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# Vaginal distribution and retention of a multiparticulate drug delivery system, assessed by gamma scintigraphy and magnetic resonance imaging

Samata Mehta[a], Hans Verstraelen[b], Kathelijne Peremans[c], Geert Villeirs[d], Simon Vermeire[c], Filip De Vos[e], Els Mehuys[a], Jean Paul Remon[a], Chris Vervaet[a,*]

[a] Laboratory of Pharmaceutical Technology, Ghent University, Harelbekestraat 72, B-9000 Ghent, Belgium
[b] Department of Obstetrics and Gynecology, Ghent University Hospital, De Pintelaan 185, B-9000 Ghent, Belgium
[c] Department of Radiology, Ghent University Hospital, De Pintelaan 185, B-9000 Ghent, Belgium
[d] Department of Medical Imaging, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, B-9820 Merelbeke, Belgium
[e] Laboratory of Radiopharmacy, Ghent University, Harelbekestraat 72, B-9000 Ghent, Belgium

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ABSTRACT

*Background:* For any new vaginal dosage form, the distribution and retention in the vagina has to be assessed by in vivo evaluation. We evaluated the vaginal distribution and retention of starch-based pellets in sheep as live animal model by gamma scintigraphy (using Indium-111 DTPA as radiolabel) and in women via magnetic resonance imaging (MRI, using a gadolinium chelate as contrast agent). A conventional cream formulation was used as reference in both studies.
*Method:* Cream and pellets were administered to sheep ($n=6$) in a two period-two treatment study and to healthy female volunteers ($n=6$) via a randomized crossover trial. Pellets (filled into hard gelatin capsule) and cetomacrogol cream, both labeled with Indium-111 DTPA (for gamma scintigraphy) or with gadolinium chelate (for MRI) were evaluated for their intravaginal distribution and retention over a 24 h period. Spreading in the vagina was assessed based on the part of the vagina covered with formulation (expressed in relation to the total vaginal length). Vaginal retention of the formulation was quantified based on the radioactivity remaining in the vaginal area (sheep study), or qualitatively evaluated (women study).
*Results:* Both trials indicated a rapid distribution of the cream within the vagina as complete coverage of the vaginal mucosa was seen 1 h after dose administration. Clearance of the cream was rapid: about 10% activity remained in the vaginal area of the sheep 12 h post-administration, while after 8 h only a thin layer of cream was detected on the vaginal mucosa of women. After disintegration of the hard gelatin capsule, the pellet formulation gradually distributed over the entire vaginal mucosa. Residence time of the pellets in the vagina was longer compared to the semi-solid formulation: after 24 h $23 \pm 7\%$ radioactivity was detected in the vaginal area of the sheep, while in women the pellet formulation was still detected throughout the vagina.
*Conclusion:* A multi-particulate system containing starch-based pellets was identified as a promising novel vaginal drug delivery system, resulting in complete coverage of the vaginal mucosa and long retention time.



## 1. Introduction

There is an increasing interest in novel vaginal drug delivery systems with special emphasis on enhancing the product distribution and retention in the vagina as these are important for locally acting drugs as well as for those intended for systemic delivery (Alexander et al., 2004; Richardson and Illum, 1992; Vermani and Garg, 2000). Currently, several conventional vaginal formulations are available on the market, mainly semisolid (creams and gels) and solid (capsules, tablets, ovules, and rings) dosage forms. However all of them are associated with specific disadvantages, contributing to poor patient compliance: semisolids are messy to administer, cause leakage and have a limited residence time (Hussain and Ahsan, 2005), while tablets and capsules results in poor disintegration and require frequent administrations due to self cleansing action of vagina. Although vaginal retention can be improved by using bioadhesive tablet, loss of these tablets has also been reported after vaginal application (Voorspoels et al., 2002). Vaginal rings are only suitable for long-term controlled drug delivery.

* Corresponding author. Tel.: +32 9 264 80 69; fax: +32 9 222 82 36.
*E-mail address:* chris.vervaet@ugent.be (C. Vervaet).

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We have recently proposed, disintegrating starch-based pellets as a new vaginal drug delivery dosage form. Evaluation of their distribution and retention in the human vagina by colposcopy indicated that these disintegrating starch-based pellets distributed evenly over the vaginal epithelium with a longer retention time compared to non-disintegrating microcrystalline cellulose pellets (Poelvoorde et al., 2009). However, since colposcopy is an invasive technique, the speculum (placed in the vagina at frequent intervals during the study) can influence disintegration of the hard gelatin capsule and distribution of the pellets. To overcome this drawback, non-invasive techniques must be used to evaluate vaginal distribution and retention of the starch-based pellets.

Non-invasive medical imaging techniques such as gamma scintigraphy and magnetic resonance imaging (MRI) have proven valuable in assessing the in vivo retention of pharmaceuticals (Mauck et al., 2008). Gamma scintigraphy has been used extensively to track the in vivo distribution of formulations following nasal, oral, rectal or pulmonary administration via non-invasive imaging with a short-lived gamma-emitting radioisotope (Davis et al., 1992; Meseguer et al., 1994; Newman and Wilding, 1999). MRI can be used as a complementary tool to gamma scintigraphy to study the in vivo drug distribution, as it offers the possibility to acquire three-dimensional information on drug distribution together with visualization of the anatomical features in the vicinity of delivery systems. MRI has been used successfully to detect the vaginal anomalies in the female pelvis (Rouariet et al., 2008), and to determine the intravaginal distribution of gels in human clinical trials (Barnhart et al., 2004c, 2005a; Omar et al., 2008; Pretorius et al., 2002b). While most of the studies have used MRI, to visualize the vaginal distribution of gels, ovules (Barnhart et al., 2004a; Menon et al., 2007) and rings (Barnhart et al., 2005b; Pretorius et al., 2002a), we used MRI to detect small sized multiparticulates and to evaluate their intravaginal distribution in women.

In the first part of this study, the multiparticulate pellet formulation and cream were administered intravaginally to sheep, and the distribution and retention (up to 24 h post dose) was monitored via gamma scintigraphy. The trial design was a two period-two treatment study in 6 sheep, administering pellets and cream with a 6 week interval. Indium-111 DTPA was selected as radiolabel, based on its half life of 2.8 days allowing data acquisitions up to 24 h. The retention and the distribution of the formulations in the vaginal area were determined over a 24 h period. During the second part of the study, the pellet and cream formulation were administered in a randomized cross-over trial to 6 healthy women and the intravaginal distribution and retention was assessed using MRI as monitoring tool. The main purpose of this study was to assess the time of disintegration of hard gelatin capsule (used to administer the pellets) and to evaluate the intravaginal distribution and retention of the pellets and cream. Both formulations were loaded with gadoterate meglumine (Dotarem®) for visualization via MRI. Comparison of the gamma scintigraphy (sheep study) and MRI data (women trial) was performed, which allowed to assess if sheep can be used as a model to evaluate the distribution and retention of vaginal drug delivery systems.

## 2. Materials and methods

### 2.1. Materials

#### 2.1.1. Starch pellets

Starch-based pellets consisted of a high amylose, crystalline and resistant starch grade (VELOX™ MCS, Henkel, New Jersey, USA) as main excipient (76.4%). Hydroxypropylmethyl cellulose (HPMC) (Methocel® E15 LV EP Pharm, Colorcon, Dartford, UK) (4.4%) was used as binder and sorbitol (Sorbidex® P 16615, Cerestar, Vilvoorde, Belgium) (9.2%) was added as plasticizer. Demineralized water was used as granulation liquid. Indium-111 DTPA ($^{111}$In) ($t_{1/2}$ = 2.8 days) (Covidien, Petten, The Netherlands) and gadoterate meglumine (Dotarem®, Guerbet, Roissy, France) were used as contrast agents for gamma scintigraphy and MRI, respectively.

#### 2.1.2. Cream

A placebo, buffered cetomacrogol cream (pH 4.5) was used as reference formulation, based on the standardized formulation listed in the 'Therapeutisch Magistraal Formularium' (a validated formulary of compounded formulations available to Belgian pharmacists): 7.2% cetostearyl alcohol, 1.8% cetomacrogol 1000, 15% white vaseline, 6% liquid paraffin and water. Sodium dihydrogen phosphate (0.3%) was added as buffering agent, and 0.02% propylparaben and 0.08% methylparaben as preservatives. All ingredients were purchased from Fagron (Waregem, Belgium).

### 2.2. Preparation of pellets

Starch-based pellets were produced by extrusion-spheronisation according to Dukic et al. (2007). The contrast agents were dissolved in demineralized water (used as granulation liquid). After dry mixing of all excipients for 15 min in a tumbling mixer (Turbula® model T2A, W.A. Bachofen, Basel, Switzerland), the powder mix was granulated with demineralized water (containing the contrast agents) using mortar and pestle. The wet mass was extruded in a bench-top single screw mini extruder (model MSE, Caleva, Dorset, UK) at an extrusion speed of 50 rpm, equipped with an extrusion screen with 0.5 mm perforations. The extrudates were spheronised at 1000 rpm for 3 min, in a Model 10 spheroniser (Caleva, Dorset, UK). After overnight drying in an oven at 40 °C, the 315–710 μm size fraction was collected by sieving and about 700 mg pellets were filled into size 00 hard gelatin capsules (Intercaps, Acapharma, Waregem, Belgium). Each capsule contained about 18.5 MBq radioactivity or 3% (w/w) gadoterate meglumine at the time of administration.

### 2.3. Preparation of cream

The hydrophilic and lipophilic phases of the cream were separately heated to 70 °C. After mixing both phases, the cream was cooled to room temperature while stirring continuously. Cream pH was adjusted to 4.5. Immediately prior to dosing, the cream was physically mixed with Indium-111 DTPA (about 18.5 MBq/5 g cream) or gadolinium chelate (final concentration: 3%, w/w), and filled into aluminum tubes coated with epoxy layer.

### 2.4. Sheep studies

#### 2.4.1. Progesterone assay

Sheep experiments were carried out under approval of the Ethical Committee for Animal Research (Ghent University). Research was adhered to the "Principles of Laboratory Animal Care" (NIH publication # 85-23, revised in 1985). Six female sheep (Texel breed) of 7–9 months old with a body weight between 30 and 35 kg were used. The sheep were housed in holding pens at the Faculty of Veterinary Sciences (Ghent University, Belgium) from 2 weeks prior to the study until the end of the study. The animals were fed an ewe meal and had free access to water. The animals were not fasted prior to the study and supply of water was also not affected. At the start of the study, the stage of oestrous cycle in all sheep was determined by measuring plasma progesterone concentration. A blood sample (8 ml) was collected in a heparinised tube, by direct venipuncture

of the jugular vein. Plasma was separated from blood by centrifugation at 3000 rpm and stored at −20 °C awaiting analysis. Plasma progesterone was measured by radioimmunoassay, using a DSL-3900 ACTIVE® Progesterone Coated-Tube Radioimmunoassay kit (Diagnostic Systems Laboratories, Texas, USA). The progesterone concentration were very low (0.1–3.5 ng/ml) in all sheep, confirming their anoestrous stage.

*2.4.2. Administration of cream and pellets to sheep*

Initially the radiolabeled cream was administered intravaginally to each sheep and after a washout period of 6 weeks a hard gelatin capsule filled with radiolabeled pellets was inserted. To facilitate dose administration, the sheep were lightly sedated with an intramuscular injection of xylazine (Xyl-M® 2%, VMD, Arendonk, Belgium) at a dose of 0.15 mg/kg body weight. An accurately weighed amount of radiolabeled cream (±5 g) was applied deep into the vagina, using applicators supplied with the marketed Gynomyk® cream. Gamma scintigraphy was performed 0, 1, 2, 4, 6, 8, 12 and 24 h post administration. After a washout period of 6 weeks, a control scintigraphy was performed to confirm the absence of radioactivity in the vagina. Afterwards a hard gelatin capsule filled with radiolabeled pellets was administered with a commercially available vaginal applicator (13 cm length) (Infemin applicator, Pierre Fabre Santé Benelux, Brussels, Belgium) and scans were taken at 0, 4, 6, 8, 12 and 24 h after administration.

*2.4.3. Gamma scintigraphy*

The distribution and retention of the cream and pellet formulation in the vagina was evaluated by gamma scintigraphy, using a Toshiba GCA-901 gamma camera. The fleece on the head and back of each sheep was marked with animal stock marker and these lines were lined-up with marks on the table to ensure that each sheep was roughly in the same position for each scintigraphic image. The sheep were restrained on the table in right lateral recumbency with the camera positioned underneath the table centered over the vaginal area and the images were obtained by registering activity for 2 min. Processing and analysis of data was performed with the Multimodality software (version 5) of nuclear diagnostics (Nuclear Diagnostic, Stockholm, Sweden).

*2.4.4. Vaginography*

To outline the vagina of the sheep a vaginography was performed. A Foley balloon catheter was vaginally inserted and 20 ml iopromide (commercially contrast fluid, Ultravist®, Bayer Healthcare Pharmaceuticals, Wayne, NJ) was injected into the balloon catheter, followed by an X-ray scan.

*2.4.5. Analysis of gamma scintigraphy scans*

The vaginal template outlined via the vaginograms was used to facilitate the drawing of the regions of interest (ROI) for all scintigraphic images. For each scintigraphic scan, three ROIs were drawn: (i) ROI corresponding to the vaginal area, (ii) background ROI and (iii) ROI corresponding to the area of initial deposition of the cream or pellet formulation. The total radioactivity (counts per minute per unit area: cpm/cell) in each vaginal ROI was calculated, by correcting for background activity and radioactive decay. To determine the vaginal retention of the formulation, the radioactivity present in the vaginal ROI at zero time was denoted as the total amount of radioactivity deposited in the vagina (100%). For each scan at subsequent times, the actual activity was expressed as the percentage of the total activity at time 0 (i.e., administered dose). Hence, the retention of each formulation in the vagina was evaluated by the decrease of activity against time. Graphs were plotted of the remaining activity at each time point for all sheep and the area under curve (AUC) was calculated. The SPSS statistical software package (version 17, Somers, New York) was used to perform the statistical analysis by paired sample *t*-test for the AUC, at a significance level of 0.05.

To assess the distribution within the vagina, the longitudinal length of vaginogram was considered as the vaginal length and based on the spread of the formulations in the vaginal ROI the distribution of the radiolabeled formulations was calculated as percentage of the vaginal length.

*2.5. Women trial*

*2.5.1. Study protocol*

Cetomacrogol cream and starch-based pellets were administered to 6 healthy female, nulliparous volunteers (age 21–28 years, mean body weight 55.5 ± 4.5 kg) in a randomized, crossover trial and MRI was performed to monitor the distribution and retention of the formulations. The study was approved by the Institutional Ethical Review Board of Ghent University (Belgium). The research followed the tenets of the Declaration of Helsinki. Subjects were recruited by written informed consent during their first screening visit and assigned a subject number. Subjects were compensated for their time and effort. Screening examinations included a brief questionnaire on medical and gynecologic history; thereby also asking for presence of any genital symptoms (subject-reported), a pregnancy test, pelvic and colposcopy evaluation, assessment of vaginal pH, microscopic examination and culture of a vaginal smear and Chlamydia test on an endocervical sample. Subjects were excluded from the trial if any infections like candidiasis, bacterial vaginosis or chlamydiosis were present. To be included in the trial, women had to meet the following criteria: good health, normal results from physical and genital/gynecological examination, signed informed consent and regular menstrual cycle. Exclusion criteria were as follows: clinically significant abnormal physical examination and/or laboratory findings; vaginitis at the time of screening, active dermatologic abnormality of the vagina (including atopic dermatitis, lichenification, etc.), history of operations and vaginal hysterectomy, diagnosed pelvic floor dysfunction, vaginal breakthrough menstrual bleeding, pregnancy and presence of an intrauterine device or a metal prosthesis, claustrophobia, use of local or systemic antibiotics (including antifungal and antiprotozoal drugs) from 3 weeks prior to start of the study.

The initial visit of volunteers was a screening visit, followed by two concurrent visits when either gadolinium-labeled pellets (filled into hard gelatin capsule) or cream was administered. A 2-week washout period was respected between both the tests. After cream application, scans were taken at 0, 1, 4, 8, 24 h, whereas for pellets scanning was done at 0, 4, 8, 12 and 24 h following initial insertion.

The formulations were administered to the study subjects by a gynecologist, when they were lying supine on the MRI scanner. The study subjects were remained supine for 6 h to mimic the dose administration of vaginal formulations during night time. To minimize activity by the subject, they were transferred in a wheel chair from the scanner to the resting room. After 6 h, the subjects were allowed to go home but urged to not do any intensive activity (no sports), during 24 h after formulation administration. Only light physical activity was allowed. Use of intimate hygiene products (spray, intimate deo, vaginal solutions) was prohibited. Plucking or trimming of pubic hair during the trial, use of tampons and intercourse during 24 h trial period were also prohibited.

*2.5.2. Magnetic resonance imaging (MRI)*

T1-weighted MRI was performed to evaluate the distribution and retention of the cream and pellets in women, using a dedicated research 3 T magnet (Magnetom Trio, Siemens, Erlangen, Germany) with Tim upgrade, Siemens Leonardo workstation and Siemens syngo VB 15 software, using a pelvic phased-array coil

(to allow small fields of view and to increase signal-to-noise ratio). Transverse and sagittal T1-weighted sequences were taken: gradient-echo (GE) T1 w sequence (256 matrix, TR/TE 168/4.8), turbo spin-echo (TSE) T1 w sequence (512 TR/TE matrix, 400/12) and T2 w images (256 matrix, TR/TE 1310/89). Digitally stored images of MRI were used to measure the spread of cream and pellets. A single MR radiologist measured the spread of formulations in the vagina for each T1 weighted data set. Processing and all measurements were performed with the internal MR scanner software (Siemens Syngo). The maximum distances between the high-intensity signal of the contrast-enhanced cream or pellet spread in the transverse and in the sagittal directions were measured. Total linear length of vagina was measured on T2 images. The linear spread of each formulation, represented by the gadolinium-enhanced area in T1 images was measured and subsequently, the distribution was calculated as a percentage of the total linear vaginal length. Graphs were plotted for vaginal spread in function of time for all subjects and the area under curve (AUC) for each graph was calculated. Using SPSS, statistical analysis was performed by paired sample *t*-test for AUC of cream and pellet curves, at a significance level of 0.05.

## 3. Results

### 3.1. Sheep studies

Fig. 1a and b shows representative gamma scintigraphy scans at each time point after cream and pellet administration, respectively. Leakage of the cream and contamination on the vulva and

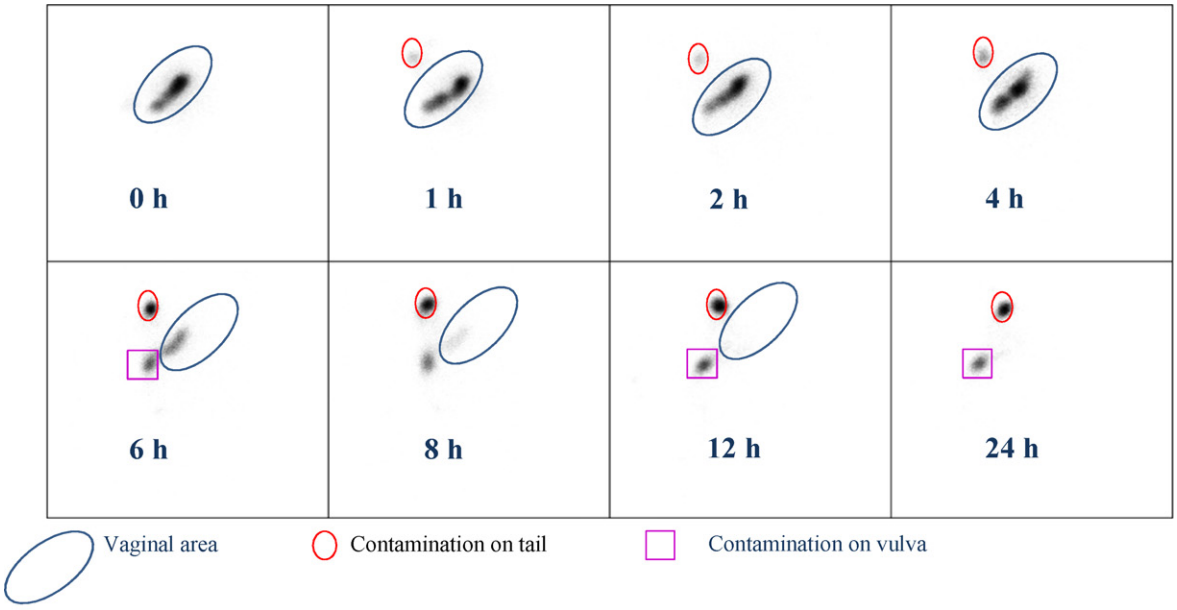


**Fig. 1.** Gamma scintigraphy scans after vaginal administration of (a) cream and (b) starch-based pellets (filled into hard gelatin capsule). These images show scans of a specific sheep (sheep 1).

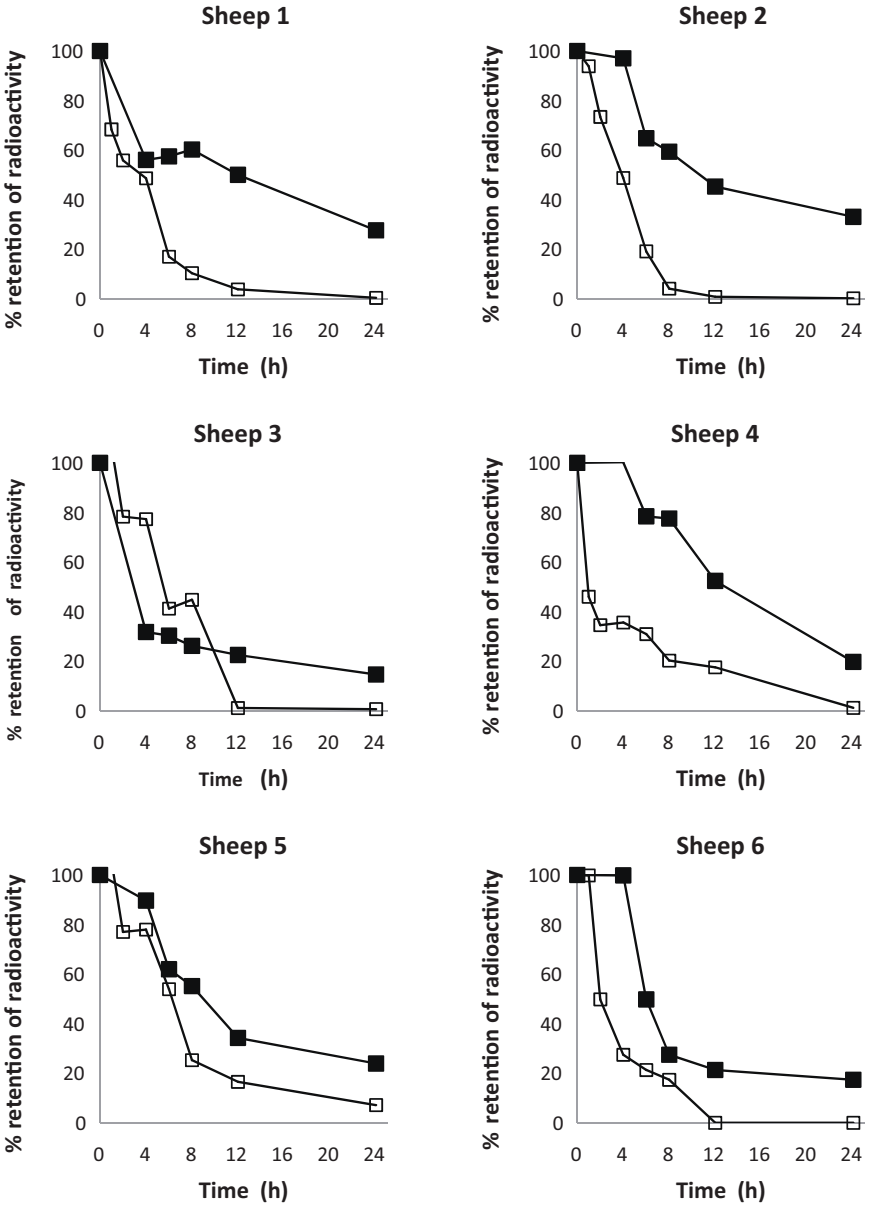


**Fig. 2.** Retention profiles of radiolabeled cream (□) and pellet (■) formulations in the vaginal area after vaginal administration to sheep ($n = 6$).

tail is clearly distinguished after application of the cream in the vagina. There was no leakage or contamination after vaginal administration of the pellets. Cream already leaked during first 1 h after administration. This observation is not apparent from the scans as the vulva and tail were cleaned prior to scanning with decontamination solution (Radiacwash, Biodex Medical Systems, New York) when leakage was visually observed, in order to get a clear image of the vaginal area in the scans.

The radioactivity remaining in the vaginal area for the cream and pellet formulations in each sheep is depicted in Fig. 2. Within 1 h of cream administration, a sharp drop in activity was observed confirming the leakage of cream from the vagina. After 6 h 31 ± 15% of the cream was retained in the vagina, while after 12 h cream could only be detected in 2 sheep (about 20% activity remaining), while in the other 4 sheep the entire dose of administered cream was cleared from the vagina. Between 35 and 100% of the pellets remained in the vaginal area 4 h after administration, with more than 90% of the pellets detected in 3 sheep. The average pellet retention after 24 h was 23 ± 7%. The average retention profiles (Fig. 3a) showed that both formulations were gradually cleared after vaginal application, but – in contrast with the cream – a significant fraction of the pellets still remained in the vagina after 24 h. Statistical analysis of the individual retention curves (based on the AUC values) confirmed the better performance of the pellets with respect to the retention in the vagina: AUC after pellet application increased by 622 units (95% CI: 246–998, $p = 0.008$, paired sample $t$-test) compared to the cream.

Distribution of the cream and pellets in the vagina (assessed via the procentual spread of the formulation over the entire vaginal length) indicated that the cream immediately distributed throughout the vagina after administration, while the pellets were initially concentrated at the application site as they were administered via a hard gelatin capsule (Fig. 3b). The cream remained distributed throughout the vagina during the measurement period. Data about the spread of cream after 12 and 24 h are not shown as the activity remaining in the vagina is too low to obtain meaningful data. The pellets (administered via a hard gelatin capsule) could only distribute in the vagina after disintegration of the capsule (4–6 h)

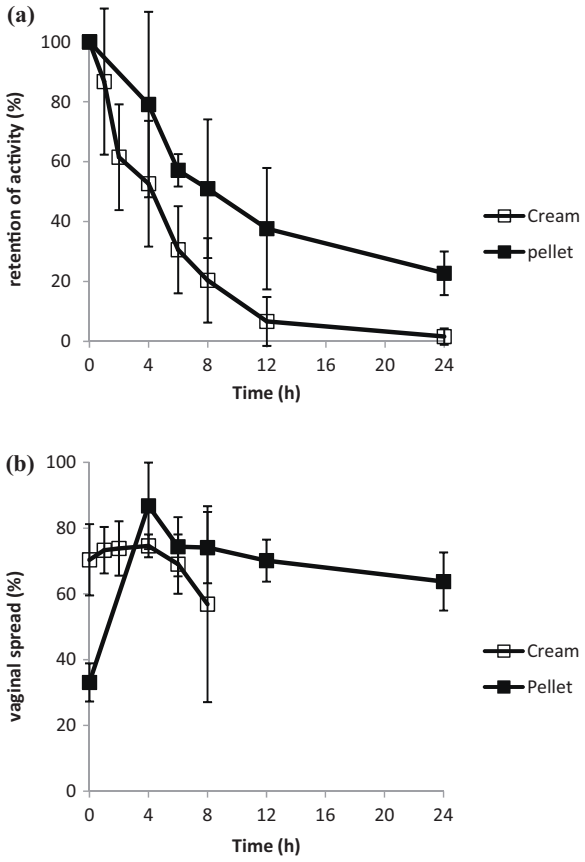


**Fig. 3.** Mean retention of activity (a) and mean spread in the vaginal area (b) after vaginal administration of cream (□) and pellet (■) formulation to sheep ($n = 6$). Error bars indicate standard deviations.

and release of pellets in the vagina. Afterwards the pellets spread throughout the vagina, and radioactivity was still detected in the entire vaginal area after 24 h.

### 3.2. Women trial

Figs. 4 and 5 show the spread of cream and pellets, respectively, on the sagittal T1-weighted three-dimensional gradient echo magnetic resonance scans. The contrast between the cream and the pelvic structures in the MRI scans was high due to gadolinium incorporated in the cream as an MRI marker. Immediately after administration (0 h), the initial bolus of cream was primarily located in the upper vagina, but over time the spread extended to the lower vagina. After 1 h the cream had already distributed to the lower vagina and at 4 h a large fraction of the cream was cleared from the vagina, with only a thin layer of cream lining the vagina at the end of the trial (24 h). All volunteers received about 5 g cream, except volunteer 4, where a second administration was required due to an initial failure of the applicator, resulting in a total administered amount of about 7 g cream. As a result, leakage at the introitus was observed, as gadolinium was detected on the MRI scan below the introitus.

The hard gelatin capsule containing the pellets was applied at the anterior fornix in 3 subjects and at the posterior fornix in the other 3, as assessed via MRI scans at 0 h. At 0 h, no high intensity (white) signal of gadolinium was detected on the MRI scans, as the marker was incorporated in the pellets as a dry powder. This created the susceptibility artifact, seen as a black spot deep in the vagina (Fig. 5, scan at 0 h). The MRI signal of the pellets only intensified after disintegration of the capsule, release of the pellets and dissolution of the gadolinium chelate label. MRI data indicated that in 5 volunteers the pellets were released from the capsule within 4 h, but that the spread was limited to upper vagina (cervical area). In one volunteer, disintegration of the capsule occurred between 4 and 8 h after administration. At the 8 h time point, the pellets had spread to the lower part of the vagina. At 12 and 24 h pellets were detected throughout the vagina. Both the pellet and cream formulation had spread into the endocervical canal of 2 volunteers.

Fig. 6A shows a sagittal MRI scan, illustrating the parts of the vagina used to measure the linear spread of cream and pellets within the vagina. The linear spread is a assessment of the part of the vagina along the axis from the cervix to the introitus covered with the formulation, expressed as a percentage of the total linear length of the vagina (Barnhart et al., 2004a). Fig. 6b and c presents the transverse images for cream (at 1 h) and pellets (at 24 h), respectively, visualizing the W-shape of the vagina confirming the complete coverage of the vaginal mucosa with cream and pellets. Fig. 7 represents the individual vaginal spread profiles over a 24 h period for both cream and pellets, in all volunteers.

In volunteer 3, the pellet spread at 12 h could not be clearly detected, however at 24 h 80% of vaginal area was covered by the pellet formulation. Discontinuous spread of the pellets in some parts of the vagina was observed, which might be due to an insufficient amount of vaginal fluid, reducing the wetting of the pellets and decreasing visualization of the formulations in the scans. The radiologist reported a very mobile uterus in volunteer 6 (e.g. anteflexion, cranioversion), which affected the reproducibility of the spread measurement in the vagina.

Although the AUC data of the linear spread vs. time profile (Fig. 8) did not indicate a significant difference between cream and pellet (mean difference: 337 unit, 95% CI: −186 to 860, $p = 0.159$), the behavior of these formulations during the first hours of the test was different. The cream immediately covered a large portion of the vagina and the spread remained fairly constant throughout the entire test period (although – as previously mentioned – the amount of cream residing in the vaginal cavity at the later time points was minimal). In contrast, the pellet formulation gradually spread throughout the vagina as disintegration of the hard gelatin capsule and wetting and disintegration of the pellets was needed before the material could distribute in the vaginal cavity. In addition variability of the pellet spread was higher (specifically at the initial time points) as intravaginal disintegration of the hard gelatin capsule will be variable.

Quantitative assessment of the MRI data (to determine the percentage of formulation remaining in the vagina at each time point) was not done as the MRI marker (gadolinium) was present in a dry form in the pellet formulation and differences in the amount of gadolinium dissolved at each time point would affect the intensity of the MRI signal, hence preventing accurate quantification of the MRI scans.

## 4. Discussion

Intravaginal formulations are used either for treatment of local infections (e.g., vaginitis, vaginal atrophy), for systemic drug delivery (e.g., delivery of steroids for hormone replacement therapy) or for preventive measures (e.g., spermicides for contraception, microbicides for HIV prevention). Based on the desired effect of treatment, the medication should be retained at a specific area or cover the entire vagina and should remain in contact with the vaginal mucosa for a significant period of time. Hence, quantification and optimization of the distribution of intravaginal formulations is essential (Barnhart et al., 2001).

The interest in using animal models other than nonhuman primates for the evaluation of vaginal formulation is evident. Sheep

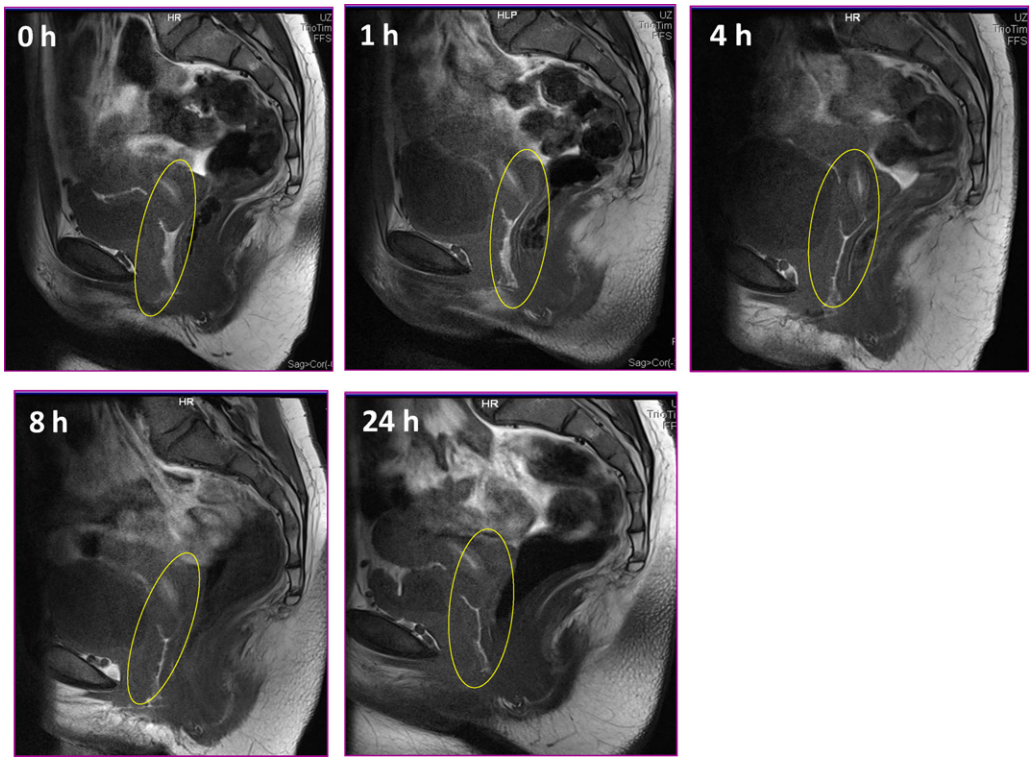


**Fig. 4.** Sagittal T1-weighted three-dimensional gradient echo magnetic resonance scans obtained 0, 1, 4, 8 and 24 h after intravaginal administration of cream. Circle identifies the vaginal area with a high contrast between cream and pelvic structures due to the incorporation of gadolinium. At 0 h cream was primarily located in the upper vagina. At 1 h, distribution had extended to the lower vagina. At 4 h most of the cream was cleared from the vagina, with only a thin layer remaining at the end of the trial (24 h).

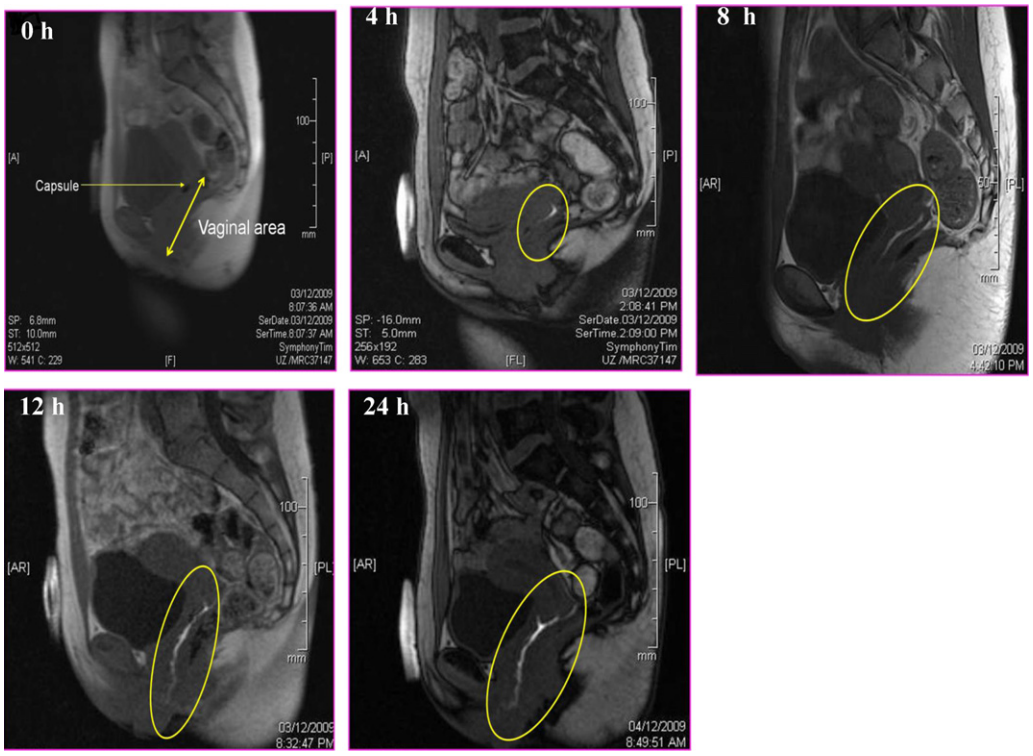


**Fig. 5.** Sagittal T1-weighted three-dimensional gradient echo magnetic resonance scans obtained 0, 4, 8, 12 and 24 h after administration of capsule filled with pellets. Circle identifies the vaginal area with a high contrast between pellets (released from disintegrated capsule) and pelvic structures due to the incorporation of gadolinium. At 0 h the capsule was intact and visible as a black spot deep into the vagina, as gadolinium was not in contact with water. At 4 h pellets were released from the capsule but the spread was limited to the upper vagina (cervical area). At 8 h, the pellet spread had extended to the lower part of the vagina. After 12 and 24 h, pellets were spread throughout the vagina.

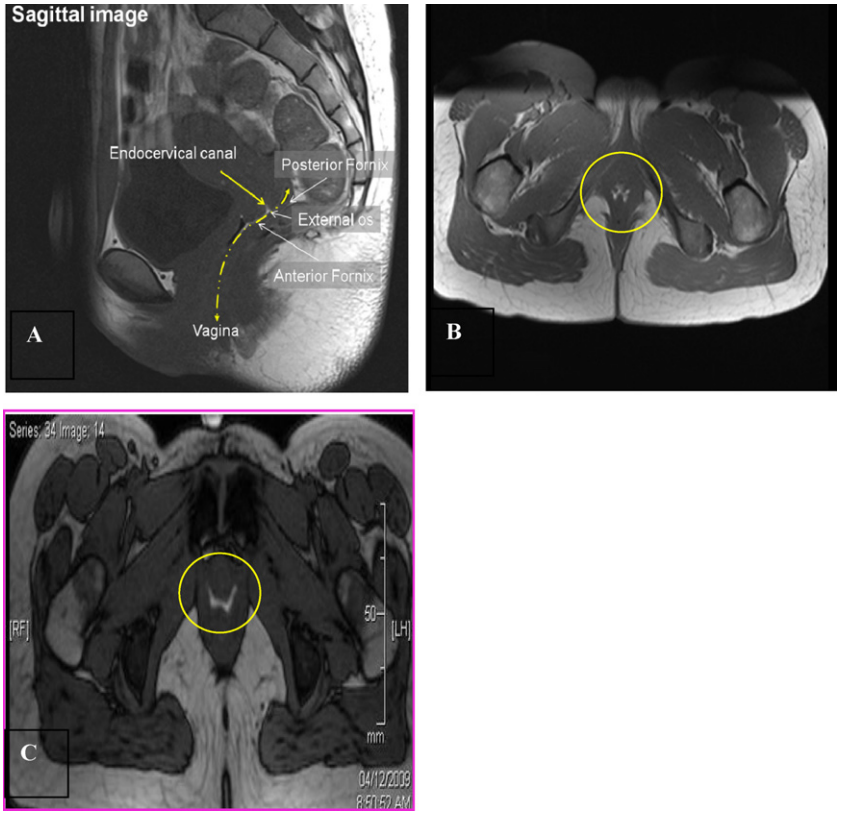


**Fig. 6.** (A) Sagittal image illustrating the parts of vagina used for measurement of the linear spread, (B) transverse image 1 h after cream administration, (C) transverse image 24 h after pellet administration. The W-shape of the vagina is identified in the transverse images, indicating complete vaginal coverage with the formulations.

are good candidates to fulfill this role based on a number of criteria. They have a size that facilitates conventional gamma camera imaging as opposed to small laboratory animals, and their vagina has comparable characteristics to the human vagina. The vaginal length is 9–13 cm in sheep and 8–12 cm in women. The cervical diameter in women is 2.3–3.3 cm, versus 1–1.15 cm in sheep. Furthermore, histological evaluation of sheep vagina and cervical epithelium indicated that the stratified squamous epithelium overlying the dense vascular submucosa is thinner but similar in structure to human epithelium. Sheep are relatively inexpensive, easy to handle, and available in large numbers with the ability to control for age, reproductive state and sexual history (Vincent et al., 2009). Also the vaginal tract in sheep is easily accessible for the administration of delivery systems and the docile nature of the animal allows the experiments to be performed without sedation or anesthesia (Richardson et al., 1996). Vincent et al. (2009) already used sheep during toxicity studies to evaluate the epithelial injury in the vaginal tract after exposure to benzalkonium chloride by colposcopy and tomography. We expanded the use of sheep as model to assess the behavior of dosage form by evaluating the intravaginal distribution and retention of a multiparticulate pellet formulation and a cream, using gamma scintigraphy as non-invasive imaging technique.

The use of radiolabeled formulations in combination with gamma scintigraphy is an elegant and relatively cheap modality to explore their intravaginal distribution and retention. This technique has already been used in sheep to monitor the retention and spreading of muco-adhesive microspheres over a 12 h period, after intravaginal administration as a dry powder or dispersed in a pessary (Richardson et al., 1996). Gamma scintigraphic evaluation of vaginal dosage forms in women indicated limited intra-subject, but considerable inter-subject variability in clearance: after 6 h between 2 and 80% of the formulations (radiolabeled pessary and polycarbophil gel) was cleared in post-menopausal women (Brown et al., 1997), while Chatterton et al. (2004) determined that the vaginal retention of a clotrimazole cream and test gel (both labeled with $^{99m}$Tc-DTPA) in pre-menopausal women after 24 h ranged 1 and 81% of the administered dose. Similar to our trial, these studies did not report migration of the formulations to the surrounding organs (uterus, urinary bladder).

The gamma scintigraphic scans indicated that the cream formulation (having a similar viscosity as commercially available creams intended for vaginal application) was immediately available within the entire vaginal cavity. However, the cream continuously leaked from the vagina, resulting in a fast clearance of the formulation which would require frequent application in a clinical situation. In contrast, vaginal residence time of the pellets was prolonged as even at the end of the evaluation period (24 h) a significant amount of tracer was detected in the vaginal area. However, a drug incorporated in the pellet formulation will not be immediately available as the hard gelatin capsule (used to administer the pellets intravaginally with the aid of an applicator) needs to disintegrate. The in vivo disintegration time of hard gelatin capsules after intravaginal administration to women was in excess of 4 h (determined via colposcopy) (Poelvoorde et al., 2009). The starch-based pellets can only disintegrate in the vaginal fluids after opening of the capsules, and afterwards the material will distribute throughout the vagina. Once the pellets were released from the hard gelatin capsule, the formulation distributed over the entire vagina. In contrast to the cream formulation, the loss of the pellets from the vagina over time was not due to continuous leaking of material, but linked to urination.

To assess the value of sheep as model to evaluate the behavior of dosage forms after vaginal application, both formulation (starch-based pellets and cetomacrogol cream) were administered to women. To avoid exposure of the volunteers to a radioactive

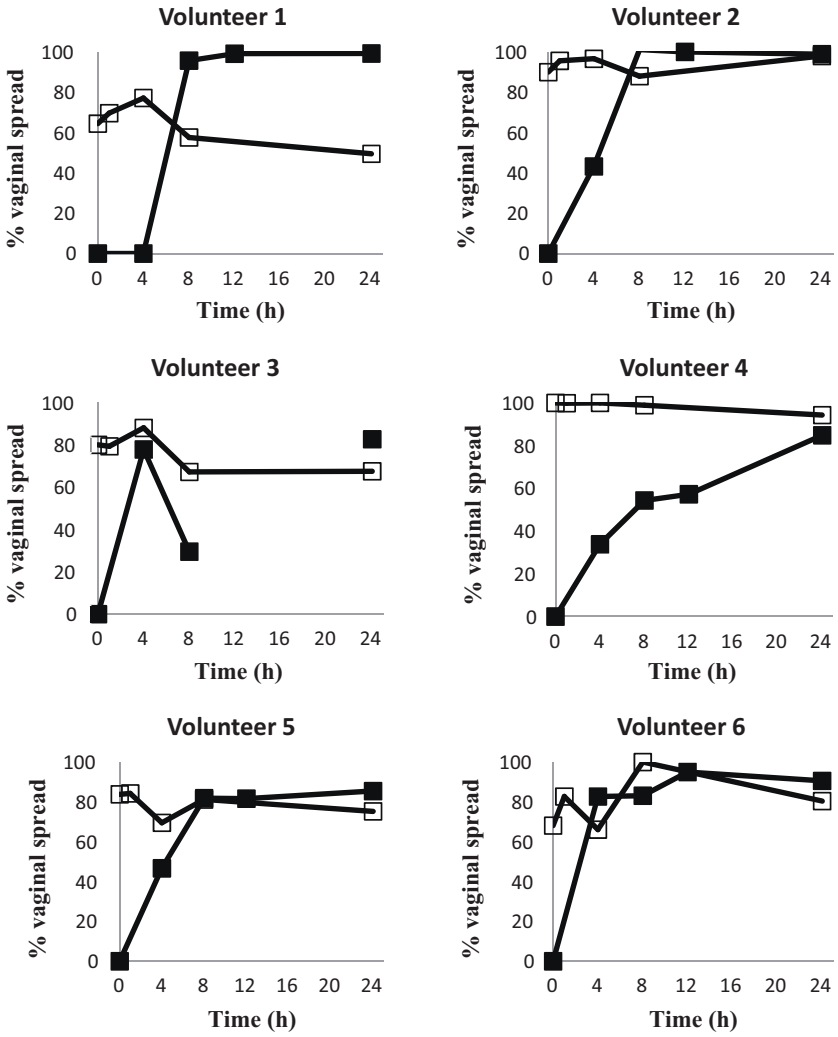


**Fig. 7.** Vaginal spread profiles over a 24 h period after intravaginal administration of the cream (□) and pellet (■) formulations to 6 volunteers. The vaginal spread is expressed as the percentage of the total vaginal length covered by the formulation.).

marker, MRI was used as non-invasive, non-ionizing imaging technique. Gadolinium was used as contrast agent as it was reported to be an accurate marker to study the intravaginal distribution of a gel since it did not leak from the formulation during distribution (Barnhart et al., 2001, 2006).

An important quantitative measure to monitor the distribution of dosage forms within the vagina is the linear spread, i.e., the total linear length along the axis of the vagina where formulation can be detected (Barnhart et al., 2001, 2005a; Pretorius et al., 2002b). This parameter indicated the immediate distribution of the cream throughout the vagina, but MRI also visualized a rapid clearance of the cream (although only qualitatively assessed based on the thin lining of contrast agent on the vaginal mucosa 8 and 24 h after cream application. These observations are consistent with our scintigraphic data obtained in sheep and with an MRI study monitoring gel distribution in women (a gradual decrease in mucosal coverage over time, with minimal coverage at 24 h after application of 3.5 ml gel) (Barnhart et al., 2005a). By contrast, the pellet formulation gradually distributed throughout the vagina (since disintegration of the capsule was required before spreading of the formulation could occur). The exact time point of capsule disintegration is not determined, but MRI scans indicated that in 5 women the capsule opened within 4 h of administration. However, at the initial time point (4 h) the pellets were mainly concentrated in the upper vagina. Based in the qualitative assessment of the MRI scans the residence time of the pellets was longer compared to the pellets as a high intensity signal was detected in the upper part of the vagina at the end of the evaluation period. This observation is consistent with our sheep study.

The W-shape of the vagina identified in the transverse images (axial plane) indicate complete coverage of the vaginal mucosa with formulation, as a thin coating of material separates the anterior and posterior walls of the vagina (Barnhart et al., 2004b). Using the cream the complete coverage was already seen at 1 h based on the rapid distribution of the cream, while the W-shape was most obvious for the pellets at 12 and 24 h.

Although different techniques were used to monitor the behavior of the formulations in sheep (gamma scintigraphy) and women (MRI) since no MRI equipment for veterinary application was available at the study site and exposure of the women to ionizing radiation was avoided, the similar trends observed for the distribution and retention (albeit based on qualitative analysis for MRI data) of the cream and pellet formulations during both studies support that sheep are a suitable animal model for future evaluation of vaginal dosage forms.

Both the sheep and women study indicated that the starch-based pellets performed better after intravaginal delivery compared to cream, as their retention time was longer (>24 h), reducing the application frequency. Compared to the cream (or semi-solids in general) starch-based pellets also provide a better option for the

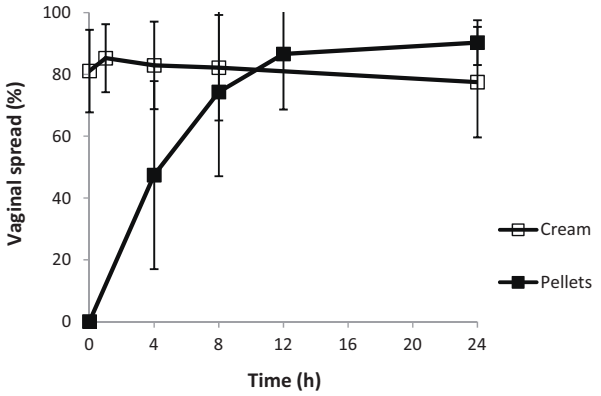


**Fig. 8.** Mean ($n = 6$) vaginal spread profile over a 24 h period after intravaginal administration of the cream (□) and pellet (■) formulations. The vaginal spread is expressed as the percentage of the total vaginal length covered by the formulation. Error bars indicate standard deviations.

patient towards patient compliance, as semi-solids are less convenient (messiness, leakage) (Hussain and Ahsan, 2005) and more prone to dosage errors upon self administration (Garg et al., 2003). However, to take full advantage of the therapeutical benefits of these starch-based pellets for vaginal drug delivery, optimization of the final dosage forms is essential since the main constraint of the evaluated formulation is the long in vivo disintegration time of the capsule (±4 h) after intravaginal administration. However, this problem could be overcome, either by using specially designed applicators (prefilled with pellets and sealed with a membrane) to allow direct introduction of the pellets in the vagina or by developing fast disintegrating tablets containing the starch-based pellets. Research in this area is ongoing.

## 5. Conclusions

Multiparticulate starch-based pellets have been identified a promising new vaginal drug delivery system, based on their complete coverage of the vaginal mucosa (similar to cream) and long retention time (>24 h) after intravaginal administration to sheep and women. We have also demonstrated that sheep have the potential to become a good model for evaluating the retention and distribution characteristic of intravaginal formulations.

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