Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

Research article

Some techniques for sex election in Wistar rats

Ali H. Jaber Al-Bdeery
Department of Surgery and Obstetrics, College of Veterinary Medicine, University of Al-Qadisiyah, Iraq
Corresponding Author Email: ali.habeeb@qu.edu.iq

(Received 12/6/2017, Accepted 25/12/2017)

Abstract

The present study carried out on (36) mature female Wister rats about (65-70) days old divided into (6) groups included (6) female which not given any treatment as control group, other (30) female treated with progesterone (20 mg/kg, s.c) daily for 7 days , after 24hrs. injected with PMSG (150 IU/kg, i.p) and after 48hrs. injected LH (75 IU/kg, i.p) and divided for (5) groups within 14-15hrs. equally, (T1) put with mature male rat (1:2) and isolated after 48hrs. for natural mating, (T2) given alkaline solution in vagina for two days during first and second day from natural mating with mature male rat and removed after 48hrs., (T3) treated with acidic solution in vagina for two days during first and second day from natural mating with mature male rat and separated after 48hrs., (T4) inseminated artificially with upper layer of solution prepared from tail of epididymis and put with castrated male rat for 48hrs. after injected with oxytocin (10 m IU/g, i.p), (T5) inseminated artificially with lower layer of solution prepared from a tail of epididymis and put with a castrated male for 48hrs. after injection of oxytocin (10 m IU/g, i.p). The results revealed T4& T2 recorded high ratio of newly born males (71.429%&63.415%), while (C, T1, T3& T5) were (28.571, 30.952, 23.810 and 25%) respectively. While the high ratio of newly born females rate recorded in (T3&T5) were (76.190% and 75%), and in (C, T1, T2 &T4) recorded (71.429, 69.048, 36.585 and 28.571%) respectively. The pregnancy rate was 100% in all hormonally treated groups compared with control group and groups inseminated artificially which recorded 50%. Addition to that, the results showed highly a significant variance (P≤0.01) in newly born numbers with a mathematic decrease in body weights of hormonally treated groups.

Keywords: Techniques, Sex, election, Wistar, Rats.

Introduction

The pre-election of sex in the animals are of essential economic purpose in many developed and developing (1). Many studies were achieved to focus the benefit of using election in sex for example in livestock industry the pre-election refer to increase number of females in dairy herds while in beef herds refer to increase in number of males (2,3,4&5). Different purposes had suggested for pre-election of embryos according to different field of advantages and these are represented to helps fill the need to increase demand for food products (6). Many breeding animal stations as well as the free clusters of animals in countryside the farmers alternative ratio of male to female for good management results (2&3).The best chooses phenotypic characteristic of animals were determined by different genetic types belong to that such as artificial insemination for election is good for males as sires of the herd, the pre- election of males had good characteristic which are very important for successful reproductive efficiency (1). Accordingly, there are some defects in many animals could prevent them for being a productive animals this technique was applied to decrease or even avoid these undesired signs where the pre-election of sex to avoid some genetic diseases, they many

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

appear in males and other diseases may appear in females (7,8&9), Besides that many rare animals were threatened by extinction, for that this situation could be avoided by application early sex election was important for the conservation of endangered animals (10&11). The fertilized embryo was come from joining sperm and ova from male and female , the newborn sex was determined by two types of sperms depended to contain x-chromosome or y-chromosome (3,12&13). Different environment could affect the spermatozoa during the trip for fertilization starting with media of vagina and cervix when found in female reproductive tract after mating (14). Theoretically, centrifugation of spermatozoa the x-sperm which is heavy will settled in the bottom while the y-sperm which is light floating on the top, and this can be explained by the mean of large head, neck as well as tail, besides the dry mass was high in x-chromosome present in sperms as compared with y-chromosome, this technique was applied successfully in both cattle and pigs (15,16,17&18).The aim of this study was to elect the sex in rats by use different vaginal solutions and artificial insemination after centrifugation of semen.

Materials and Methods

Ethical approval

The Animal Ethical Committee of Veterinary Medicine College, University of Al-Qadisiyah, Iraq, has approved the present study under permission No: 231

Thirty-six mature female Wistar rats were obtained from the animal house of College of Veterinary Medicine, University of Al-Qadisiyah; the age of these rats was range between 65-70 days. These animals were kept under suitable environmental conditions of 25-30 °C in an airconditioned room and photoperiod of 12-14 hours daily. The animals were housed in plastic cages 29×15×12 cm in dimension. Divided for 6 groups included 6 female had no any treatment as control group, other 30 female treated with progesterone (vetagesterone/ Tehran-Iran) 20mg/kg s.c once daily for 7 days and after 24hrs injected with PMSG (Folligon/ Intervet) 150 IU/Kg, i.p and then after 48hrs. injected LH (Chorulon/ Intervet) 75 IU/Kg, i.p (19&20) and divided for 5 groups within 14-15hrs. after injection 6 female rats for each group involved, (T1) put with mature male rat (1:2) and removed after 48hrs. for natural mating, (T2) were given alkaline solution (GYNOX/PH 8.0/Turkey) in vagina for two days during first and second day from natural mating with mature male rat and separated after 48hrs., (T3) treated with acidic solution (GYNOX/PH 4.2/Turkey) in vagina for two days during first and second day following the natural mating with mature male rat and isolated after 48hrs., (T4) inseminated artificially with the upper layer of solution prepared from the tail of epididymis and put with castrated male rat for 48hrs. after intraperitonealy injection of oxytocin 10 mIU/g (Espana) (21), (T5) inseminated artificially with the lower layer of solution prepared from the tail of epididymis) and put with castrated male for 48hrs. after injected with oxytocin (10 mIU/g, i.p).

Statistical analysis:

The results were viewed as mean values±SE and the results analyzed by using computerized SPSS system ($p \leq 0.01$) (19).

Results

Ratio of newly born:

The results of the study showed that the highest percentages of newly born males were in the group T4&T2 were (71.429 and 63.415%) while the lowest percentage was recorded in group T3 (23.810%), where the results were (28.571, 30.952, 63.415, 23.810, 71.429&25%) in experimental groups (C,T1,T2,T3,T4&T5) respectively, table (1), (figure 1), and (figure 4). The results revealed highest percentages of newly born females were in group T3&T5 (76.190% and

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

75%) while the lowest percentage showed in group T2 (36.585%), the results were (71.429, 69.048, 36.585, 76.190, 28.571&75%) respectively, table (1), (figure 2) and (figure 5).

Effect of hormonal programs on the pregnancy rate:

The results showed that the use of hormonal programs for estrus synchronization had a significant effect on pregnancy rate (100%) in the treated groups compared with the control group and the inseminated artificially group which recorded (50%).

Effect of hormonal programs on the newly born numbers:

The use of the hormonal programs for synchronization of estrus were positively observed in newly born numbers (42,41,42,21&20) in the treated groups (T1,T2,T3,T4&T5) respectively compared with the of newly born numbers (14) in the control group (C), table (3), (figure 4), and (figure 5) .

Effect of hormonal programs on the newly born body weights:

The results showed that the newly born body weights were affected by the hormonal programs used, the body weight levels were (22.974,23.365,22.871,24.500&23.930gm) in treated groups (T1,T2,T3,T4&T5) respectively, which decreased mathematically when compared with the newly born body weights (25.873gm) for control group (C), table (4), and (figure 6).

Artificial insemination:

The results revealed success in sex election by using AI compared with natural matting whereas the percentage of pregnancy rates by artificial insemination was 50% in T4&T5 and showed significant variances when compared with the groups which used the natural mating (T1,T2&T3) and the variances were not significant with control group, table(2), and (figure 3).

Table (1): Effect of the media on the sex ratio

Groups / New born	C%	T1%	T2%	T3%	T4%	T5%
Males	28.571 [a]	30.952 [a]	63.415 [b]	23.810 [a]	71.429 [b]	25[a]
Females	71.429 [a]	69.048 [a]	36.585 [b]	76.190 [a]	28.571[b]	75[a]

Different letters denote a significance difference (p≤0.01) .

Table (2): Effect of hormonal programs on the Pregnancy rate

Groups / Pregnancy rate	C	T1	T2	T3	T4	T5
Pregnant	3	6	6	6	3	3
Non Pregnant	3	0	0	0	3	3
Percentage	50% [a]	100% [b]	100% [b]	100% [b]	50% [a]	50% [a]

Different letters denote a significance difference (p≤0.01) .

Table (3): Effect of hormonal programs on the newly born number

Groups / New born	C	T1	T2	T3	T4	T5
Males	4	13	26	10	15	5
Females	10	29	15	32	6	15
Total	14	42	41	42	21	20

Table (4): Effect of hormonal programs on the newly born body weights in age two weeks.

Groups / New born	C	T1	T2	T3	T4	T5
Weight	25.873±0.320 [a]	22.964±1.213 [a]	23.365±1.334 [a]	22.871±1.195 [a]	24.500±0.012 [a]	23.930±0.093 [a]

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

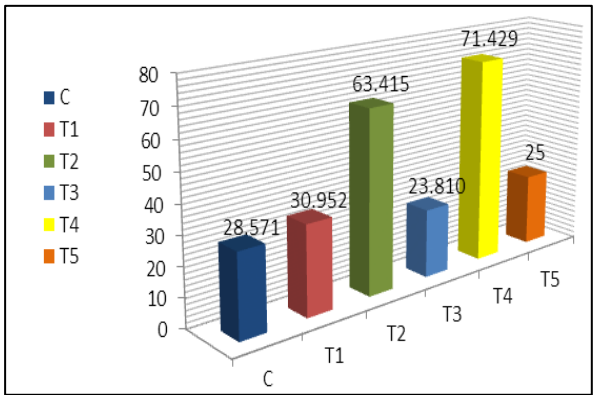

Figure (1): Effect of the media on the newly born male percentages.

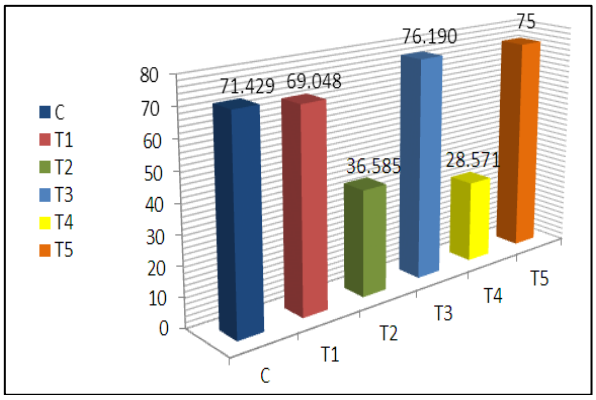

Figure (2): Effect of the media on the newly born female percentages.

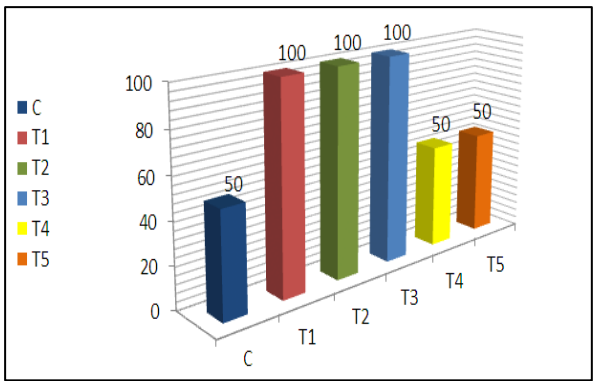

Figure (3): Effect of hormonal programs on the pregnancy rates.

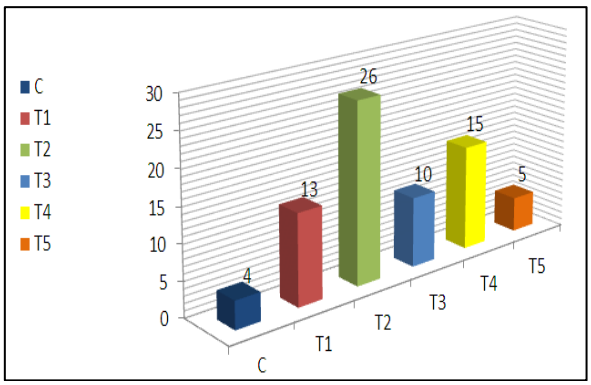

Figure (4): Effect of hormonal programs on the newly born male number.

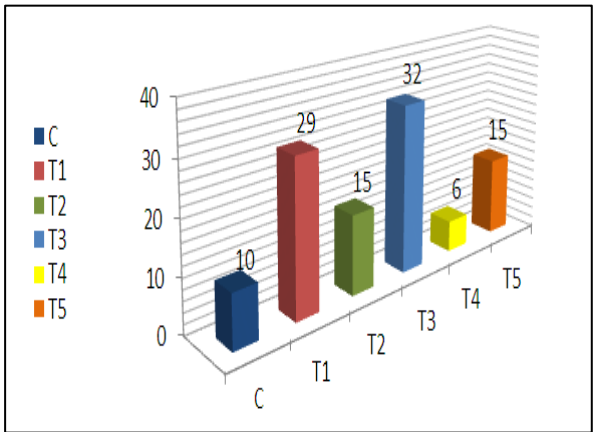

Figure (5): Effect of hormonal programs on the newly born female number.

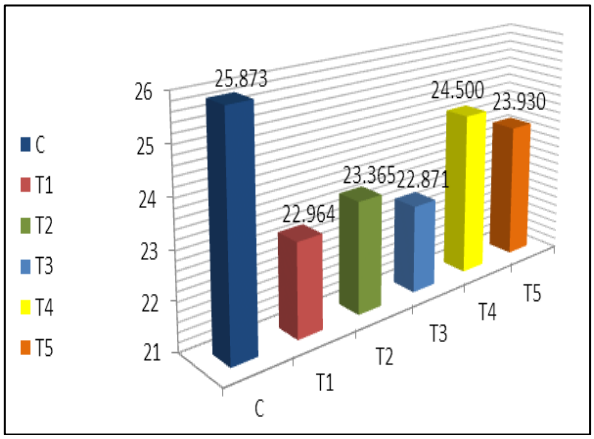

Figure (6): Effect of hormonal programs on the newly born body weights in the age two weeks

Discussion

Ratio of newborns:

This study reveal high percentage of newly born males for groupT4& T2 while T3 show a lower percentage. These percentages of our results are in accordance with (14&23) who found that the alkaline of vagina has negative effect on spermatozoa which contain x-chromosome and agree with (15&18) who found that the super net of sperm after centrifugation had the high ratio of y-chromosome. And also, the results reveal the high percentage of newly born females for T3&T5 while T2 show a lower percentage, these results agree with (24)

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

whose mentioned that acidic media has negative effect on spermatozoa which contain y-chromosome and this result accord with (15&18) who mentioned that spermatozoa in bottom after centrifugation may x-chromosome.

Effect of hormonal programs on the pregnancy rate:

Hormonal program show significant variances at $p \leq 0.01$ in pregnancy rates for each treatment groups 100% (T1, T2& T3) when compare of with C, T4& T5 50%. Our results are in accordance with (25& 26) that show a relationship between the levels of hormones and the pregnancy rates, and with (27&28) who found that the certainly ovulation by hormonal programs may increase the numbers of oocytes.

Effect of hormonal program on number of newborns:

Our results show high newly born number of treatment groups (T1,T2,T3,T4&T5) when compare them with C group, these results are accompanied with (29) who notice that superovulation by hormones treatment lead to increase of newly born number.

Effect of hormonal programs on the newly born body weights:

The results at table (4) and (fig.6) show that newly born body weights of C group higher than that of the treatment groups, these results are accompanied with (29&30) who found that superovulation may lead to increase number of embryos with less body weight.

Artificial insemination:

The results recorded the percentage of pregnancy rates by A.I. were 50% in T4&T5. Which have significant effect when compare them with (T1,T2&T3), this decrease in percentage agree with (31&32) who mention that maturation of spermatozoa in the rats needs to be completed in the females genital tract, also our results are in accordance with (33) that mention the spermatozoa stored in the epididymis possesses the efficacy that the spermatozoa remain alive in isolated epididymis for several days at 4 C˚, and closely agree with (34) who noticed pregnancy rate was 1/13 by A.I. while increased 7/12 when intraperitoneal injection of oxytocin. Our results showed success of sex selectivity by using AI after centrifugation of semen this agree with (15&18) whose reached the high ratio of y-chromosome spermatozoa in upper layer and lower layer contain high ratio from x-chromosomes spermatozoa.

References

1-Seidel J Overview of sexing sperm. Theriogenology, (2007); 68:443-46.

2-Parati K, Bongioni G, Aleandri R, Galli A, Sex ratio determination in bovine semen: A new approach by quantitative real time PCR, Theriogenology, (2006); 66: 2202-09.

3-Prasad S, Rangasamy S, Satheshkumar S. Sex preselection in domestic animals-Current status and future prospects. Veterinary World, (2010); 3:346-48.

4-Scahaw .The welfare of cattle kept for beef production, Report of the scientific committee on animal health & animal welfare adopted 25 april (2001) .

5-Oltenacu P, Algers B. Selection for increased production and the welfare of dairy cows: are new breeding goals needed? Ambio, (2005); 34:311-15.

6-Rath D, Barcikowski S, De G, Garrels W, Grossfeld R, Klein S, Knabe W, Knorr C, Kues W, Meyer H, Michl J, Moench-Tegeder G, Rehook C, Taylor U and Washausen S. Sex selection of sperm in farm animals:status report and developmental prospects . Reprod., (2013); 1470-1626 :1741-7899.

7-Kapral M, Redelmeier D. Carotid endarterectomy for women and men. Journal of Women's Health and Gender-Based Medicine, (2000); 9(9): 987-94.

8-Baruch J. Preimplantation Genetic Diagnosis. Journal of Health Law and Policy, (2008); P.245.

9-Alabama B. Sex selection and preimplantation genetic diagnosis.Vol.82.

10-Seidel J, Garner D (2002) Current status of sexing mammalian spermatozoa, Reproduction. Theriogenology, (2004) 124:733-743.

11-Seidel J, Sperm sexing technology-The transition to commercial application. An introduction to the symposium "Update on sexing mammalian sperm", Theriogenology, (2009); 71:1-3.

12-Barthakur I, Shroff G . Natural Selection of Gender of the Baby at Conception: Proposing a Scientific

Al-Qadisiyah Journal of Veterinary Medicine Sciences
(P-ISSN 1818-5746/ E-ISSN 2313-4429)
www.qu.edu.iq/journalvm

Hypothesis. Science Journal of Public Health, (2015); 3(5): 664-68.
13-Jain A, Yathish H, Jain T, Sharma A . Efficient production of sexed semen by Flow Cytometry : A Revi., Agricul. Revi., (2011) 32, 36-45 .
14-Rinehart W. Sex Preselection . N.W. Washington, USA Secon. Cla. postage paid Washing. D.C. 20009.1: (2001); Pp 21-32.
15-Wolf C, Brass K, Rubin M, Pozzobon S, Mozzaquator F, De La Corte F . The effect sperm selection by percoll or swim-up on the sex ratio of in vitro produced bovine embryos. Anim. Reprod., (2008); 5:110-15.
16-Morrell J, Dalin A and Rodringuez-Martinez H. Prolongation of stallion sperm survival by centrifugation through coated silica colloid: a preliminary study. Anim. Reprod. (2008); 5:121-26.
17-Williamson S. Sex (1st) selection ? Medical Law international. (2004); 6:185-206.
18-Kobayashi J, Oguro H, Uchida H, Kohsaka T, Sasada H, Sato E .Assessment of bovine x and y bearing spermatozoa in fractions by discontinuous procoll gradients with rapid fluorescence in situ hybridization. Jour. Reprod. Dev. (2004); 50:463-69.
19-Liao Z, Smith P. Persistent genital hyperinnervation following progesterone administration to adolescent female rats. Jou. Biolo.Reprod. (2014); 91(6):1-9.
20-Kon H, Hokao R, Shinoda M .Fertilizability of superovulated egg by estrous stage independent PMSG/hCG treatment in adult Wistar-Imamichi rats. Jour. Exp. Anim. (2014); 63(2):175-182.
21-Robinson G, Evans J .Oxytocin has a role in gonadotrophin regulation in rats. Jour.Endoc. (1990); 125:425-432.
22- Pallant J (2005) SPSS SURVIVAL MANUAL : A step by step guide to data analysis using SPSS for Windows (Version 12). 2nd edition. Printed by Ligare, Sydney. Social sciences-Statistical methods—Computer.
23-Shettles L. Factors influencing sex ratios. Jour. Gyn. and Obst. (1970); 8 (5): 643-47.
24-Haniyeh M. Choose the sex of the baby because of heredity. Jour. Islamic University, (2009); 17(1):27-48.
25-Gumen A, Keskin A, Yilmazbas-Mecitoglu G, Karakaya E, Cevik S, Balci F .Effects of GnRH, PGF2α and oxytocin treatments on conception rate at the time of artificial insemination in lactating dairy cows. Journal Czech J. Anim. Sci., (2011); 56 (6): 279–283.
26-Khodaei-Motlagh M, Zare Shahneh A, Masoumi R, Derensis F Alterations in reproductive hormones during heat stress in dairy cattle. Afric. Jour. Biotechn. (2011); 10(29): 5552-58.
27-Luo C, Zuniga J, Edison E, Palla S, Dong W and Parker-Thornburg J Superovulation strategies for 6 commonly used mouse strains. Journal Am. Assoc. Lab. Anim. Sci. (2011); 50(4):471-478.
28-Kaim M, Bloch A, Wolfenson D, Brawtal R, Rosenberg M, Voet H, Folman Y Effects of GnRH administered to cows at the onset of estrus on timing of ovulation, endocrine responses & conception. Jour. of Dairy Sci., (2003), 86: 2012-21.
29-Liljander M, Andersson A, Holmdahl R, Mattsson R Increased litter size and superovulation rate in congenic C57BL mice carryins a polymorphic fragment of NFR/N origin at the Fecq4 locus of chromosome 9. Journal Genet. Res. (Camb) (2009) 91(4):259-65.
30-Ertzeid G, Storeng R The impact of ovarian stimulation on implantation on fetal development in mice. Human Reproduction, (2001); 16, 2:221-25.
31-Jones R To store or mature spermatozoa? the primary role of the epididmis. Int. J. Androl. (1999); 22:57-67.
32-Jones R, Dacheux J, Nixon B, Ecroyd H .Role of the epididymis in sperm competition. Asian. J. Androl. (2007); 9, 493-99.
33-Guerin Y, Locatelli Y, Comizolli P, Mauget R, Mermillod P, Legendre X, Gatti J, Dacheux J .Conservation et utilisation du sperme épididymaire d'ovins et de cervidés en insémination artificielle et fécondation in vitro. In: Les Actes du BRG, BRG . Abstract .Publishers, Paris, vol (2003); 4: 173-183.
34-Nakata M, Okuda Y, Yamashita Y, Nalauchi C, Ito J, Kashiwazaki N. Successful production of offspring using cryopreserved sperm via nonsurgical artificial insemination in rats. Journal of Reproduction and Development. (2012); 58(4):501-504.