ANOGENITAL DISTANCE, SEXUAL BEHAVIOR AND PLASMA LEVELS OF TESTOSTERONE IN SYNTHETIC RABBIT BUCK

BOUMAHDI MERAD* Zoubeïda, Dalila TARZAALI, Ratiba RAIS, Mohamed BACHIR PACHA, Rafik BELABBAS, Rachid KAIDI

Laboratory of Biotechnologies related to Animal Reproduction, Institute of Veterinary Sciences, University of Blida-1, Route de Soumâa, BP 270, 09 000, Blida, Algeria

**Corresponding author: zoubidaboumahdi@yahoo.fr*

Abstract. The purpose of this study was to assess the relationship between the anogenital distance measures on certain reproductive parameters such as scent marking, sexual satiety, and testosterone level. 34 animals (22 rabbit bucks and 12 females) aged 6 and 7 months with an average weight of 3458 g were used. 54.55% of the males had an anogenital distance higher, whereas 45.45% had a lower anogenital distance. The anogenital distance, has influenced on scent marking, sexual satiety and testosterone level. There is a very significant difference in the scent marking and sexual satiety and a highly significant decrease in scent marking (72.74%; p = 0.007) after satiety (51 vs 14). Males with a high number of mounting (4 to 13) had a high plasma testosterone level compared to less mounting (1 to 3) (9.75-13.5 vs 2.57-11.33ng /mL). Males with high anogenital distance showed elevated testosterone levels (≥13.5 ng /mL). Our results suggest that a longer anogenital distance may predict normal male reproductive potential.

Keywords: Rabbit, scent marking, sexual satiety, androgens, anogenital distance.

INTRODUCTION

Rabbits provide an excellent source of protein for human consumption and may play a significant role in solving a part of meat shortage in Algeria. Rabbits are characterized by high reproduction ability and short generation interval, so they can produce high quantity of meat in a short period. Nonetheless, continuous efforts are undertaken to improve the control of breeding and performance of rabbit. In order to derive maximum benefit from the particularities of the rabbit species, several studies on the zootechnical aspect and the improvement of reproductive performances have been carried out. These reproductive performances are influenced by factors that have been studied by a large number of researchers.

In addition to the genetic factor and environmental factors, anogenital distance (AGD), the distance between the anus and the vagina or penis (Hotchkiss and Vandenbergh, 2005) is frequently used as a biomarker of natural variation in prenatal androgenization and has been proposed as a convenient predictor of intrauterine position for behavioral or physiological studies (aggression, reproductive behavior and hormone biology) in mice (Vandenbergh and Huggett, 1995, Ryan and Vandenbergh, 2002) in rats (Clemens et al., 1978), in gerbils (Clark et al., 1990) and in rabbits (Bánszegi et al., 2009).

Associations between AGD and different breeding parameters have also been investigated. Larger AGD female mice are more aggressive (Vom Saal, 1989) and less attractive to males (Rohde - Parfet et al., 1990) than females with shorter AGD and rabbit bucks showed a stronger response to scent marks of females with small AGD than to marks of females with large AGD (Bánszegi et al., 2009, Kerkoucke et al., 2013).

There is a paucity of knowledge on the association of AGD with rabbit buck behavior (scent marking, sexual satiety) or testosterone level. However, only investigations concerning the association of AGD and testosterone level have been recently conducted in men (Eisenberg et al., 2015) but to the best of our knowledge, no study has investigated the association of AGD and sexual satiety (it is the point when a buck will display a large number of mounts, intromissions, and ejaculations until sexual activity ceases). In this study, we investigated whether anogenital distance was associated with some reproductive parameters such as sexual, scent marking behavior and testosterone level in synthetic rabbit buck, and if AGD would be as a predictor of the reproductive parameters.

MATERIAL AND METHOD

The experiment was carried out at the Rabbitry Unit of the Teaching and Research Farm of the University of Blida - 1, Algeria. The animals were over six months of age, with an average weight 3458 g. A total of 34 rabbits of synthetic strains composed of 22 males and 12 receptive does were used for sexual behavior assessment. Animals provided from the ITELV experimental station of Baba Ali. The synthetic strain has been formed from the insemination of females of the local population by fresh semen of males from the INRA2666 (France). Animals were individually housed in galvanized wired cages equipped with commercial feeders and nipple drinkers. Fed pelleted commercial feed and water offered *ad libitum.* All animals were healthy and free of any external parasites or skin diseases.

Experimental procedure

Determination of anogenital distance: Anogenital distance was defined as the distance from the center of the anus to the distal end of the penis (Bánszegi et al., 2012). It was measured using stainless-steel digital calipers (Fig. 1). For each male, AGD was measured three times by different operators and the average of the three observations was calculated. The males were classified according to their average AGD in two classes (Drickamer et al., 2001). The first class concerns males with a small AGD (which has an AGD equal to or less than the average AGD). In contrast, the second class includes males with above-average AGD.

Behavioral assessment: Figure 2 summarizes the sequences of our experimental procedure that will be eventually described in details through each test.

Scent marking (SM) behavior: Scent marking behavior itself is the marking behavior when the chin gland is actively rubbed against specific objects and the excretion is smeared on the surface. It was quantified before and after the test of satiety. At the start of the experiment, we placed each rabbit male sexually

experienced, individually in an empty circular wire meshes enclosure (1 m in diameter by 43 cm high) for arena test during 5 minutes for five consecutive habituation days. Following their release into the observation arena, most rabbits seemed to show an explosion of running, probably as a result of their prior long periods of confinement in small cages. Then in the middle of arena was added three piles of 3 cm terracotta bricks each (Fig. 3), arranged in a triangle 0.5 m apart and located in a separate, quiet room. When placed in the arena containing the bricks, rabbit bucks moved around freely, sniffing at the floor and sniffing at and rearing up against the wall, or lying down in an apparently relaxed posture. Most animals started within seconds to rub their chin across the bricks, sometimes leaving clearly visible damp marks from the deposited secretion. The number of chin marks made onto of any of these bricks was recorded for the following 10 minutes ranged from 0 (just once) to up to 100 (**Hudson et al.,1990,** González-Mariscal et al., 1990). Fresh, unmarked bricks were used for each test with each animal. The bricks were then removed and the stimulus female was introduced to begin the sexual satiety test. The scent marking frequency was determined again at the end of the sexual satiety. These and all subsequent sessions were conducted throughout the day, balancing the number of morning and afternoon sessions for each individual.

Fig. 1. Recording of anogenital distance

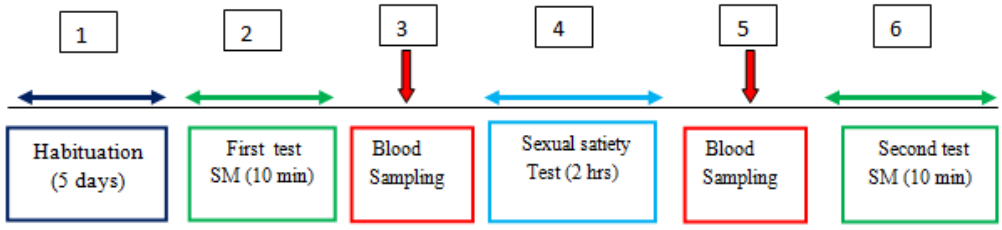


Fig. 2. (1) Habituation to the arena test; (2) First test scent marking behavior; (3) First blood taken to measure testosterone; (4) Sexual satiety test for 2 hours; (5) Second blood taken to measure testosterone; (6) Second test scent marking behavior.

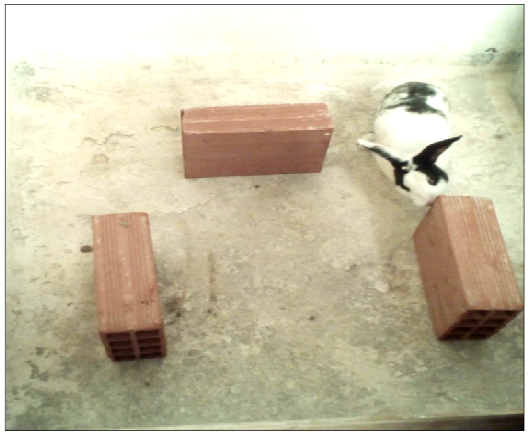


Fig. 3. Scent marking behavior in arena containing the bricks

Blood Sampling and Determination of Plasma Concentrations of Testosterone: Blood samples were collected on two subsequent occasions after the first scent marking test and sexual satiety of each animal from ear vein into heparinized tubes and were placed immediately on ice box. Plasma was obtained by blood centrifugation at 3000 rpm for 20 minutes and stored at -20°C until analysis to test whether these affected hormone concentrations. Concentrations of testosterone in the plasma were determined by Enzyme Linked Fluorescent Assay (ELFA) and VIDAS testosterone II. The sensitivity of assays for testosterone was 0.05 ng/mL.

Sexual Satiety test: A male was placed in the mating wire-mesh arena for 5 minutes before introducing the female. Thereafter, a first female sexually receptive, was introduced into the male's arena for a period during which the copulatory sexual behavior stages were recorded. The buck was allowed to mate *ad libitum* until no interest in the doe was shown for 30 minutes (Jiménez et al., 2012). At this point, the female was removed and replaced by another one, and so on, until the male showed no interest in any female for a period of 2 hours (criterion used to establish sexual satiety). This change of sexually receptive females was done to maximize the display of the buck's copulatory activity. If the buck showed no interest in the doe for 30 minutes, a new one was introduced and, from then until the end of testing. During the 2 hours of testing, we quantified the following parameters of male sexual behavior displayed towards the female: a) total number of ejaculations performed with females, b) total number of mounts that did not culminate in ejaculation. Aggressive behaviors such as biting, scratching or chasing were so rare that we have not considered them here. The criterion to establish that a male reached sexual satiety was that it did not show sexual activity for 120 minutes after repeated ejaculations. At the end of each test, the arena was thoroughly cleaned.

Statistical analysis: The statistical significance of the differences is calculated using Fisher-Student's " t " test. The statistical method was one way ANOVA test. Pearson correlation coefficients were calculated to assess the relationship of genital measures. Linear regression models were used to determine the relationship between

genital measures, scent marking and sexual satiety and other parameters under study. All data were analyzed with the PAST statistical package. Data are presented as means ± SD (Standard Deviation).

RESULTS AND DISCUSSIONS

Classification of males according to their AGD. The classification of males according to their mean AGD is reported in Table 1. The mean AGD (AGDA) for the males used in our experiment was 22.98 ± 1.98 mm. 54.55% of the males had an AGD larger (AGDL) with average AGDL = 24.50 ± 0.75 mm against 45.45% of the males having an AGD smaller (AGDS) with average AGDS = 21.16 ± 1.26 mm.

Table 1.

Classification of males according to their AGD (mean ± SD)

AGD (mm)	AGD1	AGD2	AGD3	AGDA
Rabbit (n=22)	23.44 ± 2.22	22.78 ± 2.54	22.72 ± 2.04	22.98 ± 1.98
AGDL (n=12)	24.96 ± 1.58	24.47 ± 1.75	24.07 ± 1.26	24.50 ± 0.75
AGDS (n=10)	21.63 ± 1.29	20.76 ± 1.74	21.10 ± 1.56	21.17 ± 1.26

AGDL: Large anogenital distance; AGDS: Small anogenital distance; AGDA: Average anogenital distance.

Effect of AGD on scent marking before and after sexual satiety test: The relationship between the male rabbit's AGD and its scent marking is illustrated in Table 2 and Figure 4. Our results indicate that males with large AGD score more in their territory compared to males with a small AGD. The relationship between the male rabbit's AGD and its scent marking is positive with a low correlation coefficient (r = 0.26).

Table 2.

AGD classification of males according to first and second scent marking (SM1, SM2)

	AGDA (mm)	First scent marking (min)	Second scent marking (min)
AGDL	24.50 ± 0.75	67.50 ± 43.36	14.00 ± 25.76
AGDS	21.16 ± 1.26	31.20 ± 17.45	13.80 ± 11.51

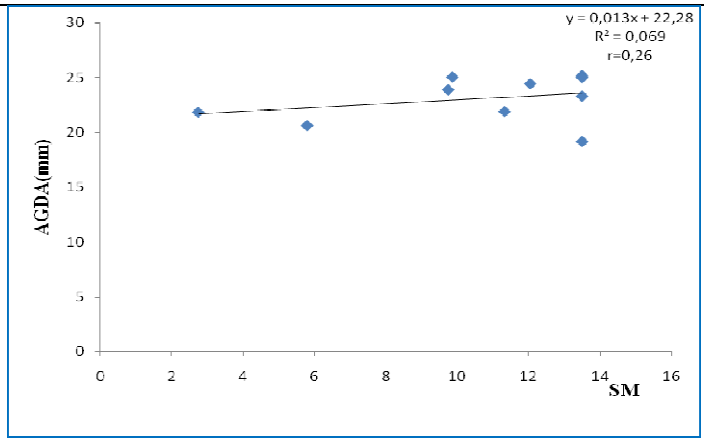
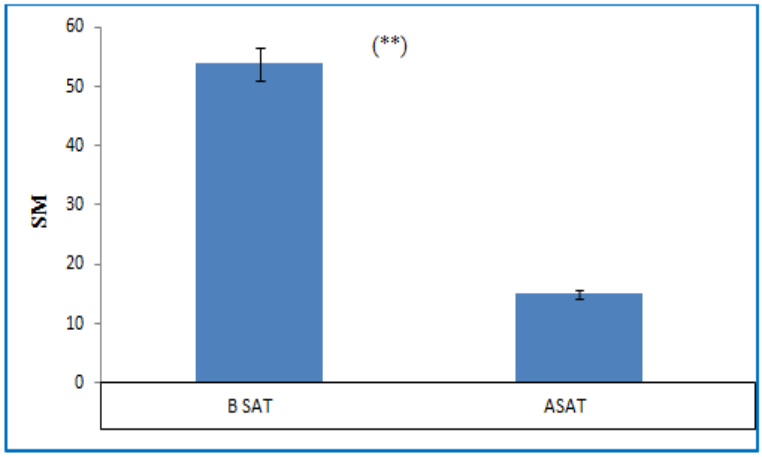


Fig. 4. Relationship between the male rabbit's AGD and its SM

Relationship between sexual satiety of rabbits and the scent marking: The variation of the scent marking as a function of the satiety of the males is presented in figure 5. Our results indicate that there is a very significant difference in the variations of the scent marking of males according to their satiety. There is a highly significant reduction in scent marking (72.74%, p = 0.007) after satiety**.**



(BSAT: Before satiety, ASAT: After satiety)

Fig. 5. Variation of scent marking according to sexual satiety of rabbits (72.74%, p= 0.007)

Effect of AGD on sexual behavior of males during the sexual satiety test: The sexual behavior of males to females inside the arena for a period of 2 hours was observed for each male and shown in Table 3 and illustrated in Figure 6. Males with a large AGD have a greater tendency to mount females (94%) and ejaculate (100%) and significant displaying mounting, in contrast to males with small AGD who showed a low sexual activity. Rabbits began urination a few minutes after they entered the observation enclosure. In addition, the frequency of urination during observations periods was often quite high. In AGDS, rabbits eventually developed the behavior pattern of spraying urine (80%) compared to AGDL who did develop spraying only (16%). In our investigation, we registered 40% of rabbits AGDS expressing behavior of shyness. In contrast, it is totally absent in rabbits AGDL. There is a highly significant reduction in scent marking (72.74%, p = 0.007) after satiety and these results are similar to those reported by González-Mariscal et al. (1997) who showed that *ad libitum* copulation significantly reduced the frequency of marking in all males at 2 hours after the last ejaculation and the marking frequency was reduced by approximately 70%. This effect was evident in all tests, regardless of their duration or the number of copulation events that were observed. Consistent with previous reports (Verberne and Blom, 1979, Martínez-Gómez et al., 1997) animals usually started marking immediately they were placed in the arena, with the most active animals sometimes marking more than 100 times during a 10 minutes test. The same observations have been described by González-Mariscal et al. (1992), in intrinsic

regulatory mechanisms in the expression of male rabbit sexual behavior. Indeed, some males needed more than one female to reach sexual satiety on the first hour of testing and even males never required more than 3 females. As scent marking has been reported to be closely related to males' willingness to copulate (González-Mariscal et al., 1992), our results suggest that males are equally motivated to engage in sexual activity at the beginning of the tests even if they are not able to reach ejaculation.

The function of urine in scent-marking in rabbits has not been investigated in detail previously although several authors have mentioned enurination of the female by the male in sexual behavior. Mykytowycz (1970) indicated that urine functions in territorial marking. The frequency of urination in general and the spraying of urine in the observation arena suggest scent-marking, although factors such as stress could not be excluded from our test situation. However, if urination were due to stress, one would expect the frequency to decline with repeated visits to the observation arena, which did not occur.

For both males and females, longer AGDL is correlated with masculinized physiology, morphology and behavior, whereas shorter AGDS are associated with a feminized phenotype (e.g. guinea pig), (Phoenix, 2009) and house mice (Ryan and Vandenbergh 2002). Male house mice with shorter AGDS are often less competitive in intrasexual interactions than males with longer AGDL (Drickamer, 1996), particularly in competitions for resources or territories (Godsall et al., 2014). A shortened AGD in rats is associated with various reproductive tract abnormalities including hypospadias, abnormal testicular descent and decreased testicular volume (Gray et al., 2001).

Table 3

Effect of AGD on sexual behavior of males

Rabbit buck (n=22)	Mounting (%)	Shyness (%)	Urination (%)	Ejaculation (%)
AGDL (n=12)	94	0	16	100
AGDS (n= 10)	71	40	80	60

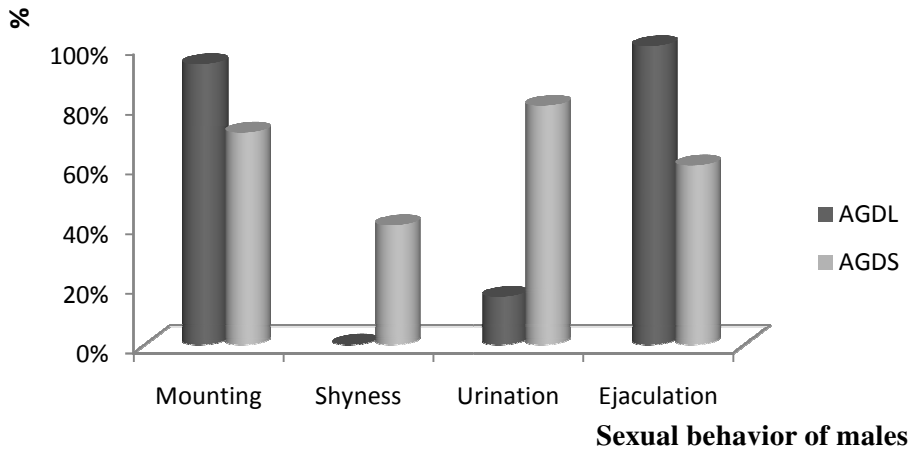


Fig. 6. Relationship between AGD and sexual behavior

Relationship between testosterone levels, AGD and scent marking: Our results indicate that males with AGDL have a high testosterone levels (9.75 to 13.5 ng/mL) and are the ones who score more in their territory compared to males with AGDS who have low testosterone levels (2.75 to 11.33 ng/mL). A positive correlation was established between scent marking and testosterone and between AGD and testosterone, by an averaged correlation coefficient (r = 0.43; 0.31 respectively). The results are shown in Table 4 and illustrated in figures 7 and 8. Testosterone levels correlated positively with the frequency of scent marking (Arteaga et al., 2008). Nor is it clear if the positive correlation found between concentrations of serum testosterone and frequency of scent marking was due to the action of testosterone itself, or rather to one or more of its metabolites as has been shown in previous studies (Girolami et al., 1997). Taken together, the present results are consistent with reports that in wild rabbits scent marking by males is associated with territoriality and social dominance (Hayes et al., 2002). Our results are thus consistent with reports that castration reduces or eliminates scent marking in rabbits while testosterone replacement restores it (González-Mariscal et al., 1993) and showed that in addition to reduced anogenital lengths, rodents exposed to certain phthalates, which are known to suppress fetal androgen levels, had altered testicular size and Sertoli cell function. Foster et al. (2001), reported that exposure of developing rats to di-n-butyl phthalate (DBP), an antiandrogen, led to reproductive tract anomalies, reduced anogenital distance, and impaired testosterone production. Furthermore, Swan et al. (2005) demonstrated that male infants of mothers exposed to increasing levels of known endocrine disruptors had shorter anogenital lengths suggesting an impairment of *in utero* male genital development. Moreover, Hsieh et al. (2008) studied young boys undergoing elective urological surgery and showed that boys with more severe genital anomalies (i.e. hypospadias and cryptorchidism) had significantly shorter anogenital lengths compared to boys with no genital anomalies.

Table 4.

Effect of Testosterone on AGD and scent marking
(Values expressed in mean±standard deviation)

AGDA (mm)	Scent marking (min)	Testosterone (ng/mL)	
		Before	After
AGDL = 24.50 ± 0.75	67.5 ± 43.36	12.03 ± 1.80	1.02 ± 0.45
AGDS = 20.90 ± 1.29	38.25 ± 8.66	8.35 ± 4.94	0.51 ± 0.15

Males with large AGD have a high testosterone levels (13.5/ mL), our results are consistent with the recent findings demonstrated by Nerli et al. (2018) in human studies. They clearly showed an association between AGD and serum testosterone levels. Eisenberg et al. (2015) were the first to report an association between perineal length and androgen levels in men. They believe that AGD may predict normal genital development in men and, therefore, could provide a novel metric to assess testicular function. Nerli et al., (2018) and Mira-Escolano et al. (2014) showed that

longer AGD was significantly associated with higher serum test levels in young women and was the first study to report an association between AGD measures and levels of reproductive hormones in women. *In utero* exposure to agents that interfere with the action of androgens in male rodents induced genital development disorders, involving, lower testosterone levels and shorter AGD in adults (Macleod et al., 2010).

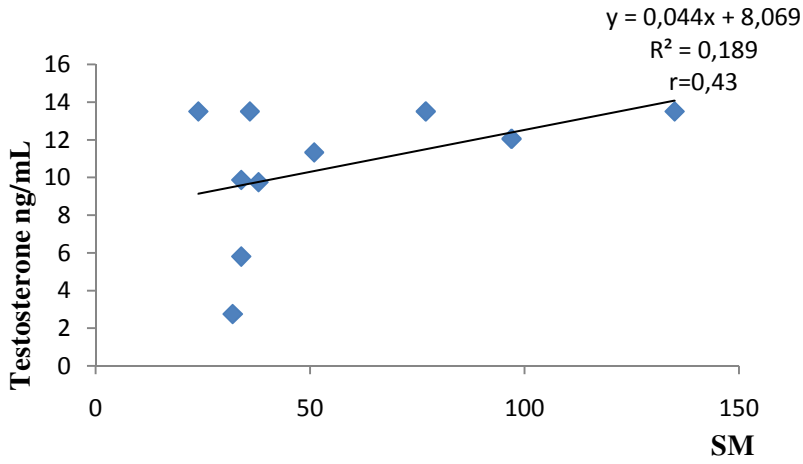


Fig. 7. Relationship between SM and testosterone levels (r = 0.43)

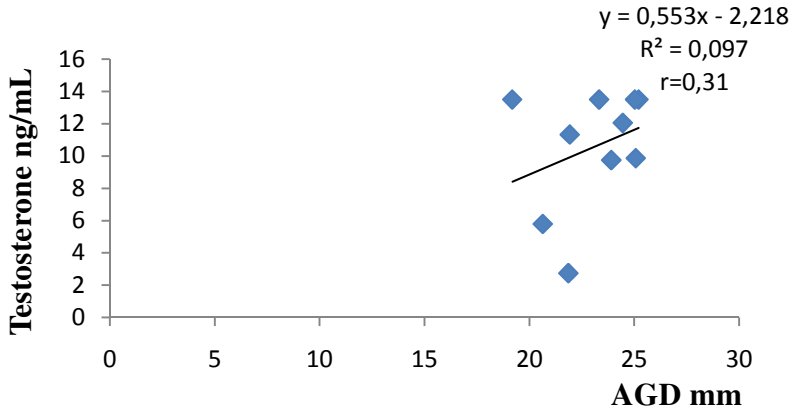
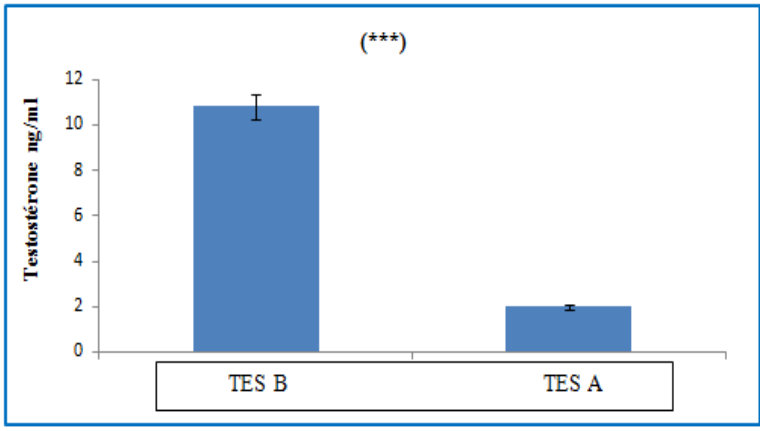


Fig. 8. Relationship between AGD and testosterone level (r = 0.31)

Effect of plasma testosterone on sexual satiety. Testosterone: Our results indicate that the level of plasma testosterone has a very important effect during the satiety of males. Males having made a mounts with ejaculations (4 to 13) have a very high testosterone plasma level (13.5 ng/mL), the males having made a smaller number of ejaculations (1 to 3) have a plasma testosterone level (2.57 ng/mL). Figure 9 clearly shows a drastic drop in testosterone levels at the end of satiety with a very significant difference (81.88%, p = 0.0001).



TES B: Testosterone before satiety; TES A: Testosterone after satiety

Fig. 9. Variation of testosterone during sexual satiety

CONCLUSIONS

Our work is the first study showing an association between AGD, scent marking, sexual satiety behavior and testosterone concentrations in rabbit male synthetic strain. Perhaps the most interesting and to our knowledge new aspect of the present findings is that clear to make a comparative study between rabbit synthetic strain and local population and show differences in scent marking and other dominance-associated behaviors apparent among individuals. Which might suggests differences either in the animals' genetic make-up, differential exposure to hormones associated with intrauterine position as has been established for various rodent species. Moreover, the current method of AGD measurement in rabbit synthetic strain or local population has not been studied, thus its accuracy and reproducibility were difficult to assess other than the performed comparison of measurements between investigators. Future studies are necessary to compare techniques for measurement as well as other anatomic locations of the AGD measurement. Our study may help in establishing a relationship between AGD and testicular function in rabbit adults and predicting sperm production.

REFERENCES

1. Arteaga, L., Bautista, A., Martínez-Gómez, M., Nicolás L., Hudson R. (2008). Scent marking, dominance and serum testosterone levels in male domestic rabbits. Physiol Behav., 94, 510–515.
2. Bánszegi, O., Altbäcker, V., Bilkó, Á. (2009). Intrauterine position influences anatomy and behavior in domestic rabbits. Physiol Behav., 98, 258–262.
3. Bánszegi, O., Szenczi, P., Dombay, K., Bilkó, A., Altbäcker V. (2012). Anogenital distance as a predictor of attractiveness, litter size and sex ratio of rabbit does. Physiol Behav., 105(5): 1226–1230.

4. Clark, M. M., Malenfant, S. A., Winter, D.A., Galef, B.G.Jr. (1990). Fetal uterine position affects copulation and scent marking by adult male gerbils. Physiol. Behav., 47, 301–305.

5. Clemens, L.G., Gladue, B.A., & Coniglio, L.P. (1978). Prenatal endogenous androgenic influences on masculine sexual behavior and genital morphology in male and female rats. Harm Behav., 10: 40–53.

6. Drickamer, L.C. (1996). Intra-uterine position and anogenital distance in house mice: consequences under field conditions. Anim Behav., 51: 925–934.

7. Drickamer, L.C., Robinson, A.S., Mossman C.A (2001). Differential responses to same and opposite sex odors by adult house mice are associated with anogenital distance. Ethology, 107.

8. Eisenberg, M.L., Larry, I., Lipshultz L.I. (2015). Anogenital distance as a measure of human male fertility. J Assist Reprod Genet., 32(3): 479–484.

9. Foster, P.M., Mylchreest, E., Gaido, K.W., Sar, M. (2001). Effects of phthalate esters on the developing reproductive tract of male rats. Hum Reprod Update, 7: 231–235.

10. Girolami, L., Fontani, G., Lodi, L., Lupo, C. (1997). Agonistic behavior, plasma testosterone, and hypothalamic estradiol binding in male rabbits. Aggr Behav., 23: 33–40.

11. Godsall, B, Coulson, T., Malo A.F. (2014). From physiology to space use: Energy reserves and androgenization explain home-range size variation in a woodland rodent. Journal of Animal Ecology, 83(1).

12. González-Mariscal, G., Melo, A.I., Zavala, A., Beyer C. (1990). Variations in chin-marking behavior of New-Zealand female rabbits throughout the whole reproductive-cycle. Physiol Behav., 48: 361–365.

13. González-Mariscal, G., Alboneti, M.E., Cuamatzi, E., Beyer C. (1997). Transitory inhibition of scent marking by copulation in male and female rabbits. Anim. Behav., 53: 323–333.

14. González-Mariscal, G., Melo, A.I., Zavala, A., Chirino, R. Beyer, C. (1993). Sex steroid regulation of chin-marking behavior in male New Zealand rabbits. Physiol Behav., 54: 1035–1040.

15. González-Mariscal, G., Melo, A.I., Zavala, A., Beyer, C. (1992). Chinmarking behavior in male and female New Zealand rabbits: onset, development, and activation by steroids. Physiol Behav., 52: 889–893.

16. Gray, L.E., Ostby, J., Furr, J., Wolf, C.J., Lambright, C., Parks, L.,Veeramachaneni, D.N., Wilson, V., Price, M., Hotchkiss, A., Orlando, E., Guillette, L. (2001). Effects of environmental antiandrogens on reproductive development in experimental animals. Hum Reprod Update, 7, 248–264.

17. Hayes, R.A., Richardson, B.J., Wyllie, S.G. (2002). Semiochemicals and social signaling in the wild European rabbit in Australia: I. Scent profiles of chin gland secretion from the field. J Chem Ecol., 28: 363–84.

18. Hotchkiss, A.K., Vandenbergh, J.G. (2005). The anogenital distance index of mice (Mus musculus domesticus): an analysis. Contemp Top Lab Anim Sci., 44 (4): 46–8.

19. Hudson, R., González-Mariscal, G., Beyer, C. (1990). Chin marking behavior, sexual receptivity, and pheromone emission in steroid-treated, ovariectomized rabbits. Hormone and Behavior. 24: 1–13.

20. Hsieh, M.H., Breyer, B.N., Eisenberg, M.L., Baskin, L.S. (2008). Associations among hypospadias, cryptorchidism, anogenital distance, and endocrine disruption. Curr Urol Rep., 9: 137–142.

21. Jiménez, P., Serrano-Meneses, M.A., Cuamatzi, E., González-Mariscal, G. (2012). Analysis of sexual Behaviour in male rabbits across successive tests leading to sexual exhaustion. World Rabbit Sci. 20: 13–23.

22. Kerkouche, T.N., Zitouni, G.H., Boumahdi, Z., Berbar, A., Kerkouche, R., Benali, N., Titouh, F. Belabbas, R. (2014). Etude des relations entre distance ano-génitale, parité et quelques caractéristiques de la reproduction de la lapine. Livestock Research for Rural Development 26.

23. Macleod, D.J., Sharpe, R.M., Welsh, M., Fisken, M., Scott, H.M., Hutchison G.R. (2010). Androgen action in the masculinization programming window and development of male reproductive organs. Int J Androl., 33: 279-287.

24. Martínez-Gómez, M., Guarneros, M., Zempoalteca, R., Hudson, R.A. (1997). Comparison of spontaneous and odor-induced chin marking in male and female domestic rabbits (Oryctolagus cuniculus domesticus). Ethology., 103: 893–901.

25. Mira-Escolano, M.P., Mendiola, J., Mínguez-Alarcón, L., Roca, M., Cutillas-Tolín, A., López-Espín, J.J., Torres-Cantero, A.M. (2014). Anogenital distance of women in relation to their mother's gynaecological characteristics before or during pregnancy. Reprod Biomed Online., 28(2): 209–215.

26. Mykytowycz, R. (1970). The role of skin glands in mammalian communication. *In*: Advances in Chemoreception Vol. 1. Communication by Chemical Signals. Eds. J. W. Johnston, D. G. Moulton, and A. Turk. (Appleton-Century-Crofts, New York), pp. 327–360.

27. Nerli, R.B., Ghagane, S.C., Hiremath, M.B., Dixit, N.S., Neelagund, S. (2018). Anogenital distance in males attending assisted reproduction center. J Sci Soc., 45:72–75.

28. Phoenix, C.H. (2009). Organizing action of prenatally administered testosterone propionate on the tissues mediating mating behavior in the female guinea pig. Horm Behav., 55(5): 566.

29. Rohde - Parfet, K.A., Ganjam, V.K., Lamberson, W.R., Rieke, A.R., Saal, F.S.V., Day, B.N. (1990). Intrauterine position effects in female swine: subsequent reproductive performance, social and sexual-behavior. Appl Anim Behav Sci., 26: 349–362.

30. Ryan, B.C., Vandenbergh, J.G., (2002). Intrauterine position effects. Neurosci Biobehav Rev 26: 665–678.

31. Swan, S.H., Main, K.M., Liu, F., Stewart, S.L., Kruse, R.L. (2005). Decrease in anogenital distance among male infants with prenatal phthalate exposure. Environ Health Perspect, 113: 1056–1061.

32. Vandenbergh, J.G., Huggett, C.L. (1995). The anogenital distance index, a predictor of the intrauterine position effects on reproduction in female house mice. Laboratory Animal Science, 45: 567–573.

33. Verberne, G., Blom, F. (1979). Scentmarking dominance and territorial behaviour in male domestic rabbits. *In:* Meyers K. & McInnes C.D. editors. Proc World Lagomorph Conf. Guelph: University of Ontario (1981), pp. 280–290.

34. Vom Saal, F.S. (1989). Sexual-differentiation in litter-bearing mammals – Influence of sex of adjacent fetuses in utero. Journal of Animal Science, 67: 1824–1840