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Original article

Use of immunocontraception in the regulation of male goat sexual activity

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Abstract

This study focused on continuous monitoring of the immunocontraceptive effect of Improvac® vaccine on the sexual activity of male goats determined by measuring plasma testosterone levels, testicular biometric and ejaculate examination. The animals in the experimental group (n=12) were administered two doses of 2 ml of Improvac® at a four-week interval; the animals in the control group (n=5) received 2 ml of saline. Blood collection, semen collection and testicular measurements were performed at 14-day intervals. A total of 8 samples were collected from each animal. In 9 animals a significant decrease ($p<0.05$) in testosterone concentration was observed two weeks after the first dose. At the end of the experiment (16 weeks), eight goats reached a testosterone concentration below the detection limit and one goat had a concentration of 0.47nmol/L. The testicular size was significantly ($p<0.01$) smaller four weeks after the first dose. At the end of the experiment, the testicular size was approximately three times smaller ($p<0.001$). Motility was 0% in two goats at the end of the experiment, 1% in one animal and 10% in one animal. The median sperm concentration was significantly lower ($p<0.01$) at the end of the study. A significant ($p<0.0001$) shift in the percentage of morphological changes was recorded eight weeks after the first administration. At the end of the study, there were five animals with azoospermia, two with 100% morphologically altered sperm, one with 99% and one with 96% morphologically altered sperm. In the three male goats, a significant skin reaction occurred after the first application, which resulted in an inadequate response to the treatment. Our results show that Improvac had a significant effect on the sexual function and sperm production in 9 out of 12 male goats.

Keywords: buck, Improvac, immunocontraception, sperm

Introduction

Castration of males of various domestic species is a common veterinary practice used worldwide to improve the quality of meat, accelerate growth and metabolism, prevent antagonistic behaviour in males and ultimately to control fertility (Lofthouse and Kemp 2002). The conventional surgical method of castration is being replaced by other, less invasive methods, due to increasing consumer awareness of animal welfare in livestock production around the world. Immunological methods involving vaccination against GnRH represent animal-friendly alternatives to surgical castration in animals (Thompson 2000, Janett et al. 2012a,b). Improvac, the first available vaccine used in the swine industry, was developed in Australia in 1998 (Zoetis). Improvac® contains a synthetic GnRH analogue conjugated to the diphtheria toxoid in the form of an aqueous adjuvant (Earl at al. 2006, Wicks et al. 2013).

The efficacy of GnRH vaccination with Improvac has been demonstrated in numerous studies in pigs. According to the results of previous studies, two vaccinations with Improvac four weeks apart significantly reduced testicular weight, boar taint, concentration of steroid hormones and improved carcass quality (Dunshea et al. 2001, Jaros et al. 2004, Zamaratskaia et al. 2008, Pauly et al. 2009). The use of immunocastration in cattle is also relatively well documented (Janett et al. 2012a, Janett et al. 2012b, Bolado-Sarabia et al. 2018, Monleón et al. 2020, Yamada et al. 2021). There is quite a lot of information on the effect of immunocastration in rams, but studies in goats are rare. The influence of immunocastration on testicular development and histology has been investigated in ram lambs using a variety of vaccines and has been successful in reducing testicular growth and seminiferous tubule development (Kiyma et al. 2000, Ülker et al. 2002, Ülker et al. 2005, Needham et al. 2019b). Although Improvac® decreases testicular size for at least 3 months after the second vaccination in lambs (Janett et al. 2003), it also reduces the scrotal circumference, the semen quality and the viability of sperm in immunocastrated lambs (Needham et al. 2019b).

Limited data are available on immunocastration in male goats. Improvac® vaccination significantly reduced antagonistic interactions among males, reduced testicular circumference and testosterone concentration in male goats (Bishop et al. 2016). One of these studies observed long-term effects (17 months) of Improvac® on testicular size, concentration of testosterone, quality of ejaculate and male odour intensity (Giriboni et al. 2020). Other immunocastration agents have also been studied in male goats. The Bopriva® vaccine (two doses four weeks apart) was effective and resulted in immuno-sterilisation expressed by suppression of testicular development and the lowering/diminishing in viable sperm concentration after the second administration regardless of the dose volume (Lents et al. 2018). Based on the results of previous studies, it is clear that immunocastration of small ruminants has a great potential. In particular, it is necessary to focus on continuous monitoring of reproductive status, i.e. on changes in the quality of ejaculate and its fertility potential.

The aim of this study was therefore to assess the effect of Improvac® on the reproductive performance of male goats, mainly on the suppression of testosterone production, and the subsequent effects on semen quality, sperm viability and morphology.

Materials and Methods

Animal selection

The experiment was carried out on male goats of the White Shorthair breed (*Capra aegagrus hircus*) housed at the Ruminant and Swine Clinic, University of Veterinary Sciences, Brno. The animals were purchased from a commercial goat dairy farm. The goats were given one month to recover from travel and to acclimatise to the new conditions. The goats were randomly selected and divided into the experimental (n=12) treated group (TG) and the control group (CG) (n=8). They were fed good quality roughage in the form of hay and ad libitum feed pellets and mineral blocks, with free access to water until the beginning of the experiment. They received 1 mL of vaccine providing protection against clostridium infection (Covexine; Zoetis Belgium SA, Louvain-La-Neuve, Belgium) administered subcutaneously, and a drench for coccidia, administered orally (diclazuril, Vecoxan; Elanco GmbH, Cuxhaven, Germany). Both were repeated four weeks after the initial doses. In the control group, three animals were excluded due to unsuitability for the experiment (2x testicular atrophy, 1x spermiogranuloma). In the experimental group, a marked skin reaction was observed in three goats after the first administration of the Improvac® vaccine. Despite booster application, these goats did not respond adequately to the treatment and were also excluded from the statistical evaluation at the end of the experiment. Thus, a total of five goats remained in the CG and nine goats in the TG. The experiment was started with goats aged 5-6 months, body weight 32.89±3.84 (± s.e.m.; range: 26.0-40.0 kg) at the beginning of the breeding season (beginning of September).

Experimental design

The animals in the experimental group were administered the GnRH vaccine (GnRH analogue and protein conjugate, Improvac®; Zoetis Belgium SA, Louvain-la-Neuve, Belgium) 2 mL (min. 300μg of conjugate in 2ml) subcutaneously, twice in four weeks. Prior to the first administration, venous blood was collected to determine testosterone concentration; testicular length and width were measured to determine the testicular size, and electroejaculation was performed to obtain sperm for the subsequent assessment. All these procedures were performed at 14-day intervals, 8 times in total. The same sampling protocol was performed in the control group, except that the saline solution was administered instead of the vaccine.

Blood sampling and hormone assay

Blood samples for testosterone (T) concentration assessment were collected from the jugular vein into plastic tubes with blood clotting granules and allowed to clot for 24 hours at room temperature, and then centrifuged. The serum was then stored at -20°C until the testosterone measurement using Chemiluminescent Microparticle Immunoassay (Alinity 2nd Generation Testosterone Reagent Kit 07P68, Abbott GmbH, Wiesbaden, Germany). The detection limit of the assay was 0.45 nml/L.

Measurement of the testicular size

The testicular length (a) and width (b) (cm) (including scrotal skin) of the left and right testes were measured using a caliper. The size of the testes was calculated, using the equation: area (cm^2) = ½a · ½b · π. The absolute testicular size was calculated as the sum of the values for the left and right testicles.

Semen collection

Semen production and quality were assessed by semen collection using an electroejaculator with a rectal probe (length 14.5cm, 2.6 cm diameter) with three longitudinal electrodes (Electro Ejaculator e320, Minitube GmbH, Tiefenbach, Germany). The procedure was performed under general inhalation anaesthesia with isoflurane and gas mixture (air and oxygen) with premedication with butorphanol (0.02 mg/kg body weight) (Butomidor, VetViva Richter GmbH, Wels, Austria) and xylazine (0.2 mg/kg body weight) (Xylazin, Ecuphar N.V., Oostkamp, Belgium). A default electroejaculator preset for small ruminants was chosen for semen collection. The initial current of 0V was incrementally raised by 0.5 with a pause of 2s. At the first sign of ejaculation, the current increase was stopped and when a sufficient amount of ejaculate was reached, the procedure was terminated. The 10V limit was never exceeded.

Semen evaluation

The collected semen was evaluated macroscopically and microscopically for semen volume and the presence of spermatozoa; sperm concentration, motility, progressive sperm motility, sperm vitality (sperm with integral membrane function) and morphologic examination were determined. Immediately after the semen collection, the ejaculated semen volume and gross motility was assessed using a light microscope (BA 310E-Bino, MoticOptical, Wetzlar, Germany). The sperm concentration was then determined using a Bürker counting chamber. The semen sample (200μl) was diluted with a TRIS-citrate extender of the same amount and stored at 4°C until the procedure was completed in the last animal. After semen collection from the last animal all samples were rewarmed to 38º C, diluted with Sperm Thyrodes lactate extender according to the rough density estimate obtained at the time of semen collection, and analysed using a computerised sperm analyser (version 12 Ceros, Hamilton Thorne, Beverly, USA). The overall motility and progressive motility were assessed. In addition, two sperm slide samples were prepared; one was stained with nigrosin-eosin to determine the sperm cell membrane integrity; the second was stained with Farelly staining to analyse the percentage of sperm with normal morphology. In both cases, 100 cells were counted per sample.

All experiments were approved by the Institutional Animal Care Committee (experiment No. 8-2020, MSMT-23924/2020-2).

Statistics

Statistical analysis was performed using R software (2021). Comparison of the treatment and control groups was carried out using a linear mixed-effects model with REML estimates. In all cases, the response depended on the group, time, and their interaction (as fixed effects), while the identifier of the buck was treated as a random effect. The differences were considered statistically significant at $p<0.05$.

Results

Adverse effects

After the first administration of the vaccine, lameness was observed in about half of the animals on the forelimb where the vaccine had been administered

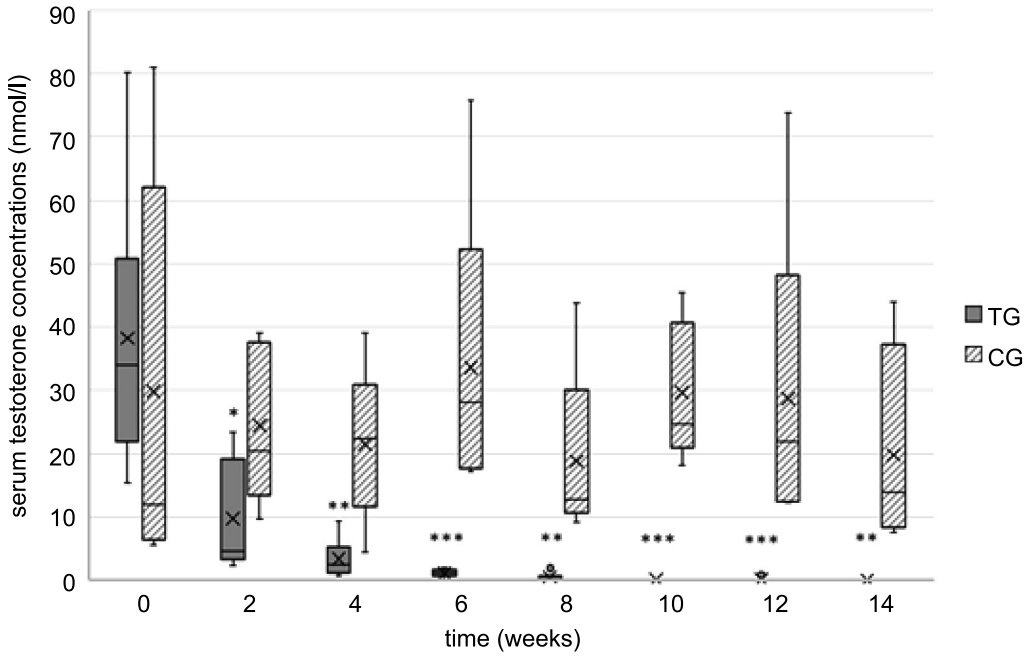

Fig.1. Serum testosterone concentrations (nmol/l) of immunocastrated goats (TG, n=9) vaccinated at week 0 and week 4 and intact control goats (CG; n=5). Rectangles represent 25-75% of the values, bars the remaining values, horizontal lines in rectangle median, cross in rectangle average, * statistically significantly different values compared to the initial value, * $p<0.05$, ** $p<0.01$, *** $p<0.0001$.

subcutaneously. In some individuals, this lameness persisted for three days. Marked necrotic skin changes at the injection site were also observed in three goats. The necrotic changes healed spontaneously, but a lower response to the vaccination was observed during the experiment, the goats had a mean sperm concentration of 1.5×10^6 and still measurable testosterone concentrations at the end of the experiment, one of which even increased significantly (18.31 nmol/L). Despite a certain suppressive effect on the spermatogenesis, evidenced by an increased percentage of morphologically impaired spermatozoa, we decided to exclude those animals from the statistical evaluation.

Serum testosterone levels

A statistically significant decrease ($p<0.05$) in testosterone concentration was observed in the TG as early as two weeks after the first dose. Ten weeks after the beginning of the experiment, eight goats had testosterone concentrations below the detection limit and one goat had a testosterone concentration of 1.19 nmol/L. At the end of the experiment this one goat had a concentration of 0.47 nmol/L. The testosterone concentration in the CG remained unchanged. There were significant differences in testosterone concentrations in the CG, both between individual animals and between individual samples. These inter-animal differences were also noted in the TG in the samples obtained at the first three examinations (Fig. 1).

Testicular biometry

Testicular size in the TG was statistically significantly ($p<0.01$) reduced four weeks after the first administration. The testicle size was approximately three times smaller at the end of the experiment (67.56±8.2 vs. 25.45±4.22). The reduction in the testicular size was also evident in the CG, statistically significant ($p<0.01$) from 8 weeks after the first dose, but this reduction at the end of the experiment (62.11±8.07 vs. 54.28±6.35) was not as demonstrably significant as in the TG (Fig. 2).

Sperm parameters

Sperm concentration in the TG decreased slightly from the beginning of the experiment and was statistically significantly lower ($p<0.05$) 12 weeks after the start of the experiment. Three goats were azoospermic, five goats had a concentration below 0.15×10^6 sperm, and one animal still had a relatively high concentration of 0.45×10^6 sperm. At the end of the experiment, the sperm concentration was statistically significantly lower ($p<0.01$) (0.79×10^6 vs. 0.09×10^6). Five goats were azoospermic, two goats had a concentration below 0.01×10^6 sperm, one goat 0.23×10^6 sperm and one 0.58×10^6 sperm. The sperm concentration in the CG remained unchanged (Fig. 3).

Motility, as determined by the CASA analyser, first showed a slight increase and then a gradual decrease, reaching 0% motility in two goats, 1% in one animal and 10% in one animal at the end of the experiment.

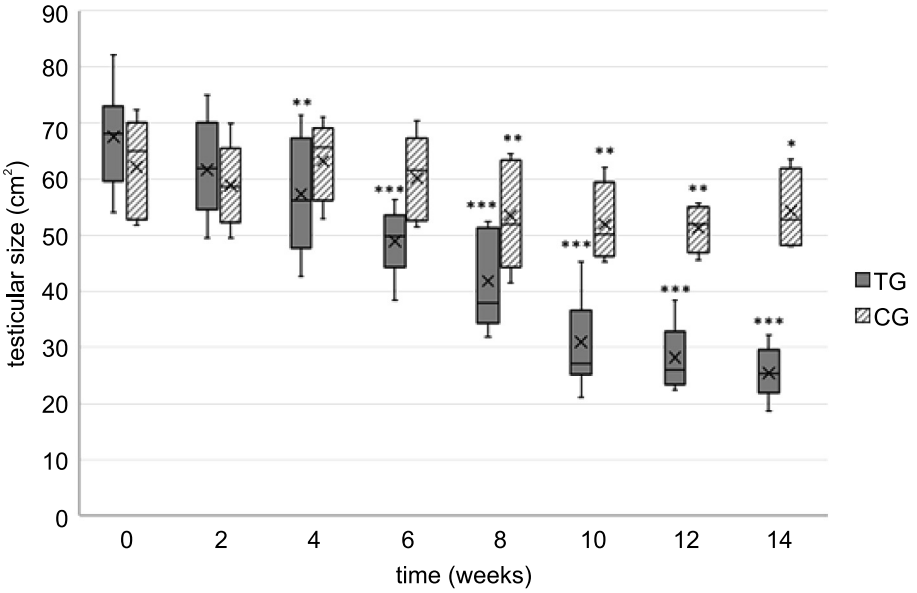

Fig. 2. Testicular size (cm^2) of immunocastrated goats (TG, n=9) vaccinated at weeks 0 and week 4 and intact control goats (CG; n=5). Rectangles represent 25-75% of the values, bars the remaining values, horizontal lines in rectangle median, cross in rectangle average, * statistically significantly different values compared to the initial value, * p<0.05, ** p<0.01, *** p<0.0001.

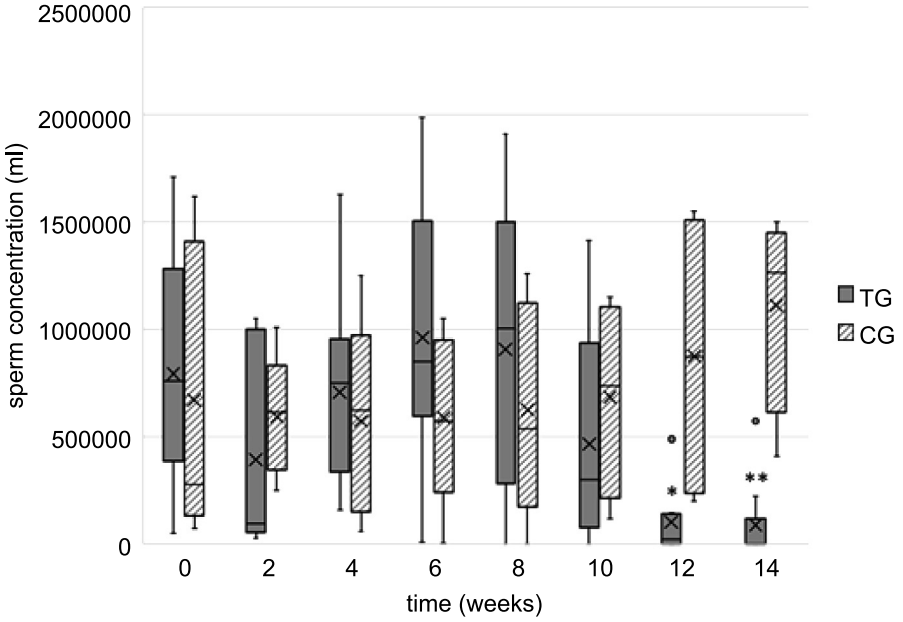

Fig. 3. Sperm concentration of immunocastrated goats (TG, n=9) vaccinated at week 0 and week 4 and intact control goats (CG; n=5). Rectangles represent 25-75% of the values, bars the remaining values, horizontal lines in rectangle median, cross in rectangle average, * statistically significantly different values compared to the initial value, * p<0.05, ** p<0.01.

Five goats were azoospermic. However, there was no statistically significant and systematic dependence of motility on vaccine administration and time. Large inter-animal differences were observed in the TG. A statistically significant decrease (p<0.05) was observed in the CG, but this decrease was not systematic (at two weeks, six weeks and 14 weeks after the beginning of the experiment) and was probably due to the small number of the control group (Fig. 4).

A similar trend to the motility was observed for the progressive straightforward movement in the TG, where a significant decrease was recorded at the end of the experiment (19.67±24.64 vs 0.25±0.5), but this decrease was not found to be statistically significant. In the CG, only a slight decrease in the last sample was observed, but even this was not found to be significant. (Fig. 5)

Sperm morphology also changed significantly during the experiment in the TG. Statistically significant (p<0.0001) morphological changes were observed 8 weeks after the first application. At the end of the experiment there were five animals with azoospermia,

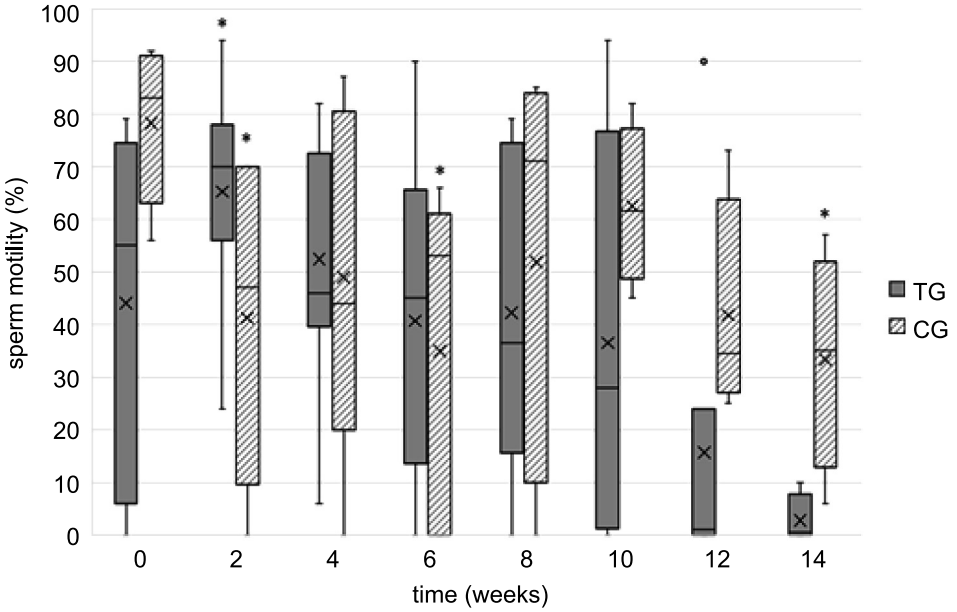

Fig. 4. Sperm motility of immunocastrated goats (TG, n=9) vaccinated at week 0 and week 4 and intact control goats (CG; n=5). Rectangles represent 25-75% of the values, bars the remaining values, horizontal lines in rectangle median, cross in rectangle average, * statistically significantly different values compared to the initial value.

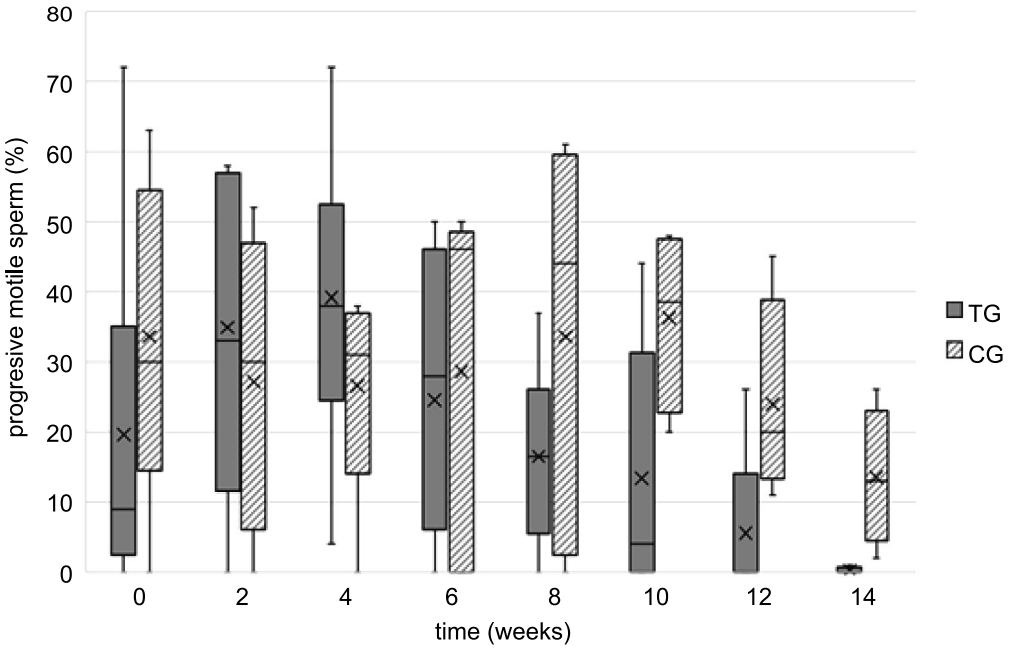

Fig. 5. Percentages of progressive straightforward sperm movement of immunocastrated goats (TG, n=9) vaccinated at week 0 and week 4 and intact control goats (CG; n=5). Rectangles represent 25-75% of the values, bars the remaining values, horizontal lines in rectangle median, cross in rectangle average.

two with 100% morphologically altered sperm, one with 96% morphologically altered sperm and two with 99% morphologically altered sperm in the TG (Fig. 6). We also observed changes in the frequency of individual defects in the TG during the experiment. To assess the dynamics of the occurrence of individual morphological changes, we divided all the observed defects into groups, namely: midpiece abnormalities, acrosome abnormalities, abnormal heads, abnormal tails, proximal droplets and loose heads. At the beginning of the experiment, tail defects and acrosome abnormalities predominated. On the other hand, at the end of the experiment, head abnormalities and loose heads were most frequently detected. In the CG, the sperm morphology was about 15% of morphologically altered sperm. A statistically significant decrease was observed only 6 and 14 weeks after the start of the experiment.

A statistically significant increase ($p<0.05$) was observed in sperm with integral membrane function in the TG two weeks after the start of the experiment,

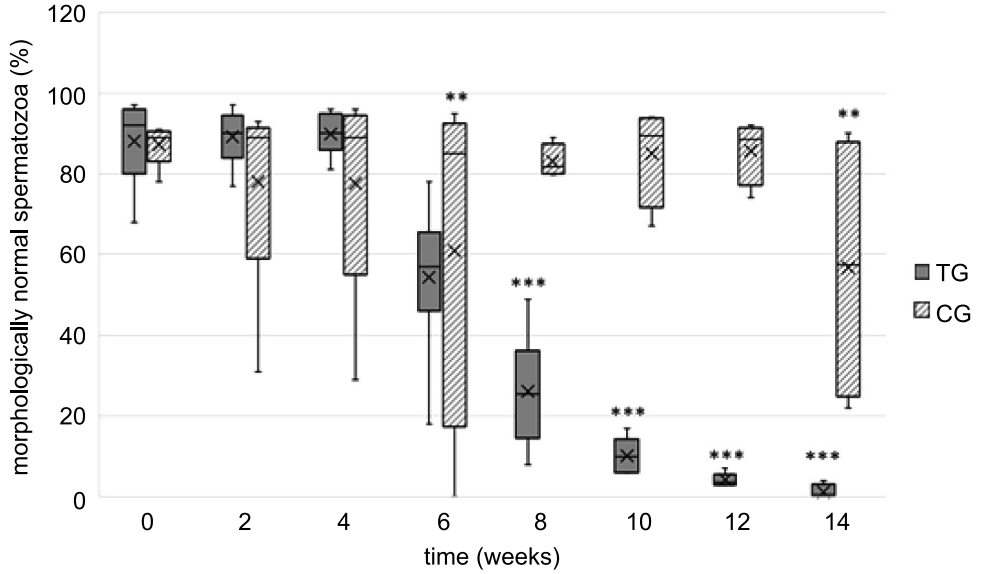

Fig. 6. Percentage of morphologically normal spermatozoa of immunocastrated goats (TC, n=9) vaccinated at week 0 and week 4 and intact control goats (CG; n=5). Rectangles represent 25-75% of the values, bars the remaining values, horizontal lines in rectangle median, cross in rectangle average, * statistically significantly different values compared to the initial value, ** p<0.01, *** p<0.0001.

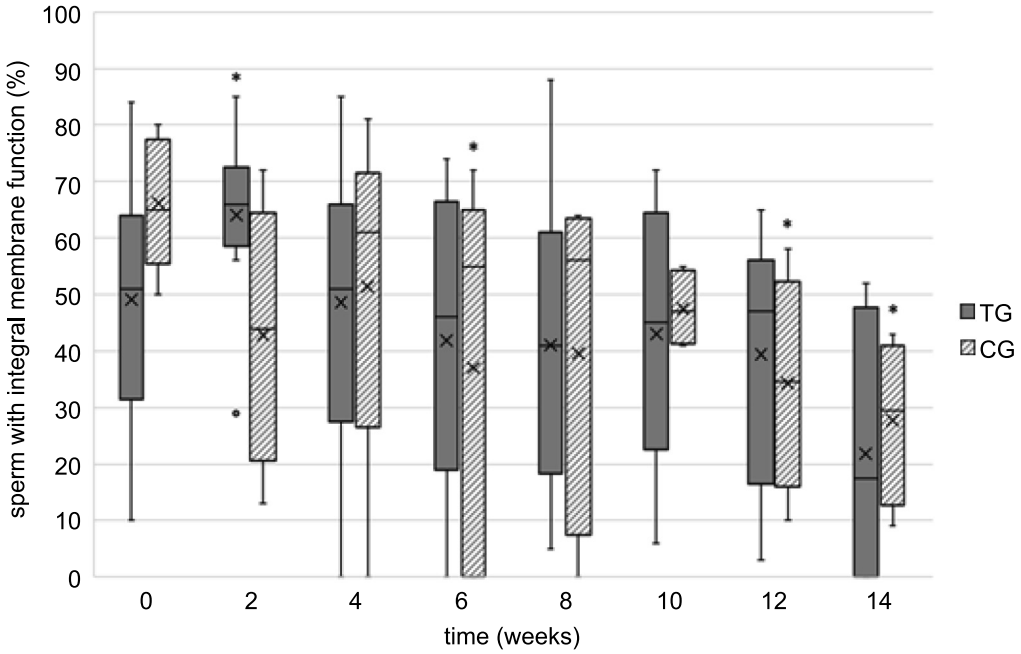

Fig. 7. Percentage of sperm with integral membrane function of immunocastrated goats (TG, n=9) vaccinated at week 0 and week 4 and intact control goats (CG; n=5). Rectangles represent 25-75% of the values, bars the remaining values, horizontal lines in rectangle median, cross in rectangle average. * Statistically significantly different values compared to the initial value.

followed by a very slow decrease which was not significant at the end of the experiment (49±23 vs 22±26). Significant changes ($p<0.05$) were also observed in the CG, but these were non-systematic (8, 14 and 18 weeks) and were again probably caused by an insufficient number of animals in the CG (Fig. 7).

Discussion

Immunocastration with a diphtheria toxoid-conjugated synthetic GnRH analogue (Improvac®) has not been well studied in small ruminants, therefore, only limited data on the effect of Improvac® treatment in goats is available (Bishop et al. 2016, Giriboni et al. 2020). For this reason, not many post-administration effects have been reported in small ruminants, as opposed to bulls, where much more research has been con-

ducted (Lents et al. 2018), describing post-vaccination apathy and a mild sensitivity at the vaccination site. In our study, we observed lameness in several goats after the first dose of the vaccine and marked skin necrosis at the injection site in three goats. These goats had a significantly slower onset of the vaccine effect compared to the other animals. The skin reactions may have been caused by improper strict subcutaneous administration and possible diphtheria toxoid reaction. Therefore, if such reactions occur after administration, the animal cannot be considered neutered and must be castrated using a standard method.

The results showed a clear statistically significant decrease in testosterone concentration. As described by Han et al. (2015), the decreased serum concentrations of LH, FSH, and testosterone significantly limit spermatogenesis, resulting in aspermia and azoospermia. According to the manufacturer of Improvac, testosterone levels in boars should decrease after the second vaccine. However, in our study, there was a significant decrease ($p < 0.05$) already 2 weeks after the first dose (38.22±20.45 vs. 9.74±8.54). Similar results were reported by Bishop et al. (2016). They found a slight decrease in serum testosterone levels of vaccinated goat bucks as early as 14 days after the first administration of Improvac and a significant decrease after 30 days. Significant individual differences between animals (43.95 vs. 7.48) were found in the present study, similar to Giriboni et al. (2020), who also found a significant decrease in testosterone levels as early as one month after the first dose. However, the return to the initial levels occurred within 3 months after the vaccination. In our study, the concentration was still at basal levels after 4 months. Janett et al. (2012b) investigated the effect of the GnRH vaccine on pre-pubertal bulls. In vaccinated calves, the testosterone concentrations decreased to values below 0.5 ng/ml serum after the booster injection and remained below the detection limit for at least 22 weeks. Theubet et al. (2010) found similar results in pre-pubertal bulls, although the testosterone levels remained low for only 10 weeks. In the present study, there were no statistically significant differences in the testosterone levels in the control group. However, a slight decrease in the testosterone levels was observed at the end of the experiment, which could be explained by the end of the mating season (end of December). The LH and testosterone concentrations, libido, and odour presence in the bucks peak in the autumn during the breeding season (Mickelsen and Memon 1997). A gradual decrease in testicular size was also observed as early as 4 weeks after the first dose, depending on the decrease in testosterone concentration. Ferro et al. (2004) stated that the reduction in the GnRH production, and subsequent reduction in the FSH and LH levels caused by the efficient blockade of the hypothalamo-pituitary axis causes a reduction in the synthesis and release of testosterone, resulting in germinal epithelium atrophy. Another effect is a reduction in the number of Sertoli cells and germline cells, which not only promotes the nuclear condensation and cytoplasmic atrophy of Leydig cells, but also directly affects spermatogenesis and consequently the semen quality. Ülker et al. (2005, 2009) tested an anti-GnRH vaccine in goats and sheep, and observed a reduced scrotal circumference in goats 8 weeks after the first dose, and 4 weeks later in sheep. A similar finding was reported by Bishop et al. (2016), where a statistically significant decrease ($p < 0.05$) was observed 4 weeks after the start of the experiment, and at 8 weeks the testicle size in the experimental group was 18% smaller than in the control group. In our experiment we found a significant decrease in testicular size even in the CG, which can be attributed to the end of the breeding season. Although the effect of Improvac has previously been tested in lambs (Needham et al. 2019b) and bucks (Bishop et al. 2016), we still lack data on the effects on semen production and quality in bucks. In a recent study, Giriboni et al. (2020) studied the effect on spermatogenesis, but the ejaculate collection was only performed at 1-month intervals. The effect of Improvac on spermatogenesis in rams was monitored in a similar way, but conversely, the effect was only observed in the course of 6 weeks after the second vaccination (Needham et al. 2019a). In our study, the parameters for the semen obtained prior to the administration of the anti-GnRH vaccine show that the animals were suitable for reproduction. The values presented are within the recommended physiological range: volume between 0.5 and 1.5 mL, sperm motility between 70 and 90%, morphologically normal sperm between 70 and 90% (Edmondson et al. 2007). Only the sperm concentration was lower ($0.793 \pm 0.550 \cdot 10^9$/mL), due to the age of the animals (6 months), the reference range should be between 2 and $5 \cdot 10^9$/mL. Our study demonstrated effective inhibition of reproductive function in goats by immuno-sterilisation with the anti-GnRH vaccine. This was characterised by a reduction in the testosterone concentration, by a reduction in the testicular size, and by a spermatogenic suppression, expressed as a reduction or total absence of spermatozoa in the ejaculate, a decrease in motility and sperm viability. These changes are associated with reduced testosterone and decreased testicular size, as this reduction affects sperm production due to the reduced germinal epithelium, caused by the blockade of the hypothalamic-pituitary-gonadal axis (Lents et al. 2018). The most statistically significant changes in our project were observed in sperm morphology. Considering that sper-

miogenesis lasts 6-8 weeks in small ruminants (Edmondson et al. 2007), the cessation of testosterone production should be reflected in changes in the ejaculate quality within two months. In our study, a significant decrease in testosterone concentration was clear already two weeks after the first vaccination. At the end of the study, a total of five animals showed azoospermia, two animals had 100% defective sperm in the ejaculate and one animal had 99% defective sperm in the ejaculate. Similar results were reported by Lents et al. (2018) and Rocha et al. (2021). Giriboni et al. (2020) presented similar results in a study comparing the effects of deslorelin and Improvac in goats, but no azoospermia was observed in experimental animals during the research. In our study, the sperm defects were divided into individual groups. The dominant morphological abnormalities in TG were tail defects and acrosomal abnormalities at the beginning of the experiment. At the end of the experiment, head abnormalities and detached heads predominated. However, there was a high inter-individual variability in the individual parameters of sperm morphology. The most common tail defect was a coiled tail. This type of defect, together with detached heads, is classified as a secondary defect resulting from altered epididymal maturation or abnormalities in the composition of the seminal plasma. On the other hand, acrosomal and head abnormalities are classified as primary defects resulting from the disruption of spermatogenesis (Kaya et al. 2014, Sathe 2014). Low values of normospermia in the control group might have been caused by the influence of long-term stress during the experiment and the age (6-9 months) of the experimental animals. The timing of the collection of the last samples, which were taken at the end of the breeding season, may also have had an effect, as a higher percentage of secondary abnormalities can be seen in semen samples (Edmondson et al. 2007, Sathe et al. 2014). Significant changes were also observed in sperm concentration. A similar finding was reported by Lents et al. (2018) in goats after Bopriva application, where 83% of the goats were azoospermic 60 days after the second dose. In our experiment, 3 goats were azoospermic 60 days after the second dose, 5 goats had a sperm concentration below 0.15×10^6, and only one goat still had a sperm concentration of 0.58×10^6. Again, there were significant differences between individuals. Consistent with our results, a high degree of individual variation in the recovery of testicular function has previously been observed in boars vaccinated with Improvac® (Claus et al. 2007, Wicks et al. 2013). After a negligible increase in sperm motility in TG at the beginning of the study, this gradually decreased and by the end of the experiment most goats were completely azoospermic or had immotile spermatozoa. The slight increase could be due to variable ejaculate quality in pubertal males (six months of age) at the beginning of the experiment. As the azoospermic males were removed from the statistical evaluation of sperm motility, the statistical significance could not be achieved due to the small number of animals remaining in the experiment. There were occasional statistically significant fluctuations in sperm concentration in the CG, probably caused by a small number of animals in the CG. At the same time, progressive straightforward movement was monitored. Again, no statistically significant changes were observed, although the values at the beginning and end of the experiment differed. This result is probably due to the increasing number of excluded azoospermic animals.

Summarising all the results regarding the semen quality, it can be said that the fertility of goats is significantly impaired 10 weeks after the administration of Improvac. The most remarkable changes were recorded in sperm morphology, preceding the changes in sperm concentration and progressive motility. Fourteen weeks after the first dose, all goats were infertile. The duration of this effect requires further research, as the available literature gives conflicting information. If the skin reaction (necrosis) occurs after administration, the animal cannot be considered neutered and must be castrated by a standard method.

Acknowledgements

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