

1 **Short-term oestrous synchronisation protocol following single fixed-time**
2 **artificial insemination and natural mating as alternative to long-term protocol in**
3 **dairy goats**

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13 **Highlights**

- 14 • Short and long-term synchronisation protocols following fixed-time artificial
15 insemination and natural mating have comparable influence on fertility in dairy goats.
16 • Short-term synchronisation protocol can be alternative to long-term protocol
17 following fixed time artificial insemination or natural mating in dairy goats.
18 • Short and long-term synchronisation protocols equally influence oestrous response,
19 onset of oestrus and duration of oestrus in dairy goats.

20
21 **Abstract**

22 This study investigated the hypothesis that the use short-term synchronisation protocol
23 following single fixed-time artificial insemination (AI) with extended cooled semen and
24 natural mating in fertility management of dairy goats could be as good as or better than
25 traditional long-term protocol. This was tested by designing an experiment using Toggenburg
26 dairy goats raised under semi-intensive production system in the tropics. Twenty-eight (28)
27 females Toggenburg dairy goats were randomly allocated to two synchronisation protocols in
28 completely randomised design and within each synchronisation protocol the animals were
29 further subdivided into two mating methods. Oestrus was synchronised using short (7 days)
30 and long-term (12 days) protocols and animals mated using natural mating and AI. The onset
31 and the duration of oestrus were monitored using two intact-aproned bucks following
32 controlled internal drug release (CIDR) devices withdrawal. The non-return to oestrus
33 method was used to determine conception rate. The onset and duration of oestrus, response to
34 oestrus and conception rate were evaluated. The onset and duration of oestrus was analysed
35 using one-way ANOVA, while response to oestrus, conception rate and kidding rate were
36 analysed by using Chi-Square test. Generally, the two protocols realised 100% response to
37 oestrus. Onset and duration of oestrus in short-term protocol were 31.75hrs and 31.70hrs,
38 respectively, while the corresponding values for long-term protocol were 33.33 and 30.93hrs.
39 The two protocols did not significantly differ in onset and duration of oestrus, conception,
40 kidding and twinning rate. Similarly, the two mating methods did not differ significantly on
41 conception, kidding and twinning rates. The current study has an overall of conception rate,
42 kidding and twinning rate of 71.42, 64.29 and 44.50%, respectively. The short-term protocol
43 following single fixed-time AI and natural mating therefore, can be alternative to long-term
44 oestrous synchronisation protocol in dairy goats.

45 **Key words:** Conception rate, oestrous response, reproductive performance, Toggenburg
46 goats

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1. Introduction

49 The traditional long-term progestagen based oestrous synchronisation protocols
50 normally range from 10-19 days (Pietroski et al., 2013; Harl, 2014). The long-term protocol,
51 however, has been associated with low fertility rates (Diskin et al., 2002; Pietroski et al.,
52 2013). This low fertility has been linked to sub luteal serum progesterone concentrations
53 which is associated with some abnormalities in follicular development, ovulation, oocyte
54 health, luteal function (Evans et al., 2001; Menchaca and Rubianes, 2001; Viñoles et al.,
55 2001). Given the above reasons and long duration of treatment for the traditional
56 synchronisation protocol, short-term protocol have been suggested (Viñoles et al., 2001;
57 Menchaca and Rubianes, 2004; 2007; Karaca et al., 2010; Menchaca et al., 2018). These
58 studies demonstrated that, the short-term protocol is associated with supraluteal levels of
59 progesterone concentrations, which positively influence follicular turnover, increases the
60 number of healthier young large follicles with the potential to ovulate and improve pregnancy
61 rate.

62 It has been documented that fertility can be influenced depending on whether fresh,
63 cooled or frozen semen is used. On the other hand, timing of insemination or natural mating
64 following synchronised oestrus plays crucial role in determining the fertility of an animal.
65 Therefore, it is important to conduct studies looking at how different types of synchronisation
66 protocols and mating methods influence fertility.

67 Comparative studies have been conducted comparing short and long-term oestrous
68 synchronisation protocols following artificial insemination (AI) and natural mating (Karaca et
69 al., 2010; Ramukhithi et al., 2012; Pietroski et al., 2013). In these studies, does were
70 inseminated with fresh raw semen using cervical or laparoscopic technique either 48 hrs
71 following CIDR removal or according to the onset of oestrus at 48 and 60 hrs following
72 sponge removal. Use of invasive technique such as laparoscopy is not convenient especially,
73 in routine breeding of animals by farmers. Also application of AI at different time intervals
74 takes long time in performing breeding activities of animals and may cause inconvenience in
75 terms of management. Therefore, use of single fixed-time AI could offer an alternative
76 convenient option in breeding of goats. There are, however, no studies to the best of our
77 knowledge comparing short and long-term synchronisation protocols following single fixed-
78 timed AI with extended cooled semen and natural mating. We reasoned that synchronising
79 oestrus using short-term protocol following single fixed-time artificial insemination with
80 extended cooled semen and natural mating could be as good as or better than long-term
81 protocol in fertility of exotic dairy goats in the tropics. This is because short-term protocol is
82 associated with high progesterone, which could lead to improved follicular development and
83 health, rate of ovulation and sperm transport. We tested this hypothesis by designing an
84 experiment comparing short and long-term oestrous synchronisation protocol following
85 single fixed-time AI and natural mating using Toggenburg dairy goats raised under semi-
86 intensive production system in the tropics.

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2. Materials and methods

2.1 Ethical approval

91 The materials and procedures of this study had been approved by Kenya Institute of
92 Primate Research under the permit No: ISERC/10/19 and National Commission of Science
93 and Technology under the permit No: NACOSTI/P/19/76927/28821.

94 **2.2 Experimental site**

95 This study was conducted between August and September, 2019 at the Tatton
96 Agriculture Park (TAP), Egerton University, Njoro. Njoro is approximately within latitude
97 00° 19'00" S and longitudes 36° 06'00" E, and at an elevation between 2168 m and 2800 m
98 above sea level. The site receives monthly total rainfall of 86.3 mm, and its temperature
99 varies between minimum of 10.2 and maximum of 23.3°C (Wangui et al., 2018).

100

101 **2.3 Experimental animals and design**

102 In this study, 28 females Toggenburg goats in their third lactation were used and
103 allocated into two synchronisation protocols with mean initial body weight (Short-
104 term=59.00±2.76 kg and Long-term= 58.50±2.76 kg) not significantly different in a
105 completely randomized design. Within each synchronisation protocol the animals were
106 further subdivided into two mating methods with mean initial weight (AI= 58.71±2.76 kg and
107 Natural mating= 58.79±2.76 kg) not significantly different. The does and the bucks were kept
108 under semi-intensive system on natural pastures, supplemented with commercial concentrate
109 and mineral licks as well as water offered *ad libitum*. Supplementation with concentrate
110 commenced one month before the start of the study for both does and the bucks.

111

112 **2.4 Synchronisation of oestrus**

113 Two synchronisation protocols were used; short and long-term progestagen treatment.
114 All does in both groups were treated intravaginal with progesterone using controlled internal
115 drug releasing device (CIDR-G) (Pfizer, New Zealand) containing 0.3 g progesterone. The
116 CIDR-G device was left for 7 days and 12 days for short and long-term progestagen
117 treatment, respectively. At CIDR removal, all does in both groups were injected with 150 µg
118 of prostaglandin F2α (PGF2α) analogue and 200 IU of equine chorionic gonadotropin (eCG)
119 (Intervet Schering-Plough Animal Health, South Africa). Literatures for this protocol
120 includes (Greyling and Van der Nest, 2000; Romano, 2004; Fonseca et al., 2005; Menchaca
121 and Rubianes, 2007; Karaca et al., 2010; Pietroski et al., 2013).

122

123 **2.5 Semen collection and evaluation**

124 *Semen collection;* Semen was collected using electro-ejaculator for small ruminants
125 (Lane Manufacturing, Denver, Colorado, USA). The collection procedures followed was
126 according to instructions from the company. Buck was restrained in a standing position and
127 the urethral opening cleaned. Then the rectal probe was lubricated with a lubricant before
128 inserted in to the rectum. A collecting tube was fitted to the artificial vagina to collect the
129 semen.

130 *Semen evaluation;* The semen samples collected were evaluated for volume, semen density,
131 mass motility, progressive motility, live sperm cells, spermatozoa concentration. Semen
132 volume and density, mass motility and progressive motility were evaluated as described by
133 Steyn, (2005) using a phase contrast microscope (Richter Optica, Model U2, China) mounted
134 with a camera. Sperm concentration was determined using the standard procedures with an
135 aid of Neubauer improved haemocytometer (Marienfeld Company, Lauda-Königshofen,
136 Germany) under phase contrast microscope at magnification power of (x40). Semen was
137 extended using OPTIXcell extender, (IMV Technologies, France) as described by Juma,
138 (2017).

139 **2.6 Monitoring and recording parameters of oestrus**

140 The onset and the duration of oestrus were monitored using two aproned intact bucks
141 with high serving capacity following CIDR withdrawal. The does were monitored for total of
142 72 hrs following CIDR withdrawal at 8-hour interval for the detection of onset and duration

143 of oestrus. Onset and duration of oestrus were detected as described by Romano et al. (2018)
144 with few modifications. Briefly, oestrus was detected once during the first 12 hrs after CIDR
145 removal and then every 4 hr thereafter, at 10:00, 14:00, 18:00, 22:00, 02:00, and 06:00 hr for
146 72 hrs.

147

148 ***2.7 Natural mating and artificial insemination***

149 Mating was done using natural mating and timed AI using diluted cooled semen. For
150 the natural mating group, one male was mated to maximum of three (3) does following CIDR
151 withdrawal for a period of 12 hrs. In the AI group, a speculum with a built-in light source and
152 pipette connected to a 1 ml syringe was used to cervically inseminate the does (Steyn, 2005).
153 All does were inseminated cervically at single fixed-time of 48 hrs following CIDR
154 withdrawal. Each doe was inseminated with 0.4 ml of diluted cooled semen with sperm
155 concentration of 500×10^6 sperm cells.

156

157 ***2.8 Pregnancy diagnosis***

158 The pregnancy diagnosis was carried out using non-return to oestrus method as
159 described by Mellado (2016) with few modifications. The does were monitored twice a day
160 using two aproned bucks (Morning and evening) to detect does that returned to oestrus from
161 day 16-26 following natural mating and artificial insemination.

162

163 ***2.9 Evaluation of reproductive parameters***

164 The following parameters were evaluated. The onset and duration of oestrus as well as
165 the response to oestrus were evaluated as described by Romano et al. (2018). The conception
166 rate as the number of does that conceived out of the total number of does synchronised
167 multiplied by 100. Kidding rate was calculated as the number of does that kidded out of the
168 total number of does synchronised multiplied by 100. Twinning rate was calculated as the
169 number of does that kidded two (2) kids per total number of does kidded. None of the does
170 among those that kidded gave birth to more than two kids.

171

172 ***2.10 Statistical analysis***

173 The onset and duration of oestrus were analysed using one-way analysis of variance
174 (ANOVA). The model fitted was: $Y_{ij} = \mu + SP_i + \epsilon_{ij}$

175 where: -

176 Y_{ij} – Observation on the dependent variables, μ – Overall mean, SP_i – Fixed effect of
177 synchronisation protocol, ϵ_{ij} – Random error

178 Response to oestrus, conception rate, kidding rate and twinning rate were analysed using Chi-
179 Square test procedures of SAS (Version 9.0; 2002). Differences were considered significant
180 at $P < 0.05$.

181

182 **3. Results**

183 Our finding confirmed the premise that, synchronising oestrus using short-term
184 protocol following single fixed-time AI with extended cooled semen and natural mating
185 would be as good as the traditional long-term protocol in fertility of dairy goats raised in the
186 tropics. This was confirmed by no observable significant different between the two protocols
187 on response to oestrus cycle, onset of oestrus, duration of oestrus, conception rate, kidding
188 rate and twinning rate when single fixed-time AI with extended cooled semen and natural
189 mating was applied.

190

191 ***3.1 Effect of synchronisation protocols on response to oestrus, onset of oestrus and***
192 ***duration of oestrus.***

193 The mean response to oestrus (%), onset and duration of oestrus (hours) following
 194 short-term and long-term oestrous synchronisation protocols are presented in (Table 1). There
 195 was no significant difference ($P>0.05$) between short and long-term protocol in terms of
 196 response to oestrus, duration of oestrus and onset of oestrus.

197
 198

199 Table 1: Response to oestrus (%), onset and duration of oestrus (hours) (LSMeans±standard
 200 error) following short-term and long-term oestrous synchronisation protocol in Toggenburg
 201 goats.

Synchronisation protocol	No of goats	Response to oestrus	Onset of oestrus	Duration of oestrus
<i>P-value</i>		NS	NS	NS
Long-term	14	100	33.33±0.86	30.93±0.54
Short-term	14	100	31.75±0.84	31.70±0.52
Overall	28	100	32.54±0.85	31.32±0.53

202 * NS: Not significant at $P > 0.05$

203 In all the two protocols, all does showed signs of oestrus at least 28 hrs after CIDR
 204 withdrawal. More does showed oestrous signs earlier in the short-term than in long-term
 205 protocol. Additionally, at 40 hrs after CIDR withdrawal all the does in different groups
 206 showed signs of oestrus.

207

208 **3.2: Effect of synchronisation protocol and mating methods on conception rate, kidding**
 209 **rate and twinning rate**

210 The current study had an overall conception, kidding and twinning rates of 71.42 %, 64.29 and 44.50%, respectively (Table 2). There was no significant difference with each
 211 synchronisation protocol and mating method. When the data were pooled based on the
 212 oestrous synchronisation protocols and mating methods, no significant difference was
 213 observed between the synchronisation protocols and mating methods on conception, kidding
 214 and twinning rates (Table 2).

216

217 Table 2: Fertility performances following different oestrous synchronisation protocols and
 218 mating methods in Toggenburg goats

Treatments	No of goats	Conception rate (%)	Kidding rate (%)	Twinning rate (%)
<i>P-value</i>		NS	NS	NS
SP				
LT	14	10/14 (71.43)	8/14(57.14)	50
ST	14	10/14 (71.43)	10/14(71.43)	40
MM				
NM	14	11/14 (78.57)	10/14(71.43)	50
AI	14	9/14 (64.29)	8/14(57.14)	38

Overall	28	71.43	64.29	44
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219 *NS: Not significant at P > 0.05*

220 **AI:** Artificial insemination, **NM:** Natural mating, **ST:** Short-term, **LT:** Long-term, **MM:** Mating method, **SP:**
 221 Synchronisation protocol

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223

224 4. Discussion

225 The findings of the current study, supported reasoning that short-term synchronization
 226 protocol could be as good as traditional long-term protocol even with single fixed-time AI
 227 with extended cooled semen and natural mating. These findings are supported by previous
 228 studies, which did not find the difference in response to oestrus, duration of oestrus and
 229 conceptions rates in goats in temperate and tropical production environments (Karaca et al.,
 230 2010; Ramukhithi et al., 2012; Pietroski et al., 2013). The onset and duration of oestrus in the
 231 current study, however, were longer compared to those reported by Pietroski et al. (2013). In
 232 their study, the onset and duration of oestrus were 26.7 and 28.5 hrs in short and 25.2 and
 233 25.2 hrs in the long-term protocol, respectively. Similarly, Karaca et al. (2010) reported onset
 234 of oestrus of 28.8 and 28.0 in long and short-term synchronisation protocol, respectively.
 235 Moreover, Ramukhithi et al. (2012) reported longer onset of oestrus (34.7 vs 33.4) and
 236 duration of oestrus (37.9 vs 35.2) in short and long-term synchronisation protocols,
 237 respectively, than in the current study. These differences could be attributed to different types
 238 of progestagen devices used, use of gonadotropins, breed of goats, nutrition, season and male
 239 presence (Orihuela, 2000; Dogan et al., 2008). They used progestagen sponges during the
 240 non-breeding season while in the current study CIDR was used during the breeding season.
 241 These devices have different concentration levels of progesterone (Motlomelo et al., 2002).

242 The lack of significant difference in conception rate between short and long-term
 243 synchronisation protocols found in this study concurs with previous studies in goats (Karaca
 244 et al., 2010; Pietroski et al., 2013) and cows (Kasimanickam et al., 2015). On the contrary, to
 245 our findings Ramukhithi et al. (2012) reported higher pregnancy rates in Boer and indigenous
 246 goats when short-term protocol was used. These differences could be attributed to differences
 247 in breed, type of progestagen and quantity of equine chorionic gonadotropin used. Although
 248 there were no significant differences between the two synchronisation protocols, short-term
 249 protocol can be a better alternative than long-term protocol. This is because short-term
 250 protocol takes few days to synchronise animals and thus reduce time spent by farmers to
 251 breed their animals. In addition, CIDR from short-term protocol can be re-used with effective
 252 oestrous synchronisation and pregnancy rate (Vilarino et al., 2011).

253 In terms of kidding rate, there was no significant difference between short and long-
 254 term protocols. Short-term protocol, however, tended to have higher kidding rate (71.43 %)
 255 than in long-term protocol (57.14 %). This tendency could be attributed to the fact that short-
 256 term protocol achieved high progesterone concentration at the end of the synchronisation
 257 protocol, normal follicular turnover and ovulation of newly formed follicles (Viñoles et al.,
 258 2001; Menchaca and Rubianes, 2007). This study concurs with the previous finding by
 259 Karaca et al. (2010) who reported kidding rate of 76.5 % and 61.1 % in short and long-term
 260 protocol, respectively.

261 On the method of mating, regardless of oestrous synchronisation protocol, there were
 262 no differences recorded on conception rate, kidding rate and twinning rate between natural
 263 mating and AI using cooled extended semen. This finding is in agreement with the previous
 264 study which reported no differences in pregnancy rate and parturition rate between natural
 265 and AI (Pietroski et al., 2013). This similarity is despite the fact that in the current study
 266 single fixed-time AI was carried out 48 hrs following CIDR withdrawal irrespective of

267 whether a doe shown signs of oestrus or not, while in the study by Pietroski et al. (2013), they
268 inseminated animals at different time intervals according to signs of oestrus (48 and 60 hrs).
269 Contrary to the current study, other studies reported significant differences on pregnancy rate
270 between natural mating and artificial insemination with 93% and 70 %, respectively
271 (Agossou and Koluman, 2018). This inconsistency could be attributed to different semen
272 types and site of semen deposition used.

273 The current study confirmed that short-term protocol could replace long-term oestrous
274 synchronisation protocol following single fixed-time AI and natural mating in dairy goats. It
275 also demonstrated that, short and long-term oestrous synchronisation protocols have no effect
276 on oestrous response, onset of oestrus and duration of oestrus and conception rates of dairy
277 goats in the tropics.

278 **Conflict of interest**

280 The authors declare no any conflict of interest.

281 **Acknowledgement**

283 This material is based upon work supported by the United States Agency for International
284 Development, as part of the Feed the Future Initiative, under the CGIAR Fund, award
285 number BFS-G-11-00002, and the predecessor fund the Food Security and Crisis Mitigation
286 II grant, award number EEM-G-00-04-00013. Also the authors acknowledged Egerton
287 University and Centre of Excellence for Livestock Innovation and Business (CoELIB)) for
288 providing the experimental animals and allowing us to use the laboratory.

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