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Novel thermosensitive *in situ* gel based on poloxamer for uterus delivery

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ABSTRACT

Side effects and drug residues are major concerns affecting hormone therapy of bovine reproductive diseases. Fertility-promoting intrauterine infusion liquid (FPL), an effective alternative to hormone therapy, is associated with short retention time and low therapeutic efficacy. To address these problems, we developed a thermosensitive *in situ* gel based on poloxamer 407 for local uterine administration. To achieve the desired gelling temperature and enhance local retention property, we added poloxamer 188 and HPMC to the formulation containing poloxamer 407 and FPL. After screening was performed, the optimized formulation showed good temperature sensitivity *in vitro* and *in vivo*. Gelation temperature was approximately 27 °C. *In vitro* release tests showed that icariin (the major active compound in FPL) was slow released from *in situ* forming gel. After the gel was locally administered, uterine and ovarian indexes were significantly increased in the gel group compared with the control group ($P < 0.05$). The serum estradiol level of the gel group was significantly higher than that of the control group ($P < 0.01$). Histological evaluation did not show mucosa irritation in the gel group. Therefore, the proposed *in situ* forming gel system based on poloxamer 407 is a promising local drug delivery system to treat bovine uterine diseases.

1. Introduction

Bovine reproductive diseases delay reproduction and decrease production. With increased costs of treatment and preventive measures, these diseases result in great economic losses (Bellows et al., 2002). Among the most important causes of bovine reproductive diseases are endometritis, persistent corpus luteum, and inactive ovarian infertility (Arthur et al., 1982; Fourichon et al., 2000). Common therapeutic strategies for persistent corpus luteum and inactive ovarian infertility disorders are treatments involving hormones, such as estrogen, follicle-stimulating hormone, and human chorionic gonadotropin (Barile, 2005; Becker and Johnson, 1992; Thatcher et al., 1993). Although steroid treatments are very effective and induce a rapid onset, such treatments elicit adverse effects (Meredith, 1979). Moreover, hormone residues in milk and meat pose potential risks to human health (Pfaff et al., 2000).

The limitations of hormone therapy in cattle can be solved by applying Chinese traditional medicine. Fertility-promoting intrauterine infusion liquid (FPL) is an extract from epimedium, safflower, and motherwort. FPL has been widely used in China and other Asian countries to treat dam infertility caused by inactive ovaries and a persistent corpus luteum. The efficacies of FPL to treat uterine infertility have been widely approved (Li et al., 2011; Liu et al., 2010). However, FPL is characterized by a short retention period in the uterus and is prone to leakage after infusion; thus, therapeutic effects are reduced.

In situ forming gel systems have been developed to address short local retention. The system is in sol form before this treatment is administered to the body. Once administered, the system undergoes gelation *in situ*. Gel formation depends on several factors, such as pH change, temperature modulation, or presence of ions. The advantages of the system include localized and site-specific action, prolonged drug delivery, decreased drug dosages, reduction of side effects, and improved patient comfort and compliance (Abashzadeh et al., 2011; Chenite et al., 2000). *In situ* gel-forming system-based eye drops (Nirmal et al., 2010; Pandey, 2010; Song et al., 2013) and cavity suppositories (Kim et al., 2010; Yuan et al., 2012) have been developed to prolong the residence time of liquid dosage forms.

Poloxamer 407 (P407), also known as Pluronic F127, is a non-toxic, non-irritable biomaterial with good tolerability. P407 has been widely used in topical, rectal, and ocular formulations (Bansal et al., 2007; Chiappetta and Sosnik, 2007; Dumortier et al., 2006). With good temperature sensitivity and

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biocompatibility, P407 is an ideal excipient to prepare temperature-sensitive *in situ* gel. *In situ* vaginal and nasal gels based on P407 have been developed to prolong drug release, reduce drug dosage, and increase bioavailability (Agrawal et al., 2010; Baloglu et al., 2011; Kim et al., 2010).

Thus far, thermosensitive *in situ* gels based on P407 have not been developed for local uterine drug delivery. Therefore, this study was conducted to develop fertility-promoting intrauterine infusion *in situ* gel (FPG), a thermosensitive *in situ* forming gel of FPL to address problems associated with FPL formulation. Gelation temperature, *in vitro* release properties, *in vivo* retention, and uterine tissue irritation, were evaluated. The effects on follicles and estrogen of rats were also investigated.

2. Materials and methods

2.1. Materials

FPL was prepared using *Epimedium,* safflower, and motherwort according to Chinese Veterinary Pharmacopoeia. According to our previous research (Lu et al., 2014), the average extraction yield and the concentration in extracting solution of Icariin are 0.223% and 2.233 mg/mL, respectively. Poloxamers (P407 and P188) were received as gifts from Nanjing Well Chemical Co., Ltd. (Nanjing, China). Hydroxypropyl methyl cellulose (HPMC) was donated by the Anhui Sunhere Pharmaceutical Excipients Co., Ltd. (Anhui, China). Sodium alginate (SA) was purchased from Chengdu Kelong Chemical Co., Ltd. (Sichuan, China). Methanol and acetonitrile (HPLC grade) were purchased from Tianjin Kemiou Chemical Reagent Co., Ltd. (Tianjin, China). All other reagents were of analytical grade.

2.2. Icariin assay

Icariin is the main active ingredient of FPG. Icariin was quantified using a Shimadzu LC-2010CHT HPLC equipped with a smart line degasser, 1000 pump, and a UV/Vis detector (Shimadzu, Japan) set at 270 nm. Chromatographic separation was achieved with a Kromasil C18 column (4.6 × 150 mm, 5 μm) equipped with a pre-filter at 25 ± 0.5 °C. The mobile phase consisted of 55% methanol and 45% water at a flow rate of 1.0 mL/min. The injection volume was 20 μL. The column was equilibrated for at least 1 h before injection was performed. The solvents were sieved using 0.22 μm Teflon membrane filters (Pozharitskaya et al., 2007). Icariin was eluted for 6.9 min, and the method showed linearity at a concentration range of 5–100 μg/mL. The limits of detection and quantification of icariin were 0.2 and 0.5 μg/mL, respectively.

2.3. In situ gel-forming system preparation

In situ forming gel was prepared using cold method, as described previously (Cafaggi et al., 2008; Choi et al., 1998). In brief, the required amount of P407, P188, HPMC, and SA were placed in a flat-bottomed vial and maintained at 4 °C until solids completely liquefied. FPG was obtained after the substances were mixed. A total of 11 formulations with different excipient amounts were prepared (Table 1). P407, P188, HPMC, and SA concentrations in the gel were presented as weight percentage (wt.%) unless otherwise specified.

2.4. Gelation temperature measurement

The FPG solutions were transferred to vials and stored at 4 °C overnight. On the next day, the vials were incubated in a water bath and equilibrated at 15 °C for 10 min. The sol–gel transitions of the solutions were evaluated by tube inversion method at a temperature range of 15–45 °C with a heating rate of 0.4 °C per min (Garripelli et al., 2010). Gelation temperature was defined as the temperature at which a FPG solution stopped flowing after the tube was inverted.

Table 1
In site gel forming compositions and its gelation temperatures.

No.	Concentration (%)					Mean $T_{sol-gel}$ ± SD. (°C, n = 3)
	P 407	P 188	HPMC	SA	FPL	
Control	20	–	–	–	–	42.5 ± 0.5
H-1	20	–	0.3	–	–	40.0 ± 0.2
H-2	20	–	0.5	–	–	40.1 ± 0.2
H-3	20	–	0.7	–	–	39.9 ± 0.3
S-1	20	–	–	0.3	–	40.2 ± 0.3
S-2	20	–	–	0.5	–	41.4 ± 0.2
S-3	20	–	–	0.8	–	39.7 ± 0.3
F-1	20	–	–	–	+	28.8 ± 0.4
F-2	19	2.5	0.3	–	+	27.3 ± 0.4
F-3	18	5	–	0.3	+	29.3 ± 0.3
F-4	18	7.5	0.3	–	+	27.9 ± 0.2

– Means no added.
+ The amount of FPL was 100 g gel containing extracts of 100 g herbal medicines.

2.5. In vitro release studies

FPG solution (3 mL) was placed in a dialysis tube (cutoff 14,000 D). The releasing medium was 200 mL phosphate buffer solution (pH = 7.2) containing 0.15% methylparaben as a preservative. The release study was performed in a shaking water bath at 37.5 °C. Sink conditions were maintained during the whole study. The medium (2 mL) was withdrawn at specific time points (0.5, 1, 2, 3, 5, 7, 10, 12, 18, 24, 30, and 36 h) and replaced with 2 mL of fresh medium. Icariin concentration was determined through HPLC, as described in Section 2.2. The concentration of Icariin in initial FPG is 1.733 mg/g. The experiment was performed in triplicate.

2.6. Gelation characterization

For *in vitro* evaluation, 10 mL of FPG was transferred to a small beaker and incubated at 37 °C. The system was monitored every 2 min for gelation. For *in vivo* evaluation, 0.5 mL of FPG was injected subcutaneously into the flank of a rat. The injection site was surgically cut open at 0.5 min post-administration. Gel formation was then observed (Abashzadeh et al., 2011).

2.7. In vivo evaluation

2.7.1. Uterus and ovary indices

Female SD rats weighing 180–200 g were purchased from the Experimental Animal Center of Chengdu (Sichuan, China). All of the rats were fed with a standard laboratory diet and tap water under climate-controlled conditions of light dark (25 °C, 55% humidity, 12 h: 12 h). The animal experimental procedures used in this study were approved by the Ethics in Animal Research Committee of the Sichuan Agriculture University.

A total of 24 female SD rats were divided into three groups. These rats were treated with 20 mL of normal saline (Group A), FPL (Group B), or FPG (Group C) via the uterus for one week. Afterward, the rats were sacrificed at 24 h after the last administration. The uterus and the ovaries were collected and weighed. Uterine index (I_U) and ovarian index (I_O) were calculated based on the following equation:

$$I_U = M_U/M_B$$

$$I_O = M_O/M_B$$