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Clinical, ultrasonographic and haematological changes in aglepristone induced early post implantation pregnancy termination in bitches

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Abstract
Therapeutic measures to control unwanted pregnancies were often limited to those beyond 30 days of gestation. However, pregnancy can be terminated as soon as implantation occurs. Hence, the present study was aimed at assessing the effect of aglepristone on early post-implantation termination. Twelve bitches were randomly allotted to two groups. Group I received aglepristone (10 mg/kg S/C, two doses 24 hours apart), and Group II served as untreated controls. Treatment efficacy and physiological responses were assessed through clinical observation, haematological analysis and Doppler ultrasonography. Pregnancy termination occurred within 5.5 ± 0.22 days, confirmed by ultrasonographic evidence of the disappearance of gestational sacs. Progesterone concentration did not decrease after the start of treatment. An increase in TLC, ALP and uterine artery RI, and PI was recorded post-treatment. Minor side effects were observed in this study, which concludes aglepristone is a safe and effective abortifacient for early post-implantation pregnancy termination in bitches.

Keywords: Bitches, early post implantation, aglepristone, TLC (Total leucocyte count), ALP (Alkaline phosphatase), RI (Resistive index) and PI (Pulsatility index)

1. Introduction
Mis-mating is a prevalent issue in canine veterinary practice, often stemming from pet owners' limited understanding of canine reproductive physiology. Unintended mating can negatively affect both owners and the health of the female dog, necessitating preventive or therapeutic measures to control pregnancies. One of the most common requests from dog owners is the termination of unwanted pregnancies. Ovariohysterectomy (OHE) is the optimal and safest method for preventing these pregnancies; however, alternatives are sought when the dog may be a potential breeder and to maintain its future fertility (Sridevi, 2015) [24] or if the owner wants to avoid surgical risks and postoperative complications (Jyothi *et al.,* 2019) [13].
Canines rely on progesterone for pregnancy maintenance, with a minimum concentration of > 2 ng/ml (Verstegen-Onclin and Verstegen, 2008) [26]. Canine embryo implantation occurs approximately 17-18 days post-fertilization (Kowalewski, 2023) [18] or 19-21 days post-ovulation (Concannon *et al.,* 2001) [5]. Early pregnancy diagnosis can be achieved through relaxin levels, detectable as early as day 18; however, clinical diagnosis typically utilizes ultrasonography, which can identify pregnancy from Day 25 post-mating for accurate gestational sac measurement (Aissi *et al.,* 2008) [1]. For early post-implantation termination of pregnancy, a regimen combining cloprostenol and cabergoline has demonstrated 100% efficacy, albeit with severe side effects such as salivation, prostration, and vomiting appearing shortly after administration and takes about 8 ± 1.2 days to terminate pregnancy in dogs (Onclin *et al.,* 1995) [21]. Alternatives like the progesterone receptor antagonist mifepristone necessitate a longer treatment duration to achieve abortion (15 days) to complete termination or increase in dose rate of up to 20 mg/kg rather than conventional dosing of 2.5 mg/kg body weight to terminate pregnancy in early gestation (Concannon *et al.,* 1990) [6].
The timing of pregnancy termination can be confirmed to prevent unnecessary treatment of

non-pregnant bitches (Wanke *et al.,* 2002) [27] and avoid adverse medication effects (Srinivas *et al.,* 2008) [25]. Ultrafast monitoring through regular ultrasound can also identify embryonic death between 25-30 days, leading to resorption rather than abortion. The current study focuses on inducing abortion via aglepristone during the early post-implantation phase, alongside the assessment of hematological parameters, serum progesterone levels, alkaline phosphatase (ALP), and uterine artery perfusion during this process.

2. Materials and Methods

The study was conducted at the Small Animal Obstetrics and Gynaecology unit of the Teaching Veterinary Hospital, Madras Veterinary College, Tamil Nadu, involving twelve female dogs. Six mis-mated bitches were treated with aglepristone (Alizin® Virbac at 10 mg/kg body weight via subcutaneous injection, two doses administered 24 hours apart) and classified as Group I. The remaining six dogs served as untreated controls in Group II. Blood samples were drawn from either the cephalic or saphenous vein on days 0, 3, and 6 after initiation of treatment for both groups to assess serum progesterone levels, alkaline phosphatase (ALP), or various haematological parameters. These parameters included haemoglobin, packed cell volume, total erythrocyte and leucocyte counts, platelet counts, and differential leucocyte counts using a haematology analyser. Serum separation was done using a centrifuge at 3000 RPM, and progesterone was measured using an RIA kit.

Bitches were examined by ultrasonography during the treatment period using a sector transducer at 3.5-7.5 MHz or a linear transducer at 7-11 MHz for pregnancy diagnosis and treatment efficiency. The follow-up evaluations were done on alternate days during treatment with ultrasound. The uterine artery perfusion was calculated via the resistive index and pulsatility index. Observations were made to monitor the general health and any side effects of the treatment. The study recorded the day of onset abortion (DSA), indicated by the first vaginal discharge, and the day of completion abortion (ESA), confirmed by the absence of foetal structures on ultrasound following treatment initiation.

Table 1: Mean ±SE of fetal heart rate, rectal temperature and resistive and pulsatility index in treated and control bitches

Parameters	Group	Day 0	Day 2	Day 4	Day 6	F value
Fetal Heart rate	I	233.83±3.8	237.0±5.0	253.33±2.6	-	1287.8**
	II	232.83±2.6	226.5±3.5	224.17±5.3	225.67±5.7	0.739^{NS}
	t value	0.220^{NS}	1.727^{NS}	4.972**	39.700**	
Resistive Index (RI)	I	0.56±0.0076	0.59±0.0088	0.61±0.0037	0.64±0.0033	29.129**
	II	0.57±0.006	0.54±0.0037	0.53±0.01	0.51±0.0076	12.798**
	t value	1.372^{NS}	5.587**	7.303**	15.800**	
Pulsatility Index (PI)	I	0.85±0.013	0.87±0.0099	0.91±0.007	0.932±0.089	0.451**
	II	0.81±0.018	0.76±0.007	0.73±0.0052	0.71±0.0085	17.283**
	t value	1.643^{NS}	8.500**	20.441**	1.536^{NS}	
Rectal Temperature (°F)	I	101.00±0.12	98.93±0.24	99.16±0.27	101.18±0.41	17.995**
	II	101.88±0.31	101.38±0.36	100.88±0.22	101.91±0.19	3.070^{NS}
	t value	2.676 *	5.586 **	5.010 **	1.627^{NS}	
(P< 0.05* and (P< 0.01** ^{NS} Non-Significant (P> 0.05)						

3. Results

In this study, Group I had a mean gestational age of 25.8±0.48 at the start of treatment. Vaginal discharge occurred within 4.3±0.21 days, marking the beginning of abortion, which spanned a range of 4 to 5 days. The mean fetal heart rates were 233.83±3.8 and 232.83±2.6 for the treated and control groups, respectively, before treatment. Post-treatment, Group I showed a significant increase in heart rate compared to the control group, with a statistically significant difference (P< 0.01) observed on days 4 and 6. No significant differences were found within Group II or between the groups on days 0 and 2. Despite vaginal discharge, fetal heart rates remained detectable without indications of a deceased foetus in the treated bitches until the complete absence of the foetus. All pregnancies were successfully terminated within 5.5±0.22 days by resorption. Resorption was noticed on ultrasonographic examinations as collapsed gestational sacs, hypoechoic embryonic fluids (Fig 1). Out of six treated bitches, 33.33 percent of them was hyporectic and 16.66 percent of bitches had enlargement of mammary gland. No pain was evinced at the site of injection.

Body temperature showed significant differences (P< 0.01) between Groups I and II on days 2 and 4, with Group I also exhibiting significant differences (P< 0.01) within the group between days. Normal temperature was established by day 6, with no significant differences noted (Fig 2). Haematological parameters, serum progesterone, and ALP values are detailed in Table 2. No significant differences were found between treated and control groups for Hb, PCV, TEC, and platelets; however, a highly significant difference (P< 0.01) for PCV was observed between days within each group. Total leukocyte count (TLC) began to increase post-treatment, showing a statistically significant difference (P< 0.01) between groups on days 3 and 6, with a similar difference also observed between the days of Group I. Differential leukocyte counts revealed significant differences (P< 0.01) for neutrophils, lymphocytes, and eosinophils between days in Group I, where neutrophils and eosinophils displayed an increasing trend, while lymphocytes decreased post-treatment. Significant differences were also noted between groups for neutrophils and lymphocytes on days 3 and 6 and for eosinophils on day 6, with no significant differences recorded for monocytes within and between the groups.

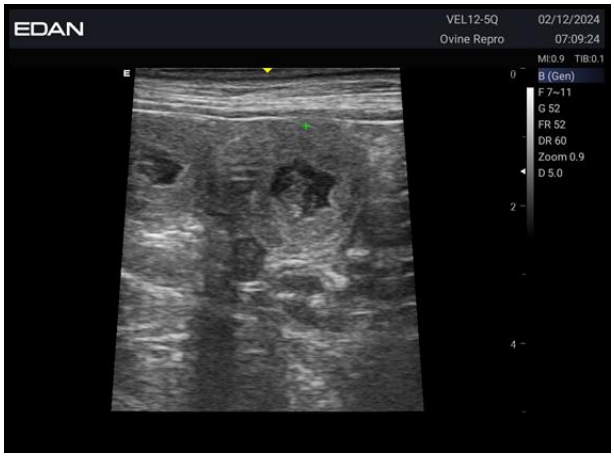

Fig 1: Empty Gestational sac with hypoechoic embryonic fluid

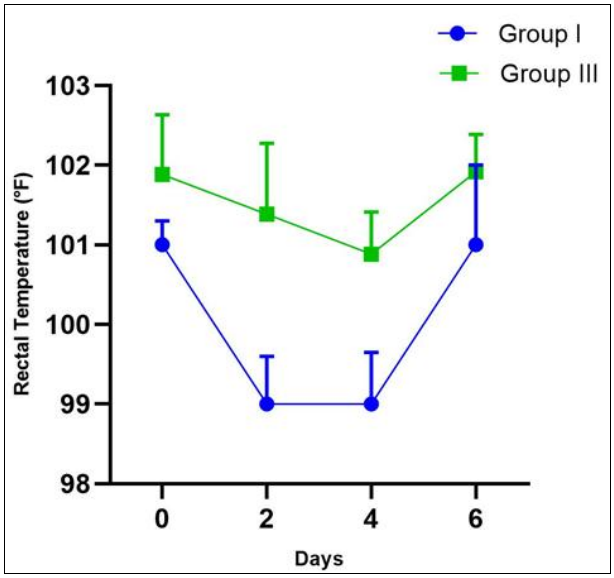

Fig 2: Body temperature in (°F)

Mean serum progesterone levels on day 3 were significantly higher in Group I compared to Group II (P<0.01). Significant differences were also observed between the days for both groups and between groups on days 3 and 6. On day 6, serum progesterone was lower in Group I (17.34±0.34) than in Group II (21.18±0.32). Additionally, serum ALP levels in Group I showed a decreasing trend post-treatment, with a notable significant difference between days and groups (P<0.01). On day 6, ALP levels were 68.67±3.4 in Group I versus 185±2.5in Group II.

Haemodynamic parameters such as the resistive index (RI) and pulsatility index (PI) of Group I and II bitches were shown in Table 1 (mean ± SE). Before the start of treatment, the RI and PI values were 0.56±0.0076 and 0.85±0.013 in Group I and 0.57±0.006 and 0.81±0.018 in Group II bitches, respectively. Whereas, post-treatment on day 6, RI and PI values were significantly higher (P< 0.01) in Group I (0.64 ± 0.0033 and 0.932±0.089) when compared to Group II (0.51±0.0076 and 0.71±0.0085) bitches, respectively.

4. Discussion

Aglepristone is a progesterone receptor blocker effective in terminating pregnancy in dogs and cats, achieving 100% efficacy within 5 to 6 days, consistent with Galac *et al.* (2000) [10]. However, the efficacy reported in other studies differs from the findings of this study. In mid-term pregnancy termination, the efficacy rates reported were 98.20% (Hubler and Arnold, 2000) [12], 96% (Fieni *et al.,* 2001) [8], and 95% (Galac *et al.,* 2000) [10]. An efficacy rate of 93.4% for early post-implantation termination was noted by Corrada *et al.* (2005) [7]. The side effects documented included hyporexia and mammary gland enlargement, corroborating findings by Fieni *et al.* (2001) [8]. The elevation of prolactin concentrations after aglepristone administration is attributed to its inhibitory effect on progesterone synthesis, leading to increased prolactin levels due to a simulated drop in progesterone (Fieni *et al*., 2001; Galac *et al*., 2000) [8, 10]. Additional side effects from aglepristone treatment included local pain, swelling, mild discomfort at the injection site, anorexia, depression, and a reduced inter-estrus interval, as reported by Hubler and Arnold (2000) [12] and Galac *et al.* (2000) [10]. A significant decrease in body temperature was observed in this study, consistent with findings by Corrada *et al*. (2005) [7], attributed to the effects of aglepristone on the hypothalamus and central nervous system. Progesterone is identified as a thermogenic hormone (Lamm and Makloski, 2012) [19]. Ultrasonographic examinations conducted every other day revealed hypoechoic embryonic fluids and changes in the gestational sac from spherical to flaccid and irregular shapes. These indicators suggest fetal resorption, similar to conditions observed in a post-partum uterus (Galac *et al*., 2000; Kahn, 1994) [10, 14].

Table 2: Mean ±SE of haematological parameters, serum ALP and serum Progesterone in treated and control bitches

Parameters	Group	Day 0	Day 3	Day 6	F value
Hb (g/dL)	I	13.31±0.23	12.08±0.25	12.12±0.29	7.399 ^{NS}
	II	13.52±0.47	12.47±0.18	12.17±0.21	5.017 ^{NS}
	t value	0.382 ^{NS}	1.246 ^{NS}	0.138 ^{NS}	
PCV (%)	I	43.87±0.37	42.02±0.4	40.7±0.45	15.243**
	II	42.92±0.78	41.17±0.64	36.88±1.3	10.718**
	t value	1.106 ^{NS}	1.124 ^{NS}	2.779 ^{NS}	
Total Erythrocyte Count (m/cmm)	I	5.88±0.23	5.58±0.22	5.32±0.098	2.215 ^{NS}
	II	6.15±0.19	5.82±0.12	5.65±0.16	2.583 ^{NS}
	t value	0.898 ^{NS}	0.938 ^{NS}	1.805 ^{NS}	
Total Leucocyte count (TLC) x 10^3/µL	I	17.6±0.48	20.41±0.56	24.19±0.31	51.342**
	II	16.32±0.71	17.73±0.44	18.11±0.39	3.128 ^{NS}
	t value	1.489 ^{NS}	3.777**	12.165**	
Platelets x 10^4/µL	I	22.83±1.2	23.77±0.46	23.48±0.48	0.366 ^{NS}
	II	21.33±0.88	23.08±0.88	23.03±0.67	1.489 ^{NS}
	t value	1.010 ^{NS}	0.688 ^{NS}	0.545 ^{NS}	
Neutrophils (%)	I	78.33±0.76	81.67±0.8	83.33±0.67	11.667**
	II	77.83±0.91	76.67±0.76	78.83±0.65	1.924 ^{NS}
	t value	0.423 ^{NS}	4.523**	4.818**	

Lymphocytes (%)	I	16.67±0.84	14.33±0.61	12.83±0.6	7.720**
	II	18±0.37	18.33±0.67	18.17±0.7	0.078 NS
	t value	1.451 NS	4.411**	5.766**	
Monocytes (%)	I	2.17±0.54	1.5±0.43	1±0.37	1.682 NS
	II	2.33±0.49	2±0.26	1.67±0.42	0.682 NS
	t value	0.227 NS	1.000 NS	1.195 NS	
Eosinophils (%)	I	2.5±0.43	2±0.26	4±0.26	10.263**
	II	2±0.26	2.67±0.42	1.33±0.21	4.615 NS
	t value	1.000 NS	1.348 NS	8.000**	
ALP (U/L)	I	138.67±5.3	87.67±4.3	68.67±3.4	67.235**
	II	140±2.10	162±4.20	185±2.50	12.193**
	t value	2.174 NS	10.422**	16.201**	
Serum Progesterone (ng/ml)	I	25.38±0.99	25.53±0.69	17.34±0.34	41.163**
	II	26.26±0.46	23.34±0.18	21.18±0.32	55.500**
	t value	0.905 NS	3.130**	6.575**	
(P< 0.05* and (P< 0.01** NS Non-Significant (P> 0.05)					

Fetal heart rate serves as a key indicator of fetal stress, typically ranging from 220 to 240 beats per minute (bpm) in dogs (Nyland and Mattoon, 2002) [20]. In the current study, it was observed that fetal heart rate did not decrease until resorption occurred; instead, it started to rise with the onset of treatment. This aligns with findings from Blanco *et al*. (2016) [4], indicating that fetal vagal cardiac control maturation is influenced by fetal weight, which diminishes due to inadequate fetal perfusion. Hypoxia leads to increased catecholamine levels, further complicating the predictive value of fetal heart rate regarding embryo status during pregnancy termination. Additionally, changes in heart rate appear to correlate with uterine blood flow, consistent with previous research (Gaikwad *et al*., 2020) [9]. An increase in resistance index (RI) and pulsatility index (PI) was noted, echoing Blanco *et al*. (2016) [4], where treatment with aglepristone in cats led to increased uterine artery resistance. This is attributed to progesterone's role in enhancing uterine blood flow, with its deprivation resulting in reduced blood flow (Blanco *et al*., 2009; Blanco *et al*., 2016) [3, 4].

Pregnancy was successfully terminated using aglepristone, with progesterone concentrations started to increase after start of treatment. Thereafter, resorption of all fetus it started to decrease. This finding was consistent with kaya *et al.* (2014) [15]. Aglepristone acts directly on the uterus to mimic a decrease in progesterone levels without affecting luteal function, as confirmed by studies of Ozalp *et al*. (2013) [23]. This methodology leads to luteolysis, primarily facilitated by $PGF_2\alpha$ secretion from the uterus following abortion or resorption (Galac *et al*., 2000) [10]. Additionally, a notable decrease in serum alkaline phosphatase (ALP) levels was observed in treated animals, contrasting with an increase in control pregnant animals, attributed to fetal hematogenesis which raises maternal serum ALP levels (Kimura and Kotani, 2018) [17]. The decreasing trend in treated animals aligns with findings from Binli *et al*. (2022) [2] and Keerthana *et al*. (2024) [16], suggesting that this is due to the disruption of fetal hepatic activity.

In this study, the total leucocyte count and neutrophil levels were observed to increase following treatment, a finding consistent with Binli *et al*. (2022) [2]. This rise is attributed to inflammatory responses during resorption (Keerthana *et al*., 2024) [16] or enhancements in non-specific immunity that could facilitate recovery post-resorption or abortion (Ozalp *et al*., 2013) [23], as well as stress linked to fetal resorption (Georgiev *et al*., 2010) [11]. Additionally, an increase in eosinophils was noted, aligning with the results reported by Ozalp *et al*. (2022) [22]. The observed changes in eosinophil levels are likely due to the activation of eosinophils involving immunoglobulins, cytokines, and platelet activating factors, or may stem from hypersensitivity reactions related to the oily preparation of aglepristone.

5. Conclusion

Based on the study results, aglepristone is an effective abortifacient during the early post-implantation gestation period, exhibiting minimal side effects. The success of pregnancy termination can be reliably evaluated through serial ultrasonographic examinations. Additionally, Doppler studies of the uterine artery serve as a trustworthy indicator of fetal stress, which may be detected prior to any reduction in heart rate-a key sign of fetal distress and potential abortion in bitches. Furthermore, aglepristone's administration did not significantly alter haematological values, except for notable changes in total leukocyte count (TLC), which do not impact the health status of the bitch.

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7. Conflict of Interest

Not available.

8. Financial Support

Not available.

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