

Effect of Semen Extender pH Value on Rahmany Ram Characteristics and on Altering Sex Ratio of Offspring

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Abstract: The objective of this study was to examine the effect of different pH extenders on semen characteristics and fetal sexing ratio of Rahmany sheep. The studying was included two trials. The first trail estimated the effect of incubation period on semen characteristics with different acidic values (6.9 to 6.4) and alkaline (7.1 to 7.6) media. Incubation semen was replicated with 18 pooled ejaculates collected from three rams and diluted with tris fructose extender (TFE). The second trial was used artificial insemination to define sex ratio of newborn using thirty ewes. The experimental ewes divided into two groups (15 each) G1 inseminated with pH 6.6, while, pH 7.3 serviced G2. Ewe's vagina washed before insemination by media 6.6 for G1 and 7.3 for G2. The results showed that sperm characteristics during incubation were higher ($P<0.05$) with pH between 6.9 and 6.6 than pH 6.5 and 6.4, while the difference in sperm characteristics from 6.9 to 6.6 was insignificant. Extenders with pH of 7.4-7.6 recorded decrease ($P<0.05$) in semen characteristics. However, pH between 7.1 and 7.3 yielded insignificant difference among semen characteristics through incubation period. Insemination with pH 6.6 and pH 7.3 produced females sex ratio 66.67% and males lambing ratio 81.33%, respectively. It could be concluded that pH of semen affected on sex ratio of newborn lambs.

Keywords: Sheep, semen pH, extenders, sex ratio.

INTRODUCTION

In animals, offspring sex ratio is a feature of economic value in livestock. Attempts to control sex ratio in farm animals depended on alteration the proportions of spermatozoa that are bearing X or Y-chromosomes. Spermatozoa of Y- chromosome are smaller and moving faster than those bearing X and prefer alkalinity media, which is in contrast with those bearing X chromosome (Murray *et al.*, 1988).

Difference in DNA content is the only measurable and validated criterion for distinguishing between X- and Y-bearing spermatozoa. Isolation of these two types of spermatozoa has been addressed using centrifugation (Ericsson *et al.*, 1973), Percoll gradients (Iwasaki *et al.*, 1988), laminar flow fractionation (McEvoy, 1992), flow cytometry (Penfold *et al.*, 1998), free flow electrophoresis (Ishijima *et al.*, 1992) and phosphatidyl cholin (PC12) (Mocé *et al.*, 2006).

Up to the knowledge of the authors no data are available for the effect of pH of semen extender on the sex ratio of newborn lambs in local sheep breeds in

Egypt. Therefore, the present study was carried out to study the effect of pH semen extenders on incubation sperm profile and sex ratio.

MATERIALS AND METHODS

The present experiment was carried out at El-Serw Experimental Farm, Animal Production Research Institute, Ministry of Agriculture and Land Reclamation, Egypt. The experimental work was conducted during breeding month September 2007.

Experimental rams:

Three healthy rams of 60 to 65 kg body weight and 19 months old of age were used in the present study. The main ration composition offered to rams was 50% concentrate feed mixture (CFM) supplemented with 50% reed grass silage (RGS) through experimental work. The composition of CMF ration was cotton seed 17.50%, yellow maize 40%, wheat bran 25%, soybean meal 7%, molasses 6%, limestone 2.5%, common salt 1.5%, minerals 0.5%. Table 1 showed chemical analysis of feedstuff.

Table (1): Component of main rations (% on dry matter basis).

Items (%)	Treatment rations	
	CFM	RGS
Dry matter (DM)	90.50	30.10
Organic matter (OM)	93.20	89.60
Crude fiber (CF)	14.80	28.90
Crude protein (CP)	15.10	10.90
Ether extract (EE)	3.50	3.10
Nitrogen free extract (NFE)	59.83	46.70
Ash	6.77	10.40

Experimental ewes:

Thirty normal and healthy ewes without any problem in estrus allocated into two groups G1 and G2 fifteen ewes in each group. CFM ration plus berseem hay presented to ewes. All experimental animals were

housed in a roofed and open-side shelter and feed according to NRC (1990) requirements. Fresh water and mineral blocks were made available during the experimental period.

Procedure of Semen Collection and Extension for incubation:

The tris fructose extender (TYE) was prepared before incubation with different pH values acidic 6.9 to 6.4 and alkaline 7.1 to 7.6 with 0.1 pH as a difference between studied pH levels. The components of TYE as following:- Tris 2.400 gm, fructose 1.25 gm, egg yolk 15%, penicillin procaine 30000 IU, penicillin sodium 10000 IU, streptomycin 0.1gm, distilled water up to 100 ml). The pH values were controlled through citric acid content in each media and precisely using pH meter (CG 837 Germany).

Semen was collected by a warm artificial vagina to replicate eighteen pooling ejaculates (2 successive ejaculates/ram/weekly up to three weeks). Semen characteristics were estimated using gradual tube for volume (ml), sperm forward motility, live spermatozoa (%), abnormal tails (%), abnormal head (%) and sperm cell concentration/ml ($\times 10^9$) according to Evans and Maxwell (1987). Each Pooled ejaculate was allocated into 12 clean test tubes contained TYE with different pH values using extension rate 1 semen: 5 extender. Semen tubes were incubated at 37°C in water bath to record sperm advanced motility % and abnormal tails % at hourly interval for 4 successive hours.

Detection of Estrus and Insemination

During the insemination season, ewes were exposed to the teaser twice daily at 6-8 am and 6-8 pm to recognized ewes in heat. Ewes that displayed estrous behavior were inseminated by a dose of 0.50 ml extended semen contained 300×10^6 mobile sperm.

Ewes in G1 were inseminated by semen of pH 6.6, while ewes of G2 were inseminated with semen of pH 7.3. Ewes vagina was washed frequently by 10 ml TFE buffer with pH of 6.6 (G1) or 7.3 (G2) at one hour before insemination and five hours after insemination to avoid reducing copious mucus and to active the target spermatozoa (X or Y sperm). Ewe were inseminated by two doses which were deposited into the entrance of the cervix, 12 and 24 hrs from onset heat. After two cycles, the ewe which did return to heat was considered pregnant. Reproductive traits and lambs' sex were calculated as:

Conception rate % = No. of ewes pregnant / No. of ewes inseminated X100.

Ewes lambd % = No. of ewes lambd / No. of ewes conceived X100.

Sex lambs % = No. of born lambs in particular sex / Total No. of born lambs X100.

Statistical Analysis

Data were analyzed using SAS (1996). The significant differences between overall means were according to Duncan (1955). The used statistical model was:

$$Y_{ijk} = u + M_i + I_j + M_{ij} + e_{ijk}$$

Y_{ijk} = the studied trait

u = overall mean

M_i : the effect of pH of extender=($i=12$)

I_j : the effect of incubation time=($j = 1,4$)

M_{ij} : interaction between pH and incubation time

e_{ijk} : random error.

RESULTS AND DISCUSSION

First trial: incubation process of acidity and alkalinity dilution

Physical characteristics of acidic or alkaline extenders are presented in Tables 2 and 3, respectively. The results found that incubated semen in TFE with pH between 6.9 and 6.6 showed better ($P < 0.05$) livability of spermatozoa compared with $pH \leq 6.5$. Regarding incubation with alkalinity TFE the differences between 7.4 and 7.6 were significant ($P < 0.05$) compared with 7.1 - 7.3 pH. This result is in agreement with Salamon and Maxwell (2000) who reported better ram sperm tolerance between pH 6.5 to 7.5. Addition of egg yolk to extender coated and protected sperm mitochondria membrane from pH alteration. These results were explained by CiDePauw *et al.* (2003) who indicated that sperm coating could preserve sperm characteristics and penetrating capacity of fresh spermatozoa stored in egg yolk containing diluent with pH 6 to 7. The ram spermatozoa diluted in Tris based extender showed high sperm motility and membrane integrity during incubation that reflected enhanced fertility result (Cheng *et al.*, 2004). Also, Benjamin *et al.* (2008) reported that ram sperm tolerated pH ranged between 5.6 and 7.2 but the optimal acidity pH ranged from 6 to 6.5.

Effect of time on physical characteristics of spermatozoa during incubation. Results showed that the first hour of incubation, spermatozoa characteristics were better ($P < 0.05$) than the subsequent hours. Decreasing in sperm characteristics may be due to a gradual decline in the ability of spermatozoa to generate ATP through aging mitochondria (Cummins *et al.*, 1994 and Khalifa, 2005). Moreover, many sperm are dead and released toxic enzyme that affect live sperm membrane (Sadhan *et al.*, 2004). Extension media produced reactive oxygen species (Graaf *et al.*, 2006). The reaction producing three free radicals are more active at ambient incubation temperature (Karunakaran *et al.*, 2007 and Muiño *et al.*, 2007).

The interaction effects between pH media and incubation time were highly significant ($P < 0.01$). These findings are in agreement with those of Khalifa *et al.* (2006). These results may be attributed to fluctuations in the sperm metabolic characteristics with different pH during incubation time.

Second trial: effect of acidic and alkaline extenders on newborn sex ratio

Results in Table 4 showed effect of AI with acidic and alkaline extenders on sex ratio of newborn. Extender semen in pH 6.6 buffer (media) produced ratio of females newborn 66.67% and 33.33% males. While, pH 7.3 yielded 81.25% male lambs and 18.75% females. This finding could be attributed to that either acidity or alkalinity extenders activate respectively X and Y motile sperm as stated by Murray *et al.* (1988). Moreover, the response of X-bearing and Y-bearing spermatozoa to acidity or alkalinity extenders may be due to the major sperm protein (MSP) that plays an essential role in sperm motility. These results are in agreement with those of King *et al.* (1994). The major role of MSP explained by Italiano *et al.* (1999) who found that physiological buffer restores MSP assembly

and disassembly of motility filaments. Also, Buttery *et al.* (2003) reported that pH media gradient forms within the pseudopod of the spermatozoon, a higher pH is observed at the leading edge, where new filaments assemble, than at the base, where disassembly occurs. Decreasing the pH by buffer treatment can either slow

or halt MSP polymerization at the leading edge of the pseudopod without affecting disassembly (Smith, 2006). The phenomenon of intensity ram sperm motility (X and Y chromosomes) was similarly related to change in pH and the relationship between pH and motility are rather metabolic (Benjamin *et al.*, 2008).

Table (2): Mean± S.E of raw semen characteristics during incubation in different acidity media.

Items %	Inc hrs	pH media						Overall mean
		6.9	6.8	6.7	6.6	6.5	6.4	
Progressive motility	1	80.6±1.32	78.3±1.46	79.4±0.36	78.9±2.02	74.4±1.32	72.5±2.00	77.4 ^A ±1.27
	2	78.9±1.12	76.1±1.40	75.6±1.56	75.0±1.89	70.6±1.56	65.0±1.88	73.5 ^B ±2.02
	3	75.6±1.31	74.4±1.32	72.8±1.71	71.1±1.64	64.4±1.32	57.8±1.90	69.4 ^C ±2.80
	4	71.7±0.84	70.0±1.19	70.6±1.56	69.4±2.14	58.9±1.64	49.4±2.59	65.0 ^D ±3.63
Overall mean		76.7 ^a ±1.97	74.7 ^a ±1.77	74.6 ^a ±1.92	73.6 ^a ±2.11	67.1 ^b ±3.42	61.2 ^c ±4.94	71.3±1.70
Abnormal tails	1	14.2±0.53	15.3±0.69	15.6±0.84	15.9±0.62	20.6±0.65	23.8±0.33	17.6 ^D ±1.52
	2	17.3±0.65	18.7±0.61	18.9±0.74	19.0±0.56	26.3±0.56	31.2±0.58	21.9 ^C ±2.25
	3	19.9±0.68	21.1±0.66	20.6±0.74	21.9±0.64	29.7±0.51	36.2±0.79	24.9 ^B ±2.67
	4	24.0±0.41	25.3±0.65	25.6±0.63	26.1±0.39	32.3±0.77	40.2±0.75	28.9 ^A ±2.54
Overall mean		18.9 ^c ±2.07	20.1 ^c ±2.11	20.1 ^c ±2.09	20.7 ^c ±2.18	27.2 ^b ±1.57	32.9 ^a ±2.19	23.3±1.56

^{a, b, c, d} values with different superscripts in the same row or ^{A, B, C, D} in the same column differ significantly (P<0.05).

Table (3): Mean± S.E of raw semen characteristics during incubation in different alkaline media.

Items %	Inc hrs	pH media						Overall mean
		7.1	7.2	7.3	7.4	7.5	7.6	
Progressive motility	1	84.1±1.09	84.8±0.95	83.7±1.14	79.7±0.63	74.8±0.80	69.7±0.86	79.5 ^A ±2.46
	2	79.4±1.41	80.1±0.94	78.8±1.49	73.2±0.62	68.8±1.08	63.0±0.56	73.9 ^B ±2.80
	3	75.2±1.47	75.4±1.07	74.1±1.57	67.2±0.73	62.2±0.62	57.3±0.67	68.6 ^C ±3.08
	4	69.7±0.97	70.4±1.26	69.3±1.34	61.8±0.82	56.8±0.73	50.6±0.77	63.1 ^D ±3.31
Overall mean		77.1 ^a ±3.08	77.7 ^a ±3.08	76.5 ^a ±3.07	70.5 ^b ±3.85	65.6 ^c ±3.91	60.1 ^d ±4.07	71.3±2.12
Abnormal tails	1	13.8±0.71	14.4±0.68	14.3±0.45	18.3±0.51	20.7±0.61	22.8±0.60	17.4 ^D ±1.54
	2	15.6±0.78	17.3±0.45	17.1±0.57	22.2±0.58	26.1±0.70	30.7±0.86	21.5 ^C ±2.41
	3	19.4±0.68	20.7±0.34	19.6±0.36	27.2±0.67	31.2±0.58	36.0±0.82	25.7 ^B ±2.81
	4	23.7±0.53	23.8±0.33	24.0±0.41	31.7±0.91	36.3±0.57	41.1±0.80	30.1 ^A ±3.04
Overall mean		18.11 ^d ±2.2	19.1 ^d ±2.03	18.8 ^d ±2.05	24.9 ^c ±2.91	28.6 ^b ±3.37	32.6 ^a ±3.92	23.7±1.73

^{a, b, c, d} values with different superscripts in the same row or ^{A, B, C, D} in the same column differ significantly (P<0.05).

Table (4): Conception rate and sexual ratio for acidity and alkalinity extender.

Items	pH of extender	
	6.6	7.3
No. of ewes inseminated	15	15
No. of ewes conceived	12	14
Conception rate %	80.00	93.33
No. of ewes lambed	10	11
Ewes lambed %	83.33	78.57
Total number of lambs	15	16
No. of males	5	13
No. of females	10	3
Males %	33.33	81.25
females %	66.67	18.75

CONCLUSION

The present study showed that pH extenders and alteration of genital female media affected both X and Y spermatozoa percentage of in turn offspring sex ratio in Rahmany sheep.

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تأثير قيمة الحموضة لمخفف السائل المنوي لكباش الرحمانى على صفات السائل المنوى وتغيير النسبة الجنسية للمواليد

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أجريت هذه الدراسة لتحديد تأثير pH المخفف على صفات السائل المنوى والنسبة الجنسية للأغنام الرحمانى. قسمت الدراسة إلى تجربتين الأولى لتقييم تأثير فترة التحضين على صفات السائل المنوى مع مستويات مختلفة من الحموضة (٦,٩ - ٦,٤) والقوية (٧,١ - ٧). واستخدمت التجربة الأولى ١٨ قذفة جمعت من ٣ كباش وخفت بمخفف الترس. والتجربة الثانية استخدم فيها التلقيح الصناعى لتحديد النسبة الجنسية للمواليد باستخدام ٣٠ نجة قسمت النعاج إلى مجموعتين لقت الأولى بسائل منوى ٦,٦ والثانية بمخفف ٧,٣. وعسل مهبل النعاج قبل التلقيح بالبينة ٦,٦ للمجموعة الأولى و ٧,٣ للمجموعة الثانية. أوضحت النتائج أن صفات الحيوان المنوى أثناء التحضين فى البيئة الحمضية غير معنوية للتركيزات ٦,٩ حتى ٦,٦ ولكن التركيزات ٦,٥، ٦,٤ و ٦ أظهرت فروق معنوية. و البينات القوية من ٧,٤ إلى ٧,٦ لها تأثير معنوى على صفات السائل المنوى والتركيزات من ٧,١، إلى ٧,٣ ليس بينها فروق معنوية. وتلقيح النعاج بتركيز ٦,٦ أنتج إناث بنسبة ٦٦,٦٧% والتركيز ٧,٣ حقق أعلى نسبة من الحملان الذكور ٨١,٣%. توصى التجربة أن pH السائل المنوى أثر على نسبة الحملان المولودة.