Uptake of exogenous bovine GH–pmKate2–N expression vector by rams spermatozoa

Waleid Mohamed El-Sayed Shakweer[1*], Yassien Mohamed Hafez[2,3], Ashraf El-Sayed[2,3], Sherif Mohamed Dessouki[2,3], Ibrahim Mohamed Awadalla[1] and Mamdouh Ibrahim Mohamed[1]

Abstract

Background: Sperm-mediated gene transfer (SMGT) is a technique that utilizes the ability of the spermatozoa to take up exogenous DNA. Growth hormone is anabolic hormone that plays an important role in muscle-building process and milk production in all animals. High blood concentration of growth hormones (GH) was observed for animals that were genetically selected for high milk production or for low carcass fatness levels. The present study aimed to investigate and enhance the capacity of ovine spermatozoa to uptake exogenous growth hormone cDNA and its impact on sperm motility. The current study is an introduction for further future studies to produce transgenic Egyptian sheep characterized with high productive performance.

Methods: The growth hormone cDNA sequence was extracted from pituitary gland of Egyptian × Holstein (EH_GH) cattle and subcloned into the pmKate2–N vector to construct the EH_GH–pmKate2–N expression vector. The complete sequence of EH_GH mRNA was registered in GenBank (AC: KP221576). A total of three groups were assessed for the sperm uptake experiment, namely, negative control, positive control, and dimethyl sulfoxide (DMSO) groups; all treated groups were incubated with the EH_GH–pmKate2–N vector. The expression of EH_GH protein was detected in DH10B cells using a fluorescence microscope and the SDS polyacrylamide gel electrophoresis.

Results: The EH_GH–pmKate2–N vector was expressed in cultured *Escherichia coli* cells, and the molecular weight of EH_GH protein was 24,558 Da. The EH_GH–pmKate2–N vector was introduced efficiently into the heads of the spermatozoa in the DMSO and positive control groups. Incubation of the spermatozoa with the vector caused a significant reduction in progressive motility compared to the negative control.

Conclusion: The present results demonstrated the ability of ovine spermatozoa to take up the exogenous vector without notable deleterious effects on sperm motility. In subsequent studies, the successful introduction of the exogenous GH expression vector into the sperm head allows for the production of GH-transgenic sheep characterized by a high growth rate in order to reduce the meat shortage in Egypt.

Keywords: Bovine growth hormone, pmKate2–N expression vector, Sperm uptake

Background

As far as I know, this study is considered as one of the few studies that were carried out in Egypt to examine and enhance the ability of sperm to uptake exogenous cDNA and its effect on sperm motility. In addition, this study is an introduction to produce genetically modified sheep with high production performance. The growth hormone gene plays an important role in growth, regulation of metabolism, and milk production (Horvat and Medrano 1995; Bauman 1999 and Lagziel et al. 1999). The cows and sheep that genetically were selected for high milk production and low carcass fatness respectively have owned high concentrations of blood growth hormone (GH) (Bonczek et al. 1988; Francis et al. 1995). Transgenesis is a promising tool for producing genetically modified animals for use in medical disease, biotechnology, and basic science researches (Menchaca et al. 2016). A sperm-mediated gene transfer (SMGT) technique aims

* Correspondence: wm.shakweer@nrc.sci.eg
[1]Animal Production Department, Agricultural and Biological Research Division, National Research Centre, 33 El Bohouth St. (Former El-Tahrir St.), Dokki, Giza, P.O. 12622, Egypt
Full list of author information is available at the end of the article

to utilize the ability of spermatozoa to take up and transfer the exogenous DNA into the oocyte at the fertilization process. Lavitrano et al. (1989) reported that the circular DNA can be introduced into spermatozoa using simple incubation. The efficiency of SMGT depends on sperm viability and progressive motility (Lavitrano et al. 2006). Foreign DNA uptake by spermatozoa has been studied in many species like ram (Castro et al. 1991), bull (Atkinson et al. 1991), cattle and chicken (Shemesh et al. 2000), salmon (Sin et al. 2000), shell fish (Tsai 2000), zebrafish (Khoo 2000), pigs (Lavitrano et al. 2002) and rabbit (Shen et al. 2006).

The sperm membrane prevents foreign DNA entry into the sperm cytoplasm in SMGT (Maione et al. 1997). Efforts have been made to increase the uptake rate of foreign DNA by spermatozoa, such as electroporation (Rieth et al. 2000 and Collares et al. 2011), DNA nano-carriers like liposomes (Kim et al. 2010), magnetic nano-particle (Shen et al. 2006), and dimethyl sulfoxide (DMSO) (Zhao et al. 2012). DMSO is an aprotic solvent that has physiological and technical characteristics of replacing the water in cells, is an effective cryoprotectant, and enhances the permeability of lipid membranes so that exogenous DNA can transport across the sperm membranes (Jacob and Herschler 1986). The present study was designed to investigate and enhance the capacity of ovine spermatozoa to uptake the exogenous EH_GH–pmKate2–N vector and its impact on sperm motility. Hence, we will be able in the future study to produce GH-transgenic local sheep characterized by a superior growth rate as a step toward reducing the meat shortage in Egypt.

Methods
Semen collection, evaluation, and cryopreservation
Three adult Rahmani rams were used for semen collection twice per week. The physical semen characteristics were evaluated according to Hafez and Hafez (2000). In brief, the semen volume (ml) was recorded based on the gradual scale of the collection tube; pH was determined using pH paper; total motility was scored from 0 to 5, where 0 is when the sperm is motionless and 5 is a very good wave motion; and the percentage of advanced motility was determined by diluting a small drop of semen with a drop of saline on a clean glass slide covered with a cover glass then examined under a microscope to estimate the advanced motility of sperm along a linear track.

The percentages of abnormalities and dead and live sperm were calculated by staining a drop of semen with a drop of Eosin-Nigrosine stain which was then smeared on a slide microscope. About 100 spermatozoa were classified as dead sperm if the red stain passed through the head sperm membrane and as live sperm if not stained. The sperm concentration ($\times 10^9$/ml) was calculated using the hemocytometer method. The semen was diluted 100 times (10 μl of semen diluted with 990 μl eosin solution) and then vortexed. The mixture was transferred to a hemocytometer chamber for sperm counting.

Tris-based extender was used to dilute semen samples for final concentration of 250×10^6/ml as described by Fukui et al. (2008). Tris-based extender consisted of 297.58 mM Tris, 96.32 mM citric acid, 82.66 mM fructose, 5% (v/v) glycerol, 15% (v/v) egg yolk (Fukui et al. 2008), and 500 μl/ml gentamycin according to Vivanco and Alarcon (1987). The diluted semen was cryopreserved according to Matsuoka et al. (2006). In brief, the diluted semen was cooled gradually to 4 °C for 2–3 h. The cooled semen was packaged in 0.25-ml straws and kept at 4 °C before freezing. They were exposed to liquid nitrogen (LN2) vapor (– 125 to – 130 °C) for 3–4 min then plunged into LN2 (– 196 °C) and stored in LN2 until used for a sperm uptake experiment. The cryopreserved straws were picked randomly for evaluation.

Isolation of bovine growth hormone cDNA
Total RNA was isolated from the pituitary gland of an Egyptian Holstein crossbred (EH) cattle using the Booze reagent kit (Bioflux®, USA), as previously described by Chomczynski (1993). Briefly, total RNA was extracted from quick-frozen anterior pituitary tissue. About 30 mg of tissue was homogenized for 60 s with 1 ml Trizol reagent (Invitrogen, USA). About 200 μl chloroform was added and mixed and incubated at room temperature for 5 min then centrifuged at 12,000g for 15 min. The aqueous phase was transferred into a new tube and about 500 μl isopropanol and incubated at – 20 °C for 10 min then centrifuged at 12,000g for 10 min. The pelleted RNA was washed with 700 μl (70% ethanol) and then centrifuged at 12,000g for 10 min. The pelleted RNA was air-dried and dissolved in 50 μl of elution buffer. The RNA quality and quantity were determined using agarose gel electrophoresis and NanoDrop. The isolated RNA was converted into cDNA using the Oligo dT18 and RevertAid First Strand cDNA Synthesis Kit (Fermentas, Canada), as previously described by Nagarajan et al. (2017). In brief, the total RNA purity and integrity was determined using gel electrophoresis before using for cDNA synthesis. The first step of the cDNA synthesis is the removal of genomic DNA from RNA samples which was done in the indicated order: 1 μg RNA, 1 μl 10× Reaction Buffer with MgCl$_2$, 1 μl DNase I, and 10 μl nuclease-free water. The mixture was incubated at 37°C for 30 min. For reaction termination, the mixture was incubated at 37°C for 30 min with 1 μL EDTA (50 mM). and incubated at 65 °C for 10 min. The second step is the first-strand cDNA synthesis that was achieved with the following reaction: 5 μg RNA

template, 1 μl Oligo (dT)18 primer, and nuclease-free water up to 12 μl incubated at 65 °C for 5 min. The vial was chilled on ice, spun down, and placed back on ice. The following components are indicated in the following order: 4 μl 5× Reaction Buffer, 1 μl RiboLock RNase Inhibitor (20 U/μl), 2 μl 10 mM dNTP Mix, 1 μl RevertAid M-MuLV RT (200 U/μl), and nuclease-free water up to 20 μl, mixed gently and centrifuged briefly and incubated for 60 min at 42 °C. The reaction was terminated by heating at 70 °C for 5 min. The reverse transcription reaction product can be directly used in polymerase chain reaction (PCR) applications or stored at − 70 °C. The EH_GH cDNA primer was designed with two restriction sides, SacI and SacII. The EH_GH_F primer was 5′-GAGCTCCAGGGTCCTGTGGACAGC-3′, and the EH_GH_R primer was 5′-CGCGGTGCGATGCAATTTCCTCAT-3′. PCR reaction was done as follow; denaturation at 94 °C for 1 min, annealing at 57 °C for 2 min and extension at 72 °C for 3 min for 29 cycles, and a final extension at 72 °C for 7 min were done. The PCR product was then electrophoresed.

Expression vector construction

The pmKate2–N is a mammalian expression vector (4700 bp) that encodes red fluorescent protein (Evrogen, Cat. No. FP182, Russia), according to Haas et al. (1996). The pmKate2–N vector (4700 bp) backbone is shown in Fig. 1. The purified EH_GH cDNA sequence and closed

pmKate2–N expression vector were digested with SacI and SacII restriction enzymes by incubating for 30 min at 37 °C (Jean Bioscience GmbH, Germany) as described by Kuspa (2006). The digested EH_GH cDNA sequence was ligated into the digested pmKate2–N vector in a reaction mixture that contained 1 μl of digested pmKate2–N vector, 2.5 μl of digested EH_GH cDNA, 2 μl of 10× ligase buffer, 1 μl T4 DNA ligase, and sterile water up to 20 μl and was incubated for 1 h at 22 °C (Cherepanov and de Vries 2001). The constructed EH_GH–pmKate2–N vector was transformed to DH10B cells (Fermentas®, USA, Rand 1996) and extracted from DH10B cells according to Birnboim and Doly (1979). In brief, the previous prepared competent DH10B cells were taken from − 80 °C and thawed on ice. A total of 2.5 μl of EH_GH–pmKate2–N vector was added to 50 μl of the chilled cells, mixed and incubated on ice for 5 min. The mixture of vector and cells was exposed to heat shock at 42 °C for 90 s and then chilled again on ice for 2 min. One milliliter of LB medium was added and incubated for 1 h at 37 °C. About 50 μl was spread on pre-warmed LB (Luria-Bertani broth) agar plates supplemented with Kanamycin X-Gal/IPTG and incubated overnight at 37 °C. About 10 ml of LB medium supplemented with Kanamycin was inoculated with a white single colony which was picked from a fresh plate and incubated at 37 °C overnight. About 5 ml of the overnight cultured cells was harvested by centrifugation at 6500g for 2 min.
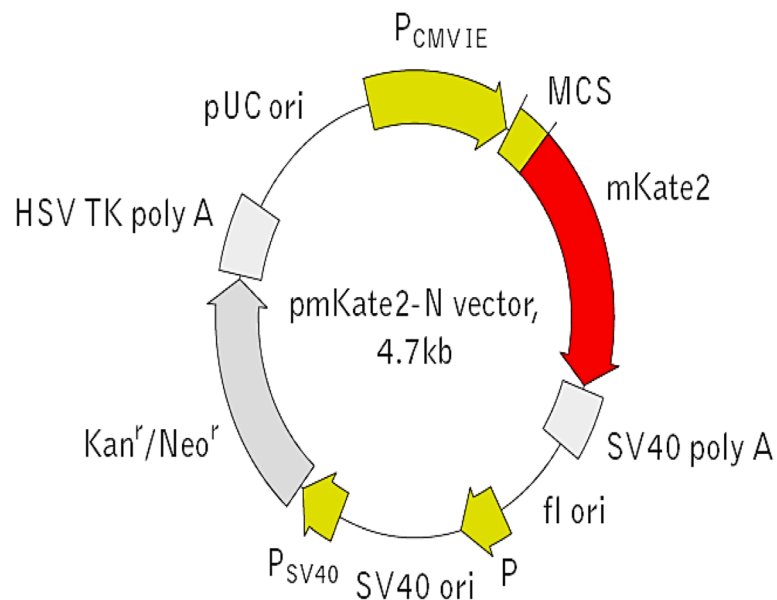
Fig. 1 The backbone of the pmKate2–N expression vector. MCS, multiple cloning site for cloned insertion; mKate2, far-red fluorescent protein; PCMV IE, immediate early promoter of cytomegalovirus; SV40 ori, origin for replication in mammalian cells; pUC ori, origin of replication for propagation in *E. coli*; f1 ori, origin for single-stranded DNA production; SV40 poly A, polyadenylation signals; PSV40, early promoter provides neomycin resistance gene (Neo^r) expression to select stably transfected eukaryotic cells using G418; P, bacterial promoter provides kanamycin resistance gene expression (Kan^r) in *E. coli*; Kanr/Neor, gene is linked with herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signals (Shcherbo et al. 2009)

The pelleted cells were re-suspended with 250 μl of re-suspension solution and lysed with 250 μl lysis solution. The lysed cells were neutralized with 350 μl of neutralization solution and centrifuged at 19,700g for 5 min. The supernatant was transferred into a spin column and centrifuged at 19,700g for 1 min. About 50 μl of elution buffer was added to the center of the spin column and centrifuged at 19,700g for 2 min. The purified plasmid DNA has been stored at − 20 °C.

Expression of the EH_GH–pmKate2–N vector into DH10B transformed cells

The well-transformed colonies were spread with sterile water drop on clean microscopic slides. The smear was allowed to air dry and then embedded in ethanol acetic acid solution (3:1) for 10 min. After fixation, the slide was washed using deionized water and allowed to air dry according to Chao and Zhang (2011). The slide was investigated for pmKate red fusion protein expression using an AxioImager.Z2 fluorescence microscope under a 62HE BFP/GFP/HcRed filter with excitation of 633 nm and emission of 588 nm.

SDS polyacrylamide gel electrophoresis (SDS-PAGE) for EH_GH protein

SDS-PAGE was performed to determine the relative molecular weight of the expressed EH_GH protein (24,558 Da) into DH10B cells according to Weber and Osborn (1969). Briefly, the transformed DH10B cells were prepared by inoculating one colony into 5 ml LB medium/ Kanamycin and incubated at 37 °C overnight. Afterwards, the cultured medium was centrifuged at 1600g for 5 min then re-suspended with 500 μl of two X-loading buffers. The sample was heated in boiling water for 3 min, allowed to cool at room temperature, and centrifuged at 2500g for 10 min to remove any insoluble materials. The 12% separating gel and 4% stacking gel were prepared. Twenty microliters of the sample was loaded into the wells, and the electrophoresis carried out at 15 mA for 7–8 h. The gel was stained with Coomassie blue R$^{− 250}$ for at least 4 h at room temperature and distained for at least 3 h by distaining solution several times at room temperature until the background became clear.

Semen preparation

The cryopreserved semen straws were thawed in a water bath for 1 min at 37 °C. The pooled semen samples (7 straws) were assayed using the computer-assisted sperm analysis (CASA) instrument (Sperm Vision™ software MiniTube, version 3.0, USA) connected to an Olympus BX 51 microscope (Olympus, Japan). The pooled semen with high percentages of progressive motility, live sperm, straightness, and linearity was washed three times using 500 μl of 0.9 NaCl to remove the seminal plasm by centrifugation at 1600g for 5 min according to Lavitrano et al. (2006). The washed semen was re-suspended with Tris extender to achieve a final concentration of 40×10^6 spermatozoa and then assayed using CASA. Sperm motion parameters, including total motility (%), progressive motility (%), distance curved line (DCL; μm), distance average path (DAP; μm), distance straight line (DSL; μm), velocity average line (VAP; μm/s), velocity curved line (VCL; μm/s), velocity straight line (VSL; μm/s), straightness (STR = VSL/VAP, %), linearity (LIN = VSL/ VCL, %), wobble (WOB = VAP/VCL), amplitude of lateral head displacement (ALH; μm), and beat cross frequency (BCF; H2), were recorded.

Sperm uptake experiments

A total of 160 μl of ovine sperm cell suspension (40×10^6 sperm) was incubated with 200 ng of EH_GH–pmKate2– N vector (Kuznetsov et al. 2000). Three experimental groups were assigned: negative control group (sperm cells incubated for 1 h at 37 °C without vector), positive control group (sperm cells incubated for 1 h at 37 °C with vector), and dimethyl sulfoxide (DMSO) group (sperm cells incubated with vector and supplemented with 1% DMSO, then vortexed and incubated for 10 min at room temperature and supplemented with 2% DMSO, then incubated for 1 h at 37 °C). All incubated groups were washed three times with 500 μl of 0.9% NaCl to remove the unbound EH_ GH–pmKate2–N vector by centrifugation at 1600g for 5 min. The sperm motion parameters were assessed for the washed semen groups using CASA.

Recognition of the pmKate2–N vector in the spermatozoa

The genomic DNA of the spermatozoa was extracted according to Jerzy et al. (2003). In brief, about 200 μl of semen was digested with 2 μl of proteinase K (25 mg/ml) and 50 μl of 10% sodium dodecyl sulfate (SDS) then incubated at 37 °C for 60 min. About 250 μl of the chloroform:phenol:isoamyl alcohol mixture (25:24:1) was added to 250 μl of digested sperm and centrifuged at 19, 700g for 5 min. The supernatant was transferred into a new tube, and 2.5 volume of 96% ethanol and 1/10 volume of 3 M sodium acetate (pH, 5.2) were added and mixed thoroughly then incubated overnight at − 20 °C. The DNA was pelleted by centrifugation at 19,700g for 20 min at 4 °C. The pelleted DNA was washed with 200 μl ethanol (70%) and centrifuged at 19,700g for 5 min then dried and diluted in 30 μl Tris-EDTA (TE) buffer and stored at − 20 °C. The specific primer was designed for the pmKate2–N red fusion protein sequence to confirm the presence of the pmKate2–N vector (690 bp) in the extracted genomic DNA of sperm cells. The forward primer was 5′-CCACTCGCTCGACTAATTCC-3′, and the reverse primer was 5′-GATCCCTCCAGCGTCATAGA-3′. The PCR condition was denaturation at 94 °C for 1

min, annealing at 59 °C for 30 s, and extension at 72 °C for 3 min, and then final extension for 5 min at 72 °C after 35 cycles. The PCR product was then electrophoresed.

Statistical analysis

The collected data of the sperm motion parameter after sperm uptake experiments were analyzed using the general linear model (SAS 2000) according to the following model:

$$Y_{ij} = \mu + T_i + E_{ij}$$

Y_{ij} = the observation ij

μ = Overall mean

T_i = Treatment ($i = 1$, negative control group, $i = 2$, positive control group, $i = 3$, DMSO group).

E_{ij} = Experimental error, i observation assumed to be randomly distributed ($0-6^2$).

The differences among means were tested (Duncan 1955).

Results

Growth hormone cDNA isolation and subcloning

The electrophoretic band of the EH_GH cDNA was 740 bp (Fig. 2). The uncut forms of EH_GH–pmKate2–N vectors are shown in Fig. 3. Three out of five forms appeared for the uncut EH_GH–pmKate2–N expression vector. The PCR electrophoresis band of the EH_GH–pmKate2–N vector is shown in Fig. 4.
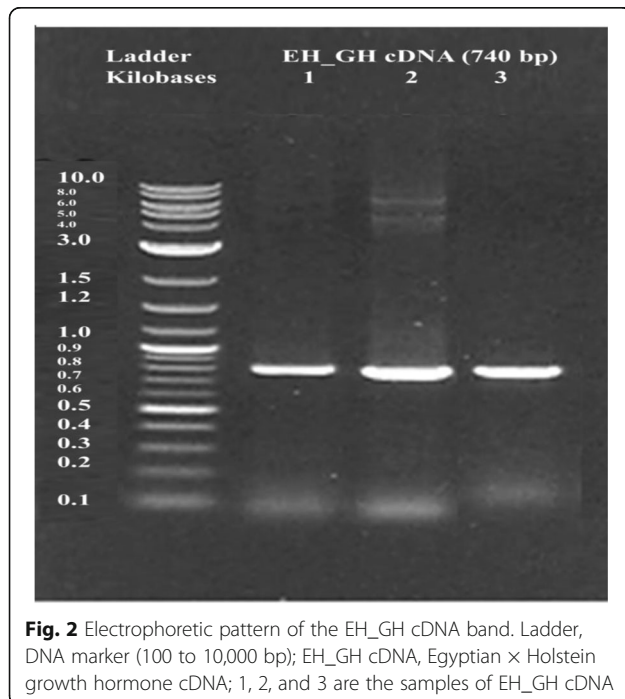
Fig. 2 Electrophoretic pattern of the EH_GH cDNA band. Ladder, DNA marker (100 to 10,000 bp); EH_GH cDNA, Egyptian × Holstein growth hormone cDNA; 1, 2, and 3 are the samples of EH_GH cDNA

Expression of the EH_GH–pmKate2–N vector and SDS-PAGE

The expression of the EH_GH–pmKate2–N vector clearly appeared in DH10B cells (Fig. 5). SDS-PAGE electrophoresis illustrated that the EH_GH protein was expressed into DH10B cells, and the molecular weight of the EH_GH protein was 24,558 Da (Fig. 6).

Sperm uptake of the EH_GH–pmKate2–N vector

The semen characteristics before cryopreservation were 84.2% for advanced motility, 369×10^9 for sperm concentration, 4.8% for abnormalities, and 81.8% for live sperm. The PCR electrophoresis of genomic spermatozoa after incubation illustrated that the EH_GH–pmKate2–N vector was introduced efficiently into the heads of spermatozoa in positive control and DMSO groups (Fig. 7). The EH_GH–pmKate2–N vector was spontaneously taken up by the spermatozoa in the positive control group after incubation for 1 h at 37 °C.

CASA

The effects of EH_GH–pmKate2–N vector incubation and treatment on sperm motility traits are shown in Table 1. The total sperm motility did not differ significantly in the positive control group compared to the negative control group (64.7% vs. 64.0%, respectively). In addition, the total sperm motility was increased ($P < 0.05$) in the DMSO group compared to those in the positive and negative control groups. However, the progressive motility was decreased ($P < 0.05$) by 3.4% in the positive control group compared to that in the negative control group (48.4% vs. 51.8%, respectively); this is due to the adverse effect of incubation of the EH_GH–pmKate2–N vector with sperm cells. Furthermore, the total and progressive motilities were enhanced ($P < 0.05$) by the addition of DMSO in the DMSO group compared to all other groups. In the positive control group, the incubation of spermatozoa with the EH_GH–pmKate2–N vector was reduced significantly in VSL, VCL, ALH, STR, and LIN parameters compared to that in the negative control. The WOB value and STR percentage in the DMSO group were decreased significantly compared to those in the positive control and negative control groups.

Discussion

Growth hormone cDNA isolation and subcloning

In the current study, three out of five forms appeared for the uncut EH_GH–pmKate2–N expression vector. Torsten et al. (1999) reported that there are five uncut plasmid forms: nicked open-circular, relaxed circular, linear, supercoiled, and supercoiled denature. The PCR electrophoresis band (740 bp) of the EH_GH–pmKate2–N vector (Fig. 4) indicated that the sequence of EH_GH cDNA was subcloned into pmKate2–N vectors successfully.
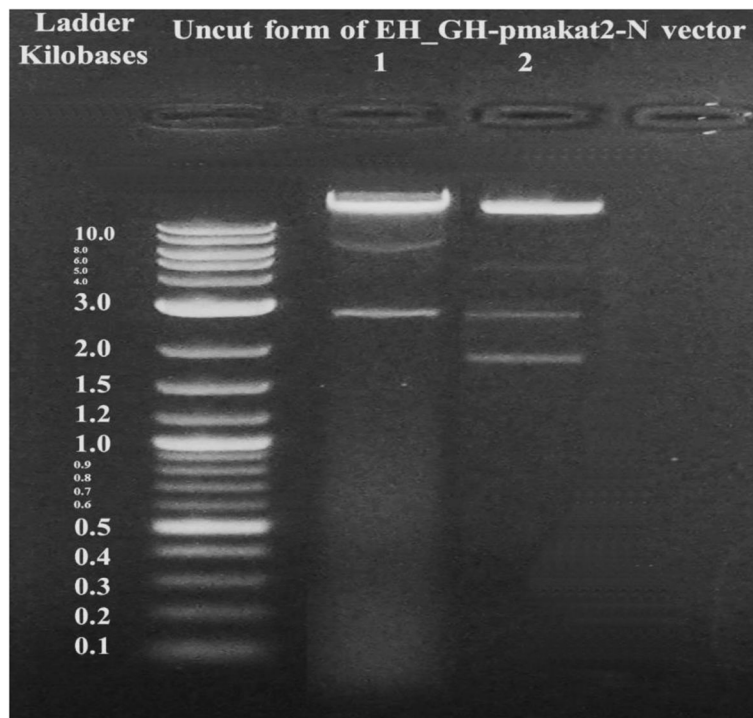
Fig. 3 Electrophoretic pattern of the uncut EH_GH–pmKate2–N expression vector. Ladder, DNA marker (100 to 10,000 bp); 1 and 2 are the samples of the uncut EH_GH–pmKate2–N expression vector

Expression of the EH_GH–pmKate2–N vector and SDS-PAGE

The expression of the EH_GH–pmKate2–N vector in DH10B cells (24,558 Da) was confirmed under a fluorescence microscope and also using SDS-PAGE electrophoresis. The production of GH protein has been reported previously in *Escherichia coli* cells, and the molecular weight of bovine growth pre-hormone was 24,562 Da (Miller et al. 1980; Gerald and Hansen 1986; Uchida et al. 1997 and Nam-Kyu et al. 1998). This expression indicated the ability of the EH_GH sequence to express in the origin host (mammalian cells) or in embryos when using transfected spermatozoa for embryo production in future studies.

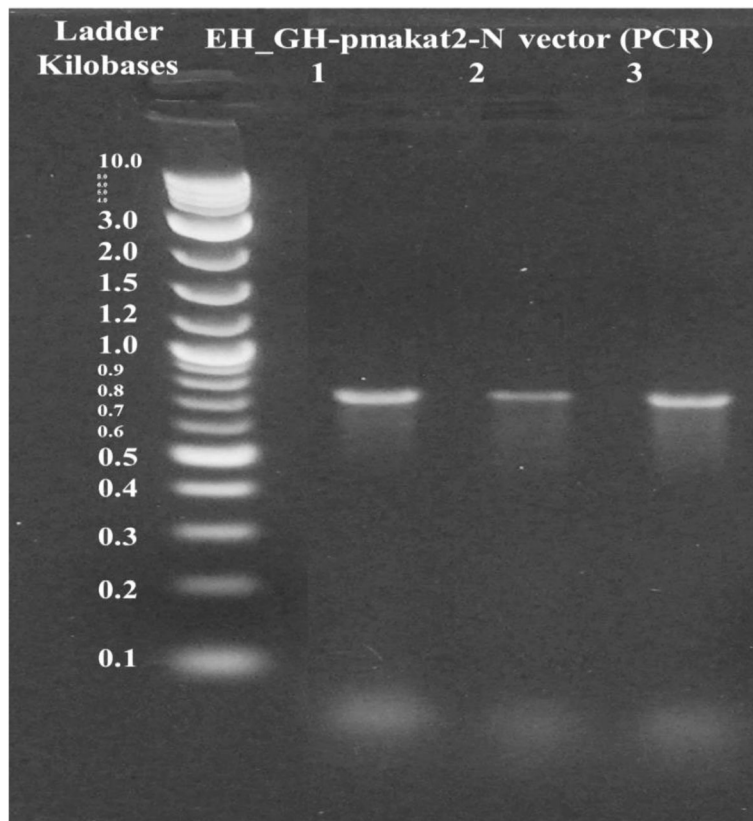### Sperm uptake of the EH_GH–pmKate2–N vector

The semen characteristics before cryopreservation were within the normal range as reported previously by Shakweer (2008). The PCR electrophoresis of genomic spermatozoa after incubation illustrated that the EH_GH–pmKate2–N vector was introduced efficiently into the head of sperm in the positive control and DMSO groups. DMSO is an aprotic solvent that has physiological and technical characteristics of replacing the water in cells, is an effective cryoprotectant, and enhances the permeability of lipid membranes so that the exogenous DNA can transport across the sperm cell

membranes (Jacob and Herschler 1986). Many studies have supported the ability of DMSO to enhance the ability of spermatozoa to uptake the exogenous DNA (Kuznetsov and Kuznetsova 1995; Kuznetsov et al. 2000; Li et al. 2006; Collares et al. 2011; Zhao et al. 2012).

CASA

The cryopreserved semen straws were pooled and washed to remove the seminal plasma inhibitory factors that may compete with DNA on the same binding sites of the sperm surface (Sasaki et al. 2000). The function of inhibitory factors might protect the spermatozoa from the dangerous molecules as exogenous DNA. The seminal plasma in most mammalian species contain inhibitory factors such as polyamines and glycosaminoglycans which are molecules that strongly bind to negatively charged molecules, such as DNA (Lee et al. 1985; Setchell and Brooks 1988). Shen et al. (2006) reported that the green fluorescent protein has been expressed in embryos produced by rabbit spermatozoa treatment with 3% DMSO. Collares et al. (2011) reported that treatment of spermatozoa with 3% DMSO could be an efficient method for transfection in chickens. This enhancement in total and progressive motilities may be due to the effect of the physiological characteristics of DMSO.
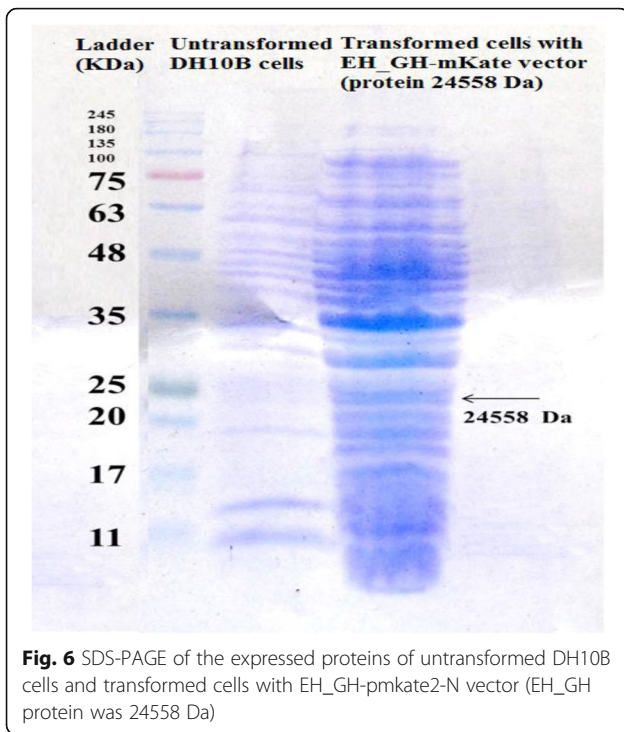
Sebastian et al. (2010) reported that certain sperm motions such as VSL (44.06% vs. 64.80%), VAP (69.39% vs.
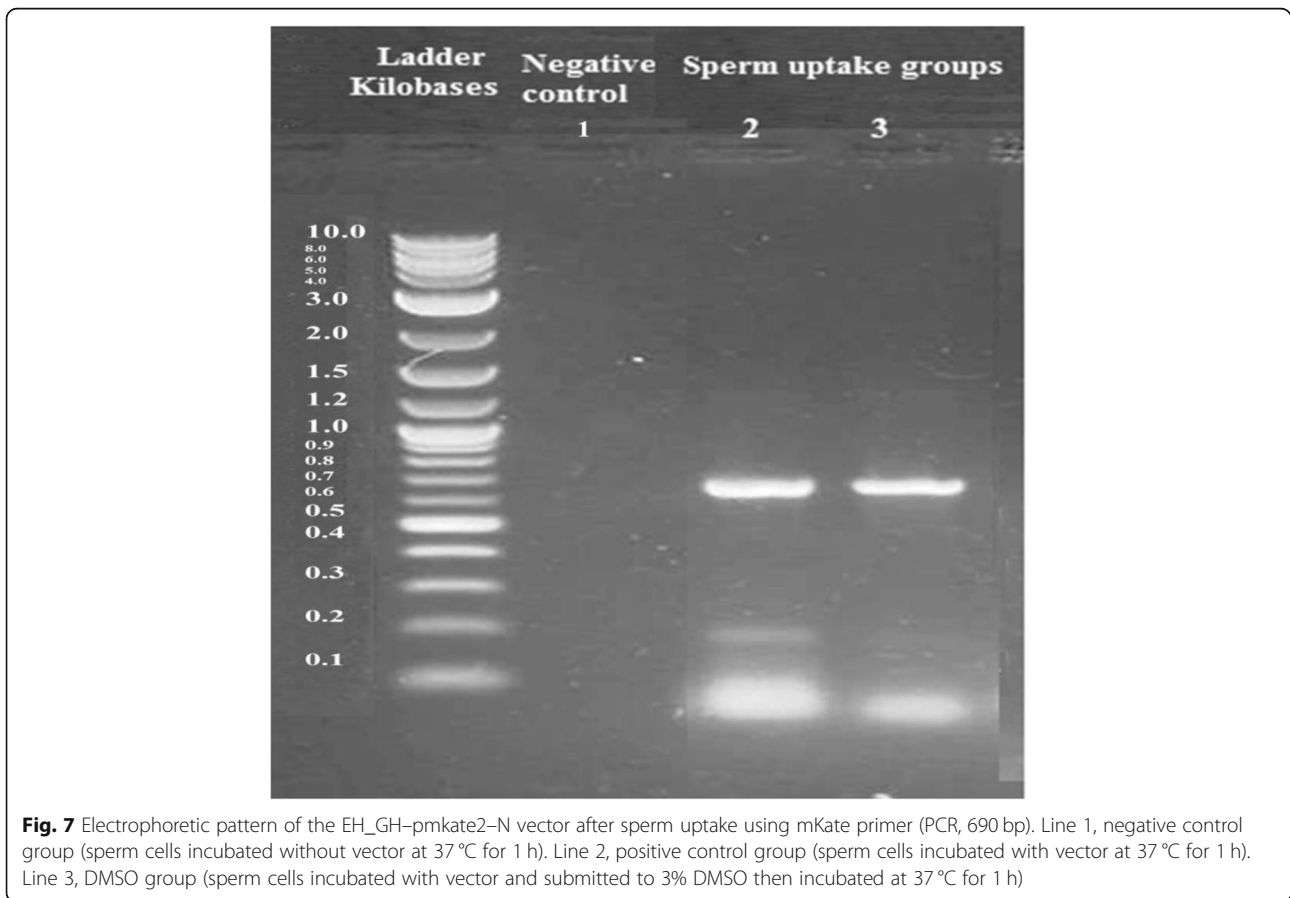
Fig. 4 Electrophoretic pattern of the EH_GH–pmKate2–N vector band (PCR, 740 bp). Ladder, DNA marker (100 to 10,000 bp); 1, 2, and 3 are the samples of the EH_GH–pmKate2–N vector
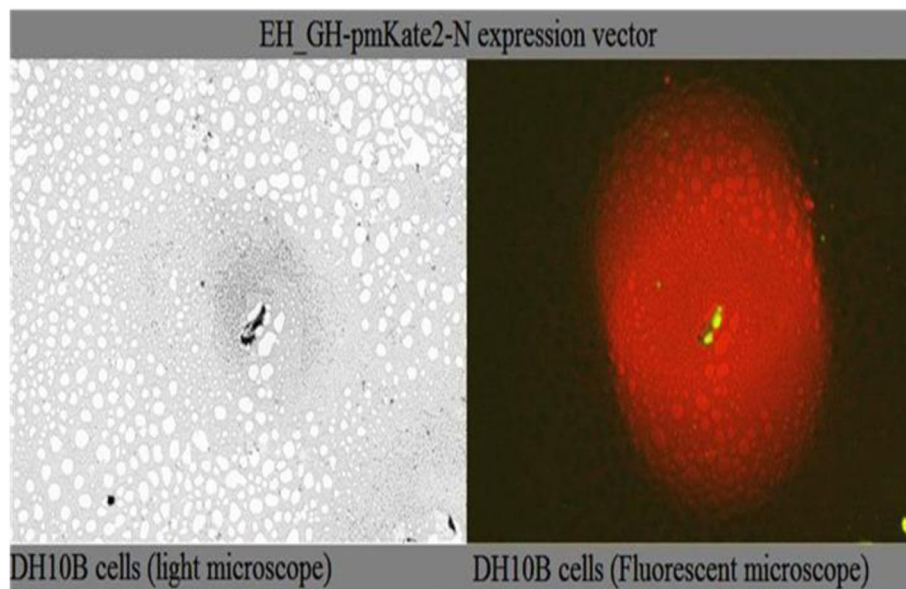
Fig. 5 Expression of the EH_GH–pmKate2–N vector in transformed DH10B cells under a fluorescence microscope. Left side: the image of the transformed DH10B cell under a light microscope. Right side: the image of the same field of the transformed DH10B cell under a red fluorescent filter (excitation 588 nm and emission 633 nm)

Fig. 6 SDS-PAGE of the expressed proteins of untransformed DH10B cells and transformed cells with EH_GH-pmkate2-N vector (EH_GH protein was 24558 Da)

89.31%), LIN (37.96% vs. 52.86%), and SRT (61.68% vs. 71.69%) were reduced ($P < 0.05$) after semen incubation with exogenous DNA compared to the control group. They concluded that such reductions in VSL, VAP, LIN, and SRT did not affect the ability of the sperm to fertilize the oocyte in vitro. The semen parameters, including VSL, VCL, ALH, STR, and LIN, were positively correlated with bull fertility (Farrell et al. 1996 and Perumal et al. 2011). On the other hand, the binding exogenous DNA reduces sperm viability as a result of the endonuclease activation process in head sperm as a natural protection that prevents the exogenous DNA to transfer to the offspring (Spadafora 1998; Anzar and Buhr 2006). The motility and velocity parameters of the spermatozoa reflect their mitochondrial function and energy status indirectly. The higher values of VCL and ALH indicate that there is a major bending of the sperm middle piece and large amplitude of lateral head displacement. These characteristics signify the hyperactivation of the spermatozoa, which in turn implies a high energy state of the spermatozoa, which is essential for sperm penetration through the cervical mucus and zona pellucida, fusion with the oocytes, and successful fertilization (Aitken et al. 1985).

Fig. 7 Electrophoretic pattern of the EH_GH–pmkate2–N vector after sperm uptake using mKate primer (PCR, 690 bp). Line 1, negative control group (sperm cells incubated without vector at 37 °C for 1 h). Line 2, positive control group (sperm cells incubated with vector at 37 °C for 1 h). Line 3, DMSO group (sperm cells incubated with vector and submitted to 3% DMSO then incubated at 37 °C for 1 h)

Table 1 Effect of the EH_GH–pmKate2–N vector uptake on sperm motility (mean ± SEM)

Parameters	*Negative control	EH_GH–pmKate2–N expression vector groups	
		**Positive control	‡‡DMSO
Total motility (%)	64.0 ± 0.8[b]	64.7 ± 0.6[b]	66.5 ± 0.6[a]
Progressive motility (%)	51.8 ± 0.8[b]	48.4 ± 0.8[c]	52.7 ± 0.7[a]
DAP (μm)	23.8 ± 0.3[a]	23.1 ± 0.3[a,b]	22.9 ± 0.3[b]
DCL (μm)	38.0 ± 0.4[a]	36.5 ± 0.5[b]	37.4 ± 0.4[a,b]
DSL (μm)	20.6 ± 0.3[a]	20.0 ± 0.4[a,b]	19.6 ± 0.2[b]
VAP (μm/s)	51.8 ± 0.6[a]	50.0 ± 0.8[b]	49.6 ± 0.6[b]
VCL (μm/s)	82.6 ± 0.9[a]	78.9 ± 1.0[c]	81.0 ± 0.6[b]
VSL (μm/s)	44.7c ± 0.6[a]	43.1 ± 0.8[b]	42.5 ± 0.5[b]
STR (VSL/VAP, %)	0.86 ± 0.00[a]	0.85 ± 0.0[b]	0.84 ± 0.00[c]
LIN (VSL/CCL, %)	0.54 ± 0.00 [a]	0.54 ± 0.0[a]	0.52 ± 0.00[b]
WOBa (VAP/VCL)	0.62 ± 0.00[a]	0.62 ± 0.0[a]	0.61 ± 0.00[b]
ALH (μm)	2.9 ± 0.04[a]	2.8 ± 0.03[b]	2.8 ± 0.02[b]
BCF (H2)	28.8 ± 0.2[a]	27.0 ± 0.28[b]	28.5 ± 0.24[a]

Mean values with different superscript letters within the same row differ (P < 0.05). *Negative control, sperm cells incubated at 37 °C for 1 h without vector. **Positive control, sperm cells incubated at 37 °C for 1 h with vector. ‡‡DMSO group, sperm cells incubated with vector and submitted to 3% DMSO then incubated at 37 °C for 1 h. DAP distance average path (microns), DCL distance curved line (microns), DSL distance straight line (microns), VAP velocity average line (microns/s), VCL velocity curved line (microns/s), VSL velocity straight line (microns/s), STR straightness (VSL/VAP), LIN linearity (VSL/VCL), WOB wobble—describes side to side movement of the sperm head (VAP/VCL), ALH amplitude of lateral head displacement (microns), BCF beat cross frequency (H2)

Conclusion

The current results demonstrated the ability of ovine spermatozoa to take up the exogenous EH_GH–pmKate2–N expression vector in treated groups without notable deleterious effects on sperm motility, especially in the DMSO group. In subsequent studies, the successful introduction of the exogenous GH expression vector into the sperm head allows the production of GH-transgenic sheep characterized by a high growth rate in order to reduce the meat shortage in Egypt.

Abbreviations

ALH: Amplitude of lateral head displacement; BCF: Beat cross frequency; bp: Basepair; CASA: Computer-assisted sperm analysis; cDNA: Complementary DNA; DAP: Distance average path; DCL: Distance curved line; DMSO: Dimethyl sulfoxide; DSL: Distance straight line; EH_GH cDNA: Egyptian × Holstein growth hormone cDNA; f1 ori: Origin for single-stranded DNA production; GH: Growth hormone; HSV: Herpes simplex virus; Kan^r: Kanamycin resistance gene expression; LIN: Linearity; MCS: Multiple cloning site; mKate2: Far-red fluorescent protein; NaCl: Sodium chloride; Neo^r: Neomycin resistance gene; P: Bacterial promoter; PCMV IE: Immediate early promoter of cytomegalovirus; PCR: Polymerase chain reaction; poly A: Polyadenylation signals; PSV40: Early promoter; pUC ori: Origin of replication for propagation in E. coli; SDS-PAGE: Sodium dodecyl sulfate polyacrylamide gel electrophoresis; SMGT: Sperm-mediated gene transfer; STR: Straightness; SV40 ori: Origin for replication in mammalian cells; SV40 poly A: Polyadenylation signals; TK: Thymidine kinase; VAP: Velocity average line; VCL: Velocity curved line; VSL: Velocity straight line; WOB: Wobble

Author details
[1]Animal Production Department, Agricultural and Biological Research Division, National Research Centre, 33 El Bohouth St. (Former El-Tahrir St.), Dokki, Giza, P.O. 12622, Egypt. [2]Department of Animal Production, Faculty of Agriculture, Cairo University, 7 Gamaa Street, Giza, 12613, Egypt. [3]Cairo University Research Park (CURP), Faculty of Agriculture, Cairo University, Giza, Egypt.

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