using species-specific swimming speed (VCL) cut-off values for six species to identify three motility subpopulations (rapid-, medium- and slow-swimming spermatozoa) within the total motile sperm population. Motility characteristics have also been successfully used to classify sperm into subpopulations by Quintero-Moreno *et al.* (2003) in stallion ejaculates and Martinez-Pastor *et al.* (2005) in Iberian red deer ejaculates.

Different subpopulations of spermatozoa coexist within any typical mammalian ejaculate (Chang and Hunter, 1975: Bedford, 1983). These are thought to owe their origins to variations in the assembly of individual spermatozoa during spermatogenesis as well as to the differential maturational status and age through mixing in the epididymis. Davis *et al.* (1995), using a complex interactive multiple regression technique to analyze human sperm motion parameters prior to freezing, were able to demonstrate the existence of subpopulations this way, reflecting the physiological status of individual cells. The notion that motility is an indicator for fertilization to occur (Fraczek *et al.*, 2014) is important, however, the degree of motility as indicated by detailed kinematic parameters is of vital importance. The value of using such sperm swimming characteristics have been indicated by Mortimer and Mortimer (2013), who reported on hyperactivation (indicator for fertilization) cut-off values for VCL, ALH and LIN.

While Tankwa goat semen in February 2017 had a significantly higher percentage rapid swimming spermatozoa (33.6%) than the other three collection periods, there was also more rapid swimming spermatozoa in the summer months (21.8 ± 21.7%) compared to the winter months (9.6 ± 13.9%). The average Tankwa goat percentage rapid swimming sperm (16.5%) was very low, but it was due to the low percentage rapid swimming spermatozoa in the winter months (ranged from 0% to 60.9%).

5.2.3 Kinematics

Sperm movement characteristics are important for spermatozoa to be able to fertilize oocytes. In earlier studies, Holt *et al.* (1994) reported that the swimming speed (VCL) of ejaculated spermatozoa measured with a semi-automatic image analysis system was strongly correlated with *in vitro* fertilization (IVF) rates. Jeulin *et al.* (1996) showed that the ALH of motile spermatozoa selected by the swim-up technique correlated positively with fertilization rates, which was also confirmed by Liu and Baker (1992) and Sukcharoen *et al.* (1995) in humans. The significance of VCL and VAP as predictors of fertilization may imply the importance of capacitation in the process of fertilization, because both of these are

enhanced after capacitation. The ALH is one of the parameters affecting the outcome of IVF (Barlow *et al.*, 1991 and Jeulin *et al.*, 1996) and the mammalian spermatozoa's ability to penetrate cervical mucus and fuse with the oocytes (Aitken *et al.*, 1982: Feneux *et al.*, 1985: Aitken *et al.*, 1986: Aitken *et al.*, 1992: Aitken and Fisher, 1994: Mortimer *et al.*, 1995). This parameter is of importance as it indicates the vigour of flagellar beating together with the frequency of cell rotation (David *et al.*, 1981: Serres *et al.*, 1984), which are probably important for the progression of spermatozoa into the cervical mucus and the peri-oocyte envelopes.

The summer Tankwa goat sperm velocity for VCL, VSL and VAP were 146.7 µm/s, 76.6 µm/s and 92.4 µm/s respectively, which were lower to the values recorded in Florida goats except for VCL (146.4 µm/s, 104.06 µm/s and 117 µm/s) .The average summer Tankwa goat sperm STR, LIN and WOB were 77.3%, 51.9%, 63.4% respectively, they were also lower than those of Florida goats except for STR (71.1%, 88.3% and 79%) as reported by (Dorado *et al.,* 2010). The average Tankwa goat sperm ALH parameter (3.4 µm) was in agreement to that observed by Dorado *et al.* (2010) (3.95 µm). Dorado *et al.* (2010) used Dulbecco phosphate buffered saline to dilute the semen and like the present study, used SCA® to analyse the kinematics.

The summer sperm kinematics for VCL (146.7 ± 31.0 µm/s) and ALH (3.4 ± 0.8 µm) were higher than winter (125.0 ± 26.3 µm/s and 2.8 ± 0.5 µm respectively). High VCL and ALH are characteristics of spermatozoa that can penetrate cervical mucus, therefore summer season indicate a superior fertilisation potential.

5.3 Sperm morphology and morphometry

The fertility potential of a male is often analysed through classifying spermatozoa according to the normality of their different components (head, midpiece and tail) and various defects such as nuclear vacuoles and cytoplasmic droplets. Morphology has become a vital structural sperm test, due to the correlation it has with other functional and structural parameters such as percentage motility and vitality (Maree, 2011). Poor semen morphology is an important indicator of decreased fertility in humans (Kruger *et al.*, 1988), stallions (Jasko *et al.*, 1990), bulls (Sekoni and Gustafsson, 1987), goats, and rams (Oshinowo *et al.*, 1988; De Jarnette *et al.*, 1992; Gravance *et al.*, 1995).

The Tankwa goat sperm morphometric values did not show any significant differences for seasonal variation with the exception of head roughness and head regularity, midpiece width

and midpiece area. The average Tankwa goat sperm head length, width, elongation and regularity (8.7 ± 0.2µm, 4.1 ± 0.1µm, 0.4 ± 0.01 and 0.8 ± 0.02 respectively) were similar to that of the Florida goats (8.47 ± 0.27µm, 4.16 ± 0.18µm, 0.34 ± 0.02 and 0.95 ± 0.03 respectively) (Vazquez *et al.*,2015).

Although significant differences were obtained for head roughness and regularity between summer and winter, the values were the same for these two sperm components and a small variation was observed. The summer season morphometric values (1.1 ± 0.1 µm) was slightly smaller than winter (1.2 ± 0.1 µm) for midpiece width, and the summer (10.9 ± 1.6 μm^2) was smaller than winter (11.8 ± 2.2 μm^2) for midpiece area.

The average Tankwa goat percentage normal morphology was lower (73.3%) when compared to the Tankwa goats from the same population (84.7%) (Ramukhithi ,2016). The difference could be due to analysis equipment used. This study used Morphology module of the SCA® while Ramukhithi employed fluorescence microscopy. The staining technique could also have influenced the results, Ramukhithi used nigrosin-eosin while the Sperm Blue was used in this study.

The Tankwa goat percentage normal sperm morphology was considerably lower in both summer (68.9 ± 18.2%) and winter (76.7 ± 15.8%) as compared to Zaraibi goats (88.6% and 81.7% respectively) as observed by Barkawi *et al.* (2005) and to Markhoz (Angora) goats (90.8% and 88.8% respectively) as observed by Talebi *et al.* (2009). Both summer and winter season were lower compared to unspecified Brazilian indigenous goats as observed by Aguiar *et al.* (2013) where normal sperm morphology was 82.8 ± 2.5% and 86.9 ± 1.8% for autumn and spring, respectively. However, it should be noted that these mentioned studies had employed subjective methods in determining sperm morphology as compared to the present study which used objective method namely CASA. Van der Horst *et al.* (2018) demonstrated the use of CASA as a superior method compared to manual techniques as CASA offers the advantage of reduced bias.

Due to human error in the morphology staining technique, unreadable Tankwa goat semen samples were encountered in February 2016, with spermatozoa either being over-stained or under-stained. This resulted in only a few (n = 8) of the stained samples (n = 22) to be usable and the majority not being correctly analysed by the SCA® CASA system and were therefore not included in the final results.

Despite the apparent higher percentage normal morphology found in the winter, no significant difference was observed between the two seasons. This is probably a result of the large variation and accordingly large standard deviation that was observed within the two seasons. As mentioned above, sample size differences could have contributed to this observation, since summer had a smaller sample size (n = 30) compared to winter (n = 39).

It should be noted that August 2015 had the highest percentage normal sperm as compared to other periods. Another factor that could have a played role in this observation are the restrictions for morphology classification, as the morphometric data for August 2015 was used to set the restriction for normal Tankwa sperm morphology. There is a possibility that the restriction settings might have been too strict for other periods.

5.4 Sperm vitality

The average percentage Tankwa goat sperm vitality of this study (72.7 ± 14.3%) was similar to the one observed by Ramukhithi (2016) in Tankwa goats from the same population (70.6 ± 2.1%). The average percentage Tankwa goat sperm vitality was higher for both summer (74.9 ± 15.3%) and winter (68.1 ± 11.1%) when compared to other indigenous goats as observed by Webb *et al.* (2012) where percentage vitality was between 37.64% and 42.5% in winter and improved to 68% in summer. Futhermore, Tankwa goat sperm vitality was lower compared to that of the British goat breeds for summer (82%) and winter (87.65%) as recorded by Ahmad and Noakes (1996), as well as to that of Zaraibi goat (87.2% and 77.2% for summer and winter respectively) as recorded by Barkawi *et al.* (2005), and to that of Markhoz (Angora) goats for summer (88.2%) and winter (84.9%) as observed by Talebi *et al.* (2009). However, the sample size in the above studies were lower (n=20 for Webb *et al.* (2012) and n=10 for the subsequent studies) as compared to that of this present study (n=75). This could have influenced the difference between these studies. Moreover, the current study had a small sample size for the winter (n=25) as compared to the summer (n=50) period. This could have influenced the results in favour of summer period in our study.

Percentage sperm vitality was lower than the percentage sperm total motility for August 2015, February 2016 and August 2016. Large variations in percentage vitality were also observed during 2016. A possible factor that played a role in the variation and anomaly mentioned above was that the vitality staining technique used during 2015-2016 period was

not ideal for the Tankwa goat spermatozoa. Difficulties were experienced whilst analysing nigrosin-eosin stained spermatozoa with the Sperm Class Analyser® CASA system. Analyses might have been inaccurate, as it was difficult to deduce whether a spermatozoon was alive or dead, possibly because of the over-staining with eosin. In addition to this, time delay and temperature control could have had a significant effect on the vitality of spermatozoa, and therefore it is possible that these factors could also have contributed to the inconsistent results.

5.5 Hyperactivation

Hyperactivation is thought to provide strong thrusting power to the spermatozoa while passing through the cells and layers surrounding the oocyte, particularly the zona pellucida (Yanagimachi, 1984). Hyperactivation is a flagellar phenomenon, even though it is often measured by changes in the movement of the sperm head. The difference between the flagellar beat patterns of hyperactivated and nonhyperactivated spermatozoa is caused by changes in the degree of bending of the axoneme, as well as changes in the propagation of the flagellar beats (Katz *et al.*, 1978; Katz *et al.*, 1986; Mortimer *et al.*, 1997). In previous studies, it has been shown that if spermatozoa are unable to become hyperactivated, they are unable to fertilize the oocytes *in vitro* (Fleming and Yanagimachi, 1982; Fleming and Kuehl, 1985). It was observed in human spermatozoa co-cultured with epithelial cells from fallopian tubes (Pacey *et al.*, 1995) and mouse spermatozoa in oviducts of naturally mated mice (Demott and Suarez, 1992) that hyperactivation helps spermatozoa to break free and move along the oviduct. The objective measure of this physiological process can serve as a biological marker to evaluate the functional capabilities of spermatozoa.

5.5.1 Determination of cut-off points for Tankwa goat sperm hyperactivation

Receiver operating curves (ROC) graphs were constructed from the kinematics of individual spermatozoa from the February 2016 data, to determine the cut-off values for Tankwa goat sperm hyperactivation. The VAP was the only parameter that did not have high sensitivity (74.8) and high specificity (32.8) and thus was not used to set the hyperactivation restriction. It should be noted that the kinematics used were the ones that were treated with procaine in February 2016 and could have influenced the results in favour of procaine.

This was a novel approach as most studies have not reported on the sperm hyperactivation in goats. Determining sperm hyperactivation is a useful aspect in the improvement of sperm functional characteristics.

5.5.2 Comparison of media for sperm hyperactivation in Tankwa goats

Individual Tankwa goat semen samples were exposed to four different media namely phosphate buffered saline (PBS), BO medium, 5 mM procaine hydrochloride and 4% lignocaine to evaluate its capability to induce sperm hyperactivation.

Procaine has been widely reported to induce hyperactivation in numerous species such as guinea pig and bovine (Ho and Suarez, 2001) and stallions (McPartlin *et al.,* 2009). According to Meyers and Baumber (2006) and Mortimer and Mortimer (1990) hyperactivation induced by procaine resembles non-progressive hyperactivation or star-spin pattern. Lignocaine has been observed to cause sperm hyperactivation in human sperm (Bennett *et al.,* 1992) and it was mainly used in this study for comparison with procaine in terms of sperm trajectories.

In the present study, procaine displayed lower mean and rapid VSL, LIN, and STR, and high mean, rapid and medium VCL than the other three media. The significant values in these kinematic parameters suggest that sperm tracks induced by procaine display a typical asymmetrical hyperactivation pattern as described by Mortimer and Mortimer (1990). Spermatozoa displaying low VSL, LIN and STR are an indication of non-progressive hyperactivation tracks (Baumber and Meyers, 2006).

Spermatozoa exposed to PBS and BO media showed more linear and progressive sperm tracks as compared to the asymmetrical and non-progressive tracks induced by procaine hydrochloride. Spermatozoa exposed to lignocaine also showed asymmetrical sperm tracks but they were not as pronounced as compared to those induced by procaine.

5.5.3 Comparison of sperm hyperactivation over three seasons

Procaine had significant low VSL, STR, LIN, WOB, and BCF averages than PBS. It also displayed higher averages for VCL, ALH and HA (%). The average for procaine was 22.9% which is higher than the cut-off point (20%) for good fertilizing potential for a spermatozoan. However, there was a large variation observed among the individual goats as is evident from the high standard deviation (22.9 ± 15.5%) and ranged from 0% to 54.5%.

The goats with the most hyperactivated spermatozoa, i.e number of spermatozoa that had a HA (%) of greater than 20%, were recorded in February 2017 (n=17), while August 2016 had the least amount of males with a HA (%) of greater than 20%. This could mean the Tankwa goat spermatozoa are more likely to be hyperactivated in the summer month, showing potentially a higher chance for fertilization in this period.

5.6 Acrosome integrity

The acrosome reaction is an essential event for mammalian fertilization. The acrosome may contain enzymes that aid in penetration of the extracellular matrix surrounding the oocyte, part of which is a secretion known as the zona pellucida (ZP) (Florman, 1994; Breitbart 2003). Further, the release of this vesicle may enable the spermatozoa to attach to the ZP via either the now exposed inner acrosomal membrane or plasma membrane (Yanagimachi and Phillips, 1984)

O'Toole *et al.* (2000), Harper *et al.* (2006), and Florman *et al.* (2008) have proposed two primary physiologically relevant triggers for this reaction *in vivo*, the zona pellucida glycoprotein (ZP3 in humans) and progesterone. Both of these agonists can induce the acrosome reaction in laboratory settings. Until recently, zona pellucida glycoproteins were thought to be the more biologically relevant agonist, as it was thought that the AR happens when the sperm binds to the egg where the ZP glycoproteins are located in successful fertilization events. It has been observed that the ZP can trigger or accelerate the acrosome reaction (Florman and Storey 1982; Bleil and Wassarman 1983; Crozet and Dumont 1984; Cherr *et al.* 1986; Uto *et al.* 1988; Tollner *et al.* 2003; Abou-haila and Tulsiani 2009; Buffone *et al.* 2009) However, recent *in vivo* mouse studies have suggested that the acrosome reaction is often initiated before reaching the ZP (Jin *et al.* 2011; Inoue *et al.* 2011; Hino *et al.* 2016; La Spina *et al.* 2016), indicating that ZP-induced acrosome reactions may not be the primary mechanism in vivo.

Although only a small number of samples were evaluated for acrosome integrity (n=6), 80%-95% acrosome intactness was observed in these samples, implying high quality spermatozoa for Tankwa goats. It should be noted that these are preliminary results and this functional test needs to be investigated further on the Tankwa goat sperm. At least this study has confirmed that the routine protocol suggested for the staining of the sperm acrosome with FITC-PNA seems to give accurate results with Tankwa goat spermatozoa.

A future experiment where the acrosome reaction is induced is needed to confirm the results and accuracy of this functional test in Tankwa goats. The assessment of the acrosomal status remains a research interest, as acrosomal status represents one of the most important approaches to evaluating the sperm's fertilizing ability (Esteves *et al.,* 2007).

Chapter 6: Conclusion

6.1 General conclusion

The CASA system has proved to be a valuable tool in assessing semen of the Tankwa goat. The number of motile and morphologically normal spermatozoa was determined. The novel approach of the study was determination of cut-off points for hyperactivation, which was successful as a number of hyperactivated sperm were quantified. Employing the SCA® has proved to be useful in this aspect.

There was distinct seasonal difference between summer and winter for various semen and sperm characteristics, although large variations were observed among individual goats. Semen samples collected in the summer season seem to have a higher percentage of functionally intact and potentially fertile spermatozoa as compared to winter. Seasonal differences and individual variations were also observed with the multivariate analysis. After exposing Tankwa goat spermatozoa to different hyperactivation-inducing media, procaine hydrochloride is believed to be the best stimulant for evaluating sperm hyperactivation. The results from this study can be used as baseline recommendation for future studies on the potential fertilising ability of the Tankwa goats. Determining sperm hyperactivation is a useful aspect in the improvement of sperm functional characteristics. Furthermore, this functional test can be optimised and include more hyperactivation-inducing media.

Limitations within the study included:

- Human error. Unreadable slides were encountered and thus, could have led to inconsistent results as evident in percentage normal sperm parameter.
- Staining techniques. In the vitality test, it was difficult to deduce whether a sperm cell was alive or dead. It is therefore recommended to optimise the staining technique in future studies.
- Sample size. In acrosome intactness tests, there was low sample size and the technique needs to be evaluated further.

References

Abou-haila, A, Tulsiani, D.R., 2009. Signal transduction pathways that regulate sperm capacitation and the acrosome reaction. *Archives of Biochemistry and Biophysics* 485(1):72–81

Abou-Haila, A., Tulsiani, D. R. P., 2000. Mammalian sperm acrosome: Formation, contents, and function. *Archives of Biochemistry and Biophysics* 379: 173-82

Abu Hassan Abu, D, Franken, D. R., Hoffman, B., Henkel, R., 2012. Accurate sperm morphology assessment predicts sperm function. *Andrologia*, 44:571–577. doi:10.1111/j.1439-0272.2011.01229.

Aguiar, G.V., van Tilburg, M.F., Catunda, A.G.V., Celes, C.K.S., Lima, I.C.S Campos, A.C.N., Moura, A.A.A. & Araújo, A.A., 2013. Sperm parameters and biochemical components of goat seminal plasma in the rainy and dry seasons in the Brazilian Northeast: the season's influence on the cooling of semen. *Arq. Bras. Medical Veterinary Zootechnology* 65: 6-12. doi: 10.1590/S0102-09352013000100002

Ahmad, N. & Noakes, D.E., 1995. Seasonal variations in testis size, libido and plasma testosterone concentrations in British bucks. *Journal of Animal Science*, 61: 553-559.

Aitken, R.J., Best, F.S.M., Richardson, D.W., Djahanbakhch, O. & Lees, M.M.,

1982. The correlates of fertilizing capacity in normal fertile men. *Fertility. Sterility.* 38: 68-76.

Aitken, R.J., Irvine, D.S. & Wu, F.C., 1992. Prospective analysis of sperm-oocyte fusion and reactive oxygen species generation as criteria for the diagnosis of infertility. *Journal of Obstetrics Gynaecology,* 164:542–551.

Aitken, R.J. & Fisher H., 1994. Reactive oxygen species generation and human spermatozoa: the balance of benefit and risk. *Bioessays* 16: 259-267.

Aitken, R.J., Sutton, M., Warner, P. & Richardson, D.W., 1986. Relationship between the movement characteristics of human spermatozoa and their ability to penetrate cervical mucus and zona-free hamsteroocytes. *Journal of Reproductive. Fertility.* 73, 441-449.

Ajao, O.A.A., 2015. Evaluation of the effectiveness of different extenders of goat buck semen under refrigerated conditions. Master's Dissertation, University of Venda, Thohoyandou.

Aller, J.F, Aguilar, D., Vera, T., Almeida, G.P. & Alberio, R.H., 2012. Seasonal variation in sexual behavior, plasma testosterone and semen characteristics of Argentine Pampinta and Corriedale rams. *Spanish Journal of Agricultural Research* 10:345-352. doi: 10.5424/sjar/2012101-389-11

Almeida, A.M., Schwalbach, L.M.J., Cardoso, L.A. & Greyling, J.P.C., 2007. Scrotal, testicular and semen characteristics of young Boer bucks fed winter veld hay: The effect of nutritional supplementation. *Small Ruminant Research* 73: 216–220.

Austin, C.R., 1951. Observations on the penetration of sperm into the mammalian egg. *Australia Journal of Science Research.* (Series B), 4, 581–596.

Austin, C.R., 1952. The 'capacitation' of mammalian sperm. *Nature,* 170, 326.

Avenarius, M.R., Hildebrand, M.S., Zhang, Y., Meyer, N.C., Smith, L.L., Kahrizi, K., Najmabadi, H. & Smith RJ., 2009. Human male infertility caused by mutations in the CATSPER1 channel protein. *American Journal of Human Genetics,* 84:505-510.

Avidan, N., Tamary, H., Dgany, O., Cattan, D., Pariente, A., Thulliez, M., Borot, N., Moati, L., Barthelme, A., Shalmon, L., Krasnov, T. & Ben-Asher, E., 2003. CATSPER2, a human autosomal nonsyndromic male infertility gene. *European Journal of Human Genetics,* 11:497-502.

Baltz, J.M., Katz, D.F. & Cone, R.A. 1988. Mechanics of sperm-egg interaction at the zona pellucida. *Biophysical Journal,* 54 (4): 643-654.

Barbas, J.P., Leahy, T., Horta, A.E. & García-Herreros, M., 2018. Sperm kinematics and subpopulational responses during the cryopreservation process in caprine ejaculates. *Cryobiology*, 82; 137-147. doi: 10.1016/j.cryobiol.2018.03.005

Barkawi, A.H., Elsayed, E.H., Ashour, G. & Shehata, E., 2006. Seasonal changes in semen characteristics, hormonal profiles and testicular activity in Zaraibi goats. *Small Ruminant Research* 66: 209-213.

Batista, M., Niño, T., Santana, M., Alamo, D., Castro, N., Reyes, R., González, F., Cabrera, F. & Gracia, A., 2011. Influence of the Preservation Temperature (37, 20, 4, -196 ˚C) and the mixing of semen over sperm quality of Majorera bucks.*Reproduction in Domestic Animals*, 46: 281-288. doi:10.1111/j.1439- 0531.2010.01659.

Björndahl, L., Söderlund, I. & Kvist, U., 2003. Evaluation of the one-step eosin- nigrosin staining technique for human sperm vitality assessment. *Human Reproduction*, 18(4), 813–816.

Bedford, J.M., 1983. Significance of the need for sperm capacitation before fertilization in eutherian mammals. *Biology Reproduction,* 28, 108–120.

Bleil, J.D. & Wassarman, P.M., 1983. Sperm–egg interactions in the mouse: sequence of events and induction of the acrosome reaction by a zona pellucida glycoprotein. *Developmental Biology.* 95(2):317–324

Blottner, S., Bertram, R. & Pitra, C., 1989. Speciesspezifische Motilitatsmuster hyperaktivierter Saugetierspermatozoen und die quantitative Auswertung der Hyperaktivierung von Bullenspermatozoen. *Andrologia*, 21, 204-213

Boatman, D.E. & Bavister, B.D., 1984. Stimulation of rhesus monkey sperm capacitation by cyclic nucleotide mediators. *Journal of Reproduction and Fertility.* 71, 357–366

Bopape, M.A., Lehloenya, K.C., Chokoe, T.C. & Nedambale, T.L. 2015. Comparison of Electro Ejaculator and Artificial Vagina on Semen Collection from South African Indigenous Goat Following Assessment by Computer Aided Sperm Analysis. *Open Journal of Animal Sciences*, 5, 210-218. doi:10.4236/ojas.2015.52023

BosHoff N.H., Lambrechts, H., Maree, L., Cloete, S.W.P., & van der Horst, G., 2018. A novel flush technique to stimulate natural dispersal of spermatozoa in the female reproductive tract and expedite motility assessment of freshly ejaculated Merino (Ovis aries) sperm. *South African Journal of Animal Science*. 48(3): 469-476 DOI 10.4314/sajas.V.4813.7

Boyazoglu J., Hatziminaglou I., & Morand-Fehr P., 2005. The role of the goat in society: Past, present and perspectives for the future. *Small Ruminant Research*, 60, 13 – 23.

Brackett, B.G., & Oliphant, G., 1975. Capacitation of rabbit spermatozoa *in vitro*. *Biology Reproduction.* 12:260.

Breitbart, H., 2003. Signaling pathways in sperm capacitation and acrosome reaction. Cell *Molecular Biology*. 49: 321–7.

Buffone, M.G., Rodriguez-Miranda, E., Storey, B.T. & Gerton, G.L., 2009. Acrosomal exocytosis of mouse sperm progresses in a consistent direction in response to zona pellucida. *Journal of Cell Physiology.* 220(3):611–620

Burkman, L.J., 1984. Characterization of hyperactivated motility by human spermatozoa during capacitation: comparison of fertile and oligozoospermic sperm populations. Archives of *Andrology.,* 13: 153–165.

Campbell Q.P., 2003. The origin and description of southern Africa's indigenous goats. *South African Journal of Animal Science*, 4, 18-22.

Carlson, A.E., Westenbroek, R.E., Quill, T., Ren, D., Clapham, D.E., Hille, B., Garbers, D.L. & Babcock, D.F., 2003. CatSper1 required for evoked Ca2+ entry and control of flagellar function in sperm. *Proceedings of National Academy of Sciences* USA; 100:14864-1

Casey, P.J, Gravance, C.G, Davis, R.O, Chabot, D.D. & Liu, I.K.M., 1997. Morphometric differences in sperm head dimensions of fertile and subfertile stallions. *Theriogenology.* 1997;47: 575–582.

Casey, N.H. & Webb, E.C., 2010. Managing goat production for meat quality. *Small Ruminant Research*. 89, 218–224. doi:10.1016/j.smallrumres.2009.12.047

Chang, M.C., 1951. Fertilizing capacity of spermatozoa deposited into the fallopian tubes. *Nature,* 168, 697–698.

Chemineau, P. Cagnié, Y. & Guérin, Y. 1991. Training manual on artificial insemination in sheep and goats. Food and Agriculture Organization of the United Nations (FAO). FAO Animal Production and Health Paper. p. 211222.

Chenoweth, P.J., 2002. Semen quality assessment. Proceedings, *The Applied Reproductive Strategies in Beef Cattle Workshop*, Manhattan, Kansas. pp 247-254

Cherr, G.N., Lambert, H., Meizel, S. & Katz, D.F., 1986. In vitro studies of the golden hamster sperm acrosome reaction: completion on the zona pellucida and induction by homologous soluble zonae pellucidae. *Development and Biology.* 114(1):119–13.

Choe, C.Y., Kim, J.G., Cho S.R., Son, D.S., Kim, Y.K., Balasubramanian, S., Choe, S.Y., Rho, G.J., 2006. Influence of seasons, extenders, slow and rapid freezing on seminal characters in Korean native bucks. *Reproduction of Domestic Animals*. 41, 55–60.

Coetzee, K., Bermes, N., Krause, W., Menkveld, R., 2001. Comparison of normal sperm morphology outcomes from two different computer-assisted semen analysis systems. *Andrologia*, 33(3), pp.159–163.

Colenbrander, B., Gadella, B., Stout, T., 2003. The predictive value of semen analysis in the evaluation of stallion fertility. *Reproduction of Domestic Anim*als. 38, 305-311.

Cooper, T.G., Atkinson, A.D. & Nieschlag, E., 1999. Experience with external quality control in spermatology. *Human Reproduction.* 14:765–769.

Cooper, T.G. & Hellenkemper, B., 2009. Method-related estimates of sperm vitality.

Journal of Andrology, 30(3), 214–218.

Corteel, J.M., 1975. The use of progestagens to control the oestrous cycle for the dairy goat. *Ann. Biology of Animal Biochemistry and Biophysics.* 15:353-363.

Cross, N.L. & Meizel, S., 1989. Methods for evaluating the acrosomal status of mammalian sperm. *Biology of Reproduction,* 41 (4), 635-641.

Crozet, N. & Dumont, M., 1984. The site of the acrosome reaction during in vivo penetration of the sheep oocyte. *Gamete Research.* 10(2):97–105

Cummins, J.M., 1982. Hyperactivated motility patterns of ram spermatozoa recovered from the oviducts of mated ewes. *Gamete Research* 6, 53–63.

DAFF, 2012. A Profile of the South African Goat Market Value Chain [online]. Department of Agriculture, Forestry and Fisheries, Pretoria, South Africa, Available: http://www.daff.gov.za/docs/AMCP/Goat2012pdf (accessed 06.03.16).

David, I., Druart, X., Lagriffoul, G., Manfredi, E., Christele, R., Bodin, L., 2007. Genetic and environmental effects on semen traits in Lacaune and Manech tête rousse AI rams. *Genetics Selection Evolution* 39, 405-419.

David, G., Serres, C. & Jouannet, P., 1986. Kinematics of human spermatozoa.

Gamete Research, 4 (2): 83-95.

Davis, R.O. & Boyers, S.P., 1992: The role of digital image analysis in reproductive biology. *Archives of Pathology and Laboratory Medicine* 116: 351– 363.

Davis, R.O. & Gravance, C.G., 1993. Standardization of specimen preparation, staining and sampling methods improves automated sperm-head morphometry analysis. *Fertility and Sterility.* 59:412-417.

Davis, R.O. & Siemers, R.J., 1995. Derivation and reliability of kinematic measures of sperm motion. *Reproductive Fertility Development* 7, 857–869.

De Jonge, C.J. & Barratt, C.L.R. 2013. Methods for the assessment of sperm capacitation and acrosome reaction excluding the sperm penetration assay. *Methods in Molecular Biology,* 927, 113-118. doi; 10.1007/978-1-62703-38-0_11

Demott, R.P. & Suarez, S.S., 1992. Hyperactivated sperm progress in the mouse oviduct. *Biology of Reproduction.* 46:779–785.

Donkin, E.F. & Boyazoglu, P.A., 2000. Milk Production from Bucks for Household and Small-Scale Farmers in South Africa. University of Pretoria, Pretoria.

Donkin, E. & Boyazoglu, P., 2004. Diseases and mortality of adult goats in a South African milk goat herd. *South African Journal of Animal Science* ,34, 258–261.

Dorado, J., Molina, I., Munoz-Serrano, A. & Hildago M., 2010. Identificaton of sperm subpopulation with defined motility characteristics in ejaculates from Florida goats. *Theriogenology*, 74, 795-804. doi: 10.1016/j.theriogenology.2010.04.004.

Dunson, D.B., Weinberg, C.R., Perreault, S.D. & Chapin, R.E., 1999. Summarizing the motion of self-propelled cells: applications to sperm motility. *Biometrics*, 55(2), pp.537–543.

Elsheik, A.S. & Elhammali, N.S., 2015. Semen quality of mature crosbred male goatsduring different seasons. *Journal of Agriculture and Veterinary Science,* 8**,** pp. 2319-2372. doi: 10.9790/2380-08920105.

Epstein, H., 1971. The Origin of the Domestic Animals of Africa*. Vol 1& 2* Revised by I L Mason. Africana Publishing Corporation. New York, London, Munich.

Esteves, S.C., Sharma, R.K., Thomas Jr, A.J. & Agarwal, A., 2007. Evaluation of acrosomal status and sperm viability in fresh and cryopreserved specimens by the use of fluorescent peanut agglutinin lectin in conjunction with hypo-osmotic swelling test. *International Brazilian Journal of Urology*, 33(3), 364-376.

Evans, G. & Maxwell, C., 1987. Salamon's artificial insemination of sheep and bucks. Sidney: Butterworth. Pp.66-76, 158-161.

Feneux, D., Serres, C. & Jouannet, P., 1985. Sliding spermatozoa: a dyskinesia responsible for human infertility? *Fertility Sterility.* 44**,**508–511.

Food and Agriculture Organization of the United Nations. 2016. FAOSTAT statistics database. Available: www.faostat.org (accessed 22.08.2018).

Fleming, A.D., & Yanagimachi, R., 1982. Fertile life of acrosome reacted guinea pig spermatozoa. *Journal of Experimental Zoology* ,220, 109–115.

Fleming A.D, & Kuehl T.J, 1985. Effects of temperature upon capacitation of guinea pig spermatozoa. *Journal of Experimental Zoology,* 233, 3405–3411.

Fleming, A.D., Yanagimachi, R. & Yanagimachi, H., 1981. Spermatozoa of the Atlantic bottlenosed dolphin, *Tursiops truncatus*. *Journal of Reproductive Fertility.* 63, 509–514.

Florman, H.M., 1994. Sequential focal and global elevations of sperm intracellular Ca2+ are initiated by the zona pellucida during acrosomal exocytosis. *Developmental Biology.* 165(1):152–164

Florman, H.M., Jungnickel, MK. & Sutton, K.A., 2008. Regulating the acrosome reaction. *International Journal of Developmental Biology.* 52(5–6):503–510

Florman, H.M. & Storey, B.T., 1982. Mouse gamete interactions: the zona pellucida is the site of the acrosome reaction leading to fertilization in vitro. *Developmental Biology.* 91(1):121– 130.

Foote, R.H., 2003. Effect of processing and measuring procedures on estimated sizes of bull sperm heads. *Theriogenology,* 59: 1765 – 1773.

Ferraz, M.A.M.M., Morató, R., Yeste, M., Arcarons, N., Pena, A.I., Tamargo, C., Hidalgo, C.O., Muiño, R. & Mogas, T. 2014. Evaluation of sperm subpopulation structure in relation to invitro sperm-oocyte interaction of frozen-thawed semen from Holstein bulls. *Theriogenology*, 81 (8), 1067-1072. doi: 10.1016/j.theriogenology.2014.01.033

Fraczek, M., Wiland, E., Piasecka, M,. Boksa, M., Gaczarzewicz, D., Szumala- Kakol, A., Kolanowski, T., Beutin, L. & Kurpisz, M. 2014. Fertilizing potential of ejaculated human spermatozoa during in vitro semen bacterial infection. *Fertility Sterility.* 102: 711-719. doi: 10.1016/j.fertnstert.2014.06.002

Fraser, L.R., 1977. Motility patterns in mouse spermatozoa before and after capacitation. *Journal of Experimental Zoology.* 202: 439–444.

Gündonğan, M., Demirci, E., Bozkurt, T. & Sönmez, M., 1997. The changes before, during and later in the breeding season in the semen characteristics of rams. *The Journal of Faculty of Veterinary Medical,* 8: 40-42.

Gago, C., Perez-Sa´nchez, F., Yeung, C. H., Tablado, L., Cooper, T. G. & Soler, C., 1998. Standardization of sampling and staining methods for the morphometric evaluation of sperm heads in the Cynomolgus Monkey (*Macaca fascicularis*) using computer - assisted image analysis. *International Journal of Andrology.* 21: 169-176.

Gardner, D. L. & Hafez, E. S. E., 2004. Spermatozoa and Seminal Plasma. In: Reproduction in farm animals. Wiley John & Sons Incorporated, Nova Jersey. p.96- 109.

Gould, K.G., Young, L.G. & Hinton, B.T., 1988. Alterations in primate sperm motility with maturation and during exposure to theophylline. *American Journal of Primatology.* 15: 325–336.

Goyal, H. O. & Memon, M. A., 2007. “Clinical reproductive anatomy and physiology of the buck” in Current Therapy in Large Animal. *Theriogenology*, 2, pp. 511–514.

Gray, S.L., Lackey, B.R. & Boone, W.R., 2016. Effects of Panax ginseng, zearalenol, and estradiol on sperm function. *Journal of Ginseng Research.* 40 pp. 251-259. doi: 10.1016/j.jgr.2015.08.004.

Gualtieri, R. & Talevi, R., 2003. Selection of highly fertilization-competent bovine spermatozoa through adhesion to the Fallopian tube epithelium in vitro

Reproduction, 125 , 251-258.

Gwatkin, R.B.L., Andersen, O.F. & Hutchinson, C.F., 1972. Capacitation of hamster spermatozoa in vitro: The role of cumulus components. *Journal of Reproductive Fertility.* 30, 389-394

Hanotte, O., Bradley, D.G., Ochieng, J., Verjee, Y., Hill, E.W. & Rege, J.E.O., 2002. African pastoralism: genetic imprints of origins and migrations. *Science*, 296(5566): 336–339.

Harper, C.V., Barratt, C.L., Publicover, S.J., Kirkman-Brown, J.C., 2006. Kinetics of the progesterone-induced acrosome reaction and its relation to intracellular calcium responses in individual human spermatozoa. *Biology of Reproduction.* 75(6):933–939

Henkel, R. Maaß, G., Bödeker, R.H., Scheibelhut, C., Stalf, T., Mehnert, C., Schuppe, H.C., Jung, A., Schill, W.B., 2005. Sperm function and assisted reproduction technology. *Reproductive Medicine and Biology,* 4, 7–30.

Hildago, M., Rodriguez, I., Dorado, J., 2006. Influence of staining and sampling procedures on goat sperm morphometry using the sperm class analyzer *Theriogenology*, 66, 996 – 1003.

Hildago, M., Rodriguez, I. & Dorado, J., 2008. The effect of cryopreservation on sperm head morphometry in Florida male goat related to sperm freezability. *Animal Reproduction Science,* 100: 61 – 72.

Hino, T., Muro, Y., Tamura-Nakano, M., Okabe, M., Tateno, H., Yanagimachi, R., 2016. The behavior and acrosomal status of mouse spermatozoa in vitro, and within the oviduct during fertilization after natural mating**.** *Biology of Reproduction.* 95(3):50–1. doi:10.1095/biolreprod.116.140400

Hirst, K. "The History of the Domestication of Goats". Available: *About.com*. (Accessed 15.07.2018).

Ho, H.C. & Suarez, S.S., 2001. An inositol 1, 4, 5-trisphosphate receptor-gated intracellular Ca2+ store is involved in regulating sperm hyperactivated motility. *Biology of Reproduction.* 65(5):1606–1615

Ho, H.C. & Suarez, S.S., 2003. Characterization of the intracellular calcium store at the base of the sperm flagellum that regulates hyperactivated motility. *Biology of Reproduction.* 68(5):1590–1596

Holt, C., Holt, W.V., Moore, H.D., Reed, H.C. & Curnock, R.M., 1997.Objectively Measured Boar Sperm Motility Parameters Correlate with the Outcomes of On-Farm Inseminations: Results of Two Fertility Trials. *Journal of Andrology,* 18: 312-323.

Holt, W., Watson, P., Curry, M. & Holt, C., 1994. Reproducibility of computer-aided semen analysis: comparison of five different systems used in a practical workshop. *Fertility and Sterility,* 62(6):1277-1282.

Huat, K.S., 1973. Semen characteristics of cross bred goats. (Kambing Kajong Jamnapari). Karjan Vet. (Malaysia Singapore) (2)

Inoue, N., Satouh. Y., Ikawa, M., Okabe, M., Yanagimachi, R., 2011. Acrosome- reacted mouse spermatozoa recovered from the perivitelline space can fertilize other eggs. *Proc Natl Acad Sci* 108(50):20008–20011. doi:10.1073/pnas.1116965108

Jaiswal, B., Eisenbach, M. & Tur-Kaspa, I., 1999. Detection of partial and complete acrosome reaction in human spermatozoa: which inducers and probes to use? *Molecular Human Reproduction*, 5(3), 214–219.

Jasko, D.J., Lein, D.H. & Foote R.H. 1990. The relationship between sperm morphological classification and fertility in the stallion. *Journal of American Veterinary Medicine Association.* 197:389-394.

Jeulin, C., Lewin, L.M., Chevrier, C. & Schoevaert-Brossault, D., 1996. Changes in flagellar movement of rat spermatozoa along the length ofthe epididymis: manual and computer-aided image analysis. *Cell Motility of Cytoskeleton*, 35**:** 147–161.

Jimenez, F., Puchades, S., Gadea, J., Vicente, J.S., Viudes-de-Castro M.P., 2005. Effect of semen collection method on pre- and post-thaw Guirra ram spermatozoa. *Theriogenology*, 64, 1756–1765.

Jimenez, F., Vicente, J.S., Viudes-de-Castro, M.P., 2008. Seminal plasma composition from ejaculates collected by artificial vagina and electroejaculation in Guirra ram. *Reproduction of Domestic Animals*. 43, 403-408.

Jin, M., Fujiwara, E., Kakiuchi, Y., Okabe, M., Satouh, Y., Baba, S.A., Chiba, K., Hirohashi, N., 2011. Most fertilizing mouse spermatozoa begin their acrosome reaction before contact with the zona pellucida during invitro fertilization. *Proc Natl Acad Sci* 108(12):4892–4896. doi:10.1073/pnas.1018202108

Johnson, L.L., Katz, D.F. & Overstreet, J.W., 1981. The movement characteristics of rabbit spermatozoa before and after activation. *Gamete Research.* 4, 275–282.

Karagiannidis, A., Varsakeli, S., Alexopoulos, C. & Amarantidis, I., 2000. Seasonal variation in semen characteristics of Chios and Friesan rams in Greece. *Small Ruminant Research.* 37: 125-130

Katz, D.F., Cherr, G.N. & Lambert, H., 1986. The evolution of hamster sperm motility during capacitation and interaction with the ovum vestments in vitro. *Gamete Research,* 14 (4), 333-346.

Katz, D.F. & Overstreet, J.W., 1981. Sperm motility assessment by videomicrography. *Fertility and Sterility,* 35 (2), 188-193.

Katz, D.F., Yanagimachi, R. & Dresdner, R.D., 1978. Movement characteristics and power output of guinea-pig and hamster spermatozoa in relation to activation. *Journal of Reproductive Fertility.* 52, 167–172.

Kraemer, M., Fillion, C., Martin-Pont, B., Auger, J., 1998. Factors influencing human sperm kinematic measurements by the Celltrak computer-assisted sperm analysis system. *Human Reproduction*, 13(3), pp.611–619.

Kruger, T.F., Acosta, A.A., Simmons, K.F., Swanson, R.J., Matta, J.F. & Oehninger S., 1988. Predictive value of abnormal sperm morphology in in vitro fertilization. *Fertility and Sterility.* 49: 112-l 17.

Kruger, T.F., DuToit, T.C. & Franken, D.R. 1993. A new computerized method of reading sperm morphology (strict criteria) is as efficient as technician reading. *Fertility and Sterility.* 59, 202–209.

Kotze, A., Grobler J.P, van Marie-Korster E., Jonker T., & Dalton D.L., 2014. The Tankwa Karoo National Park feral goat population; a genetic unique population. *African Journal of Animal Science* ,44 , 43-48. doi: 10.4314/sajas.v44i1.6

Lambert, H., 1981. Temperature-dependence of capacitation in bat sperm monitored by zona-free hamster ova. *Gamete Research.* 4, 525–533.

Larsen, L., Scheike, T., Jensen, T.K., Bonde, J.P., Ernst, E., Hjollund, N.H., Zhou, Y., Skakkebaek, N.E., Giwercman, A., 2000. Computer-assisted semen analysis parameters as predictors for fertility of men from the general population. *Human reproduction*, 15(7), 1562–1567.

Latif, A., Ijaz, A., Aleem, M. and Mahmud, A. 2005. Effect of Osmotic Pressure and pH on the Short-Term Storage and Fertility of Broiler Breeder Sperm. *Pakistan Veterinary Journal*, 25, 180-182.

La Spina, F.A., Molina, L.C.P., Romarowski A., Vitale, A.M., Falzone, T.L., Krapf, D., Hirohashi, N., Buffone, M.G., 2016. Mouse sperm begin to undergo acrosomal exocytosis in the upper isthmus of the oviduct. *Developmental Biology.* 411(2):172–182. doi: I: 10.1016/j.ydbio.2016.02.006

Lishko, P.V., Botchkina, I.L. & Kirichok, Y., 2011. Progesterone activates the principal Ca2+ channel of human sperm. *Nature,* 471:387-391. doi: 10.1038/nature09767

Lishko, P.V., Kirichok, Y., Ren, D., Navarro, B., Chung, J.J. & Clapham, D.E., 2012. The control of male fertility by spermatozoan ion channels. *Annu Rev Physiol,* 74:453-475. doi: 10.1146/annurev-physiol-020911-153258.

Liu, D.Y. & Baker, H.W., 1992. Tests of human sperm function and fertilization *in vitro*. *Fertility and Sterility.;* 58: 465–83.

López-Aliaga, I., Alférez, M.J.M., Nestares, M.T., Ros, P.B., Barrionuevo, M. & Campos, M.S., 2005. Goat milk feeding causes an increase in biliary secretion of cholesterol and a decrease in plasma cholesterol levels in rats. *Journal of Dairy Science*, 88 (3), 1024-1030.

Lu, J.C., Huang, Y.F. & Lü, N.Q., 2014. Computer-aided sperm analysis: Past, present and future. *Andrologia*, 46(4), 329–338. doi: 10.1111/and.12093

Luikart, G.L., Gielly, L., Excoffier, L., Vigne, J.D., Bouvet, J. & Taberlet, P., 2001. Multiple maternal origins and weak phylogeographic structure in domestic goats. *Proceedings of the National Academy of Sciences USA*, 98(10): 5927–5930.

Malejane, C.M., Greyling, J.P.C. & Raito, M.B. 2014. Seasonal variation in semen quality of Dorper rams using different collection techniques. *South African Journal of Animal Science*, Vol. 44(1):26-32. doi:10.4314/sajas.v44i1.4.

Manceau, V., Després, L., Bouvet, J., Taberlet, P. 1999. Systematics of the *Capra* genus inferred from mitochondrial DNA sequence data. *Molecular Phylogenetic Evolution.* 13:504–510.

Mason I.L., 1981. Wild Goats and their Domestication; Breeds. In: Goat production. Eds. Gall, C., Academic Press, London. 35 –110.

Marco-Jiménez, F., Vincente, J.S. & Vludes-De-Castro, M.P., 2008. Seminal Plasma Composition from Ejaculates Collected by Artificial Vagina and Electro- Ejaculation in Guirra Ram. *Reproduction in Domestic Animals*, 43, 403-408. doi:10.1111/j.1439-0531.2007.00923.x

Maree, C. & Plug, I., 1993. Origin of Southern African livestock and their potential role in the industry. In: Livestock Production Systems Eds. Maree, C. & Casey, NH., Agri-Development Foundation. 10 – 11.

Maree, L., 2011. Sperm mitochondria: Species specificity and relationships to sperm morphometric features and sperm function in selected mammalian species. PhD thesis, University of the Western Cape.

Maree, L., du Plessis, S.S., Menkveld, R. & van der Horst, G., 2010. Morphometric dimensions of the human sperm head depend on the staining method used. *Human Reproduction*, 25,1369-1382. doi:10.1093/humrep/deq075

Mbizvo, M.T., Thomas, S., Fulgham, D.L. & Alexander, N.J., 1990. Serum hormone levels affect sperm function. *Fertility and sterility,* 54(1), 113-120.

McBean, L.D. & Miller, G.D., 1998. Allaying fears and fallacies about lactose intolerance. *Journal of the American Dietetic Association*, 98 (6): 671-676.

McPartlin, L.A., Suarez, S.S., Czaya, C.A., Hinrichs, K. & Bedford-Guaus, S.J., 2009. Hyperactivation of stallion sperm is required for successful in vitro fertilization of equine oocytes. *Biology of Reproduction,* 81:199-206.

Menkveld, R., Holleboom, C.A.G. & Rhemrev, J.P.T., 2011. Measurement and significance of sperm morphology. *Asian Journal of Andrology*, 13(1): 59–68. doi:10.1038/aja.2010.67.

Microptic, 2016. SCA® Morphology. *Microptic - Automatic Diagnostic Systerms*. Available at: http://www.micropticsl.com/products/sperm-class-analyzer-casa- system/analysis-modules/sca-morphology/ (accessed 20.08.2016).

Microptic, 2016. SCA® Motility and concentration. *Microptic - Automatic Diagnostic Systems*. Available at: http://www.micropticsl.com/products/sperm-class-analyzer- casa-system/analysis-modules/sca-motility-and-concentration/#prettyPhoto (accessed 20.08.2016).

Mohlatlole, R. P., Dzomba, E. F., & Muchadey, F. C., 2015. Addressing production challenges in goat production systems of South Africa: The genomics approach. *Small Ruminant Research* 131, 43–49. doi: 10.1016/j.smallrumres.2015.08.003

Moreno, J.S., Coloma, M.A., Dorado, J., Pastor, A.P., Gomez-Guillamon, F., Salas-Vega, R., Gomez-Brunet, A., & Sebastian L.A., 2009. Cryopreservation of Spanish ibex (Capra pyrenaica) sperm obtained by electro ejaculation outside the rutting season. *Theriogenology*, 71, 1253-1260

Morrison, J., 2007. A Guide to the identification of the Natural Indigenous Goats of Southern Africa [Online]. Available: http://landbou.com/wp-content/uploads/ 2014/03/f2297405-a93f-4399-bdb76f3de538d75d.pdf (accessed 09.03.16.).

Mortimer, D., 1990. Objective analysis of sperm motility and kinematics. In Keel, B.A. and Webster, B.W. (eds), *Handbook of the Laboratory Diagnosis and Treatment of Infertility. CRC Press, Boca Raton, pp.97–133.*

Mortimer, D., 1994. *Practical Laboratory Andrology*. Oxford University Press, New York, pp.1–393

Mortimer, D., 1995. Sperm transport in the female genital tract. In Grudzinskas, J.G. and Yovich, J.L. (eds), *Gametes—The Spermatozoon*. Cambridge University Press, Cambridge, pp. 157–174.

Mortimer, S.T., 1997. A critical review of the physiological importance and analysis of sperm movement in mammals. *Human reproduction update,* 3(5), 403-439**.**

Mortimer, S.T., 2000. CASA—practical aspects. *Journal of andrology,* 21(4), 515-524.

Mortimer, D., Courtot, A.M. & Giovangrandi, Y., 1984. Human sperm motility after migration into, and incubation in, synthetic media. *Gamete Research.* 9, 131–144**.**

Mortimer, D. & Mortimer, S., 2013. Computer-Aided Semen Analysis (CASA) of sperm motility and hyperactivation. *Methods in Molecular Biology*, 927, 77–87.

Mortimer, D., Mortimer, S. & van der Horst, G., 2015. The future of computer-aided sperm analysis. *Asian Journal of Andrology*, 17, 545-553. doi: 10.4103/1008- 682X.154312.

Naderi, S., Rezaei, H., Pompanon, F., Blum, M.G.B., Negrini, R., Naghash, H., Balkız,Ö., Mashkour, M., Gaggiotti, O.E., Marsan, P.A., Aykut Kence, A., Vigne,

J. & Taberlet, P., 2008 The goat domestication process inferred from large-scale mitochondrial DNA analysis of wild and domestic individuals. 105 (46) 17659 -17664

doi:10.1073/pnas.0804782105

Neill, J.M. & Olds- Clarke, P., 1987. A computer- assisted assay for mouse sperm hyperactivation demonstrates that bicarbonate but not bovine serum albumin is required. *Molecular Reproduction and Development*, 18(2), 121-140.

Ngoula, F., Tebug, T.T., Kenfack, A., Defang, F.H., Tendokeng, F. & Pamo, T.E. 2012. Effect of Buck Age, Storage Duration, Storage Temperature and Diluents on Fresh West African Dwarf Semen. *Journal of Reproduction and Infertility*, 3, 58-66. doi: 10.5829/idosi.jri.2012.3.3.66216

Nieschlag, E., De Geyter, C., De Geyter, M., & Koppers, B., 1998. Diagnostic accuracy of computer-assisted sperm motion analysis. *Human Reproduction*, 13(9), 2512–2520.

Nikpoor, P., Mowla, S.J., Movahedin, M., Ziaee, S.A. & Tiraihi, T., 2004. CatSper gene expression in postnatal development of mouse testis and in subfertile men with deficient sperm motility. *Human Reproduction.* 19:124-128.

Noakes, D.E., Parkinson, T.J. & England, G.C.W., 2009. *Veterinary Reproduction and Obstetrics*. Saunders, China.

Nur, Z., Dogan, I., Gunay, U., & Soylu, M.K., 2005. Relationships between Sperm Membrane Integrity and Other Semen Quality Characteristics of the Semen of Saanen Bucks. *Bulletin of Veterinary Institute in Pulawy*, 49, 183-187.

Nuti, L.C., 2002. Goat semen collection and processing. www.ansci.cornell.edu, 3-7: Accessed on the 6th June, 2018.

Olson, S.D., Fauci, L.J. & Suarez, S.S., 2011. Mathematical modeling of calcium signaling during sperm hyperactivation. *Molecular of Human Reproduction.* 17:500-510. doi: 10.1093/molehr/gar040.

Olson, S.D., Suarez, S.S. & Fauci, L.J., 2010. A model of CatSper channel mediated calcium dynamics in mammalian spermatozoa. *Bull Mathematical Biology,* 72:1925-1946. doi: 10.1007/s11538-010-9516-5.

Ortiz-de-Montellano, M., Galindo-Maldonado, F., Cavazos-Arizpe, E.O., Aguayo- Arceo, A.M., Torres-Acosta, J.F., & Orihuela, A., 2007. Effect of Electro-Ejaculation on the Serum Cortisol Response of Criollo Bucks (Capra hircus). *Small Ruminant Research*,69,228-231. doi:10.1016/j.smallrumres.2005.12.025

O'Toole, C.M., Arnoult, C., Darszon, A., Steinhardt, R.A., Florman, H.M., 2000. Ca (2+) entry throu`gh store operated channels in mouse sperm is initiated by egg ZP3 and drives the acrosome reaction. *Molecular Biology of Cell.* 11(5):1571–1584

Paulenz, H., Soltun, K., Ådnøy, T., Berg K.A., & Söderquist, L., 2005. Effect of different extenders on sperm viability of buck semen stored at room temperature. *Small Ruminant Research* 59, 89-94.

Peña, F.J., Johannisson, A., Wallgren, M. & Rodriguez Martinez, H., 2003. Antioxidant supplementation in vitro improves boar sperm motility and mitochondrial membrane potential after cryopreservation of different fractions of the ejaculate. *Animal Reproduction Science*, 78 (1-2), 85-98.

Peña, F.J., Saravia, F., García-Herreros, M., Núñez-Martínez, I., Tapia, J.A., Johannisson, A., Wallgren, M. & Rodríguez-Martínez, H., 2005. Identification of sperm morphometric subpopulations in two different portions of the boar ejaculate and its relation to postthaw quality. *Journal of Andrology*, 26 (6); 716-723.

Peña-Martínez, A.I., 2004. Canine fresh and cryopreserved semen evaluationAnimal Reproduction Science. 82-83, 209-224.

Pérez-Llano, B., Yenes-García, P.& García-Casado, P., 2003. Four subpopulations of boar spermatozoa defined according to their response to the short hypoosmotic swelling test and acrosome status during incubation at 37°C. *Theriogenology*, 60 (8); 1401-1407.

Petrunkina, A.M. & Töpfer-Petersen, E. 2000. Heterogenous osmotic behaviour in boar sperm populations and its relevance for detection of changes in plasma membrane. *Reproduction, Fertility and Development,* 12 (5-6): 297-305.

Pieters, A., 2007. Genetic characterization of commercial goat populations in South Africa. Unpublished MSc. dissertation, University of Pretoria, South Africa.

Quezada-Casasola, A.E., Martínez-Armendáriz, K., Chávez, J., Pérez-Eguía, E., Rodríguez-Alarcón, C. & Avendaño-Reyes, L., 2016. Effect of season on scrotal circumference, semen characteristics and testosterone serum concentration in Mexican Corriente and other beef breed bulls. *Animal reproduction.* 13. 787-794. doi: 10.21451/1984-3143-AR805

Ramalho-Santos, J., Amaral, A., Sousa, A.P., Rodrigues, A.S., Martins, L., Baptista, M., Mota, P.C., Tavares, R., Amaral, S., Gamboa, S., 2007. Probing the structure and function of mammalian sperm using optical and fluorescence microscopy. *Modern Research and Educational Topics in Microscopy*, 351(239), :855–789.

Ramsay, K., Harris, L. & Kotze, A., 2000. Landrace breeds: South Africa's indigenous and locally developed farm animals. Farm Animal Conservation Trust, Pretoria.

Ramukhithi, F.V., 2011.Characterisation and Cryopreservation of South African Unimproved Indigenous and Boer Buck Semen. Master's Dissertation, Tshwane University of Technology, Pretoria.

Ramukhithi, F.V., 2016. Prediction of fertility in South African indigenous goats using semen and phenotypic parameters. Doctoral Dissertation, Tshwane University of Technology, Pretoria.

Ramukhithi, F.V., Nedambale, T.L., Sutherland, B., Lehloenya, K.C., 2011. Cryopreservation of South African indigenous goats semen. *African Journal of Biotechnology*, 4, 17989-17902. doi: 10.5897/AJB11.2034

Ren, D., Navarro, B., Perez, G., Jackson, A.C., Hsu, S., Shi, Q., Tilly, J.L. & Clapham, D.E., 2001. A sperm ion channel required for sperm motility and male fertility. *Nature*; 413:603-609.

Ritar, A.J., Mendoza, G., Salamon. S. & White, I.G., 1992. Frequent semen collection and sperm reserves of the male Angora goat (Capra hircus) *Journal of Reproduction and Infertility,* 95, 97-102.

Rosa, H.J.D. & Bryant, M.J., 2003. Seasonality of reproduction in sheep. *Small Ruminant Research* 48:155-171.

Rubio-Guillén, J., González, D., Garde, J.J., Esteso, M.C., Fernández-Santos, M.R., Rodríguez-Gíl, J.E., Madrid-Bury, N. & Quintero-Moreno, A. 2007. Effects of cryopreservation on bull spermatozoa distribution in morphometrically distinct subpopulations. *Reproduction in Domestic Animals,* 42 (4), 354-357.

Russell L. D., Peterson R. N., 1984. Determination of the elongate spermatid-sertoli cell ratio in various mammalsJournal of Reproduction and Fertility. 70, 635-64.

Santiago, M., Alvarado, J.F., López, A.G., Trujillo, G., Alvarez, M. & Mellado, M., 2018. Effects of testosterone administration and feeding level on reproductive activity in sexually inactive goat bucks. *Journal of the Hellenic Veterinary Medical Society*. 69. 991-998. doi**:**10.12681/jhvms.18032

Sekoni, V.O. & Gustafsson, B.K., 1987. Seasonal variations in the incidence of sperm morphological abnormalities in dairy bulls regularly used for artificial insemination. *British Veterinary Journal.* 143: 312-317.

Serres, C., Feneux, D., Jouannet, P. & David, G., 1984. Influence of the flagellar wave development and propagation on the human sperm movement in seminal plasma. *Gamete Research.* 9**,** 183–195.

Sharma, R. K., Bhardwaj, J. K., Ahlawat, N., 2009. Ultrastructural developments during spermiogenesis in goat (*Capra hircus*). *International Journal of Biological Science.* 38: 1-36.

Sherman, J., 1989. The relationship of abnormal semen parameters to male fertility.

Journal of Human Reproduction, 4(8): 947–953.

Shipley, C.F.B., Buckrell, B.C., Mylne, M.J.A., Pollard, J. & Hunton, J.R., 2007. Artificial insemination and embryo transfer in sheep. Ch 86. In *Youngquist RS & Threlfall WR (eds). Current Therapy in Large Animal Theriogenology* (Second Edition). *ACT Elsevier*

Singh, J.P., Babcock, D.F. & Lardy, H.A. 1983. Motility activation, respiratory stimulation, and alteration of Ca2+ transport in bovine sperm treated with amine local anesthetics and calcium transport antagonists. *Archives of Biochemistry and Biophysics.* 221: 291–30.

Smith, M.C. & Sherman, D.M., 1994. Goat medicine. Lippincott Williams and Wilkins, Baltimor, pp: 1-143.

Snoj, T., Kobal, S. & Majdic, G. 2013. Effects of season, age and breed on semen characteristics in different *Bos taurus* breeds in a 31-year retrospective study. *Theriogenology*, 847-852. doi: 10.1016/j.theriogenology.2012.12.014.

Snyman, M.A., 2014. South African goat breeds: Indigenous veld goat. Info-pack ref. 2014/004. Grootfontein Agricultural Development Institute.

Snyman, M., Olivier, J., 1999. Repeatability and heritability of objective and subjective fleece traits and body weight in South African Angora goats. *Small Ruminant Research,* 34: 103–109.

Strunker, T., Goodwin, N., Brenker, C., Kashikar, N.D., Weyand, I., Seifert, R. & Kaupp, U.B., 2011. The CatSper channel mediates progesterone-induced Ca2+ influx in human sperm. *Nature*; 471:382-386. doi: 10.1038/nature09769.

Suarez, S.S., 2004. Regulation of sperm storage and movement in the mammalian oviduct. *International Journal of Developmental Biology*, 52(5-6), 455-462.

Suarez, S.S., 2008. Regulation of sperm storage and movement in the mammalian oviduct. *International Journal of Development Biology*, 52 (5-6): 445-462.

Suarez, S.S. & Dai, X.B., 1995. Intracellular calcium reaches different levels of elevation in hyperactivated and acrosome-reacted hamster sperm. *Molecular Reproduction of Development.* 42: 325–333.

Suarez, S.S., Katz, D.F. & Overstreet, J.W., 1983. Movement characteristics and acrosomal status of rabbit spermatozoa recovered at the site and time of fertilization. *Biology of reproduction,* 29(5), 1277-1287.

Suarez, S.S. & Pacey, A.A., 2006. Sperm transport in the female reproductive tract. Human reproduction update, 12(1), 23-37.

Suarez, S.S., Varosi, S.M. & Dai, X., 1993. Intracellular calcium increases with hyperactivation in intact, moving hamster sperm and oscillates with the flagellar beat cycle. *Proceedings of the National Academy of Sciences*, 90(10), 4660-4664.

Suarez, S.S., Vincenti, L. & Ceglia, M.W., 1987. Hyperactivated motility induced in mouse sperm by calcium ionophore A23187 is reversible. *Journal o Experimental Zoology.* 244:331-336.

Sukcharoen, N., Keith, J., Irvine D.S. & Aitken, R.J., 1995. Definition of the optimal criteria for identifying hyperactivated human spermatozoa at 25 Hz using in-vitro fertilization as a functional end-point. *Human Reproduction.* 10:2928–2937

Sundararaman, M.N., Kalatharan, J., & Edwin, M.J., 2007. Attempts to Achieve Semen Collection from Incapacitated Boars by Electro-Ejaculation. *Journal of Animal Veterinary,* 4: 244-246.

Talebi, J., Souri, M., Moghaddam, A., Karimi, I. & Mirmahmoodi, M., 2009. Characteristics and seasonal variation in the semen of Markhoz bucks in Western Iran. *Small Ruminant Research,* 85: 18-22

Tollner, T.L., Yudin, A.I., Cherr, G.N., Overstreet, J.W., 2003. Real-time observations of individual macaque sperm undergoing tight binding and the acrosome reaction on the zona pellucida. *Biology of Reproduction.* 68(2):664–672

Torres-Flores, V., Picazo-Juarez, G., Hernandez-Rueda, Y., Darszon, A. & Gonzalez-Martinez, M.T., 2011. Sodium influx induced by external calcium chelation decreases human sperm motility. *Human Reproduction.* 26:2626-2635 doi: 10.1093/humrep/der237.

Toshimori, K. & Ito, C., 2003. Formation and organization of the mammalian sperm head. *Archives of Histology and Cytology* 66: 383-396.

Tulsiani, D.R.P., Zeng, H.-T. & Abou-Haila, A., 2007. Biology of sperm capacitation: evidence for multiple signalling pathways. *Society of Reproduction and Fertility supplement*, 24: 1–16.

Uto, N., Yoshimatsu, N., Lopata, A., Yanagimachi, R., 1988. Zona-induced acrosome reaction of hamster spermatozoa. *Journal of Experimental Zoology Part A; Ecology of Genetic Physiology.* 248(1):113–120

van der Horst, G., 2014. Automated sperm morphology analysis*. In NAAB 25th Technical Conference on Artificial Insemination and Reproduction*. Green Bay, Wisconsin, pp. 125–128.

van der Horst, G., 2014. Sperm motility subpopulations and fertility. *In The NAAB 25th Technical conference on Artificial Insemination and Reproduction*. Green Bay, Wisconsin, pp. 73–77.

van der Horst, G. Luther, I., Goeritz, F., Hermes, R., Dierich, J., Lueders, I., Tordiff, A., Bartels, P., Potier, R., Baker, B., Theison, W., van Altena, J.J., Grobler, D. & Hildebrandt, T., 2010. Computer-Aided Semen Analysis of Loxodonta africana sperm. In *International Elephant Conservation and Symposium*. Pretoria.

van der Horst, G. & Maree, L., 2009. SpermBlue: a new universal stain for human and animal sperm which is also amenable to automated sperm morphology analysis. *Biotechnic & Histochemistry*, 84 ,299-308.

van der Horst, G., Maree, L. & du Plessis, S.S. 2018. Current perspectives of CASA applications in diverse mammalian sperm. *Reproduction of Fertility and Development.* doi: 10.1071/RD17468.

van der Horst, G., Skosana, B., Legendre, A., Oyeyipo, P. & du Plessis, S.S., 2018. Cut-off values for normal sperm morphology and toxicology for automated analysis of rat sperm morphology and morphometry, *Biotechnic & Histochemistry,* 93(1):49-58. doi: 10.1080/10520295.2017.1380842.

Van Marle-Koster, E., & Nel, L., 2003. Genetic markers and their application in livestock breeding in South Africa: a review: review article*. South African Journal of Animal Science*, 33: 1–10.

Van Marle-Koster, E., Visser, C. & Berry, D., 2013. A review of genomic selection— implications for the South African beef and dairy cattle industries. *South African Journal of Animal Science* , 43: 1–17.

Varner, D.D. & Johnson, L., 2007. From a sperm's eye view-revisiting our perception of this intriguing cell. *53th Annual Convention of the American Association of Equine Practioners*, 104-177.

Vasantha, I., 2016. Physiology of Seasonal Breeding: A Review. *J Veterinar Sci* Techno 7: 331. doi:10.4172/2157-7579.1000331

Verstegen, J., Iguer-Ouada, M. & Onclin, K., 2002: Computer assisted semen analyzers in andrology research and veterinary practice. *Theriogenology* ,57:149-179

Vikaspedia, 2018. Reproductive system in sheeps and goats. [online] Available at: http://vikaspedia.in/agriculture/livestock/sheep-and-goat-farming/castration-of- ruminants-1#section-2. (Accessed 16/07/2018).

Visser, C., Snyman, M., Van Marle Koster, E. & Bovenhuis, H., 2009. Genetic parameters for physical and quality traits of mohair in South African Angora goats. *Small Ruminant Research,* 87: 27–32.

Wassarman, P.M., 1995. Sperm–egg recognition mechanisms in mammals. *Curr. Top. Dev. Biol.,* 30, 1–19.

Webb, E.C., Dombo M.H. & Roets, M., 2004. Seasonal variation in semen quality of Gorno Altai cashmere goats and South African indigenous goats. *South African Journal of Animal Science,* 34, 240–243.

Williams, A.C. & Ford, W.C.L., 2001. The role of glucose in supporting motility and capacitation in human spermatozoa. *Journal of Andrology*, 22(4), 680-695.

Yanagimachi, R., 1970. Hyperactivated motility of sperm. *Journal of Reproduction and Fertility*, 23,193.

Yanagimachi, R., 1970. The movement of golden hamster spermatozoa before and after capacitation. *Journal of Reproduction and Fertility.,* 23, 193–196.

Yanagimachi, R., 1981. Mechanisms of fertilization in mammals. *Fertilization and Embryonic Development In Vitro.* Plenum Press, New York, 81–182.

Yanagimachi, R., 1982. Requirement of extracellular calcium ions for various stages of fertilization and fertilization-related phenomena in the hamster. *Gamete Research.* 5: 323–344.

Yanagimachi, R., 1984. Zona-free hamster eggs: their use in assessing fertilizing capacity and examining chromosomes of human spermatozoa. *Gamete Research.* 10: 187–232.

Yanagimachi, R., 1988. Mammalian fertilization. In Knobil, E., Neill, J.D., Ewing, L.L. *et al.* (eds), The Physiology of Reproduction, Volume 1. Raven Press Ltd. New York, pp. 135–185.

Yanagimachi, R., 1994. Mammalian fertilization. In: Knobil E, Neill J (eds) The Physiology of Reproduction. Raven Press, New York

Yanagimachi, R. & Mahi, C.A., 1976. The sperm acrosome reaction and fertilization in the guinea pig: a study in vivo. *Journal of Reproduction and Physiology.* 46, 49–54.

Yanagimachi, R. & Mahi, C.A., 1978. Capacitation, acrosome reaction, and egg penetration by canine spermatozoa in a simple defined medium. *Gamete Research,* 1 (2): 101-109.

Yanagimachi, R. & Phillips, D.M., 1984. The status of acrosomal caps of hamster spermatozoa immediately before fertilization in vivo. *Gamete Research* 9(1):1–19

Zamiri, M.J., Khalili, B., Jafaroghli, M. & Farshad, A., 2010. Seasonal variation in seminal parameters, testicular size, and plasma testosterone concentration in Iranian Moghani rams. *Small Ruminant Research* 94:132-136. doi:10.1016/j.smallrumres.2010.07.013

Zarazaga, L.A., Guzman, J.L., Dominguez, C., Perez, M.C. & Prieto, R., 2009. Effects of season and feeding level on reproductive activity and semen quality in Payoya goat bucks. *Theriogenol*ogy 71: 1316-1325.

Zeder, M.A. & Hesse, B. 2000. The initial domestication of goats (*Capra hircus*) in the Zagros Mountains 10,000 years ago. *Science* 287:2254–2257.