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The Use of Management and Hormonal Oestrous Synchronisation on Indigenous Sheep Reared Extensively

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Abstract: As the global population increases, demand for animal products will result in increased livestock-related greenhouse gas emissions that contribute to climate change. One of the strategies for reducing this impact would be the improvement of indigenous livestock reproductive efficiency that are hardy and well adapted to harsh environmental regions. The use of reproductive technologies increases efficiency of animals necessary for profitable production. This study evaluated the effects of age and body condition score on the response and duration of oestrus in synchronized Bapedi ewes, conserved ex-situ *in vivo* and in situ. Study ethics procedures were approved by the agricultural research council Irene animal ethics committee. Ninety-one Bapedi ewes (<2 and 3-6 years of age) were used in the study from five different research stations (Towoomba = 12, Tompi Seleka = 19, Mara research station = 20 and Agricultural research council Irene = 40). Ewes were chosen based on the Body Condition Scores (BCS) <3 and BCS≥ 3 on a scale of 1-5 during breeding season (March-June). For estrous synchronization, Controlled Intravaginal Drug Release (CIDR®) dispensers were inserted for 9 days and 300 IU of equine chorionic gonadotrophin was injected intramuscularly after CIDR removal. Oestrus detection was done for a period of 72 h, from CIDRs withdrawal with a vasectomized ram and ewes that were receptive to the ram were hand mated with Bapedi rams. Data was subjected to GLIMMIX procedure and Chi-square in SAS 2009. The results were measured significant when $p<0.05$. There were no significant differences observed in the oestrus response of ewes regardless of age ($p>0.05$) and method of conservation. The oestrus response was higher for ewes with BCS≥ 3 compared to the lower BCS group ($p<0.05$). Old and lower BCS ewes showed oestrus signs earlier (23±2.8; 21±4.1); (22±4.1; 20±5.3) and with a shorter duration (23±8.2; 20±6.2); (22±4.0; 23±3.2) compared to young and higher BCS groups (onset of oestrus: 34±2.0; 32±2.4); (36±1.3; 35±2.3) duration (30±1.3; 29±1.5); (33±5.0; 32±6.0) ($p<0.05$). Higher oestrus was observed on ewes with BCS ≥3. Young and high BCS ewes had a delayed onset of oestrus that lasted longer compared to old and lower BCS ewes. The conception rate was 65, 67, 53 and 70% for ARC, Towoomba, Tompi Seleka and Mara Research Station respectively ($p>0.05$). Towoomba farm had a significantly lower litter size recorded compared to all the other farms. The prolificacy of Bapedi sheep was 1.30±0.6 1.28±1.3; 1.29±0.8 and 1.31±0.5 for ARC, Towoomba, Tompi Seleka and Mara farms respectively. It was concluded that the conservation method did not affect the reproductive performance of Bapedi

sheep. Bapedi ewes can be synchronized successfully with an acceptable conception rate without supplementary feeding. It is recommended that flush feeding should be done to improve the litter size.

Keywords: Age, Body Condition, Oestrous Synchronization, Indigenous Sheep, Conservation

Introduction

South Africa has 23.71 million sheep but remains an importer of mutton (Ngcobo *et al*., 2022). To grow and diversify production from livestock in South Africa, sheep farming might be the best choice as indigenous sheep are well known for adaptability, survivability and disease tolerance. Production of mutton locally needs to be increased to meet the increasing demand. Improving the reproductive performance of locally adapted breeds would be a great strategy and only then will the aim to secure food for the future be a reality (Aké-Villanueva *et al*., 2017; Cavalcanti *et al*., 2012). As the global population grows and income rises, the demand for animal products is predicted to increase as well. Reproductive biotechnologies can assist initiatives to speed up the process of improving reproductive performance and thus meet the demand. These Assisted Reproductive Technologies (ARTs) improve the breeding efficiency of livestock and reduce the number of non-productive animals (Cavalcanti *et al*., 2012).

It is well known that the blackhead Persian has a yearlong estrus activity, with Merinos and Dorsets having an extended estrus period such that they could have two lamb crops per year (Debaca *et al*., 1954). Nevertheless, exotic breeds cannot tolerate harsh South African environmental conditions and South Africa is known as a water-scarce country (Cop 28, 2023). Indigenous sheep breeds such as Bapedi, Namaqua Afrikaner and Zulu sheep of South Africa can walk longer distances in search of food and water (Mavule *et al*., 2016). Bapedi sheep is a naturally selected, minimum care veld sheep that thrive well in harsh environmental conditions (Ngcobo *et al*., 2022). They produce lean meat because fat is centralized in the tail (Maqhashu *et al*., 2020). However, this breed is becoming threatened (DAD-IS, 2024) with its population decline because of unsupervised crossbreeding due to its body size and slow growth rate (Ngcobo *et al*., 2022). As a result, the application of assisted reproductive biotechnologies in the conservation programs/research farms may improve population growth and hence save this breed from extinction.

Estrus synchronization in ewes is an effective reproductive technology that favors productivity, eases management and offers homogeneous groups of lambs at suitable seasons to take advantage of niche markets, feed supplies and rising price trends (IIDA *et al*., 2004). In addition, estrous synchronization, by virtue of increasing the reproductive efficiency of a flock, contributes to sustainable livestock production. This is due to an increase in the lambing rate of a flock when estrous synchronization is used. The better the management of a flock, the more accurate estrus detection is and subsequently, the ewes are then bred at optimal times (Engle, 1965). It is notable that the ovarian response of ewes to estrous synchronization changes according to the type of intravaginal device, kind of progestogen, body condition, presence of stress factors, environmental aspects, male effect, age of the ewes and breed (Cavalcanti *et al*., 2012). Body Condition Score (BCS) is a management tool used to monitor the nutrition of flocks, especially when they are on pastures (Maqhashu *et al*., 2020). Body condition scores are scored on a scale of 1 -5. Animals with scores of 1-2 are thin and it is easy to feel the bones in the loin region, firm pressure is needed to feel the bones in condition scores of 3-4. In animals with a BCS of 5, it is difficult to feel the bones (Jalilian and Moeini, 2013). Body condition is used to predict negative energy balance severity, where the loss of energy is associated with suppressing the luteinizing hormone (Aké-Villanueva *et al.*, 2017; Yilmaz *et al*., 2011; Wang *et al*., 2016). Body condition scores are associated with many reproduction disorders such as delayed puberty, reduced ovulation rates, pregnancy rates and postpartum anestrous. Age is another important factor reported to affect ovulation rates in ewes, adult ewes are said to have higher fertility compared to young and maiden ewes (Scaramuzzi and Radford, 1983; Santolaria *et al*., 2011).

The reproductive performance of indigenous sheep breeds such as Bapedi, Zulu and Namaqua Afrikaner has not been fully studied and hence there are no strategies in place for their conservation and utilization (Nsoso *et al*., 2004; VanLeeuwen *et al*., 2021). The research into the use of these breeds for sustainable livestock production must be a priority considering their suitability for harsh environmental conditions that will be typical during climate change. Assisted reproductive technologies such as estrous synchronization may be adopted to improve ovulation rates. Animals of different ages have different physiological developments and respond differently to hormonal treatments; this might reduce the impact of reproductive technologies on livestock improvement programs (Lehloenya and Greyling, 2010). The importance of studying the approximate time of onset of estrous in ewes assists not only for mating on time but also for having a uniform lambing time. This in turn contributes to a more

productive flock with fewer animals compared to a less productive flock with more animals and subsequently, less greenhouse gas emissions (Cavalcanti *et al*., 2012). Therefore, the objective of this study was to evaluate the effects of age and BCS on the response and duration of estrous in synchronized indigenous Bapedi ewes.

Materials and Methods

The study was conducted at four indigenous animal conservation farms in the Gauteng and Limpopo provinces of South Africa., namely: Agricultural Research Council (n = 40), Mara research farm (n = 20), Toowoomba research farm (n = 12) and Tompi Seleka college of agriculture farm (n = 19) selected based on availability. The ewes used in this study had an average body weight between 48-68 kg and age 3-6 years. The study ewes were grazing on kikuyu grass pasture and clean drinking municipality water was provided ad libitum. Ewes were grouped according to their body condition on a scale of 1-5. Ewes with the body condition scores of BCS of <3 were group 1 (n = 41) and ≥3 group 2 (n = 50). The ewes' body condition ratings were recorded on a scale of 1-5 according to Matar and Aljummah (2023). In summary, BCS 1 was an ewe with no fat between the skin and bones, BCS 2 was a ewe with a light coating of fat on the bones, BCS 3 was an ewe with a smooth appearance layer of fat, BCS 4 was a ewe with a plump appearance layer of fat and BCS 5 was an obese ewe with excessive body fat. Age groups were determined by counting the number of permanent incisors as described by Kunene *et al*. (2009) and farm records.

Oestrous Synchronization and Natural Mating

The short-term progestagen protocol as described by Ramukhithi *et al*. (2012) was employed to synchronize oestrus in this study with few amendments. In brief, a Controlled Internal Drug Release device (CIDR) (0.3 g progesterone) was inserted in the vagina of the ewe for 9 days and were injected intramuscularly with 300 IU of eCG at progestogen removal of CIDR. At 0, 12, 24, 36, 48, 60 and 72 h after progestogen (12 h interval), withdrawal ewes were observed for signs of heat for 2 h using a vasectomized ram. Ewes that were on heat were separated and put together with a ram at a ratio of 1:5 and observed to ensure that mating occurred. All rams were tested for breeding soundness and declared fertile (+90% total sperm motility and +90% normal sperm morphology) before taking part in breeding. Ewes that were on heat were left with fertile rams until they rejected to be mounted by the rams.

Data Recorded

The data was recorded for the response of ewes to hormonal synchronization, conception rate, gestation length and multiple births, using the following formulas:

$$Conception\ rate\ = \frac{Number\ of\ ewes\ responded}{Number\ of\ Ewes\ synchronized} \times 100$$

$$Gestation\ Period = lamibng\ day - \ mating\ day$$

$$Litter\ size\ = \frac{Number\ of\ lambs\ born}{number\ of\ Ewes\ mated} \times 100$$

Birth weight was measured using the weighing scale as soon as lambing or parturition was complete. Weaning weight was measured using a weighing scale 3 months after lambing. The effect of dam weight before mating, litter size and sex of the lamb on birth weights and neonatal loss were also studied.

Conception Rate

Ewes were observed closely for return to oestrous and none returned to oestrous ewe were then subjected to pregnancy diagnosis by an ibex pro transabdominal ultrasound scanning machine between 30-35 days following mating (EI Medical, USA). The conception rate was then calculated as the number of ewes pregnant in relation to the number of ewes exposed to the fertile rams.

Statistical Analysis

Data regarding the effect of BCS and age on oestrous onset and duration were analyzed using the GLIMMIX procedure of SAS version 9.1, while the oestrous response was analyzed with the aid of the Chi-square test SAS 2009. The results were measured significant when $p<0.05$.

Results

This study evaluated the use of management and hormonal estrous synchronization on indigenous sheep reared extensively. Table 1 represents the effect of age on the estrus synchronization response in both ex-situ and in-situ. The oestrous response of Bapedi ewes was not influenced ($p>0.05$) by the conservation method and age. The time interval between CIDR® removal and onset of oestrous was significantly ($p<0.05$) earlier with short duration for the old ewes in compared to the young ewes in both ex-situ *in vivo* and in situ conservation methods as shown in Table 1.

The current study further evaluated the influence of body condition on the oestrous response, onset of estrous and duration and the results are set out in Table 2. The oestrous response, onset of estrus and duration of estrus were higher for BCS<3 compared to BCS≥3 in both conservation methods (ex-situ and in-situ).

Table 1: Effect of Age on the estrous synchronization response

Conservation method		N	Estrous response (%)	Onset of estrus (h)	Duration of estrus (h)
Ex situ-*in vivo*	Young (1-2)	18	83 (15/18)	34±2.0[a]	30±1.3[a]
	Old (3-6)	22	82 (18/22)	23±2.8[b]	23±5.2[b]
In situ	Young (1-2)	20	80 (18/20)	32±2.4[a]	29±1.5[a]
	Old (3-6)	31	84 (26/31)	21±4.1[b]	20±6.2[b]

[ab]Means within the same column with different superscripts differ significantly ($p<0.05$). h: Hours

Table 2: Effect of BCS on the estrous synchronisation response

Conservation method		N	Estrous response (%)	Onset of estrus (h)	Duration of estrus (h)
Ex situ-*in vivo*	BCS<3	15	73 (11/15)[a]	22±4.1[a]	22±4.0[a]
	BCS≥3	25	92 (23/25)[b]	36±1.3[b]	33±5.0[b]
In situ	BCS<3	22	73 (16/22)	20±5.3[a]	23±3.2[a]
	BCS≥3	29	90 (26/29)	35±2.3[b]	32±6.0[b]

[ab] Means within the same column with different superscripts differ significantly ($p<0.05$). h: Hours

Table 3: Expression of oestrus (%), conception rate (%), gestation length (Mean ± SE) of indigenous Bapedi sheep following oestrous synchronization and natural mating on *in situ* and *ex-situ in vivo* conservation methods

Parameters	Farms	N	Expression of estrus %	Conception rate %	Gestation length	#Lambs
Ex-situ in vivo	ARC	40	39/40 (98)	26/40 (65)	148.9±1.2	1.3±0.2
In-situ	Towoomba	12	10/12 (83)	8/12 (67)	150.1±0.4	1.0±0.0[a]
	Tompi Seleka	19	19/19 (100)	10/19 (53)	149.2±0.6	1.2±0.4
	Mara	20	17/20 (85)	14/20 (70)	146.8±2.9	1.2±0.3

Table 4: Lambing rates (%), prolificacy (Mean ± SE), multiple birth rate (%), ewe and lamb motility rates (%), sex of lamb (%) birth and weaning weights of lambs (Mean ± SE)

Parameters	Farms	N	LR	Prolificacy	MBR	EMR	LMR	ML	FL	FBW	MBW
Ex-situ in vivo	ARC	40	70	1.30±0.6	7.50	0	0	83	17	2.9±0.9	3.6±1.2
In-situ	Towoomba	12	67	1.28±1.3	0.00	0	0	98	2	3.2±0.3	3.6±0.6
	Tompi Seleka	19	68	1.29±0.8	15.07	0	0	84	16	3.0±0.6	3.5±0.6
	Mara	20	85	1.31±0.5	15.04	0	0	88	12	3.0±0.4	3.8±0.7

LR; Lambing Rates, MBR; Multiple Birth Rates, EMR; Ewe Mortality Rates, LMR; Lamb Mortality Rates, ML; Male Lamb, FL; Female Lamb FBW; Female Birth Weights; MBW; Male Birth Weights

The expression of estrus (%), conception rate (%) and gestation length of indigenous Bapedi sheep following estrous synchronization and natural mating on in situ and ex situ *in vivo* conservation methods was also evaluated and the results are depicted in Table 3. Among the research stations used in this study, the Tompi Seleka farm had the lowest conception rate (53%) when compared to other research farms, with the Mara research farm scoring the highest 70% of conception rate. No significant differences were observed between the in-situ and the ex-situ *in vivo* conserved Bapedi sheep with regard to gestation length ($p>0.05$). Towoomba farm only had single births compared to all the other stations even though the percentage of multiple births was low in all the farms.

The Lambing rates (%), prolificacy, multiple birth rate (%), ewe and lamb motility rates (%), sex of lamb (%) birth and weaning weights of lambs were evaluated and the results can be obtained in Table 4. Mara farm had a higher number of lambs born alive compared to all the other stations, even though in general the prolificacy of all the Bapedi sheep farms was acceptable all above 50%. Bapedi sheep had lower numbers of multiple births in all the farms with Towoomba farm having only single births. It was highlighted that Bapedi sheep had no lamb mortalities or ewe mortalities in both conservation methods. In all the Bapedi sheep on both the conservation methods most of the lambs born were male compared to females.

Discussion

Effect of Age on the Estrous Synchronisation Response

In the current study, no significant differences were observed in the estrus response of ewes in all age groups. These results agree with findings by Ramukhithi *et al*. (2012); Webb *et al*. (2010) where the age of the ewe did not affect estrous. The onset of estrus was earlier on the old (3-6 years) ewes with a shorter duration compared to young (1-2) ewes. A similar degree of synchrony was obtained in other studies when evaluating the onset of estrus adult ewes recorded a shorter time from CIDR® removal to first signs of heat compared to the young ones (Simonetti *et al*., 1999; Omontese *et al*., 2013). Results obtained from this study are comparable to results

obtained from work done in goats by (Lehloenya and Greyling, 2010), where the time to onset of oestrus was shorter for the adults compared to young does. Moreover, Debaca *et al*. (1954) also reported similar findings. On the other hand, Kenyon and Corner-Thomas (2022) reported conflicting findings where it was reported that maiden ewes have a shorter and less intense estrus than old ewes. Contentious, to these findings no differences were reported about the onset of estrous when adult and lamb ewes were synchronized using medroxyprogesterone and pregnant mare serum gonadotrophin over 14 days (Simonetti *et al*., 1999). Age influences ovulation rate and lambing performance, it was reported that adult ewes produced multiple births than younger ewes and that the percentage of multiple births increased with the age of the ewe. Furthermore, in another study, it was observed that yearlings and 5-7-year-old ewes had lower ovulation rates compared to 4-6-year-old ewes (McKenzie and Terrill, 1937) and concluded that lower percentages of multiple births in yearlings were as a result of lower ovulation rates.

Effect of BCS on the Estrous Synchronization Response

There is limited information on the influence of BCS on the synchronization response of ewes. Body condition affects the estrous, endocrine and follicle development and conception of both livestock and human beings. Ewes that are adequately nourished and maintained in good body condition respond most rapidly to oestrous synchronization with an increase in ovulation rate (Santolaria *et al*., 2011; Nedelkov *et al*., 2015). Results obtained from this study indicated that body condition influences the response, onset and duration of oestrous in Bapedi ewes. Higher BCS (≥3) ewes responded better to oestrous synchronization (92%) compared to lower BCS (<3) (73%) and the duration of estrus was longer on the high BCS compared to lower BCS ewes. Ewes with low BCS onset of estrus from CIDR removal was shorter compared to the high BCS group. Similar results were obtained when BCS 2 and 3 were synchronized for estrous and BCS 2 showed estrous signs earlier (25-60 h) after CIDR® removal compared to the higher BSC group (30-70 h), however, synchrony and duration were significantly higher and longer in do on the BCS 3 group compared to lower BCS group (Widayati *et al*., 2011). Nedelkov *et al*. (2015) obtained similar results, where ewes of different body condition scores were synchronized by the introduction of a teaser ram and the high BCS ewes responded better compared to ewes with BCS<2. There is limited information on the influence of BCS on the response of onset and duration of ewes to oestrous synchronization with exogenous hormones. Yilmaz *et al*. (2011) reported that BCS of >2.1 was positively effective on reproductive performance and lower BCS ewes during mating resulted in poor pregnancies, lower litter size and were most likely to abort. Similarly, in Malpura ewes BCS 3-3.5 was reported as an optimum score for improving fertility (Sejian *et al*., 2010). According to Yilmaz *et al*. (2011), BSC affects the circulation of reproductive hormones in goats and does with BCS 3 had a higher concentration of circulating progesterone, estradiol-17ß and luteinizing hormone. The fertility of BCS 3 was better compared to BCS 2 and delayed puberty and stopped estrous in cycling heifers.

Expression of oestrus (%), conception rate (%), gestation length of indigenous Bapedi sheep following estrous synchronization and natural mating on in situ and ex situ *in vivo* conservation methods.

Hormonal treatment for synchronization of Bapedi sheep was successful and a conception rate of 53, 65, 67 and 70% was obtained from Tompi Seleka, ARC, Towoomba and mara research station respectively following natural mating. Conception rates obtained in this study were lower compared to 83% obtained from a non-supplemented group of Bangladesh indigenous ewes (Zohara *et al*., 2014). But was comparable to findings by (Lehloenya *et al*., 2005) done in South African Nguni goats. The percentage of oestrous response and the conception rate in this study were comparable to findings from other studies (Zohara *et al*., 2014). The gestation length of Bapedi sheep ranged from 146.8±2.9-150.1±0.4 and there were no significant differences among the farms and within individual conservation methods. The gestation period for multiple and single births was similar in all the Bapedi sheep breeds in all the farms. The gestation length of Bapedi sheep was longer compared to the gestation length from the Bangladesh indigenous ewes which ranged from 141-145 days. The gestation length was also like that obtained from South African Boer and Nguni does. Nutrition during pregnancy has been reported to be a major influencer of gestation length, with maternal undernutrition resulting in longer pregnancies. Lower-energy diets reduced pregnancy rates (Zohara *et al*., 2014; Kusina *et al*., 2001; El-Hag *et al*., 2007). Towoomba farm had a smaller ($p<0.05$) litter size compared to other farms with ewes giving birth to only one lamb per ewe. There were no significant differences in the number of lambs/ewe between ARC, Mara and Tompi Seleka farms. These findings might be because litter size is influenced by several factors such as genotype, parity, season and ewe body weight at mating (Mukasa-Mugerwa and Lahlou-Kassi, 1995). Litter size like the results of this study was also obtained by Ashebir *et al*. (2016).

Lambing rates (%), prolificacy, multiple birth rate (%), ewe and lamb motility rates (%), sex of lamb (%) birth and weaning weights of lambs.

Mara research station had a higher lambing rate compared to all other farms ($p<0.05$) with an 85% lambing rate, compared to 68, 67 and 70% for Tompi Seleka, Towoomba and ARC respectively. The results from this study were lower compared to lambing rates of (85.3%) brown and (89.7%) black-faced Awassi ewes kept under the same traditional management conditions. These results were similar to indigenous ewes of Bangladesh where 75% lambing rates were obtained. The prolificacy of Bapedi sheep was similar to that obtained from the brown and black head Awassi, the Horro ewes from western Ethiopia and Begayt ewes (Kridli *et al*., 2009; Ashebir *et al*., 2016; Sakatani, 2022). Bapedi sheep had the lowest number of multiple births compared to Awassi and Begyat ewes. The twinning or percentage of triplets can be improved by flush feeding and better management, through selection. The potential of the reproductive performance of Bapedi sheep has not been exploited properly. There were no ewe and lamb mortalities in all the farms. This can be attributed to the adaptation traits of the Bapedi sheep. Surprisingly Bapedi ewes gave birth to mostly male lambs than females. Ram lambs had higher birth weights than ewe lambs, these findings were like the results of indigenous Tswana goats (Nsoso *et al*., 2004).

Conclusion

It was concluded that the conservation method did not affect the reproductive performance of Bapedi sheep. Bapedi ewes can be synchronized successfully with an acceptable conception rate without supplementary feeding. It is recommended that flush feeding should be done to improve the number of offspring per ewe. When considering the relevance of reproductive biotechnologies and particularly estrus synchronization to conservation, it is important to note that the Bapedi sheep breed and other indigenous breeds in the hands of smallholder farmers, may be the key to meeting the increased global demand for animal products. By innately being hardier and more disease tolerant, Bapedi sheep are expected to be able to cope with the changing and sometimes drastic environmental changes that accompany climate change. Their reproductive physiology and its manipulation must be studied for their optimal utilization.

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Author's Contributions

Ayanda Maqhashu: Conceptualized the study, wrote original draft.

Jabulani Nkululeo Ngcobo: Wrote original draft and edited manuscript.

Hester Adri O'Neill, Olivia Mapholi, Rimbilana Shingange, Khathutshelo Agree Nephawe and Tshimangadzo Lucky Nedambale: Edited manuscript.

Phokgedi Julius Sebei and Fhulufhelo Vincent Ramukhithi: Data collection, edited manuscript.

Ethics

Authors would like to confirm that this manuscript is the original work of the authors and does not contain any unpublished material. The corresponding author further confirms that all the authors read and approved this study and no ethical matters are involved.

Conflict of Interest

The authors declare that there are no competing interests.

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