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# CAMEL SEMEN QUALITY AND ENZYMATIC ACTIVITY OF SPERMATOZOA UNDER REFREGERATION CONDITIONS

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## ABSTRACT

The camel is an important livestock species that can uniquely adapted to hot and arid environments. Success of Artificial Insemination (AI) which in turn is dependent on the quality of semen obtained and its capacity for dilution and storage with minimum loss of fertilizing ability. The aim of the current work was to investigate the effects of varying ages and storage time on male Maghrebi camel's spermatozoa parameters during refrigeration at 5ºC for three days. Semen was collected using an artificial vagina (AV), which was then extended using lactose-yolk-citrate extender (LYC) then evaluated for motility, viability, morphology, DNA integrity and spermatozoa's in vitro response and cervical mucus penetration occurrence. The results showed that both age or storage time resulted in a reduction (P<0.01) in total motile spermatozoa. The highest sperm quality was recorded on the first days of storage at 5°C During three days of refrigerated storage at 5ºC, the proportions of dead, aberrant, acrosome damaged, and chromatin damaged spermatozoa increased (P<0.01) in all animals ages. At camel aged 4–9 and 9–14 years, the seminal plasma's AST and ALT enzyme activity had decreased (P<0.01). However, pH levels remained relatively unaffected at different ages. During incubation at 37ºC for up to 4 hours, spermatozoa at ages 4 to 14 and 9 to 14 years had a greater (P<0.01) capacity to permeate cervical mucus than those at ages 14 to 19. In conclusion, varying ages of Maghrebi camels and duration of storage times resulted in a reduction in spermatozoa motility.

***Keywords:*** Camels, Ages, Storage time, Semen quality, Penetration score.

## INTRODUCTION

The camel (Camelus dromedarius) is a notable livestock animal that is uniquely adapted to thrive in hot, arid conditions. In Egypt, common camel breeds include the Sudani, Maghrebi, Fellahi, and Al-Mowalled camel breeds. Al-Sudani and Al-Maghrebi breeds were bred for their meat and milk, Al-Fellahi breed is dominant in the Nile delta region but does not flourish in desert conditions, and Al-Mowalled breed is much better suited as farm and desert animals (Wilson, 1997).

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Creating a management strategy that optimizes reproductive efficiency requires an understanding of the camel's reproductive biology (Zeidan *et al*., 2001). Achieving high reproductive performance depends critically on the effectiveness of artificial insemination (AI) and its capacity to dilute and store with little loss of fertilizing potential (Tibaryand Anouassi 1997). When chilled, spermatozoa usually survived a few days (2–5 degrees Celsius). However, one day of storage does not usually produce sufficient reproductive results in dromedary camels (Zeidan *et al*., 2008 and Matter 2019).

The rutting season, which varies slightly by breed and location, starts around the age of five or six years in nomadic herds of male dromedary camels and lasts until fourteen or fifteen of age (El-Wishy 1988) Reproductive management of female parameters at first service can affect the rate of conception, the rate of calving, and the interval between successive calvings (Khanna 1993).

This study was conducted to investigate the effects of varying male Maghrebi camel ages and storage time on spermatozoa parameters while they are refrigerated at 5ºC for three days. The Animal Production Research Institute, Ministry of Agriculture, Egypt, authorized the Guidelines for the Care and Use of Animals, which were followed in all trials (Code number: 331429).

## MATERIALS AND METHODS

**1. Experimental animals:**
Nine male Camels (Camelus dromedarius) weighing 600–700 kg were used; they were classified according to age into three groups (4-9 Y, n=3), (9-14 Y, n=3) and (14-19 Y, n=3). All of the camels in the herd were healthy, had an excellent reproductive history, and were clinically free of external and internal parasites.

**2. Management and feeding:**
Throughout the study, fresh water was always accessible, and the camels were fed in compliance with (Banerjee 1988). There was a concrete floor with a covered water basin and a communal feeding trough in the yard where the camels were housed.

**Methods:**
**1. Semen collection:**
Using artificial vagina, seven semen ejaculates were collected from each male camel during rutting season between 8:00 to 9:00 a.m. (Zeidan 2002).

**2. Semen extension.**
Male camel semen samples were collected then pooled each group, and extended using lactose-yolk-citrate (LYC) (Zhang *et al*., 2002) with a dilution rate of 1 ml semen: 3 ml extender (Musa *et al*., 1992)

**3. Evaluation of cooling semen quality:**
**3.1. Sperm motility (%).**
High power magnification (X40) was used to calculate the percentage of sperm motility (Agarawal *et al*., 2004).

**3.2. Hydrogen-ion concentration (pH).**
Seminal pH value was measured according to the method of (Karras 1952).

**3.3. Dead spermatozoa (%)**
The percentage of dead spermatozoa was assayed according to (Hackett and Macpherson 1965).

**3.4. Abnormal spermatozoa (%)**
The morphology of aberrant spermatozoa was assessed according to (Watson 1975).

**3.5. Acrosome integrity of sperm (%)**
Assessment of the percentage of acrosome damage was done according to (Zedain *et al*., 2007)

**3.6. Chromatin damage (%)**
300 sperm cells in each smear were evaluated in order to quantify the amount of chromatin damage (Erenpreiss *et al*., 2004).

**4. Biochemical analysis of seminal plasma.**

Semen samples were cooled at 5ºC for 0, 1, 2, and 3 days as storage period and after each storage period the cooled semen was centrifuged for 15 minutes at 8000 rpm. Using the procedure outlined by (Reitman and Frankle 1957), seminal plasma was isolated and kept at -20ºC until the aspartate-amino transaminase (AST) and alanine-amino transaminase (ALT) enzymes were measured.

**5. Sperm penetration (score):**

A rank score was used to evaluate the penetration of sperm into the cervical mucus of she-camels at several ages (Hanson 1982).

**6. Statistical analysis**

SAS's General Linear Model (GLM) technique was used to statistically analyze the data (SAS 2006). Significant variations between means were found using Duncan's multiple range test (Duncan 1955). Prior to statistical analysis, percentage data were converted to arc-sin values. The Chi-square test was used to analyze the penetration score.

## RESULTS

**1. Camel semen quality during chilled storage at 5ºC.**

Table (1) shows a reduction (P<0.01) in motile spermatozoa extended with LYC extender. With varying camel ages, the change in seminal pH (table2) was negligible. Acrosome damage (Table 5), chromatin damage (Table 6), dead spermatozoa, aberrant spermatozoa (Table 4), AST enzymes (Table 7), and ALT enzyme (Table 8) all shown substantial increases (P<0.01) at various ages.

The proportion of motile spermatozoa was significantly (P<0.01) reduced as storage duration at 5ºC increased (Table 1). However, there was an increase (P<0.05) in the percentage of dead spermatozoa, aberrant spermatozoa, acrosome damage, chromatin damage, AST, and ALT (Tables 3,4,5,6,7, and 8).

Additionally, the seminal pH concentration declined insignificantly (P<0.01) when the age of camel prolonged, while it significantly affected by various stored periods.

**2. Penetrating ability of the camel spermatozoa**

The penetrating mucus test evaluation of the extended camel spermatozoa at different ages was affected significantly (P<0.01) Figure 1. With varying incubation times at 37ºC and ages, the penetration rate into she-camel cervical mucus was significantly (P<0.01) lower when the incubation time was extended up to 4 hours at 37ºC.

**Table 1:** Influence of different camel ages on the percentage of motile spermatozoa during chilled storage at 5ºC for 3 days (Means ± SE).

| chilled storage (day) | Age (years) | | | Mean |
|---|---|---|---|---|
| | 4 – 9 | 9 - 14 | 14 - 19 | |
| 0 | 67.25±0.86 | 65.11±0.82 | 61.94±0.68 | 64.76±0.80$^{A}$ |
| 1 | 58.17±0.74 | 58.05±0.73 | 50.82±0.61 | 55.68±0.73$^{B}$ |
| 2 | 41.72±0.52 | 40.14±0.50 | 36.17±0.49 | 39.34±0.48$^{C}$ |
| 3 | 18.91±0.14 | 16.20±0.12 | 11.36±0.10 | 15.49±0.017$^{D}$ |
| Mean | 46.51±0.81$^{a}$ | 44.87±0.80$^{a}$ | 40.07±0.73$^{b}$ | 43.81 |

a-b Values with different superscripts within a row are significantly different (P<0.01).
A-D Values with different superscripts within a column are significantly different (P<0.01).

**Table 2:** Influence of different camel ages on spermatozoa's seminal hydrogen ion during chilled storage at 5ºC for 3 days (Means ± SE).

| chilled storage (day) | Age (years) | | | Mean |
|---|---|---|---|---|
| | 4 – 9 | 9 - 14 | 14 - 19 | |
| 0 | 7.86±0.08 | 7.63±0.09 | 7.98±0.36 | 7.82±0.08$^{A}$ |
| 1 | 6.53±0.06 | 6.27±0.08 | 6.30±0.30 | 6.36±0.07$^{B}$ |
| 2 | 6.81±0.05 | 6.75±0.08 | 5.62±0.04 | 6.39±0.06$^{B}$ |
| 3 | 3.72±0.04 | 4.69±0.05 | 4.83±0.03 | 4.41±0.03$^{C}$ |
| Mean | 6.23±0.03 $^{a}$ | 6.33±0.08$^{a}$ | 6.18±0.07$^{a}$ | 6.24 |

a-b Values with different superscripts within a row are significantly different (P<0.01).
A-D Values with different superscripts within a column are significantly different (P<0.01).

**Table 3:** Influence of different camel ages on dead spermatozoa during chilled storage at 5ºC for 3 days (Means ± SE).

| chilled storage (day) | Age (years) | | | Mean |
|---|---|---|---|---|
| | 4 – 9 | 9 - 14 | 14 - 19 | |
| 0 | 18.32±0.13 | 21.76±0.12 | 21.53±0.16 | 20.53±0.15$^{D}$ |
| 1 | 19.53±0.14 | 23.11±0.16 | 30.56±0.18 | 24.40±0.21$^{C}$ |
| 2 | 37.31±0.18 | 41.60±0.18 | 48.18±0.24 | 42.53±0.38$^{B}$ |
| 3 | 58.16±0.25 | 65.42±0.31 | 72.85±0.35 | 65.49±0.75$^{A}$ |
| Mean | 33.45±0.27$^{c}$ | 37.98±0.21$^{b}$ | 43.28±0.82$^{a}$ | 38.23 |

a-b Values with different superscripts within a row are significantly different (P<0.01).
A-D Values with different superscripts within a column are significantly different (P<0.01).

**Table 4:** Influence of different camel ages on aberrant spermatozoa during chilled storage at 5ºC for 3 days (Means ± SE).

| chilled storage (day) | Age (years) | | | Mean |
|---|---|---|---|---|
| | 4 – 9 | 9 - 14 | 14 - 19 | |
| 0 | 9.15±0.13 | 9.13±0.14 | 10.46±0.19 | 9.58±0.12$^{D}$ |
| 1 | 10.17±0.14 | 11.24±0.18 | 11.63±0.23 | 11.01±0.15$^{C}$ |
| 2 | 12.43±0.18 | 13.06±0.23 | 16.25±0.27 | 13.91±0.19$^{B}$ |
| 3 | 18.32±0.21 | 20.17±0.27 | 21.84±0.35 | 20.11±0.27$^{A}$ |
| Mean | 12.51±0.11$^{c}$ | 13.40±0.20$^{b}$ | 15.04±0.23$^{a}$ | 13.65 |

a-b Values with different superscripts within a row are significantly different (P<0.01).
A-D Values with different superscripts within a column are significantly different (P<0.01).

**Table 5:** Influence of different camel ages on percentage of spermatozoa acrosome damage during chilled storage at 5ºC for 3 days (Means ± SE).

| chilled storage (day) | Age (years) | | | Mean |
|---|---|---|---|---|
| | 4 – 9 | 9 - 14 | 14 - 19 | |
| 0 | 3.15±0.04 | 4.13±0.14 | 5.26±0.17 | 4.18±0.05$^{D}$ |
| 1 | 3.40±0.07 | 4.72±0.16 | 5.85±0.21 | 4.65±0.08$^{C}$ |
| 2 | 4.16±0.14 | 5.18±0.18 | 6.71±0.23 | 5.17±0.19$^{B}$ |
| 3 | 5.07±0.18 | 6.14±0.22 | 7.92±0.38 | 6.58±0.27$^{A}$ |
| Mean | 3.94±0.06$^{c}$ | 5.04±0.10$^{b}$ | 6.48±0.15$^{a}$ | 5.14 |

a-b Values with different superscripts within a row are significantly different (P<0.01).
A-D Values with different superscripts within a column are significantly different (P<0.01).

**Table 6:** Influence of different camel ages on percentage of spermatozoa chromatin degradation during chilled storage at 5ºC for 3 days (Means ± SE).

| chilled storage (day) | Age (years) | | | Mean |
|---|---|---|---|---|
| | 4 – 9 | 9 - 14 | 14 - 19 | |
| 0 | 0.68±0.04 | 1.32±0.02 | 2.46±0.03 | 1.48±0.01$^{B}$ |
| 1 | 1.05±0.03 | 1.54±0.06 | 2.61±0.03 | 1.73±0.05$^{B}$ |
| 2 | 1.20±0.07 | 1.87±0.08 | 2.90±0.06 | 1.99±0.06$^{AB}$ |
| 3 | 1.43±0.09 | 2.96±0.10 | 3.28±0.11 | 2.55±0.07$^{A}$ |
| Mean | 1.09±0.02$^{c}$ | 1.92±0.05$^{b}$ | 2.81±007$^{a}$ | 1.94 |

a-b Values with different superscripts within a row are significantly different (P<0.01).
A-D Values with different superscripts within a column are significantly different (P<0.01).

**Table 7:** Influence of different camel ages on percentage of aspartate-aminotrans aminase enzyme activity (U/L) during chilled storage at 5ºC for 3 days (Means ± SE).

| chilled storage(day) | Age (years) | | | Mean |
|---|---|---|---|---|
| | 4 – 9 | 9 - 14 | 14 - 19 | |
| 0 | 26.85±0.42 | 27.11±0.46 | 29.52±0.53 | 27.76±0.40D |
| 1 | 27.16±0.48 | 30.86±0.53 | 36.18±0.61 | 31.40±0.56C |
| 2 | 38.62±0.67 | 41.90±0.72 | 50.74±0.85 | 43.75±0.83B |
| 3 | 49.52±0.96 | 53.45±1.13 | 62.57±1.25 | 55.18±1.18A |
| Mean | 35.53±0.68b | 38.33±0.70b | 44.70±0.86a | 39.52 |

a-b Values with different superscripts within a row are significantly different (P<0.01).
A-D Values with different superscripts within a column are significantly different (P<0.01).

**Table 8:** Influence of different camel ages on percentage of alanine-aminotrans aminase enzyme activity (U/L) during chilled storage at 5ºC for 3 days (Means ± SE).

| chilled storage(day) | Age (years) | | | Mean |
|---|---|---|---|---|
| | 4 – 9 | 9 - 14 | 14 - 19 | |
| 0 | 39.46±0.29 | 43.60±0.46 | 45.18±0.52 | 42.74±0.46D |
| 1 | 43.62±0.38 | 45.17±0.50 | 48.23±0.53 | 45.67±0.54C |
| 2 | 49.75±0.63 | 50.23±0.78 | 53.17±0.82 | 51.05±0.81B |
| 3 | 57.28±0.92 | 60.75±1.14 | 68.56±1.12 | 62.19±1.23A |
| Mean | 47.52±0.58b | 49.93±0.61b | 53.78±0.84a | 50.41 |

a-b Values with different superscripts within a row are significantly different (P<0.01).
A-D Values with different superscripts within a column are significantly different (P<0.01).

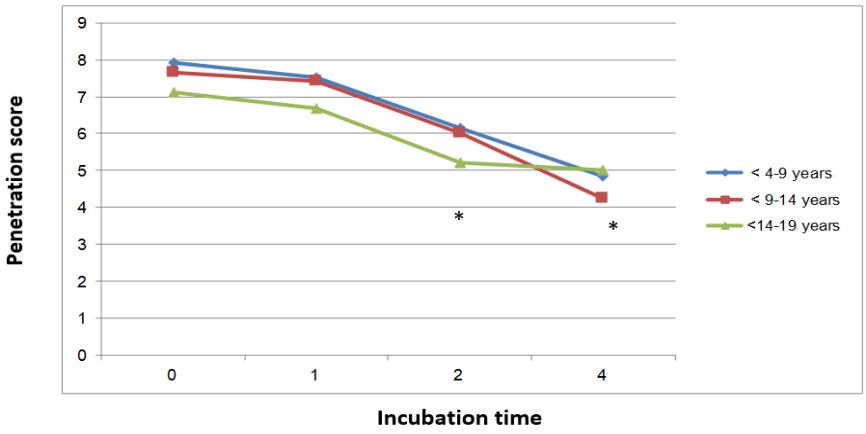


**Figure 1:** Shows the penetration score values of male dromedary camel spermatozoa into the cervical mucus of she-camel of varying ages after up to 4 hours of incubation at 37ºC. substantial (P<0.01) difference from control at the same period.

## DISCUSSION

In the world's arid regions, the camel is an essential socioeconomic resource that sustains millions of people. For many pastoralists, camel milk is their only source of nutrition. In times of extreme drought, the camel has shown to be the most resilient domestic animal, not only surviving but also maintaining high levels of productivity and reproduction under heat stress environmental condition (Wardeh 1989).

In India, the dromedary camel's testes exhibit seasonal fluctuations, with a high concentration of active spermatozoa in the winter and a relative lack of activity during the summer (Singh and Bharadwaj 1978). Camels under Egyptian condition showed similar patterns (Zeidan *et al*., 2001 and Matter 2019). variations in sperm production and motility contribute to corresponding oscillations in sexual activity and spermatozoa numbers.

Even after just one day of camel semen storage, good reproductive outcomes are not always obtained (Zeidan *et al*., 2001 and Matter 2019).

In the current study, the proportion of motile camel spermatozoa in the current study was significantly higher ($P<0.01$), particularly for camels aged 4 to 9 years. However, the dromedary camels aged 9 to 14 followed by 14 to 19 years had significantly higher ($P<0.01$) percentages of dead spermatozoa, abnormal sperm-atozoa, acrosome damage of spermatozoa, chromatin damage of spermatozoa, and aspartate (AST) and alanine aminotrans aminase (ALT) enzymes (IU/L). Further-more, the concentration of seminal hydrogen ions (pH) was negligible. In dromedary camel a study (El-Mahdy 2019) discovered that the spermatozoa's chromatin damage percentage was consid-erable ($P<0.01$). Numerous variations were found in spermatozoa's damaged, Deoxyribonucleic Acid (DNA) negatively impact the spermatogenesis process, apoptosis, and reactive oxygen species (Baiee *et al.,* 2017 and Ahmadi, 2020).

Semen quality was significantly ($P<0.01$) better in the dromedary camels aged 4 to 9 or 9 to 14 years in the current study, consistent with previous findings (Zeidan *et al*., 2001 and Matter 2019). This improvement may be attributed to the increased activity of antioxidant enzymes such as glutathione peroxidase, which is present in the seminal plasma of rams, goats, bulls, and humans, (Zhang *et al*., 2002).

The percentages of dead, aberrant, and chromatin-damaged camel spermatozoa at ages 4 to 9 years were considerably ($P<0.01$) lower in this study than those at ages 9 to 14 or 14 to 19. The reduction of necrotic and apoptotic spermatozoa may be the cause of the dromedary camel's high semen quality between the ages of 4 and 9 years. It is noteworthy that camels under the age of 14 to 19 years had a negative impact on viable sperm and a higher number of apoptotic and neurotic sperm (Khalil *et al*., 2018).

Additionally, spermatozoa stored at low temperatures exhibited structural damage to the acrosome, possibly as a result of cold shock. A decrease in the percentage of spermatozoa with an intact acrosome attribute with increase in the release of enzymes into the extracellular media accompanies these alterations (Zeidan *et al*., 2001). However, the decline in adenosine triphosphate, which activates the dromedary camels' capacity to resynthesize spermatozoa linked to fructolysis (Zeidan *et al*., 2001, El-Mahdy 20019 and Matter 2019), may be the cause of the decline in semen quality with camel age increases.

According to our findings, the proportion of motile spermatozoa was reduced ($P<0.05$) when storage duration was extended to three days at 5ºC. However,

the percentages of chromatin damage, acrosome damage, dead spermatozoa, and aberrant spermatozoa were considerably (P<0.01) higher in the extended camel spermatozoa with LYC diluter at various ages. These outcomes could be caused by an increase in metabolic rate, which could raise lactic acid levels, decrease glutathione peroxidase activity, which would then raise lipid peroxidase levels and ultimately result in poor semen quality (Zeidan *et al*., 2001), or decrease protein synthesis while increasing protein degradation (Khalil *et al.,* 2018).

Similar to what was shown in dromedary camels by (Zeidan *et al*., 2007), the aging of camels can lead to a loss in fertility and an increase in acrosome and chromatin damage of spermatozoa. The extension of the motile camel spermatozoa was likewise significantly (P<0.01) reduced with age. The concentration of seminal hydrogen ions, was, decreased negligibly. Similar results in dromedary camels were reported by (Zeidan *et al*., 2008).

## CONCLUSION

During storage at 5ºC, camel spermatozoa aged 4 to 9 or 9 to 14 years showed improvements in semen quality and penetrating capacity. These findings suggest that camel age is positively associated with reproductive efficiency and, in turn, dromedary camel fertility rates. Lastly, the first day of storage at 5°C resulted in noticeably better sperm quality.

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# جودة السائل المنوي للجمال والنشاط الانزيمى اثناء الحفظ بالتبريد

***علاء السيد زيدان بلاسي ، حسن السيد عبده المتولي ، احمد موسي عامر ،***
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تعتبر الجمال اهم انواع الماشبة الفريدة فى التاقلم مع ظروف البيئية الصحراوية كما ان نجاح عملية التلقيح الصناعى تعتمد على جودة السائل المنوى وقابليته للتخفيف والحفظ دون فقد فى قدرته على التخصيب. ولقد اجريت هذه الدراسة لمعرفة تاثير عمر الجمال على جودة السائل المنوى اثناء الحفظ بالتيريد لاستخدامه فى التلقيح الصناعى حيث تمت دراسة تأثير المراحل العمرية المختلفة لذكر الإبل المغربي على الحيوانات المنوية أثناء تخزينها عند درجة حرارة ٥ مئوية لمدة ٣ أيام. ولقد تم جمع السائل المنوي بواسطة المهبل الاصطناعي (AV)، وتم تخفيفه باستخدام مخفف اللاكتوز والصفار والسيترات (LYC) وتقييمه. تم تقدير الاستجابة المختبرية للحيوانات المنوية وقدرتها على اختراق مخاط عنق الرحم. أوضحت النتائج المتحصل عليها أن النسبة المئوية لحركة الحيوانات المنوية في الجمال انخفضت معنويا ($P<0.01$) بزيادة المرحلة العمرية وكذلك زيادة مدة التخزين. بينما كانت نسبة الحيوانات الميتة وغير الطبيعية وتلف الاكروسوم والكروماتين تزداد زيادة معنوية ($P<0.01$) مع التقدم في العمر وفى فترات التخزين عند درجة حرارة ٥ مئوية لمدة ٣ أيام. بالإضافة إلى انخفاضاً معنوياً ($P<0.01$) فى نشاط إنزيمات (AST) و(ALT) في البلازما المنوية في الجمال عند اعمار من ٩ إلى ١٤ ومن ١٤ إلى ١٩ سنَّة. اما بالنسبة لتركيز أيون الهيدروجين في البلازما المنوية (PH) للجمال كان غير معنوى بالنسبة للاعمار المختلفة. وبالنسبة لقدرة الحيوانات المنوية على اختراق مخاط عنق الرحم كانت المجموعتين من ٤ إلى ٩ ومن ٩ إلى ١٤عامًا أفضل معنويا ($P <0.01$) عن المجموعة من ١٤ إلى ١٩ عامًا بعد التحضين علي ٣٧ درجة مئوية لمدة ٤ساعات. لقد تبين من هذه الدراسة أن الجمال التي كانت أعمارها من ٤ إلى ٩ ومن ٩ إلى ١٤ سنة كانت أفضل في جودة السائل المنوي المحفوظ بالتبريد عند درجة ٥ درجة مئوية وكذلك قدرتها علىى اختراق مخاط عنق الرحم مقارنة بعمر من ١٤ إلى ١٩ سنة وكذلك قدرة السائل المنوى بالاحتفاظ بجودته عند تبريده لدرجة ٥ درجة مئويه لمدة لا تزيد عن يوم .