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ORIGINAL ARTICLE

Influence of estrous stages on electrocardiography, clinical pathology and ovarian weight of experimental beagle dogs: a retrospective analysis

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ABSTRACT

Estrous cycle is a repetitive phenomenon occurring during the reproductive life of a female dog. The duration of the canine estrous cycle is considerably longer than one in the most of the other animals and is broadly grouped into follicular phase (proestrus and estrus), luteal phase (diestrus) and non-seasonal anestrus. Dogs in the same stage of cycle can be inadvertently assigned to same group during routine safety and metabolic studies leading to possible erroneous interpretation of test-item related effects. This retrospective analysis was conducted by analyzing data of 86 female beagle dogs from control/placebo treated groups to correlate any possible effect of estrous stages with electrocardiography, clinical pathology and ovarian weight. Different estrous cycle stages of beagles were confirmed histologically by evaluating ovary, uterus, vagina and mammary glands. The incidence of beagles in diestrus was the highest, followed by anestrus, proestrus and estrus. No significant effect was noticed on heart rate, P–A, P–D, RR, QRS and QT intervals across different stages of estrous cycle. However, significantly higher PQ (PR) interval in dogs in proestrus stage was observed compared to dogs in anestrus and estrus. Marginally higher WBCs, neutrophils, lymphocytes, RBCs, hemoglobin, AST and lower hematocrit, lipid profile (total cholesterol, HDL, LDL, triglycerides), ALP level was evident in estrous period. Relative ovary weight was significantly higher in dogs in diestrus stage. Considering these results, one may need to exercise caution while interpreting experimental data from female beagle dogs.

KEY WORDS: estrous cycle; electrocardiography; clinical pathology; ovarian weight; beagle dogs

Introduction

Estrous cycle is a repetitive phenomenon occurring during the reproductive life of a female that involves a patterned sequence of structural, functional and hormonal changes in the reproductive system (Butinar *et al.*, 2004). Compared with the other laboratory animals, there are numerous reproductive features which are inimitable in female dogs. The duration of the canine estrous cycle is considerably longer than one in the mostof the other animals (Butinar *et al.*, 2004). The canine estrous cycle consists of 4 phases: proestrus, estrus, diestrus and anestrus. Broadly these different stages of estrous cycle can be grouped into follicular phase (proestrus and estrus), luteal phase (diestrus) and non-seasonal anestrus (Chandra & Adler, 2008). Anestrus length and ovarian cycle intervals, variable within and among bitches, are likely affected by neuroendocrine components of an endogenous circannual cycle. This is linked and controlled by cyclical fluctuations in the levels of FSH, LH, estrogen and progesterone (Butinar *et al.*, 2004). Hardly any organ in the body remains unaffected by these large hormonal fluctuations. Repeated cyclical changes and variations in the estrogen and progesterone level in the blood during different phases could affect the blood/plasma volume, cardiac activity and may also affect electrocardiographic pattern and reproductive organ weight. In routine safety studies and certain metabolic studies, due to small group size (3 to 6 dogs per dose group), dogs in the same stage of the cycle can be inadvertently

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assigned to same group potentially leading to erroneous interpretation of drug-induced effects on the electrocardiography, clinical pathology and reproductive organ weight when the test chemical being tested has either direct or indirect effect on the female reproductive system/hormones. Recording of ECG in non-rodents has a particular importance during preclinical assessment of drugs: to extrapolate the potential cardiovascular risk in humans (Hammond *et al.*, 2001; Finley *et al.*, 2003). Multiple factors have been described to affect the ECG parameters (De Ponti *et al.*, 2002; Luo *et al.*, 2004). There is a paucity of literature which describes effect of estrous on ECG parameters in animals.

Keeping in view of all the above factors, the present retrospective analysis in beagle dogs from control/placebo groups of routine preclinical toxicity studies was undertaken to correlate and summarize any possible effect of estrous stage with electrocardiography, clinical pathology and reproductive organ weight.

Materials and methods

This retrospective analysis was conducted using data from 86 female beagle dogs which were used as control (vehicle/placebo treated) animals in toxicity studies

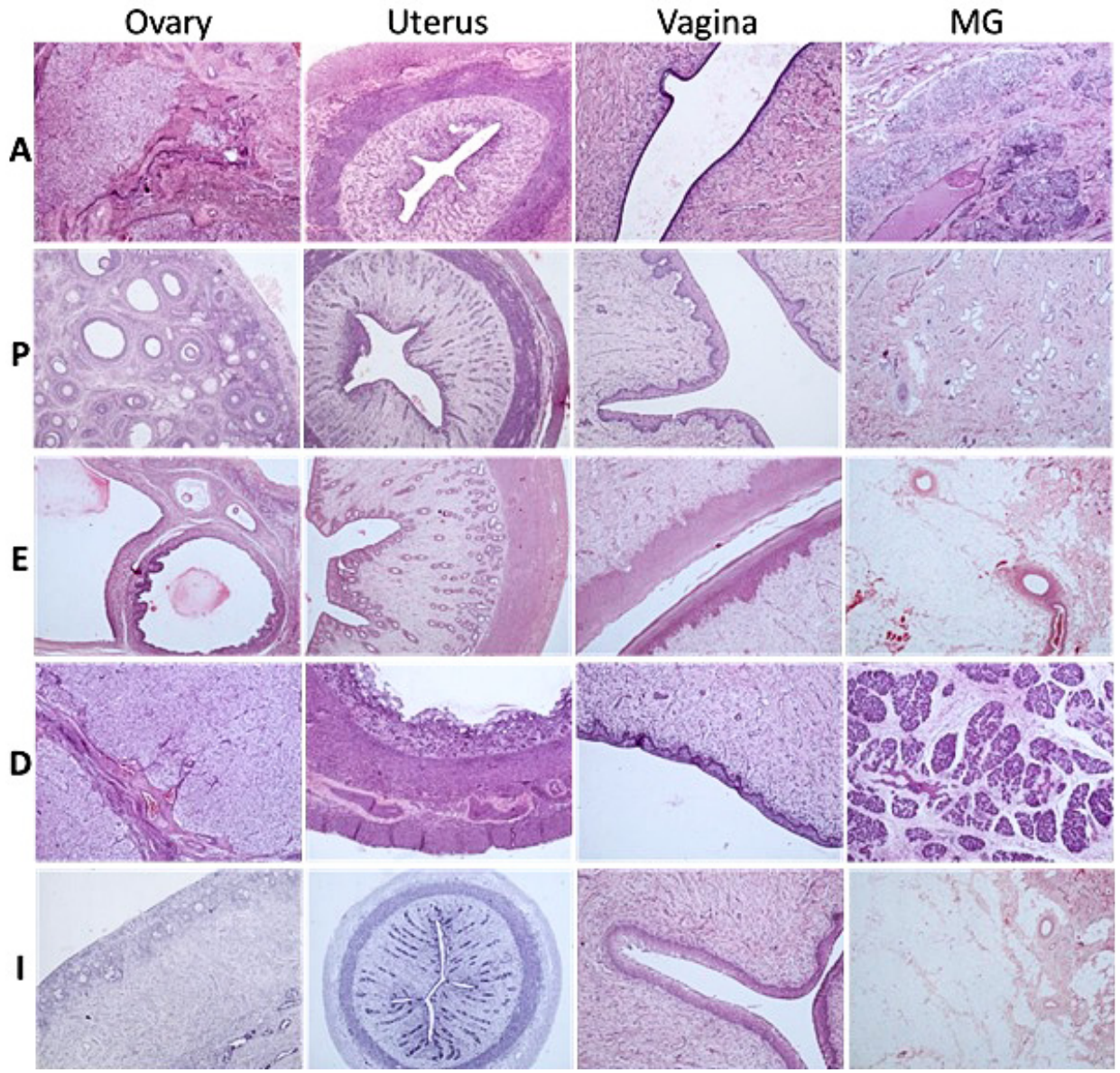

Figure 1. Typical histological feature (H&E Stain) of the ovary (40×), uterus (40×), vaginal mucosa (100×) and mammary gland (MG) (40×) in anestrus (A), proestrus (P), estrus (E), diestrus (D) and immature (I) beagle dogs.

conducted between year 2005 and 2014 at Zydus Research Centre, Ahmedabad, India. Age at termination was in the range of 10–21 months. All dogs were supplied by Animal Research Facility of Zydus Research Centre and were housed individually in kennels, under identical housing and husbandry conditions at 22±3 °C temperature and 30% to 70% relative humidity with 12/12 hours light/dark cycle. Comingling was permitted daily for few hours. Standard dog feed (Pedigree, Mars International India Pvt. Ltd.) and purified water was provided to dogs. Periodical quality checking of feed and water were performed to ensure proper nutrient content and acceptable limits of total dissolved solute in water and microbial contamination as per Standard Operating Procedures of Zydus Research Centre.

Electrocardiographic (ECG) examination was performed on all dogs during the terminal stage of each study using CARDIOVIT AT-1(VET) Electrocardiograph Machine, Schiller AG, Switzerland. The ECG parameters viz. heart rate, P–A, P–D, RR, QRS, QT, QTc and PR intervals were recorded from Lead II. Blood was collected from cephalic/saphenous vein for hematology (Cell-DYN® 3700, USA) and clinical chemistry (Randox Daytona analyzer, Randox Laboratories Ltd., USA and EasyLyte electrolyte analyzer, Medica Corporation, USA). Animals were euthanized by intravenous injection of overdose of Thiopentone Sodium. At termination, animals were subjected to gross pathological examinations and tissues (ovary, uterus, vagina and mammary gland) were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 µm thickness and stained with hematoxylin and eosin. Stage of estrous was confirmed by histological examination of ovary, uterus, vagina and mammary glands and further data was grouped into proestrus, estrus, diestrus, anestrus and immature stages (Figure 1 and Table 1) (Harleman & Foley, 2001; Rehm *et al.*, 2007; Chandra & Adler, 2008).

The studies were conducted in AAALAC (Association for Accreditation and Assessment of Laboratory Animal Care) accredited facility, in compliance with Indian regulations (Committee for the Purpose of Control and Supervision of Experiments on Animals, 2005) governing the housing and use of animals and all the procedures used in these studies were reviewed and approved by the Institutional Animal Ethics Committee.

Results

Incidences of estrous stages

The percentage incidence of diestrus, anestrus, proestrus and estrus were 33.73, 26.51, 26.51 and 4.82 respectively. In addition, few dogs (8.43%) were found to be immature during histological evaluation (Table 2).

Electrocardiography

The electrocardiographic parameters such as P–A, P–D, RR, QRS and QT intervals did not reveal any significant changes at different stages of estrous cycle. In addition, no distinct pattern of variation in heart rate was evident at different phases of estrous cycle. However, higher PQ (PR) interval was observed in dogs of proestrus stage compared to dogs in anestrus and estrus stages (Table 3).

Table 1. Overview of estrous cycle with duration and hormonal dominance.

Stage	Duration (in days)	Hormonal dominance
Proestrus	9	Estrogen, FSH
Estrus	9	Estrogen, LH, FSH
Diestrus	60	Progesterone, Prolactin
Anestrus	100–150	FSH, Prolactin

FSH-Follicle Stimulating Hormone, LH- Luteinizing hormone

Clinical Pathology

There were no statistically significant changes in hematology and clinical biochemistry parameters. However, certain non-significant fluctuations such as marginally higher WBCs, neutrophils, lymphocytes, RBC counts, hemoglobin, AST and lower hematocrit, lipid profile (total cholesterol, HDL, LDL, triglycerides) and ALP level were evident in estrus stage. Lower triglycerides, HDL cholesterol, total protein and globulin and higher serum inorganic phosphorous were observed in immature animals (Tables 4 and 5).

Organ weight

The absolute and relative ovary weight was higher in diestrus stage and lower in immature animals.

Discussion

The beagle is essentially monoestric (Anderson & Simpson, 1973; Chastain & Ganjam, 1986; Concannon, 1993) with considerable variation in intervals between cycles and in hormonal profile during different stages of estrous cycle (Table 6). Non-rodent safety and metabolic studies are frequently performed in purpose-bred beagle dogs with a limited (three to six per group) number of dogs assigned randomly to individual treatment groups based on body weight.

The assigned stage of cycle for each bitch in the present analysis was based completely on the histomorphological findings in the reproductive organs viz. ovary, uterus, vagina and mammary glands (Harleman & Foley, 2001; Rehm 2007; Chandra & Adler, 2008). The present retrospective analysis indicated that diestrus stage was the most frequent in bitches at the time of termination in toxicity studies followed by anestrus, proestrus and estrus. The higher chances of occurrence of bitches in anestrus-diestrus are expected due to their longer duration in the cycle (Table 1) (Chandra & Adler, 2008; Van Cruchten *et al.*, 2003).

Table 2. Key histological features during different stages of estrous cycle in beagle dogs.

Organs	Proestrus	Estrus	Diestrus	Anestrus	Immature
Ovary	Moderately large ovarian antral follicles. Liquor folliculi lined by multiple layers of granulosa cells. Small, shrunken (atretic) corporal lutea (CL) with vacuolated cells.	Larger and cystic follicles. Two or three large tertiary follicles lined by stratified layers of elongated granulosa cells. Follicles lined by thick layers of rounded luteinizing cells amid loose stroma, and the cystic space containing eosinophilic material.	Large with large CL. Up to two large CLs with closely packed luteal cells containing amphophilic to eosinophilic cytoplasm. Late phase luteal cells rarefy to vacuolated cytoplasm.	In the early phase, small CLs with irregular outline, luteal cells containing cytoplasmic vacuoles and prominent vasculature (arterioles) and stroma. Late phase: shrunken (atretic) CLs containing lipofuscin pigment. Early follicular development with many primary and secondary follicles.	Absence of CL or its remnants.
Uterus	Clear and edematous endometrial stroma. Proliferation of superficial and deep endometrial glands. Thicker myometrium with hypertrophied eosinophilic smooth muscle cells.	Thick and eosinophilic endometrial stroma. Glandular and myometrial features similar to those in proestrus.	Thick myometrium composed of hypertrophied smooth muscle cells and thick endometrium. Initially, the superficial epithelial cells are columnar and eosinophilic, followed by a fine vacuolated appearance in the later part of this phase.	Atrophic Small cross-shaped lumen. Basophilic endometrial stroma. Compact myometrium.	–
Vagina	Five to seven layers of squamous epithelium.	Five to seven layers of squamous epithelium covered by four to six layers of keratin (hyperkeratosis and parakeratosis).	Variable histological features. Mucosa lined by three to four layers of cuboidal epithelium with neutrophils between the cell layers.	Thinner than in diestrus. Generally two cell layers thick. Conspicuous absence of leukocytes.	–
Mammary gland	Quiescent and inactive ducts. Hemosiderin pigment and occasional apoptosis. No mitosis. Compact stroma and inactive glands.	Inactive (quiescent) glandular tissue to very slight stromal/periductal edema.	Phase I: Stromal and ductal proliferation. Phase II: Early lobular development with branching ducts and alveolar proliferation. Phase III: Abundance of glandular tissue with large lobules containing secretory material. Phase IV: Early regression, increased interlobular connective tissue, and eosinophilic secretions in distended ducts and acini.	In the early part, ducts distended with secretions and acinar regression not complete. Apoptosis of acinar epithelium. In late anestrus, lobular architecture of the glandular tissue barely evident. Abundant mature connective tissue, and collapsed ducts.	Glandular tissue barely discernible in dermis.

Table 3. Electrocardiographic changes in different stages of estrous cycle.

Parameters	Follicular Phase		Luteal Phase	Sexually inactive	
	Estrus (e)	Proestrus (p)	Diestrus (d)	Anestrus (a)	Immature (i)
RR (msec)	480.7±96.6	473.7±92.8	547.3±138.7	457.3±76.4	496.6±70.4
HR (bpm)	127.4±25.6	131.5±27.7	118.2±30.2	135.1±26.5	122.8±16.0
P–A (mV)	0.3±0.0	0.2±0.1	0.2±0.1	0.3±0.1	0.2±0.1
P–D (msec)	40.0±2.0	42.6±5.7	43.3±12.0	41.8±4.4	37.1±4.9
QT (msec)	185.0±7.1	181.2±18.3	192.3±16.5	174.4±14.7	180.0±15.3
QTc(B) (msec)	268.4±16.8	265.5±26.7	266.1±24.3	259.2±14.5	256.1±15.7
QTc(F) (msec)	237.0±6.9	233.5±20.8	238.4±15.4	226.9±11.8	227.6±14.1
QTc(V) (msec)	230.2±1.3	227.0±15.7	228.2±19.6	213.1±18.8	221.8±16.5
QRS (msec)	50.0±2.2	51.8±4.4	49.8±8.8	48.4*^{i}±2.3	60.0*^{a}±1.0
PQ/PR (msec)	80.0*^{p}±14.1	107.8*^{a,e}±8.3	94.0±13.4	91.4*^{p}±12.2	94.3±15.1

All values in mean±SD, *: Statistically significant at $p<0.05$ as compared to respective phase superscripted

Table 4. Hematological changes in different stages of estrous cycle.

Parameters	Follicular Phase		Luteal Phase	Sexually inactive	
	Estrus (e)	Proestrus (p)	Diestrus (d)	Anestrus (a)	Immature (i)
WBC (10^3 cells/µl)	10.9±1.2	9.1*[i]±1.3	8.2±2.5	8.9*[i]±2.2	8.5*[p,a]±2.7
RBC (10^6 cell/µl)	7.0±1.4	6.5±0.8	6.2±0.9	6.4±0.8	6.2±0.3
Hemoglobin (g/dl)	16.2±2.1	14.8±1.2	14.7±1.6	15.4±1.6	14.5±1.0
Hematocrit (%)	40.0±2.0	43.6±4.1	42.7±5.4	45.1±5.2	41.6±3.0
MCV (fl)	68.4±3.46	67.1±2.7	68.7±3.3	70.8±3.6	67.3±2.0
MCH (pg)	23.4±1.8	22.8±1.1	23.8±1.3	24.2±1.2	23.4±0.6
MCHC (g/dl)	34.3±0.9	33.9±0.8	34.6±1.0	34.1±0.8	34.8±0.7
Platelets (10^3 cells/µl)	346.5±29.0	384.5±108.2	333.0±79.1	307.6±126.7	255.7±29.7
Neutrophils (10^3 cells/µl)	7.2±0.6	6.2±0.9	5.6±1.7	6.7±2.2	4.8±1.6
Lymphocytes (10^3 cells/µl)	3.2±0.1	2.4±0.5	2.1±0.9	1.8±0.8	2.8±1.1
Monocytes (10^3 cells/µl)	0.4±0.6	0.4±0.4	0.4±0.3	0.4±0.2	0.7±0.3
Eosinophils (10^3 cells/µl)	0.003±0.003	0.007±0.005	0.006±0.009	0.008±0.008	0.036±0.081
Basophils (10^3 cells/µl)	0.06±0.09	0.02*[i]±0.02	0.03±0.03	0.02*[i]±0.02	0.08*[p,a]±0.06
PT (sec)	8.2±1.1	8.1±0.9	9.1±3.3	7.94±0.5	8.1±0.7
APTT (sec)	10.6±0.7	11.7±1.4	11.2±1.2	12.5±1.9	11.3±0.9
Reticulocyte (%)	0.5±0.2	0.7±0.2	0.5±0.3	0.5±0.3	0.8±0.5
Reticulocytes (10^3 cells/µl)	26.0±11.2	43.3±17.7	34.2±23.5	43.0±23.5	46.6±30.8

All values in mean±SD, *: Statistically significant at $p<0.05$ as compared to respective phase superscripted.

Recording of ECG has a particular importance on cardiac repolarization during preclinical assessment of drugs. Dog shares certain similarities with the human electrical conduction system and hence used to evaluate the potential risk of arrhythmia in humans (Hammond *et al.* 2001; Finley *et al.* 2003), which cannot be assessed by other methods and have no morphological correlates visible in histopathological examination (Detweiler, 1981). Owing to association with Torsades de Pointes, drug-induced QT interval prolongation has been and remains a significant hurdle to the development of safe and effective drug. Drug regulatory agencies have showed increasing interest in the QT interval because certain drugs can prolong the QT interval to a level that produces ventricular arrhythmias. QT interval is a dynamic physiological variable that can be affected by the velocities of both the ventricular conduction and repolarization (Moss, 1999; Sheridan, 2000; De Ponti *et al.*, 2002). Multiple factors have been described to affect the QT interval such as cardiac cycle length, autonomic nervous system activity, age, gender, circadian rhythm, plasma electrolyte concentrations and variations in ion channels involved in cardiac repolarization (De Ponti *et al.*, 2002; Luo *et al.*, 2004). However, effect of estrous on QT and other ECG parameters have not been reported in experimental beagle dogs. In the present analysis, it was found that there was no statistically significant influence of estrous on QT interval in bitches.

The PQ (sometimes referred to as the PR interval as a Q wave is not always present) interval indicates how fast the action potential is transmitted through the atrioventricular node (AVN) from the atria to the ventricles. A prolonged PQ interval is a sign of a degradation of the conduction system or increased vagal tone (Bezold-Jarisch reflex), or it can be pharmacologically induced, characterized as 1st , 2nd or 3rd degree AV block depending on the severity (Hanton & Rabemampianina, 2006). In present analysis, PQ interval was found to be higher in proestrus dogs as compared to dogs in anestrus and estrus; nonetheless all the values observed were within historical data range. Reports suggest that in addition to the differences in PQ interval between genetic strains, there shall be high inter- and intra-animal (beat to beat) variability in PQ interval and values up to 169 ms may occur in healthy animals (Hanton & Rabemampianina, 2006). Review of various literatures suggests that PQ interval may get affected by heart rate in dogs which could be due to drug treatment or experimental conditions, in particular stress and excitation (Ettinger & Suter, 1970; Ganz & Knappen, 1976). In our study, no such findings correlate to fact that the prolonged PQ interval is associated with heart rate, as no changes were evident in heart rate among different

Table 5. Serum biochemical changes in different stages of estrous cycle.

Parameters	Follicular Phase		Luteal Phase	Sexually inactive	
	Estrus (e)	Proestrus (p)	Diestrus (d)	Anestrus (a)	Immature (i)
Glucose (mg/dl)	96.6±1.1	88.7±8.7	91.5±8.9	93.0±12.6	100.5±10.0
Triglycerides (mg/dl)	60.8±49.4	96.7±49.0	92.4±44.2	155.0*i±106.8	48.3*a±6.0
Total Cholesterol (mg/dl)	239.9±76.4	289.8±79.0	292.3±82.4	288.5±76.8	216.0±31.2
HDL Cholesterol (mg/dl)	187.0±68.5	184.3±21.8	191.0±35.0	196.4*i±35.5	148.7*a±26.9
LDL Cholesterol (mg/dl)	9.4±7.2	21.6±17.3	21.6±14.6	23.2±17.6	9.3±5.9
ALT (U/l)	50.0±2.2	28.5±5.2	26.9±7.1	31.6±6.6	35.6±11.2
AST (U/l)	46.2±1.2	39.2±21.3	37.5±29.6	33.3±12.9	32.3±11.2
ALP (U/l)	80.1±16.1	177.6±119.0	108.7±69.3	173.6±82.9	86.0±16.0
GGT (U/l)	1.6±0.5	2.7±1.1	3.3±1.7	2.8±1.3	2.2±1.5
Creatine Kinase (U/l)	120.2±34.6	205.4±124.9	188.7±116.9	198.8±69.6	241.2±73.7
Total Bilirubin (mg/dl)	0.3±0.3	0.1±0.1	0.2±0.1	0.2±0.1	0.3±0.2
Total Protein (g/dl)	6.0±0.6	5.8±0.6	6.0±0.5	6.2*i±0.3	5.3*a±0.3
Albumin (g/dl)	3.3±0.3	3.1±0.5	3.2±0.3	3.2±0.2	3.5±0.5
Globulin (g/dl)	2.7±0.4	2.8*i±0.4	2.8*i±0.4	2.9*i±0.3	1.9*p,d,a±0.6
A/G ratio	1.3±0.1	1.1*i±0.2	1.2*i±0.2	1.1*i±0.2	2.1*p,d,a±1.0
Urea (mg/dl)	23.1±3.0	22.7±4.3	24.4±8.4	26.8±17.0	30.6±4.9
Creatinine (mg/dl)	0.9±0.1	0.8±0.1	0.8±0.2	0.8±0.3	0.9±0.2
Phosphorus (mg/dl)	4.9±0.5	4.2±0.7	4.0*i±0.8	4.8±1.1	5.3*d±0.8
Calcium (mg/dl)	10.1±0.6	10.1±0.8	10.3±0.8	10.5±0.5	10.6±1.0
Sodium (mmol/l)	147.3±2.7	146.7±1.9	147.3±2.1	147.3±2.5	146.4±4.0
Potassium (mmol/l)	4.6±2.7	4.5±1.9	4.5±2.1	4.5±2.5	4.4±4.0
Chloride (mmol/l)	111.9±1.1	111.5±2.0	112.7±1.9	113.3±4.3	114.2±1.0

All values in mean±SD, *: Statistically significant at $p<0.05$ as compared to respective phase superscripted.

Table 6. Ovarian weight in different stages of estrous cycle.

Parameters	Follicular Phase		Luteal Phase	Sexually inactive	
	Estrus ^{e}	Proestrus ^{p}	Diestrus ^{d}	Anestrus ^{a}	Immature ^{i}
Absolute Ovary weight (grams)	1.22±0.11	1.52±0.65	1.94*i±0.63	1.32±0.55	0.85*d±0.12
Ovary weight (relative to body weight)	0.007±0.002	0.009*d±0.004	0.015*a,i,p±0.006	0.010*d±0.003	0.008*d±0.001
Ovary weight (relative to brain weight)	1.36±0.15	1.81±0.79	2.52*i±0.84	1.74±0.66	1.09*d±0.10

All values in mean±SD, *: Statistically significant at $p<0.05$ as compared to respective phase superscripted.

stages of estrous and all dogs used in the present analysis were from control group.

The cyclic changes that occur in the female reproductive tract are stimulated and regulated by ovarian steroid hormones, estrogen and progesterone, that in turn are controlled by an integrated hypothalamic-pituitary-ovarian (HPO) axis through release of FSH and LH. The ovary plays a pivotal role in the estrous cycle (Evans, 2009). Numerous studies have been undertaken to examine the fluctuation in clinical pathological parameters during the estrous cycle in dogs and other laboratory animal species. The present study reveals that hematological determinants show hardly any variation during the different stages of estrous cycle. However, total WBC, neutrophils, lymphocytes, RBC counts and hemoglobin values showed non-significant higher values at estrus

stage. Contrastingly, Willson *et al.,* 2012 reported lower hematocrit, RBC count and hemoglobin values in diestrus dogs. Interestingly, our analysis did not reveal any changes in the circulating eosinophil numbers in relation to the estrous stages. However, Willson *et al.,* 2012 reported that there was a 45.8% higher circulating eosinophils in diestrus dogs. Corroborative to our findings, few authors (Castrodale *et al.*, 1941; Crafts, 1948; Gaunt & Pierce, 1986) also reported increase in neutrophil production in the bone marrow, resulting in an increase in white blood cells in the peripheral blood after administration of exogenous estrogen.

All the minor changes in clinical pathological parameters during the estrous cycle may be associated with the presumptive changes in blood estrogen, progesterone, gonadotrophic hormones and/or body temperature. Similar to our findings, Günzel-Apel *et al.*, 1997 also reported that PT, APTT and hematocrit were unaffected during the estrous cycle in bitches, however, he concluded that the luteal phase of the nonpregnant and pregnant bitches exhibited significantly increased fibrinogen, the large number of platelets and the decreased antithrombin III activity which were attributed to direct or indirect effects of the high peripheral progesterone concentrations. Landshman & Bleiberg, 1979 reported that estrogen enhanced megakaryocytopoiesis and erythropoiesis in the bone marrow and spleen in mice. Rüberg *et al.,* 1990 studied the fluctuations in blood coagulation parameters during estrous cycle in experimental dog models and indicated that higher blood estradiol and progesterone were accompanied by impaired and enhanced coagulation respectively. They also observed increased platelet count during proestrus and estrus but they concluded it impossible to relate changes in coagulation to a given stage of estrous or ovulation due to individual variation.

With some limitations, the stage of the cycle can be determined through a combination of vaginal smears and serum hormone levels prior to initiating safety/toxicity studies (Fowler *et al.*, 1971; Vermeirsch *et al.*, 2001)

Conclusion

The long duration of the estrous cycle, monoestric behavior, and variable duration of individual estrous stages can be confounding factors in interpreting xenobiotic-induced effects on the female reproductive system in beagle dog. In conclusion, one should consider the overall endocrine system and unique reproductive features to correlate electrocardiographical, clinical pathological and ovarian weight data when interpreting data from safety/toxicity studies.

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