PHYSIOLOGY & REPRODUCTION

THE EFFECT OF INSEMINATION TIMING ON FERTILIZATION AND EMBRYO GENDER IN ZARAIBI GOATS

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SUMMARY

The purpose of this study was to evaluate the effect of pH of cervical mucus and vaginal temperatures from onset of oestrus up to insemination time on sex ratio in Zaraibi goats. Three Zaraibi bucks were used for semen collection. Semen was evaluated and immediately diluted with tris-yolk fructose (TYF) extender. Nanny goats (n=44) were artificially inseminated with 1 ml extended semen containing $600x10^6$ motile spermatozoa. Nanny goats were allocated into four groups (11 females each). The interval time to estimate pH of cervical mucus and vaginal temperatures during oestrus period was 6 hrs. Artificial insemination was performed as following: group (G1) goats were artificially inseminated at onset of oestrus (zero time) which considered as control group. The groups G2, G3 and G4 were artificially inseminated at 12, 24 and 36 hours (hrs) after beginning of heat , respectively. Oestrus was detected eight times daily with three hours interval and determined when doe stand to be mounted. The results indicated that the lowest ($P<0.05$) value of pH of cervical mucus and vaginal temperatures were recorded at 24 and 30 hours of heat. The kidding rates were 54.5, 81.8, 100 and 100 in groups 1, 2, 3 and 4, respectively. Goats inseminated at 0 hr (G1) gave 50% males and 50% females. Goats inseminated at 12 hr produced 62.50% males and 37.50% females. The highest female ratio of offspring (84.62% vs. 15.38% male) were obtained in G3. In contrast, G4 recorded 73.08% male and 26.92% female kids.

It is proved that the period between onset of oestrus to insemination time is able to alter forward motility for either X or Y chromosomes to change the sex kind of fetal gender in Zaraibi nanny goats.

Key Words: *goats, artificial insemination, interval time, sex ratio, fertility.*

INTRODUCTION

Control of sex ratio of newborns is quite important for genetic improvement and farm economics. Special attention have been paid to control sex of offspring

throughout the manipulation of insemination time (Gutiérrez-Adán et al. (1999) in sheep; Roelofs et al., (2006) in cattle; Smits et al. (2005) in human and Vega et al. (2008) in rabbits). The previous results revealed that optimal pregnancy rate (PR) could reach its maximum when insemination taking place from mid-oestrus until a few hours after the end of oestrus (Foote, 1979). The duration of oestrus in does is usually extended for 20-40 hrs, while ovulation occurs shortly after the end of the heat phase or 30 to 36 hours after the onset of heat (Evans and Maxwell, 1987). Life span of ova was found to be 16-24 hrs and 30-48 hrs for spermatozoa. The best circumstances for fertilization is when spermatozoa quickly reached the site of fertilization within 15-30 minutes of mating and insemination done at 12-36 after onset of oestrus (Hafez, 1987). The newborn sex ratio has been altered for several species by varying the sperm media. Khalifa et al. (2009) found that sex ratio could be controlled by changing pH in sperm extender. Acidic extender (6.6) gave 66.67% female lambs, while alkaline extender (7.3) resulted in 81.25 % male lambs.

The objectives of the present work were to study the effects of eitherchanges in the pH of cervical mucus or vaginal temperatures through oestrus period and delaying insemination time on sex ratio of obtained offspring in Zaraibi goats.

MATERIALS AND METHODS

Experimental animals and feeding

This experiment was executed in the Animal Production Research Station, El-Serw Domietta Governorate, Animal Production Research Institute, throughout the autumn season of 2008.

Forty-four healthy Zaraibi does of age 30-36 months were allocated into four equal groups (11 each). Does were nourished 50% concentrate feed mixture (CFM) and 50% berseem hay (BH). The composition of CFM was cotton seed (17.50%), yellow maize (40%), wheat bran (25%), soybean meal (7%), molasses (6%), limestone (2.5%), common salt (1.5%) and minerals (0.5%). Three healthy bucks with age of 30 - 34 months was

Reed forage of 50-100 cm height was mowed to be used for feeding.

fed 50% CFM and 50% reed grass silage (RGS) were used to inseminate the experimental females. Fresh water was available for experimental animals. The chemical analysis of feed stuffs used in this experiment is shown in Table 1

Table 1: Chemical composition and nutritive values of the experimental feeds.

Ingredients (%)	Feed stuffs		
	CFM	BH	RGS
Dry matter	90.50	89.4	30.10
Organic matter	93.20	87.5	89.60
Crude fiber	14.80	28.4	28.90
Crude protein	15.10	13.9	10.90
Ether extract	03.50	02.3	03.10
Nitrogen free extract	59.83	42.9	46.70
Ash	06.77	12.5	10.40

RGS: The reed forage was collected from El- Manzalla lake, Dakahliya Governorate.

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Semen collection and extension

Semen was collected after 54 days of feeding bucks RGS, using artificial vagina. Semen ejaculates were evaluated immediately after collection. Forward sperm motility of > 75 %, abnormal spermatozoa <15% and sperm cells concentration > 2.90 x 10^9 were extended with tris-yolk fructose (TYF). The chemical composition of TYF that give pH 7.0 consists of Tris (4.50 gm), fructose (1.25 gm), citric acid (2.60 gm), egg yolk (2.5%), penicillin procaine (10000 IU), penicillin sodium (10000 IU), streptomycin (0.1gm), distilled water up to 100 ml. Does were artificially inseminated with 1 ml extended semen containing 600x10^6motile spermatozoa.

Oestrus detection and AI groups

Oestrus was observed naturally for 8 times through 24 hrs by teaser buck at 3 hrs interval. Oestrus detection extent for two hours then approximately 60 minutes for resting teaser buck. The onset of oestrus was determined when the nanny goat stood to be mounted. Goats in heat were artificially inseminated by extended semen which deposited into vaginal tract by speculum in the Os-cervix. The pH of cervical mucus and vaginal temperatures had been recorded every 6 hrs from onset of oestrus to the time of insemination. The insemination process was performed at 0 hrs (G1 which served as control), 12 hrs (G2), 24 hrs (G3) and 36 hrs (G4) from onset of heat.

Cervical mucus pH and vaginal temperature during heat

The pH of cervical mucus was measured by pH meter (CG 837 Germany). pH was measured via inserting clean electrode against the vaginal wall at a point of

approximately 8 cm depth into vagina until Os-cervix mucus. When reading of pH meter being stable reading was recorded. The electrode was rinsed with 70% ethanol, then distilled water, and dried between insertions (Lewis and Newman**,** 1984).

Vaginal temperature was measured by a digital readout thermistor thermo meter (Cole-Parmer Instrument Co., Chicago, IL). The probe was inserted into the anterior portion of the vagina, and held in place until reaching a stable reading. The probe was cleaned as above.

Fertility traits and kid's sexing were calculated as follow:

Kidding rate %= No. of pregnant goats / No. of inseminated goats X100.

Nanny goat kids % = No. of nanny goat kided / No. of pregnant goats X100.

Sex of kid (male/female) % =No. of born kids in particular sex/Total No. of born kids X100.

Statistical analysis

Data were analyzed using SAS (1999). The differences among means were evaluated by Duncan Multiple Range test (Duncan, 1955). The differences between group in sex ratio were statistically analyzed by x^2.

RESULTS AND DISCUSSION

Cervical mucus pH and vaginal temperature during oestrus hours

Results in Table (2) indicate significance ($P<0.05$) of changesin pH of cervical mucus and the vaginal temperature during heat. At the onset of heat, pH value was more than 7.0 and decreased to 6.60 up to 30 hrs from onset of heat. This is similar to that reported by Schilling and Zust (1968). The difference in vaginal pH may be attributed to the changes of electrical resistance of vaginal mucus that act as indicators for oestrus and ovulation (Leidl and Stolla, 1976). The results revealed that pH decreased with advance of oestrus which coincide with ovulation. These findings are in accordance with the results of Evans and Maxwell (1987). At the end of heat, the mucus pH retrained to alkaline (Rezac et al., 2001). Reductions of vaginal pH depends upon an influx of ions, such as hydrogen, sodium and chloride, into vagina, which accompanied by accumulation of glycogen and proteins (Khadiga et al., 2005) and coincide with the LH surge (Schofield et al., 1991 and Blaszczyk, et al., 2004).

Vaginal temperature around oestrus were increased approximately 0.4 degree for 6 consecutive hours up to18 hrs from onset of heat. Vaginal temperature decreased for the next 18 hrs, and then declined steadily from 24 hrs until 36 hrs before end of

oestrus. Also, data indicate that vaginal temperature was elevated at 18 hrs (mid-period). Kyle et al. (1998) reported that vaginal temperature was decreased 3 days prior oestrus and significantly elevated at mid-heat period in cows. The temperature was fluctuated during the oestrus period. It may be due to secretion of estrogen that increased blood flow to the reproductive tract, causing an increase in hydration of vaginal tissue (Smith et al., 1989). During oestrus period, the low levels of progesterone and high levels of estrogen before ovulation had caused changes in the vaginal temperature (Dhalia et al., 2005). Vaginal temperature was affected by modifying the activity of proteolytic enzymes that needed to depolymerize the follicle wall, distensibility and dissociation of tissues, remodelling of the basement membrane and coagulability of follicular fluid (Hunter et al., 2006 and 2007). Moreover, Hunter (2009) reported that biosynthesis of gonadal steroid hormones would be sensitive to vaginal temperature regulation. He also indicated that the vaginal temperature was being high on the day of heat, low again at the time of ovulation, and high during the luteal phase of the oestrus.

Table2: Mean±S.E of pH of cervical mucus and vaginal temperature during different times of oestrus.

Items	groups	Time interval between onset of oestrus /hrs						
		0	6	12	18	24	30	36
pH of cervical mucus	G1	7.28 ±0.14	-	-	-	-	-	-
	G2	7.26^{a} ±0.11	7.09^{b} ±0.15	6.87^{c} ±0.19	-	-	-	-
	G3	7.27^{a} ±0.08	7.06^{b} ±0.06	6.89^{c} ±0.06	6.79^{d} ±0.05	6.59^{e} ±0.04	-	-
	G4	7.25^{a} ±0.13	7.11^{b} ±0.12	6.88^{c} ±0.11	6.78^{d} ±0.09	6.58^{e} ±0.07	6.60^{e} ±0.07	7.13^{b} ±0.09
Vaginal temperature (^{o}C)	G1	38.27 ±0.29	-	-	-	-	-	-
	G2	38.26^{c} ±0.24	38.66 ^{b} ±0.23	39.06^{a} ±0.15	-	-	-	-
	G3	38.25^{d} ±0.20	38.65^{c} ±0.21	39.05^{b} ±0.22	39.46^{a} ±0.19	38.19^{e} ±0.23	-	-
	G4	38.24^{d} ±0.21	38.65^{c} ±0.11	39.04^{b} ±0.12	39.44^{a} ±0.24	38.18^{e} ±0.21	38.17^{e} ±0.18	38.25^{d} ±0.19

Values with different superscripts in the same row significantly different (P<0.05).

The fertility traits and kid's sexing

The obtained results in Table 3 show that the mean kidding rates in nanny goats artificially inseminated 0, 12, 24 and 36 hrs (G1, G2, G3 and G4) after the onset of oestrus were 54.55, 81.82, 100 and 100 %, respectively. The lowest kidding rate observed in G1 was combined with the earliest insemination . The results show that G3 and G4 surpass G2 in kidding rates. The nanny goats in G1 that shortly inseminated and produced fewer kids might due to possible decrease of a healthy sperm contacting a healthy ovum at fertility site. Changing vaginal temperature could influence the activity of both nucleus and cytoplasm and thus the characteristics of nuclear and cell membranes, including the remodelling of structural proteins (Rajamahendran et al., 1989 and Hunter, 2008); defect of pH of cervical mucus (Elrod and Butler 1993) and prolong time from insemination up to ovulation over 12 h after the end of oestrus period (Goel and Agrawal, 2003). Moreover, Santos et al. (2004) showed hormonal interaction such as estradiol-17ß (E_2) might facilitate sperm migration into the oviduct and that progesterone (P_4) was antagonized the sperm motility toward the site of fertility. They also reported that interaction between E_2 and P_4 might stimulate adhesion of spermatozoa to the oviduct epithelium. The vaginal temperature influences spermatozoa that already has been made, besides motility and membranous modification as primary consideration (Eisenbach and Giojalas, 2006).

In respect to newborn kids, the total newborn kids increased ($P<0.05$) with nanny goats artificially inseminated at 24 hrs and 36 hrs after the onset of heat. The data show that delayof insemination time had altered offspring ratio which depend on ovulation time. These results are in agreement with those of Gutiérrez-Adán et al. (1999) who found significant differences in the sex ratio obtained when ewes inseminated during 5 h preceding ovulation (more females) compared with those inseminated during 5 h after ovulation (more males). Moreover, skewing of sex ratio to females or males was attributed respectively to acidity (pH=6.59) and alkalinity (pH =7.13) at both 24 and 36 hrs that rapidly forward movement of either X or Y chromosome-bearing spermatozoa to fertility side. These results are explained by Smith (2006) who found that both decreasing and increasing pH can affect majority of sperm protein that plays an important role in sperm motility at the leading edge, where new filament assemble disassembly occurs. In addition, Y chromosome-bearing spermatozoa are capacitated before X chromosome-bearing spermatozoa. Delayed insemination to 36 hrs near the ovulation time would cause X chromosome-bearing spermatozoa to lose their ability to fertilize and allow Y chromosome-bearing to fertilize oocyte (Martineza et al., 2004, Atsushi et al., 2007 and Orkun et al., 2007). Alteration of embryo sex ratio tendency to females by moving the timing of insemination to 24 hrs may due to skewing mucus in vagina to acidic which activate X chromosome-bearing spermatozoa to reach fertility site faster than Y chromosome-bearing spermatozoa. These results are in agreement with that reported by Khalifa et al. (2009) who reported that gradient in pH extender at 6.6 and 7.3 produced 66.67 and 81.25% female and male lambs, respectively.

Table 3: Kidding rate, total number of kids and sex ratio of the does artificially inseminated with different times of insemination after oestrus detection.

Items	Time of insemination after oestrus detection (hrs)			
	0	**12**	**24**	**36**
No. of goat does inseminated	11	11	11	11
No. of goat does pregnant	6	9	11	11
Kidding rate %	54.55	81.82	100	100
No. of goat does kids	5	9	11	11
Goat does kids (%)	83.33	100	100	100
Total number of kids	10	16	26	26
No. of males	5	10	4	19
No. of females	5	6	22	7
Sex ratio :				
Males (%)	50.00^{c}	62.50^{b}	15.38^{d}	73.08^{a}
Females (%)	50.00^{b}	37.50^{c}	84.62^{a}	26.92^{d}

Values with different superscripts in the some row, differ significantly ($P<0.05$).

CONCLUSION

It is recommended to organize artificial insemination to be performed close to the time of ovulation in females. Accurate and careful detection of oestrus and control of oestrus and ovulation are necessary to reach a satisfactory fertilization level and skew the sex of offspring, especially during insemination at 24 hours from onset of oestrus in goats.

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تأثير الفترة الزمنية بين بدء الشياع والتلقيح على الإخصاب و النسبة الجنسية في الماعز

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الملخص العربى

الهدف من هذة الدراسة هو تحديد درجة حموضة مخاط المهبل وحرارة المهبل خلال ساعات الشياع لمجاميع التلقيح وتوضيح تأثيروقت التلقيح الإصطناعى على النسبة الجنسية للمواليد فى الماعز الزرايبى. تم تجميع السائل المنوى من ٣ تيوس عن طريق المهبل الإصطناعى وخففت القذفات بمخفف الترس فراكتوز. تم تلقيح ٤٤ عنزة إصطناعيا وكانت جرعة التلقيح لكل عنزة ١ مل سائل منوى مخفف يحتوى على ٦٠٠x١٠^٦ حيوان منوى متحرك . قسمت مجاميع التلقيح الى ٤ مجموعات (١١ عنزة بكل مجموعة). تم قياس حموضة مخاط المهبل ودرجة حرارة المهبل أثناء ساعات الشياع كل ٦ ساعات. ولقحت المجاميع إ صطناعيا كالتالى: المجموعة الأولى (مقارنة) عند بداية الشياع،المجموعة الثانية بعد ١٢ساعة من الشياع، المجموعة الثالثة بعد ٢٤ ساعة من الشياع والمجموعة الرابعة بعد ٣٦ ساعة من الشياع. اوضحت النتائج أن اقل درجة حموضة وحرارة للمهبل (بمعنوية ٥ %كانت عند ٢٤ -٣٠ ساعة من بداية الشياع. وكان معدل الحمل ٥٤.٥٥ ، ٨١.٨٢ ، ١٠٠ ، ١٠٠ % لمجاميع التلقيح١،٢،٣،٤ على التوالى. أعطت الماعز الكنترول مواليد ذكور ٥٠% وأناث ٥٠%. والمجموعة الثانية اعطت ٥٠و٦٢ % ذكور ، ٥٠و٣٧ % اناث (بعد ١٢ ساعة من الشياع). أعلى نسبة مواليد إناث ٨٤.٦٢% مع أقل نسبة ذكور١٥.٣٨% كانت لصالح المجموعة الثالثة التى لقحت بعد ٢٤ ساعة من الشياع وعلى العكس المجموعة الرابعة التى لقحت بعد ٣٦ ساعة من الشياع أعطت ٠٨و٧٣% جداء ذكور ، ٩٢و٢٦% أناث وكان عدد المواليد الناتجة من التلقيح الإصطناعى اقتصادى بالنسبة للمجموعتين ٣، ٤ . وتوصى الدراسة بأن الفترة الزمنية بين ملاحظة الشياع ووقت التلقيح قادرة على تعديل حركة الكروموسوم x ، y بما يسمح فى االتحكم فى النسبة الجنسية لمواليد الماعز الزرايبى.