CHAPTER 4

4.0 RESULTS

A total of 35 surgery sessions of embryo recovery through oviduct (3 sessions) and uterine (32 sessions) flushing methods were conducted in this study. The effects of 4 factors (the dosage and pattern of the gonadotrophin (oFSH) administrated, breed and body weight) on embryo recovery were evaluated. Specific parameters measured were ovarian response; quantity, quality and the stages of embryo development obtained during the embryo recovery procedure. The effects of ICSI- and *in vivo*-derived embryos on embryo transfer performance were evaluated.

4.1 EFFECT OF DOSAGE LEVEL AND ADMINISTRATION PATTERNS OF oFSH ON OVARIAN RESPONSE IN SUPEROVULATED DONOR DOES

The effect of oFSH dosage and patterns administered on the number of total responded follicles, ovulation, embryos and ova recovered were determined in this study. A total of 590 oFSH-responded follicles were examined, in which 338 (57%) corpora lutea (CL) and 252 (43%) anovulatory follicles were detected. The corpora lutea appeared as highly vascularised body, which developed and emerged beyond the surface of the ovary (Figure 4.1). Meanwhile, the anovulatory follicles appeared as clear fluid-filled sac, 5 to 10 mm in diameter, and slightly protruded from the ovary (Figure 4.2). In all treatment groups, the number of CL and AF per doe varied from 0 to 40 and 0 to 16, respectively.

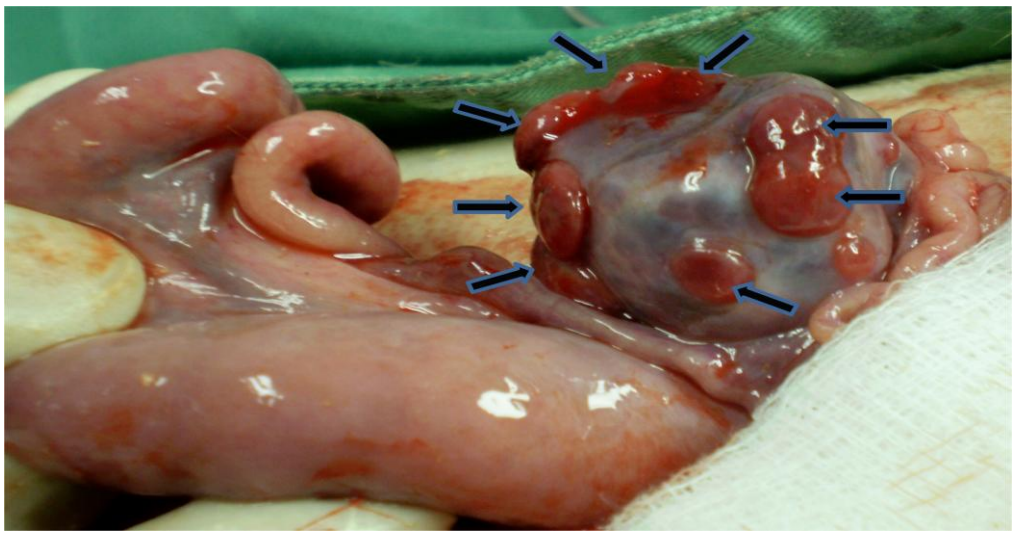

Figure 4.1. Corpus Lutea on the ovary of superovulated donor doe
*CL appeared as highly vascular body which develops beyond the surface of the ovary

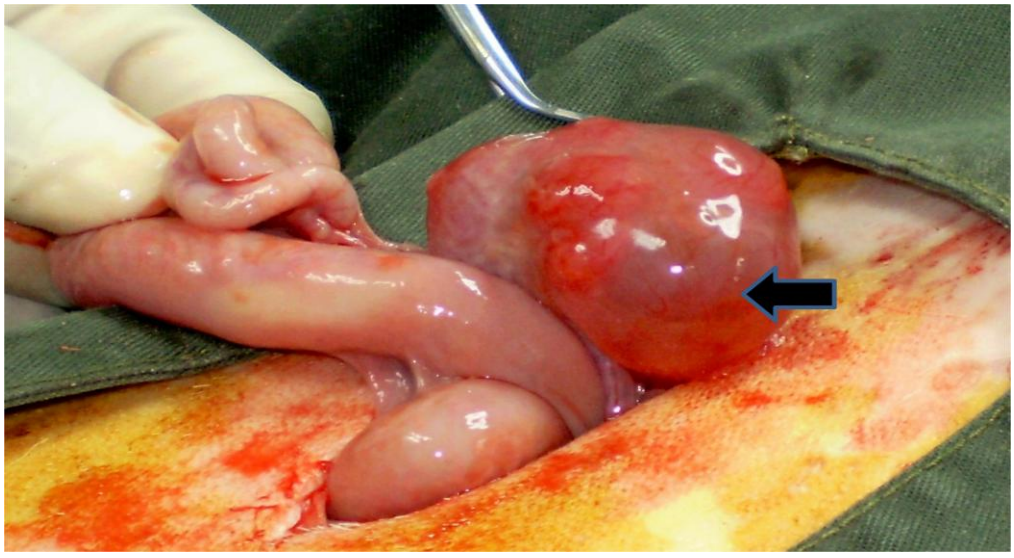

Figure 4.2. Anovulatory follicle on the ovary of superovulated donor doe
* Anovulatory follicle appeared as clear fluid-filled sac
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Table 4.1. Effect of oFSH treatments on ovarian response (mean ± SEM) in superovulated donor does

Parameters	S Single administration of 8.8 mg oFSH	M_1 Multiple administration of 8.8 mg oFSH	M_2 Multiple administration of 14.1 mg oFSH
Number of animals (n)	13	13	9
Ovulated does (%)[1]	69 (9)	100 (13)	100 (9)
Superovulated does (%)[2]	38 (5)	100 (13)	100 (9)
Total ovarian response per doe (CL plus AF/doe)	8.5 ± 1.4^{a}	20.0 ± 1.5^{b}	23.7 ± 4.4^{b}
Structures recovery per doe (embryo plus ova/doe)	0.5 ± 0.5^{a}	3.2 ± 1.2^{ab}	5.4 ± 2.4^{b}
Unfertilised plus degenerated ova per doe	0.5 ± 0.5^{a}	0.8 ± 0.5^{a}	3.4 ± 2.0^{a}
Viable embryos per doe	0.1 ± 0.1^{a}	2.4 ± 1.0^{a}	2.0 ± 1.4^{a}

[abc] Mean value within rows with different superscripts were significantly different $P<0.05$ level

[1] doe with at least 1 CL

[2] doe with more than 2 CL

Two factors that affect the ovarian response in superovulated does, namely the dosage and the patterns of oFSH administered, were observed and recorded in current study. Two dosages of multiple intramuscular (i.m.) injections of oFSH were administered into 2 does groups for 4 consecutive days starting 2 days before the end of CIDR treatment. Does in Group M_1 (n=13) received of 8.8 mg oFSH (low dosage group) and M_2 does (n=9) received 14.1 mg oFSH (high dosage group). For the patterns of oFSH administration, the ovarian responses of M_1 does were compared to Group S does (n=13). Does in S treatment group received same amount of oFSH as M_1 (8.8 mg) but in single injection (i.m.) upon progestagen (CIDR) removal. The ovarian response of 35 does that were treated in 3 different oFSH superovulatory treatments were evaluated during laparotomy sessions on days 3 and 7 after CIDR withdrawal.

The ovarian responses of the does following 3 different oFSH superovulatory treatments are presented in Table 4.1. All the does (100%) that received multiple administration of oFSH, regardless the amount of oFSH administration (M_1 and M_2) were responded to the treatments by ovulating with at least 1 corpus luteum. However, only 69% (9/13) of the does in single administration group (S) ovulating following to the treatment. As for superovulated does, all does in multiple administration groups were successful superovulated with more than 2 corpora lutea, except only 38% of single administration group. The total ovarian responses (CL plus AF) to superovulation treatment were significantly ($P<0.05$) higher in multiple administration group (M_2 and M_1) than S group, with ovarian response (CL plus AF) of 23.7 ± 4.4, 20.0 ± 1.5 and 8.5 ± 1.4, respectively. The structures recovery rates (embryos plus ova/doe) were also significantly ($P<0.05$) higher in M_2 (5.4 ± 2.4) group compared to M_1 (3.2 ± 1.2) and S (0.5 ± 0.5) groups; however, there was no significant difference between M_1 and M_2 groups. No significant differences ($P<0.05$) were observed in number of unfertilised ova and viable embryos in all groups recorded.

Table 4.2. Effect of oFSH treatments on CL formation (mean ± SEM) in superovulated donor does

Parameters	S Single administration of 8.8 mg oFSH	M_1 Multiple administration of 8.8 mg oFSH	M_2 Multiple administration of 14.1 mg oFSH
Number of animals (n)	13	13	9
Percent Ovulation (%)	35.5 ± 10.0^{a}	58.8 ± 3.9^{b}	72.4 ± 7.6^{b}
CL/doe	2.7 ± 0.7^{a}	11.6 ± 1.0^{b}	16.9 ± 3.9^{b}
Number of CL on left ovary/doe	1.0 ± 0.5^{a}	6.3 ± 0.7^{b}	8.8 ± 1.8^{b}
Number of CL on right ovary/doe	1.6 ± 0.5^{a}	5.3 ± 0.6^{b}	9.6 ± 1.9^{c}

[abc] Mean value within rows with different superscripts were significantly different $P<0.05$ level

Table 4.3. Effect of CL categories on ovarian response (mean ± SEM) in oFSH superovulated donor does

	CL Categories			
	1-2	**3-10**	**11-18**	**>18**
Number of animals (n)	4	14	10	3
Total ovarian response per doe (CL plus anovulatory follicles/doe)	7.6 ±2.0[a]	13.6 ±1.3[a]	21.4 ±1.3[b]	39.7± 4.9[c]
Percent ovulation (%)	21.5±12.7[a]	57.8 ±5.1[b]	67.2 ±6.6[b]	74.9 ±6.8[b]
CL/doe	0.9 ±0.4[a]	7.2 ±0.5[b]	13.8 ±0.9[c]	30.3 ±6.1[d]
Structure recovery per doe (embryo plus ova/doe)	0.0 ±0.0[a]	1.3±0.9[a]	7.7± 1.9[b]	1.0 ±1.0[a]
Unfertilized plus degenerated ova per doe	0.0 ±0.0[a]	1.1± 0.8[a]	2.9 ±1.7[a]	1.0± 1.0[a]
Viable embryo per doe	0.0 ±0.0[a]	0.1 ±0.1[a]	4.8 ±1.5[b]	0.0± 0.0[a]

[abcd] Mean value within rows with different superscripts were significantly different P<0.05 level

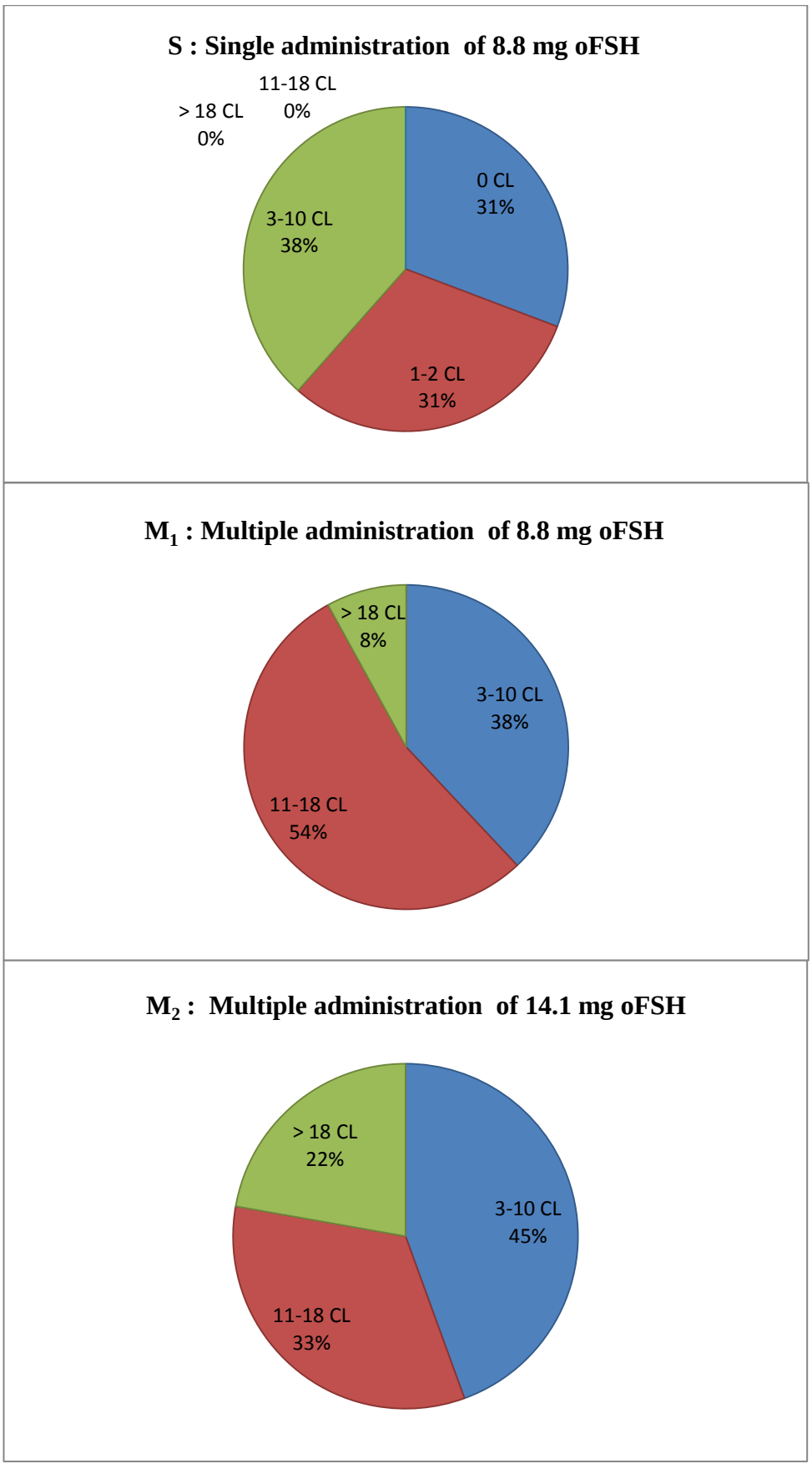


Figure 4.3. Distribution of the CL categories in donor does following different oFSH superovulation protocols

Table 4.2 shows the ovarian responses following single and multiple dosage superovulation treatments. Although no significant differences (P>0.05), the percent ovulation and CL per doe in multiple administration groups increased with increase in total oFSH dosage; in which, it was higher in 14.1 mg than 8.8 mg oFSH treated does (72.4±7.6 vs.58.8±3.9, 16.9±3.9 vs.11.6±1.0, respectively). However, does treated with same oFSH dosage, the percent ovulation, CL per doe and number of CL on left and right ovaries were significantly lowest (p>0.05) in single administration group (35.5±10.0, 2.7±0.7, 1.0±0.5 and 1.6±0.5, respectively).

Table 4.3 shows the effect of CL categories on ovarian response of superovulated does using oFSH, regardless involvement of other factors. The total ovarian responses were significantly increased (P<0.05) with CL categories, in which it was the highest in more than 18 CL followed by 11-18 CL, 3-10 CL and 1-2 CL categories (39.7±4.9 vs. 21.4±1.3 vs.13.6±1.3 vs.7.6±2.0, respectively). Similar pattern was observed for percent ovulation and CL per doe (74.9±6.8 vs. 67.2±6.6 vs. 57.8±5.1 vs. 21.5±12.7; 30.3±6.1 vs. 13.8±0.9 vs. 7.2±0.5 vs. 0.9±0.4 respectively). The structure recovery and viable embryos per doe tended to increase with CL categories, in which, it was significantly the highest (P<0.05) in does with 11-18 CL than 3-10 CL, while reduced for more than 18 CL and none for 1-2 CL categories, with the values of 7.7±1.9 vs. 1.3+0.9 vs. 1.0±1.0 vs. 0.0±0.0 and 4.8±1.5 vs. 0.1±0.1 vs. 0.0±0.0 vs. 0.0±0.0 respectively. Conversely, no significant differences in number of unfertilised plus degenerated ova in all CL categories with same distribution pattern were observed (2.9±1.7 vs. 1.1±0.8 vs. 1.0±1.0 vs. 0.0±0.0, respectively).

Figure 4.3 illustrates the distribution pattern of superovulated does in 4 categories of ovulation in the different treatment groups. In multiple administration treatment groups, the increased in the dosage of oFSH increased the number of CL formed per doe in more than 18 CL category (8% vs. 22%, respectively). Similar trend

Table 4.4. Effect of oFSH treatments on AF formation (mean+SEM) in superovulated donor does

Parameters	S Single administration of 8.8 mg oFSH	M_1 Multiple administration of 8.8 mg oFSH	M_2 Multiple administration of 14.1 mg oFSH
Number of animals (n)	13	13	9
Number of does with AF (%)	85(11)	92(12)	78(7)
AF percentage (%)	60.7 ± 11.1^{b}	41.2 ± 3.9^{ab}	28.2 ± 7.8^{a}
AF/doe	6.2 ± 1.5^{a}	8.4 ± 1.1^{a}	7.0 ± 1.7^{a}
Number of AF on left ovary/doe	3.2 ± 0.8^{a}	3.3 ± 0.6^{a}	4.3 ± 1.3^{a}
Number of AF on right ovary/doe	3.1 ± 1.1^{a}	5.1 ± 0.9^{a}	4.1 ± 1.0^{a}

[ab] Mean value within rows with different superscripts were significantly different $P<0.05$ level

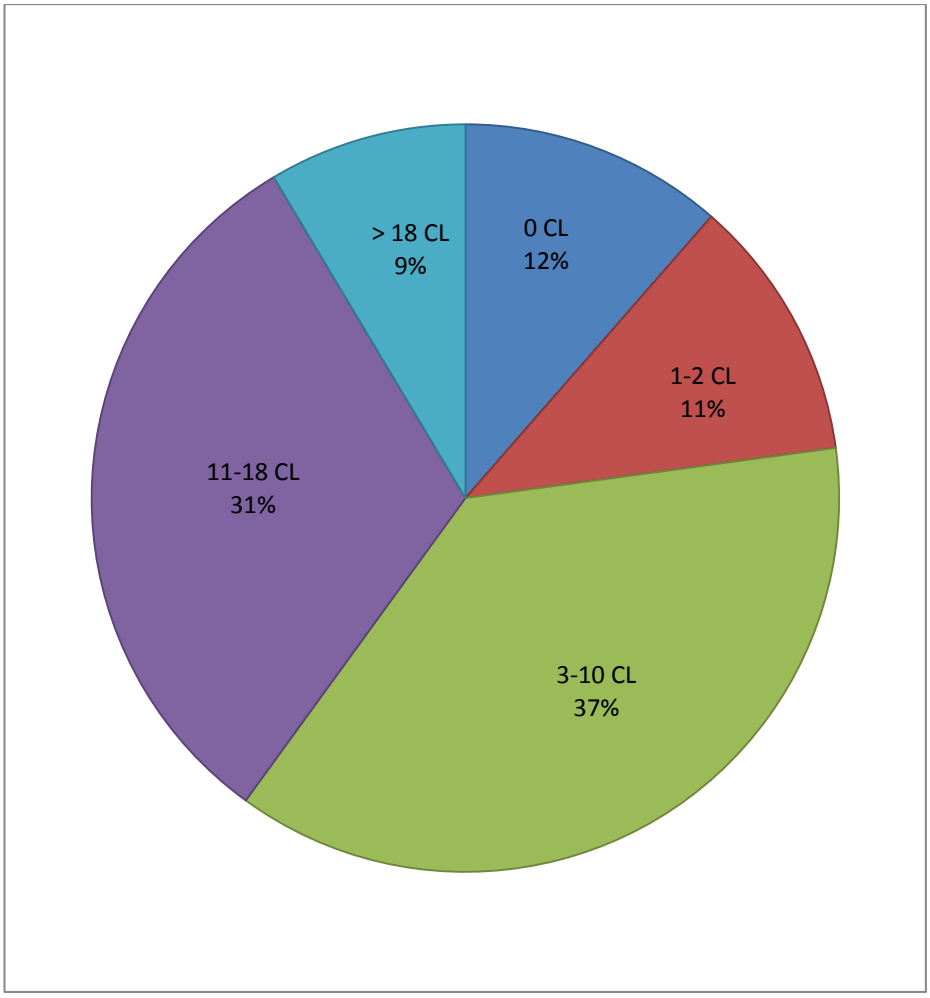


Figure 4.4. Overall distribution of the CL categories in superovulated donor does

was observed for the 3-10 CL category (45% vs. 38%, respectively). Conversely, the increase in the dosage of oFSH reduced the number of CL per doe formed in 11-18 CL category (54% vs. 33% respectively). In does treated with same dosage of oFSH (8.8 mg), for single administration, the distribution of CL per doe was relatively similar for CL categories (0 CL, 31%; 1-2 CL, 31%; 3-10 CL, 38%). However, no doe ovulated more than 10 CL was observed. For multiple administrations, the distribution of CL per doe was different from that of single administration with more CL per doe were observed in the former (3-10 CL, 38%; 11-18 CL, 54%; and more than 18 CL, 8%). Regardless hormonal administration treatments, the overall distribution of CL formation in all treated does were highest in 3-10 CL category, followed by 11-18 CL, 0 CL, 1-2 CL and > 18 CL with the values of 37% vs. 31% vs. 12% vs. 11% vs.9% per doe, respectively (Figure 4.4).

The occurrences of anovulatory follicles following 3 different superovulation treatments in this present study are shown in Table 4.4. Regardless the dosage and pattern of oFSH superovulatory treatment, 86% of oFSH superovulated does formed at least one anovulatory follicle at embryo recovery. A total of 252 anovulatory follicles were detected, represented 43% of the total ovarian response in all treatment groups that developed follicles to preovulatory size, and subsequently could reach the ovulation stage in response to oFSH treatment. In low oFSH treatments groups, the percentage of does with anovulatory follicles was higher in multiple administered group, M_1 (92%) compared to single administered group, S (85%). The incidence of anovulatory follicles was lowest in higher dosage of oFSH (M_2, 78%) of multiple administration groups. The percent anovulatory follicles for oFSH treatment was singnificantly highest ($P<0.05$) in single administration groups (60.7 ± 11.1). In does received multiple administration, although no differences were observed, the presence of anovulatory follicles was lower in high oFSH dosage group compared to low dosage group (28.2 ± 7.8 vs. 41.2 ± 3.9

Table 4.5. Effect of anovulatory follicles categories on ovarian response (mean ± SEM) in oFSH superovulated donor does

Parameters	AF Categories			
	0	**1-2**	**3-10**	**11-18**
Number of animals (n)	5	2	19	9
Total ovarian response (CL plus anovulatory follicle)	9.4± 3.0^{ab}	3.0±1.0^{a}	16.0±2.5^{bc}	25.7±2.6^{c}
Percent ovulation (%)	100±0.0^{b}	25.0±25.0^{a}	47.6±5.8^{a}	46.2±7.0^{a}
Percent anovulatory (%)	0.0±0.0^{a}	50.0±50.0^{b}	52.2 ±5.8^{b}	54.5±7.0^{b}
CL/doe	9.4±3.0^{a}	1.0±1.0^{a}	9.0±2.0^{a}	13.0±2.9^{a}
AF/doe	0.0±0.0^{a}	2.0 ±0.0^{a}	7.1± 0.7^{b}	12.7±0.7^{c}
Structure recovery per doe (embryo plus ova)	4.6±2.2^{a}	0.0±0.0^{a}	3.1±1.3^{a}	1.7±1.0^{a}
Unfertilized plus degenerated ova per doe	1.2±1.2^{a}	0.0±0.0^{a}	2.1±1.0^{a}	0.3±0.3^{a}
Viable embryo per doe	3.4±2.4^{a}	0.0±0.0^{a}	1.0±0.7^{a}	1.4±1.0^{a}

^{abcd} Mean value within rows with different superscripts were significantly different P<0.05 level

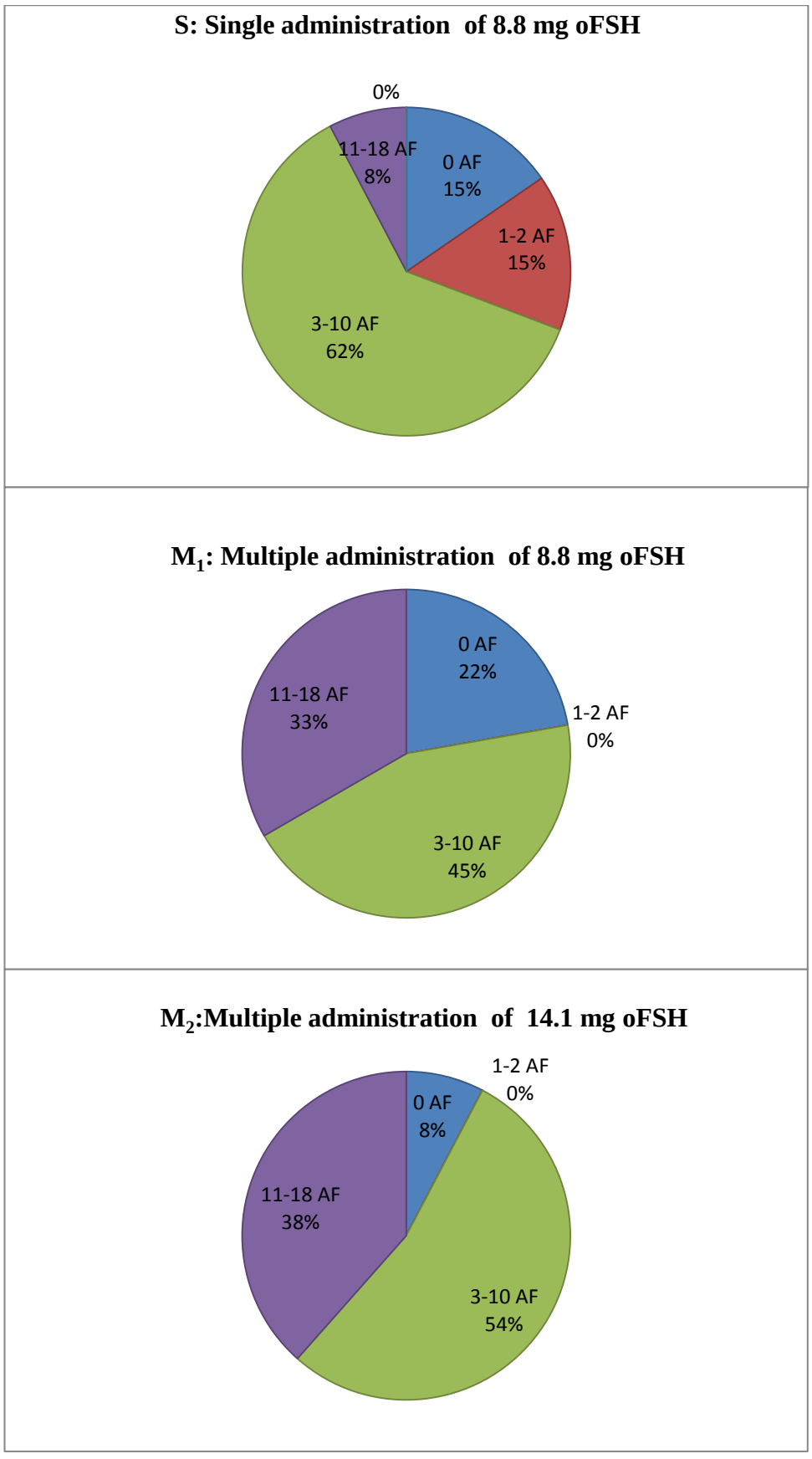


Figure 4.5. Distribution of the AF categories in donor does following oFSH superovulation protocols

respectively). However, no difference ($p>0.05$) was observed in number of anovulatory follicles per doe for the three treatment groups (6.2±1.5, 8.4±1.1, 7.0±1.7 in S, M_1 and M_2, respectively).

Table 4.5 shows the effects of AF categories on ovarian response parameters of oFSH superovulated donor does. The donor does were categorised according to AF formation on theirs ovaries. No doe was observed to have > 18 AF in this experiment. Total ovarian response (number of CL plus number of AF per doe) and AF per doe were significantly increased ($P<0.05$) with the AF categories, with the values of 3.0±1.0, 16.0±2.5, 25.7±2.6 and 2.0±0.0, 7.1±0.7, 12.7±0.7 for the 1-2 AF, 3-10 AF and 11-18 AF, respectively. Conversely, all does in 0 AF category responded to oFSH superovulation treatment (i.e. 100% ovulation) with highest number of total ovarian response (all CL plus 0 AF), CL per doe, structure recovery (viable plus non-viable embryos) and viable embryos (9.4±3.0, 9.4±3.0, 4.6±2.2 and 3.4±2.4 respectively). As for other categories (1-2 AF, 3-10 AF and 11-18 AF), no singnificant differences ($P>0.05$) were observed between categories for percent ovulation, percent anovulatory follicles and CL per doe, although the values were higher with increasing AF categories (25.0±25.0 vs. 47.6±5.8 vs. 46.2±7.0; 50.0±50.0 vs.52.3±5.8 vs. 53.8±7.0; 1.0±1.0 vs. 9.0±2.0 vs. 13.0±2.9, respectively). Even though no singnificant differences ($P>0.05$) were observed, highest structure recovery and viable embryo per doe were highest in 0 AF category, with the values of 4.6±2.2 vs. 0.0±0.0 vs. 3.1±1.3 vs.1.7±1.0 and 3.4±2.4 vs. 0.0±0.0 vs. 1.0±0.7 vs.1.4±1.0 in 0 AF, 1-2 AF, 3-10 AF and 11-18 AF categories, respectively.

Figure 4.5 illustrates the distribution pattern of superovulated does in 4 categories of anovulatory follicles (AF) in different oFSH superovulation regimes. In multiple administration treatment groups, increase in dosage of oFSH reduced the number of does without any AF (0 AF category) from 22% in 8.80 mg oFSH to 8% in

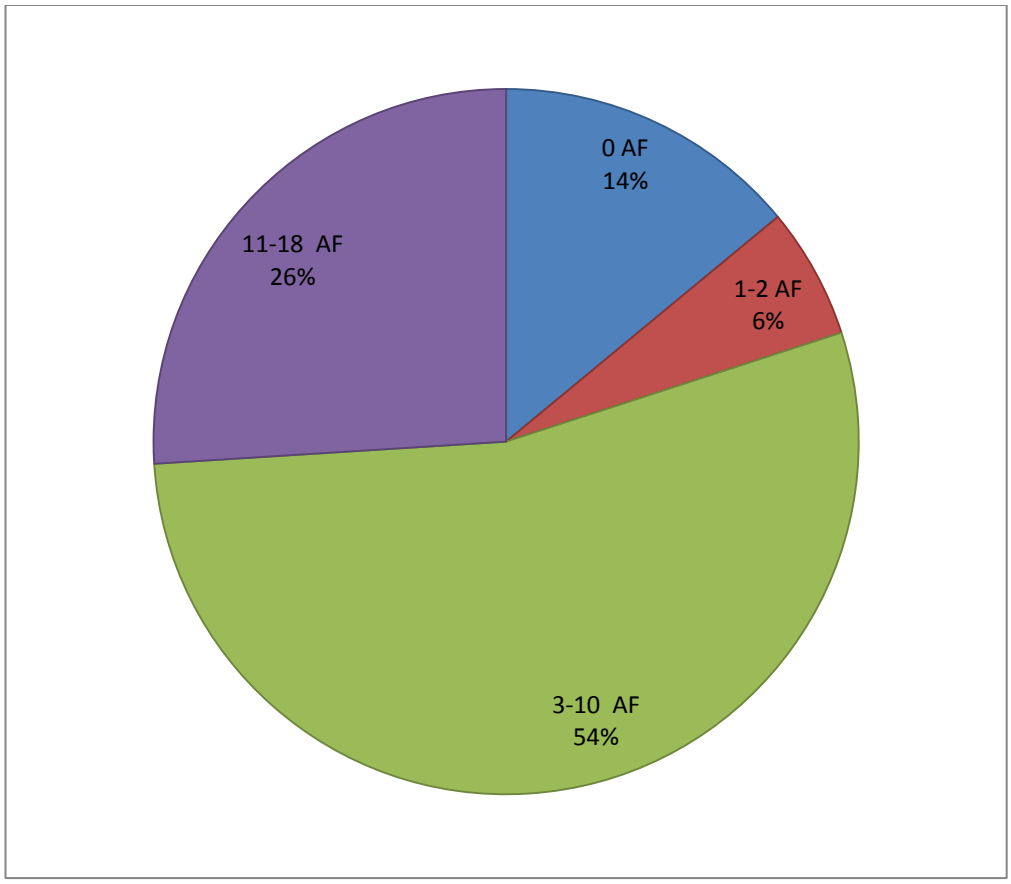


Figure 4.6. Overall distribution of AF categories in superovulated donor does

14.08 mg oFSH group. Opposite trend was observed, respectively, in 1-2 AF (15% vs. 0%), 3-10 AF (45% vs. 54%) and 11-18 AF (33% vs. 38%). However, no doe in both 8.80 mg and 14.08 mg oFSH treatments group had 1-2 AF. For the does treated with same dosage of 8.08 mg oFSH (S and M_1), does in multiple injection group without AF (22% vs. 15%, respectively) and 11-18 AF (33% vs. 8%, respectively) than in single dosage group. However, the percentage of does with 3-10 AF was higher in single administration group (62% vs. 45%, respectively). Regardless of hormonal administration treatments, the overall distributions of AF formation in all treated does were highest in 3-10 AF category, followed by 11-18 AF, 0 AF and 1-2 AF with the values of 54% vs. 26% vs. 14% vs. 6% per doe, respectively (Figure 4.6).

4.2 EFFECT OF THE BREED ON OVARIAN RESPONSE IN SUPEROVULATED DONOR DOES

Three goat breeds, consisted of Boer crossbred, Jamnapari and local mixed-breed, were used in this study. The overall ovarian responses of the breeds following oFSH superovulatory treatments are presented in Table 4.6. The results have shown that Boer does (94%) responded better to hormonal treatment with the presence of at least 1 CL compared to the local mixed breed(83%) and Jamnapari does (83%). Except for local mixed breed(83%), however, Boer crossbred and Jamnapari showed less responsive to hormonal treatments whereby the superovulated does (more than 2 CL) had the values of 82 and 75%, respectively. The total ovarian response per doe, total structures recovery per doe, numbers of unfertilised plus degenerated ova and viable embryos per doe were not significantly different ($P>0.05$) between breeds with the values of 21.6 ± 3.3 vs. 16.7 ± 3.0 vs. 14.5 ± 2.0; 3.3 ± 1.2 vs. 3.2 ± 1.4 vs. 1.9 ± 1.2; 1.2 ± 0.7 vs. 2.2 ± 1.2 vs. 0.3 ± 0.3 and 2.2 ± 1.4 vs. 1.1 ± 0.1 and 1.5 ± 1.1 for the local mixed-breed, Boer crossbred and Jamnapari does, respectively. Although no difference, local mixed breed recorded highest values in total ovarian response per doe, total structures recovery.

The effects of oFSH superovulation treatment on CL formation in Boer crossbred, Jamnapari and local mixed breed are shown in Table 4.7. No differences ($P>0.05$) were observed in the percent ovulation, number of CL per doe, number of CL on left and right ovaries per doe for all the breeds studied. The percent ovulation was highest in Boer crossbred and followed by Jamnapari and local mixed-bred with the values of 57.1 ± 7.7, 52.4 ± 8.9 and 46.3 ± 9.7, respectively. Conversely, the number of CL per doe was highest in local mixed-bred compared to Boer crossbred and Jamnapari with values of 11.2 ± 2.6, 10.5 ± 2.7 and 7.7 ± 1.5, respectively.

Table 4.6. Effect of the breed on the ovarian response (mean ± SEM) in oFSH superovulated local mixed-breed, Boer crossbred and Jamnapari donor does

Parameters	Local mixed-breed	Boer crossbred	Jamnapari
Number of animals (n)	6	17	12
Ovulated does (%)[1]	83 (5)	94 (16)	83 (10)
Superovulated does (%)[2]	83 (5)	82 (14)	75 (9)
Total ovarian response per doe (CL plus AF/doe)	21.6±3.3[a]	16.7±3.0[a]	14.5±2.0[a]
Structures recovery rate per doe (embryo and ova/doe)	3.3±1.2[a]	3.2±1.4[a]	1.9±1.2[a]
Unfertilised plus degenerated ova/doe	1.2±0.7[a]	2.2±1.2[a]	0.3±0.3[a]
Viable embryo per doe	2.2±1.4[a]	1.1±0.1[a]	1.5±1.1[a]

[a] Mean value within rows with same superscripts were not significantly different P>0.05 level
[1] doe with at least 1 CL
[2] doe with more than 2 CL

Table 4.7. Effect of oFSH treatments on the CL formation (mean ± SEM) in oFSH superovulated local mixed-breed, Boer crossbred and Jamnapari donor does

Parameters	Local Mixed-breed	Boer Crossbred	Jamnapari
Number of animals (n)	6	17	12
Percent ovulation (%)	46.3±9.7[a]	57.1±7.7[a]	52.4±8.9[a]
CL/doe	11.2±2.6[a]	10.5±2.7[a]	7.7±1.5[a]
Number of CL on left ovary/doe	6.0±1.7[a]	5.1±1.4[a]	4.3±0.9[a]
Number of CL on right ovary/doe	5.2±1.2[a]	6.1±1.4[a]	3.4±0.7[a]

[a] Mean value within rows with same superscripts were not significantly different P>0.05 level

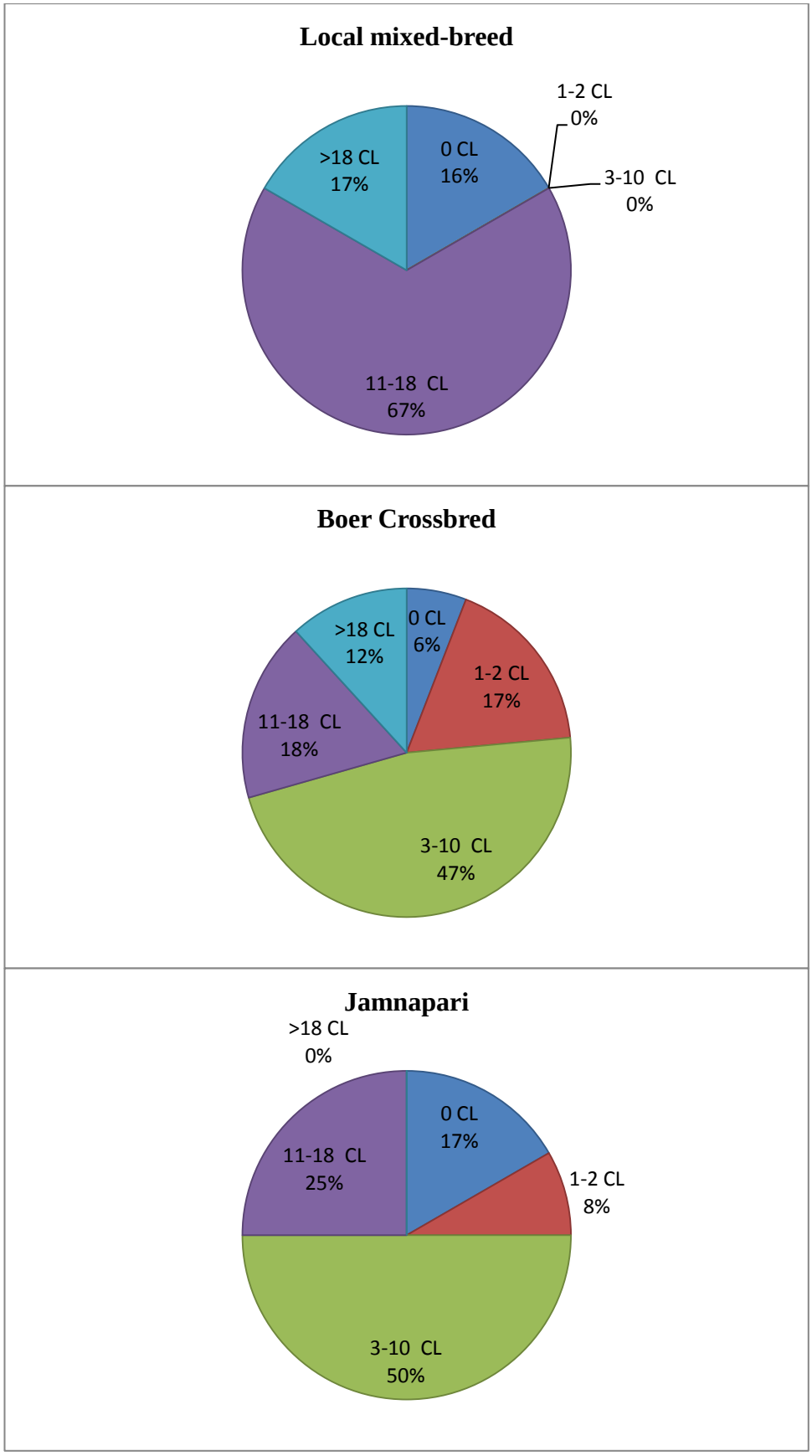


Figure 4.7. Distribution of the CL categories in oFSH superovulated local mixed-breed, Boer crossbred and Jamnapari breed donor does

Table 4.8. Effect of oFSH treatments on the AF formation (mean ± SEM) in superovulated local mixed-breed, Boer crossbred and Jamnapari donor does

Parameters	Local mixed-breed	Boer crossbred	Jamnapari
Numbers of animals (n)	6	17	12
Percent anovulatory(%)	53.7±9.7[a]	40.6±8.1[a]	47.6±8.9[a]
AF/doe	10.0±1.0[a]	6.5±1.2[a]	6.8±1.5[a]
Numbers of AF on left ovary	4.0±0.7[a]	3.8±0.8[a]	2.9±0.7[a]
Numbers of AF on right ovary	6.0±0.6[a]	3.5±0.9[a]	3.9±1.0[a]

[a] Mean value within rows with same superscript were not significantly different (P>0.05) level.

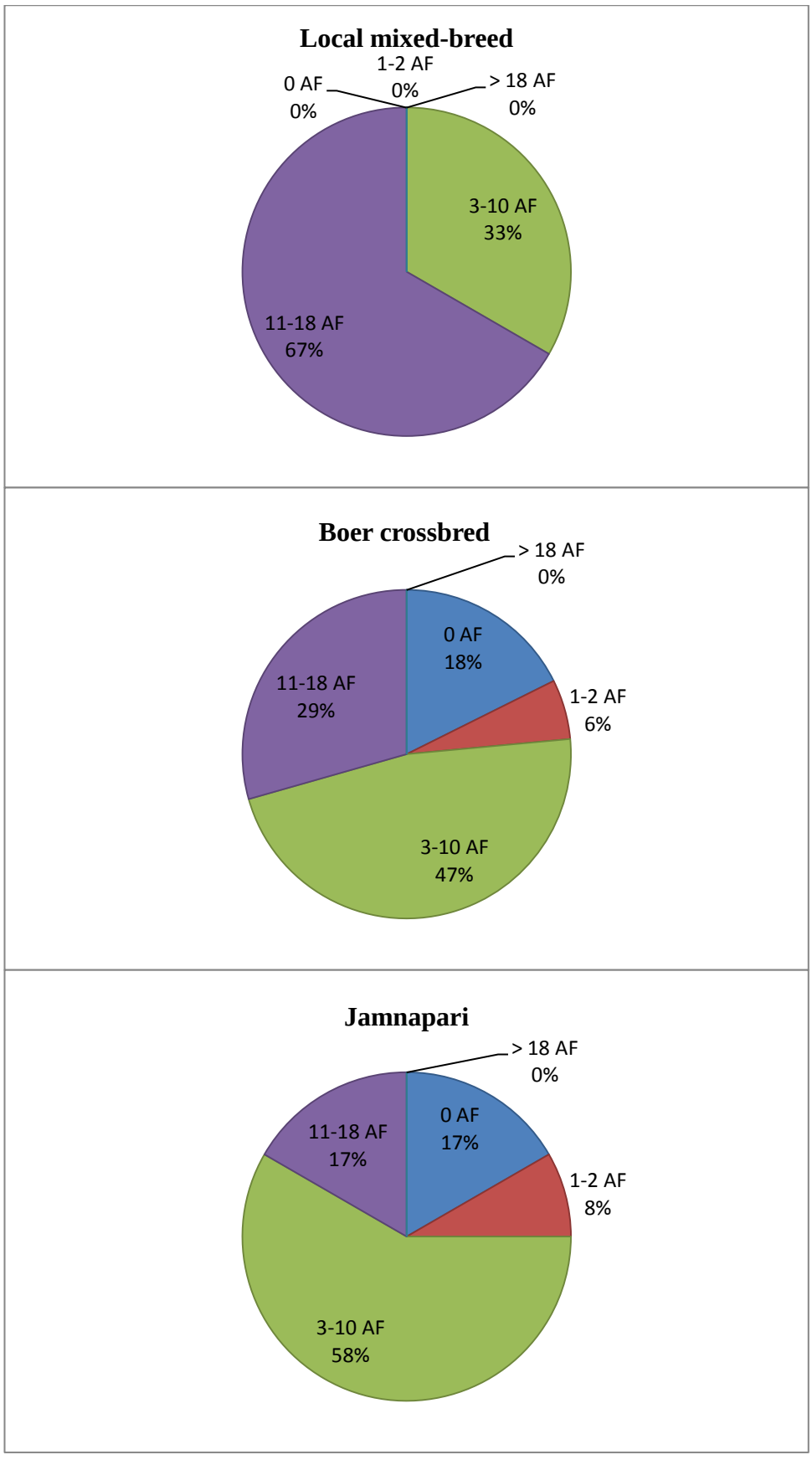


Figure 4.8. Distribution of the AF categories in oFSH superovulated local mixed-breed, Boer crossbred and Jamnapari breed donor does

Figure 4.7 illustrates the distribution pattern of superovulated does in 5 categories of ovulation for the 3 different breeds studied. A total of 16, 6 and 17% of the local mixed-breed, Boer crossbred and Jamnapari does failed to response (0 CL) to superovulation treatments. It is interesting to note that the local mixed-bred had excellent response to superovulation treatment with 67% and having 11-18 CL with no 1-2 CL and 3-10 CL categories. The corresponding responses for Boer crossbred and Jamnapari were 47 and 50% for 3-10 CL category, respectively. As for >18 CL category, the responses were between 17% and 12% for local mixed breed and Boer crossbred.

The effect of oFSH treatments on the AF formation in superovulated local mixed breed, Boer crossbred and Jamnapari donor does are shown in Table 4.8. Although local mixed breed had excellent response to superovulation treatment (Table 4.7), the does had the highest percent AF, number of AF per doe and number of AF on left and right ovary than Boer crossbred and Jamnapari donor does, with the values of 53.7$\pm$9.7, 40.6$\pm$8.1, 47.6$\pm$8.9; 10.0$\pm$1.0, 6.5$\pm$1.2, 6.8$\pm$1.5; 4.0$\pm$0.7, 3.8$\pm$0.8, 2.9$\pm$0.7; 6.0$\pm$0.6, 3.5$\pm$0.9, 3.9$\pm$1.0, respectively. No significant difference ($P>0.05$) was observed between breeds. The distributions of the AF categories in local mixed breed, Boer crossbred and Jamnapari oFSH superovulated donor does are illustrated in Figure 4.8. None of the doesfor all breeds formed more than 18 AF per doe. In local mixed breed groups, high percentage of the donor does had 11-18 AF than Boer crossbred and Jamnapari (67% vs. 47% vs. 25%, respectively). Conversely, high percentage of Jamnapari does had 3-10 AF than Boer cross breed and local mixed breed (58% vs. 47% vs. 33%, respectively). None of the local mixed breed does had less than 3AF. Conversely, same distribution pattern were observed for 0 AF and 1-2 AF in Boer crossbred and Jamnapari with the values of 18% vs. 17% and 6% vs. 8%, respectively.

4.3 EFFECT OF THE BODY WEIGHT ON OVARIAN RESPONSE IN SUPEROVULATED DONORS DOES

Twenty-eight oFSH superovulated does were studied in this experiment for 2 ranges of body weight (20-30 kg and 30-50 kg), regardless hormonal treatment received and breed involved. The parameters observed for 2 groups of body weight are shown in Table 4.9. All does (100%) with body weight 20-30 kg ovulated with at least 1 CL compared to 93% for the does in 30-50 kg body group. For the successful superovulated does, all does (100%) with body weight 20-30 kg ovulated with more than 2 CL compared to 87% of the does in 30-50 kg body weight group. Although no difference ($P>0.05$) for all parameters observed, the superovulated does in 30-50 kg body weight group recorded higher values of the total ovarian response per doe, total structures recovery per doe, and numbers of unfertilised and degenerated ova than low body weight group with the values of 20.9$\pm$3.0 vs. 16.7$\pm$2.1; 4.0$\pm$1.6 vs. 2.9$\pm$1.1; 2.3+1.3 vs. 1.0$\pm$0.6, respectively. Conversely, superovulated does in 30-50 kg body weight group produced lower viable embryos per doe than body weight 20-30 kg group (1.7$\pm$0.9 vs. 1.9$\pm$1.1, respectively).

The effects of oFSH superovulation treatment on CL formation in 20-30 kg and 31-50 kg body weight are shown in Table 4.10. No difference ($P>0.05$) was observed in the percent ovulation, number of CL per doe, number of CL on left and right ovary per doe of both body weights. The percent ovulation was higher in 20-30 kg body weight group with the values of 65.0$\pm$5.8 vs. 60.2$\pm$6.9, respectively. Conversely, mean numbers of the CL per doe, number of CL on left and right ovary per doe were higher for 31-50 kg body weight group with the values of 12.9+2.8 vs. 10.2+1.3; 6.2+2.8 vs. 5.9+1.0 and 6.9+1.4 vs. 5.0+0.5, respectively.

Figure 4.9 illustrates the distribution pattern of superovulated does in 5 ovulation categories in 2 different body weight groups. All does in 20-30 kg body weight responded to superovulation treatments (0% in 0 CL and 1-2 CL categories, respectively). However, considerable numbers of the does in 31-50 kg body weight group failed to ovulate (0 CL; 7%) or superovulated (1-2 CL; 7%), following superovulation treatments. Of both body weights, the highest percent of the does in 20-30 kg body weight had 3-10 CL than does in 31-50 kg body weight (77% vs. 40%, respectively). Conversely, does with higher body weight had 11-18 CL and more than 18 CL than low body weight, with the values of 33% vs. 15% and 13% vs. 8%, respectively.

Table 4.11 shows the AF formation in superovulated donor does in 2 groups of body weight. Although no difference ($P>0.05$) was observed, the does in 31-50 kg body weight group shown higher percent anovulatory, number of AF per doe and number of AF on left and right ovaries than light body weight donor does (20-30 kg), with the values of 39.8 ± 6.9 vs. 35.0 ± 5.8; 8.1 ± 1.3 vs. 6.5 ± 1.2; 4.2 ± 0.8 vs. 3.3 ± 0.8; 4.7 ± 0.9 vs. 3.4 ± 0.9, respectively. The distributions of the AF categories in 20-30 kg and 31-50 kg body weight groups of oFSH superovulated donor does are illustrated in Figure 4.10. None of the does (0%) in both body weight groups had 1-2 AF and more than 18 AF categories. Majority of the does in 20-30 kg body weight were without AF (0 AF category) than 31-50 kg body weight (23% vs. 13% respectively). Higher percentage of does in heavier body weight group (31-50 kg) were observed to have 3-10 AF (46% vs. 54%, respectively) and 11-18 AF category (33% vs. 31%, respectively) than lower body weight.

Table 4.9. Ovarian response (mean ± SEM) in superovulated donor does with different size of body weight

Parameters	20- 30 kg	31 – 50 kg
Number of animals (n)	13	15
Ovulated does (%)[1]	100 (13)	93 (14)
Superovulated does (%)[2]	100 (13)	87 (13)
Total ovarian response (CL plus AF/doe)	16.7±2.1 [a]	20.9±3.0[a]
Structures recovery rate ((embryo plus ova/doe)	2.9±1.1[a]	4.0±1.6[a]
Unfertilised and degenerated ova/doe	1.0±0.6[a]	2.3±1.3[a]
Viable embryo/doe	1.9±1.1[a]	1.7±0.9[a]

[a] Mean value within rows with same superscript were not significantly different (P>0.05) level.

[1] doe with at least 1 CL
[2] doe with more than 2 CL

Table 4.10. Effect of oFSH treatments on the CL formation (mean ± SEM) in different size of donor body weight

Parameters	20-30 kg	31-50 kg
Number of animals (n)	13	15
Percent ovulation (%)	65.0±5.8[a]	60.2±6.9[a]
CL/doe	10.2±1.3[a]	12.9±2.8[a]
Number of CL on left ovary/doe	5.9±1.0[a]	6.2±1.4[a]
Number of CL on right ovary/doe	5.0±0.5[a]	6.9±1.4[a]

[a] Mean value within rows with same superscript were not significantly different (P>0.05) level.

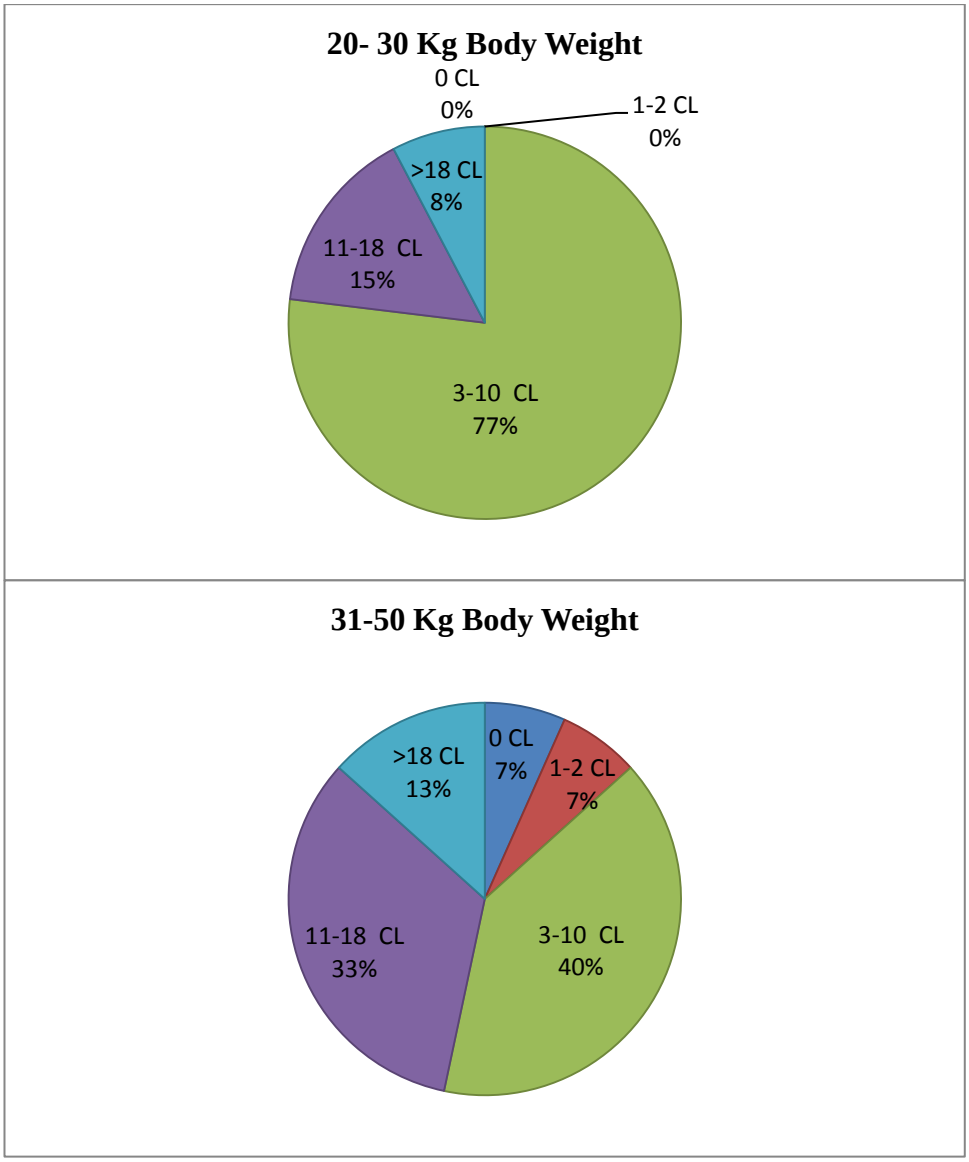


Figure 4.9. Distribution of CL categories in two different body size of oFSH superovulated donor does

Table 4.11. Effect of oFSH treatments on the AF formation (mean ± SEM) in different size of donor body Weight

Parameters	20-30 kg	31-50 kg
Number of animals (n)	13	15
Percent anovulatory (%)	35.0±5.8[a]	39.8±6.9[a]
AF/doe	6.5±1.2[a]	8.1±1.3[a]
Number of AF on left ovary/doe	3.3±0.8[a]	4.2±0.8[a]
Number of AF on right ovary/doe	3.4±0.9[a]	4.7±0.9[a]

[a] Mean value within rows with same superscript were not significantly different (P>0.05)

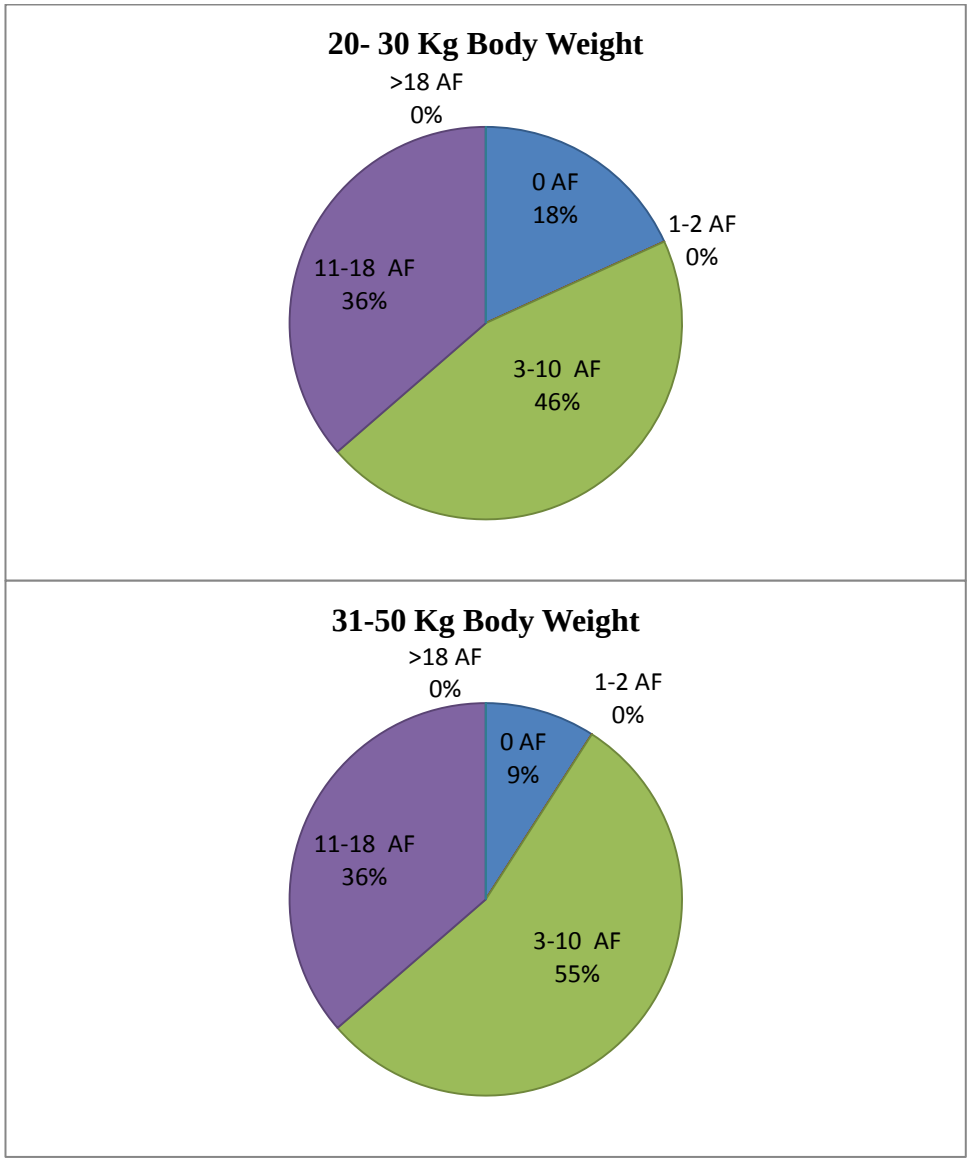


Figure 4.10. Distribution of the AF categories in two different body size of oFSH super ovulated donor does

4.4 EFFECT OF THE DAY OF EMBRYO RETRIEVAL ON THE STAGES OF EMBRYONIC DEVELOPMENT AND THE QUALITY OF THE EMBRYOS

The detailed assessment of the stages of development and the quality of the embryos retrieved from oFSH-superovulated goats that were successfully flushed on Day 3 or Day 7 after CIDR withdrawal are presented in Tables 4.12 and 4.13 From 12 successfully superovulated does, 233 stimulated follicles were recorded following superovulation treatments, 67 percent (157) of them ovulated (ovulatory follicles). A total of 98 structures (embryos plus ova) were recovered to give the overall structures recovery rate of 62%. The structures (embryos plus ova) recovery rate was high on day 3 recovery (90%) compared to day 7 recovery (56%). Beside high recovery rate, 50% of day 3 structures retrieved were degenerated ova/embryos compared to 34% for on day 7 retrieval. The percentage of non-viable ova/embryos was 49% for all of the donors flushed.

The stages of development for recovered embryos are shown in Table 4.14. Twenty five percent of the embryos flushed on day 3 after CIDR withdrawal were at 2 cell stage with subsequent development of 14 and 4 % for 4 cell and 8 cell. For the day 7 recovery, a large proportion of the embryos recovered were at morula (27%), followed by blastocyst (10%) and hatched blastocyst (4%) of development stage. No slow growth embryo (8 and less cell stages) was observed on day 7 recovery. Schematic photographs of the *in vivo* embryonic development stages of the embryos recovered on days 3 and 7 are presented in Figure 4.11. Meanwhile representative photographs on undeveloped aging oocytes and unfertilised as well as degenerated oocytes and embryos are depicted in Figures 4.12 and 4.13.

The embryos that were retrieved in this experiment were classified into 4 categories according to their quality (Table 4.14). Twenty five percent and 47.14% of the embryos retrieved on days 3 and 7 after CIDR withdrawal were categorised as Grade 1. Correspondingly, 7.12, 7.14 and 60.71% of the day 3 embryos were categorised as Grades 2, 3 and 4, respectively. Similarly, for day 7 embryos, 4.08, 3.06 and 52.04% categorised as Grades 2, 3 and 4, respectively. Based on total embryos recovered in this experiment, 40.82, 4.08, 3.06 and 52.04% categories as Grade 1, 2, 3 and 4, respectively.

Table 4.12. Overall embryo recovered from oFSH-superovulated does on day 3 and 7 after CIDR withdrawal

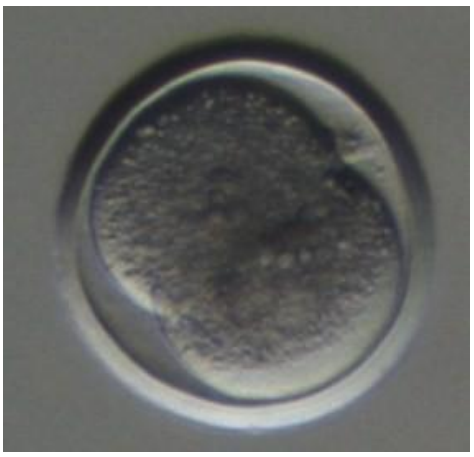
	Days after CIDR withdrawal		
	Day 3	**Day 7**	**Total**
Number of does flushed	3	9	12
Number of CL's	31	126	157
Number of anovulatory follicles	20	56	76
Number of follicles induced	51	182	233
Ovulation Percentage (%)	61	69	67
Number of structures (embryo/ova) recovered	28	70	98
Percent Structure recovered (%)	90	56	62
Percent of viable embryos (%)	50	51	51
Percent of non-viable embryos (%)	50	34	50

Table 4.13. The developmental stages of the *in vivo* embryo recovered from oFSH-superovulated goats on day 3 and 7 after CIDR withdrawal

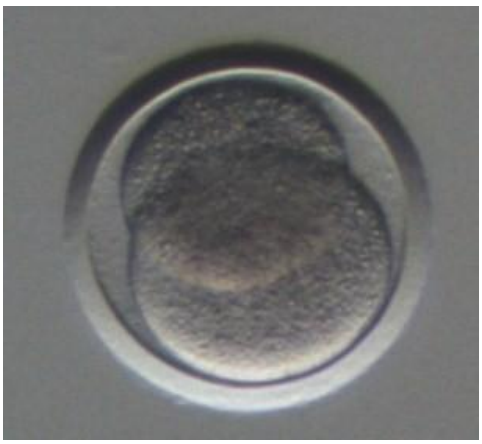
	Days after CIDR withdrawal		
	Day 3	**Day 7**	**Total**
Number of does flushed	3	9	12
Unfertilized ova (%)	14 (50)	34 (49)	48 (49)
2 cell (%)	7 (25)		7 (7)
3 cell (%)	2 (7)		2 (2)
4 cell (%)	4 (14)		4 (4)
8 -16 cell (%)	1 (4)		1 (1)
Morula (%)		26 (27)	26 (27)
Blastocyst (%)		7 (10)	7 (7)
Hatched blastocyst (%)		3 (4)	3 (3)

Table 4.14. The quality of *in vivo* embryo recovered from oFSH-superovulated goats on day 3 and 7 after CIDR withdrawal

Parameters	Days After CIDR Withdrawal		
	Day 3	**Day 7**	**Total**
Number of does flushed(n)	3	9	12
Total number of CL's(CL)	31	126	157
Total number of recovered structures (RS)	28	70	98
Rate of structures recovery rate (RS /CL %)	90	56	62
Rate of viability(Grade 1,2,3 embryos/RS %)	39	51	48
Rate of degenerated embryos(Grade 4/RS %)	61	49	52
Grade 1(%)	7 (25)	33 (47)	40 (41)
Grade 2(%)	2 (7)	2 (3)	4 (4)
Grade 3(%)	2 (7)	1 (1)	3 (3)
Grade 4(%)	17 (61)	34 (49)	51 (52)



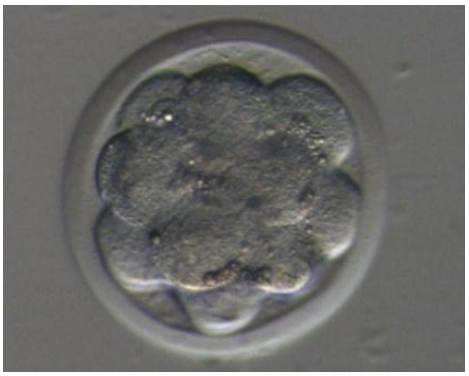
a. First cleavage stage in progress



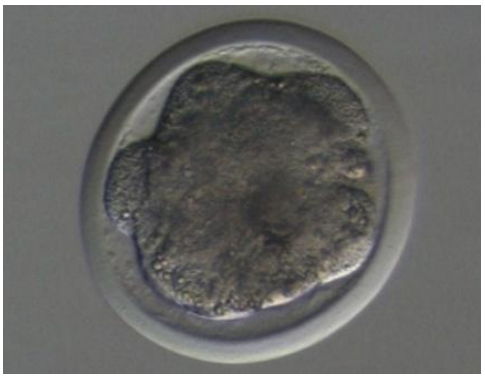
b. Two-cell stage

c. Second cleavage stage (4 cell stage)

d. 12-cell stage



e. Initial stage of compaction



f. Final stage of compaction

Figure 4.11. The development stages of *in vivo* embryos recovered on days 3 (a,b,c)and 7 (d,e,f,g,h,i,j,k,l) after CIDR removal from oFSH superovulated donor does

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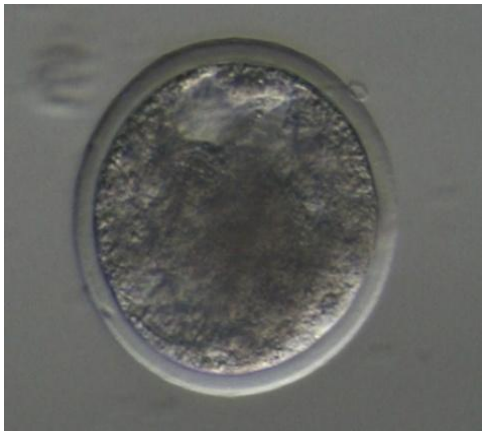
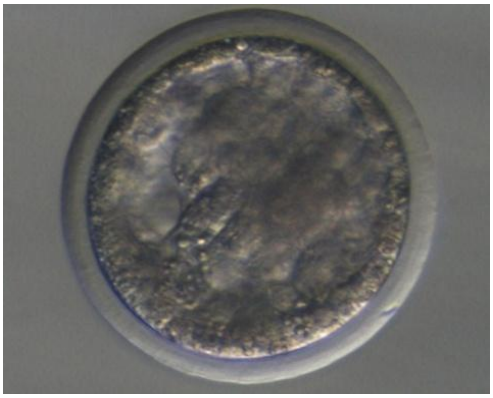
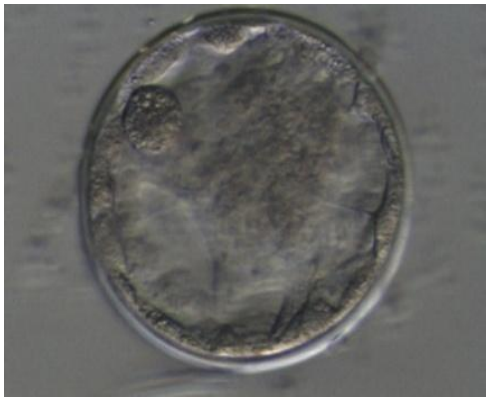
g. Decompacted and cavitating morula



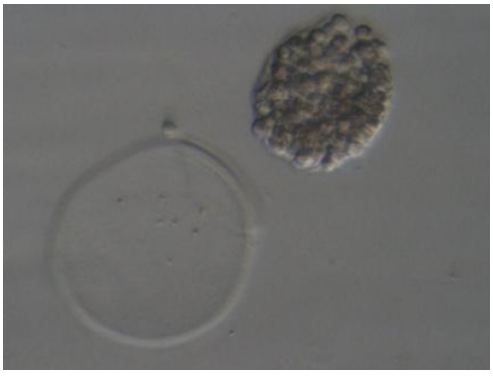
h. Stage 3 begins (early blastocyst, blastocoel less than half of the blastocyst)



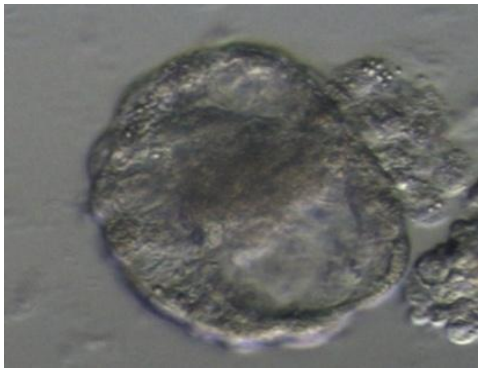
i. Early blastocyst (blastocoels more than half of the blastocyst)



j. Fully expanded blastocyst (increases in size, single ring of trophoblastic cell, zona stretch very thin, clear inner cell mass at 10 o'clock

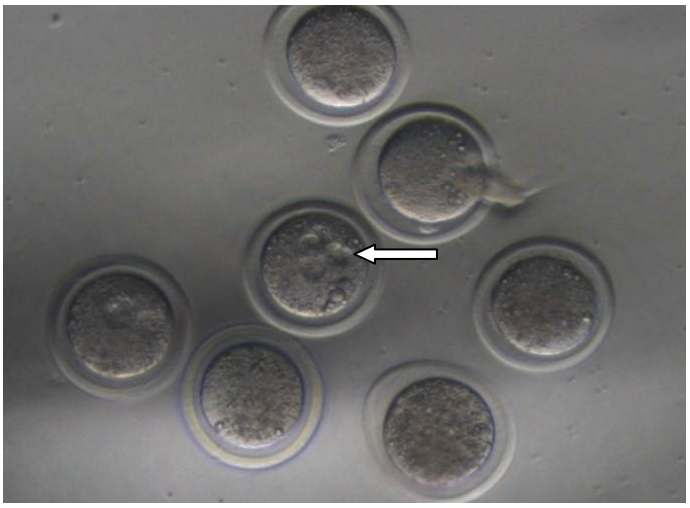


k. Hatched blastocyst leave its empty zona pellucida

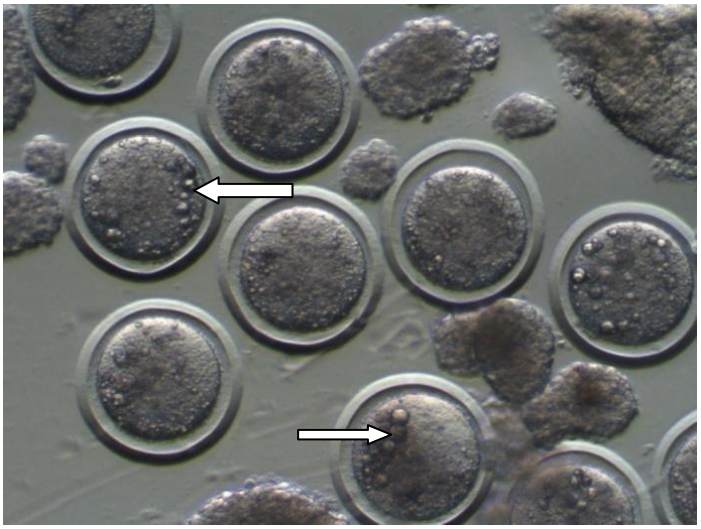


l. Hatched blastocyst ready for implantation

a.



b.



c.

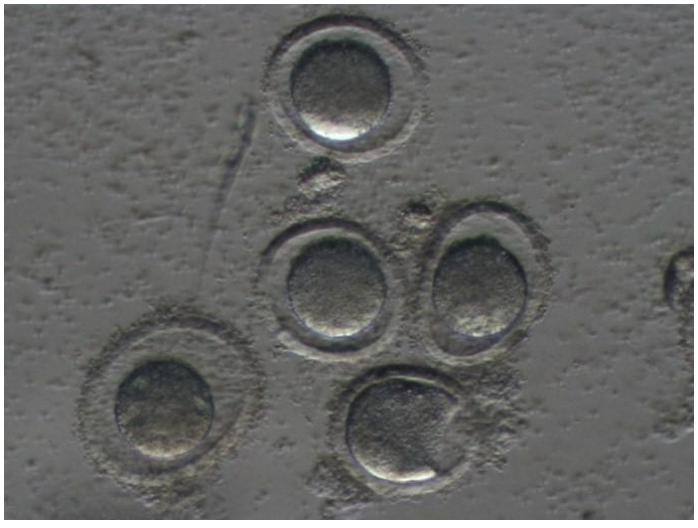


Figure 4.12. Some structures of the undeveloped aging oocytes recovered on day 3 and 7 after CIDR removal from oFSH superovulated goats.(a) Oocytes diplaying poly pronuclei cause of abnormal fertilisation. (b) Oocytes with extreme granularity. (c) Degenerated oocytes.

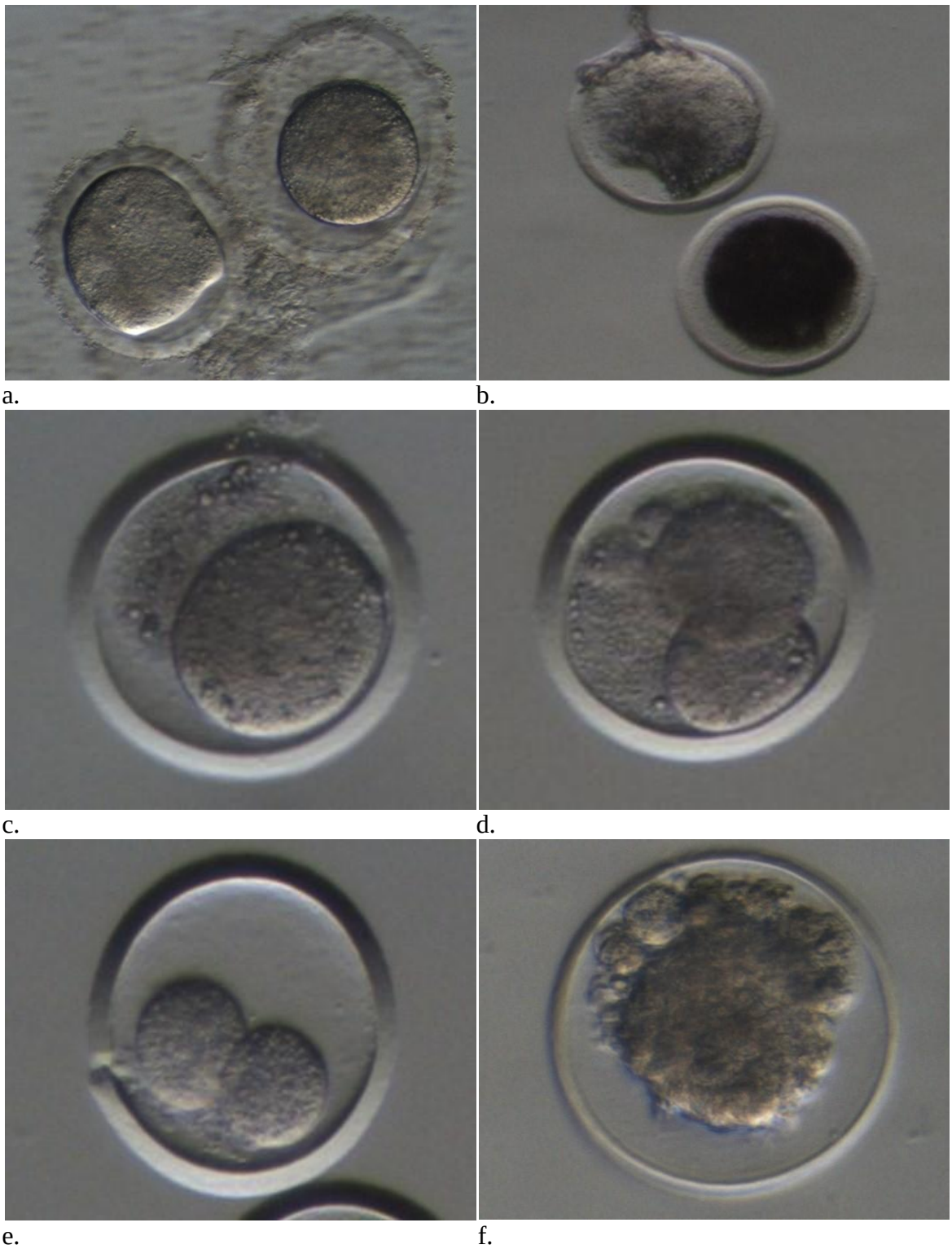


Figure 4.13. Some structures of unfertilised oocytes (a, b, c), and degenerated embryos (d, e, f) recovered on day 3 and 7 after CIDR removal from oFSH superovulated donor does.

4.5 EFFECT OF SOURCES OF THE EMBRYOS ON GOATS EMBRYO TRANSFER

Thirteen does with at least 1 CL present on either side of the ovaries were used as recipients in this experiment. Each recipient doe received not more that 4 embryos in each transfer session. The results of the embryo transfer are shown in Table 4.15. Out of 13 recipient does, 5 females received a total of 8 ICSI embryos at morula stage of development and 8 recipient goats received 32 *in vivo* fertilised embryos at different stages of development. Four (80%) recipient goats that were implanted with ICSI embryos were positive to ultrasound pregnancy diagnosis at 60 days after embryo transfer compared to 5 (14%) recipient does of *in vivo* fertilised embryos. One recipient doe carried full term pregnancy resulted in twin kids (Figure 4.14). Unfortunately, the kids died soon after birth.

Table 4.15. The results of embryo transfer of ICSI and *in vivo* fertilised embryos

	Sources of embryos	
	ICSI embryos	***in vivo* fertilised embryos**
Number of recipient	5	8
Number of embryos transferred	8	32
Number of recipient does with positive ultrasound result at 60 days	4 (80%)	5 (62%)
Kids born	0	2
Percentage Kidding (%)	0	20

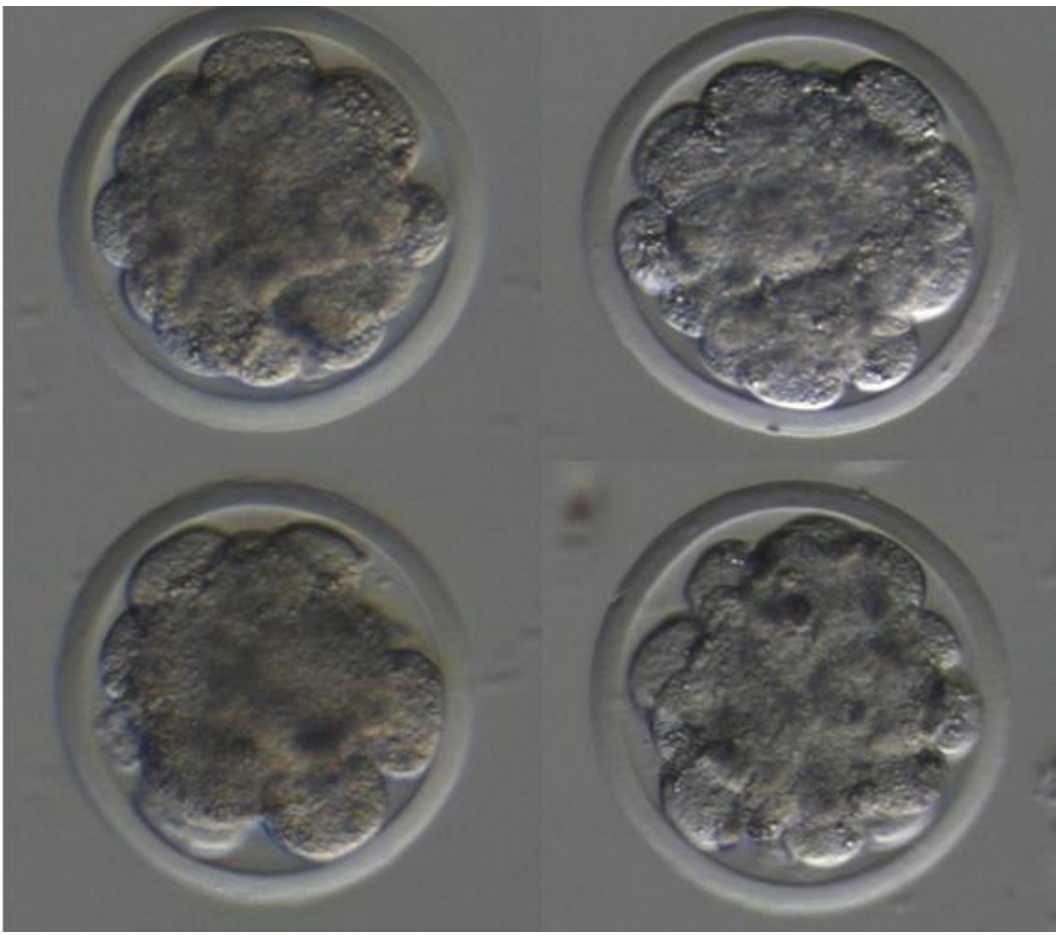
a.

b.


Figure 4.14. Successful embryo transfer

(a) Four embryos photographed and transferred on day 7 into a Boer recipient doe and led to a twin gestation. (b)The kids die after born.