

Effect of *Echinops spinosus* (Teskra) and PGF_{2α} on reproductive performance in cows with clinical endometritis



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Abstract

The efficacy of *Echinops spinosus* (Teskra) and PGF_{2α} in the treatment of clinical endometritis (CE) on clinical recovery and reproductive performance was evaluated. A total of 20 Holstein-Friesian cows with CE that were 21-40 days in milk (DIM) were assigned to one of two experimental groups. Cows in the PGF group ($n=10$) received two doses of d-cloprostenol (analogue of PGF_{2α}) 14 days apart. Cows in the Teskra group ($n=10$) received a decoction of Teskra root administered orally once daily for three days. The control group consisted of 20 clinically healthy cows ($n=20$; HE group). The clinical recovery rate was 70% and 40% in PGF and Teskra groups, respectively ($P>0.05$). The proportion of pregnant cows at first service was 35%, 40%, 20% in the HE, PGF, and Teskra groups, respectively ($P>0.05$). However, the conception rate until 120 DIM was significantly higher in the HE group compared to the

Teskra group ($P<0.05$). The mean days from calving to the first service interval (CFSI) were significantly lower in the HE group compared to the PGF and Teskra groups ($P<0.001$). Cows in the Teskra group conceived approximately 35 d and 26 d later than cows in the HE and PGF groups, respectively ($P<0.05$). Cows in the Teskra group required about 2.4 services to become pregnant, compared to 1.8 and 1.6 for those in the HE and PGF groups, respectively ($P>0.05$). Except for CFSI, no significant differences were found between the HE and PGF groups for any of the tested parameters. The results indicate that endometritic cows treated with PGF_{2α} had nearly the same reproductive performance as healthy cows and Teskra was not effective in treating CE in dairy cows.

Key words: clinical endometritis; Teskra; PGF_{2α}; recovery rate; reproductive performance

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Introduction

A healthy uterus is one of the key factors influencing reproduction success (Đuričić et al., 2012; Kočila et al., 2013; Boudelal and Niar, 2020; Mimoune et al., 2021). One of the most important reproductive disorders affecting cows is clinical endometritis (CE), which is known to alter the fertility of dairy cows by reducing reproductive efficiency (Đuričić et al., 2014; Dobos et al., 2022), thus increasing rearing costs (Giuliodori et al., 2013; Boudelal et al., 2020). Bacteria are often present in the bovine uterine lumen after parturition, which in most cows is remedied by a series of uterine defence mechanisms (Sheldon et al., 2008). However, the persistence of these pathogenic bacteria leads to uterine diseases. CE is commonly diagnosed by rectal palpation and vaginal examination (Knutti et al., 2000; LeBlanc et al., 2002). It is defined as the presence of purulent (>50% pus) or mucopurulent (approximately 50% pus, 50% mucus) uterine fluids detectable in the vagina, 21 days or more postpartum, without systemic signs (Williams et al., 2008). This disease is most commonly associated with the presence of several bacteria species, including *Trueperella pyogenes*, *Fusobacterium necrophorum*, *Prevotella* spp., and *Escherichia coli* (Williams et al., 2005). Multiple approaches have been used to treat CE, most commonly antibiotic drugs (LeBlanc et al., 2002; Tison et al., 2017; Ahmadi et al., 2018). Otherwise, several alternatives to antibiotics have been tried, including enzymes (Drillich et al., 2005), dextrose 50% (Ahmadi et al., 2019), antiseptics (Mido et al., 2016), and liquid paraffin (Ahmadi et al., 2019), with conflicting reports. PGF_{2α} and its analogues are widely used as a therapeutic agent for clinical endometritis in dairy cows (Borchardt et al., 2018; Boudelal et al., 2022), with some controversial results (Haimerl et al.,

2018). The use of herbal remedies for the treatment of bovine endometritis and as a prophylaxis has also been reported (Arlt et al., 2009; Rautela et al., 2018).

Due to increasing concerns about antibiotic resistance and drug residues in food products (i.e., milk and meat), the use of alternative therapies in livestock production has risen and become a subject of recent scientific investigations (Huang et al., 2018). Plants are frequently used as natural remedies in human and animal healthcare (Rautela et al., 2018; Miara et al., 2019). Among these medicinal plants, *Echinops spinosus* (*Asteraceae*), vernacularly called Teskra, is a traditional remedy recommended in Algeria for reproductive disorders in sheep and cattle, and also for humans (Miara et al., 2018, 2019). Indeed, this plant is used to accelerate and facilitate delivery, to expel the placenta and clean the uterus, leading to earlier postpartum recovery and faster return to normal cyclicity (Miara et al., 2018, 2019; Bouzabata et al., 2018). Also, Teskra can be used as an abortifacient remedy (Bouzabata et al., 2018). Phytochemical tests of Teskra have revealed the presence of flavonoids, alkaloids, tannins, reducing sugars, and quinines (Gheffour et al., 2015). Many sesquiterpenoids have been isolated from the roots of *Echinops spinosus* (Dong et al., 2008; Yahyaoui et al., 2018). Moreover, pharmacological studies have demonstrated that Teskra has anti-inflammatory, antioxidant, and antimicrobial activity (Gheffour et al., 2015; Bouzabata et al., 2018; Yahyaoui et al., 2018).

In North Africa, especially Algeria, few clinical studies have been carried out on the efficacy of medicinal plants as an effective and safe alternative to modern medication, despite their various properties. This is indeed the case of *Echinops spinosus*, and its clinical efficacy against various diseases that it is traditionally used for has not been

widely tested. This study therefore aimed to test the ethnobotanical data obtained from the study of Miara et al. (2019) on the use of this plant as a postpartum recovery accelerator. To the best of our knowledge, no study has been published in this precise context, and this study is the first approach.

The aim of the study was to obtain a better understanding of the pharmacological action of Teskra by evaluating and comparing its efficacy against PGF_{2α} on clinical recovery rate and reproductive performance in cows with CE compared with healthy cows.

Materials and methods

Animals

The study was conducted from September 2018 to April 2019 on a commercial dairy herd in Ksar El Boukhari, Algeria (35°53'08"N, 2°45'03"E). With the owner's agreement, we applied the experimental protocol by keeping cows in free stall barns, in which a zero-grazing feeding system is followed. Animals receive a ration of corn silage with vetch oats hay, supplemented with grain mixture, and with free access to water. Parity of cows ranged from 1 to 4 lactations and their body condition score varied between 2.75 and 3.50 according to the model defined by Ferguson et al. (1994). Cows were milked twice daily and the average daily milk production was 17 ± 2.40 kg/day/cow. All cows were dried 60 days before the expected date of calving.

Plant Material

Specimens of *Echinops spinosus* spontaneously growing in the province of Sougueur (Tiaret) were collected wild around the city. Botanical determination was performed by Dr. Miara using the available literature and a voucher specimen was deposited in the herbarium of the Department of Ecology, University

of Tiaret, Algeria (voucher number: AST/20/31). The roots were washed and air-dried in a dark place at room temperature for one month.

Gynaecological examination and assignment to treatment

Each cow that was 21-40 days in milk (DIM; Exam 1) was subjected to rectal palpation to determine the size of the cervix, location and size of uterine horns, and to vaginoscopy to detect the possible presence of abnormal discharge in the vagina. The vulva was dry wiped, and cleaned with an antiseptic solution and the vaginoscope was inserted into the vagina. The nature of the vaginal contents was visually inspected with a torch and scored using a 4-point scale as described by Williams et al. (2005), where 0 indicates clear mucus, 1 mucus containing flecks of pus, 2 discharge containing less than 50% pus, and 3 discharge containing more than 50% pus. Affected cows were those with a vaginal discharge score equal to or greater than 1 (Boudelal et al., 2020). Cows with vaginal laceration at calving, those that had caesarean section and those that received any medication for at least 15 days prior to allocation were excluded from the trial.

Cows that required treatment between the date of allocation and re-examination were suspended. Cows with signs of CE were assigned to one of two treatment groups according to the last digit of their ear tag number (odd ear tag numbers were enrolled in the PGF group, and even ear tag numbers were enrolled in Teskra group). Cows in the PGF group ($n=10$), received two doses of 150 µg d-cloprostenol (Dalmazin®, FATRO S.p.A. Veterinary Pharmaceutical Industry, Ozzano dell'Emilia (Bologna), Italy) at an interval of 14 days. The second dose of PGF_{2α} was administered irrespective of the uterine status of cows upon re-examination two weeks later. The roots (100 g) of *Echinops spinosus*

were decocted in water and the cooled decoction was administered orally to afflicted cows ($n=10$) once daily for three days. Twenty ($n=20$; HE group) clinically healthy cows were left as a control. The lack of a negative control group was based on ethical grounds. After two weeks, all treated cows were re-examined (35-54 DIM; Exam 2). Clinical recovery was assessed by the absence of abnormal discharge upon re-examination. Cows that were uncured at the 2nd exam were re-treated according to their group and were subjected to a 3rd exam two weeks later. Cows that were culled or sold for reasons other than reproductive disorders before their first service were removed from the analysis. All cows were bred by natural service. The voluntary waiting period was set at 50 days and pregnancy controls were performed by ultrasonography at 30 ± 2 days post service.

Ethical approval

The owner's agreement was obtained beforehand after explanation of the context, objectives and risks of the study according to the Code of ethics and veterinary conduct of the Algerian state. The application of the experimental protocol and the recording of herd data was carried out with the verbal consent of the owner of the animals. The administration of drugs was taken by a qualified veterinarian, in a professional manner and respecting animal welfare. No animals were euthanized and the study was approved by the ethical review board of the Institute of veterinary science of the University of Tiaret in Algeria.

Reproductive parameters and statistical analysis

The reproductive parameters are shown in Table 1. Clinical recovery rate, first service conception rate (FSCR), and conception rate until 120 DIM were compared among the experimental groups using the Chi-square analysis

and Fisher's exact test. Reproductive parameter data (calving to the first service interval (CFSI), Calving to conception interval (CCI), and number of services per conception (NSP) are expressed as means \pm standard deviation (SD) and were compared using the Mann-Whitney test. Differences with $P < 0.05$ were considered statistically significant. Statistical analyses were performed using IBM SPSS, version 24.0.

Results

The obtained results show that there was no difference between the treatment groups for the clinical recovery rate, which was 70% for the PGF group and 40% for the Teskra group ($P > 0.05$). The proportion of pregnant cows at first service did not differ among the control and both treatment groups ($P > 0.05$). The proportions of pregnant cows at first service were 35%, 40%, and 20% in the control, PGF, and Teskra groups, respectively. However, the conception rate until 120 DIM was significantly higher in the HE group compared to treatment groups ($P < 0.05$), and was 70%, 50% and 20%, in HE, PGF and Teskra groups, respectively. In both treatment groups, CFSI was significantly higher compared to the HE group ($P < 0.001$) (Table 2). The mean CCI was significantly higher in the Teskra group compared to the HE and PGF groups ($P < 0.05$). Cows that received a decoction of Teskra root conceived approximately 35 d and 26 d later than healthy cows and those treated with PGF_{2 α} , respectively. NSP was slightly lower in the PGF group compared to the control group (1.6 *vs.* 1.8), whereas the highest value was found in the Teskra group (2.4), though the differences between groups were not significant ($P > 0.05$). Except for CFSI, no significant differences were found between HE and PGF groups for any of the tested parameters (Table 2).

Table 1. Description of reproductive indicators in cows in the healthy control group and two treatment groups

Parameters	Trait description
Clinical recovery rate (%)	number of cured cows on day 14 divided by the number of treated cows in each group*100
Calving to first service interval (CFSI)	number of days from calving to the first service
Calving to conception interval (CCI)	number of days from calving to successful service
Number of services per conception (NSP)	number of services divided by the number of pregnant cows
First service conception rate (FSCR %)	number of pregnant cows divided by the number of mated cows*100
Conception rate until 120 DIM	number of pregnant cows until 120 DIM divided by the number of cows enrolled *100

Table 2. Detailed reproductive performance analysis results for control and Teskra or PGF_{2α} treated cows

Parameters	Healthy	PGF	Teskra	P
Calving to Exam 1	27.80±05.04	31.60±10.02	32.60±07.32	0.34
Clinical recovery rate %	-	70 (7/10)	40 (4/10)	0.185
FSCR%	35 (7/20)	40 (4/10)	20 (2/10)	0.58
Conception rate until 120 DIM % [n]	70 (14/20) ^a	50 (5/10) ^a	20 (2/10) ^b	0.035
FCSI (mean±SD) days	63.65±13.96 ^a	77.50±08.11 ^b	88.20±11.71 ^c	<0.001
CCI (mean±SD) days	81.10±20.85 ^a	90.70±09.14 ^a	116.40±30.21 ^b	0.005
NSP (mean±SD) days	1.8	1.6	2.4	0.14

Values in lines with different letters (a, b, c) vary significantly ($P<0.05$).

Discussion

Clinical endometritis is a reproductive health problem often tackled by researchers to explore an effective drug against this disease. The evaluation of uterine discharge detected in the vagina is the most common method for diagnosing CE, as it is a rapid, inexpensive and informative technique (LeBlanc et al., 2002; Sheldon et al., 2008) and the standard established by Williams et al. (2005) has been applied in several studies (Drillich et al., 2006; Borchardt et al., 2018; Boudelal et al., 2022).

In this study, we found that the clinical recovery rate and FSCR did not differ between groups ($P>0.05$). The indices cited above were numerically the lowest in the Teskra group. Even though these indices were not statistically different, the treatment with Teskra appears to be ineffective. In the absence of a negative control group (i.e., untreated control cows) and due to the short interval between treatment and the first service conception, Lefebvre and Stock (2012) reported that FSCR would be the best measure to evaluate the

treatment effect. In addition, it has been reported that CE has a tendency to be recovered spontaneously (Hoedemaker, 1998; LeBlanc et al., 2002). Yahyaoui et al. (2018) found that the essential oils in *Teskra* roots had less antibacterial activity against *E. coli*, a pathogen involved in CE (Williams et al., 2005). The conception rate until 120 DIM was significantly higher in the HE compared to the *Teskra* group (70% vs. 20%). Huang et al. (2018) reported that the cumulative conception rate is the better parameter of reproductive performance. In general, for a true comparison between groups, it seems necessary to include a negative control group.

We also found that CFSI was significantly shorter in the control group compared to both treatment groups ($P < 0.001$) and the mean CCI showed a significant difference between groups ($P < 0.05$). In general, fertility is restored in the healthy cow from 40 to 50 DIM (Hoedemaker, 1998). In contrast, CE may be associated with more serious endometrial damage that needs treatment and more time to promote tissue regeneration and re-establish regular cyclicity before restoring fertility (Tison et al., 2017). Kim and Kang (2003) reported that the intervals from calving to first service and conception were prolonged by 23 and 36 days, respectively, in affected cows compared with healthy cows. CE also has detrimental effects on reproductive efficiency, as affected cows take longer to get pregnant (Giuliodori et al., 2013), which agrees with the results presented here.

PGF_{2α} has been commonly used as a therapeutic substance for postpartum diseases (Sheldon et al., 2008; Boudelal et al., 2022). PGF_{2α} can be used to restore uterine function by promoting luteolysis of the corpus luteum (CL) that then initiates a new cycle, oestrus emergence and subsequent uterine contractility, as well as stimulating

uterine defence mechanisms and leading to uterine clearance (Kasimanickam et al., 2005). Even though the CL is absent, PGF_{2α} increased uterine leukotriene B4 secretion that supports chemotaxis, cell-mediated cytotoxicity, phagocytosis, and lymphocyte function which improves uterine immune function (Slama et al., 1993). Eslami et al. (2015) evaluated the effect of PGF_{2α} on the clinical recovery rate in cows with ultrasonographically diagnosed endometritis at 31±3 DIM, according to the presence of a CL on their ovaries and found that recovery rate did not differ between groups. In their experiment, the cure rate was 54.46% and 62.61% for cows without and with CL, respectively. Likewise, PGF_{2α}-treated endometritis cows have a comparable recovery rate and reproductive performance, irrespective of their ovarian status (i.e., presence or absence of a palpable CL) (Knutti et al., 2000; Okawa et al., 2017). On the contrary, LeBlanc et al. (2002) reported that PGF_{2α} is ineffective unless a CL is present. In the current study, we found that affected cows treated with two doses of PGF_{2α} two weeks apart had a nearly identical reproductive performance with those in the control group. This is in agreement with the findings of Drillich et al. (2006), that cows with CE treated with two doses of PGF_{2α} 14 days apart had very similar reproductive performance near to healthy cows. With the application of one or two injections of PGF_{2α} 10 to 14 days apart, about 90% of cows with uterine infection are more likely to heal and have negative bacterial cultures (Hoedemaker, 1998). Multiparous cows with signs of CE treated with PGF_{2α} had a higher likelihood to become pregnant at the first AI (Borchardt et al., 2018). There are still contradictory reports about this therapy option on reproductive efficiency (Eslami et al., 2015). In a meta-analysis, Haimerl et al. (2013 and 2018) reported that PGF_{2α} had no significant effect on

reproductive performance. The time of PGF_{2α} administration according to parturition, presence or absence of CL, and the degree of uterine infection are the main factors affecting PGF_{2α} efficacy in dairy cows during the postpartum period (LeBlanc et al., 2002; Eslami et al., 2015). Regarding the effect of Teskra, the academic literature provides no science-based reports with which to compare our results.

Conclusions

In comparison with healthy cows, the Teskra group had a lower clinical recovery rate, FSCR and conception rate until 120 DIM, higher FCSI, CCI, and NSP. However, very similar reproductive performance was displayed in PGF_{2α}-treated endometritis cows. Teskra appears to be ineffective and should be avoided as alternative therapy option for CE in dairy cows. Further studies with more administration routes using other parts of the plant are required to complete this research, with a large sample size per group and the inclusion of a negative control group, which would provide more accurate results.

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Učinak *Echinops spinosus* (Teskra) i PGF_{2α} na reproduktivnu učinkovitost krava s kliničkim endometritisom

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Procijenjena je učinkovitost *Echinops spinosus* (Teskra) i PGF_{2α} u liječenju kliničkog endometritisa (CE), s obzirom na klinički oporavak i reproduktivnu sposobnost. Ukupno 20 holštajn-frizijskih krava u danima od 21.-40. proizvodnje mlijeka u laktaciji (engl. *days in milk- DIM*) s kliničkim endometritisom (CE) podijeljeno je u dvije eksperimentalne skupine. Krave u skupini PGF ($n=10$) primile su dvije doze d-kloprostenola (analog PGF_{2α}) u razmaku od 14 dana. Za Teskra skupinu ($n=10$), uvarak Teskra korijenja primjenjivan je jednom dnevno tijekom tri dana oralno. Dvadeset ($n=20$; HE skupina) klinički zdravih krava ostalo je kao kontrolna skupina. Klinička stopa oporavka bila je 70 % i 40 % za PGF, odnosno Teskra skupinu ($P>0,05$). Omjer gravidnih krava nakon prvog osjemenjivanja bio je 35 %, 40 %, 20 % za HE, PGF, odnosno Teskra skupinu, ($P>0,05$). Međutim, stopa koncepcije do 120. dana proizvodnje mlijeka u laktaciji (DIM) bila je značajno veća u HE

skupini u usporedbi s Teskra skupinom ($P<0,05$). Prosječni broj dana od teljenja do prvog osjemenjivanja (CFSI) bio je značajno niži u HE skupini u usporedbi s PGF i Teskra skupinama ($P<0,001$). Krave u Teskra skupini koncipirale su oko 35 dana i 26 dana kasnije nego krave u HE, odnosno PGF skupinama ($P<0,05$). Za krave u Teskra skupini trebalo je oko 2,4 osjemenjivanja za koncepciju, u usporedbi s 1,8 i 1,6 za one u HE, odnosno PGF skupinama ($P>0,05$). Osim za prosječni broj dana od teljenja do prvog osjemenjivanja (CFSI), nisu pronađene značajne razlike između HE i PGF skupina za niti jedan ispitani pokazatelj. Rezultati ukazuju na to da su krave s endometritisom liječene s PGF_{2α} imale gotovo istu reproduktivnu učinkovitost u usporedbi sa zdravim kravama, a da uvarak Teskre nije bio učinkovit u liječenju kliničkog endometritisa mliječnih krava.

Ključne riječi: klinički endometritis, Teskra, PGF_{2α}, stopa oporavka, reproduktivna učinkovitost