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Intravaginal Controlled Administration of Flurogestone Acetate: (IV) In Vitro - In Vivo Correlation for Intravaginal Drug Delivery from Rate-Control Vaginal Pessary+

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ABSTRACT

One hundred fifty sheep received various types of Rate-Control vaginal pessaries for a period of up to 19 days at various geographic locations. As predicted from the *in vitro* studies, a constant (Q - t) absorption profile was also observed in *in vivo*. The effect of the loading dose of flurogestone acetate on the *in vitro* and *in vivo* absorption profiles were examined and minimum effective loading dose was determined. An excellent *in vitro* - *in vivo* correlation was established, which permitted

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the prediction of the long-term (19-day) in vivo absorption profiles from a short-term (3-day) in vitro absorption study.

INTRODUCTION

For many centuries, various pastes, jellies and mixtures have been applied into the vagina to prevent conception (1). However in 1918, a literature review suggested that there were many cases of deaths as a result of vaginal administration of toxic substances, including arsenic, mercury, and others used for local effect (2). The first evidence of vaginal absorption was also reported by Macht from the studies conducted in animals (2). Later, the possibility of using vaginal cavity as a site of drug administration for systemic effect was then investigated (3). The effectiveness of vaginal absorption was illustrated, just over two decades ago, by the intravaginal administration of progesterone via a suppository formulation (4). A literature review in 1983 indicated that a wide variety of both organic and inorganic compounds, including most steroids, prostaglandins, penicillins, sulfa drugs, proteins, nonoxynol-9, and methadone are well absorbed from the vaginal mucosa (1).

The advantages of using vagina as the route of administration for contraceptive steroids include: Ease for the insertion and removal of medical device; continuous "infusion" of an effective dose without the peak and valley effects of conventional dosage forms; avoidance of the hepatic "first-pass metabolism", since

the blood from the vagina bypasses the liver (5,6). The surface of the vaginal wall is well suited for absorption, as it is composed of numerous flat longitudinal folds which extend throughout the length of vagina (7).

Flurogestone acetate (FGA) is an ideal candidate for intravaginal controlled drug delivery, since it is potent, lipophillic compound with short duration of activity and good vaginal absorptivity (8). In light of these advantages, Robinson developed and patented a long-acting FGA-releasing pessary to replace the daily FGA injection for estrus synchronization in the sheep (9). The goal of estrus synchronization is to establish a stage at which one can induce fertile estrus as desired and can also make the female animal fertilizable in a desired season (i.e., control breeding) (10). The sheep regain their estrus and ovulate 2-4 days after cessation of the 15-day treatment with vaginal pessary. At this time artificial insemination can be carried out at a pre-determined schedule, which makes the female animal more fertilizable and also reduces the difficulty and time-consuming labor of heat detection by the farmers (11). The FGA-releasing vaginal pessary was then marketed as Syncro-Mate vaginal pessary (G. D. Searle & Co.) and Chronogest vaginal pessary (Intervet S.A.)

Recently, an *in vitro* intravaginal release and permeation IRP system, which was capable of determining simultaneously the rate of FGA release and vaginal absorption from the vaginal

pessary, was developed in this laboratory (12). Using this IRP system, the *in vitro* release and vaginal absorption profiles of FGA from various vaginal pessaries were measured and the mechanisms and rate profiles were characterized (13). Results indicated that the release and vaginal absorption profiles of FGA from the Syncro-Mate vaginal pessaries marketed follow the Q vs. $t^{1/2}$ relationship. Several Rate-Control vaginal devices were developed and their *in vitro* release and vaginal absorption profiles were also evaluated and observed to follow a zero-order (Q vs. t) kinetics.

In this investigation, the *in vivo* studies were conducted to study the intravaginal release and absorption profiles of FGA from various Rate-Control vaginal pessaries, using the marketed Syncro-Mate vaginal pessaries as the reference for comparison, in the sheep to determine their clinical efficacy and to establish the *in vitro* - *in vivo* relationship. A simple mathematical model was also derived to relate the *in vivo* rates of release and absorption with the *in vitro* data.

THEORETICAL ANALYSIS

Physical Model: - A physical model is developed to analyze the intravaginal release and absorption of flurogestone acetate (FGA): In this model, a Rate-Control vaginal pessary, which is a polyurethane sponge coated with a laminate of FGA-dispersing silicone device containing a rate-controlling membrane, is inserted into the vaginal cavity (Figure 1). The Rate-Control vaginal

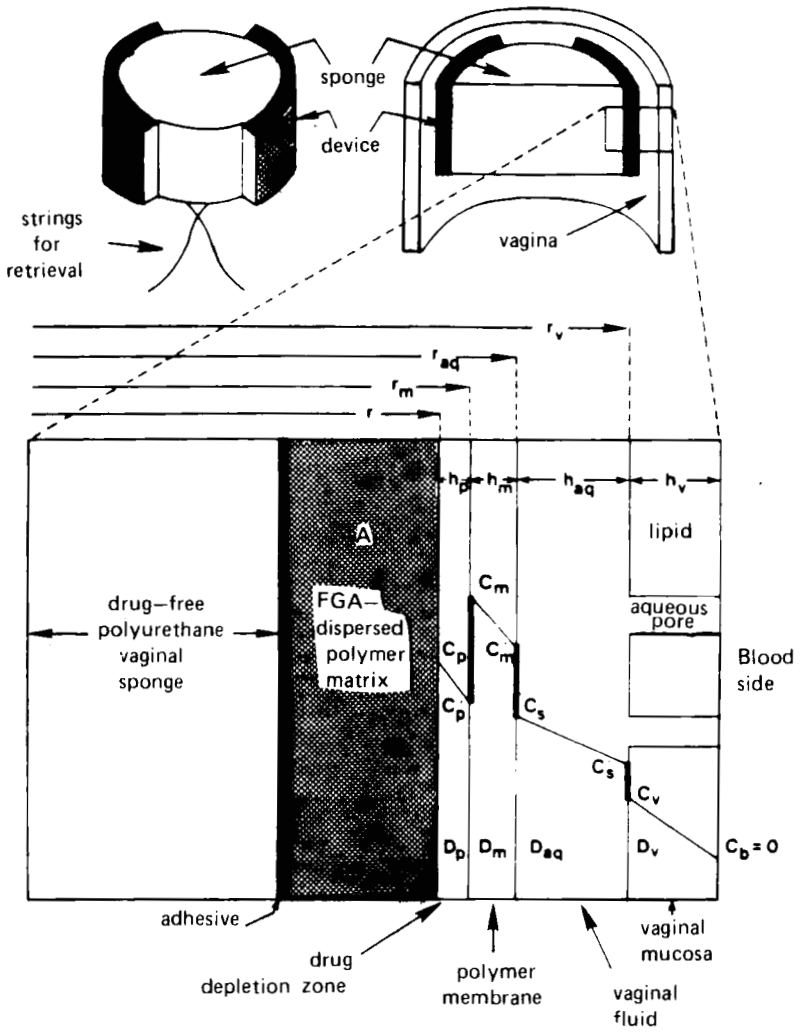

Figure 1.

A physical model for the intravaginal absorption of flurogestone acetate from the in situ Rate-Control vaginal pessary (Type II). See text for the definition of various parameters.

pessary consists of a receding interface of drug dispersion zone/drug depletion zone in the device as FGA is released through the rate-limiting polymeric membrane. An aqueous diffusion layer exists between the vaginal pessary and the vaginal epithelium. The vaginal mucosa, which is composed of lipid continuum with interdispersed pores or aqueous shunt pathways, is surrounding around the vaginal pessary.

The absorption of FGA from this vaginal pessary is dependent upon a series of steps in sequence: (1) The dissolution of finely-divided, well-dispersed drug particles into the surrounding polymer matrix continuum, (2) diffusion of FGA molecules through the polymer matrix to the matrix/membrane interface, (3) partitioning of FGA molecules from the polymer matrix onto the rate-controlling membrane, (4) release of drug molecules from the rate-controlling membrane into the aqueous diffusion layer, (5) diffusion of drug molecules across the vaginal mucosa and, finally, (6) transport and distribution of drug molecules by circulating blood and/or lymph to a target tissue.

The following assumptions were made in this physical model:

1. The drug in the matrix is finely divided and uniformly dispersed such that matrix dissolution is not a rate limiting factor.

2. A sharp boundary is maintained between the drug dispersion region and drug depletion region within the device, which recedes into the core of the device as time passes.

3. The FGA has a finite solubility in the matrix, C_p, and that the loading dose per unit volume, A, including the dissolved and undissolved drug, is much greater than C_p.

4. The FGA molecules reach the matrix surface by diffusion through the matrix continuum rather than the pores; and the end diffusion is negligible.

5. In view of the thick coat of silicone adhesive applied to glue the device onto the sponge, the inward diffusion of FGA molecules into the drug-free sponge is negligible.

Under these assumptions, an activity gradient for FGA will be established beginning at the receding interface within the drug-dispersed polymer matrix and essentially terminating at the outer reach of the vaginal microcirculation, where the FGA molecules are picked up and transported to the target tissues. This gradient is depicted in Figure 1, as a series of discontinuous concentration gradients.

The flux across a unit area of the drug depletion zone (J_p):

$$J_p = \frac{D_p}{h_p} (C_p - C_p') \qquad \text{[Eq. 1]}$$

Where, D_p is the drug diffusivity in the drug depletion zone with a thickness of h_p; C_p and C_p' are the drug solubility in the polymer matrix and the drug concentration on the matrix side of the polymer matrix/rate-controlling membrane interface, respectively.

The flux across a unit area of the rate-limiting membrane (J_m):

$$J_m = \frac{D_m}{h_m} (C_m - C_m') \qquad \text{[Eq. 2]}$$

Where, D_m is the drug diffusivity in the rate-limiting membrane with a thickness of h_m; C_m and C_m' are the drug concentrations at the membrane/matrix interface and membrane/solution interface, respectively.

The flux across a unit area of the aqueous diffusion layer (J_{aq}):

$$J_{aq} = \frac{D_{aq}}{h_{aq}} (C_s - C_s') \qquad \text{[Eq. 3]}$$

Where, D_{aq} is the drug diffusivity in the aqueous diffusion layer (with a thickness of h_{aq}) between the rate-limiting membrane and the vaginal epithelium; C_s and C_s' are the drug concentrations at the rate-limiting membrane/aqueous solution layer interface and at the aqueous solution layer/vaginal epithelium interface, respectively.

The flux across a unit area of the vaginal mucosa (J_v):

$$J_v = \frac{D_v}{h_v} (C_v - C_b) = \frac{D_v}{h_v} C_v, \text{ (since } C_b \simeq 0) \qquad \text{[Eq. 4]}$$

Where, D_v is the drug diffusivity in the vaginal mucosa with a thickness of h_v; C_v is the drug concentration at the vaginal mucosa/aqueous solution layer interface.

The partition coefficient for the interfacial partitioning between the polymer matrix and the rate-limiting membrane (K_m):

$$K_m = \frac{C_m}{C_p'} = \frac{S_m}{C_p} \qquad \text{[Eq. 5]}$$

Where, S_m is the solubility of drug in the rate-limiting membrane.

The partition coefficient for the interfacial partitioning between the rate-limiting membrane and the vaginal fluid (K_s):

$$K_s = \frac{C_s}{C'_m} = \frac{S_{aq}}{S_m} \qquad \text{[Eq. 6]}$$

Where, S_{aq} is the solubility of drug in the vaginal fluid.

The partition coefficient for the interfacial partitioning between the vaginal fluid and the lipoidal biophase in the vaginal mucosa (K_v):

$$K_v = \frac{C_v}{C'_s} = \frac{S_v}{S_{aq}} \qquad \text{[Eq. 7]}$$

where S_v is the drug solubility in the vaginal mucosa.

Since the fluxes across the series of barriers will reach a quasi-steady state.

So, $$J_p = J_m = J_{aq} = J_v = J \qquad \text{[Eq. 8]}$$

Where J is the net flux of vaginal absorption from the vaginal pessary.

Incorporating Eq. (7) for C_v ($= K_v C'_s$) in Eq. (4):

$$J_v = \frac{D_v K_v}{h_v} C'_s = (P_v) C'_s \qquad \text{[Eq. 9a]}$$

and $$P_v = (P_1 + P_a) = \left[(f)\left(\frac{D_1 K_v}{h_v}\right) + (1 - f)\frac{D_a}{h_v}\right] \qquad \text{[Eq. 9b]}$$

where P_v, P_1 and P_a are the permeability coefficients of vaginal mucosa, the lipoidal biophase and aqueous pores in the vaginal

mucosa, respectively; f is the fraction of lipoidal biophase; D_l and D_a are the drug diffusivities in the lipoidal biophase and in the aqueous pores, respectively.

Rearranging Eq. (9a):

$$C_s' = \frac{J_v}{P_v} \qquad \text{[Eq. 9c]}$$

Inserting Eq. (9c) for C_s' in Eq. (3) yields:

$$J_{aq} = \frac{D_{aq}}{h_{aq}} \left[C_s - \frac{J_v}{P_v} \right] \qquad \text{[Eq. 10a]}$$

Expanding Eq. (Eq. 10a):

$$J_{aq} = \left[\frac{D_{aq} C_s}{h_{aq}} \right] - \left[\frac{D_{aq} J_v}{h_{aq} P_v} \right] \qquad \text{[Eq. 10b]}$$

or $$J_{aq} + \left[\frac{D_{aq} J_v}{h_{aq} P_v} \right] = \left[\frac{D_{aq} C_s}{h_{aq}} \right] \qquad \text{[Eq. 10c]}$$

Since $$J_{aq} = J_v = J \quad \text{(Eq. 8)}$$

so, $$J\left[1 + \frac{D_{aq}}{h_{aq} P_v} \right] = \frac{D_{aq} C_s}{h_{aq}} \qquad \text{[Eq. 10d]}$$

multiplying both sides of Eq. (10d) by (h_{aq}/D_{aq}) gives

$$C_s = J\left[\frac{h_{aq}}{D_{aq}} + \frac{1}{P_v} \right] \qquad \text{[Eq. 10e]}$$

Substituting Eq. (10e) for C_s in Eq. (6) and solving for C_m' $(=C_s/K_s)$ yield

$$C_m' = \frac{J}{K_s} \left[\frac{h_{aq}}{D_{aq}} + \frac{1}{P_v} \right] \qquad \text{[Eq. 11]}$$

Inserting Eq. (11) for C_m' in Eq. (2) gives

$$J_m = \left(\frac{D_m}{h_m}\right)\left[C_m - \frac{J}{K_s}\left(\frac{h_{aq}}{D_{aq}} + \frac{1}{P_v}\right)\right] \qquad \text{[Eq. 12a]}$$

Expanding Eq. (12a) yields

$$J_m = \left[\frac{D_m C_m}{h_m} - \frac{J D_m}{h_m K_s}\left(\frac{h_{aq}}{D_{aq}} + \frac{1}{P_v}\right)\right] \qquad \text{[Eq. 12b]}$$

or $$J_m + \frac{J D_m}{h_m K_s}\left(\frac{h_{aq}}{D_{aq}} + \frac{1}{P_v}\right) = \frac{D_m C_m}{h_m} \qquad \text{[Eq. 12c]}$$

Since $J_m = J$ (Eq. 8)

so, $$J\left[1 + \frac{D_m}{h_m K_s}\left(\frac{h_{aq}}{D_{aq}} + \frac{1}{P_v}\right)\right] = \frac{D_m C_m}{h_m} \qquad \text{[Eq. 12d]}$$

Multiplying both sides of Eq. (12d) by (h_m/D_m) gives

$$C_m = J\left[\frac{h_m}{D_m} + \frac{1}{K_s}\left(\frac{h_{aq}}{D_{aq}} + \frac{1}{P_v}\right)\right] \qquad \text{[Eq. 12e]}$$

Inserting Eq. (12e) for C_m in Eq. (5) and solving for C_p' $(= C_m/K_m)$ yield

$$C_p' = \frac{J}{K_m}\left[\frac{h_m}{D_m} + \frac{1}{K_s}\left(\frac{h_{aq}}{D_{aq}} + \frac{1}{P_v}\right)\right] \qquad \text{[Eq. 13]}$$

Substituting Eq. (13) for C_p' in Eq. (1) gives

$$J_p = \left(\frac{D_p}{h_p}\right)\left(C_p - \frac{J}{K_m}\left[\frac{h_m}{D_m} + \frac{1}{K_s}\left(\frac{h_{aq}}{D_{aq}} + \frac{1}{P_v}\right)\right]\right) \qquad \text{[Eq. 14a]}$$

Expanding Eq. (14a) produces

$$J_p = \left(\frac{D_p C_p}{h_p}\right) - \frac{J D_p}{K_m h_p}\left[\frac{h_m}{D_m} + \frac{1}{K_s}\left(\frac{h_{aq}}{D_{aq}} + \frac{1}{P_v}\right)\right] \qquad \text{[Eq. 14b]}$$

or $$J_p + \frac{J D_p}{K_m h_p}\left[\frac{h_m}{D_m} + \frac{1}{K_s}\left(\frac{h_{aq}}{D_{aq}} + \frac{1}{P_v}\right)\right] = \frac{D_p C_p}{h_p} \qquad \text{[Eq. 14c]}$$

Since $J_p = J$ (Eq. 8)

so, $$J\left(1 + \frac{D_p}{K_m h_p}\left[\frac{h_m}{D_m} + \frac{1}{K_s}\left(\frac{h_{aq}}{D_{aq}} + \frac{1}{P_v}\right)\right]\right) = \frac{D_p C_p}{h_p} \qquad \text{[Eq. 14d]}$$

Multiplying both sides of Eq. (14d) by h_p yields:

$$J\left(h_p + \frac{D_p}{K_m}\left[\frac{h_m}{D_m} + \frac{1}{K_s}\left(\frac{h_{aq}}{D_{aq}} + \frac{1}{P_v}\right)\right]\right) = D_p C_p \qquad \text{[Eq. 14e]}$$

or $$J = \frac{D_p C_p}{[h_p + Y_1]} \qquad \text{[Eq. 14f]}$$

where, $$Y_1 = \frac{D_p}{K_m}\left[\frac{h_m}{D_m} + \frac{1}{K_s}\left(\frac{h_{aq}}{D_{aq}} + \frac{1}{P_v}\right)\right] = \text{constant} \qquad \text{[Eq. 15]}$$

The rate of drug release (dQ/dt), which is equal to the rate of change in the thickness of the drug depletion layer (dh_p/dt) in the polymer matrix, is defined as:

$$J_p = \frac{dQ}{dt} = A\left(\frac{dh_p}{dt}\right); \text{ if } A \gg C_p \qquad \text{[Eq. 16]}$$

where, Q is the cumulative amount of drug released, t is the time and A is the initial drug loading in a unit volume of polymer matrix. The other terms have been previously defined in Eq. (1).

Letting Eq. (14f) equal to Eq. (16) and rearranging, Eq. (17a) is resulted:

$$dh_p(h_p + Y_1) = \left[\frac{D_p C_p}{A}\right] dt \qquad \text{[Eq. 17a]}$$

Expanding Eq. (17a) gives:

$$h_p dh_p + Y_1 dh_p = \left[\frac{D_p C_p}{A}\right] dt \qquad \text{[Eq. 17b]}$$

Integrating Eq. (17b) between $h_p = 0$ and $t = 0$ and $h_p = h_p$ at $t = t$, the change in the thickness of drug depletion zone with time is then given by:

$$\frac{h_p^2}{2} + Y_1 h_p = \left[\frac{D_p C_p}{A} \right] t \qquad \text{[Eq. 18]}$$

Integrating Eq. (16), the cumulative amount of drug released is:

$$Q = A h_p \qquad \text{[Eq. 19]}$$

Incorporating Eq. (19) for h_p in Eq. (18):

$$\frac{Q^2}{2A^2} + Y_1 \frac{Q}{A} = \left[\frac{D_p C_p t}{A} \right] \qquad \text{[Eq. 20]}$$

Multiplying both sides of Eq. (20) by "A" and rearranging give

$$\frac{1}{2A} Q^2 + Y_1 Q - (D_p C_p t) = 0 \qquad \text{[Eq. 21]}$$

Solving the above quadratic equation:

$$Q = \frac{-Y_1 + [Y_1^2 + 4(1/2A)\ (D_p C_p\ t)]^{1/2}}{2(1/2A)} \qquad \text{[Eq. 22]}$$

Simplifying Eq. (22):

$$Q = A \left[-Y_1 + \left(Y_1^2 + \frac{2 D_p C_p t}{A} \right)^{1/2} \right] \qquad \text{[Eq. 23]}$$

Differentiating Eq. (23) with time yields

$$\frac{dQ}{dt} = \frac{A}{2} \left[Y_1^2 + \frac{2 D_p C_p t}{A} \right]^{-1/2} \left[\frac{2 D_p C_p}{A} \right] \qquad \text{[Eq. 24a]}$$

Rearranging Eq. (24a) gives

$$\frac{dQ}{dt} = D_p C_p \left[Y_1^2 + \frac{2 D_p C_p t}{A} \right]^{-1/2} \qquad \text{[Eq. 24b]}$$

After plugging in the constant (Y_1) from Eq. (15), the *in vivo* rate of drug release from the Rate-Control vaginal pessary is defined by:

$$\frac{dQ}{dt} = (D_p C_p)\left[\left(\frac{D_p}{K_m}\left[\frac{h_m}{D_m} + \frac{1}{K_s}\left(\frac{h_{aq}}{D_{aq}} + \frac{1}{P_v}\right)\right]\right)^2 + \left(\frac{2C_p D_p}{A}\right)t\right]^{-1/2} \quad \text{[Eq. 24c]}$$

since $R = h/DK = 1/P$

and R, h, D, K, and P are defined as the diffusional resistance, thickness, diffusivity, partition coefficienct and permeability coefficient across each barrier, respectively.

So, Eq. (24c) becomes

$$\frac{dQ}{dt} = (C_p D_p)\left[\left(\frac{D_p}{K_m}\left[K_m R_m + \frac{1}{K_s}(K_s R_{aq} + R_v)\right]\right)^2 + \left(\frac{2C_p D_p}{A}\right)t\right]^{-1/2} \quad \text{[Eq. 24d]}$$

Rearranging Eq. (24d) gives

$$\frac{dQ}{dt} = (C_p D_p)\left[\left(D_p\left[R_m + \frac{1}{K_m}\left(R_{aq} + \frac{R_v}{K_s}\right)\right]\right)^2 + \left(\frac{2C_p D_p}{A}\right)t\right]^{-1/2} \quad \text{[Eq. 24e]}$$

Where, R_m, R_{aq} and R_v are the diffusional resistances of drug across the rate-limiting membrane, the aqueous solution layer (or vaginal fluid) and the vaginal mucosa, respectively.

Eq. (24e) can be rewritten as:

$$\frac{dQ}{dt} = \frac{X}{(Y^2 + Zt)^{1/2}} \quad \text{[Eq. 24f]}$$

in which, $X = C_p D_p$ [Eq. 25]

$$Y = D_p\left[R_m + \frac{1}{K_m}\left(R_{aq} + \frac{R_v}{K_s}\right)\right] \quad \text{[Eq. 26]}$$

$$Z = 2D_pC_p/A \qquad \text{[Eq. 27]}$$

where X, Y, and Z are constants.

For cases where the rate-limiting membrane, the aqueous diffusion layer and/or the vaginal mucosa are rate controlling, Eq. (24e) is reduced to:

$$\frac{dQ}{dt} = \left(C_p \right) \left[R_m + \frac{1}{K_m} \left(R_{aq} + \frac{R_v}{K_s} \right) \right]^{-1} \qquad \text{[Eq. 28]}$$

and for cases where the permeation of drug across the rate-limiting membrane is the primary rate-determining step, Eq. (28) is further simplified to:

$$\frac{dQ}{dt} = \frac{C_p}{R_m} = \text{constant} \qquad \text{[Eq. 29a]}$$

or,
$$\frac{dQ}{dt} = \frac{C_pD_mK_m}{h_m} \qquad \text{[Eq. 29b]}$$

since $K_m = S_m/C_p$ (Eq. 5)

$$\frac{dQ}{dt} = S_mD_m\left[\frac{1}{h_m} \right] \qquad \text{[Eq. 29c]}$$

A zero-order drug release profile is resulted. The rate of drug release is thus dependent upon the drug solubility (S_m) and drug diffusivity (D_m) in the rate-controlling polymeric membrane and its thickness (h_m).

In the case where the Rate-Control vaginal pessary contains no rate-limiting membrane around the external surface of the

drug-dispersed polymer matrix (Figure 2), the mathematical expression for the intravaginal rate of drug absorption can be derived by following the same mathematical derivations from Eq. 1 thru 24e:

$$\frac{dQ}{dt} = (D_p C_p) \left([D_p(R_{aq} + \frac{R_v}{K_s'})]^2 + [\frac{2C_p D_p}{A}]t\right)^{-1/2} \qquad \text{[Eq. 30]}$$

Where K_s' is the partition coefficient for the interfacial partitioning between the polymer matrix and the aqueous diffusion layer and is defined as:

$$K_s' = \frac{C_s}{C_p'} = \frac{S_{aq}}{C_p} \qquad \text{[Eq. 31]}$$

where, S_{aq} and C_p are the drug solubilities in the aqueous diffusion layer and polymer matrix, respectively. When C_p/A is very small, the rate is controlled by the diffusional resistance across the aqueous diffusion layer and/or vaginal mucosa (R_{aq} and R_v, respectively), so Eq. (30) is reduced to:

$$\frac{dQ}{dt} = [C_p] [R_{aq} + \frac{R_v}{K_s'}]^{-1} = \text{constant} \qquad \text{[Eq. 32]}$$

When C_p/A is very large or the residence time in the vagina has increased sufficiently, the matrix diffusion-controlled process becomes dominant and Eq. 30 is reduced to:

$$\frac{dQ}{dt} = D_p C_p [\frac{2D_p C_p t}{A}]^{-1/2} \qquad \text{[Eq. 33]}$$

Integration of Eq. (33) gives:

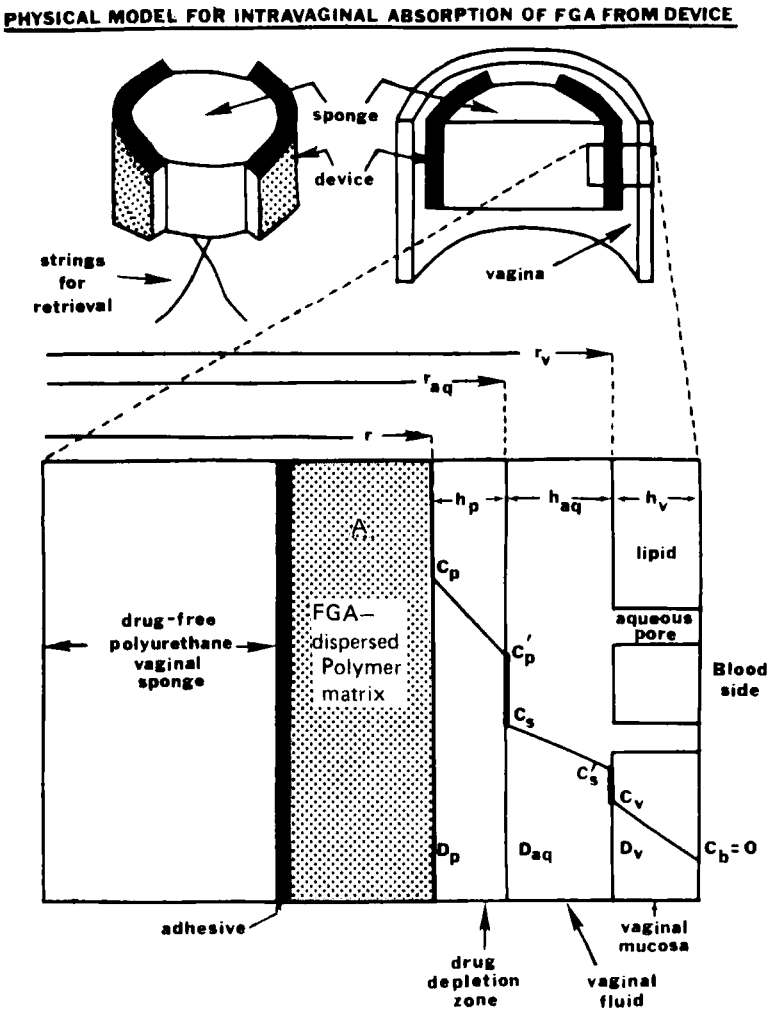

Figure 2.

A physical model for the intravaginal absorption of fluogestone acetate from in situ Rate-Control vaginal pessary (Type I). See text for the definition of various parameters.

$$\frac{Q}{t^{1/2}} = [2AD_pC_p]^{1/2} \qquad \text{[Eq. 34]}$$

A matrix-type (Q vs. $t^{1/2}$) vaginal absorption profile is resulted.

EXPERIMENTAL

A. Materials:

1. Chemicals and reagents: Flurogestone acetate (FGA)[1], anhydrous sodium phosphate[2], anhydrous citric acid[2], polyethylene glycol (PEG) 400[2], glass-distilled methanol[3], acetone[2], and absolute ethanol[4] were used as received. HPLC-grade water[5] was freshly prepared and used throughout the studies.

2. Silicone polymers: Silastic 382 (medical grade) elastomer[6], silicone fluid 360 (medical grade)[6], catalyst M (stannous octanoate)[6], silastic adhesive (silicone Type A, medical grade)[6], Silastic sheeting (medical grade, non-reinforced)[6], were used to prepare the Rate-Control vaginal pessaries.

3. Non-medicated vaginal sponges: Cylinder-shaped polyurethane sponges, hard grade 800 [gray (#375), 30mm (h) X 42 mm (d)][7] or soft grade 300 [white (#373), 30mm (h) X 40 mm(d)][7] were used to prepare Syncro-Mate or Rate-Control vaginal pessaries.

4. Animal model: Sheep was used in the in vivo experiments and the sheep vagina[8] freshly removed from the sheep was used in the in vitro studies.

B. Preparations:

1. Preparation of vaginal pessaries:

1) Preparation of Syncro-Mate vaginal pessary: The polyurethane virgin sponges (800 grade) were each impregnated, using a syringe and hypodermic needle, with 2 ml of FGA solution (10 mg/ml) in a combination of acetone and ethanol (1:4) at the center of the sponges (13). The sponges were then suspended on a horizontal bar and allowed to dry overnight (Figure 3E)

2) Preparation of Rate-Control FGA-releasing Vaginal pessaries (Type II): The medicated silicone sheet (containing 1% FGA and a thickness of 1mm) laminated with 2 x 0.125 mm rate-limiting membrane was fabricated by the same method as described earlier (13) and cut into rectangular strips (3 cm X 13 cm X 0.1 cm) with a scalpel. Silicone adhesive was then applied to one side of the strip and also the entire circumference surface of the non-medicated vaginal sponge (800 grade). The strip was glued onto the sponge and held in place by an aluminum foil and cardboard holder (both 3 cm X 13 cm). The pessaries were then cured in a humidity chamber (with a 100% R.H.) for 24 hours and dried at 45°C for another 12 hrs. to remove any residual acetic acid released during the adhesive curing process. After proper crosslinking, the cardboard holder and aluminum foil were removed and

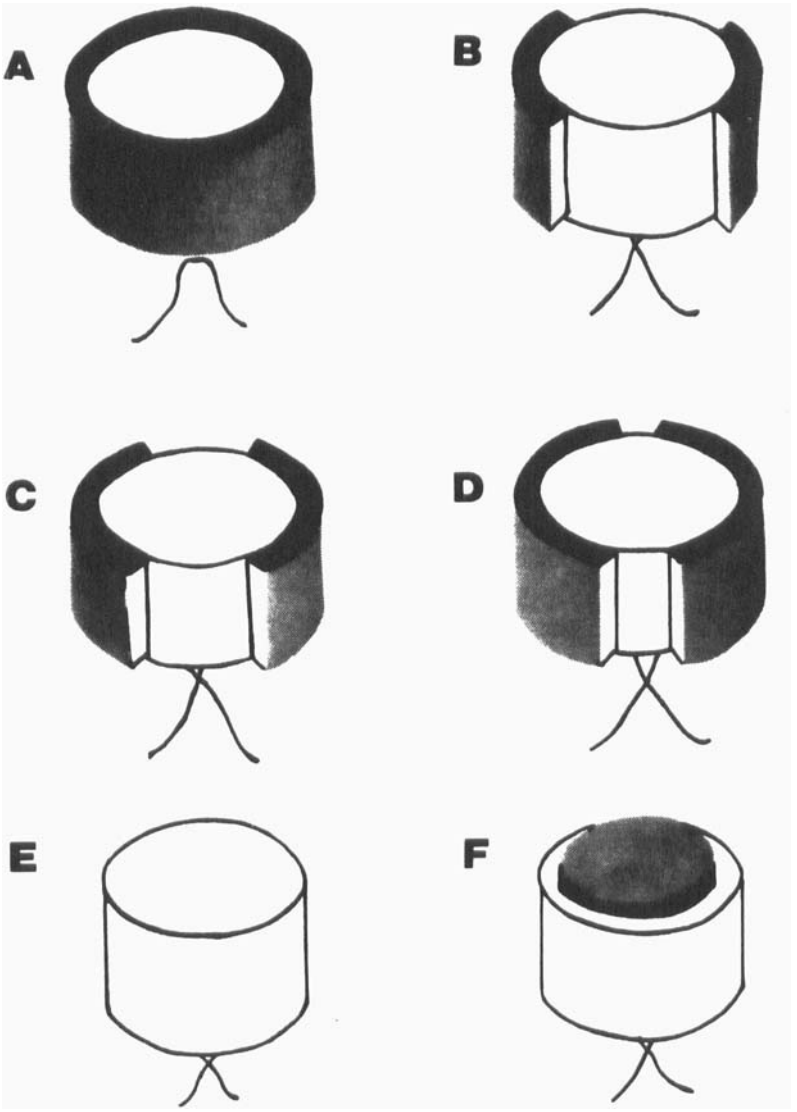

Figure 3.

Various types of vaginal pessaries used in the *in vitro* and the *in vivo* studies: (A) Rate-Control vaginal pessary (Type II): It consists of 39 cm^2 FGA-releasing device surrounding the circumference surface of a non-medicated hard grade sponge; (B) Rate-control vaginal pessary (Type I): It consists of 12-cm^2 FGA-releasing device surrounding the circumference surface of a soft grade sponge; (C) same as (B), except that it contained 18-cm^2 FGA-releasing device; (D) same as (B), except that is contained 24-cm^2 FGA-releasing device; (E) Syncro-Mate pessary prepared by impregnating FGA into the hard grade sponge; (F) same as (B), except that it contained 10-cm^2 FGA-releasing device on the top surface of a hard grade sponge.

the vaginal pessaries were stored until the in vivo studies in the sheep (Figure 3A).

3) Preparation of Rate-Control FGA-releasing vaginal pessaries (Type I):

(a) With the drug-releasing device on the circumference surface: The medicated silicone sheet (containing 1 - 2% FGA and a thickness of 1 mm) with no rate-limiting membrane was fabricated by the same method as described earlier (13) and cut into strips of either 2 cm X 3 cm, 3 cm X 3 cm or 4 cm X 3 cm using a scalpel. Two strips of each size (containing a drug/surface area of 13.3 mg/12 cm^2, 20 mg/18cm^2 or 26.7 mg/24 cm^2) were then glued onto the circumference surface (Figure 3B, C & D) of the non-medicated vaginal sponge (grade 300), using silicone adhesive. The 2 strips were positioned at an equal distance and held in place by wraping in an aluminum foil and cardboard holder (3 cm X 13 cm). The pessaries were then cured in a humidity chamber (with 100 % RH) for 24 hours and dried at 45°C for additional 12 hours to remove any residual acetic acid released during the curing process. After proper crosslinking, the cardboard holder and aluminum foil were removed and the vaginal pessaries were stored until the in vivo studies in the sheep.

(b) With the drug-releasing device on the top surface: The medicated silicone sheet (containing 2 % FGA and a thickness of 1 mm) with no rate-limiting membrane was fabricated by

the same method as described earlier (13) and cut into circular discs of 10 cm^2 with a scalpel. The circular disc was glued onto the sponge using silicone adhesive and then compressed between two porcelain plates and placed in a humidity chamber (with 100% RH) for 24 hours. The vaginal pessaries formed were then dried at 45°C for additional 12 hours to insure a complete removal of the moisture and acetic acid released during the curing process (Figure 3F).

2. Preparation of elution medium:

The simulated vaginal fluid (SVF) containing 20% PEG 400 was prepared by adding 200 ml of polyethylene glycol (PEG) 400 to a mixture of 135 ml citric acid (0.02M) and 365 ml of $NaHPO_4$ (0.04M) and then q.s. to 1000 ml with HPLC-grade distilled water (pH 7.3 ± 0.5). The PEG 400 was added into the SVF to increase the solubility of FGA, so a sink condition can be maintained throughout the *in vitro* drug release studies.

3. Preparation of vaginal mucosa:

Fresh vagina of adult sheep[8] was obtained from local slaughter house. It was cut open vertically and the vaginal mucosa was separated from the wall by cutting off the connective tissue and placed in a beaker of distilled water to prevent drying. Within about half an hour, the mucosa was removed and laid down on a paper towel resting on a glass plate with serosal side up and the excess water was removed by dabbing with an absorbent paper. The blood vessels along with any adhering subcutaneous

connective tissues were carefully removed. Precaution was taken not to damage or puncture the vaginal membrane. The resulting piece of vaginal mucosa was washed again to remove any foreign matter and loose tissue that may be left in the process. It was then laid down on another absorbent paper towel with mucosal side up and the excess water was once again removed by dabbing with an absorbent paper. The vaginal mucosa was sandwiched between two microscope slides and its thickness was determined by a caliper. It was then transferred onto a Teflon-coated silicone "O" ring. After cutting off the excess mucosa to the desired size, the whole assembly was mounted onto the intravaginal release and permeation (IRP) system[9].

C. Experimental Procedure:

1. In Vitro Simultaneous Release and Absorption Studies:

The same procedure as outlined earlier (13) was used in these studies. Basically, a 28 cm^2 circular-shaped medicated silicone disc (containing 1 - 10% FGA) was placed in intimate contact with the vaginal mucosa and sandwiched between the two compartments of the intravaginal release/permeation (IRP) system (Figure 4) (12) and then clamped together. The medicated silicone disc and the serosal side of the vaginal mucosa were each facing the donor and receptor compartments, respectively. Six hundred and fifty (650) ml of the 38°C elution medium containing 20% PEG 400 was filled into each compartment and the whole assembly was then thermostated at 38°C by a circulating waterbath[10] with the magnets rotating at a constant speed of 330 rpm. At scheduled

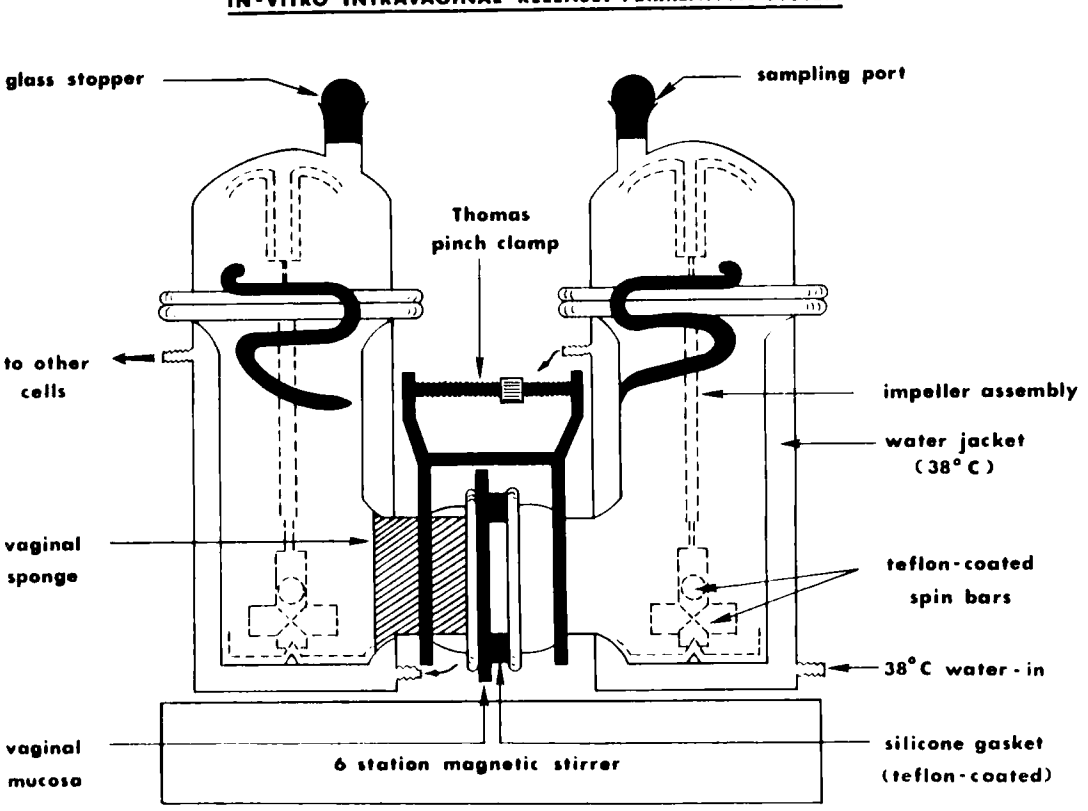

Figure 4.

Schematic illustration of a unit of the in vitro intravaginal release and permeation (IRP) system. Each unit consists of a donor (mucosal) and receptor (serosal) compartments, and is maintained at 38°C by circulating a thermostated water through the water jacket. The solution hydrodynamics in each compartment is kept at constant by a matched pair of impeller assembly rotating at a synchronous rate, ranging from 60 to 330 rpm, by a six-station magnetic stirrer. It is designed for simultaneous drug release and vaginal absorption studies.

intervals, aliquots (1 - 2 ml) of the elution medium were withdrawn from each compartment and assayed for FGA by the HPLC method described later. The simultaneous in vitro drug released and vaginal absorption profiles were monitored by determining the cumulative amount of drug in the donor and receptor solutions, respectively, as a function of time.

2. Intravaginal FGA Absorption and Clinical Efficacy Studies:

Several types of the FGA-releasing vaginal pessaries were fabricated (Figure 3) and sent for in vivo evaluations (for clinical efficacy and intravaginal release and absorption studies) at various geographical locations, following the protocols outlined in Table 1. One unit of the vaginal pessary was inserted deeply into the Sheep's vagina with the top surface in close contact with the cervix and the bottom surface (with a retrieval string) facing the vaginal opening. Clinical efficacy (Type A) study was determined by measuring the blocking of cycle and the synchronization of estrus in the sheep. The rate of intravaginal release (Type B) study was determined by withdrawing the pessaries from one group of 6 ewes after a predetermined period of vaginal residence by pulling the string and then assaying the residual FGA content in the pessaries. The medicated silicone devices were separated from the sponges and sectioned into small pieces and then extracted 4 times with 25 ml of acetone by constant shaking[11] for 12 hours and then q.s. to 100 ml. The extractions were diluted 10 folds with methanol and then assayed for FGA content by the HPLC method outlined later. In the case of the Syncro-Mate vaginal pessaries, the sponges, after removal from the sheep, were cut into small pieces and also extracted 4 times with 60 ml absolute ethanol by constant shaking[11] for 12 hours and then q.s. to 250 ml. The extractions were then diluted 5 folds with methanol and assayed for FGA content by HPLC. The cumulative amount of FGA released from the Rate-Control or

Table I: Protocol for Clinical Efficacy (Type A) and Intravaginal Absorption (Type B) Studies

Study Protocol	Type of Study	Rate-Control Pessary Type (h_m)	Pessary Area/Location	Flurogestone acetate (mg)	Ewe Breed	Ewe Number	Duration (days)
#1	A	Fig. 3A (0.125 mm)	39 cm^2/side	40	Adult & Cyclic [a]	6 X 5	6, 9, 12 15, 19
#2a	A + B	Fig. 3C (0 mm)	18 cm^2/side	40	Adult & Cyclic [a]	6 X 5	6, 9, 12 15, 19
#2b	A + B	Fig. 3F (0 mm)	10 cm^2/top	20	Adult & Cyclic [a]	6 X 5	6, 9, 12 15, 19
#3a	B	Fig. 3C (0 mm)	18 cm^2/side	20	Adult [b]	6 X 5	6, 9, 12 15, 19
#3b	B	Fig. 3C (0 mm)	18 cm^2/side	40	Adult [b]	6	19
#3c	B	Fig. 3C (0 mm)	18 cm^2/side	80	Adult [b]	6	19
#4a	B	Fig. 3B (0 mm)	12 cm^2/side	13.3	Adult [c]	6	10
#4b	B	Fig. 3C (0 mm)	18 cm^2/side	20	Adult [c]	6	10
#4c	B	Fig. 3D (0 mm)	24 cm^2/side	26.7	Adult [c]	6	10

a) France (Ile de France)
b) Large Animal Research Center, Rutgers University
c) Huntington Research Center, Cambridgeshire, U.K. (Clun X Suffolk)

Syncro-Mate vaginal pessaries was calculated by subtracting the amount remaining after a period of vaginal insertion from the initial drug loading dose in the medicated silicone devices or in the polyurethane sponges.

D. Analytical Procedures:

An aliquot (up to 100 µl) of the sample was injected into a microprocessor-controlled high performance liquid chromatograph[12], which is equipped with a variable wavelength detector, an automatic sampler, a variable-volume injector, a

dual-head reciprocating pump and a dual solvent system. The solvent and column temperatures were both maintained at ambient. A combination of methanol and water (50:50 to 63:35) was used as the mobile phase. Flurogestone acetate in the sample was separated by a C_{18} resolve column[13] (5 μ, 3.8 X 15 cm) and detected at a wavelength of 240 nm. At a flow rate of 0.8 - 1.0 ml/min., the specific peak for FGA was shown at a retention time of 4-6 minutes.

E. Data Analysis:

The peak height for FGA was directly measured from the chromatogram and the concentration was calculated by comparing it to a standard curve. The cumulative amount of FGA (mcg/cm^2) released was then computed and plotted as a function of time (in hours or days) or the square root of time (in $hours^{\frac{1}{2}}$ or $days^{\frac{1}{2}}$) for the determination of the fluxes of drug release.

RESULTS AND DISCUSSION

A. Preliminary Studies of Clinical Efficacy:

Based on the *in vitro* drug release and viginal absorption studies conducted earlier (13), 30 units of the Rate-Control vaginal pessaries (Type II), each containing 39 cm^2 FGA-releasing silicone device laminated with a rate-limiting membrane (h_m = 0.125 mm)(Figure 3A), were prepared and submitted for preliminary clinical efficacy studies in the sheep; even though the *in vitro* studies predicated that these pessaries were able to deliver

only about 200 mcg/day of FGA, which is considered to be a subeffective dose for estrus synchronization in the sheep. After following the protocol #1, as outlined in Table I, several problems were encountered in the group of animals treated for a duration of longer than 9 days. Namely, for the 12-, 15-, and 19-day groups, the treatment had to be terminated at 9 days after pessary insertion, since some uterine inflammation was observed in the treated animals. Also, the pessaries were found to be difficult for insertion deep into the vagina, because they were too rigid to be deformed for insertion through a pessary insertor. Furthermore, all the pessaries withdrawn were also noted to be highly deformed. The results also demonstrated that these pessaries were not effective in synchronizing the estrus in the sheep as predicted from the in vitro studies.

From these observations it became apparent that the irritation observed may have been resulted from the use of hard grade 800 sponge in the fabrication of vaginal pessaries. The strip of medicated silicone device, which was glued around the total circumference surface of the sponge, also added an extra diameter and rigidity to the pessaries. Additionally, the circular-shape of the sponge could not be well retained, if it is too rigid, as a result of the dynamic constriction of the vaginal wall. The deformation of the pessary also reduced the area of FGA-releasing surface in contact with the absorption surface of the vaginal epithelium, which could lead to a further reduction in the amount of drug being delivered.

Hence, one of the major problems need to be resolved is, therefore, considered to be the development of an irritation-free and clinically efficacious vaginal pessary. The irritation-free vaginal device could be produced by reducing the diameter of the pessary slightly from a diameter of 4.2 cm to 4.0 cm, by using a virgin sponge of a softer grade (300 instead of 800), by decreasing the surface area of the pessary and/or completely evaporating off any possible residual acetic acid, which has been released during the curing of silicone adhesive, from the pessary before uses.

On the other hand, to obtain a clinically efficacious vaginal pessary, a series of in vitro and in vivo studies were conducted, using the (Type I) Rate-Control vaginal pessaries, to study the effect of drug loading dose, surface location and surface area on the intravaginal release and absorption of FGA.

B. In Vitro Simultaneous Release and Vaginal Absorption Studies:

1. Effect of loading dose: A series of in vitro drug release and vaginal absorption studies were conducted, using Rate-Control vaginal pessaries (Type I), to study the effect of drug loading dose and to determine the optimum loading dose required for the estrus synchronization in the sheep. The in vitro release and vaginal absorption of flurogestone acetate from the Rate-Control vaginal pessaries containing 2% of FGA were reported earlier to follow a linear Q - t relationship (13). In the present investigation, in order to determine the effect of drug loading

dose on the rate profiles of the release and vaginal absorption of FGA, the Rate-Control vaginal pessaries were fabricated to contain 1%, 2%, 4% or 10% of FGA. The results of the evaluations indicated that, as expected from the theoretical model (13), the magnitude of Q/t values calculated are found to be very much independent of the loading dose in the range of 1% to 10% FGA (Figure 5). The mean values of the drug release and vaginal absorption rates determined from the data in Figure 5 were found to be 18.63 ± 1.04 and 15.17 ± 0.94 mcg/cm^2/day, respectively. These results support the physical model proposed earlier (13) that as the loading dose is relatively large, the drug release and vaginal absorption profiles from the Rate-Control vaginal sponge will be at constant rates, which should be independent of the loading dose. These findings suggested that vaginal pessaries can now be fabricated from the Type I controlled-release silicone device to contain only 1% of FGA, which still yields the same magnitude of release and absorption rates as the device containing 10% of FGA. If the device containing 2% of FGA is clinically effective, 1% loading dose may also be effective as well in blocking the cycle and in synchronizing the estrus in the sheep. The daily FGA dose released from a device with a drug-releasing surface of 18 cm^2 was calculated to be 335.3 ± 18.7 mcg/day, which is within the dose range of 300 - 400 mcg/day needed for the estrus synchronization in the sheep.

Furthermore, the data in Figure 5 indicated that the magnitude of the vaginal absorption rates is slightly lower than that of

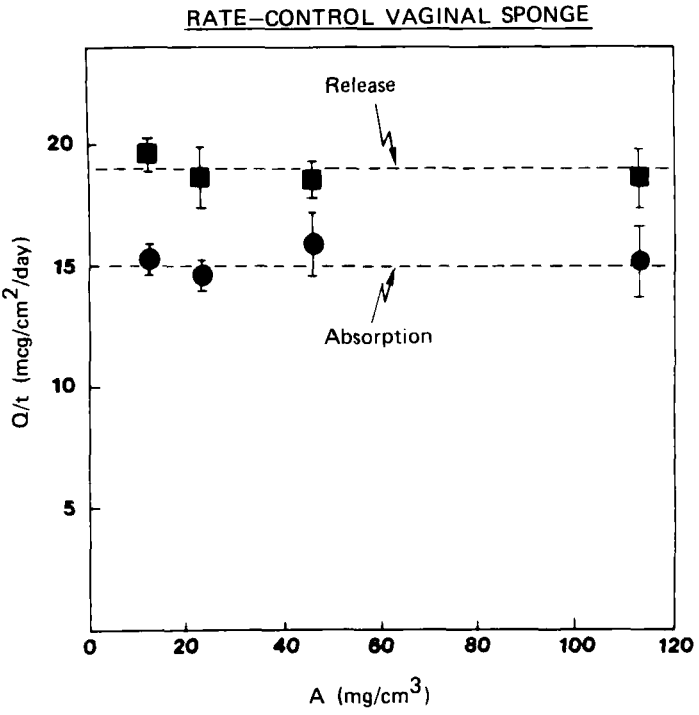

Figure 5.

Effect of drug loading dose on the in vitro simultaneous drug release and vaginal absorption rate profile of the FGA from Rate-Control vaginal pessary (Type I). The rates of drug release and vaginal absorption (Q/t) were found to be independent of the loading dose (A) between 11.4 and 114 mg/cm^3. The mean values (± standard deviation) for the drug release and vaginal absorption rates, calculated from the 4 groups of data points, are 18.63 ± 1.04 and 15.17 ± 0.94 mcg/cm^2/day, respectively. Each group of data points represents the mean ± standard error of 3 determinations for each loading dose.

the release rates. The difference can be explained if one compares the difference in the diffusional resistances between in vitro and in vivo conditions. In the in vitro drug release studies, the elution medium was always maintained at sink conditions throughout the experiment. In this case, only a hydrodynamic boundary layer exist between the drug-releasing surface of the

vaginal pessary and the bulk of elution medium (13). However, in the case of in vitro vaginal absorption studies, two additional barriers are also present, namely, the aqueous diffusion layer (between the drug-releasing surface of the pessary and the absorption surface of the vaginal mucosa) and the vaginal mucosa, which could certainly add more diffusional resistance on the surface of the vaginal pessary. These additional diffusion barriers are expected to reduce the amount of drug absorbed at any given time (i.e., the lower the magnitude of Q/t). This fact should be taken into consideration when one intends to make any in vitro - in vivo correlation.

2. Rate-Control pessary versus Syncro-Mate pessary: The in vitro vaginal absorption profiles of FGA from a Type I Rate-Control pessary and a Syncro-mate pessary, both contain a same loading dose (20 mg of FGA), were evaluated simultaneously. The results clearly indicated the difference in the mechanisms of vaginal absorption from these two types of vaginal pessaries. A constant (Q vs. t) vaginal absorption profile was observed from the Rate-Control pessaries, while a non-linear (Q vs $t^{\frac{1}{2}}$) relationship was observed for the Syncro-Mate pessaries (Figure 6). For the initial 47 hours, the vaginal absorption profile of FGA from the Syncro-Mate vaginal pessary was greater than from the Rate-Control vaginal pessary. After 47 hours, the vaginal absorption profile from the Syncro-Mate vaginal pessary became smaller than from the Rate-Control vaginal pessary. The difference in the absorption rates between these two types of vaginal

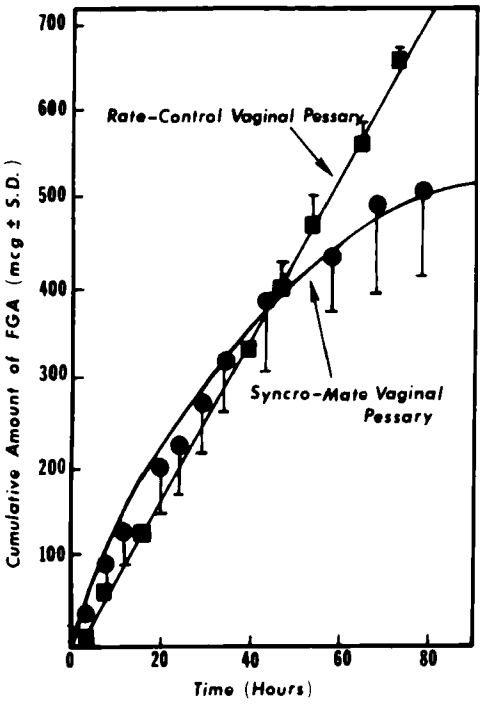

Figure 6.

Comparison in the time course for the vaginal absorption profiles of FGA from the Rate-Control vaginal pessary (Type I) and from Syncro-Mate vaginal pessary (both containing 20 mg of FGA). The Rate-Control vaginal pessary (■) showed a constant (Q - t) relationship with the Q/t value of 275 ± 5 mcg/cm^2/day, while Syncro-Mate pessary (●) showed a non-linear (Q - t$^{1/2}$) relationship with the Q/t$^{1/2}$ value of 397 ± 60 mcg/cm^2/day$^{1/2}$. Each data point represents the mean value ± one standard deviation of 3 determinations.

pessaries were also apparent (Figure 7). The results indicated that the rate of vaginal absorption of FGA from the Rate-Control vaginal pessary reaches the steady-state at around 40 hours and then remains at the steady-state rate of 0.64 ± 0.01 mcg/cm^2/hr throughout the studies, while the rate from the Syncro-Mate pessary is not constant and continues to decrease as a function of time.

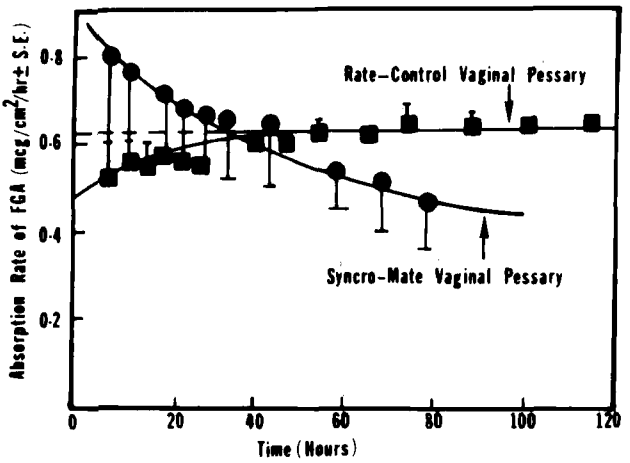

Figure 7.

Comparison in the time course for the rates of vaginal absorption of FGA from the Rate-Control and Syncro-Mate vaginal pessaries. The absorption rate profile increased and reached a plateau and constant value of 15.28 ± 0.28 mcg/cm^2/day at around 40 hours for the Rate-Control vaginal pessaries (■), while the absorption rate for the Syncro-Mate pessary (●) continued to decrease in a curvilinear fashion ($Q/t^{1/2}$ = 27.95 ±4.22 mcg/cm^2/day$^{1/2}$).

C. In Vivo Drug Release Studies:

1. Effect of loading dose: Since the release of flurogestone acetate from the Rate-Control vaginal pessary residing in the vaginal tract of the sheep is expected to be affected by the diffusional resistance across the vaginal fluid and vaginal mucosa (14), the cumulative amount of drug released (Q) in the sheep's vagina is, therefore, defined by Eq. (32). Equation (32) suggests that the cumulative amount of flurogestone acetate released from a unit surface area of the device should be linearly proportional to the duration of vaginal residence as shown in Figure 8. The rate of intravaginal drug release (Q/t), following the protocol

#2a (Table I), was calculated from the slope of the Q vs t plots as 15.48 mcg/cm^2/day for the Rate-Control vaginal pessary containing a 18 cm^2 drug-releasing device (2% FGA) on the circumference surface of the sponge. The Rate-Control vaginal pessary containing the same surface area of the drug releasing device but with only 1% FGA (protocol #3a, Table I) was found to yield a slightly lower rate of intravaginal release (13.3 mcg/cm^2/day). This difference, however, was observed to be rather insignificant.

Additional studies were also conducted to determine the cumulative amount of FGA in the same surface area of the drug releasing device (protocols #3b and #3c,Table I). After 19 days of intravaginal administration, the total amount of FGA released was found to be within the drug release profiles obtained for 1% and 2% FGA (Figure 8). These in vivo data further support the in vitro observations (Figure 5) that the rate of drug release from this Rate-Control vaginal pessary is constant and relatively independent of the drug loading dose as predicted from Eq. (32).

2. Effect of Surface Location: The intravaginal release profiles of FGA from the Rate-Control vaginal pessary with the drug-releasing device on either the circumference surface or on the top surface were evaluated (protocol #2, Table I). The results indicated that the intravaginal release profile of FGA from the vaginal pessary containing a 10 cm^2 device on the top surface shows a rather scattering in the individual data(Figure 9), while the rate of intravaginal drug release calculated (13.75

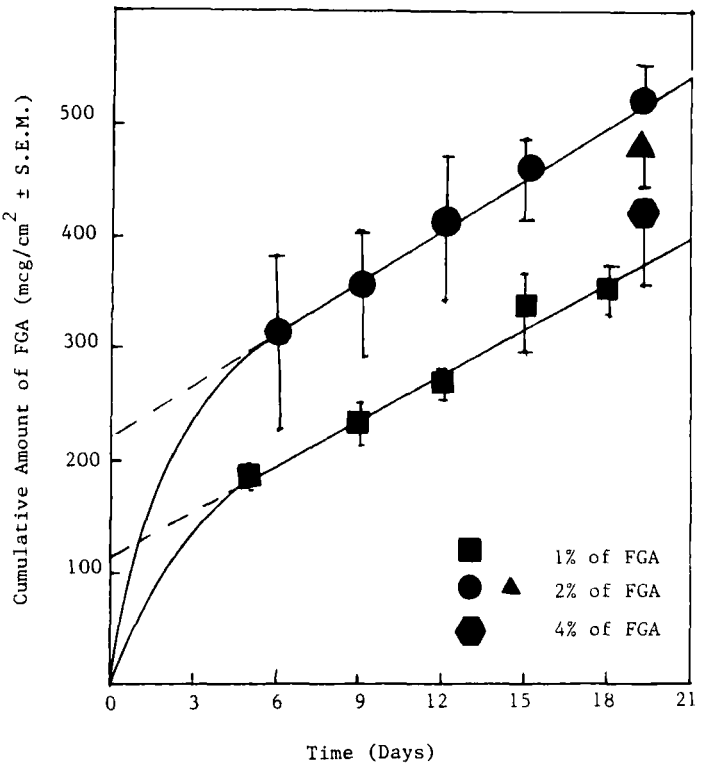

Figure 8.

The time course for the in vivo vaginal absorption of FGA from the Rate-Control vaginal pessry (Type I with a loading dose from 1 to 4% of FGA) in the sheep's vagina for 19 days. A linear relationship was established with the vaginal absorption rates ranging between 13.33 and 15.48 mcg/cm^2/day for 1% and 2% FGA, respectively. Keys:(■), 1%; (●,▲), 2%; and (⬢), 4% FGA.

mcg/cm^2/day) is only slightly lower than the rate from the pessary with a 18 cm^2 device on the circumference (side) surface (15.48 mcg/cm^2/day). Therefore, the results suggested that the rate of intravaginal drug release is relatively independent of the location of the drug-releasing device on the pessary as long as a good contact is maintained between the drug-releasing surface and the absorption surface of the vaginal mucosa. However, the

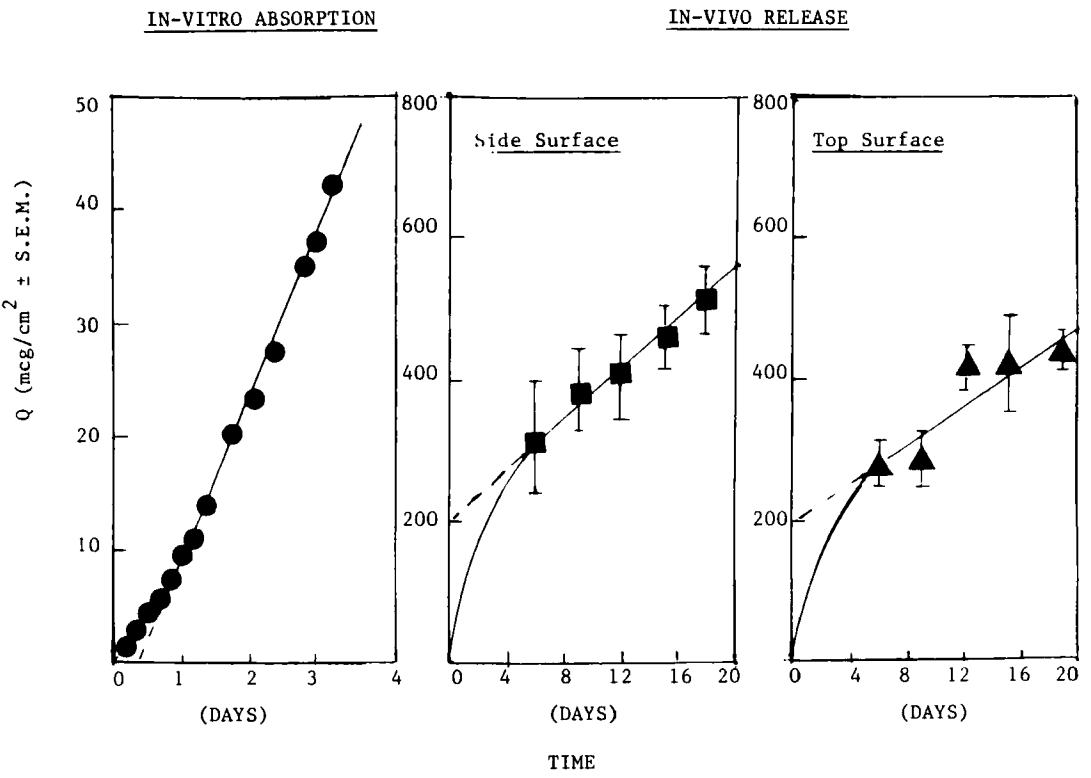

Figure 9.

Comparison in the time course for the in vitro and in vivo release of FGA from the Rate-Control vaginal pessary (Type I) with the drug-releasing surface located on various surfaces of the non-medicated polyurethane sponges. The constant (Q-t) linearity was obtained. The 18-cm^2 device on the circumference surface yielded an in vivo release rate of 15.48 mcg/cm^2/day, which was in a good agreement with the in vitro absorption rate of 14.6 mcg/cm^2/day, as did the 10 cm^2 device on the top surface of the sponges, which gave an in vivo release rate of 13.75 mcg/cm^2/day. Each data point represents the mean value ± one standard error of 4-6 determinations.

overall daily dose of FGA (137.5 mcg/day) released from the pessaries with the drug-releasing device on the top location would be too small to be clinically effective, since the maximum area available on the top surface of the sponge was rather limited (only 12.6 cm^2).

3. Effect of Surface Area: The dependency of the amount of intravaginal drug release on the surface area of the drug-releasing device located on the circumference surface of the Rate-Control vaginal pessary was evaluated following protocol #4, (Table I). As shown in Figure 10, the cumulative amount of FGA released from the Rate-Control vaginal pessary in the sheep's vagina was observed to be linearly dependent upon the surface area of the drug-releasing device surrounding the circumference of the sponge. This linearlity permits one to determine the surface area of the drug-releasing device needed for delivery of a therapeutically effective daily dose.

From this linear relationship, one can also predict the amount of FGA could have been released from a 10 cm^2 device after 10-day intravaginal insertion in the sheep. A value of 259 mcg/day was determined, which was 188% greater than the daily dose (137.5 mcg/day) delivered by the device positioned on the top. The observed lower daily dose (137.5 mcg/day) delivered could be a result of the fact that the drug-releasing device located on the top surface of the vaginal sponge may not be in an intimate contact with the cervix as does the drug-releasing device on the circumference surface of the sponge, which is closely surrounded by the vaginal epithelium as a result of the constant constriction of the vaginal wall.

D. In Vitro - In Vivo Correlation of the Intravaginal FGA Release:

For the analysis of in vitro-in vivo relationship, the FGA molecules released from the Rate-Control vaginal pessary are

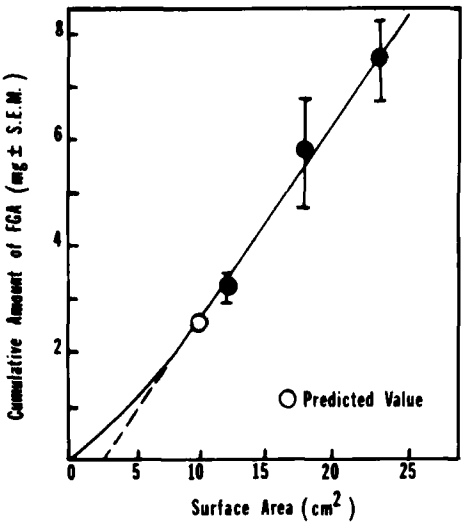

Figure 10.

Linear relationship between the cumulative amount of FGA absorbed and the surface area of the drug-releasing device (12, 18 and 24cm^2) located on the circumference surface of the Rate-Control vaginal pessary (Type I), which was inserted in the sheep's vagina for 10 days. Each data point represents the mean value ± one standard error of 4-5 determinations.

presumed to be first dissolved in the surrounding vaginal fluid and are then transported across the vaginal mucosa and finally distributed into a biological sink maintained by hemoperfusion (Figure 1 and 2). In such case, the rate of drug absorption (dQ/dt) by the vaginal mucosa should be directly proportional to the drug concentration (C_s') in the vaginal fluid (as the driving force for drug absorption) and inversely proportional to the diffusional resistances encountered by the drug molecules along the way across the vaginal mucosa. So rearranging Eq. (32) (since $K_s'C_p = S_{aq}$):

$$\left[\frac{dQ}{dt}\right]_{in\ vivo} = \frac{S_{aq}}{[R_{aq}K_s' + R_v]} = \frac{S_{aq}}{\Sigma R_{in\ vivo}} \qquad \text{[Eq. 35]}$$

Where, $\Sigma R_{in\ vivo}$ is the diffusional resistance across the composite of the aqueous diffusion layer and vaginal mucosa. Eq. (35) also indicates that since the drug solubility (S_{aq}) in the vaginal fluid is constant and invariant with time, a constant rate of vaginal absorption should be obtained (dQ/dt = constant).

In the in vitro vaginal absorption studies, the elution medium was maintained at sink conditions throughout the experiment. In this case, an additional hydrodynamic boundary layer exists between the serosal surface of the vaginal mucosa and the bulk of the elution medium. In earlier studies (13), it was found that the rate of in vitro vaginal absorption from the Rate-Control vaginal pessary (Type I) was defined by the following relationships:

$$\frac{dQ}{dt} = (C_p) \left[R_{aq} + \frac{1}{K_s'} \left(R_v + \frac{R_d}{K_v} \right) \right]^{-1} \qquad \text{[Eq. 36]}$$

So, rearranging Eq. (36) (since $K_s' C_p = S_{aq}$):

$$\left[\frac{dQ}{dt} \right]_{in\ vitro} = \frac{S_{aq}}{[(R_{aq} K_s' + R_v) + R_d/K_v]} = \frac{S_{aq}}{\sum R_{in\ vitro}} \qquad \text{[Eq. 37]}$$

Where, $\sum R_{in\ vitro}$ is the diffusional resistance across the composite of aqueous diffusion layer, vaginal mucosa and hydrodynamic boundary layer.

Currently, measurement or calculation of the $\sum R_{in\ vivo}$ value can be done only with great difficulty. Extensive instrumentation has to be applied and many approximations have to be assumed

(15). On the other hand, the prediction of the in vivo release profile from an in vitro study can be done, without knowing the magnitude of $\sum R_{in\ vivo}$ value, by establishing the relationship between in vitro and in vivo in the mechanism and the rate of drug release (15). This relationship may be established by studying the mechanisms and the rates of drug release from the same type of drug delivery system under both in vitro and in vivo conditions. It was exemplified by the intravaginal absorption studies of ethynodiol diacetate in rabbits (14). A proportionality, called correlation factor (γ), is established by comparing Eq. (35) with Eq. (37). If the S_{aq} values are identical, then it yields:

$$\frac{(Q/t)_{in\ vivo}}{(Q/t)_{in\ vitro}} = \frac{[(R_{aq}K'_sK_v + R_vK_v) + R_d]}{[R_{aq}K'_sK_v + R_vK_v]} = \frac{\sum R_{in\ vitro}}{\sum R_{in\ vivo}} = \gamma \quad \text{[Eq. 38]}$$

The only difference between the $\sum R_{in\ vitro}$ and $\sum R_{in\ vivo}$ is the presence of R_d term or the diffusional resistance across the hydrodynamic boundary layer on the serosal surface of the vaginal mucosa.

In the past, scientists used to compare the in vitro release with the in vivo absorption rates for establishing an in vitro-in vivo correlation. Usually, the in vitro releases rates were much greater, so this was accounted for by increasing the hydrodynamic boundary layer on the surface of the device to match the in vivo data (16), However, much better fit can be achieved if the in vitro vaginal absorption data are compared with in

vivo absorption data. This way, the barrier properties of the biological layers can be taken into account, since their diffusional resistance properties reduce the absorption rate. Also, due to a snug fit between the vaginal pessary and the vaginal mucosa, the thickness of the aqueous diffusion layer, e.g., vaginal fluid, is too small to produce any significant effect on the absorption rate (17).

In this study, both the in vitro release rate and in vitro vaginal absorption rates were determined and compared with the in vivo absorption rates (Table II) for three types of vaginal pessaries. The results indicated that the correlation factor between the in vitro vaginal absorption rate and the in vivo absorption rate shows a much better fit, which is almost close to the unity (0.94 - 1.19), than the correlation factor for the in vitro release rate and the in vivo absorption rate. The best in vitro - in vivo correlation was obtained for the Rate-Control vaginal pessary with a 18 cm^2 drug-releasing device on the circumference surface (γ = 1.06). This correlation factor of 1.06 was found very useful in the translation of the in vitro data to the in vivo drug absorption profiles, since the in vitro vaginal absorption studies were conducted under the controlled conditions (i.e., $\sum R_{in\ vitro}$ is constant). This correlation factor was also very useful in the development of a long-acting drug delivery system. As soon as this relationship is established, the proportionality of (γ) can be applied to translate all the data generated in the short-term in vitro vaginal absorption

Table II: In Vitro - In Vivo Correlation on Intravaginal Administration of Flurogestone Acetate

Vaginal Pessary	$(Q/t)_{in\ vitro}$ (mcg/cm^2/t) Release	Absorption	$(Q/t)_{in\ vivo}$ (mcg/cm^2/t)	Correlation Factor (γ) A	B
Syncro-Mate (40 mg)	814 ± 134 day$^{-1/2}$	46 ± 8 day$^{-1/2}$	54.8 day$^{-1/2}$	0.067	1.19
Rate-Control (2 %)					
Side location	18.6 ± 1.2 day^{-1}	14.6 ± 0.5 day^{-1}	15.48 day^{-1}	0.832	1.06
Top location	18.6 ± 1.2 day^{-1}	14.6 ± 0.5 day^{-1}	13.75 day^{-1}	0.739	0.94

A) $(Q/t)_{in\ vivo}$ / $(Q/t)_{in\ vitro,\ release}$

B) $(Q/t)_{in\ vivo}$ / $(Q/t)_{in\ vitro,absorption}$

studies (e.g., 3-day in vitro testing in this investigation) to the results expected from the long term in vivo studies (e.g., 19-day intravaginal absorption in this investigation). Therefore, following the establishment of this in vitro - in vivo correlation, the in vitro data can be utilized to formulate a Rate-Control drug delivery device with an optimal rate of drug release for a long-term medication.

E. Clinical Efficacy Studies:

Sixty (60) units of the Type I, Rate-Control vaginal pessary were fabricated to contain 10 to 18 cm^2 of the drug-releasing device (2% FGA) on either the top or the circumference surface of the polyurethane sponge (Figure 3C & F) and sent for animal testing, following the protocol #2 (Table I) for 19 days. These Rate-Control vaginal pessaries were found to be easily introduced into the sheep's vagina. Moreover, only one out of the 60 pessaries tested was lost during the entire clinical trials. The most important observation was that the Rate-Control pessaries with the 18-cm^2 drug-releasing device on the circumference surface are observed to be clinically effective in blocking the cycle during the 19-day treatment and then synchronizing the estrus following the termination of the treatment. All the ewes treated were detected to ovulate normally by endoscopic examination of the ovaries a few days later. Artificial insemination could then be carried out during this period to achieve a greater rate of conception.

On the other hand, the Rate-Control vaginal pessary with the 10 cm^2 drug-releasing device on the top surface of the sponges was found not effective in blocking the estrus, which may be due to lack of a good contact between the FGA-releasing surface and the vaginal mucosa and a small surface area for FGA release. Results indicated that only 50% of the ewes treated are detected to ovulate normally after the treatment, which is certainly not satisfactory.

CONCLUSIONS

A Rate-Control vaginal pessary, which consists of a drug-releasing silicone device surrounding a drug-free polyurethane sponge was developed to improve the kinetics and reproducibility of drug release from the Syncro-Mate vaginal pessary currently marketed. Several types of Rate-Control vaginal pessaries were fabricated and submitted for clinical efficacy and FGA absorption studies in the sheep and the effect of various parameters. A pessary made with 39 cm^2 drug-releasing device (with 1% FGA and 0.125 mm silicone coating membrane) on the circumference surface was found to be clinically ineffective in blocking the cycle as a result of low release rates. On the other hand, the pessaries made with 18 cm^2-device (2% FGA, with no rate-limiting membrane) on the same location were found effective in blocking the cycle and in synchronizing the estrus in the sheep as the result of delivering a constant daily dose of FGA (278.6 mcg/day). The pessaries made with 10 cm^2-device on the top surface were

found not effective, which could be due to the low daily dose delivered as a result of small surface area available for drug release. The location of the drug-releasing device on the sponge did not affect the absorption profile, but the rate of absorption. As the surface area of the drug-releasing device increased, the amount of drug absorbed by the vaginal mucosa also increased. Both the *in vitro* and *in vivo* absorption rates were found to be independent of the loading dose when more than 1% of FGA was incorporated, as predicted from the proposed physical model (Eq. 32). This indicates that the FGA dose can be reduced, while an effective synchronization is still maintained. An excellent *in vitro-in vivo* correlation with a factor of 1.06 was obtained for the controlled intravaginal absorption of FGA from this Rate-Control vaginal pessary, so the intravaginal rate of absorption of FGA in a 19-day treatment could be predicted from a 3-day *in vitro* study.

The major advantages of the Rate-Control vaginal pessary over Syncro-mate FGA-releasing vaginal pessaries include: Precise release and predictable absorption patterns have been achieved; intravaginal drug bioavailability has been maximized due to the minimization of dose required for effective estrus synchronization and the reduction of drug wastage due to washing off by the vaginal secretions.

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FOOTNOTES

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2. Fisher Scientific Co., Fairlawn, New Jersey.
3. Burdick & Jackson Lab., Inc., Muskagon, Michigan.
4. Pharmko,Publick Industries Co., Linfield, Pennsylvania.
5. Nanopure, Sybron/Barnstead, Boston, Massachusetts.

6. Dow Corning Corporation, Midland, Michigan.
7. Synkron Corporation, Paris, France.
8. Dealeman Meats, Warren Township, New Jersey.
9. Bellco Glass, Inc., Vineland, New Jersey.
10. Waterbath Model 80, Fisher Scientific Company, Springfield, New Jersey.
11. Wrist Action Shaker, Burlin Corporation, Pittsburgh, Pennsylvania.
12. HP Model 1084B HPLC, Hewlett Packard, Palo Alto, California.
13. Resolve C-18 column, Water Associates, Milford, Massachusetts.

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