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SOME FACTORS AFFECTING FERTILIZNIG CAPACITY OF FROZEN GOAT SEMEN

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ABSTRACT

The present study aimed to study some factors affecting the successful artificial insemination (AI) including optimal level of egg yolk and dilution rate of extender, semen collection month and AI procedures of crossbred buck semen. Semen was collected from10 fertile goat bucks (1/2 Damascus x 1/2 Baladi) throughout all months of the year. ♂Ejaculates with mass motility ≥80% were pooled, diluted with Tris-citric acid-glucose-glycerol extender containing different levels of egg yolk (2.5, 5.0, 7.5, 10.0, 15.0 and 20%) or diluted with different rates (1:4, 1:5, 1:8, 1:10 and 1:16) with the best level of egg yolk (2.5%), then semen was frozen with the best level of egg yolk (2.5%) and dilution rate (1:5) during all months of the year for all treatments. Sperm motility was evaluated and recovery rate was calculated for each treatment. Fertility trail was conducted using sixty four synchronized does by Estrumate. Does were inseminated (intra-vaginal *vs.* intra-uterine) with inseminated dose (100 *vs.* 200 x 10^6) after 48 or 52 h of Estrumate-injection. Results revealed that sperm motility percentage was the highest ($P<0.05$) in post-thawed semen diluted with Tris-extender containing 2.5% egg yolk (54.8%) and at a dilution rate of 1:5 (54.5%). Post-thaw sperm motility percentage was the highest during autumn months, in particular, in November and the lowest during summer months (June-Aug.). Fertility rate (FR) was the highest ($P<0.05$) of does intra-uterine inseminated with high dose of insemination after 48 h of Estrumate-injection (62.5%), while the lowest FR was recorded for the control does (43.75%). Does inseminated intra-uterine with low dose after 48 h of Estrumate-injection and those inseminated intra-uterine with high dose after 52 h of Estrumate-injection had moderate rates of fertility, being 50.0 and 56.25%, respectively.

In conclusion, dilution of buck semen with Tris-based extender containing 2.5% egg yolk at a rate of 1:5 and frozen in pellets form will be more efficient in maintaining post-thaw sperm motility. The best season of collection and freezing goat semen is autumn. Intra-uterine insemination with high dose of motile spermatozoa (200 x 10^6) could yield acceptable fertility rates of goat does.

Keywords: Goat, egg yolk, dilution rate, season, intra-uterine insemination, fertility

INTRODUCTION

Artificial insemination (AI) with fresh, chilled or frozen-thawed semen is a basic tool in goat breeding. As in other domestic animals, the freezing process reduces the viability of goat spermatozoa and consequently decreasing kidding rate after AI with frozen-thawed semen, ranging between 30 and 70% (Ritar *et al*., 1990; Leboeuf *et al*., 2003 and Dorado *et al*., 2007). With transcervical insemination, a low proportion of spermatozoa traverse the cervical canal, meaning a relatively higher number of sperm cells are necessary to get reasonable pregnancy rates (Roca *et al*., 1997 and Gacitua

and Arav, 2005).Different studies have noted this fact as a hand cap in AI in small ruminants (Gacitua and Arav, 2005 and Kaabi *et al*., 2006).

Several factors are affecting sperm motility and livability in frozen semen, Goat spermatozoa are very sensitive to cold shock and can't be cooled to 5 ^{o}C in the absence of egg yolk (Salamon and Maxwell, 1995). Egg yolk is common constituent in semen extenders to protect sperm cells against cold chock and has been thought to confer protection during freezing and thawing (Simpson *et al*., 1986). Egg yolk level in semen extenders plays a major role during the freezing steps of goat spermatozoa (Aboagla and Terada, 2004). However, egg yolk might show negative effect on frozen goat semen due to the presence of phospholipase A enzyme in the seminal plasma which catalyzes the hydrolyses of lecithin in egg yolk to fatty acids and lysolecithins, which are toxic to spermatozoa (Iritani and Nishikawa, 1972). In this respect, some authors found that removal of the seminal plasma from the semen (Corteel, 1975) or Cowper's glands from the animals (Corteel, 1981) is beneficial when the spermatozoa are frozen in a diluent containing egg yolk.

Semen is diluted to protect spermatozoa during preservation, but the rate of dilution is often changed for technical reasons (Salamon and Maxwell, 1995). Some studies have indicated that semen production in bucks is influenced by seasonal changes (Loubser *et al*., 1983).

Successful attempts to dilate the cervix of ewes were reported to facilitate the passage of inseminating catheter using oxytocin (Khalifa *et al*., 1992; Khalifa, 1993 and Fateh El-Bab *et al*., 2000). However, no available information are found on the use of oxytocin in dilation of the cervix of goats. Therefore, the objective of this study was to assess optimal egg yolk level, dilution rate and collection month as well as site of deposition of semen intra-vagina or intra-uterine with high or low semen dose that allow increasing the success rate of AI and consequently increasing fertility of goat does with frozen semen of Damascus x Baladi goat bucks.

MATERIALS AND METHODS

The present study was carried out at Sakha Research Station, Animal Production Research Institute, Agricultural Research Center.

Semen collection and dilution:

Semen was collected from 10 fertile 1/2 Damascus x 1/2 Baladi goat bucks (3-5 years old) with an artificial vagina twice weekly between 0.8 to 0.9 a.m. during all months of the year. On each collection day, ejaculate of each buck was taken immediately to the laboratory, then evaluated and held in a water-bath at 35-37^{o}C. Only, ejaculates showing mass motility ≥80% were pooled, extended with Tris (hydroxymethyl-aminoethane)-citric acid extender containing 375.0 mM Tris (Sigma Chemical Co., St. Louis, MO, USA), 124.0 mM citric acid (Sigma), 41.0 mM glucose (Sigma Aldrich), 5% glycerol and different levels of egg yolk according to the experimental procedures. Egg

yolk was separated from the albumin and added to the extender. Also, semen was diluted at different rates according to the experimental procedures.

Freezing and post-thaw motility evaluation:

Semen was frozen as pellets form (0.25 ml/pellet) on a fluorethene plate with holes engraved in the surface and cooled to -79^{o}C, then to -140^{o}C by immersion in liquid nitrogen (**Evans and Maxwell, 1987**). After 2 to 3 minutes, the frozen pellets were transferred into liquid nitrogen and stored for use. Glycerol was added to the cooled semen and was left at 5^{o}C for 2 hours as equilibration period before freezing process. Frozen semen was thawed using thawing-solution 1:3 as thawing medium (2.9 sodium citrate/100 ml water) for 120 seconds at 37^{o}C.

Percentage of sperm progressive motility was determined in fresh semen, pre-freezing (post-dilution) and post-thawed semen. Recovery rate (RR%) was calculated according the following formula:

RR (%) = {Post-thaw sperm motility (%)/ fresh motility (%)} × 100.

Fertility trail:

Estrus synchronization of does:

Estrus was synchronized in all does of each group by 0.5 ml Estrumate (125 µg colprostenol, Agropharm Inc., Willowdale, Ontario Canada) and 200 IU PMSG (Folligon, Intervet International B.V. Boxmeer, Holland) as intramuscular injection per doe 10-12 days post the onset of natural heat. Starting from twenty-four hours post injection, estrus detection was performed by teaser bucks at 4 h interval and onset of estrus was recorded.

Artificial insemination

Total of sixty four mature 1/2 Damascus x 1/2 Baladi goat does (3-5 year of age and 30-35 kg body weight) were artificially inseminated during September breeding season with frozen semen diluted in Tris-based extender containing 2.5% egg yolk at a dilution rate of 1:5 in pellets form. Does were assigned into 4 similar groups (16 in each). Does in the 1st group were artificially inseminated by the conventional method (at Os cervix) with a high dose (200 x 10^{6} motile sperm), while those in the other groups were injected intravenously with 5 IU oxytocin/doe to dilate the cervix and transcervically inseminated (the insemination tube was penetrated all the cervical canal into the uterus of does) with different semen doses and times of insemination within 8 min of oxytocin injection. In this study, different oxytocin levels (15, 10 and 5 IU/doe) were used in this study to dilate the cervix. It is of interest to obtain that 5 IU of oxytocin was an appropriate level of cervix dilation.

Does in the 2nd were inseminated with a low dose (100 x 10^{6} motile sperm), while does in 1st, 3rd and 4th groups were inseminated with a high dose (200 x 10^{6} motile sperm). Insemination time was after 48 h of Estrumate-injection in the 1st, 2nd and 3rd groups and after 52 h of Estrumate-injection in the 4th group.

Fertility rate (FR %) was calculated as the following:

FR (%) = (Number of kidded does/number of inseminated does) x 100.

Statistical analysis:

Data were analyzed by one-way analysis of variance according to Snedecor and Cochran (1982). The statistical model was: $Y_{ij} = \mu + A_i + e_{ij}$, where: Y_{ij} = observed values, μ = overall mean, A_i = level of egg yolk, dilution rate or month of the year, and e_{ij} = random error. Fertility rate results were analyzed using Chi-square test.

Duncan multiple range test (Duncan, 1955) was used to test the differences among means. Significance level was tested at $P<0.05$.

The percentage values were subjected to arcsine transformation before performing the analysis of variance. Means were presented after being recalculated from the transformed values to percentages.

RESULTS AND DISCUSSION

Effect of egg yolk level:

Results presented in Table (1) show that post-thaw sperm motility and recovery rate were negatively affected by increasing egg yolk (EY) level in Tris- extender. Sperm motility percentage insignificantly decreased from 54.8 to 50.4% with increasing EY level from 2.5 up to 10%. However, increasing EY level more than 10% significantly ($P<0.05$) decreased sperm motility to 45.4% with 15% EY and to 39.0% at 20% EY. Similar trend was observed for the changes in recovery rate of sperm motility with different EY levels.

Similar results were reported by Ritar and Salamon (1982), who found that sperm motility of Angora goats after thawing was 35.50, 36.00, 26.80, 19.80 and 15.30% with levels of 0, 1.5, 3.0, 6.0 and 12.0% EY, respectively. Also, El-Maghraby (2007) found that sperm motility of goat semen was the optimal percentages with a level of 2.5% EY in Tris-based extender.

Table (1): Effect of egg yolk level in Tris-based extender on sperm motility percentage and recovery rate in post-thawed semen.

Egg yolk level (%)	Post-thaw sperm motility (%)	Recovery rate (%)
2.5	54.8±1.67^a	65.9±0.25^a
5	51.3±1.93^a	61.7±0.12^a
7.5	50.8±1.65^a	61.1±0.23^a
10	50.4±1.68^a	60.6±0.20^a
15	45.4±1.51^b	54.6±0.15^b
20	39.0±1.90^c	46.9±0.16^c

a, b and c: Means having different superscripts within the same column differ significantly at P<0.05.

The observed reduction in sperm motility by increasing EY level from 2.5 up to 20% in this study may be attributed to that increasing EY level increases lecithin concentration in semen extender, consequently the increased hydrolysis of lecithin by the activity of the egg yolk coagulating

enzyme (phospholipase) in the buck seminal plasma, which leads to further deterioration of sperm motility.

On the other hand, many extenders containing 20% EY were used for washed semen with accepted post-thawed semen quality (Deka and Rao, 1986; Tuli *et al.*, 1991). At studying the suitable EY level with Tris-egg yolk citric acid-fructose glycerol extender for freezing washed buck semen, Deka and Rao (1986) found that sperm motility in post-thawed semen with 20% EY was better than 10 or 7% EY (68.35, 67.35 and 58.40%, respectively), while percentage of spermatozoa with damaged acrosome was 11.70, 16.80 and 24.10%, respectively.

Effect of dilution rate:

Results presented in Table (2) show that post-thaw sperm motility and recovery rate were significantly (P<0.05) affected with dilution rate. Dilution rate of 1: 5 significantly (P<0.05) showed the highest sperm motility percentage (54.5%) and recovery rate (65.7%) as compared to other dilution rates. It is of interest to note that increasing dilution rate to more than 1:8 significantly (P<0.05) decreased post-thaw sperm motility and recovery rate.

Table (2): Effect of dilution rate with Tris-based extender containing 2.5% egg yolk on sperm motility percentage and recovery rate in post-thawed semen.

Dilution rate	Post-thaw sperm motility (%)	Recovery rate (%)
1:4	53.7±1.67^{a}	64.8±0.24^{a}
1:5	54.5±1.74^{a}	65.7±0.16^{a}
1:8	52.0±1.70^{ab}	62.7±0.17^{a}
1:10	47.9±1.64^{b}	57.8±0.23^{b}
1:16	45.4±1.83^{c}	54.8±0.15^{b}

a, b and c: Means having different superscripts within the same column are significantly different at P<0.05.

In accordance with the present results, Ritar and Salamon (1982) indicated that best sperm characteristics obtained when frozen goat semen was diluted at ranges from 1:4 to 1:8. Also, Singh *et al.* (1995) found the highest post-thaw sperm motility (47.8%) and live sperm percentage (67.1%) in goat semen diluted at a rate of 1:5. Moreover, Salamon and Maxwell (1995) recorded the highest survival rate (64.3%) at dilution rate of 1:7, followed by 1: 5 (63.1%) compared with 1: 9 (54.2%) in goat semen stored at refrigerator temperature.

The success of deep freezing depends, to a notable degree, on the rate of dilution of semen. Semen is diluted to protect spermatozoa during freezing and thawing, but the rate of dilution was often changing for technical reasons (Salamon and Maxwell, 1995). In this study, increasing dilution rate more than 1:5 led to a severe effect on the cation concentration of spermatozoa (Robertson and Watson, 1987) and consequently on sperm motility.

Effect of month of the year:

Results shown in Table (3) revealed that percentage of post-thaw sperm motility and recovery rate significantly (P<0.05) increased in autumn months (Sept, Oct. and Nov.), compared with other months of the year. The lowest values have been reached in summer in July and August. El-Maghraby (2007) found the highest percentage of sperm motility, in fresh, pre-freezing and post-thawed semen for different goat breeds, during autumn.

Table (3): Sperm motility percentage and recovery rate in post-thawed semen diluted with 2.5% egg yolk in Tris-based extender at a rate of 1:5.

Season	Month	Post-thaw sperm motility (%)	Recovery rate (%)
Spring	March	46.90±1.14^{de}	56±0.01^{e}
	April	49.28±2.97^{bcde}	60±0.03^{c}
	May	41.42±0.92^{ef}	56±0.01^{d}
Summer	June	41.48±0.80^{ef}	56±0.01^{d}
	July	32.85±3.59^{g}	43±0.05^{i}
	August	35.71±2.64^{fg}	51±0.04^{h}
Autumn	eptember	57.14±2.64^{ab}	67±0.03^{b}
	October	52.85±4.20^{bc}	62±0.05^{e}
	ovember	62.85±1.84^{a}	78±0.02^{a}
Winter	ecember	47.85±2.14^{cde}	59±0.02^{h}
	January	46.42±2.82^{bcd}	58±0.03^{f}
	February	44.28±2.02^{cde}	56±0.03^{g}

a, b and c: Means having different superscripts within the same column are significantly different at P<0.05.

Effect of site of semen deposition, and dose and time of insemination:

Fertility rates (FRs) of inseminated does as affected by AI procedures including site of semen deposition, dose of insemination, time of insemination and their interaction are presented in Table (4).

The obtained results show that only effect of site of semen deposition and its interaction with dose and time of insemination were significant (P<0.05) on FRs of does. However, the effect of dose and time of insemination was not significant. These findings indicated that FRs significantly (P<0.05) increased by increasing the depth of semen deposition within the uterus of does. However, when the control does were conventionally inseminated, FR was significantly (P<0.05) lower (43.75%) than in those intra-uterine inseminated (56.25%, Table 4).

On the other hand, increasing insemination dose from 100 to 200 x 10^6 motile sperm insignificantly increased FR from 50 to 54.17%. Also, FR of does inseminated after 48 h of Estrumate-injection insignificantly increased to 56.25% as compared to those inseminated after 52 h of Estrumate-injection (52.08%, Table 4).

Table (4): Effect of site of semen deposition, sperm concentration in semen dose and insemination time on fertility rate of does.

Variable	Inseminated does (n)	Kidded does (n)	Fertility rate (%)
Effect of site of semen deposition:			
Control (Os. Cervix)	16	7	43.75
Intra-uterine (within cervix)	48	27	56.25
Significance			*
Effect of sperm concentration in semen does:			
Low dose (100 x 10^6 motile sperm)	16	8	50.0
High dose (200 x 10^6 motile sperm)	48	26	54.17
Significance			NS
Effect of insemination time:			
48 h post-Estrumate injection	16	9	56.25
52 h post-Estrumate injection	48	25	52.08
Significance			NS
Effect of interaction:			
Control x high dose x 48 h	16	7	43.75^{c}
Intra-uterine x low dose x 48 h	16	8	50.0^{bc}
Intra-uterine x high dose x 48 h	16	10	62.50^{a}
Intra-uterine x high dose x 52 h	16	9	56.25^{ab}
Significance			*

a, b and c :Means with different litters within the same column are significantly different at P<0.05. * Significant at P<0.05. NS: Not significant.

The interaction effect of site of semen deposition with dose and time of insemination was significant, reflecting significantly (P<0.05) the highest FR of does inseminated intra-uterine with high dose after 48 h of Estrumate-injection (62.5%) and the lowest FR of the control does (43.75%). However, does intra-uterine inseminated with low dose after 48 h of Estrumate injection or intra-uterine with high dose after 52 h of Estrumate-injection resulted in moderate rates of fertility, being 50.0 and 56.25%, respectively (Table 4).

The present FR of does inseminated intra-uterine by frozen semen in this study is higher than that obtained by several authors, being 43.2% in Majorera goats (Batista *et al*., 2009), 39% in Cashmere goats (Ritar *et al*., 1990), 39-42% in Saanen goats (Gacitua and Arav, 2005) and 47.6% in Florida goats (Dorado *et al*., 2007). However, similar FRs to that obtained in this study were obtained by other authors, being 51% in Angora goats (Ritar and Salamon, 1991), 57% in Murciano-Granadina goats (Salvador *et al*., 2005), and (57 and 61%) in Saanen and Alpine goats, respectively (Leboeuf *et al*., 2000).

The high fertility rate in the intra-uterine inseminated does is believed to be due to the availability of high sperm concentration close to the site of fertilization. The cervix constitutes a barrier to the passage of the spermatozoa. It causes a limitation in the availability of sperm cells and hence reduces conception percentage.Several investigators reported that the needed does of motile sperm cells not less than 150-200 x 10^6/intra-vaginal insemination as compared to 5-10 x 10^6 motile sperm/cervical or uterine insemination in cattle. After intra-vaginal insemination, semen drainage from

the vagina to the exterior accounts for the great loss of spermatozoa from the ewe reproductive tract (Hawk and Conly, 1971 & 1972; Fateh El-Bab, 1975).

CONCLUSION

In conclusion, dilution of ram semen with Tris-based extender containing 2.5% egg yolk at a rate of 1:5 and frozen in pellets form will be more efficient in maintaining post-thaw sperm motility. The best season of collection and freezing goat semen is autumn. Intra-uterine insemination with high dose of motile spermatozoa (200 x 10^6) could yield acceptable fertility rates of goat does.

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بعض العوامل المؤثرة على القدرة الاخصابية للسائل المنوى المجمد للماعز

عبد العزيز عبد العظيم سلام

معهد بحوث الانتاج الحيوانى _مركز البحوث الزراعيه - دقى- جيزه - مصر

تهدف هذه الدراسة الى دراسة بعض العوامل المؤثرة على نجاح التلقيح الصناعى متضمنة المستوى المثالى لصفار البيض ، ومعدل التخفيف ، والشهر الذى تم جمع السائل المنوى فيه ، واجراءات التلقيح الصناعى للسائل المنوى للتيوس الخليط. تم جمع السائل المنوى من 10 تيوس خليط 2/1 دمشقى × 2/1 بلدى على مدار العام ، تم خلط القذفات المنوية المحتوية على حيوية اكثر من 80% فقط لاستخدامها وتم تخفيفها بمخفف ترس – حامض الستريك – الجلوكوز – الجليسرول بمستويات مختلفة من صفار البيض (2.5- 5 -7.5- 10 - 15- 20%) وتم التخفيف بمعدلات مختلفة (1 : 4 ، 1: 5 ، 1: 8 ، 1: 10 ، 1: 16) بأفضل مستوى لصفار البيض (2.5%) . حينئذ تم تجميد السائل المنوى بأفضل مستوى صفار بيض (2.5%) ومعدل تخفيف (1: 5) خلال اشهر العام لكل المعاملات. تم تقييم حيوية الحيوان المنوى لكل معاملة ،وتم اجراء حساب الخصوبة على 64 عنزة تم تنظيم الشبق لها بواسطة الحقن بالاسترواميت . تم تلقيح الماعز (داخل الرحم مقارنة بداخل المهبل) بجرعة سائل منوى (100 مقابل 200 × 10 ^{6}) بعد 48 أو 52 ساعة من حقن الاسترواميت. أوضحت النتائج ان اعلى نسبة مئوية لحركة الحيوان المنوى ($P<0.05$) بعد الاسالة للسائل المنوى المخفف بمخفف الترس محتويا على 2.5% صفار بيض كانت (54.8%) وعند معدل تخفيف 1: 5 (54.5%) وكانت اعلى نسبة حركة للحيوانات المنوية بعد الاسالة خلال فصل الخريف ، على وجة الخصوص فى شهر نوفمبر وكانت اقل قيمة خلال فصل الصيف (يونيو-اغسطس). كان اعلى معدل خصوبة ($P<0.05$) للماعز الملقحة داخل الرحم بجرعة سائل منوى عالية بعد 48 ساعة من حقن الاسترواميت (62.5%) بينما كانت اقل قيمة لمعدل الخصوبة سجلت للماعز الكنترول (43.75%) . الماعز الملقحة داخل الرحم بجرعة سائل منوى عالية بعد 52 ساعة من حقن الاسترواميت كانت لها معدلات خصوبة متوسطة 50.5 ، 56.25% على الترتيب.

اوضحت النتائج ان تخفيف السائل المنوى للتيوس بمخفف الترس محتويا على 2.5% صفار بيض وبمعدل تخفيف 1: 5 ومجمد فى شكل كريات (حبيبات) يكون اكثر كفاءة فى الحفاظ على حيوية عالية للحيوات المنوية بعد الاسالة ، وان افضل فصول السنة لجمع السائل المنوى للماعز لتجميدة هو فصل الخريف ، وان التلقيح داخل الرحم بجرعة عالية من الحيوانات المنوية (200 × 10 ^{6}) يمكن ان تعطى نتيجة مقبولة من معدلات الخصوبة لاناث الماعز.

قام بتحكيم البحث

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