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Research Article

Oestrus Synchronization in West African Dwarf Goat: Effect of Prostaglandin Synchronized Oestrus on *In-Vitro* Cervical Mucus Sperm Characteristics

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Abstract

Oestrus synchronization is a tool that has assisted in maximizing reproductive efficiency in livestock. This study investigated the *in-vitro* sperm mucus penetration test (ISMPT) of prostaglandin synchronized West African Dwarf goats using eight does and two bucks. The doe goats were randomly divided into two groups namely: Group A (given 1ml of distilled water twice at 11 days intervals) and Group B (treated with 5mg/kg of Prostaglandin $F_{2\alpha}$ twice at 11 days interval). Oestrus detection was done using aproned bucks and vaginal cytology. Cervical mucus was collected at oestrus using standard procedure. Semen was collected via the electroejaculation method. Its evaluation was carried out before and after the introduction of cervical mucus according to standard procedure. The mean value for sperm motility (88.75±3.75 and 50.00 ± 15.14%), liveability (96.00 ± 2.00 and 82.00 ± 11.02%) and total sperm abnormalities (28.71 ± 2.55 and 21.56 ± 2.81%) for Group A before and after ISMPT were not significantly different ($p > 0.05$). The mean value for motility (87.50 ± 2.50 and 60.00 ± 9.13%), liveability (96.50 ± 0.87 and 77.00 ± 9.95%) and total sperm abnormalities (23.03 ± 2.17 and 18.71 ± 2.56%) for Group B before and after ISMPT were not significantly different ($p > 0.05$). The mean value for motility, liveability and total sperm abnormalities for Group A and B before and after ISMPT were not significantly different ($p > 0.05$). This observation shows that the cervical mucus from prostaglandin synchronized doe goats had no deleterious effect on sperm characteristics *in-vitro*.

Keywords: Cervical Mucus; Oestrus; Synchronization; Prostaglandin; Goat

Introduction

Oestrus synchronization is an assisted reproductive technique that is continually been employed in livestock breeding to maximise of their reproductive efficiency. This technique involves the manipulation of the oestrous cycle to bring a group of females to heat at a specific period. The oestrous cycle is controlled by hormones from the hypothalamus-pituitary-ovarian axis and oestrous synchronization can be used to alter this axis. Alteration of the oestrous cycle could involve either lengthening or shortening it using hormones. Different hormones such as prostaglandin, gonadotropin-releasing hormone, oestrogen, equine chorionic gonadotropin, human chorionic gonadotropin, and progesterone, have been used for oestrus and ovulation synchronization. Prostaglandin helps in shortening the oestrous cycle length due to its effect on the corpus luteum [1]. This hormone lysis the corpus luteum which is responsible for producing progesterone that maintains the luteal phase of the cycle and even pregnancy. The cervical mucus, a secretion of the cervix, varies in quality and quantity during the various stages of the oestrous cycle. The cervical mucus produced at oestrus is usually clear and stringy which is conducive to sperm penetration and conception, as opposed to turbid cervical mucus [2]. Although produced in large quantity during oestrus, its physicochemical properties have a great influence on spermatozoa activity in the female reproductive tract following coitus. The physicochemical properties of the cervical have been shown to influence the conception rate in Murrah buffaloes [3] and even humans [4]. Also, the report from Tsiligianni., *et al.* [5] has shown that a significant difference exists

in the enzyme composition of cervical mucus from spontaneous and induced oestrus in the cow. The West African Dwarf goat is the most important livestock species in the rainforest zone of Southern Nigeria due to its relative trypanotolerant trait [6]. They serve as a source of protein and income for homes in the rainforest zone. This study investigated the effect of prostaglandin synchronized oestrus on invitro cervical mucus sperm characteristics in West African Dwarf goats.

Materials and Methods

Study location

The study was carried out at the Theriogenology Laboratory, Department of Theriogenology, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Oyo State.

Study animals and management

Ten West African Dwarf (WAD) goats were purchased from a livestock market in Ibadan. Eight female WAD does and two WAD bucks weighing between 9kg-15kg, and 12-14 months of age with a body condition score of 3 (scale of 5) were used for this study. They were housed in well-ventilated pens with no mixing of sexes and allowed to acclimatize for 14 days at the small ruminant unit of the Faculty of Veterinary Medicine. Animals were dewormed, vaccinated against Peste de Petit ruminatum (PPR) with Peste de Petit ruminatum vaccine (NVRI VOM) and placed on multivitamins during the period of acclimatization. They were fed daily with concentrates and cassava/yam peels after they have returned from grazing in the morning. Water was provided *ad libitum*.

Ultrasound imaging of WAD does

Ultrasound examination was carried out to ascertain the reproductive status of the doe goats. The non-sedated doe goats were placed on lateral recumbency with both hind legs held apart by two assistants and ultrasonographic evaluation was done using the Draminski® portable ultrasound machine with a 5MHz sectorial transducer. The ovaries and uterus were checked for evidence of cyclicity and pregnancy status were determined.

Breeding soundness evaluation of WAD bucks

The WAD bucks were subjected to general physical and clinical examination to ascertain their fertility. Evaluation of semen was done after collection with the electroejaculation method in both bucks. Libido testing was done by introducing the doe goats to the aproned bucks [7,8].

Semen Analysis

The semen of the bucks was analyzed to determine the percentage motility, liveability and morphological abnormalities as described by Zemjanis [9,23] and Oyeyemi., *et al.* [10], before and after the invitro sperm penetration test.

Sperm motility

This was evaluated after a drop of semen plus a drop of 2.9% buffered sodium citrate placed on a clean grease-free warm glass slide, covered with a glass coverslip and then viewed under the microscope at a magnification of X40. Motility rating in percentage was based on the movement of sperm cells in unidirectional progressive motion across the microscopic field.

Sperm liveability

This was evaluated after mixing a drop of semen with a drop of Eosin- Nigrosin stain on a clean grease-free warm glass slide and making a thin smear from the mixture on a clean grease-free warm glass slide. The thin smear was air-dried and viewed under the microscope at a magnification of X100. The percentage of the invitro live sperm cells as opposed to the dead sperm cells was recorded [22].

Sperm morphology

This was determined after mixing a drop of semen with two drops of Wells and Awa on a clean grease-free slide. A thin smear was made from the mixture, smeared air-dried and viewed under the microscope at a magnification of X100. Head, midpiece and tail abnormalities across the microscopic fields were recorded in percentages. Total sperm morphological abnormality was also noted.

Study design

The WAD does were divided into two groups of four each.

- **Group A:** Doe goats were given 1ml of distilled water intravenously on day 0 and day 11.
- **Group B:** Doe goats were administered prostaglandin $F_{2\alpha}$ (Estroplan®) at 5mg/kg intramuscularly on day 0 and day 11.

Oestrus detection

Use of aproned bucks

Detection of oestrus was achieved by introducing the doe goats to the proven aproned buck four times daily after the second treatment for both groups.

Vaginal cytology

Vaginal cytology was also carried out to detect oestrus based on the percentage of superficial cells in the vaginal smears collected from all the females daily after the second treatment in both groups. The doe goats were restrained in a standing position by an assistant before the collection of vaginal smears. The vulva

was cleaned with clean water, wiped with tissue paper and then a long sterile cotton-tipped swab stick was gently inserted into the vagina, after parting the vulva lips, at an angle of 45^0 to avoid entering the urethral diverticulum. The cotton-tipped swab was rolled against the anterior vaginal mucosa and pulled straight out. The swab was then rolled on a clean grease-free glass slide to make a smear. The vaginal smear was fixed with 70% methanol for 15 minutes, stained with Giemsa stain for 45 minutes and air-dried. Microscopic viewing of the stained smear was done at a magnification of X100. The superficial epithelial cell percentage was estimated [11].

Cervical mucus collection

Cervical mucus was collected from doe goats that stood to be mounted by aproned buck and had a vaginal cytology picture of over 70% superficial epithelial cells as confirmation for oestrus. Restraint of the doe goats in a standing position was achieved by an assistant. The vulva of the doe goats was cleaned and mucus was collected with Tuberculin syringes by gentle aspiration from the cervix [12].

In vitro sperm mucus penetration test

After collection of the cervical mucus from the WAD doe goats of both groups, the mucus was aspirated into labelled capillary tubes by positive displacement and inserted into a well-labelled collecting tube containing extended fresh buck semen through a hole drilled axially on the rubber bung. The ends of the capillary tubes were adjusted to approximately 5 mm below the surface of the extended semen and incubated at 37^0C for 90 minutes to aid the dissolution of the cervical mucus. Careful removal of the capillary tubes was done and mucus was expelled into a clean grease-free glass slide for sperm motility, liveability and morphological analysis [13].

Data analysis

Data were analyzed using Statistical Package for Social Sciences (IBM SPSS Statistics) version 20. Paired T-test was used to compare the sperm characteristics before and after the invitro sperm mucus penetration test (ISMPT). An Independent T-test was used to compare the two groups. The Mean and Standard error of the mean (SEM) for sperm motility, liveability and morphology was computed and represented in bar charts. The P-value for testing the level of significance was $P > 0.05$.

Results

Figure 1 shows that the mean ± SEM values of sperm motility (%) before and after the invitro sperm mucus penetration test (ISMPT) in Group A were 88.75 ± 3.75 and 50.00 ± 15.14. The difference in the mean values was not statistically significant ($P > 0.05$). Figure 2 shows that the mean ± SEM values of sperm motility (%) before ISMPT and after ISMPT in Group B were 87.50 ± 2.50 and 60.00 ± 9.1. The difference in the mean values was not statistically significant ($P > 0.05$). Figure 3 shows that the comparison between mean ± SEM values of sperm motility (%) after ISMPT for both Group A (50.00 ± 15.14) and Group B (60.00 ± 9.1) was not statistically significant ($P > 0.05$). Figure 4 shows that the mean ± SEM values of sperm liveability (%) before ISMPT and after ISMPT in Group A were 96.00 ± 2.00 and 82.00 ± 11.02. The difference in the mean values was not statistically significant ($P > 0.05$). Figure 5 shows that the mean ± SEM values of sperm liveability (%) before ISMPT and after ISMPT in Group B were 96.50 ± 0.87 and 77.00 ± 9.95. The difference in the mean values was not statistically significant ($P > 0.05$). Figure 6 shows that the comparison between mean ± SEM values of sperm liveability (%) after ISMPT for both Group A (82.00 ± 11.02) and Group B (77.00 ± 9.95) was not statistically significant ($P > 0.05$). Figure 7 shows that the mean ± SEM values of total sperm abnormalities (%) before ISMPT and after ISMPT in Group A were 28.71 ± 2.55 and 21.56 ± 2.81. The difference in the mean values was not statistically significant ($P > 0.05$). Figure 8 shows that the mean ± SEM values of total sperm abnormalities (%) before ISMPT and after ISMPT in Group B were 23.03 ± 2.17 and 18.71 ± 5.56. The difference in the mean values was not statistically significant ($P > 0.05$). Figure 9 shows that the comparison between mean ± SEM values of total sperm abnormalities (%) after ISMPT for both Group A (21.56 ± 2.81) and Group B (18.71 ± 5.56) was not statistically significant ($P > 0.05$). Table 1 shows the sperm morphological abnormalities (%) before and after ISMPT for Group A. There was no statistically significant difference ($P > 0.05$) between the sperm morphological abnormalities before and after ISMPT for abaxial midpiece, curved midpiece, rudimentary tail, bent tail, detached tail, incomplete tail and kinked tail. A statistically significant difference ($P < 0.05$) was shown with detached heads, looped tails and coiled tails. Table 2 shows the sperm morphological abnormalities (%) before and after ISMPT for Group B. There was no statistically significant difference ($P > 0.05$) between the sperm morphological abnormalities before and after ISMPT for detached heads, abaxial midpiece, curved midpiece, bent tail, looped tail, coiled tail, detached tail, incomplete tail and kinked tail. A statistically significant difference ($P < 0.05$) was shown with a rudimentary tail. Table 3 shows the comparison between sperm morphological abnormalities (%) after ISMPT for Group A and Group B. There was no statistically significant difference ($P > 0.05$) between the sperm morphological abnormalities after ISMPT for detached heads, abaxial midpiece, curved midpiece, rudimentary tail, bent tail, looped tail, and coiled tail, incomplete tail and kinked tail. A statistically significant difference ($P < 0.05$) was shown with a detached tail.

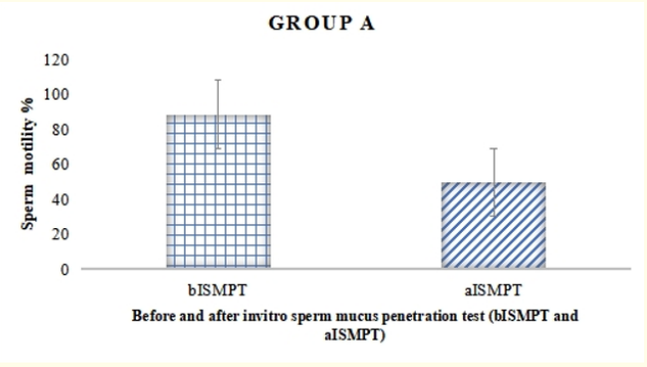

Figure 1: Mean ± SEM for sperm motility before and after the *invitro* sperm mucus penetration test in Group A.

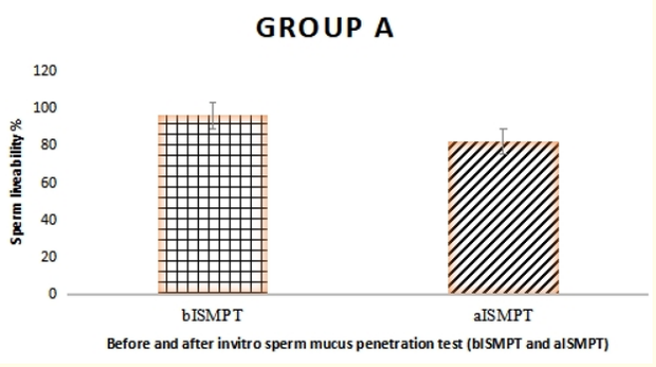

Figure 4: Mean ± SEM for sperm liveability before and after the *invitro* sperm mucus penetration test in Group A.

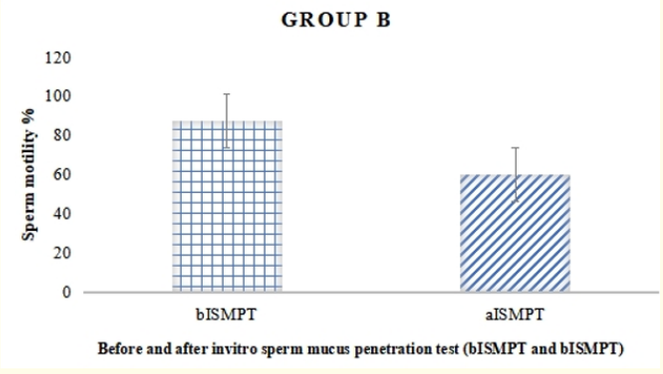

Figure 2: Mean ± SEM for sperm motility before and after the *in vitro* sperm mucus penetration test in Group B.

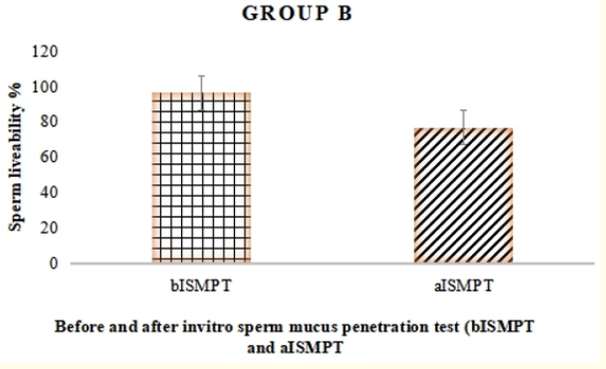

Figure 5: Mean ± SEM for sperm liveability before and after the *invitro* sperm mucus penetration test in Group B.

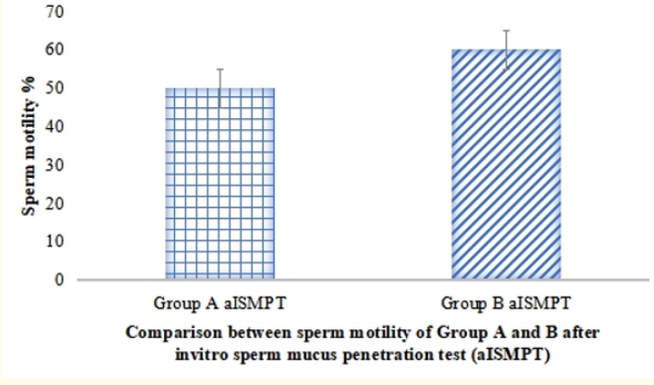

Figure 3: Comparison between Mean ± SEM for sperm motility of Group A and B after *invitro* sperm mucus penetration test.

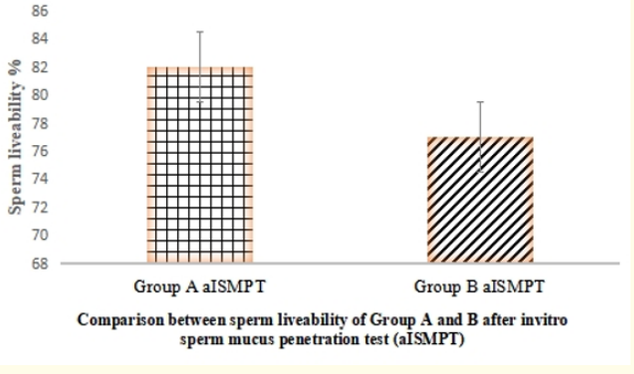

Figure 6: Comparison between Mean ± SEM for sperm liveability of Group A and B after the *invitro* sperm mucus penetration test.

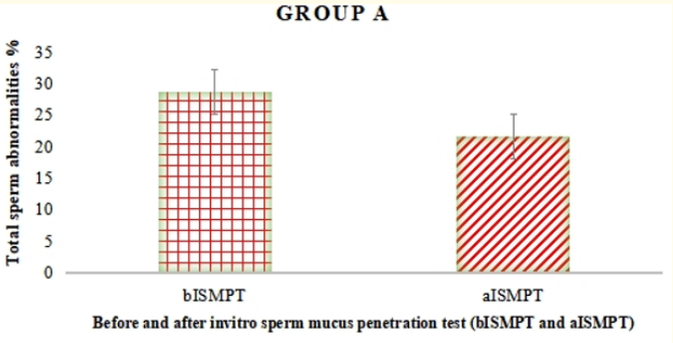

Figure 7: Mean ± SEM for total sperm abnormalities before and after the *invitro* sperm mucus penetration test in Group B.

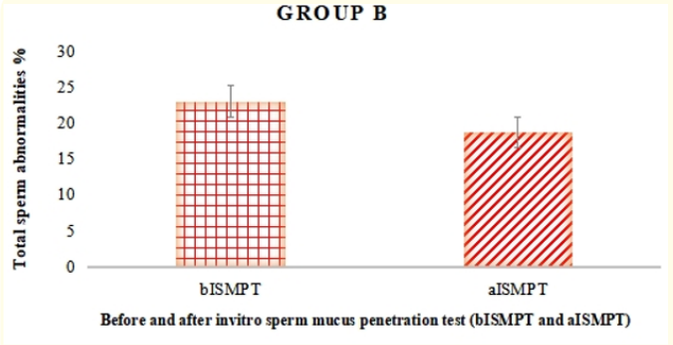

Figure 8: Mean ± SEM for total sperm abnormalities before and after the *invitro* sperm mucus penetration test in Group B.

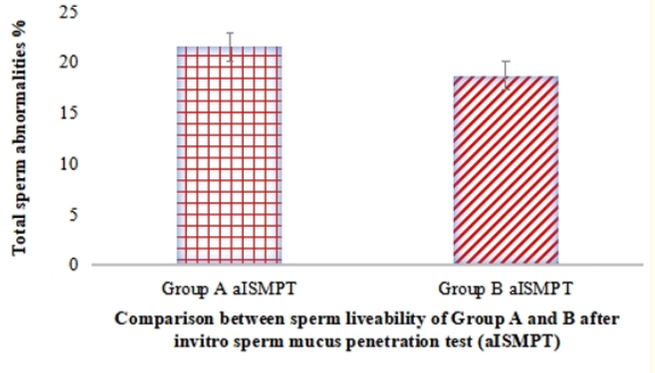

Figure 9: Comparison between Mean ± SEM for total sperm abnormalities of Group A and B after *invitro* sperm mucus penetration test.

Sperm morphological abnormalities	bISMPT (%)	aISMPT (%)
Detached heads	1.73 ± 0.13[a]	0.63 ± 0.30[b]
Abaxial midpiece	0.93 ± 0.41[a]	0.13 ± 0.07[a]
Curved midpiece	4.56 ± 1.99[a]	1.38 ± 0.16[a]
Rudimentary tail	0.51 ± 0.29[a]	0.32 ± 0.16[a]
Bent tail	1.77 ± 0.66[a]	2.38 ± 0.94[a]
Looped tail	14.96 ± 3.50[a]	13.38 ± 2.30[b]
Coiled tail	3.23 ± 0.31[a]	1.13 ± 0.16[b]
Detached tail	1.36 ± 0.10[a]	0.32 ± 0.12[a]
Incomplete tail	0.51 ± 0.07[a]	1.50 ± 0.42[a]
Kinked tail	0.42 ± 0.24[a]	0.44 ± 0.36[a]
Total abnormal sperm cells	28.71 ± 2.55[a]	21.56 ± 2.81[a]

Table 1: Sperm morphological abnormalities before and after invitro sperm mucus penetration test in Group A.

Note: Mean values with the same superscript when comparing bISMPT and aISMPT are not statistically significant (P > 0.05).

Sperm morphological abnormalities	bISMPT (%)	aISMPT (%)
Detached heads	2.76 ± 1.13[a]	1.13 ± 0.52[a]
Abaxial midpiece	0.30 ± 0.11[a]	0.58 ± 0.22[a]
Curved midpiece	3.22 ± 1.93[a]	1.87 ± 0.83[a]
Rudimentary tail	0.80 ± 0.25[a]	0.19 ± 0.12[b]
Bent tail	2.82 ± 0.53[a]	2.54 ± 0.53[a]
Looped tail	8.06 ± 2.49[a]	7.35 ± 3.20[a]
Coiled tail	1.78 ± 0.35[a]	1.63 ± 0.99[a]
Detached tail	2.82 ± 0.95[a]	1.57 ± 0.41[a]
Incomplete tail	0.67 ± 0.32[a]	1.29 ± 0.78[a]
Kinked tail	0.12 ± 0.07[a]	0.58 ± 0.33[a]
Total abnormal sperm cells	23.03 ± 2.17[a]	18.71 ± 5.56[a]

Table 2: Sperm morphological abnormalities before and after invitro sperm mucus penetration test in Group B.

Note: Mean values with the same superscript when comparing bISMPT and aISMPT are not statistically significant (P > 0.05).

Sperm morphological abnormalities after ISMPT	Group A (%)	Group B (%)
Detached heads	0.63 ± 0.30[a]	1.13 ± 0.52[a]
Abaxial midpiece	0.13 ± 0.07[a]	0.58 ± 0.22[a]
Curved midpiece	1.38 ± 0.16[a]	1.87 ± 0.83[a]
Rudimentary tail	0.32 ± 0.16[a]	0.19 ± 0.12[a]
Bent tail	2.38 ± 0.94[a]	2.54 ± 0.53[a]
Looped tail	13.38 ± 2.30[a]	7.35 ± 3.20[a]
Coiled tail	1.13 ± 0.16[a]	1.63 ± 0.99[a]
Detached tail	0.32 ± 0.12[a]	1.57 ± 0.41[b]
Incomplete tail	1.50 ± 0.42[a]	1.29 ± 0.78[a]
Kinked tail	0.44 ± 0.36[a]	0.58 ± 0.33[a]
Total abnormal sperm cells	21.56 ± 2.81[a]	18.71 ± 5.56[a]

Table 3: Comparison between sperm morphological abnormalities after invitro sperm mucus penetration test for Group A and Group B.

Note: Mean values with the same superscript when comparing bISMPT and aISMPT are not statistically significant (P > 0.05).

Discussion

The findings from this study showed a decrease in the percentage of sperm motility after the invitro sperm mucus penetration test when compared with the percentage of sperm motility before the test in both groups which had no statistical significance (P > 0.05). The decrease in percentage sperm motility after the invitro sperm mucus penetration test could be due to the sperm filtering property of cervical mucus. The report of Barros., *et al.* [14] and Cortes., *et al.* [15,21] revealed that the cervical mucus facilitates normal spermatozoa movement while inhibiting those with morphological abnormalities which may be the reason for our present findings of decreased percentage sperm motility after ISMPT in both groups. The percentage of sperm motility after ISMPT for Group B was slightly higher than that of Group A, though not statistically significant (P > 0.05). The reason for this observation is unclear but it may suggest that the prostaglandin $F_2\alpha$ had no effect on the cervical mucus which is in agreement with Akusu and Egbunike›s [16] report that prostaglandin $F_2\alpha$ induced oestrus had no observable deleterious effect on gestational length, percentage of goats kidding, the total number of kids and their birth weights in doe goats.

The percentage of sperm liveability after ISMPT decreased slightly more than that before ISMPT in both groups although of no statistical significance (P > 0.05). The observation of a slight decrease in percentage sperm liveability after ISMPT may be due to the sperm filtration property of the cervical mucus [15,21]. When comparing the percentage of sperm liveability of both groups after ISMPT, Group A was slightly higher than that of Group B though not statistically significant (P > 0.05). The reason for this is unclear. The observation of percentage sperm liveability may suggest that prostaglandin $F_2\alpha$ induced oestrus had no deleterious effect on the cervical mucus evidenced by the relatively high percentage of sperm liveability in both groups after ISMPT.

Findings with the percentage of total sperm abnormalities showed a decrease after ISMPT when compared with before ISMPT in both groups though not statistically significant (P > 0.05). The reason for the high percentage of total sperm abnormalities before ISMPT is unclear but may be due to the semen handling and processing. The decrease in the percentage of total sperm abnormalities after ISMPT may be due to the cervical mucus immune-mediated mop-up of morphologically abnormal spermatozoa [17,18]. Furthermore, the percentage of total sperm morphological abnormalities after ISMPT in Group A was slightly above the maximum range of 15-20% allowing for optimal productivity in the semen of fertile bucks as described by Evans and Maxwell [19] and Olurode., *et al.* [20]. The reason for this is unclear. Furthermore, a decrease was observed after ISMPT in some sperm morphological abnormalities except for the bent tail and kinked tail in Group A, and abaxial midpiece, incomplete tail and kinked tail in Group B. The reason for these variations is unclear but it may be due to other factors unconnected to the agent used for synchronization.

Conclusion

The present findings may suggest that prostaglandin $F_2\alpha$ had no deleterious effect on sperm motility, liveability and morphology within the scope of this study using the invitro sperm mucus penetration test. This implies that prostaglandin $F_2\alpha$ is safe for use as an oestrous synchronization agent in West African dwarf doe goats.

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