SOME STUDIES TO IMPROVE QUALITY AND FERTILIZING ABILITY OF COOLED BUFFALO SPERMATOZOA

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SUMMARY

This work aimed to assess the influence of removal of seminal plasma (RSP) and caffeine supplementation upon quality and fertilizing ability of Egyptian buffalo-bulls' spermatozoa post 6 days of hypothermic preservation at 5ºC. Semen samples were collected from five buffalo-bulls pooled, then separated into two main parts. First part was centrifuged (washed) twice to remove the seminal plasma, then the sperm plugs were re-suspended in camel skim milk extender to a volume equal to that of semen pre-washing. Second part was non-centrifuged (unwashed). Both washed and unwashed semen were diluted (1:4) with camel skim milk extender containing 10% egg yolk supplemented with caffeine at different concentrations (0,5,10 and 15 mM) and gradually cooled to 5°C then preserved at this temperature for 6 days. Data elaborated that RSP enhanced (P<0.01) sperm motility, head-to-head agglutinated spermatozoa and conception rate percentages, while dead, abnormalities and acrosomal damage percentages of spermatozoa , as well as, leakage of ALT, AST, hyaluronidase and ALP enzymes to extracellular fluids of spermatozoa were reduced (P<0.01) post-RSP . The best (P<0.01) semen characteristics, enzymatic activities and fertilizing efficiency of buffalo-bulls' spermatozoa were registered with washed diluted buffalo-bulls semen supplemented with 10 mM caffeine. Conclusively, removal of seminal plasma is a simple, effective, and easily practicable technique, which supplies a helpful alternative to semen preserved in liquid status, especially when administrated with 10mM caffeine for optimizing quality and fertilizing efficiency of buffalo spermatozoa that can be useful for artificial insemination in Egyptian buffaloes.

***Keywords**: Buffalo semen, Caffeine, Washed semen, Conception rate.*

INTRODUCTION

The improvement of breeding practices in buffalos has not received enough attention despite its importance for the production of meat, milk and leather (Sansone *et al*., 2000). Recent studies indicated that buffalo-bulls' spermatozoa can be preserved just like bovine spermatozoa, but there is limited information on its semen characteristics (El-Nagar, 2021 and Chavda *et al.*, 2022). Unfortunately, buffalo-bulls' spermatozoa are more susceptible to hazards during freezing than cattle spermatozoa (Sansone *et al*., 2000 and Chaudhari *et al*., 2015), which minimize its suitability in the field due to the unsatisfactory resulted conception rates (El-Sisy *et al*., 2016). This can be controlled if sperm quality is able to be well maintained in the liquid state for several days using appropriate diluting media (Kumar *et al*., 1992 and Cheng *et al*., 2022). Some studies proposed that hypothermic preservation can be a substitute for cryopreservation to overcome the possible severe cryoinjury (Bahgat, 2008 and Pini *et al*., 2018). The most effective hypothermic preservation strategies are the replacement of seminal plasma with extenders and temperature-induced metabolic restriction (Cheng *et al*., 2022). Also, hypothermic preservation of semen is technically easier than freezing and its shipment is cost-effective (Singh *et al*., 2012).

Some inhibitory factors in seminal plasma, which secreted from the accessory organs of the male reproductive tract, cause an apparent reduction in glucose uptake by spermatozoa (Zeidan *et al.*, 2008). It has been reported that decapacitation factors obtained from the seminal plasma of different species can inhibit corona penetrating enzyme which is an acrosomal enzymes involved in the passage of fertilizing spermatozoa through the corona radiate surrounding the ovum (Flipse, 1954 and Ahmad *et al*., 1996).

Therefore, RSP is a practical, simple and effective technique that provides a useful alterative for storage of cooled liquid semen (El-Kishk, 2003 , EL-Harairy *et al*., 2006 and Bahgat, 2008) or frozen states (Goyal *et al*., 1996 and Daramola, 2017). It was found that RSP enhanced the percentage of sperm motility, grade of the progressive motility in buffaloes (EL-Harairy *et al*., 2006). Also, RSP by washing the goat spermatozoa prior to extension improved the survival of cells during preservation (Roca *et al*., 1997, Zeidan *et al*., 2008 and Daramola, 2017).

Interestingly, supplementation of theophylline and/or caffeine to semen extender markedly improved respiration, motility and viability of goat, rabbit, buffalo and bovine spermatozoa (Sinha *et al*., 1995; López and Alvariño, 2000; Shukla and Misra, 2014 and Barakat *et al*., 2015). Addition of caffeine to semen extender was reported to be more effective in maintaining sperm motility and membrane stability as well as increasing penetrating ability of spermatozoa into cervical mucus (Aitken *et al*., 1983, Abd El-Kariem, 2004 and Barakat *et al*., 2015). This work aimed to investigate the impact of RSP and addition of various levels of caffeine upon semen characteristics, and enzymatic activities of buffalo-bulls spermatozoa post 6 days of hypothermic preservation at 5°C. Also, the conception rate following artificial insemination was assessed.

MATERIAL AND METHODS

Bulls and feeding:

Five healthy Egyptian buffalo bulls at 3 - 4 years old were used in this study. Bulls were clinically free of diseases and internal or external parasites with a sound history of fertility in the herd. Examination of the external genitalia demonstrated that the bulls were typically normal. Animals were housed individually under semi-open sheds and were individually fed rice straw and concentrate feed mixture according to (National Research Council, 2001). Dietary allowances were given individually to bulls twice daily at 08.00 a.m. and 14.00 p.m., while clean and fresh water and minerals block were available all day times. Proximate analysis of feedstuff was done according to (Cunniff, 1995). Table 1 is showing display the proximate nutrients composition of the concentrate feed and rice straw.

Table (1): Chemical analysis of concentrate feed and rice straw.

Item	Concentrate feed mixture	Rice straw
Chemical composition (% on DM basis):		
Dry matter, DM	92.06	89.95
Organic matter, OM	92.10	83.81
Crude protein, CP	16.33	3.40
Crude fiber, CF	16.22	34.90
Ether extract, EE	4.15	1.40
Nitrogen free extract, NFE	55.40	44.11
Ash	7.90	16.19

Semen collection:

Semen samples were collected once weekly between 07.00 - 08.00 a.m. by means of an artificial vagina (IMV, France) maintained at appropriate temperature. Two successive ejaculated were taken from each bull and just incubated in a water bath at 37°C till examination. Only semen samples with ≥ 70% sperm motility were used for further processing.

Semen extension and preservation:

Semen samples were pooled, then separated into two main equal parts. The first part was washed by centrifugation two times at 1000 X g for 15 minutes at room temperature (Ahmad *et al*., 1996). After RSP the sperm plugs were re-suspended in camel skim milk extender (CSM) to a volume equal to that of semen pre-washing. The second part was left without centrifugation (un-washed). Both washed and unwashed semen were diluted (1:4, v/v) with camel skim milk extender containing 10% egg yolk (Table 2). Both washed and un washed diluted semen were administrated with various levels of caffeine (0,5,10

and 15 mM) and gradually cooled to 5°C in a refrigerator over 2 hours, and thereafter preserved at this temperature for 6 days.

Table (2): Ingredients of buffered yolk extender used in semen dilution.

Ingredients	Camel skim milk extender (CSM)
Citric acid anhydrous (gm)	0.04
Sodium citrate dehydrate (gm)	2.90
Egg yolk (ml)	10.00
Camel skim milk (ml)	10.00
Streptomycin sulphate (mg/ml)	500
Penicillin (Iu/ml)	500
Distilled water/ml up to	100

Semen evaluation:

Semen characteristics and enzymatic activities were estimated after 6 days of hypothermic preservation at 5°C. Sperm motility, sperm abnormalities, dead spermatozoa percentages were measured (Salisbury *et al*., 1978). Also, the percentage of head-to-head agglutinated spermatozoa was performed as described by (Harayama *et al*., 2000). Acrosomal damage of spermatozoa was determined using a Giemsa stain procedure as described by (Watson, 1975) and inspected by a phase contrast microscope. Activity of alkaline phosphatase (ALP), alanine (ALT) and aspartate (AST) transaminases enzymes was measured colorimetrically (Reitman and Frankel, 1957). The activity of hyaluroindase enzyme was estimated post suitable dilution with distilled water according to (Foulkes and Watson, 1975). Based on the result of semen evaluation, 10mM caffeine was selected as the optimal concentration for further experiment.

Conception rate:

Total of 100 normally cyclic lactating Egyptian buffaloes (in private farms) were randomly allocated into four groups (25/each) as follows: (1) the control group, which was inseminated with unwashed semen without caffeine, (2) buffaloes were inseminated with washed semen without caffeine, (3) buffaloes were inseminated with unwashed semen administrated with 10mM caffeine, and (4) buffaloes were inseminated with washed semen administrated with 10mM caffeine. Each buffalo-cow in normal estrous and heat was injected intramuscularly with 10 μg buserelin (2.5 ml of Receptal, MSD Animal Health, New Cairo, Egypt) to induce ovulation and immediately inseminated. Buffalo-cows received a second insemination after 10-12 hours interval, with 0.5 ml of cooled liquid semen, by recto-vaginal insemination technique according to (Salisbury *et al*., 1978). All inseminations were carried out by the same inseminator. Conception rate was assessed on the basis of pregnancy diagnosis by rectal palpation post 45 days from the day of insemination.

Statistical analysis:

All data were analyzed using the SPSS software (IBM, SPSS Statistics, Version 22, USA). The data were expressed as mean ± standard error of means (SEM). Two-way ANOVA was used to compare the effect RSP and caffeine supplementation upon semen characteristics and enzymatic activities. Duncan Multiple Range test (Duncan, 1955) was used to differentiate between significant means at $P < 0.05$. The percentage values were transferred into arc-sing prior being analysis and computed as means. The conception rates were analyzed using Chi-square test.

RESULTS AND DISCUSSION

Impact of seminal plasma removal and/or caffeine supplementation on semen quality:

The results obtained in Table 3 indicated that treatment of buffalo-bulls semen with different concentrations of caffeine, especially with 10mM caffeine significantly ($P<0.01$) increased the percentages of sperm motility and head-to-head agglutinated spermatozoa, and depressed ($P<0.01$) the percentages of dead, abnormalities and acrosomal damage of spermatozoa. Additionally, RSP

significantly (P<0.01) improved the percentages of sperm motility and head-to-head agglutinated spermatozoa, and depressed (P<0.01) the percentages of dead, abnormalities and acrosomal damage of spermatozoa. Generally, the optimal (P<0.01) percentages of sperm motility, dead, abnormalities and acrosomal damage of spermatozoa, as well as, head-to-head agglutinated spermatozoa were observed with washed buffalo semen diluted with CSM extender, especially when supplemented with 10mM caffeine.

These findings agrees with previous reports indicated that RSP of buffalo semen improved the percentages of sperm motility, acrosomal integrity and live of spermatozoa (Ahmad *et al*., 1996, El-Kishk, 2003 and EL-Harairy *et al*., 2006) and the viability of buffalo spermatozoa was improved in the presence of half of the normal volume of seminal plasma (Ibrahim *et al*., 1981). The reason behind that may be due to washing of semen prior extension results in removal of high molecular weight fractions, which had adverse effects on semen quality during preservation, while low molecular weight components retained with spermatozoa (Ahmad *et al*., 1996 and Daramole, 2017). As high molecular weight fractions in seminal plasma was reported to reduce percentages of sperm motility, viability and absolute index of viability (Jasko *et al*., 1991 and Bahgat, 2008). In addition, the presence of phospholipase A, an egg yolk coagulating enzyme produced by the cowper's glands, in buffalo seminal plasma catalyzes the hydrolysis of lecithins in egg yolk to fatty acids and lysolecithins, which are harmful to spermatozoa in unwashed semen samples containing egg yolk (Roy, 1957, Roca *et al*., 1997, El-Kisk, 2003 and EL-Harairy *et al*., 2006).

Table (3): Some characteristics of washed and unwashed buffalo bull semen administrated with various levels of caffeine, post 6 days of hypothermic preservation at 5°C.

Characteristics of spermatozoa	Caffeine levels (mM/100ml)	Type of semen		Overall means
		Washed semen	Unwashed semen	
Motility (%)	0	50.00 ± 1.27	30.20 ± 2.88	40.10 ± 4.35^{c}
	5	59.00 ± 1.34	35.40 ± 3.02	47.20 ± 3.11^{b}
	10	65.00 ± 1.30	56.00 ± 1.27	60.50 ± 1.35^{a}
	15	50.60 ± 1.40	41.60 ± 1.35	46.10± 1.57^{b}
Overall means		56.15 ± 1.24^{A}	40.80 ± 2.70^{B}	48.48 ± 2.05
Dead spermatozoa (%)	0	40.60 ± 2.11	49.50 ± 2.50	45.05 ± 3.22^{a}
	5	32.50 ± 2.30	41.00 ±2.40	36.75 ± 2.11^{b}
	10	20.50 ± 0.60	29.30±1.20	24.90 ± 0.91^{c}
	15	30.80 ± 1.08	39.20± 2.20	35.00 ± 1.81^{b}
Overall means		31.10 ± 0.77^{B}	39.75 ± 1.85^{A}	35.43 ± 1.77
Abnormalities (%)	0	25.90 ± 2.81	32.50 ±3.11	29.20 ±2.95^{a}
	5	17.60 ± 1.08	22.80 ±1.26	20.20 ±1.88^{b}
	10	11.80 ± 0.31	17.50 ± 0.55	14.65 ±0.45^{c}
	15	20.40 ± 0.99	24.50 ± 1.05	22.45 ±1.95^{b}
Overall means		18.93 ± 0.63^{B}	24.33 ± 1.34^{A}	21.63 ± 0.99
Acrosmal damage (%)	0	20.70 ± 1.99	27.50 ± 2.23	24.10 ± 1.83^{a}
	5	11.50 ± 0.83	17.80 ± 0.91	14.65± 0.67^{b}
	10	6.40 ± 0.43	12.70 ± 0.67	9.55 ± 0.33^{c}
	15	13.10 ± 0.66	18.90 ± 0.78	16.00 ± 0.68^{b}
Overall means		12.93 ± 0.36^{B}	19.23 ± 1.93^{A}	16.08 ± 0.85
Head to Head agglutination (%)	0	51.40 ± 2.61	30.10 ± 3.15	40.75 ± 3.40^{c}
	5	60.60 ± 1.71	35.70 ± 2.80	48.15 ± 2.61^{b}
	10	69.50 ± 0.95	57.10 ± 1.31	63.30 ± 0.63^{a}
	15	51.00 ± 1.88	41.10 ± 2.11	46.05 ± 1.90^{b}
Overall means		58.1 ± 0.99^{A}	41.00 ± 2.88^{B}	49.56 ± 2.11

a-c Means within each column have various superscripts, differ significantly at (P<0.01) level.
A-B Means within each row have various superscripts, differ significantly at (P<0.01) level.

The improved sperm motility post addition of caffeine to extended semen may be related to the methylxanthines group which acts as a phosphodiesterase inhibitor. In line with our results, caffeine promoted the sperm's motility, vitality and enhanced fertilization and early in vitro development of mouse embryos (Nabavi *et al*., 2013). The mechanism by which caffeine improves motility of

spermatozoa through the inhibition of phosphodiesterase enzyme results in accumulation of cyclic adenosine monophosphate (cAMP) enhancing motility of spermatozoa through direct acting on the axoneme in the tail or indirectly acting on the plasma membrane as a secondary messenger (Schoenfeld *et al*., 1975 and Aitken *et al*., 1983).

Singh and Raina (2000) who found that enhanced intracellular cAMP concentrations led to activate glycogen phosphorylase and/or convert glycogen to simple sugars, hence improving sperm's motility. Shukla and Misra (2014) and Spalekova *et al.* (2014) reported that influence of caffeine on motility of spermatozoa may be related to species-specific and/or dose-dependent. Furthermore, Spalekova *et al.* (2014) and Hamid and Ibrahim (2019) in ram, Barakat *et al.* (2015) in bovine and Shukla and Misra (2014) and Chavda *et al.* (2022) in buffalo spermatozoa, recorded a significant improvement in sperm characteristics using caffeine as an additive to semen extender.

Impact of seminal plasma removal and/or caffeine supplementation on enzymatic activities:

As shown from Table 4, the addition of caffeine at different concentrations, especially with 10mM caffeine reduced (P<0.01) the release of transferees (ALT and AST), hyaluronidase and alkaline phosphatase (ALP) enzymes from intracellular to extracellular fluids of spermatozoa. In addition, unwashed buffalo-bulls' semen had higher (P<0.01) values of ALT, AST, hyaluronidase and ALP enzymes. Generally, lowest (P<0.01) leakage of ALT, AST, hyaluronidase and ALP enzymes into extercellular medium of spermatozoa were observed with washed buffalo semen diluted with CSM extender, especially when supplemented with 10 mM caffeine.

As expected, the values of ALT, AST, ALP and hyaluronidase enzymes were significantly reduced upon addition of caffeine due to the stabilizing impact of caffeine on sperm cellular membrane (Abd El-Kariem, 2004 and Shukla and Misra, 2014). Matching with our results, addition of caffeine to the diluted cooled buffalo bull spermatozoa decreased the amounts of AST, ALT and ALP enzymes released into the extracellular medium (Zeidan *et al*., 2006).

Table (4): Enzymatic activities (u/10^9 spermatozoa) of washed and unwashed buffalo bull semen administrated with various levels of caffeine, post 6 days of hypothermic preservation at 5°C.

Enzymatic activities	Caffeine levels (mM/100ml)	Type of semen		Overall means
		Washed semen	Unwashed semen	
Alanine aminotransferase (ALT)	**0**	52.20 ±2.11	60.60 ± 3.15	56.40 ± 4.93^{a}
	5	22.70 ±1.99	34.60 ±2.15	28.65 ±3.80^{b}
	10	11.40 ±0.81	26.30 ±1.51	18.85 ±1.04^{c}
	15	28.00 ± 1.71	39.10 ± 1.98	33.55 ± 2.80^{b}
Overall means		28.58 ± 1.81^{B}	40.15 ± 2.75^{A}	34.36 ± 2.95
Aspartate aminotransferase (AST)	**0**	74.60 ± 3.11	118.00 ± 5.15	96.30 ± 4.66^{a}
	5	37.90 ± 2.11	71.60 ± 4.05	54.75 ±3.11^{b}
	10	19.20 ± 1.87	33.10 ±3.03	26.15 ±2.16^{c}
	15	53.50 ± 1.77	79.00 ± 2.15	66.25 ± 3.77^{b}
Overall means		46.30 ± 1.03^{B}	75.43 ± 3.11^{A}	60.86 ± 3.11
Alkaline phosphatase (ALP)	**0**	111.30 ±4.11	143.60 ± 6.13	127.45 ±5.60^{a}
	5	55.40 ±2.71	85.20 ± 3.35	70.30 ± 2.18^{b}
	10	22.50 ±1.99	49.40 ±2.11	35.95 ±0.99^{c}
	15	60.40 ± 2.19	80.70 ± 2.80	70.55 ± 1.77^{b}
Overall means		62.40 ± 1.05^{B}	89.73 ± 2.99^{A}	76.06 ± 2.01
Hyaluronidase	**0**	46.50 ± 2.50	76.80 ±4.60	61.65 ± 4.99^{a}
	5	24.20 ±1.59	41.00 ±2.30	32.60 ±3.77^{b}
	10	12.40 ±0.99	32.00 ±1.98	22.20 ±1.05^{c}
	15	26.90 ± 1.81	45.20 ± 2.05	36.05 ± 2.99^{b}
Overall means		27.50 ± 1.77^{B}	48.75 ± 3.19^{A}	38.13 ± 1.99

a-c Means within each column have various superscripts, differ significantly at (P<0.01) level.
A-B Means within each row have various superscripts, differ significantly at (P<0.01) level.

Activities of glutathione-S-transferases (GSTs) were decreased (P<0.01) with all caffeine samples compared with the control sample (El-Kishk, 2003 and Chavda *et al.*, 2022). Results recorded that RSP reduced ALT, AST, ALP and hyaluronidase enzymes which may be related to the removal of the inhibitory factors presented in seminal plasma that causes the breakdown of sperm membrane and ease of enzymatic leakage from intracellular to extracellular fluids of spermatozoa (Sengupta *et al*., 1976, Zeidan *et al*., 2004, EL-Harairy *et al*., 2006, Bahgat, 2008 and Daramola, 2017).

Impact of seminal plasma removal and/or caffeine supplementation on conception rates of buffalo-cows:

Table 5 declared that supplementation of 10 mM caffeine to diluted-bulls' semen improved (P<0.01) the conception rate of buffaloes compared with non-supplementation of caffeine (88% vs. 60%, respectively) Furthermore, it is worth noting that the conception rate of buffaloes inseminated with washed semen was greater (P<0.01) compared with those inseminated with unwashed semen (82% vs. 66%, respectively). Generally, conception rate of the Egyptian buffaloes inseminated with washed semen administrated with 10mM caffeine was significantly (P<0.01) optimal, while it was significantly (P<0.01) the lowest with unwashed semen without caffeine (96% vs. 52%, respectively).

One of the most severe challenges facing spermatozoa is the fertilization of mature oocyte. Here we used the Egyptian buffaloes to clarify that treatment with caffeine improved the conception rate following insemination with buffalo spermatozoa preserved at 5°C in refrigerator for 6 days after treatment with caffeine. Early studies showed that the highest percentage of cumulus-intact ova was fertilized when caffeine was present in the fertilization medium (Dodds and Seidel Jr, 1983, Cai and Marik, 1989 and Nabavi *et al*., 2013). Other studies proofed that caffeine can enhance the fertilization rate of oocytes by increasing capability of spermatozoa (Homonnai *et al*., 1976 and Niwa and Ohgoda, 1988). High percent of spermatozoa with normal membrane and/or acrosome at the site of fertilization is necessary for optimizing the fertilizing capacity of spermatozoa (Spalekova *et al.*, 2014). Caffeine administration improved intracellular calcium levels leading to hyper-activation of ram spermatozoa (Colas *et al.*, 2010). In the current work, the grater conception rate of buffaloes inseminated with washed semen was may be attributed to RSP by washing spermatozoa prior to extension which enhanced sperm motility, survival rate and fertilizing capacity of spermatozoa during hypothermic preservation at 5°C (Martinus *et al*., 1991 and El-Kishk, 2003). In this context, close correlation between movement of human spermatozoa and its penetrating ability into cervical mucus was recorded (Aitken *et al*., 1983).

Table (5): Conception rates for Egyptian buffaloes inseminated with washed or unwashed buffalo bull semen with and without 10mM of caffeine.

Caffeine level (10mM)	Type of semen		Mean
	Washed semen	Unwashed semen	
Without	(17/25), 68%	(13/25), 52%	(30/50), 60%^{b}
With	(24/25), 96%	(20/25), 80%	(44/50), 88%^{a}
Mean	(41/50), 82%^{A}	(33/50), 66%^{B}	(74/100), 74%

a-b Means within each column have various superscripts, differ significantly at (P<0.01) level.
A-B Means within each row have various superscripts, differ significantly at (P<0.01) level.

CONCLUSION

Conclusively, removal of seminal plasma by centrifugation of buffalo-bull semen is a simple, inexpensive, effective, and easily practicable technique, which supplies a helpful alternative to semen preserved in liquid status, especially when administrated with 10mM caffeine for improving survival rate, viability, maintenance of enzymatic activities of spermatozoa as well as conception rate that can be useful for spreading the tool of artificial insemination programs in lactating Egyptian buffaloes, especially in those village or desert regions in Egypt where liquid nitrogen may not be available for long time freezing of semen.

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بعض الدراسات لتحسين الجودة والمقدرة الاخصابية للحيوانات المنوية المبردة من الطلائق الجاموسي

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تم تصميم هذه التجربة لدراسة تأثير نزع بلازما السائل المنوي و إضافة الكافيين بمستويات مختلفة علي خصائص السائل المنوي و النشاط الإنزيمي و المقدرة الإخصابية للحيوانات المنوية من طلائق الجاموس المصري وذلك بعد عملية الحفظ بالتبريد علي درجة حرارة 5ºم لمدة 6 أيام ، تم تجميع السائل المنوي من خمس طلائق جاموسي و قسمت العينات في كل مرة جمع إلي قسمين متساويين. القسم الأول تم نزع بلازما السائل المنوي منه بالطرد المركزي مرتين ، و القسم الثاني للمقارنة بدون نزع بلازما السائل المنوي ، ثم خفف كل من السائل المنوي المنزوع و غير المنزوع بمخفف لبن الابل الفرز المحتوي علي 10% صفار البيض ثم أضيف الكافيين بمستويات مختلفة (صفر ، 5 ، 10 ، 15 مللي مول) وتم الحفظ بالتبريد علي درجة حرارة 5ºم لمدة 6 أيام. أظهرت النتائج أن نزع بلازما السائل المنوي لطلائق الجاموس أدي إلي زيادة النسبة المئوية لحركة الحيوانات المنوية و الحيوانات المنوية ملتصقة الرأس و نسبة الخصوبة بينما أدي إلي إنخفاض النسبة المئوية للحيوانات المنوية الميتة و الغير طبيعية ذات الأكروسوم الغير سليم بدرجة معنوية (علي مستوى 0.01) بالاضافة إلي إنخفاض معدل إرتشاح كل من الإنزيمات الناقلة لمجموعة الأمين (ALT, AST) و الهيالورونوديز و الفوسفات القاعدي ALP إلي البيئة الخارجية بدرجة معنوية (علي مستوي 0.01) ، كذلك أظهرت النتائج أن أفضل خصائص للسائل المنوي و كذلك النشاط الإنزيمي و أعلي كفاءة إخصابية للحيوانات المنوية لطلائق الجاموس قدرت للسائل المنوي منزوع البلازما و المضاف له الكافيين بمستوي 10 مللي مول.

وخلصت هذه الدراسة إلي أن نزع بلازما السائل المنوي لطلائق الجاموس طريقة عملية و تطبيقية و غير مكلفة عند حفظ السائل المنوي في الحالة السائلة مع إضافة الكافيين بمستوي 10 مللي مول و ذلك لتحسين جودة و معدل بقاء الحيوانات المنوية وثبات النشاط الانزيمي وتعزيز المقدرة الاخصابية للحيوانات المنوية عند إجراء برامج التلقيح الاصطناعي في الجاموس المصري خاصة في المناطق القروية و الصحراوية والتي لا يتوافر فيها النيتروجين السائل اللازم لتجميد السائل المنوي لحفظه لفترات طويلة.