*Egyptian Journal of Rabbit Science, 25(2): 169 – 184(2015)*

# EFFECT OF α–CHYMOTRYPSIN AND GELATIN SUPPLEMENTATION IN RABBIT SEMEN EXTENDER ON ANTI-SPERM ANTIBODYS AND FERTILITY

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## ABSTRACT

*Three experiments were carried out to study the effects of α-chymotrypsin with gelatin on anti-sperm antibodies (ASAs) in rabbit's semen and fertilization rate. In the first experiment, all rabbits males (20 bucks) were tested for the presence of ASAs in seminal plasma. The second experiment, aimed to define the effect of different levels of α-chymotrypsin supplementation (0, 100, 200 and 400 mg/100ml extender) with gelatin in tris-based extender in presence ASAs on progressive sperm motility%, live and abnormal spermatozoa%. In the third experiment, does were inseminated with semen diluted in tris-based extender (1:3) as described in experiment II. Insemination was carried out using 80 does divided into five equal experimental groups (16 does/group) and inseminated artificially by diluted semen containing $50x10^6$ spermatozoa. Kindling rate and litter size were calculated.*

***Results showed** significant (P<0.05) decreases in progressive sperm motility, while sperm abnormalities and dead spermatozoa increased (P<0.05) in the ejaculates with ASAs - positive (titer>1/10) as compared with ASAs-negative ejaculates (titer<1/10). Semen ejaculates positive-ASAs supplemented with gelatin and α-chymotrypsin were exhibited ASAs titer 1/10 in seminal plasma that represents a significant change (P<0.05) compared with control group (C). Most amino acids were significantly (P<0.05) increased by the inclusion of gelatin alone or gelatin plus α-chymotrypsin. Supplementation of gelatin and α-chymotrypsin significantly (P<0.05) decreased ammonia in semen, ALT and AST activities in seminal plasma, TBARS concentration, sperm abnormalities, dead spermatozoa, glutathione and superoxide dismutase, while, increased percentages of progressive linear motility as compared to the control. Kindling rate was improved as compared with the control group. In addition, the total and live litter size and bunny weights at birth were considerably higher for supplemented groups α-chymotrypsin and*

*gelatin than those of the control group. The levels of α-chymotrypsin 200 and 400 mg/100 ml extender with gelatin represented the same significant differences with other experimental groups.*

***In conclusion**, supplementation of rabbit semen with gelatin and α- chymotrypsin not only enhanced the semen quality but it also improved anti-sperm antibodies status and reproductive performance.*

**Key words:** Rabbits semen, anti-sperm antibodies, α-chymotrypsin, gelatin, fertility.

## INTRODUCTION

Immunology is a powerful tool for studying both normal fertility and infertility of farm animals (Valentovicov *et al.,* 2005). Anti-sperm antibodies are protein molecules made by the woman's or man's body that are attracted to a specific site on the sperm and interfere with the spermatozoa activity. Anti-sperm antibodies can either: prevent sperm from attaching to the ova, stop sperm from moving normally, prevent sperm from passing through the cervical mucus, make sperm clump together and reduce fertility (Brunner-Agten *et al.,* 2013). Moreover, Anti-sperm antibodies formation can be induced primarily during infectious and non-infectious inflammations, or by obstruction of testicular different duct. In the male and female, anti-sperm antibodies (ASAs) may be found systemically in the blood and lymph and in local secretions in seminal or cervico–vaginal fluids (Agrawal *et al.*, 2009). Also, incidence of ASAs was also induced by an accident (Zhang *et al.*, 1990), very low temperature (Fayemi *et al.*, 1992), cryptorchism Pinart *et al.*, (1999), vasectomy (Jessop and Ladds, 1995) and by excessive male exploitation (Wicher *et al.,* 1987). From another point of view, chymotrypsin treatment of sperm bound with auto-antibodies was found to improve both fertilization and pregnancy rates following conventional insemination of oocytes (Katsoff *et al*., 1995). Thus, chymotrypsin treatment seems to neutralize to some degree antibodies to proteins that inhibit the fertilization process.

Therefore, the aim of this study was to evaluate the level of anti-sperm antibodies (ASAs) in rabbits semen and investigate the effect of α-chymotrypsin and gelatin supplementation to semen extenders, on sperm parameters, antioxidant status and fertility.

## MATERIALS AND METHODS

The present study was carried out at El-Sabahia Poultry Research Station, Alexandria Governorate, Animal Production Research Institute, Agricultural Research Center, Egypt. Three experiments were carried out to study the effect of inclusion of α-chymotrypsin with gelatin on anti-sperm antibodies (ASAs) in rabbits semen and fertilization rate. In the first experiment, semen was collected from 20 sexually mature V. Line (VL) bucks twice a week for a period of ten successive weeks using an artificial vagina. Gel plugs were removed from the ejaculates immediately after collection. The ejaculates were individually evaluated microscopically for percentage of progressive linear motility, dead and abnormal spermatozoa. All bucks were tested for the presence of anti-sperm antibodies (ASAs) in rabbit seminal plasma. Detection of ASAs was carried out by an enzyme immune analytical method (ELISA) at Physiology Department, Faculty of Veterinary Medicine, Alexandria University as described by Crowther (1995). In the second experiment, ejaculates were collected and evaluated as in the experiment 1. Semen was diluted with tris-based diluent at a rate of (1 semen:3 diluent) which was supplemented with 1 gm gelatin plus 0, 100, 200 and 400 mg α-chemotrypsin/100 ml extender. Total free amino acids were determined according to the method described by Hamilton (1962). Individual free amino acids were measured using a method described by Spackman *et al.,* (1958) using amino acid analyzer system (Hitachi L-8500, Tokyo, Japan). Samples were analyzed biweekly for thiobarbituric acid-reactive substances (TBARS) in the diluted seminal plasma using the method of Tappel and Zalkin (1959).

Glutathione (GSH) was determined using commercial glutathione reduced kits according to the method of Beutler *et al.,* (1963). Superoxide dismutase (SOD) activity was assayed according to Misra and Fridovich (1972). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were detected according to Reitman and Frankel (1957).

In the third experiment, eighty V-line rabbits does (16 does each group) were randomly assigned for each testing groups according to the result of experiment II. Only receptive females (red color of vulvar lips) were inseminated with about 50 million spermatozoa in three sequence parities. Does were artificially inseminated with the control and the other tested supplementations. Does were injected with 0.8 mg (0.2 ml) of gonadotropin-releasing hormone analogue (Buserelin, Suprefact®, Hoechst-Roussel, Germany (Receptal)) after two hours at the time of insemination according to (Boussin, 1989). Does were inseminated artificially with semen extenders, which described previously. The insemination procedure

was as described by (Adams, 1981). Kindling rate, litter size and bunny weight at birth were determined.

All rabbits were housed in a naturally ventilated building and kept in individual wire galvanized cages (60×55×40 cm) equipped with feeders, stainless steel nipple and an external nest-box. Rabbits were fed *ad libitum* with a commercial pelleted diet containing 17.18% CP, 13.05% CF and 2519 kcal DE/kg diet. Clean tape water was provided as free choice. All the experimental animals were healthy and clinically free from internal and external parasites and kept under the same managerial and hygienic conditions.

All data were subjected to analysis of variance according to the statistical analysis system described by SAS (2002). The differences among groups means were tested by using Duncan's multiple rang test (Duncan, 1955).

## RESULTS AND DISCUSSION

***1. Semen quality and anti-sperm antibodies:***

Table 1 shows significant ($P<0.05$) decreases in progressive motility percentage. Contrariwise abnormal and dead spermatozoa were increased ($P<0.05$) in ejaculates with ASAs-positive titer >1/10 as compared with ASAs-negative ejaculates titer <1/10. The presence of anti-sperm antibodies in secretions of reproductive organs and blood is often associated with infertility in farm animals (Waziri and Fayemi, 2000). The bonding of those antibodies to the surface of sperm antigens, which are predominantly functional molecules such as receptors and enzymes, can induce enhanced sperm agglutination, modulates acrosome reaction (Bohring *et al.,* 2001), inhibits metabolic processes of spermatozoa and thus decreases their motility and markedly reduced the likelihood of natural conception (Bohring and Krause, 2003) and ability to penetrate into oocyte (Bohring *et al*., 2001). The presence of ASAs was associated with adverse effects on motility, normal and live spermatozoa. Milovanovic *et al*., (2005) gave the hypothesis that immune mechanism may be involved in reproductive disturbances due to high level of ASAs of IgA class. Recently, a study of Jarora *et al*., (2014) indicated that higher percentage of IgG-ASAs and IgA-ASAs in cervical mucus of cross bred cows reduced *in vitro* penetration of spermatozoa through cervical mucus in the tested animals. It can be concluded that higher level of ASAs, especially IgA class antibodies in seminal plasma reduced post-thaw motility, *in vitro* capacitation, acrosome reaction and cervical mucus penetration of spermatozoa.

**Table 1**: Relationship between the presence of ASAs in seminal plasma of rabbit males and certain semen parameters.

| Semen ejaculate | Sperm characteristics (%) | | |
|---|---|---|---|
| | Progressive motility | Abnormal spermatozoa | Dead spermatozoa |
| **ASAs-Negative (Titer<1** | 85 ±0.70 | 13±0.46 | 11±0.17 |
| **ASAs-Positive (Titer>1/** | 72 ±1.45 | 15±0.45 | 13±035 |
| **Significance** | * | * | * |

* = (P < 0.05)

***2. α-Chymotrypsin and anti-sperm antibodies:***

Table 2 demonstrated that in semen ejaculates positive-ASAs supplemented with gelatin and α-chymotrypsin (0, 100, 200 and 400) exhibited ASAs titer 1/10 in seminal plasma that represents a significant change (P<0.05) compared with control group (C). Also, treatment with α-chymotrypsin exhibited ASAs titer 1/10 in seminal plasma that represents significant decrease (P<0.05) as compared with the other groups. In control group, no significant changes were found in the seminal plasma titer>1/10 of ASAs in relation to the initial sample.

The positive effect of α-chymotrypsin treatment may be explained by its proteolytic activity which destroys the anti-sperm antibodies (Bollendorf *et al*., 1994). Centenaro *et al.,* (2011) reported that treatment of semen with α-chymotrypsin may perform proteolysis and hydrolyses amid bonds in protein and peptides. The limited proteolysis caused by α-chymotrypsin cannot destroy the spermatozoa, because the cells are protected by an external phospholipid membrane that cannot be cleaved by the protease (Chen *et al*., 2006). Further, it was demonstrated that α-chymotrypsin had no effect on the detection of sperm parameters and biochemistry markers, and could be used to treating non-liquefied samples before semen analysis in the andrology laboratory (Chen *et al*., 2006).

3. Chymotrypsin and Gelatin:

Table 3 showed that most amino acids were significantly (P<0.05) increased by the inclusion of gelatin alone or gelatin plus α-chymotrypsin. The predominant amino acids in rabbits seminal plasma were therionine, glutamic and leucine. Total free amino acids were 16.35, 42.44, 64.26, 104.14 and 174.12 mg/100 ml extended semen for C, 0, 100, 200 and 400, respectively. Similar results are in agreement with that reported by Centenaro *et al.,* (2011) who reported that the increase of total free amino acids in extended semen treated with α-chymotrypsin may be due to the digestive enzyme activity that can perform proteolysis and hydrolyses amid bonds in protein and peptides (gelatin).

**Table 2**: Occurrence of ASAs in rabbits semen supplemented with gelatin and α-chymotrypsin to semen extender.

| Treatments | Positive ASAs | | |
|---|---|---|---|
| | No of ejaculates | No | % |
| **ASAs-Positive (Titer>1/10)** | **10** | **10** | **100** |
| Control (C) | 10 | 10 | 100 |
| Gelatin (0)* | 10 | 9 | 91 |
| Gelatin (100)** | 10 | 00 | 00 |
| Gelatin (200)** | 10 | 00 | 00 |
| Gelatin (400)** | 10 | 00 | 00 |
| **Significance** | | **P<0.05** | |

Ammonia decreased (P<0.05) in extended semen supplied with gelatin and different levels of α-chymotrypsin. This in association with the results of Bilodeau *et al.*, (2009) demonstrated that the accumulation of ammonia can be reduced in the medium by supplementation with the dipeptides L-alanyl-L-glutamine and L-glycyl-L-glutamine, which can play an important role in motility.

***4. Semen quality:***

Results represented in Table 4 indicated that supplementation of gelatin and α-chymotrypsin had a significant (P<0.05) effect on sperm. Adding gelatin and α-chymotrypsin to tris-buffer extender significantly (P<0.05) increased percentages of advanced motility and decreased abnormal spermatozoa (P<0.05) and dead spermatozoa as compared to the control.

Collectively, increasing total free amino acids due to supplementation with gelatin and α- chymotrypsin was associated with improved semen quality. Several mechanisms have been proposed for the roles of amino acids during cryopreservation. Oltjen *et al.*, (1971) showed that high amounts of total free amino acids are important for the semen quality and the fertility of the animals. Ibrahim and Boldizsár (1981) also noted that there is some evidence that the amino acids present in the seminal plasma play an important role in survival of spermatozoa. The function of seminal .....اكمل

**Table 3**. Effect of α-chymotrypsin and gelatin supplementation in rabbits semen extended on free amino acids composition (Mean±SE).

| Amino acids | Gelatin (1 g/100 ml)+α-chymotrypsin level (mg /100ml) | | | | |
|---|---|---|---|---|---|
| | Control | 0 | 100 | 200 | 400 |
| **Aspartic acid** | 0.60±0.89$^{e}$ | 0.74±0.96$^{d}$ | 0.98±0.11$^{c}$ | 1.16±0.12$^{b}$ | 1.29±0.12$^{a}$ |
| **Threonine** | 8.20±0.11$^{d}$ | 16.16±0.14$^{c}$ | 16.24±0.13$^{c}$ | 23.84±0.18$^{b}$ | 34.57±0.25$^{a}$ |
| **Serine** | 0.14±0.00$^{e}$ | 0.47±0.00$^{d}$ | 1.11±0.00$^{c}$ | 1.58±0.00$^{b}$ | 1.87±0.01$^{a}$ |
| **Glutamic acid** | 3.42±0.14$^{d}$ | 6.58±0.16$^{c}$ | 8.61±0.18$^{b}$ | 8.24±0.17$^{b}$ | 9.21±0.19$^{a}$ |
| **Proline** | 0.66±0.08$^{c}$ | 1.86±0.11$^{b}$ | 1.96±0.13$^{b}$ | 2.11±0.12$^{a}$ | 2.15±0.12$^{a}$ |
| **Glycine** | 0.14±0.00$^{e}$ | 0.21±0.00$^{d}$ | 0.87±0.00$^{c}$ | 1.12±0.00$^{b}$ | 1.38±0.00$^{a}$ |
| **Alanine** | 0.50±0.00$^{e}$ | 3.13±0.11$^{d}$ | 16.24±0.14$^{c}$ | 28.19±0.17 | 65.87±2.14$^{a}$ |
| **Cystine** | 0.54±0.02 $^{d}$ | 4.43±0.06$^{c}$ | 5.53±0.08$^{c}$ | 19.54±0.14$^{b}$ | 23.15±0.16$^{a}$ |
| **Valine** | 0.45±0.00$^{e}$ | 1.42±0.11$^{d}$ | 2.48±0.13$^{c}$ | 4.36±0.15$^{b}$ | 7.14±0.16$^{a}$ |
| **Methionine** | 0.61±0.07$^{e}$ | 1.21±0.10$^{d}$ | 1.81±0.12$^{c}$ | 2.02±0.14 $^{b}$ | 3.10±0.17$^{a}$ |
| **Isoleucine** | 0.30±0.12$^{e}$ | 1.73±0.39$^{d}$ | 1.99±0.47$^{c}$ | 2.41±0.59 $^{b}$ | 9.05±0.78$^{a}$ |
| **Leucine** | 1.60±0.06$^{e}$ | 1.85±0.13$^{d}$ | 2.38±0.15$^{c}$ | 2.96±0.15$^{b}$ | 3.22±0.18$^{a}$ |
| **Tyrosine** | 0.31±0.00$^{e}$ | 0.55±0.00$^{d}$ | 0.86±0.00$^{c}$ | 2.79±0.071$^{b}$ | 4.50±0.10$^{a}$ |
| **Phenylalanine** | 0.94±0.08$^{e}$ | 0.96±0.08$^{d}$ | 1.89±0.12$^{c}$ | 2.07±0.14$^{b}$ | 2.43±0.15$^{a}$ |
| **Histidine** | 0.77±0.07$^{d}$ | 0.88±0.07$^{c}$ | 0.92±0.09$^{c}$ | 1.18±0.10$^{b}$ | 4.51±0.16$^{a}$ |
| **Lysine** | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| **Arginine** | 0.17±0.00$^{e}$ | 0.26±0.00$^{d}$ | 0.39±0.00$^{c}$ | 0.57±0.00$^{b}$ | 0.68±0.00$^{a}$ |
| **Total free amino acids** | 16.35±1.79$^{e}$ | 42.44±2.15$^{d}$ | 64.26±2.75$^{c}$ | 104.14±3.3$^{b}$ | 174.12±5.3$^{a}$ |
| **Ammonia** | 2.56±0.10$^{a}$ | 1.88±0.08$^{b}$ | 1.56±0.07$^{c}$ | 1.38±0.06$^{d}$ | 0.26±0.00$^{e}$ |

$^{ab}$ Means with different superscripts in the same row significantly (P<0.05) differ, *n= 25 samples

Plasma free amino acids is shown to act as fuels for the spermatozoa, to create favorable conditions for cell survival and to be probably involved in detoxifying function. Kundu *et al.*, (2001) suggested that the protective effects of amino acids may stem from their ability to form a layer on the spermatozoa surface, as these positively charged molecules can combine with the phosphate groups of sperm plasma membrane phospholipids. Also, Atessahin *et al.*, (2008) showed that addition of amino acids to extender improved sperm motility, viability, acrosomal integrity and membrane integrity in goat and boar. Moreover, cysteine has been shown to improve motility and morphology of ram (Uysal and Bucak, 2007) and goat sperm, and (Buck *et al.,* 2008) to maintain the viability, the chromatin structure and membrane integrity of boar sperm (Sariozkan *et al.,* 2009).

Zavos *et al., (*1997) reported that the of high viscosity semen samples using α-chymotrypsin resulted in the recovery of both a better quality and a higher number of spermatozoa which can be used for assisted reproduction. Hyper viscous seminal fluid has been shown to have a negative impact of sperm motility and semen quality (Elzanaty *et al.,* 2004 and Zakaria and Mohammed, 2014). Likewise, Nagy *et al.*, (2003) reported

that gelatin addition to extender semen had a positive effect on preserved semen quality. They found high percentage of live cells in semen preserved as compared to free gelatin. On the other hand, some investigators found no differences in goat and sheep semen motility when fresh semen extender was supplemented with gelatin immediately after semen collection (Salvador *et al.*, 2006 and Elspeiy and Al-Hanoun, 2015).

**Table 4:** Effect of gelatin and α-chymotrypsin supplementation in rabbits semen extender and sperm characteristics.

| Treatments | Sperm characteristics (%) | | |
|---|---|---|---|
| | **Progressive motility** | **Abnormal spermatozoa** | **Dead spermatozoa** |
| **Control (C)** | $69.64^{b}$±2.22 | $23.43^{a}$±.88 | $16.92^{a}$±0.96 |
| **Gelatin (0)** | $72.00^{b}$±1.27 | $21.00^{b}$±0.61 | $14.01^{b}$±0.56 |
| **Gelatin (100)** | $74.25^{a}$±0.80 | $20.34b^{b}$±0.41 | $13.93^{b}$±0.46 |
| **Gelatin (200)** | $78.74^{a}$±0.79 | $20.18^{b}$±0.89 | $13.21^{b}$±035 |
| **Gelatin (400)** | $77.92^{a}$±0.81 | $20.24^{b}$±0.43 | $13.36^{b}$±039 |
| **Significance** | * | * | * |

[ab]Means in the same column within a category with different superscripts significantly differ (P<0.05).

### *5. Transaminase Enzyme Activities:*

Table 5 revealed that exhibit alanine-aminotransferase (ALT) and aspartate-aminotransferase (AST) activities in seminal plasma being reduced (P<0.05) in treated groups than control. The low release of these two metabolic enzymes in supplemented extenders could be attributed to the protective effects of free amino acids and peptides on the integrity of the sperm cell membranes. These observations are in agreement with that found in goat semen (Kundu *et al.*, 2001). They concluded that addition of glutamine, glycine and cysteine in conventional storage medium improved membrane and acrosomal integrity of spermatozoa. However, Sariozkan *et al.*, (2009) demonstrated that amino acid enhanced the defense of mammalian cell membrane and improved cell membrane integrity during sperm storage. Furthermore, change of biochemical factors have been recognized during cryopreservation, including depletion of amino acids and lipoproteins release of AST (Barbas and Mascarenhas, 2009). Moreover, Numan *et al.,* (2010) showed that storage generates sub lethal injury to the sperm due to chemical, osmotic, thermal, and mechanical stresses which may result in loss of viability, motility, damage of deoxyribonucleic acid.

**Table 5**: Effect of gelatin and α-chymotrypsin supplementation in rabbits semen extender on biochemical of seminal plasma extender (ALT and AST).

| Treatments | Biochemical of seminal plasma extender | |
|---|---|---|
| | ALT (U/L) | AST (U/L) |
| **Control (C)** | $62.92^{a}$±1.40 | $78.55^{a}$±1.50 |
| **Gelatin (0)** | $59.25^{b}$±0.82 | $72.77^{b}$±0.85 |
| **Gelatin (100)** | $57.99^{b}$±0.76 | $71.84^{b}$±0.77 |
| **Gelatin (200)** | $57.59^{b}$±0.67 | $71.04^{b}$±0.82 |
| **Gelatin (400)** | $57.66^{b}$±.66 | $71.11^{b}$±0.79 |
| **Significance** | * | * |

$^{ab}$Means in the same column within a category with different superscripts significantly differ (P<0.05).

***6. Seminal plasma antioxidants:***

Inclusion of α-chymotrypsin and gelatin in rabbits semen extender decreased (P<0.05) TBARS concentration but significantly increased (P<0.05) glutathione and superoxide dismutase as compared with control (Table 6). Agarwal *et al.,* (2007) mentioned that spermatozoa are susceptible to reactive oxygen species (ROS) attack. The imbalance between the production of ROS and biological systems ability to readily detoxify the reactive intermediates or easily repair the resulting damage is known as oxidative stress (Agarwal *et al.,* 2003). Oxidative stress is induced by ROS, or free radicals. However, ROS have been shown to be required for sperm capacitation, hyper activation and sperm-oocyte fusion. Aitken *et al.,* (2004) reported that excessive levels of ROS can negatively impact sperm quality. Since, increased levels of ROS have been correlated with decreased sperm motility; increased sperm DNA damage (Barroso *et al.,* 2000), sperm cellular membrane lipid peroxidation and decreased efficacy of oocyte-sperm fusion (Agarwal *et al.,* 2007).

In the present study, the abundance of cysteine and the other amino acids in the extender significantly improved sperm quality parameters such as sperm motility and viability. These findings are similar to results obtained by Scanchez-Partidata *et al.,* (1992) who demonstrated that increase glutathione (GSH) and decrease malonylaldhyed (MAD) addition of low concentration of proline and betaine glycine (a component related to amino acids) to a medium containing egg yolk and glycerol improved the motility of ram spermatozoa. Buck *et al.,* (2008) showed a positive effect of cysteine on motility and membrane integrity. Raji *et al.,* (2003) reported that low sperm motility and high percentage of abnormal spermatozoa level have been associated with fertility reduction. Sariozkan *et al.,* (2009) also demonstrated that cysteine is one of the additives that have been used in freezing extender of human, boar, goat and bull to improve post-thaw

sperm parameters. Addition of cysteine and lipoic acid to the semen freezing extender, may prevent cryodamage to spermatozoa metabolism and antioxidant capacities (Rahim Beheshti *et al.,* 2011).

**Table 6**: Effect of α-chymotrypsin and gelatin supplementation in rabbits semen extender on (TBARS, GSH and SOD).

| Treatments | Sperm characteristics (%) | | |
|---|---|---|---|
| | **TBARS(nmol/ml)** | **GSH (mg/dl)** | **SOD (IU)** |
| **Control (C)** | $1.53^{a}$±0.04 | $422.6^{d}$±10.34 | $1.22^{d}$±0.05 |
| **Gelatin (0)** | $1.50^{a}$±0.03 | $450.46^{c}$±7.53 | $1.39^{c}$±0.04 |
| **Gelatin (100)** | $1.34^{c}$±0.02 | $454.52^{b}$±7.22 | $1.40^{b}$±0.02 |
| **Gelatin (200)** | $1.29^{c}$ ±0.02 | $453.96^{b}$±7.82 | $1.44^{b}$±0.02 |
| **Gelatin (400)** | $1.33^{c}$ ±0.02 | $460.10^{a}$±6.95 | $1.48^{a}$±0.01 |
| **Significance** | * | * | * |

[ab]Means in the same column within a category with different superscripts significantly differ (P<0.05).

***7. Reproductive Performance:***

Table 7 indicated that kindling rate of females artificially inseminated with semen extender supplemented with different levels of α-chymotrypsin and gelatin was improved as compared with the control group. In addition, the total and live litter sizes at birth and bunny weight at birth were higher for groups of α-chymotrypsin and gelatin than those of the control one. The levels of α-chymotrypsin 200 and 400 mg/100 ml with gelatin represented the same significant differences with other experimental groups. The improvement of the previous mentioned parameters of rabbits reproductive performance may be due to the enrichment of semen extender with α-chymotrypsin and gelatin which improved semen characteristics. The positive effect of amino acid and α-chymotrypsin as an enhancer of reproductive capacity of rabbit bucks could be attributed to its ability to protect mammal cells from oxidation (**Alvarez and Storey, 2005**). **Zavos *et al.,* (1997)** reported that the high viscosity semen samples using α-chymotrypsin resulted in the recovery of both a better quality and a higher number of spermatozoa which can be used for assisted reproduction. **Bollendorf *et al., (*1994)** found that treatment of spermatozoa (positive-ASAs) with chymotrypsin and galactose increased pregnancy rates.

**Table 7:** Effect of enrichment the rabbit semen extender with α-chymotrypsin and gelatin on reproductive performance.

| **Parameters** | **control** | **Gelatin (1 g/100ml) + α-chymotrypsin (mg/100ml)** |
|---|---|---|

| | C | 0 | 100 | 200 | 400 |
|---|---|---|---|---|---|
| **Kindling rate (%)** | $55.7^{c}$±0.75 | $66.3^{b}$±0.69 | $79.7^{a}$±0.96 | $85.8^{a}$±0.87 | $81.3^{a}$±0.83 |
| **Litter size at birth (n.)** | $5.38^{c}$±0.54 | $7.13^{b}$±0.63 | $7.03^{b}$±0.58 | $10.41^{a}$±0.6 | $9.87^{a}$±0.57 |
| **Live litter size at birth (n.)** | $4.10^{c}$±0.53 | $6.42^{b}$±0.65 | $6.63^{b}$±0.66 | $9.00^{a}$±0.57 | $8.61^{a}$±0.62 |
| **Bunny weight at birth (g)** | $44.9^{c}$±0.82 | $53.5^{b}$±0.93 | $55.2^{b}$±0.81 | $68.2^{a}$±0.73 | $65.2^{a}$±091 |

[ab] Means in the same row within a category with different superscripts significantly differ (P<0.05).

***In conclusion,*** rabbits semen extender supplementing with gelatin and α-chymotrypsin not only enhanced semen quality but it also improved anti-sperm antibodies (ASAs) status and reproductive performance.

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## تأثير إضافة ألفاكيموتربسين والجيلاتين لمخفف سائل منوى الأرانب على الأجسام المضادة للحيوان المنوى والخصوبة

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أجريت ثلاثة تجارب لدراسة تأثير إضافة ألفاكيموتربسين والجيلاتين إلي مخفف سائل منوى الأرانب على الأجسام المضادة للحيوان المنوى ومعدل الأخصاب. أستخدم في هذه الدراسة ٢٠ ذكر عمر ٦ شهور، ٨٠ أنثى أرنب فى لاين. فى التجربة الأولى تم تصنيف كل الذكور بعد إختبارها حسب وجود الأجسام المضاده للحيوان المنوى من عدمه فى بلازما السائل المنوى. والتجربة الثانيه صممت لدراسة تأثير مستويات مختلفه من ألفاكيموتربسين (صفر،١٠٠، ٢٠٠، ٤٠٠مجم/١٠٠مل مخفف) مع الجيلاتين فى مخفف الترس وذلك مع السائل المنوى المحتوى على الأجسام المضادة للحيوان المنوى على كل من صفات نسبة الحركة الفردية التقدمية، نسبة الحيوانات المنوية الحية، نسبة الحيوانات المنوية المشوهة، أما التجربة الثالثة فقد تم إجرائها إعتمادا على نتائج التجربة الثانية حيث تم تلقيح إناث الأرانب بسائل منوى مخفف بمخفف الترس بمعدل ٣:١ حيث تم تقسيم الإناث إلى ٥ مجموعات بكل منها ١٦ أنثى لقحت إصطناعيا بجرعة تحتوى على ٥٠ مليون حيوان منوى، وتم حساب معدل الولادات وحجم البطن لكل أنثى.

أوضحت النتائج وجود إنخفاض معنوى (علي مستوى احتمال معنويه ٥% ) فى الحركة التقدمية الفردية للحيوان المنوى مع زيادة فى الحيوانات المنوية المشوهة والميتة فى القذفات الموجبة للأجسام المضادة للحيوان المنوى مقارنة بالقذفات السالبة. كما لوحظ أن إضافة ألفا كيموتربسين الى المخفف حسن من حالة الأجسام المضادة للحيوان المنوى في عينات السائل المنوى الموجبة مقارنة بالكنترول. زادت معظم الأحماض الأمينية معنويا سواء فى وجود الجيلاتين فقط أو مع ألفاكيموتربسين.

كذلك وجد أن إضافة الجيلاتين مع ألفاكيموتربسين خفضت مستوى الأمونيا فى السائل المنوى وكذلك خفضت نشاط إنزيمات ALT و AST و TBARS فى بلازما السائل المنوى. كما إرتفعت نسبة الحركه التقدميه وخفضت من نسبة الحيوانات المنوية المشوهة معنويا والحيوانات المنوية الميتة، الجلوتاثيون، سوبرأكسيد ديسميوتاز مقارنة بمجموعة الكنترول. أيضا لوحظ تحسن فى معدل الولادات فى المجاميع المعامله بألفاكيموتربسين والجيلاتين مقارنة بالكنترول، وكان مجموع الخلفات المولودة، والعدد المولود حى ووزن الخلفة الفردى أعلى فى مجاميع المعاملات عنه فى الكنترول.

**التوصية**: من النتائج السابقة يمكن التوصية بإضافة الجيلاتين وألفاكيموتربسين لمخفف سائل منوي الأرانب لم يحسن فقط من نوعية السائل المنوي، ولكنه أدى إلى تحسين كل من حالة الأجسام المضادة للحيوان المنوي والأداء التناسلي للأرانب.