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Intra-Epididymal Zinc Arginine Administration and Its Effect on Testicular Dimensions and Hematological Parameters in Dogs

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ABSTRACT

Male sterilization by chemical agents is a nonsurgical contraceptive approach designed to induce azoospermia and, therefore, infertility. Intratesticular injection of zinc gluconate for sterilization of dogs has been described, but its use in cats remains limited. The objective of the present study was to evaluate, intra-epididymal zinc arginine administration and its effect on testicular dimensions and hematological parameters in dogs. A highly significant difference in the mean length and width of left and right testicle in the treated group. However, there was a significant difference (P<0.01) in the mean values of these parameters in the treated group when the values at the time of injection and castration were compared. This difference reflects the growth of the testicles with time as the dogs grew older. This also indicates that Zinc arginine does not have any deleterious effects on the growth of testicles.

Key words: *Zinc arginine, Hematological parameters, Contraceptive approach*

INTRODUCTION

The existence of free-roaming dogs raises important issues in animal welfare and in public health. A proper understanding of these animals' ecology is useful as a necessary input to plan strategies to control these populations. The abandonment and breeding of dogs in unrestricted environments have been attributed to behavioural, religious, cultural, ecological and socioeconomic factors, constituting important issues in public health and animal welfare[1,2]. Unrestricted dogs, in general, have their psychological and physical health compromised, are more likely to acquire infectious diseases and have a lower life expectancy compared to pet dogs[3,4,5].

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Their presence can be detrimental to humans since they are associated with the occurrence of biting incidents, transmission of diseases, damage to wild animal populations, accidents and pollution [6,7].

Since males are always potentially capable of impregnating a number of receptive females, so sterilizing number of males could conceivably decrease the number of pregnant female to the point of reversing population growth. It is imperative that the technique should be simple, non- surgical, least invasive, inexpensive, permanent, reliable, painless and simple for field functionaries and require no post- treatment hospitalization and care. Non-surgical method would facilitate sterilization of large population of stray dogs in shortest possible time because it will not require commitment of technical expertise, equipment and expenses.

Various chemicals that have been used for sterilization of male dogs are 10% silver nitrate, 3.6% formaldehyde[8]; methalibure, dexamethasone, metopiron, alpha chlorohydrin, danazole Dixit *et al*.[9]; chlorohexidine gluconate[10,11] calcium chloride Koger[12]; Dutta *et al*.[13], cadmium chloride Murty and Sastry[14], zinc arginine Fahim *et al*.[15]; Tepsumethanon *et al*.[16], and 70% Glycerol Immegart and Treefall[17]. However out of all these chemicals, chlorohexidine gluconate and zinc arginine have proved to be promising. The later has been approved by FDA of USA for sterilization of young pups and is being marketed in USA with the trade name of Neutersol. But no work has been done on this aspect in India. Keeping these facts and findings in mind, the present study was planned to study the effects of intra epididymal zinc arginine administration on haematological parameters, testicular dimensions, weight of epididymides in pre-pubertal dogs.

MATERIAL AND METHODS

The present study was conducted on 22 apparently healthy pre-pubertal stray community dogs weighing 6-8 kilograms. Dogs were caught from the nearby local colonies and were kept under observation for one week prior to the start of the experiment. All the dogs were housed in individual cages. For experimental studies, permission from Institutional Animal Ethics Committee was taken. For eliminating the diseased dogs from the healthy ones standard haematological, parasitological and biochemical examination of blood, faeces and urine were conducted. Gross examination of the scrotum, penis and epididymis was performed. Testicles of each dog were examined for smoothness, firmness, tone, size and adhesion if any. Selected animals were weighted, treated for external and internal parasites and immunized against rabies. Shaving of the scrotal area was performed and surgical spirit solution was applied to disinfect the area. Before giving intra epididymal injection dogs were anaesthetized with an intra-muscular injection of Ketamine HCl (2.4 mg/kg B.Wt.) and Xylazine (10 mg/kg B.Wt.). The Cauda epididymis was located by tightening the skin of the scrotum and by pushing the testis in the scrotal sac. Zinc arginine solution@ 0.2 ml per each cauda epididymis was injected per-cutaneously using 26 gauge, ½ inch needle in 18 dogs. This zinc arginine solution was a 0.4 M solution of zinc gluconate neutralized by equimolar solution of L-arginine, achieving a final concentration of 100 mg/ml of zinc arginine of solution. The pH of the solution was adjusted to 7 using N/10 HCl. Four dogs kept as untreated control. Two out of four dogs completed the experiment. Animals were examined regularly upto 96 hrs for inflammation, swelling, oedema, pain etc. in the scrotum and cauda epididymis after the injection of zinc arginine solution.

The different parameters which were recorded are listed in the table:

When evaluated	Parameters
Day -1: Pre- treatment	Physical examination Testicular dimensions
Day-0: Injection Day	Physical examination Testicular dimensions, Complete Blood Cells Count
Day-0: 2, 4, 6 hours post-injection	Reaction to injection, if any
Days1-4: Post injection follow up	Appetite, ability to walk, scrotal pain, scrotal evaluation
Day of castration	Testicular dimensions, Complete Blood Cells Count, weight of epididymides.

Scrotal evaluation included: Scrotal pain, scrotal irritation, biting and licking, scrotal swelling, scrotal irritation and dermatitis, scrotal ulceration, scrotal infection, dry scrotal skin, scrotal bruising and adhesions (at the time of castration). On day 10,20,30,40,50,60,70,80 and 90 post injection two separate animals were subjected to surgical removal of testis and epididymis. Testicular length and width were measured with Vernier calliper. Before castration dogs were weighed again.

RESULTS AND DISCUSSION

Physical Observations:

Injection Reactions: No reactions 2 and 4 hours post intra-epididymal Zinc-arginine injection were observed. Six dogs were noticed biting and licking the scrotum,6-8 hrs post injection (Table 1).

Appetite and ability to walk: None of the dogs displayed any problem during walking or had an abnormal appetite.

Scrotal Pain: Ten dogs displayed scrotal pain on days 1,2 and 3 post injection. The majority of these dogs displayed the pain on days 1 and 2 post injection when their cauda epididymis was palpated.

Scrotal evaluations i.e. irritation and dermatitis: One dog displayed scrotal irritation on day 2 post injection and the same dog displayed irritation and dermatitis on day 7 post injection.

Scrotal reactions: Mild scrotal swelling was noticed in 4 dogs between days 1 to 4 post injection. Scrotal ulceration was noticed in one dog on day 3 post injection which eventually healed after the application of local antiseptics. Adhesions between epididymis and parietal tunica vaginalis lining of scrotal sac were observed in Nine dogs at the time of castration (Table 1).

Table 1: Physical observations:

Adverse Reactions	No. of Animals (n=18)	Percentage (%)
Scrotal pain	10	55.5
Scrotal irritation	1	5.5
Biting and licking	6	33.3
Scrotal swelling	4	22.2
Scrotal irritation and Dermatitis	1	5.5
Scrotal ulceration	1	5.5
Dry Scrotal skin	Nil	-
Scrotal bruising	Nil	-
Preputial Swelling	Nil	-
Epididymal adhesions	9	50

In the present study it was observed that single intra epididymal injection of Zinc arginine did not cause any severe reactions in the treated dogs. Biting and licking of scrotum was observed in Six dogs, ten dogs displayed pain when cauda epididymis was palpated, mild swelling of scrotum was observed in Four dogs.

Body Weight: The mean body weight of treated group at the time of Zinc arginine injection was 7.31 ±0.11 kg and that of control group was 6.65±0.10 kg. The mean body weight of treated and control group at the time of castration was 9.14±0.33 kg and 11.17±1.07 kg, respectively. There was no significant difference in the mean body weight of treated and control group at the time of castration (P>0.05). However, there was a highly significant difference in the mean body weight of treated group when value at the time of injection and at the time of castration, were compared (P<0.01). This increase in body weight is the reflection of growth with time as the dogs used in this study were pre-pubertal. But no significant difference in the mean body weight of treated and control group was observed. These findings reflect that a single injection of Zinc arginine does not affect the weight gain in the treated animals or in other words the changes induced by time were independent of treatment. Pineda *et al.*[10] while conducting similar type of studies in pre-pubertal Beagle dogs with chlorohexidine gluconate also observed that treatment had no effect on body weight gain in the treated dogs. Similar findings were also reported by Brijlal[11] in Indian stray community dogs.

HAEMATOLOGICAL PARAMETERS:

Haemoglobin (Hb), Total Leukocyte count (TLC) and Packed Cell Volume (PCV): The mean values of Hb, TLC, PCV in treated dogs at the time of Zinc arginine injection were 8.01±0.22,7.91±0.41 and 23.72±0.74, respectively and in control group were 7.25±0.25,6.00±0.40 and 25±3.00,respectively whereas the mean values of these parameters in treated group at the time of castration were 8.36±0.17,8.17±0.39 and 25.04±0.54, respectively and in control group were 9.55±0.25,7.4±0.25 and 29.50±0.50, respectively. There was no significant difference in the mean values of Hb, TLC and PCV in treated group when the values of these parameters were compared at the time of injection to the values at the time of castration.

Differential Leukocyte Count(DLC): The mean values of neutrophils, lymphocytes, monocytes, eosinophils and basophils in treated at the time of Zinc arginine injection were 66.66±1.16,24.55±0.48,2.88± 0.22,5.77±0.93 and 0% respectively and in control group were 62.50±2.50,25.50±0.50, 2.50±0.50,9.50±1.50 and 0% respectively whereas the mean values of these leukocytes in treated group at the time of castration were 69.94±0.72,23.22±0.65,3.11±0.15,3.72±0.68 and 0 % respectively and in control group the values were 75.50±3.50,17.50±1.50,3.50±0.50, 3.50±2.50 and 0 % respectively. The mean values of these parameters did not differ significantly between treated and control groups at the time of castration(P>0.05).

These findings are in accordance with the finding of Fahim *et al.*[15].

TESTICULAR DIMENSIONS:

The mean length and width of the left testicle at the time of injection of Zinc arginine in treated group were 1.77 ±0.00 cm and 1.59 ±0.00 cm, respectively and in control group were 1.80 ±0.00 and 1.60±0.00 cm, respectively. The mean length and width of left testicle at the time of castration in treated group were 1.98 ±0.03 cm and 1.74 ±0.02 cm and in control 2.07 ±0.06 and 1.86 ± 0.07cm, respectively. The mean length and width of the left testicle did not differ significantly between control and treated groups at the time of castration (P>0.05).

The mean length and width of the right testicle at the time of injection of Zinc arginine in the treated group were 1.77 ±0.00 cm and 1.59±0.00cm, respectively and in control group were 1.80±0.00 and 1.60±0.00cm, respectively. The mean length and width of right testicle at the time of castration in treated group was 1.98±0.03 cm and 1.75±0.02 cm and in control 2.08±0.06 and 1.86±0.07 cm, respectively. The mean

length and width of the right testicle did not differ significantly between control and treated groups (P>0.05). However, there was a highly significant difference in the mean length and width of left and right testicle in the treated group. However, there was a significant difference (P<0.01) in the mean values of these parameters in the treated group when the values at the time of injection and castration were compared. This difference reflects the growth of the testicles with time as the dogs grew older. This also indicates that Zinc arginine does not have any deleterious effects on the growth of testicles. So, the effect of Zinc arginine was confined locally to the epididymis only. Similar findings were also recorded by Fahim *et al.*[15] with intra epididymal injection of Zinc arginine in adult dogs. Pineda *et al.*[10] reported similar findings while conducting a similar type of experiment in pre-pubertal dogs with chlorohexidine gluconate.

WEIGHT OF THE EPIDIDYMIDES:

The mean weight of left epididymis at the time of castration in treated group was 0.79 ±0.01 gm and in control group was 0.88±0.18 gm. The mean weight of the right epididymis in treated group at the time of castration was 0.78 ±0.02gms and in control group was 0.89 ±0.15 gm, respectively. The mean weight of both epididymides in treated group was 1.57±0.04 gm and in control group was 1.78±0.34 gm, respectively. The mean weight of left epididymis, right epididymis and total weight of both epididymides did not differ significantly between treated and control groups (P>0.05). The findings are in agreement to that of Fahim *et al.*[15] in adult dogs.

CONCLUSION

The present investigation was carried out on twenty-two pre-pubertal stray community dogs, of which eighteen dogs were injected 0.2 ml of 0.4M solution of zinc arginine into each cauda epididymis to study the effect of zinc arginine on to evaluate, intra-epididymal zinc arginine administration and its effect on testicular dimensions and hematological parameters in dogs. No significant difference in the mean body weight and hematological parameters of treated and control group was observed. But in testicular dimensions highly significant difference in the mean length and width of left and right testicle in the treated group. However, there was a significant difference (P<0.01) in the mean values of these parameters in the treated group when the values at the time of injection and castration were compared. This difference reflects the growth of the testicles with time as the dogs grew older. Therefore, it is concluded that single intra-epididymal injection of zinc arginine can cause an irreversible loss of fertility in dogs.

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