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Effect of GnRH analogue, buserelin acetate, on semen quality of subfertile male dogs#

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Abstract

The study was undertaken to evaluate the effect of GnRH analogue, buserelin, on the semen quality of subfertile male dogs. Six male dogs between two and seven years of age with a history of subfertility presented for breeding soundness evaluation (BSE) at the University Veterinary Hospital, Kokkalai were selected for the study. A comprehensive BSE with emphasis on previous six month breeding history and current semen quality was performed for all animals to confirm subfertility. Estimation of libido score, serum testosterone and semen evaluation parameters viz., volume and pH of sperm-rich fraction, sperm progressive motility, concentration, viability, abnormality, HOS response and alkaline phosphatase (ALP) in seminal plasma of sperm-rich fraction of each of the subfertile dogs was performed before (Day 0) and after treatment (Days 60 and 90). The animals were treated with intramuscular administration of buserelin at the rate of 10 µg per dog, weekly once, for six weeks. Significant improvement was observed in libido score, serum testosterone levels and all the semen parameters except semen pH on Day 60. However, there was a moderate decline in the aforementioned parameters except semen volume on Day 90.

Keywords: *GnRH analogue, buserelin, Subfertile, BSE, ALP*

Subfertility is one of the prevalent fertility problems in male dogs and could be suspected if a stud dog failed to produce litters in over 75 per cent of breedings when bred to normal bitches

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by observing adequate breeding management protocols (Feldman and Nelson, 1996). Almost about 15-20 per cent of dogs used for breeding are affected by subfertility (Domosławska *et al*., 2019). The inability to consistently produce litters could be terminal for the breeding career of a male dog, causing huge economic loss to the breeder and detrimental to the canine breeding industry. Subfertility may result from a variety of factors such as lack of libido, inability to perform breeding and abnormalities of the semen. Poor semen quality is one of the major aetiologies that lead to subfertility and this is caused by congenital anomalies, hormonal disturbances, testicular or prostatic disorders, infections, autoimmune disorders, oxidative stress, nutritional deficiencies, toxins, adverse environment and lifestyle (Freshman, 2001).

Testosterone has a crucial role in regulating spermatogenesis, functions of the accessory sex glands, male libido, maturation of spermatozoa, secondary sexual characteristics and negative feedback on LH secretion. The lack of testosterone can lead to deterioration in canine semen quality. The GnRH analogues stimulate the synthesis and secretion of gonadotrophins and in turn testosterone secretion. These hormones may be used to improve the male reproductive potential (Fraser and Lincoln, 1980). Consequently, the objective of the current study was to ascertain the effect of administering GnRH analogue, buserelin, on the semen quality of subfertile male dogs.

Six male dogs (2-7 years of age), that had been previously used for breeding and currently with a history of subfertility and presented for breeding soundness evaluation (BSE) at the University Veterinary Hospital, Kokkalai, were selected for the study. A detailed breeding history collection of these animals was performed. The collection of semen and the study of sexual behaviour during collection were carried out in the presence of female dog in oestrus. A female dog in oestrus was used during semen collection to evaluate male libido. It was graded according to the libido index as follows: 1 - No interest, 2 - Interest with hesitation, 3 - Little interest, 4 - Quick mounting, 5 – Eager and 6 – Uncontrolled (Price *et al*., 1992; El-mamlouk *et al*., 2020). Semen was collected by digital manipulation as described by Simon (1997) in the presence of a bitch in oestrus. Initial sperm motility was measured as soon as the sperm-rich fraction of the semen was obtained. The volume and pH of the sperm-rich fraction of semen were estimated using graduated glass collection tubes and narrow range (5-7.5) pH indicator paper. The alkaline phosphatase (ALP; IU/L) in the seminal plasma of the sperm-rich fraction was estimated using semi-automatic analyser (MasterT biochemistry analyser, Hospitex diagnostics, Italy) as per the methodology of Strzezek *et al*. (2015). The Neubauer haemocytometer was employed to evaluate the sperm concentration (Allen, 1991). The sperm viability and abnormalities were determined using the eosin-nigrosin staining technique (Hancock and Rowlands, 1949). The functional membrane integrity of the spermatozoa was analysed using the hypo-osmotic solution (150 mOsm) as per Goericke-Pesch and Failing (2013).

Blood samples were collected from all the animals by cephalic venipuncture and transferred into heparin vacutainer tubes for serum testosterone estimation. Serum testosterone (ng/mL) levels were measured by electrochemiluminescence immunoassay (ECLIA) method using Roche cobas e 411 analyser (Mannheim, Germany). Based on the breeding history and libido index the animals were diagnosed as subfertile. These animals were treated with intramuscular administration of buserelin acetate at the rate of 10 µg per dog, weekly once, for six weeks. Sexual rest was advised for these animals during the treatment period and a comprehensive BSE with emphasis on semen evaluation and aforementioned parameters was performed before (Day 0) and after the onset of treatment (Days 60 and 90).

Records were enclosed in a database and statistically analysed using the SPSS version: 24.0 statistics software package. Between-days comparison of all parameters except libido score was done by using repeated measures ANOVA followed by the least significant difference (LSD) test. In the case of libido score, between-days comparison was done by using Friedman's test followed by pair-wise comparison.

The mean libido score on Days 0, 60 and 90 were presented in Table 1. A highly significant ($p < 0.01$) improvement in mean libido score was observed between Days 0 and 60 while a significant decline was noticed on Day 90 (with respect to libido score on Day 60). However, no significant difference was noticed between Days 0 and 90 (Table 1). These observations were in concurrence with the findings of Purswell *et al*. (1993), Hess (2006) and El-mamlouk *et al*. (2020) who reported an improvement in the libido of male dogs following GnRH therapy. The increase in testosterone levels following GnRH administration might be the cause of the improvement in libido (Purswell *et al*., 1993; Hess, 2006). This might be due to the pituitary desensitisation which occurs when the pituitary cells are continuously exposed to GnRH following a brief period of stimulation. LH concentrations drop to undetectable levels after the pituitary cells begin to desensitise to GnRH, which prevents the production of testosterone and spermatozoa (Tremblay *et al*., 1984; Vickery *et al*., 1984; Dubé *et al*., 1987; Driancourt and Briggs, 2020).

The mean serum testosterone levels on days 0, 60 and 90 were presented in Table 1. A significant ($p < 0.05$) improvement in mean serum testosterone levels was observed between Days 0 and 60 as well as a significant ($p < 0.05$) decline between days 60 and 90. However, no significant difference was noticed between Days 0 and 90 (Table 1). These observations were in agreement with the findings of Kawakami *et al*. (2005), Hess (2006), Kawakami *et al*. (2009) and El-mamlouk *et al*. (2020) who reported an improvement in serum testosterone levels in male dogs following GnRH therapy. This might be due to the pituitary desensitisation which occurs when the pituitary cells are continuously exposed to GnRH following a brief period of stimulation. LH concentrations drop to undetectable levels after the pituitary cells begin to desensitise to GnRH, which prevents the production of testosterone and spermatozoa (Tremblay *et al*., 1984; Vickery *et al*., 1984; Dubé *et al*., 1987; Driancourt and Briggs, 2020).

The mean volume of the sperm-rich fraction of semen ejaculates on Days 0, 60 and 90 were presented in Table 2. The mean volume of sperm-rich fraction of semen ejaculate differed significantly between Days 0 and 60, although no significant difference was observed between Days 0 and 90 or Days 60 and 90 (Table 2). These observations were in concurrence with the findings of Kawakami *et al*. (1997) and El-mamlouk *et al*. (2020) who reported an improvement in semen volume following GnRH therapy.

The mean pH of the sperm-rich fraction of semen on Days 0, 60 and 90 were presented in Table 2. There was no significant difference in the mean pH of the sperm-rich fraction of semen after treatment and, was found to be within the normal physiological range (6.3-7.0) as recorded by Gradil *et al*. (2006) and Robert *et al*. (2016) in healthy male dogs.

A highly significant increase in mean sperm progressive motility was observed on Day 60 when compared to Day 0, followed by a significant decrease on Day 90 (Table 3). These observations were in agreement with the findings of Kawakami *et al.* (1997), Kawakami *et al.* (2005), Kawakami *et al.* (2009) and El-mamlouk *et al*. (2020) who reported an improvement in sperm motility parameters following GnRH therapy.

Table 1. Between-days comparison of libido score and serum testosterone levels before and after treatment

Parameter	Day 0 (Before treatment)	Day 60 (Post-treatment)	Day 90 (Post-treatment)	p-value
Libido score	$2.33^{B} \pm 0.42$	$4.50^{A} \pm 0.43$	$2.17^{B} \pm 0.40$	0.006**
Testosterone (ng/mL)	$2.03^{B} \pm 0.67$	$3.46^{A} \pm 0.55$	$2.91^{B} \pm 0.52$	0.013*

** Significant at 0.05 level; ** Significant at 0.01 level*

Means having different capital letters as superscripts differ significantly within a row

Table 2. Between-days comparison of macroscopic semen parameters (sperm-rich fraction) before and after treatment

Parameter	Day 0 (Before treatment)	Day 60 (Post-treatment)	Day 90 (Post-treatment)	p-value
Volume (mL)	$1.50^{B} \pm 0.15$	$1.73^{A} \pm 0.12$	$1.58^{AB} \pm 0.16$	0.012*
pH	6.42 ± 0.15	6.42 ± 0.08	6.50 ± 0	0.751^{ns}

** Significant at 0.05 level; ns non-significant*

Means having different capital letters as superscripts differ significantly within a row

The mean sperm concentration on Days 0, 60 and 90 were presented in Table 3. A significant improvement in the mean sperm concentration was noticed between Days 0 and 60 as well as between Days 0 and 90. However, no significant difference in the mean sperm concentration was observed between Days 60 and 90 (Table 3). These observations were in concurrence with the findings of Purswell *et al.* (1993), Kawakami *et al.* (1997), Kawakami *et al.* (2005), Hess (2006), Kawakami *et al.* (2009) and El-mamlouk *et al*. (2020) who reported an improvement in canine sperm concentration following GnRH therapy.

The mean per cent of sperm viability on Days 0, 60 and 90 were presented in Table 3. A significant improvement in mean sperm viability was observed between Days 0 and 60, Days 60 and 90 as well as between Days 0 and 90 (Table 3). These observations were in agreement with the findings of Kawakami *et al.* (1997) and El-mamlouk *et al*. (2020) who reported an improvement in sperm viability following GnRH therapy.

The mean per cent of sperm abnormalities on Days 0, 60 and 90 were presented in Table 3. A significant reduction in mean sperm abnormalities was observed between Days 0 and 60 as well as between Days 0 and 90. However, no significant difference was noticed between Days 60 and 90 (Table 3). These observations were in concurrence with the findings of Kawakami *et al.* (1997) and El-mamlouk *et al*. (2020) who reported a reduction in sperm abnormalities following GnRH therapy.

The mean per cent of HOS-positive sperms was on Days 0, 60 and 90 were presented in Table 3. A significant improvement in mean HOS-positive sperm per cent was observed between Days 0 and 60, Days 60 and 90 as well as between Days 0 and 90 (Table 3). These observations were in agreement with the findings of Sieme *et al*. (2003) and Giriboni *et al.* (2018) who reported an improvement in sperm functional membrane integrity following GnRH therapy which could be due to the improvement in the production of LH and FSH and in turn, testosterone, a key hormone in maintaining spermatogenic homeostasis.

The mean ALP levels in seminal plasma of the sperm-rich fraction on Days 0, 60 and 90 were presented in Table 3. A significant difference in the mean ALP levels in seminal plasma of sperm-rich fraction was noticed between Days 0 and 60. However, no significant difference was observed between Days 0 and 90 as well as between Days 60 and 90 (Table 3). The mean ALP levels observed before and after treatment were in concurrence with the reference range in mixed breeds of fertile dogs reported by Strzezek *et al*. (2015) as 6065.25 ± 999.65 IU/L. Contrary to the present study, Gobello *et al*. (2002) and Raghavendra *et al*. (2022) reported high levels of ALP in the seminal plasma of canine semen.

In the present study, significant improvement was noticed in serum testosterone levels, libido score and semen parameters viz., semen volume, sperm motility, sperm viability, sperm concentration, HOST positive sperm per cent, ALP in seminal plasma and sperm morphology on days 60 post-treatment. These improvements might be due to the increase in serum testosterone levels following GnRH therapy (Purswell *et al*., 1993; Kawakami *et al*., 2005; Hess, 2006; Kawakami *et al*., 2009; El-mamlouk *et al*., 2020). It is interesting to

Table 3. Between-days comparison of microscopic semen parameters before and after treatment

Parameter	Day 0 (Before treatment)	Day 60 (Post-treatment)	Day 90 (Post-treatment)	p-value
Sperm progressive motility (%)	$48.33^{B} \pm 4.41$	$66.67^{A} \pm 4.22$	$55.83^{B} \pm 3.52$	<0.001**
Sperm concentration (million/mL)	$128.83^{B} \pm 24.71$	$193.00^{A} \pm 34.86$	$179.17^{A} \pm 27.75$	0.001**
Sperm viability (%)	$60.83^{C} \pm 4.67$	$78.83^{A} \pm 4.11$	$70.67^{B} \pm 2.79$	<0.001**
Sperm abnormality (%)	$11.67^{A} \pm 1.23$	$6.50^{B} \pm 0.85$	$8.67^{B} \pm 0.88$	0.001**
HOS response (%)	$39.33^{C} \pm 4.44$	$59.17^{A} \pm 4.35$	$49.33^{B} \pm 3.13$	<0.001**
ALP (IU/L)	$6313.83^{B} \pm 275.29$	$6930.5^{A} \pm 448.98$	$6540.33^{AB} \pm 325.11$	0.050*

*** Significant at 0.01 level; * Significant at 0.05 level*

Means having different capital letters as superscripts differ significantly within a row

note that there was a moderate decline in serum testosterone levels, libido and some semen parameters *viz*., sperm motility, sperm viability, sperm concentration, HOS-positive sperm per cent and sperm morphology except semen volume on days 90 post-treatment (when comparing the values of aforementioned BSE parameters on days 60). These findings were in accordance with the observations of Kawakami *et al*. (2005) who reported a transient improvement in semen quality followed by a gradual deterioration in dogs treated with GnRH injection. This might be due to the pituitary desensitisation which occurs when the pituitary cells are continuously exposed to GnRH following a brief period of stimulation. LH concentrations drop to undetectable levels after the pituitary cells begin to desensitise to GnRH, which prevents the production of testosterone and spermatozoa (Tremblay *et al*., 1984; Vickery *et al*., 1984; Dube *et al*., 1987; Driancourt and Briggs, 2020).

Summary

According to the findings in the present study, it can be concluded that administration of GnRH analogue, buserelin could produce a transient improvement in semen quality on day 60 post-therapy followed by a moderate decline (with respect to semen quality on day 90 post-therapy). Moderate deterioration in semen quality indicates that further studies are warranted to ascertain the ideal dosage, duration of GnRH administration and sustainability of semen quality after GnRH therapy.

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Conflict of interest

The authors declare that they have no conflict of interest.

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