RESEARCH ARTICLE

Prevention of Unwanted Pregnancy using Diethylstilbestrol in Mismated Bitches

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Abstract

Efficiency of diethylstilbestrol (DES) was evaluated for preventing unwanted pregnancy following misalliance in eight bitches of different breeds, age, and bodyweight along with use of vaginal cytology on the day of case registration for retrospective confirmation of stage of estrous cycle and time of mating. All the bitches were injected with diethylstilbestrol (DES) @ 2.0 mg/kg b. wt., i/m (Max. 20 mg) on day three and five post-mating and were subjected to blood examination on day zero and seven of DES injection. An ultra-sonographic scan was carried out in each of the bitch on day 30-35 post-mating to rule out the pregnancy status of the animal and success of therapy. All the bitches were followed up for 70-100 days post-treatment for any untoward effects. The major exfoliative cells found in vaginal cytology were of large intermediate/anuclear cells (94.37 ± 0.57 %) characteristics of a peak estrus phase, and 6 of 8 bitches also showed presence of sperm in the smear evinced mating. All the bitches were found to be non-pregnant on USG scan 30-35 days post-treatment and had no serious side effect during the follow-up period. The pre- and post-treatment mean values of hemoglobin (g/dL), WBCs ($\times 10^3$/µL) and RBCs ($\times 10^6$/µL) were 15.58 ± 0.59 vs. 15.33 ± 0.60, 9.10 ± 0.44 vs. 11.33 ± 1.60 and 6.94 ± 0.12 vs. 6.83 ± 0.22, respectively, differing non-significantly. It was concluded that the exfoliative vaginal cytology in bitches having fully cornified superficial/anuclear cells as predominant cells with sperm presence indicates misalliance during estrus phase. Injection diethylstilbestrol twice could prevent the conception and establishment of pregnancy in bitches having misalliance, with minimal effect on their health.

Keywords: Bitch, Diethylstilbestrol, Misalliance, Prevention of conception, Success rate.

Ind J Vet Sci and Biotech (2021): 10.21887/ijvsbt.17.1.4

Introduction

Population of pet dogs is increasing day by day and unintended matting of bitches with stray dogs is common. The incidence of mismating in female dogs is increasing due to the promiscuous behaviour of bitch and longer estrus period. Further, the roaming behavior of the estrus bitch, and her tendency to accept multiple male partner increases the chances of mis-mating. In such cases prevention of unwanted pregnancy is required. Hiemstra *et al.* (2001), Mshelia *et al.* (2001) and Haji *et al.* (2018) reported that vaginal cytology provides sufficient information to detect the stages of the estrous cycle in bitches, and characterized the estrus based on the presence of predominant superficial (>90.00 %) cells in smears and even misalliance by presence of sperm (Antonov, 2017).

Over the last few decades, many new drugs have been used for prevention and/or termination of pregnancy in canines, but it must be used based on the safety, efficacy, convenience, compliance in treatment, and cost (Eilts, 2002). Abhilash *et al.* (2012) reviewed that injection of DES, @ 2 mg/kg b.wt., up to 25 mg once or twice within 5 days of mating is highly effective in terminating the pregnancy. McLauchlan and Ramsey (2008) reviewed side effects of DES mainly the pyometra and bone marrow suppression with other minor side-effects like signs of estrus, lethargy, diarrhea, vomiting, vaginal discharge, polydipsia and polyuria. The objective of the present study was to evaluate the efficacy and side effects of the diethylstilbestrol for prevention of conception in mismated bitches following confirmation by vaginal cytology.

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How to cite this article: Parmar, B.M., Panchal, M.T., Shukla, T.B., & Patel, J.A. (2021). Prevention of Unwanted Pregnancy using Diethylstilbestrol in Mismated Bitches. Ind J Vet Sci and Biotech, 17(1): 16-19.

Source of support: Nil

Conflict of interest: None.

Submitted: 20/10/2020 **Accepted:** 12/01/2021 **Published:** 25/01/2021

Materials and Methods

The bitches (n = 8) with misalliance during last 5 days were registered and treated for prevention of conception. All the bitches were subjected to exfoliative vaginal cytology prior to injecting medicines to ascertain the estrous cycle stage and retrospective confirmation of mating. The smears were stained by Field stain as usual for standard cytological studies (Bowen,

2001). All the bitches were injected with DES @ 2 mg/kg b. wt. (maximum 20 mg), i/m, on day three and five post-mating. All the bitches were also subjected to blood examination on day 0 (pre-treatment) and day 7 (post-treatment) for assessing the effect of DES on their health. These bitches were scanned transabdominally using ultrasound during 30 to 35 days post-treatment to ascertain presence or absence of fetal pups on day 70-100 for pyometra and other untowards responses in order to assess effect of DES treatment.

Results and Discussion

Determination of Stage of Estrous Cycle

Among the vaginal epithelial exfoliative cells found (Fig. 1), majority (94.37 ± 0.57%) were of large intermediate or anuclear cells characteristics of a peak estrus phase. Besides, these smears had the types of cells to be either small intermediate and parabasal ranging from 3 to 6 and 0 to 2, respectively, with their corresponding mean values of 4.12 ± 0.34 and 0.87 ± 0.26 (Table 1). The neutrophils and RBCs were found in mild (1-5) to moderate numbers (>5 to 20), which confirmed that the majority of the bitches were presented during either early or peak estrus. The present findings corroborated well with the reports by Hiemstra *et al.* (2001), Mshelia *et al.* (2001) and Noakes *et al.* (2018). They also reported that vaginal cytology provides sufficient information to detect the stages of the estrous cycle in bitches, and characterized the estrus based on the presence of predominant superficial (>90.00 %) cells in smears.

All the eight bitches were confirmed to be in the estrus at the time of misalliance based on evidence of majority proportion (>90%) of anuclear or large superficial exfoliated cells (Fig. 1). Rao *et al.* (2011) and Antonov (2017) also opined that vaginal cytology is a simple and useful method for determining the stages of estrous cycle with respect to the optimal breeding time with more than 80% anucleated cells. The present findings of getting predominantly anuclear cell types along with sperm in bitches with misalliance suggested that their breeding took place in optimum estrus phase (Fig. 1). Haji *et al*. (2018) reported the percentages of parabasal, intermediate and superficial cells to be 0.56 ± 0.03, 5.68 ± 0.03, and 92.29 ± 0.03, respectively, in estrus, with the vaginal smears having clear background and absence of neutrophils with scanty erythrocytes in estrus.

Confirmation of Mating by Vaginal Cytology

The bitches presented for treatment had a history of misalliance on the previous day or on the same day of case registration within the time period of 4 to 24 hrs. The smears from six out of eight bitches showed the presence of sperm cells, as evidence of successful mating and ejaculation, indicative of likelihood of conception and establishment of pregnancy. The presence of spermatozoa is diagnostic of mating, but an absence of spermatozoa does not necessarily indicate that mating has not occurred. These findings were in accordance with the observations reported by Whitacre *et al.* (1992) and Antonov (2017), who stated that spermatozoa could be found in vaginal smears for 24 to 36 hrs after mating;

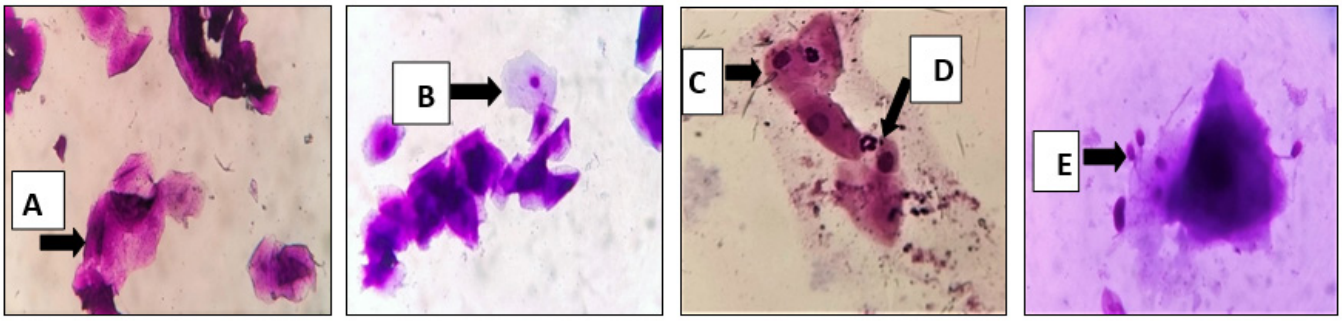

Fig. 1: Types of cells found in the vaginal cytology of mismated bitches (40X)
A: Anuclear/Superficial cell; **B:** Small intermediate cell; **C:** Parabasal cell; **D:** Neutrophil; **E:** Sperm cell

Table 1: Exfoliative epithelial cells and sperms in post-mating vaginal smears of bitches (n = 8)

	Post-mating vaginal cytology of bitches								
Types of cells	*Case 1*	*Case 2*	*Case 3*	*Case 4*	*Case 5*	*Case 6*	*Case 7*	*Case 8*	*Mean ± SE*
Large intermediate/ Superficial anuclear	95	94	95	92	95	97	94	93	94.37 ± 0.57
Small intermediate	4	4	3	5	3	3	5	6	4.12 ± 0.34
Para basal	1	0	1	2	1	0	1	1	0.87 ± 0.26
Neutrophils	+	++	++	+	+	+	+	+	+
RBCs	+	++	+	++	+	+	+	+	+
Spermatozoa	Ab	Ab	P	P	P	P	P	P	6/8

+: Mild Numbers (1-5); ++: Moderate Numbers (>5 to 20), Ab: Absent; P: Present.

Table 2: Mean (± SE) haemoglobin, WBCs, and RBCs values in bitches pre- and post-treatment with DES*

	Blood parameters (n=8)		
Days of blood collection	*Haemoglobin (g/dL)*	*WBCs ($\times 10^3/\mu L$)*	*RBCs $\times$ ($10^6/\mu L$)*
Pre-treatment (day 0)	15.58 ± 0.59	9.10 ± 0.44	6.94 ± 0.12
Post-treatment (day 7)	15.33 ± 0.60	11.33 ± 1.60	6.83 ± 0.22
'P' value	0.75	0.39	0.69

None of the parameter differed statistically between days ($p > 0.05$).
DES*: Diethylstilbestrol (Maximum dose of 20 mg, i/m, twice on day 3 and day 5 post-mating)
Day 0= Day of 1st DES Injection, Day 7= 7th Day of 1st DES Injection

however, lack of sperm does not eliminate mating.

Prevention of Conception in Bitches

None of the bitches (n = 8), subjected to DES treatment for prevention of conception and scanned transabdominally using ultrasound during 30 to 35 days post-treatment, revealed presence of fetal pups in the uterus and thus indicated role of DES in prevention of conception. The present findings indicated at very first sight, 100% efficacy of DES to prevent conception/pregnancy in bitches. However, it is not worth to say the treatment to be 100 % effective (effective to the tune of 75.00 %), in view of the two bitches who had no sperm in their vaginal smears, which raised a sense of doubt with respect to fertilization taking place or not, while attempting prevention of conception. These findings were supported by Kutzer's (2003) observations that DES when administered @ 0.5 mg/kg, i/m, once in the beginning 24 to 48 hrs after mating is having better efficacy for preventing pregnancy in bitches. Abhilash *et al.* (2012) also reviewed that injection of DES, @ 2 mg/kg b. wt., up to 25 mg once or twice within 5 days of mating is found to be highly effective in terminating pregnancy.

Effects of DES Treatment on Health of Bitches

Out of eight bitches covered for DES treatment, three showed the signs of mild vaginal mucus discharge as a resultant side effect. No other side effects of DES were noticed during the follow up period of 70 to 100 days. McLauchlan and Ramsey (2008) and Forney (2015), described pyometra and bone marrow suppression which may progress to a fatal aplastic anemia as side effects of DES. Other side-effects reported by them were signs of estrus, lethargy, diarrhea, vomiting, vaginal discharge, polydipsia and polyuria. However, none of these side effects were noticed in present study with the exception of estrus-like signs being exhibited in 3 out of 8 treated bitches. Forney (2015) stated that the risk of adverse effects increases with multiple treatments using diethylstilbestrol. The present findings of minimum side effects observed were attributed to a shorter treatment protocol duration with a minimum dosage of DES administered. The mean pre- and post-treatment hematological values of Hb, RBCs and WBCs (Table 2) in bitches were within the normal physiological range and did not differ significantly, indicative of fact that use of DES post-mating did not cause adverse effect on their health.

Conclusion

It was concluded that the exfoliative vaginal cytology in bitches having fully cornified superficial or anuclear cells as predominant cells with the presence of sperm is indicative of the fact that the bitches had misalliance during their estrus phase and that the diethylstilbestrol could prevent the conception and establishment of pregnancy effectively in bitches having misalliance when administered @ 2.0 mg/kg b. wt., i/m (max. 20 mg) on day 3 and 5 post-mating, with practically no or minimal adverse effects/ complications.

Acknowledgment

The authors are grateful to the Dean, College of Veterinary Science and Animal Husbandry, Anand, and Deputy Director of Animal Husbandry, Veterinary Polyclinic, (GOG), Vadodara for providing the necessary facilities to undertake the present study.

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