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Characterization of Certain Reproductive Parameters in Chippiparai Bitches of Tamil Nadu

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ABSTRACT

Background: This study was conducted to establish the certain reproductive parameters of Chippiparai bitches, which are indigenous dog breed of Southern Tamil Nadu in India to augment the fertility of Chippiparai bitches using vaginal exfoliative cytology and progesterone assay as diagnostic tools.

Methods: The data was collected by a questionnaire from (105 female dogs) pet owners to understand the breeding practices and the reproductive performance of Chippiparai breed. On the basis of Vaginal Exfoliative Cytology (VEC) (n=100) and combination of VEC with progesterone assay (n=12) the optimum time of breeding, ovulation, conception rate and litter size were estimated.

Result: Management and reproductive performance of Chippiparai female was recorded as other medium sized hound breeds. This study established the stages of estrous cycle in Chippiparai bitches on the basis of vaginal cytology and combination of VEC with progesterone estimation improved the conception rate and litter size and also helped in identification of the fertile period in Chippiparai bitches.

Key words: Chippiparai, Conception rate, Indigenous dog breed, Progesterone assay, Reproductive parameters, VEC.

INTRODUCTION

In general, different dog breeds in the world are classified based on their utility like protection/guarding, herding, flocking, mountain, companion, fighting, scent, toy *etc*. In India, some of the noted breeds of dogs are *viz*., Caravan Hound, Combai, Chippiparai, Rajapalayam, Rampur Hound, Kanni, Mudhol Hound, Indian Mastiff (Bulli), Himalayan sheep dog, Bhutia dogs (Gandhi, 2010) *etc*., contributing to the domestic animal biodiversity of our country. Chippiparai is the indigenous dog breed of Tamil Nadu (Thiruvenkadan *et al.,* 2012). Indigenous dog breeds are mainly utilized for guarding and shepherding of livestock and agriculture farm. But due to inflow of exotic canine breeds, the indigenous dogs were ignored by scientists, dog breeders and pet owners.

The Chippiparai breed is a medium sized dog, known as the “Greyhound of the South” was thought to be a descendant of the Saluki breed (Srinivasan, 2011). Bred by royal families in Chippiparai village in Virudhanagar District of Tamil Nadu and kept as a symbol of royalty and dignity by the rulers of Tirunelveli and Madurai (Srinivasan, 2011).

Karthickeyan *et al.* (2015) reported that the population size of Chippiparai dog breed is meagre in the breeding tract. Further, the pet owners and dog breeders of this native breed should be encouraged for increasing the population and to conserve this unique dog breed. Hence, this study was undertaken to characterize certain reproductive parameters of Chippiparai dog in its breeding tract at Tirunelveli District.

The estrous cycles of exotic bitches such as German Shepherd, Rottweiler, Labrador have been described by many researchers using exfoliated vaginal cytology (Hewitt and England, 2000; Gobello *et al.,* 2004) and hormonal profiles (Hewitt and England, 2000) but there has not been any record for the Chippiparai breed of bitches in Tamil Nadu. This study was carried out to investigate the length of estrous cycle, optimum time for mating and improvement of conception rate and litter size in Chippiparai dog by using vaginal exfoliative cytology and progesterone profiles as breeding tools.

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MATERIALS AND METHODS

The present study was carried out in Small Animal Veterinary Gynaecology and Obstetrics Section and Centralized Clinical Laboratory, Veterinary Clinical Complex, Veterinary College and Research Institute, Tirunelveli (8.7139°N, 77.7567°E), Tamil Nadu, India.

Experiment 1

To study the reproductive parameters and breeding practices of Chippiparai bitches, a questionnaire was designed and collected from pet owners of 105 female dogs (Group I) regarding the breeding management and estimation of reproductive performance (Table 5).

Experiment 2

Vaginal exfoliative cytology was obtained from 100 bitches (Group II) as per Aydin *et al.* (2011) from the first day of bloody vaginal discharge (proestrus) up to the last day of acceptance of mating (estrus). The smear was stained with the Leishman stain (https://www.vetstream.com/treat/staining-techniques-leishman-apos;s-stain). The evaluation of vaginal smears was performed with a light microscope at magnifications of 10X, 40x and 100x. The cells were differentiated into parabasal, intermediate, superficial and squamous cells (Oslon, 1989; Leigh *et al.,* 2013). The stages of the sexual cycle of the bitches were determined according to the cell types and their proportion on the vaginal smear (Klaas, 1985; Leigh *et al.,* 2013).

$$\% \text{ of x cells (on a slide)} = \frac{\text{Number of (x cells) on a slide}}{\text{Total number of cells on the slide}} \times 100$$

$$\text{Superficial cell index} = \frac{\text{Number of cells from superficial and squamous cell layers}}{\text{Total number of epithelial cells}} \times 100$$

$$\text{Karyopyknotic Index} = \frac{\text{Number of Squamous cells or cells with pyknotic nuclei}}{\text{Total number of superficial cells}} \times 100$$

The animals were allowed for natural breeding in three days interval until female showed refractoriness, when the percentage of superficial and squamous cells in vaginal smears was more than 80%, the Superficial cell Index (SCI) and Karyopyknotic Index (KPI) was 100% and 80%, respectively (Klaas, 1985; Srinivas *et al.,* 2004). Diestrus was recognized by the appearance of parabasal cells, neutrophils and few superficial and squamous cells that decreased to 20%.

Experiment 3

Blood samples were collected in vacutainer without anticoagulant for serum progesterone estimation using ichroma™ reader immune analyser (Boditech Med Inc.) from 12 bitches (Group III) from day one of proestrus bleeding, followed by VEC revealed 60%, 80%, 100% SCI, Day 0, 3,6 of 100% KPI, day of refractoriness, three blood samples were collected during cytological diestrus (Day 15,30 and 60 from 100% KPI) and two samples were collected during cytological anestrus (Day 90 and 120 from 100% KPI). Stages and length of each stage of estrous cycle were differentiated on the basis of VEC and progesterone level. The bitches were allowed for natural breeding, when the serum progesterone level elevated to ≥4 ng/ml (Arlt, 2018).

Pregnancy was diagnosed by trans abdominal ultrasonography on day 30 from last mating (Sonoray Model: C361-2) in Group II and III. The data was analyzed by using SPSS Software.

RESULTS AND DISCUSSION

On the basis of data collected from questionnaire in Group I, the optimum age of puberty in Chippiparai bitches was 10.104±0.199 months which ranged between 7-15 months and age of first mating was 3.85±0.12 years and ranged between 1.5-6.5 years. Owners maintaining the female for breeding purpose allowed first mating at 1½ to 2 years of age. But the owners rearing this breed for hunting and sporting purposes allowed mating at around 4-6 years of age, subsequently the number of parities per female was 3.33±0.11 times, which was in agreement with the observation of Karthickeyan *et al.* (2015) who reported that the decreasing population of Chippiparai dog was to be conserved by educating the pet owners by eliminating the myths involved in breeding. Present study revealed that the inter-estrus period in Chippiparai female was 11.57±0.099 months (10-14 months) which is in agreement with the finding of Karthickeyan *et al.* (2015) who reported that Chippiparai bitches whelp only once in a year. In present study, length of proestrus bleeding was recorded as 7.37±0.24 days (3-15 days). Vulval bleeding was a characteristic proestrus sign with a duration of 6 to 11 days (9 days on average) but could be used as a reliable parameter to determine the optimal time of mating (Fledman and Nelson, 2007). As per data of Group I, the number of matings allowed by the Chippiparai pet owners was 1.35±0.06 times with a range of 1-3 times per cycle. Some of the pet owners allowed one mating per cycle and believed that frequent mating reduced the hunting performance of male or female dogs. In Group I, the length of gestation period was recorded as 60.8±0.2 days (58-68 days) which was in agreement with the findings of Mutembei *et al.* (2000) and mean litter size was recorded as 4.028±0.214 pups ranging from 1-12 pups per whelping. The owners adopting one mating per cycle had low litter size, on the other hand breeders who followed multiple matings had 8-10 pups per whelping which was in disagreement with the observation of Karthickeyan *et al.* (2015) who recorded the litter size as 4-10 pups in this breed. Linde-Forsberg (1994) reported that litter size was highly correlated to optimum timing for breeding and less number of matings could be the reason of small litter size under orthodox breeding system. In this study the sex ratio male:female was estimated as 1.6:1. Sorribas *et al.* (2018) reported 1.08 males were born per female born in Labrador breed. In the present study 91.7%,

4.8% and 3.5% of pregnant bitches experienced eutocia, dystocia and caesarean section, respectively (Table 1). The incidence of dystocia (8.3%) was lower compared to Labrador (15.5%) (Sorribas *et al.,* 2018), Golden Retriever (9.1%), Pekingese (85.7%) (Gill, 2002) and Boxer (32%) breeds (Linde Forsberg and Persson, 2007). The low frequency of dystocia in Chippiparai dog compared with these breeds was probably due to its size and body characteristics (mesomorphic and mesocephalic), which made delivery easier than other breeds. Live percentage of pups in present study was 83.7%, followed by still birth (2.8%) and pups that died before weaning (13.5%), the incidence of pup mortality was more in the litters that underwent dystocia was in agreement with the finding of Rajendran *et al.* (1992). In Group I, the conception rate was 53.57% (Table 1) due to the myth that pregnancy reduced the hunting and racing performance of young bitches. This finding was in agreement with the observation of Fontbonne (2011) who reported 50% conception rate in French Kennel Club with incidence of infertility as 40% to 80% due to mistimed mating. This study revealed that the dogs were maintained mostly on non-vegetarian food, along with rice, egg and milk. Majority of the breeders (80.5%) followed twice a day feeding (morning and night). Present study revealed that 60% of Chippiparai dog owners maintain this breed for sport and hunting purposes due to its heat tolerance capability and higher resistance from communicable infectious diseases (Karthickeyan *et al.,* 2015). This breed was generally reared in backyard with no special housing system and 2-5 animals were maintained together. Karthickeyan *et al.* (2015) reported that no scientific housing and management were adopted for this breed. Present study revealed that the mating with litter mate (11.5%), within village (63.8%) and outside village (24.7%) (Table 1) were being adopted by the Chippiparai dog owners. Mating with litter mate and within village resulted in inbreeding. These findings are in agreement with the observations of Karthickeyan *et al.* (2015) who reported the mating with litter mate was a common practice in the breeding management of this breed.

In Group II, the length of the stages of estrous cycle had been differentiated according to the percentage of parabasal, intermediate, superficial and squamous cells (Table 2). In present study the average length of cytological proestrus (Fig 1 and 2), estrus (Fig 3 and 4), diestrus (Fig 5) and anestrus (Fig 6) were 7.64±0.129, 8.95±0.184, 64.74±0.41 and 213±1.42 days, respectively. Under estrogen influence, during proestrus and early estrus, the cells of the vaginal epithelium begin to proliferate, differentiate, thicken and exfoliate (Klaas, 1985). The inter-estrus interval was estimated as 294.57±1.4 days, which was in agreement with the observations of Raja *et al.* (2017) and Karthickeyan *et al.* (2015). In Group II the percentage of superficial and squamous cells in vaginal smears were above 80% (Fig 3) on day 6.68±0.184. The animals were allowed for natural breeding, when the superficial cell Index (SCI) and karyopyknotic Index (KPI) was 100% and 80% (Fig 4), respectively on day 9.37±0.21. Average number of breedings allowed were 3.44±0.074 at three days interval until the female showed refractoriness (onset of diestrus), as recognized by the appearance of parabasal cells (>80%), neutrophils and superficial and squamous cells decreased to 20% (Fig 5). These findings were in agreement with the

Table 1: Reproductive traits as per questionnaire.

Trait	Result
Age of puberty	10.104±0.199 months
	7-15 months
Age of first mating	3.85±0.12 years
	1.5-6.5 years
Inter estrus period	11.57±0.099 months
	10-14 months
No. of mating allowed	1.35±0.06 times
per estrus	1-3 times
Frequency of mating	Alternate days
Parity per female	3.33±0.11 times
Age of male used	4.93±0.13 years
for mating	2-7 years
Gestation length	60.8±0.2 days
	58-68 days
Litter size	4.028±0.214 pups
	1-12
Length of proestrus	7.37±0.24 days
bleeding	3-15 days
Sex ratio (No. of male/	1.60
no. of female foetus)	
Nature of whelping	
Eutocia	65.7%
Dystocia	27.6%
CS	6.66%
Pup loss	
Live pups	91.7%
Still birth	4.8%
Pups died before weaning	3.5%
Conception rate	53.57%
	Daily twice
	Rice, egg, milk
Diet	Weakly chicken or
	mutton or beef
	Backyard
Rearing	No special housing system
	2-5 dogs reared together
Purpose of rearing	
Breeding	30%
sport or hunting	60%
companionship	10%
Breeding management	
Mating with litter mate	11.5%
Mating within village	63.8%
Mating outside the village	24.7%

reports of Leigh *et al.* (2013) and Arlt (2018). Klaas, (1985) who also found that, as proestrus progresses the SCI will rapidly approach 100% and remained high throughout the estrus. The KPI increased slowly and reached 80% by estrus and then increased to 100% at ovulation. Both SCI and KPI would fall sharply after ovulation at the onset of diestrus, which was in agreement with our study. In Group II, the conception rate and litter size were considerably improved to 85% and 7.54±.141, respectively (Table 4) which were significantly higher than Group I. Optimum time of mating and frequency of mating played a key role in improving the conception rate and litter size (Zoldag *et al.,* 1993; Johnston *et al.,*1994; Antonov, 2017).

In Group III, the serum concentration of progesterone in Chippiparai bitches increased from 0.58±.05 ng/ml (Table 3 to 5) on the first day of proestrus bleeding to 1.02±.04 ng/ml

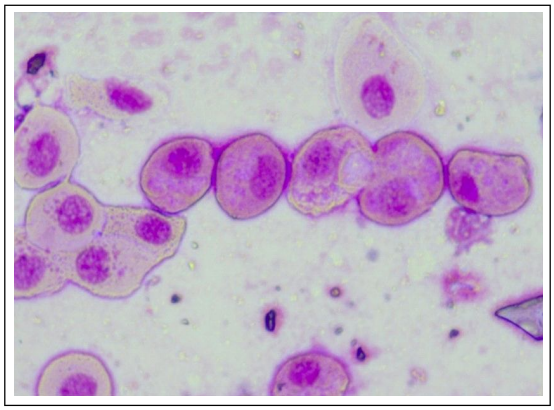

Fig 1: Parabasal cells during early proestrus (100×).

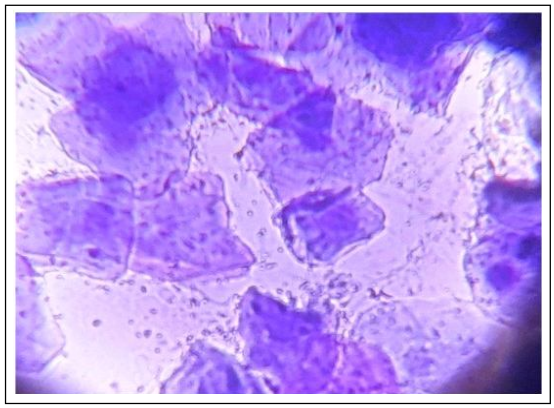

Fig 4: 100% Superficial cells Index and 80% Karyopyknotic Index during estrus (100×).

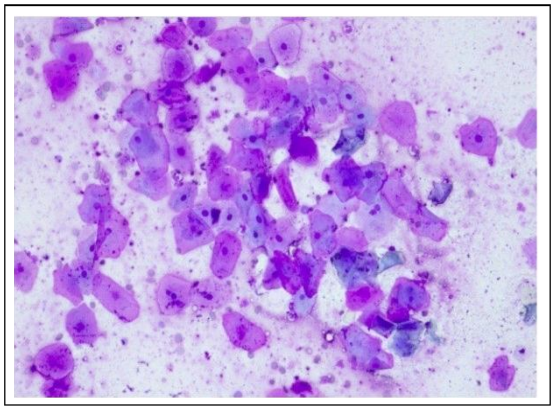

Fig 2: Large intermediate cells and superficial cells during late proestrus (40×).

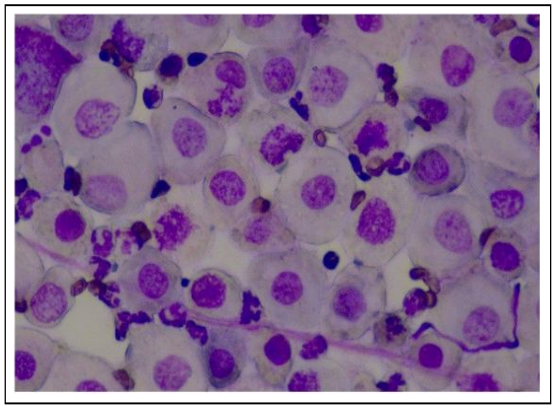

Fig 5: 100% Parabasal cells with numerous neutrophils during diestrus (100×).

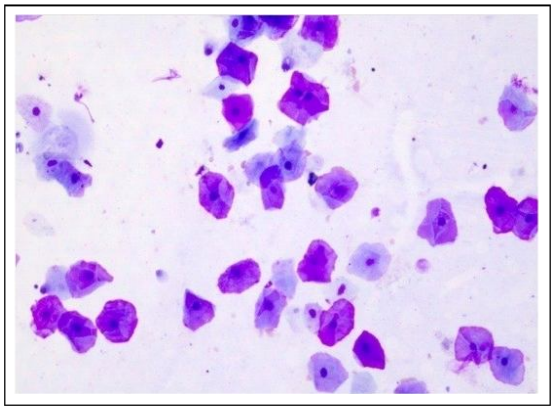

Fig 3: >80% superficial cells index numerous superficial with some squamous cells during early estrus (40×).

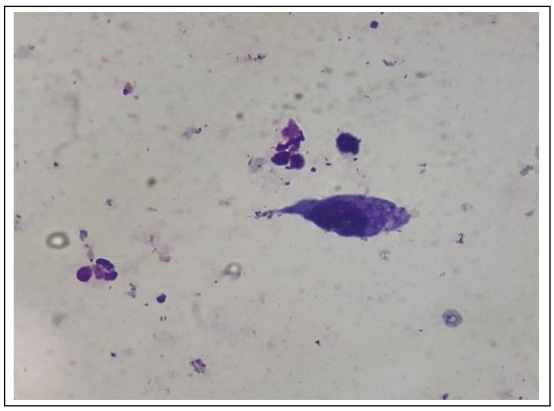

Fig 6: Debris and some parabasal cells during anestrus (40×).

by late proestrus, these findings gained support from the reports of Reddy *et al.* (2011) who reported the plasma progesterone level as 0.37±0.07 ng/ml on the first day of proestrus and recorded it as 1.55±0.19 ng/ml on day 8 of proestrus. The serum progesterone concentration in Chippiparai females was recorded as 1.57±0.073 and 2.56±0.1 ng/ml (80% SCI and 100% SCI, respectively) during early estrus followed by 6.88±0.315 and 8.9±0.28 ng/ml (Day 0 and Day 3 of 100% KPI) and concentration of progesterone increased to 11.05±0.36 ng/ml (Day 6 of 100% KPI), these findings are in agreement with the observations of Jeffocate (2004), who reported rise in progesterone level at the time of LH surge to 1-2 ng/ml and reached to 2-4 ng/ml two days later (on the day of ovulation) and on the day four from the LH surge (onset of the fertile period) circulating progesterone increased up to 6-10 ng/ml. Further, the level of progesterone had risen to 18.15±0.7 ng/ml on day one of diestus stage, which was identified by the refractoriness of female and cytologically >80% parabasal cell and numerous neutrophils. On the day 15, 30 and 60 from 100% KPI the mean progesterone concentration was 55.72±4.5, 97.98±4.5 and 26.08±2.4 ng/ml, respectively and serum progesterone concentration on day 90 was 1.035±0.12 ng/ml and reduced to undetectable level of <1 ng/ml on day 120 (Table 3) which coincided with the reports of Concannon *et al.* (1975) and Jeffocate (2004), who reported low progesterone level of 1 ng/ml by day 90 after LH peak and further reduced to baseline (<1 ng/ml) during inter-estrus interval.

In Group III, natural mating was allowed when the blood progesterone level was > 4 ng/ml and <12 ng/ml at three days intervals. Arlt (2018) reported that, it was more reliable to assess vaginal exfoliative cytology repeatedly and based on its findings progesterone was estimated to accurately predict the time of ovulation (2-4 ng/ml) and fertile period in bitches. In present study, 100% SCI and KPI during VEC were used as landmark for onset of estrus and ovulation, respectively (Klaas, 1985) and were prerequisite for progesterone estimation. Further, the conception rate

Table 2: Stages of estrous cycle on the basis of VEC (n=100).

Types of vaginal epithelial cells	Stages of estrus cycle							
	EP	MP	LP	EE	ME	LE	D	A
PBC	96.87±0.31	62.4±1.8	17.79±0.43	2.82±0.12	NIL	NIL	81.33±0.24	16.2±0.62
SIMC	5.61±0.177	55.43±0.78	16.01±0.28	3.39±0.089	NIL	NIL	15.78±0.32	NIL
LIMC	NIL	5.28±0.20	44.62±1.38	15.07±0.33	13.24±0.24	NIL	3.45±1.07	NIL
SC	NIL	2.03±0.116	53.47±0.65	56.12±0.61	32.42±0.84	18.04±0.56	NIL	NIL
SQC	NIL	NIL	9.61±0.384	17.28±0.27	60.93±0.59	83.36±0.58	NIL	NIL
RBC	+++	++	+	NIL	NIL	NIL	NIL	NIL
Neutrophils	+						+++	+

EP: Early proestrus, MP: Mid proestrus, LP: Late proestrus, EE: Early estrus, ME: Mid estrus, LE: Late estrus, D: Dioestrus, A: Anoestrus, PBC: Para basal cells, SIMC: Small intermediate cells, LIMC: Large intermediate cells, SC: Superficial cells, SQC: Squamous cells, RBC: Red blood cells.

Table 3: Serum progesterone concentration (n=12).

Stages of estrous cycle	Serum P4 concentration	Mean±S.E (ng/ml)
Early proestrus	Proestrus bleeding	0.58±0.05
Late proestrus	60% SCI	1.02±0.04
Early estrus	80% SCI	1.57±0.073
	100% SCI	2.56±0.1
Mid estrus	100%KPI (Day 0)	6.88±0.315
	100% KPI (Day 3)	8.9±0.28
Late estrus	100% KPI (Day 6)	11.05±0.36
Early diestrus	Day of refractoriness>80%Parabasal cells	18.15±0.7
Mid diestrus	100% KPI (Day 15)	55.72±4.5
	100% KPI (Day 30)	97.98±4.5
Late diestrus	100% KPI (Day 60)	26.08±2.4
Early anestrus	100% KPI (Day 90)	1.035±0.12
Mid and late anestrus	100% KPI (Day 120)	<1

SCI: Superficial cell index, KPI: Karyopyknotic cell index.

Table 4: Comparative reproductive performance parameters.

Groups	n	No. of mating	Conception rate	Litter size
Control (data by Questionaire)	105	1.35±0.06[a]	53.57%	4.028±0.214[a]
VEC	100	3.44±0.074[b]	85%	7.54±0.14[b]
VEC with P4	12	2.66±0.136[c]	91.66%	7.9±0.34[c]
		P<0.05		P<0.01

VEC: Vaginal exfoliative cytology, P4: Serum progesterone assay.

Table 5: Questionnaire to assess the reproductive parameters and breeding practices of chippiparai bitches.

Name of the owner	Animal Identification.
Resent weight:	Present age:
Age at the time of puberty:	Diet:
Date of proestrus bleeding starts:	Length of proestrus bleeding in last cycle:
No. of days mating allowed:	Frequency of mating:
Gestational length: (No. of days after last mating)	Inter-estrus period:
Season of whelping:	No. of parity:
Litter size:	No. of live pups:
Mortality per litter:	Still birth per litter
Nature of whelping:	Sex ratio of foetus:

Information of the male dog used for the mating purpose.

and litter size increased up to 91.66% and 7.9±0.34 pups, respectively by using VEC and blood progesterone concentration as the diagnostic tool to estimate optimum breeding time in Chippiparai bitches which was in agreement with the findings of Johnston *et al.* (2001) and Antonov (2017).

In this study the conception rate recorded in Group II (87%) and Group III (91.66%) was significantly higher than Group I (53.57%) which were in agreement with Reddy *et al.* (2011) and Arlt (2018).

CONCLUSION

On the basis of present study, certain reproductive parameters of Chippiparai female had been characterized. Delayed age of first mating, subsequent less number of parities, less number of mating per cycle and mating with litter mates are the myths involved in the breeding practices which resulted in reduction in the number of this majestic breed in the breeding tract and reduced its reproductive performance. From the present study it was concluded that VEC and progesterone estimation could be used as effective diagnostic tools to augment the conception rate, litter size and also helped in identification of fertile period in Chippiparai bitches.

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Conflict of interest

All authors declare that they have no conflict of interest.

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