonoxynol-9 showed a ''shivering'' pattern of motility after 1 minute of exposure (7–9). Sperm exposed to the tablet drug delivery system containing polyvinylpyrrolidone/nonoxynol-9 were killed within 3–4 minutes of exposure (Figs. 1 and 2). Sperm exposed to a similar tablet drug delivery system containing polyvinylpyrrolidone only were killed by 3 hours of exposure (Figs. 1 and 2).

The results obtained from the vaginal delivery (in vivo study) of spermicidal formulations in the form of tablet drug delivery systems are summarized in Table 1. These results showed that the onset of pregnancy was not reduced significantly ($P > 0.05$) when the tablet drug delivery system containing polyvinylpyrrolidone or polyvinylpyrrolidone/nonoxynol-9 was used and insemination was performed immediately after tablet insertion (time 0) compared with when the tablet drug delivery system containing polyvinylpyrrolidone/nonoxynol-9 was used and insemination was performed at 3 to 360 minutes after insertion.

The PR was 100% in rabbits that received one tablet drug delivery system containing polyvinylpyrrolidone only and were inseminated immediately after its insertion (time 0). Pregnancy rates were reduced significantly in rabbits that received the tablet drug delivery system containing polyvinylpyrrolidone/nonoxynol-9 and were inseminated at 3, 30, 180, and 360 minutes after tablet insertion. Pregnancy rates were reduced the most between 30 and 180 minutes after tablet insertion.

DISCUSSION

Approaches to vaginal contraception have included the delivery of spermicidal materials (i.e., nonoxynol-9, Triton X-100) and antifertility agents (i.e., sperm acrosin inhibitors) using drug delivery systems such as creams, suppositories, foams, and gels (14–19). The vaginal spermicidal potency of these drug delivery systems is dependent on the concentration of the active ingredient present in each preparation (14, 16, 17). In addition, the drug delivery system base could affect the spermicidal activity of the active ingredient (i.e., spermicide release, vaginal environment interaction) (16).

Alexander et al. (16) suggested that a sequence of tests should be performed before clinical testing in humans, including in vitro tests (i.e., Sander-Cramer and cervical mucus penetration tests), followed by breeding experi-

TABLE 1

Spermicidal efficacy and pregnancy outcome in artificially inseminated rabbits (n = 42) at various intervals after vaginal insertion of the tablet drug delivery system.

Mating group	Drug delivery system	Number of tablet drug delivery systems used*	Timing of artificial insemination† (min)	PR
1	Tablet, polyvinylpyrrolidone‡	1	0	100‖
2	Tablet, polyvinylpyrrolidone‡	2	0	67¶
3	Tablet, polyvinylpyrrolidone/nonoxynol-9§	2	0	83‖¶
4	Tablet, polyvinylpyrrolidone/nonoxynol-9§	2	3	33**
5	Tablet, polyvinylpyrrolidone/nonoxynol-9§	2	30	17#
6	Tablet, polyvinylpyrrolidone/nonoxynol-9§	2	180	17#
7	Tablet, polyvinylpyrrolidone/nonoxynol-9§	2	360	50††

* Tablet drug delivery systems consisted of an inner core and an outer core containing polyvinylpyrrolidone or polyvinylpyrrolidone/nonoxynol-9.

† Timing of artificial insemination after vaginal insertion of the tablet drug delivery system.

‡ Does received tablet drug delivery systems containing polyvinylpyrrolidone.

§ Does received tablet drug delivery systems containing polyvinylpyrrolidone/nonoxynol-9.

‖,¶,**,#,†† Pregnancy rate values with different symbols are significantly different ($P < 0.05$).

ments (in vivo testing) using one or more animal models. Animal models used in the in vivo testing of drug delivery systems and spermicidal agents include rabbits and nonhuman primates (14–18). In previous studies (6–8), spermicidal formulations consisting of polyvinylpyrrolidone/nonoxynol-9, in the form of a thin layer, were tested in vitro by the performance of the Sander-Cramer and cervical mucus penetration tests using rabbit and human sperm to determine the efficacy of the formulations to be developed further and tested as vaginal contraceptives.

In this study, previously developed spermicidal formulations consisting of polyvinylpyrrolidone/nonoxynol-9 were prepared for vaginal delivery using a tablet drug delivery system (6–8). The tablet drug delivery system contained a total of approximately 10–11 mg of polyvinylpyrrolidone/nonoxynol-9. The tablet drug delivery system was designed in this manner for continuous release of the spermicidal formulations after insertion of the tablet drug delivery system and semen deposition at the time of artificial insemination. It was believed that this approach would provide enhanced contraceptive efficacy of those formulations because of [1] the continuous availability of nonoxynol-9 over short (outer core release) and long (inner core release) periods and [2] the possible role of polyvinylpyrrolidone in providing vaginal and cervical surface-coating properties (4, 5).

Use of the tablet drug delivery system containing polyvinylpyrrolidone/nonoxynol-9 significantly reduced the PR but did not eliminate completely the onset of pregnancy in rabbits that were inseminated at 3, 30, 180, and 360 minutes after tablet insertion. Pregnancy rates were reduced the most between 30 and 180 minutes after tablet drug delivery system insertion and artificial insemination.

The results suggest that the tablet drug delivery system could be considered an efficient method of contraception and of vaginal delivery of spermicidal agents with short- and long-term protective activity. Future studies, however, will be performed to delineate further the short- and long-term contraceptive qualities of the tested formulations and to obtain a better understanding of the slight increase in PRs that occurred when 360 minutes elapsed between insertion of the tablet drug delivery system and performance of artificial insemination.

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