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Ginger Extract as a Promoter of Sub-Fertility in Buffalo Bulls

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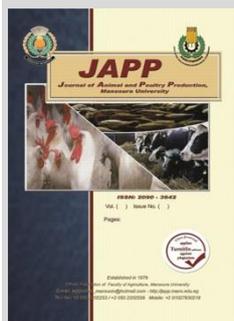


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ABSTRACT

This study aimed to evaluate the effect of ginger extract (GIN) on reproductive performance of buffalo bulls. Sub-fertile buffalo bulls (n=8) with body weight ranging from 400-450 kg and 22-25 mo of age were divided into two groups (n=4 in each). The 1st group was fed on the basal diet without treatment, but the basal diet was spread with 10 ml distilled water and served as a control. Bulls in the 2nd group were fed the basal diet spread with liquid GIN extract (200 mg GIN dissolved in 100 ml/kg diet) for 4 mo (2 mo as a preliminary period and 2 mo as a collection semen period). Semen was collected twice/week for 8 weeks. Blood samples were taken at the end of experiment. Results showed that GIN improved ejaculate volume, motility types, vitality, membrane integrity, concentration and total sperm output, while decreased (P<0.001) reaction time, and acrosomal damage. Also, GIN increased (P<0.001) testosterone by 103.7%, RBCs, PCV, and hemoglobin, while decreased WBCs count (P<0.01). Plasma concentration of total proteins, albumin, globulin significantly increased, while urea and creatinine decreased (P<0.001). Enzyme activity of AST and ALT significantly decreased in GIN group. Oral dose of 200 mg ginger in 100 ml distilled water/kg diet improved sexual activity and semen quality of buffalo bulls with low reproductive efficiency.

Keywords: Buffalo bull, low fertility, sperm characteristics, plasma testosterone.



INTRODUCTION

The use of herbal medicines (medicinal plants or phytotherapy) has recently gained popularity in the world and there is an increased interest on plant-derived chemicals on the reproductive organs activity and endocrine-hypothalamic axis (Kooti *et al.*, 2015). Ginger, GNG (*Zingiber officinale Roscoe*, *Zingiberaceae*) is a spice used in human feed. Polyphenol compounds such as 6-gingerol and its derivatives are found in this plant make it as a high antioxidant activity (Herrman, 1994; Khaki *et al.*, 2009), anti-inflammatory, anti-hepatotoxic agents (Khaki *et al.*, 2009) and androgenic properties (Kamthoung *et al.*, 2002).

Low reproductive performance is one of the major problems (Ghalehkandi, 2014), and sub-fertility in males is a multi-parametric phenomenon, representing about 30% of infertilities, which are related to a male factor (Vincent *et al.*, 2012), and about 40-50% of sub-fertility include in spermatogenesis within the testes and quality of semen stored in the epididymis (Mazaheri *et al.*, 2014). Also, the sub-fertility of males are mainly in relation with low motility, abnormality and vitality, and reduced sperm cell concentration (Raji *et al.*, 2003). Several factors affect spermatogenesis and semen production, including oxidative stress. In rats, GIN extract might be promoter to sub-fertility in males which might be a consequence of both its potent antioxidant properties and androgenic activities (Morakinyo *et al.*, 2008). In this respect, Khaki *et al.* (2009) reported that GIN treatment at a level of 100 mg/kg improved motility and vitality of spermatozoa and in testosterone in blood serum of rats.

In the literature, the researches on the effect of GIN on male reproductive efficiency are scar. Therefore the present study aimed to evaluate the effect of ginger extract as a natural antioxidant on sexual desire, semen characteristics and blood parameters of buffalo bulls with low reproductive performance.

MATERIALS AND METHODS

This experiment was conducted at Mahlt Mouse Animal Production Research Station, belonging to the Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture in cooperation with the Department of Animal Production, Faculty of Agriculture, Kafrelsheikh University, during the period from January to Jun 2019.

Animals:

A number of 8 sub-fertile buffalo bulls with an average body weight of 400-450 kg and 22-25 mo were used in this study. The sub-fertility of bulls was determined by production of semen with low sperm motility (<50%), sperm vitality (70%), sperm abnormality (>30%), and sperm cell concentration (2x10⁶/ml). All experimental bulls with normal external genitalia and were free of physical defects and infected diseases. Bulls were housed individually under semi-open sheds. They were fed according to the recommendations of Animal Production Research Institute (APRI, 2002) throughout the whole period of the experiment.

Extract preparation:

Ginger powder was prepared from the commercial product of ginger by grounding and about 50 g of the powder was soxhleted with distilled water. Then, the ginger extract was lyophilized, weighed and stored at refrigerator (4-5°C) until using.

Feeding system:

The experimental bulls were fed individually on a ration containing concentrate feed mixture (CFM, 16% CP, 11% CF and 62% NFE), fresh berseem at a rate of 70: 30% beside feeding on *ad libitum* rice straw. The ingredients of the CFM included un-decorticated cottonseed cake (35%), ground yellow corn (25%), wheat bran (20%), rice bran (10%), linseed cake (5%), molasses (3%), limestone (1%) and common salt (1%).

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Experimental design:

At the beginning of experimental period, a total of eight bulls were assigned into 2 groups (4 bulls in each). Bulls in the 1st group were given the control ration, but their CFM was spread with 10 ml distilled water during feeding and served as a control group. In another group, the same amount of CFM offered for the control ration was spread with liquid GIN extract (100 ml distilled water containing 200 mg GIN extract). The experimental period lasted for 4 months (2 months as a preliminary period and another two months as a collection semen period).

Semen collection and evaluation:

Semen was collected by conventional artificial vagina tool. Semen was collected twice a week during the collection period (16 ejaculates per bull). The collected semen was maintained in a water bath (37 oC), then transported immediately to the laboratory. In the laboratory, semen was individually evaluated to determine the physical characteristics of fresh semen.

During semen collection, the time elapsed between the introducing a bull to complete ejaculation (reaction time) was recorded using stop-watch. Ejaculate volume was recorded in a graduated collection glass tube. Different semen characteristics were tested by the same observatory. Percentages of total motility, progressive motility, and immotility of spermatozoa were measured according to Amman and Hammerstedt (1980). Percentages of sperm vitality, abnormality, and acrosomal status were examined and calculated according to Hackett and Macpherson (1965), Blom (1983) and Yanagimachi (1982), respectively. Hypo-osmotic swelling test (HOS-t) was performed at osmolarity level of 50 mOsm/l for 30 minutes to determined sperm membrane integrity after the method of El-Sherbieny (2004) on buffalo semen. Sperm cell concentration/ml per ejaculate was counting according to Khan (1994) by using haemocytometer. Total sperm production per ejaculate was computed using the following formula:

$$\text{Total sperm production/ejaculate} = \text{Ejaculate volume (ml)} \times \text{sperm concentration (x } 10^6/\text{ml)}.$$

Analytical procedures of blood:

Blood samples were taken from bulls in each group (n=4) at the last week of the collection period morning before feeding from the jugular vein. Blood samples were collected into dry clean glass tubes with heparin to prevent blood clotting. Each sample was divided into two parts; the first part was centrifuged for 15 minutes at 3000 rpm to obtain blood plasma for determination of some blood biochemical. However the 2nd part of the blood sample as left as a whole blood for some hematological parameters. Plasma samples were kept in deep freezer at -20°C till chemical analysis.

The hematological parameters were directly determined in the whole blood using CBC analyzer. These parameters included count of red (RBCs) and white (WBCs) blood cells, concentration of hemoglobin, and packed cell volume (PCV%). However, the blood biochemicals, including concentrations of total protein and albumin (Tietz 1990 and 1994), creatinine (Bartles *et al.*, 1972) and urea-N (Patton and Crouch, 1977) were determined in blood plasma. However, concentration of globulin was calculated by subtracting the albumin form the total protein concentration.

In addition, activities of asprate transaminase (AST) and alanin transaminase (ALT) in blood plasma samples were assayed by the methods of Young (1990), while concentration of plasma testosterone was determined by the radioimmunoassay

(RIA). All blood biochemicals were measured by using spectrophotometer and commercial kits of Diagnostic Products Corporation, Los Angeles, USA according to

Statistical analysis:

Data were statistically analyzed by ANOVA one way design using statistical analysis system user's guide, version 9 (SAS, 2002) to evaluate the effect of GIN on parameters of libido, semen characteristics, and liver and kidney functions. The statistical model used for analyzing all parameters was as the following: $Y_{ij} = \mu + T_i + e_{ij}$. Where, Y_{ij} = observed values, μ = overall mean, T_i = treatment effect (i: 1-2), e_{ij} = random error. The percentage values were subjected to arcsine transformation before performing the analysis of variance. Means were presented as mean \pm SE.

RESULTS AND DISCUSSION

Results

Libido parameters:

Results in Table 1 showed that ginger (GNG) administration significantly improved libido parameters, in term of decreasing (P<0.001) reaction time by about 53% and increasing (P<0.05) plasma testosterone level by about 73% as compared to control group.

Table 1. Reaction time and plasma testosterone profile of buffalo bulls as affected by ginger treatment.

Item	Experimental group		Significance	Change (%)
	Control	Ginger		
Reaction time (second)	120 \pm 8.012	56 \pm 6.175	***	-53
Testosterone (ng/ml)	3.24 \pm 0.814	5.61 \pm 0.723	*	73

* Significant difference at a level of P<0.05.

*** Significant difference at a level of P<0.001.

Semen parameters:

Effect of GIN administration was highly significant (P<0.01; P<0.001) on all semen parameters (Table 2), including ejaculate volume (EV), sperm concentration (SC), total sperm output (TSO), and percentages of all types of sperm motility, sperm vitality (SV), sperm abnormality (SA), membrane integrity (MI), and intact acrosome (IA). Administration of GNG significantly increased EV, SC, TSO, and percentages of total motility, progressive motility, SV, MI, and IA, and significantly decreased percentages of non-progressive motility, sperm immotility and sperm abnormality. The most improvement was observed in term of increasing TSO by 117% and progressive motility by 74%, and decreasing sperm abnormality by 46.2% (Fig. 1).

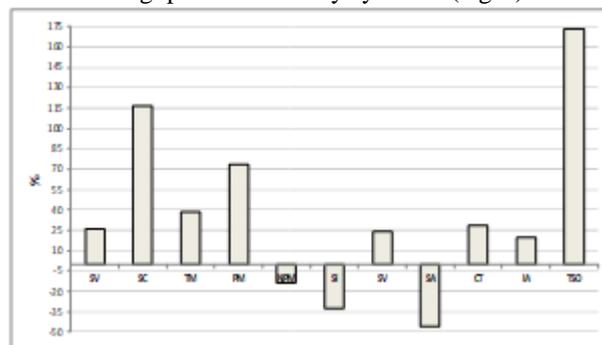


Fig. 1. Rate of change (%) in semen characteristics in control and ginger groups.

Blood hematology:

Administration of GIN significantly (P<0.001) affected count of red blood cells (RBCs), concentration of hemoglobin (Ht) and packed cell volume (PCV) value, while

count of white blood cells (WBCs) was not affected by GNG. Count of RBCs, Ht concentration and PCV value was

significantly ($P < 0.001$) higher in GNG group than in control one by about 23.6, 21.7 and 12.6%, respectively (Table 3).

Table 2. Effect of ginger administration on different semen parameters of buffalo bulls.

Semen parameter	Experimental group		Significance	Change (%)
	Control	Ginger		
Semen volume (ml)	2.10±0.12	2.64±0.09	***	25.7
Sperm concentration (x10 ⁶ /ml)	1.06±0.07	2.30±0.04	***	117
Total motility (%)	47.93±0.17	66.31±0.49	***	38.3
Progressive motility (%)	28.64±0.24	49.84±0.32	***	74.0
Non- progressive motility (%)	19.26±0.08	16.54±0.26	***	-14.1
Sperm immotility (%)	51.98±0.21	34.89±0.14	***	-32.9
Sperm vitality (%)	71.72±1.21	89.27±0.09	**	24.5
Sperm abnormality (%)	31.24±1.21	16.80±1.02	**	-46.2
Sperm membrane integrity (%)	57.92±5.14	74.40±6.31	***	28.5
Intact acrosome (%)	70.88±3.41	84.48±5.12	***	19.2
Sperm output (x10 ⁶ /ejaculate)	2.23±0.31	6.10±0.20	***	173.5

** Significant difference at a level of $P < 0.01$. *** Significant difference at a level of $P < 0.001$.

Table 3. Effect of ginger administration on some hematological parameters of buffalo bulls.

Parameter	Experimental group		Significance	Change (%)
	Control	Ginger		
Red blood cells (x10 ⁶ /mm ³)	6.321±0.251	7.81±0.202	***	23.6
White blood cells (x10 ³ /mm ³)	8.60±0.392	7.88±0.471	NS	-
Hemoglobin (g/dl)	10.20±0.684	12.41±0.523	***	21.7
PCV (%)	37.74±1.34	42.48±1.02	***	12.6

NS: Not significant. *** Significant difference at $P < 0.001$.

Blood biochemicals:

The effect of GIN administration on total proteins (TP), albumin (AL) and globulin (GL) concentrations in blood plasma of bulls was significant (Table 4). Administration of GIN significantly increased concentration of plasma TP ($P < 0.01$), AL ($P < 0.05$) and GL ($P < 0.001$), while decreased AST ($P < 0.05$) and ALT ($P < 0.01$) activities. The pronounced effect of GNG treatment on increasing TP concentration (20.4%, $P < 0.01$) was associated with low increase in AL concentration (10.2%, $P < 0.05$) and obvious elevation in GL concentration (87%, $P < 0.001$). Concerning the enzyme activity, GNG treatment significantly reduced activity of AST by about 9.2% ($P < 0.05$), being lower than that on activity of ALT (20.2%, $P < 0.01$, Table 4).

Treatment with GIN significantly ($P < 0.001$) reduced concentration of creatinine and urea-N in blood plasma of buffalo bulls, but the rate of reduction was higher for creatinine (29.8%) than urea (18.6%) as illustrated in Fig. 2.

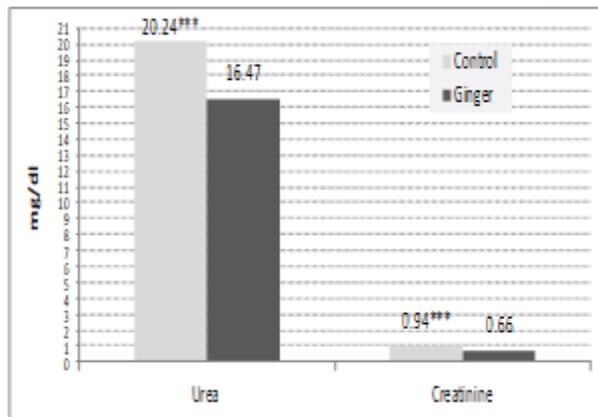


Fig. 2. Concentration of creatinine and urea in blood plasma of buffalo bulls in control and ginger groups. (***) Significant difference at a level of $P < 0.001$.

Table 4. Effect of ginger administration on liver function markers in blood plasma of buffalo bulls.

Parameter	Experimental group		Significance	Change (%)
	Control	Control		
Total proteins (g/dl)	5.93±0.29	7.14±0.21	**	20.4
Albumin (g/dl)	2.95±0.21	3.25±0.20	*	10.2
Globulin (g/dl)	2.08±0.17	3.89±0.15	***	87.0
AST (IU/l)	60.42±4.21	54.48±3.12	*	9.8
ALT (IU/l)	44.36±3.47	35.38±3.14	**	20.2

* Significant difference at $P < 0.05$. ** Significant difference at $P < 0.01$.

*** Significant difference at $P < 0.001$.

Discussion

The present study aimed to evaluate the effect of ginger extract, as a natural antioxidant, on sexual desire, semen characteristics and blood parameters of buffalo bulls with low reproductive performance. The obtained results indicated that GIN administration significantly ($P < 0.001$) decreased the reaction time (RT) by 53% as compared to control group. In agreement with the present results, the RT of Egyptian buffalo bulls was significantly reduced by treatment of bulls with different natural antioxidant as compared to the control group (El-Hawary, 2010). It is of interest to note that this reduction in RT was in association with increasing plasma testosterone concentration by about 73% as compared to control, reflecting effective role of GIN treatment on sexual desire (libido) of Egyptian buffalo bulls. The observed increase in plasma testosterone concentration may be due to the androgen biosynthesis as evidenced by a significant increase in blood testosterone in rats (Morakinyo *et al.*, 2008). Also, testosterone concentration significantly increased by oral treatment with GIN as compared to the control group (Afzali and Ghalehkandi, 2018). In general, several authors (Amr and Hamza *et al.* 2006; Rekha *et al.* 2010) indicated the androgenic action of GIN extract, in terms of increasing the production of spermatozoa in the seminiferous tubules and testosterone in semen of rats and serum testosterone (Mares and Najam, 2012). In similar parallel with increasing semen quality of buffalo-bulls, Morakinyo *et al.* (2008) found that aqueous GIN extract as an oral treatment significantly improved epididymal and testicular weights, epididymal sperm counts, and motility, viability and morphology of spermatozoa. Improving sperm production may be due to increasing in the absolute testicular and epididymal weights. This increase was associated with marked increase in testosterone concentration. The development, growth and normal function of the testis and the accessory sex glands in males are controlled by androgens and some authors showed a positive correlation between androgen level and weight of the testicular, epididymal, seminal vesicles and prostate (Setty *et al.*,

1977; Prins *et al.*, 1991). As affected by GIN, Kamtchouing *et al.* (2002) found improvement of weight of the testes and all accessory glands in association with increasing the level of testosterone in rats, which may be the reason of the observed increase in ejaculate volume of buffalo bulls treated with GIN. Zhang *et al.* (2001) reported the direct inclusion of testosterone in the development of spermatozoa, derangements in function of Leydig cells, and imbalance in steroidogenesis in the testes. So, the pronounced increase in the sperm characteristics of GIN treated buffalo bulls may be in relation with favorable increase in activity of spermatogenesis under control of increasing plasma testosterone concentration. It is well known that sperm maturation in the epididymis leads to increasing sperm viability and fertilizability acquisition. Therefore, GIN administration may improve the epididymal activity, which could have increased the progressive sperm motility of buffalo bulls as reported in rats by Morakinyo *et al.* (2008).

In accordance with improvement in semen quality by GIN treatment, Khaki *et al.* (2009) reported increased sperm viability and motility in rat treated with GIN for 20 consecutive days. In male albino rats, Donkor *et al.* (2018) observed significant increase in sperm count, motility, viability and morphology by administration of ethanolic GIN extract for 30 consecutive days. It is reported administration of 100 mg/kg of GIN increased motility, viability, and serum testosterone in rat (Khaki *et al.* 2009). Also, GIN increased sperm motility via protective effect as antioxidant (Amr and Hamza *et al.* 2006). These findings indicated that, GIN extract may be potentially useful in enhancing healthy sperm characteristics and the management of male infertility especially in those with low sperm count.

In accordance with the present results, Olayaki *et al.* (2007) found significant increase RBCs and WBCs counts and PCV in diabetic rats treated with GIN extract. The impact of GIN administration on the haematological parameters of buffalo bulls may be due to the effect of GIN as antioxidant on lowering the lipid peroxidation in RBCs membrane and decreasing RBCs susceptibility to hemolysis (Olayaki *et al.* 2007). However, the observed decrease in WBCs is involved in body defense system against infection (Ganong, 1999) which is disturbed due to the disturbed the function of WBCs (Yenigun, 1997). Although Afzali and Ghalehkandi (2018) found insignificant changes in plasma albumin and urea levels in rat treated with GIN extract, the present results on buffalo bulls resulted in improving protein metabolism in term of increasing the level of plasma total proteins and their fractions, and reducing urea-N and creatinine concentrations in GIN treated bulls. This may indicate the beneficial effects of GIN on increasing protein utilization and normality of liver and kidney functions. In this respect, Ajith *et al.* (2007) reported that GIN extract has strong antioxidant action on protection of nephron activity because GIN contains polyphenols and flavonoids. This action may be responsible for the observed reduction in plasma urea-N and creatinine levels in treated buffalo bulls. In agreement with the present results, Gholampour *et al.* (2017) reported marked improvement in the hepatic and renal functions, in rats treated with GIN hydroalcoholic extract. The protective effects of GIN on liver and kidney function could be in relation to the potentiality of GIN as an antioxidant to scavenge the free radicals. Also, Mallikarjuna *et al.* (2008) found that oral treatment with GIN ethanolic extract for 15-21 days significantly reduced activity of AST, ALT and tissue lipid peroxidation.

Generally, the several impacts of GIN may be due to that GIN contains many bioactive phytochemicals such as gingerols,

shogaols, paradols, gingerdiols, and zingerone (Baliga *et al.*, 2013). It has antioxidant, antiemetic, antithrombotic, antihepatotoxic, anti-inflammatory and cholagogue actions (El-Morsy Ibrahim and Al-Shathly, 2015). The GIN has the ability to stimulate the activity of enzymes of the antioxidant system such as super oxide dismutase, glutathione peroxides and catalase in rats (Khaki *et al.* 2009). The GIN extract contains Zingerone, gingerdiol, Zingiberene, gingerols and shogaols (Morakinyo *et al.* 2008), which reduced level of malondialdehyde (MDA), as a lipid peroxidation marker, in blood serum and the seminal fluid (Mares and Najam, 2012). In GIN, [6]-gingerol is endowed with strong anti-oxidant action both *in vivo* and *in vitro* (Kim *et al.*, 2007). Also, it has protective role in reproductive toxicities such as cyclophosphamide, cisplatin, malathion and diabetes (Riaz *et al.* 2017). Since years ago, there are increasing interests focus on verification of pharmacological and physiological actions in GIN, as a therapeutic agent (Daily *et al.* 2015). The obtained results indicated that GIN extract has pro-fertility properties. Also it has a beneficial effect on reproduction in Egyptian buffalo bulls.

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مستخلص الزنجبيل كمحفز لطلائق الجاموس منخفضة الخصوية

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هدفت هذه الدراسة إلى تقييم تأثير مستخلص الزنجبيل المائي على الأداء التناسلي لطلائق الجاموس. تم تقسيم ثمانية طلائق من الجاموس المصري منخفض الخصويه (وزن الجسم ينراوح بين 400-450 كجم وعمر 22-25 شهر) إلى مجموعتين (ن = 4 في كل منهما). تم تغذية المجموعة الأولى على النظام الغذائي الأساسي بدون أي معاملة، ولكن تم رش الطليق بـ 100 مل من الماء المقطر أثناء التغذية (مجموعه ضابطه) وتم تغذية الطلائق في المجموعة الثانية بنفس النظام الغذائي الأساسي مع رش العليقه بالمستخلص المائي للزنجبيل (200 مل من مستخلص الزنجبيل المذاب في 100 مل/كجم من العليقه). وكانت الفترة التجريبية لمدة 4 شهور (2 شهر كفترة أولية و 2 شهر كفترة جمع السائل المنوي). تم جمع السائل المنوي مرتين في الأسبوع لمدة 8 أسابيع. تم أخذ عينات الدم في نهاية التجربة. أوضحت نتائج الدراسة ان معاملة العليقه بمستخلص الزنجبيل المائي أدى الى: -1تحسن معنوي لكل من حجم العلقه والنسبه المنويه لأنواع الحركة التقدميه، الحيويه، سلامة الغشاء، التركيز، الإنتاج الكلي للحيوانات المنويه مع انخفاض معنوي في السواد وتحطم الاكرووسوم. -2انخفاض معنوي في زمن رد الفعل وزيادة تركيز هرمون التستوستيرون في بلازما الدم بنسبة 103.7 %، -3زياده معنويه في عدد كرات الدم الحمراء، حجم المكونات الخلويه للدم، تركيز الهيموجلوبين مع انخفاض معنوي في عدد خلايا الدم البيضاء. -4. زيادة معنويه في تركيز البيروتينات الكلية والألبومين والجلوبيولين في بلازما الدم، وانخفاض معنوي في تركيز اليوريا والكرياتينين. -5انخفاض معنوي في نشاط إنزيم AST و ALT بشكل ملحوظ. وتوصي الدراسة بان رش العليقه ب 200 ملجم من مستخلص الزنجبيل المائي/100 مل ماء مقطر من الزنجبيل حسن الكفاءة التناسليه وجودة السائل المنوي لطلائق الجاموس ذات الكفاءة التناسليه المنخفضة.