APPENDICES

APPENDICES

APPENDIX 1: LIST OF MATERIALS

Appendix Table 1.1: List of equipment and instruments

Equipment/instruments	Model No.	Manufacturer
Autoclave	HA- 300 MII	Hirayama Hiclave, Japan
Centrifuge	D37520	Heraeus, Germany
CIDR applicator	-	Pharmacia and Upjohn Limited, Auckland, New Zealand
CO_2 incubator	HeraCell 240	Heraeus, Germany
CO_2 insufflator system	PG001	Aesculap®, Germany
Digital balance	AB104	Mettle Toledo, Switzerland
Dissecting microscope	SZH10	Olympus, Japan
Flushing and aspiration system:	KMAR-5100	Cook, Australia
a) Aspiration system	KMAR-4000	Cook, Australia
b) Flushing system	K-FTH-1012	Cook, Australia
c) Test tube heater system	6210-72525OB	Herga Electric Limited, UK
d) Pedal		
Haemacytometer	L4247I	Neubauer, Germany
Hand-controlled pipette	-	Self- assembled
Heating stage (Thermoplate)	HATS-U55R30	Tokai Hit, Japan
Impulse sealer	KF-300H	Khind, Taiwan
Inverted microscope	IX71	Olympus, Japan
Laminar flow cabinet	HLF-120	Gelman Sciences, Australia
Laparoscopic set:		Aesculap®, Germany
a) Endoscopic system	PV431	
b) CCD camera	PV430	
c) Laparoscope (7 mm)	PE688A	
d) Light probe	OP913	
e) Light system (300 W)	OP927	
Liquid nitrogen tank	SC2/1 V	MVE, USA
Micropipette	-	Eppendorf, Germany
Oocyte respiration needle	FAS set 2	Cook, Australia
Oven	40050-1P20	Memmert, Germany
pH meter	HI-122	Hanna Instruments, Singapore
Refrigerator and freezer	SR-21NME	Samsung, Korea
Spirit burner	-	Shanghai Machinery, China
Stereomicroscope	SZH10	Olympus Optical, Japan
Surgical set	-	Aesculap®, B.Braun, Germany
Surgical table	-	Syarikat Copens Enterprise, Malaysia
Thermo flask (Mega®)	MA043	Megatrade Instruments, USA
Trocar and cannula	EJ456, EJ457	Aesculap®, Germany
Ultrapure water purification system (Milli-Q PF Plus)	-	Millipore Corporation, USA
Vapour pressure osmometer	5520	Vapro Wescor, USA
Veress needle	PG3	Cook, Australia
Vortex mixer	MS1	IKA, Germany
Water bath	GMP-GC-19	Memmert, Germany

Appendix Table 1.2: List of glass-wares, lab-wares and disposables

Glasswares/labwares/disposables	Manufacturer
Aluminium foil	Reynolds Consumer Products, Richmond, Virginia, USA
Autoclave bag	Megalab supplies, Malaysia
Blade	Gillette, USA
Catgut suture	aesculap®, Germany
Cover slips	hirschmann® Laborgerete, Germany
Culture Petri dishes (35 mm, 60 mm, 4-well dish)	Nunc, Roskilde, Denmark
Disposable gloves	Protex, Malaysia
Disposable pipette (5 ml)	LP Italiana SPA, Italy
Falcon™ conical tube (15 ml, 50 ml)	Becton Dickinson, USA
Glass bottle (50 ml, 250 ml, 500 ml)	Schott Duran, UK
Glassware (beakers, flasks, measuring cylinders, etc.)	Pyrex, UK
Haematocrit capillary (75 mm/ µl)	Hirschmann®Labogerete, Germany
Lens tissue	Kimwipes®, Kimberly-Clark, Canada
Microcentrifuge tube (0.5 ml, 1.5 ml)	Elkay, Costelloe
Micropipette tip without filter (blue, yellow)	Axygen Scientific, USA
Microscope slide	Sail Brand, China
Needle (18 G, 23 G, 26 G)	Terumo® Needle, Japan
Parafilm	Pechiney Plastic Packaging, Chicago, USA
Serological pipette (1 ml, 5 ml)	LP Italian SPA, Italy
Sterile gloves	Ansell International, Malaysia
French straw (0.25 ml)	Cryo Bio System, France
Syringe (1 ml, 5 ml, 25 ml, 50 ml)	Terumo® Syringe, Philipines
Syringe filter (0.22 µm)	Schleicher & Schuell, Germany
Vacutainer® blood collection tube	Becton Dickinson, Plymouth, UK

Appendix Table 1.3: List of chemicals, reagents and media

Chemicals/ reagents/ media	Catalogue no.	Manufacturer
6-dimethylaminopurine (6-DMAP)	D2629	Sigma-Aldrich, USA
70% ethanol	-	Prepared from absolute ethanol
Ethyl alcohol 99.8% (absolute ethanol)	ET150-50	System ChemAR®, Poland
Hibiscrub (antiseptic)	HK-06770	SSL International Plc, UK
BME amino acids solution (50X)	B6766	Sigma-Aldrich, USA
Bovine serum albumin (BSA)	A6003	Sigma-Aldrich, USA
Calcium chloride ($CaCl_2.2H_2O$)	C7902	Sigma-Aldrich, USA
Calcium ionophore (Ca^{2+} ionophore)	C7522	Sigma-Aldrich, USA
Cleaning solution 7X®-PF	-	FlowLab™, Australia
Cloprostenol (Estrumate®)	-	Schering- Plough, Australia
Cystein	C2529	Sigma-Aldrich, USA
Dimethyl sulphoxide (DMSO)	D2650	Sigma-Aldrich, USA
Disinfectant Gigasept® FF	-	Schülke & Mary GmbH, Germany

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Chemicals/ reagents/ media	Catalogue no.	Manufacturer
Estrumate®	-	Schering-Plough Animal Health, Baulkham Hills, NSW, Australia
Ethylene glycol (EG)	E9129	Sigma-Aldrich, USA
Ovagen™ (FSH)	-	ICPbio Limited, Auckland, New Zealand
Gentamycin sulphate salt	G3632	Sigma-Aldrich, USA
Goat/ sheep pellet feed	-	KMM Berhad, Malaysia
Heparin	H0777	Sigma-Aldrich, USA
HEPES	H3784, H6147	Sigma-Aldrich, USA
Hydrochloric acid	HY450-70	System ChemAR®, Poland
Intravaginal progesterone release device	-	Pharmacia and Upjohn, New Zealand
Ketamil injection (ketamine hydrochloride)	L10077	Troy Laboratories, Australia
K-Y Lubricating Jelly	-	Pharmedica Lab, South Africa
L-glutamine	G3126	Sigma-Aldrich, USA
Liquid nitrogen	-	Mox Gases Berhad, Malaysia
Magnesium chloride hexahydrate ($MgCl_2.6H_2O$)	M2393	Sigma-Aldrich, USA
MEM (100X)	M7145	Sigma-Aldrich, USA
Mineral oil	M8410	Sigma-Aldrich, USA
Oestradiol-17β	E8875	Sigma-Aldrich, USA
Oestrus goat serum, heat-inactivated	-	Prepared “in-house”
Ovidrel® PreFilled Syringe	-	Laboratories Serono, Switzerland
PBS Dulbecco A tablets	P4417	Oxoid, England
Phenol red	P3532	Sigma-Aldrich, USA
Phenol red solution (0.5%)	15100-043	Gibco BRL, USA
Potassium chloride (KCl)	P5405	Sigma-Aldrich, USA
Sodium bicarbonate ($NaHCO_3$)	S5761	Sigma-Aldrich, USA
Sodium chloride (NaCl)	S5886	Sigma-Aldrich, USA
Sodium DL-lactate (60% syrup)	L7900	Sigma-Aldrich, USA
Sodium pyruvate	P4562	Sigma-Aldrich, USA
TCM-199	M4530	Sigma-Aldrich, USA
Weak iodine solution	-	ICN Biomedicals, USA
Xylazine hydrochloride (Ilium Xylazil-20)	L10600	Troy Laboratories, Australia

APPENDIX 2: STATISTICAL DATA

Appendix Table 2.1: Number of oocytes per ovary in bovine *in vitro* fertilisation

Descriptives

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
GradeA	23	8.2239	5.66382	1.18099	5.7747	10.6731	1.25	25.00
GradeB	23	6.0443	4.36211	.90956	4.1580	7.9307	.00	16.00
GradeC	23	3.1283	3.34765	.69803	1.6806	4.5759	.00	12.50
Total	69	5.7988	4.95804	.59688	4.6078	6.9899	.00	25.00

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	300.685	2	150.342	7.238	.001
Within Groups	1370.900	66	20.771		
Total	1671.584	68			

Duncan[a]

VAR00001	N	Subset for alpha = 0.05	
		1	2
GradeC	23	3.1283	
GradeB	23		6.0443
GradeA	23		8.2239
Sig.		1.000	.110

Appendix Table 2.2: Percent distribution of bovine oocyte in *in vitro* fertilisation

Descriptives

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
GradeA	23	49.0926	22.03616	4.59486	39.5635	58.6218	5.00	100.00
GradeB	23	33.7500	14.36984	2.99632	27.5360	39.9640	.00	55.56
GradeC	23	17.0609	15.04728	3.13758	10.5539	23.5678	.00	50.00
Total	69	33.3012	21.69760	2.61208	28.0888	38.5135	.00	100.00

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	11806.322	2	5903.161	19.281	.000
Within Groups	20207.111	66	306.168		
Total	32013.433	68			

Duncan[a]

VAR00001	N	Subset for alpha = 0.05		
		1	2	3
GradeC	23	17.0609		
GradeB	23		33.7500	
GradeA	23			49.0926
Sig.		1.000	1.000	1.000

Appendix Table 2.3: Maturation rate of different grades of oocytes in bovine *in vitro* fertilisation

Descriptives

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
GradeA	23	56.7839	31.18868	6.50329	43.2969	70.2709	11.11	100.00
GradeB	22	46.5764	27.76634	5.91980	34.2655	58.8873	4.44	100.00
GradeC	18	31.2878	30.16442	7.10982	16.2874	46.2882	.00	100.00
Mixed	6	74.6917	16.37347	6.68444	57.5088	91.8746	50.00	90.00
Total	69	48.4354	31.03776	3.73651	40.9793	55.8914	.00	100.00

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	11108.165	3	3702.722	4.424	.007
Within Groups	54399.126	65	836.910		
Total	65507.291	68			

Duncan[a,,b]

VAR00001	N	Subset for alpha = 0.05		
		1	2	3
Grade C	18	31.2878		
Grade B	22	46.5764	46.5764	
Grade A	23		56.7839	56.7839
Mixed	6			74.6917
Sig.		.185	.374	.121

Appendix Table 2.4: Fertilisation rate of different grades of oocytes in bovine *in vitro* fertilisation

Descriptives

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
GradeA	23	70.4865	31.01585	6.46725	57.0743	83.8988	11.11	100.00
GradeB	22	47.2727	46.56033	9.92669	26.6290	67.9164	.00	100.00
GradeC	18	21.1111	40.85684	9.63005	.7935	41.4287	.00	100.00
Mixed	6	68.1750	18.19100	7.42644	49.0847	87.2653	39.29	86.67
Total	69	50.0035	42.62387	5.13131	39.7641	60.2428	.00	100.00

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	26820.886	3	8940.295	6.008	.001
Within Groups	96721.113	65	1488.017		
Total	123541.999	68			

Duncan[a,,b]

VAR00001	N	Subset for alpha = 0.05	
		1	2
Grade C	18	21.1111	
Grade B	22	47.2727	47.2727
Mixed	6		68.1750
Grade A	23		70.4865
Sig.		.090	.155

Appendix Table 2.5: Cleavage rate of bovine *in vitro* fertilisation with different grades of oocytes in different embryo developmental stages

Descriptive

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
cell2	GradeA	23	90.1087	14.25380	2.97212	83.9449	96.2725	50.00	100.00
	GradeB	22	50.2068	46.30117	9.87144	29.6780	70.7356	.00	100.00
	GradeC	18	15.2778	35.49947	8.36731	-2.3757	32.9312	.00	100.00
	Mixed	6	87.0250	12.36891	5.04959	74.0446	100.0054	63.64	97.50
	Total	69	57.5971	44.49445	5.35650	46.9084	68.2858	.00	100.00
cell4	GradeA	23	63.8657	33.24165	6.93136	49.4909	78.2404	.00	100.00
	GradeB	22	22.9164	40.33049	8.59849	5.0348	40.7979	.00	100.00
	GradeC	18	8.3333	25.72479	6.06339	-4.4593	21.1260	.00	100.00
	Mixed	6	76.7600	6.81437	2.78195	69.6088	83.9112	63.64	83.33
	Total	69	37.4439	41.15566	4.95456	27.5572	47.3306	.00	100.00
cell8	GradeA	23	18.5843	22.10882	4.61001	9.0238	28.1449	.00	60.00
	GradeB	22	12.4241	27.22482	5.80435	.3533	24.4949	.00	100.00
	GradeC	18	1.3889	5.89256	1.38889	-1.5414	4.3192	.00	25.00
	Mixed	6	35.6533	20.88422	8.52595	13.7367	57.5700	.00	54.17
	Total	69	13.6187	22.78207	2.74264	8.1458	19.0915	.00	100.00

morula	GradeA	23	1.2683	4.24876	.88593	-.5690	3.1056	.00	16.67
	GradeB	22	.7577	3.55406	.75773	-.8181	2.3335	.00	16.67
	GradeC	18	1.3889	5.89256	1.38889	-1.5414	4.3192	.00	25.00
	Mixed	6	3.8900	5.04649	2.06022	-1.4060	9.1860	.00	12.50
	Total	69	1.3649	4.58034	.55141	.2646	2.4652	.00	25.00

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
cell2	Between Groups	62945.305	3	20981.768	19.027	.000
	Within Groups	71678.079	65	1102.740		
	Total	134623.384	68			
cell4	Between Groups	45227.778	3	15075.926	14.009	.000
	Within Groups	69949.851	65	1076.152		
	Total	115177.629	68			
cell8	Between Groups	6203.902	3	2067.967	4.621	.005
	Within Groups	29089.640	65	447.533		
	Total	35293.542	68			
morula	Between Groups	46.592	3	15.531	.732	.537
	Within Groups	1380.014	65	21.231		
	Total	1426.607	68			

cell2

Duncan[a,,b]

VAR00001	N	Subset for alpha = 0.05		
		1	2	3
GradeC	18	15.2778		
GradeB	22		50.2068	
mixed	6			87.0250
GradeA	23			90.1087
Sig.		1.000	1.000	.815

cell4

Duncan[a,,b]

VAR00001	N	Subset for alpha = 0.05	
		1	2
GradeC	18	8.3333	
GradeB	22	22.9164	
GradeA	23		63.8657
mixed	6		76.7600
Sig.		.264	.323

cell8

Duncan[a,,b]

VAR00001	N	Subset for alpha = 0.05	
		1	2
GradeC	18	1.3889	
GradeB	22	12.4241	
GradeA	23	18.5843	
mixed	6		35.6533
Sig.		.055	1.000

morula

Duncan[a,,b]

VAR00001	N	Subset for alpha = 0.05
		1
GradeB	22	.7577
GradeA	23	1.2683
GradeC	18	1.3889
mixed	6	3.8900
Sig.		.121

Appendix Table 2.6: Cleavage rate of bovine *in vitro* fertilisation in different grades of oocytes

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
GradeA	2cell	23	90.1087	14.25380	2.97212	83.9449	96.2725	50.00	100.00
	4cell	23	63.8657	33.24165	6.93136	49.4909	78.2404	.00	100.00
	8cell	23	18.5843	22.10882	4.61001	9.0238	28.1449	.00	60.00
	morula	23	1.2683	4.24876	.88593	-.5690	3.1056	.00	16.67
	Total	92	43.4567	41.23553	4.29910	34.9171	51.9964	.00	100.00
GradeB	2cell	22	50.2068	46.30117	9.87144	29.6780	70.7356	.00	100.00
	4cell	22	22.9164	40.33049	8.59849	5.0348	40.7979	.00	100.00
	8cell	22	12.4241	27.22482	5.80435	.3533	24.4949	.00	100.00
	morula	22	.7577	3.55406	.75773	-.8181	2.3335	.00	16.67
	Total	88	21.5762	37.82271	4.03191	13.5624	29.5901	.00	100.00
GradeC	2cell	18	15.2778	35.49947	8.36731	-2.3757	32.9312	.00	100.00
	4cell	18	8.3333	25.72479	6.06339	-4.4593	21.1260	.00	100.00
	8cell	18	1.3889	5.89256	1.38889	-1.5414	4.3192	.00	25.00
	morula	18	1.3889	5.89256	1.38889	-1.5414	4.3192	.00	25.00
	Total	72	6.5972	22.59294	2.66260	1.2881	11.9063	.00	100.00

Mixed	2cell	6	87.0250	12.36891	5.04959	74.0446	100.0054	63.64	97.50
	4cell	6	76.7600	6.81437	2.78195	69.6088	83.9112	63.64	83.33
	8cell	6	35.6533	20.88422	8.52595	13.7367	57.5700	.00	54.17
	morula	6	3.8900	5.04649	2.06022	-1.4060	9.1860	.00	12.50
	Total	24	50.8321	35.99640	7.34773	35.6321	66.0320	.00	97.50

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
GradeA	Between Groups	114802.944	3	38267.648	84.335	.000
	Within Groups	39930.656	88	453.757		
	Total	154733.600	91			
GradeB	Between Groups	29450.921	3	9816.974	8.680	.000
	Within Groups	95007.545	84	1131.042		
	Total	124458.466	87			
GradeC	Between Groups	2387.153	3	795.718	1.598	.198
	Within Groups	33854.167	68	497.855		
	Total	36241.319	71			
Mixed	Between Groups	26496.826	3	8832.275	53.444	.000
	Within Groups	3305.217	20	165.261		
	Total	29802.043	23			

GradeA

Duncan[a]

VAR00001	N	Subset for alpha = 0.05			
		1	2	3	4
morula	23	1.2683			
8cell	23		18.5843		
4cell	23			63.8657	
2cell	23				90.1087
Sig.		1.000	1.000	1.000	1.000

GradeB

Duncan[a]

VAR00001	N	Subset for alpha = 0.05		
		1	2	3
morula	22	.7577		
8cell	22	12.4241	12.4241	
4cell	22		22.9164	
2cell	22			50.2068
Sig.		.253	.304	1.000

GradeC

Duncan[a]

VAR00001	N	Subset for alpha = 0.05
		1
8cell	18	1.3889
morula	18	1.3889
4cell	18	8.3333
2cell	18	15.2778
Sig.		.092

Mixed

Duncan[a]

VAR00001	N	Subset for alpha = 0.05		
		1	2	3
morula	6	3.8900		
8cell	6		35.6533	
4cell	6			76.7600
2cell	6			87.0250
Sig.		1.000	1.000	.182

Appendix Table 2.7: Maturation rate of bovine *in vitro* fertilisation in different insemination durations

Descriptives

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
8-14hr	16	54.5225	30.34239	7.58560	38.3542	70.6908	7.00	100.00
18-24hr	13	50.8492	25.09602	6.96038	35.6839	66.0146	22.50	90.00
Total	29	52.8759	27.68729	5.14140	42.3442	63.4075	7.00	100.00

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	96.777	1	96.777	.122	.729
Within Groups	21367.631	27	791.394		
Total	21464.408	28			

Appendix Table 2.8: Fertilisation rate of bovine *in vitro* fertilisation in different insemination durations

Descriptives

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
8-14hr	16	77.3669	26.08103	6.52026	63.4693	91.2645	22.22	100.00
18-24hr	13	52.0300	37.36090	10.36205	29.4530	74.6070	5.26	92.31
Total	29	66.0090	33.57174	6.23412	53.2390	78.7790	5.26	100.00

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4604.383	1	4604.383	4.612	.041
Within Groups	26953.343	27	998.272		
Total	31557.726	28			

Appendix Table 2.9: Cleavage rate of bovine *in vitro* fertilisation with different insemination durations in different embryo developmental stages

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
cell2	8-14 hr	16	86.1750	12.39066	3.09767	79.5725	92.7775	60.71	100.00
	18-24 hr	13	89.8100	16.04328	4.44960	80.1151	99.5049	50.00	100.00
	Total	29	87.8045	13.99788	2.59934	82.4800	93.1290	50.00	100.00
cell4	8-14 hr	16	63.8944	27.21498	6.80375	49.3925	78.3962	.00	100.00
	18-24 hr	13	60.6646	33.47952	9.28555	40.4331	80.8961	.00	100.00
	Total	29	62.4466	29.66189	5.50808	51.1638	73.7293	.00	100.00
cell8	8-14 hr	16	21.1356	21.56129	5.39032	9.6464	32.6248	.00	64.29
	18-24 hr	13	20.8338	25.21968	6.99468	5.5937	36.0739	.00	60.00
	Total	29	21.0003	22.83980	4.24124	12.3126	29.6881	.00	64.29
morula	8-14 hr	16	1.6519	3.97093	.99273	-.4641	3.7678	.00	14.29
	18-24 hr	13	1.7954	3.83214	1.06284	-.5204	4.1111	.00	12.50
	Total	29	1.7162	3.84009	.71309	.2555	3.1769	.00	14.29

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
cell2	Between Groups	94.771	1	94.771	.475	.497
	Within Groups	5391.568	27	199.688		
	Total	5486.339	28			
cell4	Between Groups	74.818	1	74.818	.082	.776
	Within Groups	24560.365	27	909.643		
	Total	24635.183	28			
cell8	Between Groups	.653	1	.653	.001	.973
	Within Groups	14605.726	27	540.953		
	Total	14606.379	28			
morula	Between Groups	.148	1	.148	.010	.922
	Within Groups	412.748	27	15.287		
	Total	412.896	28			

Appendix Table 2.10: Cleavage rate of bovine *in vitro* fertilisation in different insemination durations

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
hr8to14	2cell	16	86.1750	12.39066	3.09767	79.5725	92.7775	60.71	100.00
	4cell	16	63.8944	27.21498	6.80375	49.3925	78.3962	.00	100.00
	8cell	16	21.1356	21.56129	5.39032	9.6464	32.6248	.00	64.29
	morula	16	1.6519	3.97093	.99273	-.4641	3.7678	.00	14.29
	Total	64	43.2142	38.30406	4.78801	33.6461	52.7823	.00	100.00
hr18to24	2cell	13	89.8100	16.04328	4.44960	80.1151	99.5049	50.00	100.00
	4cell	13	60.6646	33.47952	9.28555	40.4331	80.8961	.00	100.00
	8cell	13	20.8338	25.21968	6.99468	5.5937	36.0739	.00	60.00
	morula	13	1.7954	3.83214	1.06284	-.5204	4.1111	.00	12.50
	Total	52	43.2760	40.90752	5.67285	31.8872	54.6647	.00	100.00

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
hr8to14	Between Groups	71811.045	3	23937.015	69.643	.000
	Within Groups	20622.619	60	343.710		
	Total	92433.664	63			
hr18to24	Between Groups	60996.895	3	20332.298	40.084	.000
	Within Groups	24347.788	48	507.246		
	Total	85344.682	51			

hr8to14

Duncan[a]

		Subset for alpha = 0.05			
VAR00001	N	1	2	3	4
morula	16	1.6519			
8cell	16		21.1356		
4cell	16			63.8944	
2cell	16				86.1750
Sig.		1.000	1.000	1.000	1.000

hr18to24

Duncan[a]

		Subset for alpha = 0.05			
VAR00001	N	1	2	3	4
morula	13	1.7954			
8cell	13		20.8338		
4cell	13			60.6646	
2cell	13				89.8100
Sig.		1.000	1.000	1.000	1.000

Appendix Table 2.11: Maturation rate of bovine oocytes *in vitro* fertilisation with different gradings in different insemination durations

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
GradeA	8-14 hr	15	64.7087	27.33826	7.05871	49.5692	79.8481	18.18	100.00
	18-24 hr	8	39.9775	17.05196	6.02878	25.7217	54.2333	22.86	80.00
	Total	23	56.1065	26.70532	5.56844	44.5583	67.6548	18.18	100.00
GradeB	8-14 hr	14	52.1029	28.09797	7.50950	35.8796	68.3261	4.44	100.00
	18-24 hr	8	36.9050	26.05227	9.21087	15.1248	58.6852	20.00	100.00
	Total	22	46.5764	27.76634	5.91980	34.2655	58.8873	4.44	100.00
GradeC	8-14 hr	10	25.8710	32.73314	10.35113	2.4551	49.2869	.00	83.33
	18-24 hr	8	38.0587	27.16316	9.60363	15.3498	60.7677	14.29	100.00
	Total	18	31.2878	30.16442	7.10982	16.2874	46.2882	.00	100.00

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
GradeA	Between Groups	3191.116	1	3191.116	5.362	.031
	Within Groups	12498.711	21	595.177		
	Total	15689.827	22			
GradeB	Between Groups	1175.872	1	1175.872	1.566	.225
	Within Groups	15014.493	20	750.725		
	Total	16190.365	21			
GradeC	Between Groups	660.183	1	660.183	.713	.411
	Within Groups	14807.985	16	925.499		
	Total	15468.169	17			

Appendix Table 2.12: Maturation rate of bovine oocytes *in vitro* fertilisation with different insemination durations in different oocyte gradings

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
hr8to14	Grade A	15	64.7087	27.33826	7.05871	49.5692	79.8481	18.18	100.00
	Grade B	14	52.1029	28.09797	7.50950	35.8796	68.3261	4.44	100.00
	Grade C	10	25.8710	32.73314	10.35113	2.4551	49.2869	.00	83.33
	Total	39	50.2251	32.23968	5.16248	39.7742	60.6760	.00	100.00
hr18to24	Grade A	8	39.9775	17.05196	6.02878	25.7217	54.2333	22.86	80.00
	Grade B	8	36.9050	26.05227	9.21087	15.1248	58.6852	20.00	100.00
	Grade C	8	38.0587	27.16316	9.60363	15.3498	60.7677	14.29	100.00
	Total	24	38.3138	22.83193	4.66055	28.6727	47.9548	14.29	100.00

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
hr8to14	Between Groups	9127.191	2	4563.596	5.410	.009
	Within Groups	30369.897	36	843.608		
	Total	39497.088	38			
hr18to24	Between Groups	38.541	2	19.271	.034	.967
	Within Groups	11951.292	21	569.109		
	Total	11989.833	23			

hr8to14

Duncan[a,,b]

		Subset for alpha = 0.05	
VAR00001	N	1	2
Grade C	10	25.8710	
Grade B	14		52.1029
Grade A	15		64.7087
Sig.		1.000	.283

hr18to24

Duncan[a]

		Subset for alpha = 0.05
VAR00001	N	1
Grade B	8	36.9050
Grade C	8	38.0587
Grade A	8	39.9775
Sig.		.811

Appendix Table 2.13: Fertilisation rate of bovine oocytes *in vitro* fertilisation with different gradings in different insemination durations

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
hr8to14	Grade A	15	82.9340	19.46888	5.02684	72.1525	93.7155	40.00	100.00
	Grade B	14	65.7143	42.81592	11.44303	40.9931	90.4355	.00	100.00
	Grade C	10	18.0000	38.23901	12.09224	-9.3545	45.3545	.00	100.00
	Total	39	60.1028	42.39054	6.78792	46.3614	73.8442	.00	100.00
hr18to24	Grade A	8	47.1475	36.20445	12.80020	16.8798	77.4152	11.11	100.00
	Grade B	9	22.5922	39.92213	13.30738	-8.0946	53.2791	.00	100.00
	Grade C	8	25.0000	46.29100	16.36634	-13.7002	63.7002	.00	100.00
	Total	25	31.2204	40.79138	8.15828	14.3825	48.0583	.00	100.00

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
hr8to14	Between Groups	25986.256	2	12993.128	11.058	.000
	Within Groups	42298.158	36	1174.949		
	Total	68284.414	38			
hr18to24	Between Groups	3008.936	2	1504.468	.896	.422
	Within Groups	36925.545	22	1678.434		
	Total	39934.481	24			

hr8to14

Duncan[a,,b]

VAR00001	N	Subset for alpha = 0.05	
		1	2
Grade C	10	18.0000	
Grade B	14		65.7143
Grade A	15		82.9340
Sig.		1.000	.215

hr18to24

Duncan[a,,b]

VAR00001	N	Subset for alpha = 0.05
		1
Grade B	9	22.5922
Grade C	8	25.0000
Grade A	8	47.1475
Sig.		.261

Appendix Table 2.14: Fertilisation rate of bovine oocytes *in vitro* fertilisation with different insemination durations in different oocyte gradings

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
GradeA	8-14 hr	15	82.9340	19.46888	5.02684	72.1525	93.7155	40.00	100.00
	18-24 hr	8	47.1475	36.20445	12.80020	16.8798	77.4152	11.11	100.00
	Total	23	70.4865	31.01585	6.46725	57.0743	83.8988	11.11	100.00
GradeB	8-14 hr	14	65.7143	42.81592	11.44303	40.9931	90.4355	.00	100.00
	18-24 hr	9	22.5922	39.92213	13.30738	-8.0946	53.2791	.00	100.00
	Total	23	48.8404	46.10696	9.61397	28.9023	68.7786	.00	100.00
GradeC	8-14 hr	10	18.0000	38.23901	12.09224	-9.3545	45.3545	.00	100.00
	18-24 hr	8	25.0000	46.29100	16.36634	-13.7002	63.7002	.00	100.00
	Total	18	21.1111	40.85684	9.63005	.7935	41.4287	.00	100.00

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
GradeA	Between Groups	6681.775	1	6681.775	9.689	.005
	Within Groups	14481.856	21	689.612		
	Total	21163.632	22			
GradeB	Between Groups	10186.894	1	10186.894	5.848	.025
	Within Groups	36581.847	21	1741.993		
	Total	46768.741	22			
GradeC	Between Groups	217.778	1	217.778	.124	.730
	Within Groups	28160.000	16	1760.000		
	Total	28377.778	17			

Appendix Table 2.15: Cleavage rate of bovine oocytes *in vitro* fertilisation with different gradings and different insemination durations in different embryo developmental stages

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
cell2	A_8-14 hr	15	89.8333	12.08485	3.12030	83.1410	96.5257	66.67	100.00
	A_18-24 hr	8	90.6250	18.60060	6.57630	75.0745	106.1755	50.00	100.00
	B_8-14 hr	14	64.6107	41.16765	11.00252	40.8412	88.3802	.00	100.00
	B_18-24 hr	8	25.0000	46.29100	16.36634	-13.7002	63.7002	.00	100.00
	C_8-14 hr	10	7.5000	23.71708	7.50000	-9.4662	24.4662	.00	75.00
	C_18-24 hr	8	25.0000	46.29100	16.36634	-13.7002	63.7002	.00	100.00
	Total	63	54.7944	45.46668	5.72826	43.3438	66.2451	.00	100.00
cell4	A_8-14 hr	15	70.7847	27.96814	7.22134	55.2964	86.2729	.00	100.00
	A_18-24 hr	8	50.8925	40.16859	14.20174	17.3107	84.4743	.00	100.00
	B_8-14 hr	14	28.8686	43.00582	11.49379	4.0378	53.6994	.00	100.00
	B_18-24 hr	8	12.5000	35.35534	12.50000	-17.0578	42.0578	.00	100.00
	C_8-14 hr	10	5.0000	15.81139	5.00000	-6.3108	16.3108	.00	50.00
	C_18-24 hr	8	12.5000	35.35534	12.50000	-17.0578	42.0578	.00	100.00
	Total	63	33.6995	41.11111	5.17951	23.3458	44.0532	.00	100.00

cell8	A_8-14 hr	15	23.0200	22.13353	5.71485	10.7629	35.2771	.00	60.00
	A_18-24 hr	8	10.2675	20.86216	7.37589	-7.1737	27.7087	.00	57.14
	B_8-14 hr	14	12.3807	22.89599	6.11921	-.8390	25.6005	.00	75.00
	B_18-24 hr	8	12.5000	35.35534	12.50000	-17.0578	42.0578	.00	100.00
	C_8-14 hr	10	2.5000	7.90569	2.50000	-3.1554	8.1554	.00	25.00
	C_18-24 hr	8	.0000	.00000	.00000	.0000	.0000	.00	.00
	Total	63	11.5202	21.96852	2.76777	5.9875	17.0529	.00	100.00
morula	A_8-14 hr	15	1.9447	5.19212	1.34060	-.9306	4.8200	.00	16.67
	A_18-24 hr	8	.0000	.00000	.00000	.0000	.0000	.00	.00
	B_8-14 hr	14	1.1907	4.45524	1.19071	-1.3817	3.7631	.00	16.67
	B_18-24 hr	8	.0000	.00000	.00000	.0000	.0000	.00	.00
	C_8-14 hr	10	2.5000	7.90569	2.50000	-3.1554	8.1554	.00	25.00
	C_18-24 hr	8	.0000	.00000	.00000	.0000	.0000	.00	.00
	Total	63	1.1244	4.50335	.56737	-.0097	2.2586	.00	25.00

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
cell2	Between Groups	66606.501	5	13321.300	12.334	.000
	Within Groups	61561.067	57	1080.019		
	Total	128167.568	62			
cell4	Between Groups	38748.482	5	7749.696	6.689	.000
	Within Groups	66039.152	57	1158.582		
	Total	104787.634	62			
cell8	Between Groups	3889.642	5	777.928	1.703	.149
	Within Groups	26032.552	57	456.711		
	Total	29922.194	62			
morula	Between Groups	59.419	5	11.884	.565	.726
	Within Groups	1197.953	57	21.017		
	Total	1257.372	62			

cell2

Duncan[a,,b]

VAR00001	N	Subset for alpha = 0.05	
		1	2
C_8-14 hr	10	7.5000	
B_18-24 hr	8	25.0000	
C_18-24 hr	8	25.0000	
B_8-14 hr	14		64.6107
A_8-14 hr	15		89.8333
A_18-24 hr	8		90.6250
Sig.		.273	.103

cell4

Duncan[a,,b]

VAR00001	N	Subset for alpha = 0.05		
		1	2	3
C_8-14 hr	10	5.0000		
B_18-24 hr	8	12.5000		
C_18-24 hr	8	12.5000		
B_8-14 hr	14	28.8686	28.8686	
A_18-24 hr	8		50.8925	50.8925
A_8-14 hr	15			70.7847
Sig.		.163	.158	.201

cell8

Duncan[a,,b]

VAR00001	N	Subset for alpha = 0.05	
		1	2
C_18-24 hr	8	.0000	
C_8-14 hr	10	2.5000	2.5000
A_18-24 hr	8	10.2675	10.2675
B_8-14 hr	14	12.3807	12.3807
B_18-24 hr	8	12.5000	12.5000
A_8-14 hr	15		23.0200
Sig.		.257	.062

morula

Duncan[a,,b]

VAR00001	N	Subset for alpha = 0.05
		1
A_18-24 hr	8	.0000
B_18-24 hr	8	.0000
C_18-24 hr	8	.0000
B_8-14 hr	14	1.1907
A_8-14 hr	15	1.9447
C_8-14 hr	10	2.5000
Sig.		.300

Appendix Table 2.16: Cleavage rate of bovine oocytes *in vitro* fertilisation with different insemination durations and oocyte gradings

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
A8to14hr	2cell	15	89.8333	12.08485	3.12030	83.1410	96.5257	66.67	100.00
	4cell	15	70.7847	27.96814	7.22134	55.2964	86.2729	.00	100.00
	8cell	15	23.0200	22.13353	5.71485	10.7629	35.2771	.00	60.00
	morula	15	1.9447	5.19212	1.34060	-.9306	4.8200	.00	16.67
	Total	60	46.3957	40.18834	5.18829	36.0139	56.7774	.00	100.00
A18to24hr	2cell	8	90.6250	18.60060	6.57630	75.0745	106.1755	50.00	100.00
	4cell	8	50.8925	40.16859	14.20174	17.3107	84.4743	.00	100.00
	8cell	8	10.2675	20.86216	7.37589	-7.1737	27.7087	.00	57.14
	morula	8	.0000	.00000	.00000	.0000	.0000	.00	.00
	Total	32	37.9463	43.23700	7.64329	22.3576	53.5349	.00	100.00
B8to14hr	2cell	14	64.6107	41.16765	11.00252	40.8412	88.3802	.00	100.00
	4cell	14	28.8686	43.00582	11.49379	4.0378	53.6994	.00	100.00
	8cell	14	12.3807	22.89599	6.11921	-.8390	25.6005	.00	75.00
	morula	14	1.1907	4.45524	1.19071	-1.3817	3.7631	.00	16.67
	Total	56	26.7627	39.38528	5.26308	16.2152	37.3101	.00	100.00

B18to24hr	2cell	8	25.0000	46.29100	16.36634	-13.7002	63.7002	.00	100.00
	4cell	8	12.5000	35.35534	12.50000	-17.0578	42.0578	.00	100.00
	8cell	8	12.5000	35.35534	12.50000	-17.0578	42.0578	.00	100.00
	morula	8	.0000	.00000	.00000	.0000	.0000	.00	.00
	Total	32	12.5000	33.60108	5.93989	.3855	24.6145	.00	100.00
C8to14hr	2cell	10	7.5000	23.71708	7.50000	-9.4662	24.4662	.00	75.00
	4cell	10	5.0000	15.81139	5.00000	-6.3108	16.3108	.00	50.00
	8cell	10	2.5000	7.90569	2.50000	-3.1554	8.1554	.00	25.00
	morula	10	2.5000	7.90569	2.50000	-3.1554	8.1554	.00	25.00
	Total	40	4.3750	14.85777	2.34922	-.3767	9.1267	.00	75.00
C18to24hr	2cell	8	25.0000	46.29100	16.36634	-13.7002	63.7002	.00	100.00
	4cell	8	12.5000	35.35534	12.50000	-17.0578	42.0578	.00	100.00
	8cell	8	.0000	.00000	.00000	.0000	.0000	.00	.00
	morula	8	.0000	.00000	.00000	.0000	.0000	.00	.00
	Total	32	9.3750	29.61446	5.23515	-1.3022	20.0522	.00	100.00

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
A8to14hr	Between Groups	75059.511	3	25019.837	69.254	.000
	Within Groups	20231.568	56	361.278		
	Total	95291.079	59			
A18to24hr	Between Groups	41189.497	3	13729.832	22.933	.000
	Within Groups	16763.089	28	598.682		
	Total	57952.587	31			
B8to14hr	Between Groups	32167.448	3	10722.483	10.491	.000
	Within Groups	53148.566	52	1022.088		
	Total	85316.014	55			
B18to24hr	Between Groups	2500.000	3	833.333	.718	.550
	Within Groups	32500.000	28	1160.714		
	Total	35000.000	31			
C8to14hr	Between Groups	171.875	3	57.292	.244	.865
	Within Groups	8437.500	36	234.375		
	Total	8609.375	39			
C18to24hr	Between Groups	3437.500	3	1145.833	1.351	.278
	Within Groups	23750.000	28	848.214		
	Total	27187.500	31			

A8to14hr

Duncan[a]

VAR00001	N	Subset for alpha = 0.05			
		1	2	3	4
morula	15	1.9447			
8cell	15		23.0200		
4cell	15			70.7847	
2cell	15				89.8333
Sig.		1.000	1.000	1.000	1.000

A18to24hr

Duncan[a]

VAR00001	N	Subset for alpha = 0.05		
		1	2	3
morula	8	.0000		
8cell	8	10.2675		
4cell	8		50.8925	
2cell	8			90.6250
Sig.		.408	1.000	1.000

B8to14hr

Duncan[a]

VAR00001	N	Subset for alpha = 0.05		
		1	2	3
morula	14	1.1907		
8cell	14	12.3807	12.3807	
4cell	14		28.8686	
2cell	14			64.6107
Sig.		.359	.178	1.000

B18to24hr

Duncan[a]

VAR00001	N	Subset for alpha = 0.05
		1
morula	8	.0000
4cell	8	12.5000
8cell	8	12.5000
2cell	8	25.0000
Sig.		.191

C8to14hr

Duncan[a]

VAR00001	N	Subset for alpha = 0.05
		1
8cell	10	2.5000
morula	10	2.5000
4cell	10	5.0000
2cell	10	7.5000
Sig.		.513

C18to24hr

Duncan[a]

VAR00001	N	Subset for alpha = 0.05
		1
8cell	8	.0000
morula	8	.0000
4cell	8	12.5000
2cell	8	25.0000
Sig.		.127

Appendix Table 2.17: Number of oocytes per ovary in caprine *in vitro* fertilisation

Descriptives

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
GradeA	30	1.9550	1.25940	.22993	1.4847	2.4253	.00	6.00
GradeB	30	1.8500	1.49805	.27351	1.2906	2.4094	.00	7.00
GradeC	30	2.3203	1.59251	.29075	1.7257	2.9150	.88	6.00
Total	90	2.0418	1.45448	.15332	1.7371	2.3464	.00	7.00

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3.657	2	1.829	.862	.426
Within Groups	184.624	87	2.122		
Total	188.281	89			

Duncan[a]

VAR00001	N	Subset for alpha = 0.05
		1
GradeB	30	1.8500
GradeA	30	1.9550
GradeC	30	2.3203
Sig.		.243

Appendix Table 2.18: Percent distribution of caprine oocyte in *in vitro* fertilisation

Descriptives

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Grade A	30	37.7777	21.61196	3.94579	29.7076	45.8477	.00	100.00
Grade B	30	37.4993	27.66677	5.05124	27.1684	47.8303	.00	100.00
Grade C	30	44.7227	26.98259	4.92632	34.6472	54.7981	10.00	100.00
Total	90	39.9999	25.49772	2.68770	34.6595	45.3403	.00	100.00

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1004.870	2	502.435	.769	.467
Within Groups	56857.018	87	653.529		
Total	57861.888	89			

Duncan[a]

VAR00001	N	Subset for alpha = 0.05
		1
Grade B	30	37.4993
Grade A	30	37.7777
Grade C	30	44.7227
Sig.		.307

Appendix Table 2.19: Maturation rate of different grades of oocytes in caprine *in vitro* fertilisation

Descriptives

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
GradeA	29	74.1862	31.18579	5.79106	62.3238	86.0486	.00	100.00
GradeB	26	54.6554	37.83040	7.41915	39.3754	69.9354	.00	100.00
GradeC	30	42.5010	39.42739	7.19842	27.7786	57.2234	.00	100.00
Total	85	57.0291	38.28249	4.15232	48.7717	65.2864	.00	100.00

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	15015.092	2	7507.546	5.695	.005
Within Groups	108091.050	82	1318.184		
Total	123106.141	84			

Duncan[a,,b]

		Subset for alpha = 0.05	
VAR00001	N	1	2
GradeC	30	42.5010	
GradeB	26	54.6554	
GradeA	29		74.1862
Sig.		.212	1.000

Appendix Table 2.20: Fertilisation rate of different grades of oocytes in caprine *in vitro*fertilisation

Descriptives

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
GradeA	29	40.5407	44.30925	8.22802	23.6864	57.3950	.00	100.00
GradeB	26	34.8077	42.97898	8.42887	17.4481	52.1673	.00	100.00
GradeC	30	16.2590	32.82931	5.99379	4.0003	28.5177	.00	100.00
Total	85	30.2171	41.09963	4.45788	21.3521	39.0820	.00	100.00

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	9483.487	2	4741.744	2.937	.059
Within Groups	132407.629	82	1614.727		
Total	141891.116	84			

Duncan[a,,b]

VAR00001	N	Subset for alpha = 0.05	
		1	2
GradeC	30	16.2590	
GradeB	26	34.8077	34.8077
GradeA	29		40.5407
Sig.		.087	.593

Appendix Table 2.21: Cleavage rate of caprine *in vitro* fertilisation with different grades of oocytes in different embryo developmental stages

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
cell2	GradeA	29	38.3907	47.89713	8.89427	20.1716	56.6098	.00	100.00
	GradeB	26	35.8977	47.06805	9.23080	16.8865	54.9089	.00	100.00
	GradeC	30	10.8333	27.95313	5.10352	.3955	21.2712	.00	100.00
	Total	85	27.9020	43.07711	4.67237	18.6105	37.1935	.00	100.00
cell4	GradeA	29	18.5059	36.02903	6.69042	4.8012	32.2106	.00	100.00
	GradeB	26	30.1285	43.72137	8.57447	12.4690	47.7879	.00	100.00
	GradeC	30	10.2780	27.65468	5.04903	-.0484	20.6044	.00	100.00
	Total	85	19.1571	36.48569	3.95743	11.2873	27.0268	.00	100.00
cell8	GradeA	29	6.1493	20.09449	3.73145	-1.4942	13.7928	.00	100.00
	GradeB	26	6.4104	23.13134	4.53643	-2.9326	15.7533	.00	100.00
	GradeC	30	4.4443	19.04471	3.47707	-2.6671	11.5557	.00	100.00
	Total	85	5.6274	20.49004	2.22246	1.2078	10.0470	.00	100.00
morula	GradeA	29	.4597	2.47532	.45966	-.4819	1.4012	.00	13.33
	GradeB	26	.0000	.00000	.00000	.0000	.0000	.00	.00
	GradeC	30	.0000	.00000	.00000	.0000	.0000	.00	.00
	Total	85	.1568	1.44584	.15682	-.1550	.4687	.00	13.33

cell2

Duncan[a,,b]

VAR00001	N	Subset for alpha = 0.05	
		1	2
GradeC	30	10.8333	
GradeB	26		35.8977
GradeA	29		38.3907
Sig.		1.000	.823

cell4

Duncan[a,,b]

VAR00001	N	Subset for alpha = 0.05
		1
GradeC	30	10.2780
GradeA	29	18.5059
GradeB	26	30.1285
Sig.		.052

cell8

Duncan[a,,b]

VAR00001	N	Subset for alpha = 0.05
		1
GradeC	30	4.4443
GradeA	29	6.1493
GradeB	26	6.4104
Sig.		.740

morula

Duncan[a,,b]

VAR00001	N	Subset for alpha = 0.05
		1
GradeB	26	.0000
GradeC	30	.0000
GradeA	29	.4597
Sig.		.265

Appendix Table 2.22: Cleavage rate of caprine *in vitro* fertilisation in different grades of oocytes

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
GradeA	2cell	29	38.3907	47.89713	8.89427	20.1716	56.6098	.00	100.00
	4cell	29	18.5059	36.02903	6.69042	4.8012	32.2106	.00	100.00
	8cell	29	6.1493	20.09449	3.73145	-1.4942	13.7928	.00	100.00
	morula	29	.4597	2.47532	.45966	-.4819	1.4012	.00	13.33
	Total	116	15.8764	34.46446	3.19994	9.5379	22.2149	.00	100.00
GradeB	2cell	26	35.8977	47.06805	9.23080	16.8865	54.9089	.00	100.00
	4cell	26	30.1285	43.72137	8.57447	12.4690	47.7879	.00	100.00
	8cell	26	6.4104	23.13134	4.53643	-2.9326	15.7533	.00	100.00
	morula	26	.0000	.00000	.00000	.0000	.0000	.00	.00
	Total	104	18.1091	36.94901	3.62315	10.9235	25.2948	.00	100.00
GradeC	2cell	30	10.8333	27.95313	5.10352	.3955	21.2712	.00	100.00
	4cell	30	10.2780	27.65468	5.04903	-.0484	20.6044	.00	100.00
	8cell	30	4.4443	19.04471	3.47707	-2.6671	11.5557	.00	100.00
	morula	30	.0000	.00000	.00000	.0000	.0000	.00	.00

	Total	120	6.3889	22.02772	2.01085	2.4072	10.3706	.00	100.00

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
GradeA	Between Groups	24536.889	3	8178.963	8.175	.000
	Within Groups	112059.974	112	1000.535		
	Total	136596.863	115			
GradeB	Between Groups	24068.161	3	8022.720	6.883	.000
	Within Groups	116550.444	100	1165.504		
	Total	140618.606	103			
GradeC	Between Groups	2384.324	3	794.775	1.665	.178
	Within Groups	55356.930	116	477.215		
	Total	57741.254	119			

GradeA

Duncan[a]

VAR00001	N	Subset for alpha = 0.05		
		1	2	3
morula	29	.4597		
8cell	29	6.1493	6.1493	
4cell	29		18.5059	
2cell	29			38.3907
Sig.		.495	.140	1.000

GradeB

Duncan[a]

VAR00001	N	Subset for alpha = 0.05	
		1	2
morula	26	.0000	
8cell	26	6.4104	
4cell	26		30.1285
2cell	26		35.8977
Sig.		.500	.544

GradeC

Duncan[a]

VAR00001	N	Subset for alpha = 0.05
		1
morula	30	.0000
8cell	30	4.4443
4cell	30	10.2780
2cell	30	10.8333
Sig.		.081

Appendix Table 2.23: Maturation rate of caprine *in vitro* fertilisation in different insemination durations

Descriptives

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
8-14hr	20	66.0335	25.67073	5.74015	54.0192	78.0478	27.27	100.00
18-24hr	16	55.2944	33.00893	8.25223	37.7052	72.8836	.00	100.00
Total	36	61.2606	29.22315	4.87052	51.3729	71.1482	.00	100.00

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1025.145	1	1025.145	1.208	.280
Within Groups	28864.583	34	848.958		
Total	29889.728	35			

Appendix Table 2.24: Fertilisation rate of caprine *in vitro* fertilisation in different insemination durations

Descriptives

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
8-14hr	20	39.8230	36.58066	8.17968	22.7027	56.9433	.00	100.00
18-24hr	16	33.5938	43.89790	10.97448	10.2022	56.9853	.00	100.00
Total	36	37.0544	39.52397	6.58733	23.6815	50.4274	.00	100.00

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	344.920	1	344.920	.216	.645
Within Groups	54330.140	34	1597.945		
Total	54675.060	35			

Appendix Table 2.25: Cleavage rate of caprine *in vitro* fertilisation in different insemination durations

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
hr8to14	2cell	20	49.3610	46.57228	10.41388	27.5645	71.1575	.00	100.00
	4cell	20	33.7735	39.19444	8.76414	15.4299	52.1171	.00	100.00
	8cell	20	7.3255	22.62355	5.05878	-3.2626	17.9136	.00	100.00
	morula	20	.5555	2.48427	.55550	-.6072	1.7182	.00	11.11
	Total	80	22.7539	37.55904	4.19923	14.3955	31.1122	.00	100.00
hr18to24	2cell	16	27.9169	43.49349	10.87337	4.7408	51.0929	.00	100.00
	4cell	16	22.2919	37.05316	9.26329	2.5476	42.0361	.00	100.00
	8cell	16	2.0831	8.33250	2.08313	-2.3570	6.5232	.00	33.33
	morula	16	.0000	.00000	.00000	.0000	.0000	.00	.00
	Total	64	13.0730	30.74806	3.84351	5.3923	20.7536	.00	100.00

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
hr8to14	Between Groups	31203.477	3	10401.159	9.851	.000
	Within Groups	80240.378	76	1055.794		
	Total	111443.855	79			
hr18to24	Between Groups	9552.143	3	3184.048	3.820	.014
	Within Groups	50010.763	60	833.513		
	Total	59562.906	63			

hr8to14

Duncan[a]

VAR00001	N	Subset for alpha = 0.05	
		1	2
morula	20	.5555	
8cell	20	7.3255	
4cell	20		33.7735
2cell	20		49.3610
Sig.		.512	.133

hr18to24

Duncan[a]

VAR00001	N	Subset for alpha = 0.05		
		1	2	3
morula	16	.0000		
8cell	16	2.0831	2.0831	
4cell	16		22.2919	22.2919
2cell	16			27.9169
Sig.		.839	.052	.584

Appendix Table 2.26: Cleavage rate of caprine *in vitro* fertilisation with different insemination durations in different embryo developmental stages

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
cell2	8-14 hr	20	49.3610	46.57228	10.41388	27.5645	71.1575	.00	100.00
	18-24 hr	16	27.9169	43.49349	10.87337	4.7408	51.0929	.00	100.00
	Total	36	39.8303	45.87977	7.64663	24.3068	55.3538	.00	100.00
cell4	8-14 hr	20	33.7735	39.19444	8.76414	15.4299	52.1171	.00	100.00
	18-24 hr	16	22.2919	37.05316	9.26329	2.5476	42.0361	.00	100.00
	Total	36	28.6706	38.15522	6.35920	15.7607	41.5804	.00	100.00
cell8	8-14 hr	20	7.3255	22.62355	5.05878	-3.2626	17.9136	.00	100.00
	18-24 hr	16	2.0831	8.33250	2.08313	-2.3570	6.5232	.00	33.33
	Total	36	4.9956	17.73650	2.95608	-1.0056	10.9967	.00	100.00
morula	8-14 hr	20	.5555	2.48427	.55550	-.6072	1.7182	.00	11.11
	18-24 hr	16	.0000	.00000	.00000	.0000	.0000	.00	.00
	Total	36	.3086	1.85167	.30861	-.3179	.9351	.00	11.11

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
cell2	Between Groups	4087.560	1	4087.560	1.997	.167
	Within Groups	69585.820	34	2046.642		
	Total	73673.380	35			
cell4	Between Groups	1171.802	1	1171.802	.800	.377
	Within Groups	49781.928	34	1464.174		
	Total	50953.730	35			
cell8	Between Groups	244.289	1	244.289	.771	.386
	Within Groups	10766.132	34	316.651		
	Total	11010.421	35			
morula	Between Groups	2.743	1	2.743	.795	.379
	Within Groups	117.260	34	3.449		
	Total	120.003	35			

Appendix Table 2.27: Cleavage rate of caprine oocytes *in vitro* fertilisation with different gradings and different insemination durations in different embryo developmental stages

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
cell2	A_8-14hr	17	47.8429	48.77576	11.82986	22.7648	72.9211	.00	100.00
	A_18-24hr	12	25.0000	45.22670	13.05582	-3.7357	53.7357	.00	100.00
	B_8-14hr	14	45.2386	48.22893	12.88972	17.3920	73.0851	.00	100.00
	B_18-24hr	12	25.0000	45.22670	13.05582	-3.7357	53.7357	.00	100.00
	C_8-14hr	17	15.1959	33.36384	8.09192	-1.9582	32.3500	.00	100.00
	C_18-24hr	13	5.1285	18.49093	5.12846	-6.0455	16.3024	.00	66.67
	Total	85	27.9020	43.07711	4.67237	18.6105	37.1935	.00	100.00
cell4	A_8-14hr	17	25.6865	39.57712	9.59886	5.3378	46.0351	.00	100.00
	A_18-24hr	12	8.3333	28.86751	8.33333	-10.0082	26.6749	.00	100.00
	B_8-14hr	14	34.5243	43.59147	11.65031	9.3553	59.6933	.00	100.00
	B_18-24hr	12	25.0000	45.22670	13.05582	-3.7357	53.7357	.00	100.00
	C_8-14hr	17	14.2159	33.04101	8.01362	-2.7722	31.2040	.00	100.00
	C_18-24hr	13	5.1285	18.49093	5.12846	-6.0455	16.3024	.00	66.67
	Total	85	19.1571	36.48569	3.95743	11.2873	27.0268	.00	100.00

cell8	A_8-14hr	17	10.4900	25.65639	6.22259	-2.7013	23.6813	.00	100.00
	A_18-24hr	12	.0000	.00000	.00000	.0000	.0000	.00	.00
	B_8-14hr	14	11.9050	30.95988	8.27438	-5.9707	29.7807	.00	100.00
	B_18-24hr	12	.0000	.00000	.00000	.0000	.0000	.00	.00
	C_8-14hr	17	5.8824	24.25356	5.88235	-6.5877	18.3524	.00	100.00
	C_18-24hr	13	2.5638	9.24408	2.56385	-3.0223	8.1500	.00	33.33
	Total	85	5.6274	20.49004	2.22246	1.2078	10.0470	.00	100.00
morula	A_8-14hr	17	.7841	3.23300	.78412	-.8781	2.4464	.00	13.33
	A_18-24hr	12	.0000	.00000	.00000	.0000	.0000	.00	.00
	B_8-14hr	14	.0000	.00000	.00000	.0000	.0000	.00	.00
	B_18-24hr	12	.0000	.00000	.00000	.0000	.0000	.00	.00
	C_8-14hr	17	.0000	.00000	.00000	.0000	.0000	.00	.00
	C_18-24hr	13	.0000	.00000	.00000	.0000	.0000	.00	.00
	Total	85	.1568	1.44584	.15682	-.1550	.4687	.00	13.33

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
cell2	Between Groups	20656.627	5	4131.325	2.414	.043
	Within Groups	135216.882	79	1711.606		
	Total	155873.508	84			
cell4	Between Groups	8819.883	5	1763.977	1.353	.251
	Within Groups	103001.365	79	1303.815		
	Total	111821.248	84			
cell8	Between Groups	1836.816	5	367.363	.868	.506
	Within Groups	33429.890	79	423.163		
	Total	35266.707	84			
morula	Between Groups	8.362	5	1.672	.790	.560
	Within Groups	167.237	79	2.117		
	Total	175.598	84			

cell2

Duncan[a,,b]

VAR00001	N	Subset for alpha = 0.05	
		1	2
C_18-24hr	13	5.1285	
C_8-14hr	17	15.1959	15.1959
A_18-24hr	12	25.0000	25.0000
B_18-24hr	12	25.0000	25.0000
B_8-14hr	14		45.2386
A_8-14hr	17		47.8429
Sig.		.256	.066

cell4

Duncan[a,,b]

VAR00001	N	Subset for alpha = 0.05
		1
C_18-24hr	13	5.1285
A_18-24hr	12	8.3333
C_8-14hr	17	14.2159
B_18-24hr	12	25.0000
A_8-14hr	17	25.6865
B_8-14hr	14	34.5243
Sig.		.063

cell8

Duncan[a,,b]

VAR00001	N	Subset for alpha = 0.05
		1
A_18-24hr	12	.0000
B_18-24hr	12	.0000
C_18-24hr	13	2.5638
C_8-14hr	17	5.8824
A_8-14hr	17	10.4900
B_8-14hr	14	11.9050
Sig.		.189

morula

Duncan[a,,b]

VAR00001	N	Subset for alpha = 0.05
		1
A_18-24hr	12	.0000
B_8-14hr	14	.0000
B_18-24hr	12	.0000
C_8-14hr	17	.0000
C_18-24hr	13	.0000
A_8-14hr	17	.7841
Sig.		.222

Appendix Table 2.28: Cleavage rate of caprine oocytes *in vitro* fertilisation with different insemination durations and oocyte gradings

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
A_8to14hr	2cell	17	47.8429	48.77576	11.82986	22.7648	72.9211	.00	100.00
	4cell	17	25.6865	39.57712	9.59886	5.3378	46.0351	.00	100.00
	8cell	17	10.4900	25.65639	6.22259	-2.7013	23.6813	.00	100.00
	morula	17	.7841	3.23300	.78412	-.8781	2.4464	.00	13.33
	Total	68	21.2009	37.70888	4.57287	12.0734	30.3284	.00	100.00
A_18to24hr	2cell	12	25.0000	45.22670	13.05582	-3.7357	53.7357	.00	100.00
	4cell	12	8.3333	28.86751	8.33333	-10.0082	26.6749	.00	100.00
	8cell	12	.0000	.00000	.00000	.0000	.0000	.00	.00
	morula	12	.0000	.00000	.00000	.0000	.0000	.00	.00
	Total	48	8.3333	27.93102	4.03150	.2230	16.4437	.00	100.00
B_8to14hr	2cell	14	45.2386	48.22893	12.88972	17.3920	73.0851	.00	100.00
	4cell	14	34.5243	43.59147	11.65031	9.3553	59.6933	.00	100.00
	8cell	14	11.9050	30.95988	8.27438	-5.9707	29.7807	.00	100.00
	morula	14	.0000	.00000	.00000	.0000	.0000	.00	.00
	Total	56	22.9170	39.38481	5.26302	12.3696	33.4643	.00	100.00

B_18to24hr	2cell	12	25.0000	45.22670	13.05582	-3.7357	53.7357	.00	100.00
	4cell	12	25.0000	45.22670	13.05582	-3.7357	53.7357	.00	100.00
	8cell	12	.0000	.00000	.00000	.0000	.0000	.00	.00
	morula	12	.0000	.00000	.00000	.0000	.0000	.00	.00
	Total	48	12.5000	33.42187	4.82403	2.7953	22.2047	.00	100.00
C_8to14hr	2cell	17	15.1959	33.36384	8.09192	-1.9582	32.3500	.00	100.00
	4cell	17	14.2159	33.04101	8.01362	-2.7722	31.2040	.00	100.00
	8cell	17	5.8824	24.25356	5.88235	-6.5877	18.3524	.00	100.00
	morula	17	.0000	.00000	.00000	.0000	.0000	.00	.00
	Total	68	8.8235	26.58264	3.22362	2.3892	15.2579	.00	100.00
C_18to24hr	2cell	13	5.1285	18.49093	5.12846	-6.0455	16.3024	.00	66.67
	4cell	13	5.1285	18.49093	5.12846	-6.0455	16.3024	.00	66.67
	8cell	13	2.5638	9.24408	2.56385	-3.0223	8.1500	.00	33.33
	morula	13	.0000	.00000	.00000	.0000	.0000	.00	.00
	Total	52	3.2052	13.62413	1.88933	-.5878	6.9982	.00	66.67

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
A_8to14hr	Between Groups	21445.280	3	7148.427	6.197	.001
	Within Groups	73826.011	64	1153.531		
	Total	95271.292	67			
A_18to24hr	Between Groups	5000.000	3	1666.667	2.316	.089
	Within Groups	31666.667	44	719.697		
	Total	36666.667	47			
B_8to14hr	Between Groups	17912.085	3	5970.695	4.606	.006
	Within Groups	67401.873	52	1296.190		
	Total	85313.958	55			
B_18to24hr	Between Groups	7500.000	3	2500.000	2.444	.077
	Within Groups	45000.000	44	1022.727		
	Total	52500.000	47			
C_8to14hr	Between Groups	2655.222	3	885.074	1.268	.293
	Within Groups	44689.438	64	698.272		
	Total	47344.660	67			
C_18to24hr	Between Groups	235.073	3	78.358	.407	.748
	Within Groups	9231.385	48	192.321		
	Total	9466.457	51			

A_8to14hr

Duncan[a]

VAR00001	N	Subset for alpha = 0.05		
		1	2	3
morula	17	.7841		
8cell	17	10.4900	10.4900	
4cell	17		25.6865	25.6865
2cell	17			47.8429
Sig.		.408	.197	.062

A_18to24hr

Duncan[a]

VAR00001	N	Subset for alpha = 0.05	
		1	2
8cell	12	.0000	
morula	12	.0000	
4cell	12	8.3333	8.3333
2cell	12		25.0000
Sig.		.479	.135

B_8to14hr

Duncan[a]

VAR00001	N	Subset for alpha = 0.05		
		1	2	3
morula	14	.0000		
8cell	14	11.9050	11.9050	
4cell	14		34.5243	34.5243
2cell	14			45.2386
Sig.		.386	.102	.435

B_18to24hr

Duncan[a]

VAR00001	N	Subset for alpha = 0.05
		1
8cell	12	.0000
morula	12	.0000
2cell	12	25.0000
4cell	12	25.0000
Sig.		.086

C_8to14hr

Duncan[a]

VAR00001	N	Subset for alpha = 0.05
		1
morula	17	.0000
8cell	17	5.8824
4cell	17	14.2159
2cell	17	15.1959
Sig.		.131

C_18to24hr

Duncan[a]

VAR00001	N	Subset for alpha = 0.05
		1
morula	13	.0000
8cell	13	2.5638
2cell	13	5.1285
4cell	13	5.1285
Sig.		.398

Appendix Table 2.29: Number of oocytes per ovary in bovine parthenogenetic activation

Descriptives

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
GradeA	4	6.4175	3.74485	1.87242	.4586	12.3764	4.00	12.00
GradeB	4	4.9575	1.97414	.98707	1.8162	8.0988	2.50	7.00
GradeC	4	1.6250	1.97379	.98689	-1.5157	4.7657	.00	4.00
Total	12	4.3333	3.21537	.92820	2.2904	6.3763	.00	12.00

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	48.274	2	24.137	3.319	.083
Within Groups	65.451	9	7.272		
Total	113.724	11			

Duncan[a]

VAR00001	N	Subset for alpha = 0.05	
		1	2
GradeC	4	1.6250	
GradeB	4	4.9575	4.9575
GradeA	4		6.4175
Sig.		.114	.463

Appendix Table 2.30: Percent distribution of bovine oocyte in parthenogenetic activation

Descriptives

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
GradeA	4	35.4325	27.58975	13.79487	-8.4689	79.3339	.00	63.16
GradeB	4	38.2125	9.94664	4.97332	22.3852	54.0398	25.00	48.15
GradeC	4	13.3925	15.53285	7.76643	-11.3237	38.1087	.00	28.57
Total	12	29.0125	20.85352	6.01989	15.7628	42.2622	.00	63.16

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1479.363	2	739.682	2.015	.189
Within Groups	3304.198	9	367.133		
Total	4783.561	11			

Duncan[a]

		Subset for alpha = 0.05
VAR00001	N	1
GradeC	4	13.3925
GradeA	4	35.4325
GradeB	4	38.2125
Sig.		.113

Appendix Table 2.31: Maturation rate of bovine oocyte in parthenogenetic activation

Descriptives

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Grade A	4	68.7500	37.50000	18.75000	9.0791	128.4209	25.00	100.00
Grade B	4	77.9750	33.86114	16.93057	24.0944	131.8556	28.57	100.00
Grade C	2	70.0000	42.42641	30.00000	-311.1861	451.1861	40.00	100.00
Mixed	12	59.1283	17.18852	4.96190	48.2073	70.0494	29.17	88.46
Total	22	65.2927	25.72738	5.48510	53.8858	76.6996	25.00	100.00

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1191.485	3	397.162	.563	.647
Within Groups	12708.380	18	706.021		
Total	13899.865	21			

Duncan[a,,b]

VAR00001	N	Subset for alpha = 0.05
		1
Mixed	12	59.1283
Grade A	4	68.7500
Grade C	2	70.0000
Grade B	4	77.9750
Sig.		.388

Appendix Table 2.32: Cleavage rate of bovine oocyte in parthenogenetic activation in different embryo developmental stages

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
cell2	Grade A	4	76.6675	29.05894	14.52947	30.4282	122.9068	40.00	100.00
	Grade B	4	50.0000	57.73503	28.86751	-41.8693	141.8693	.00	100.00
	Grade C	2	50.0000	70.71068	50.00000	-585.3102	685.3102	.00	100.00
	Mixed	12	63.8050	25.90819	7.47905	47.3437	80.2663	22.73	100.00
	Total	22	62.3786	35.64196	7.59889	46.5759	78.1814	.00	100.00
cell4	Grade A	4	53.7500	43.46934	21.73467	-15.4194	122.9194	.00	100.00
	Grade B	4	50.0000	57.73503	28.86751	-41.8693	141.8693	.00	100.00
	Grade C	2	37.5000	53.03301	37.50000	-438.9827	513.9827	.00	75.00
	Mixed	12	53.8508	23.31619	6.73080	39.0364	68.6652	13.64	83.33
	Total	22	51.6459	34.46768	7.34853	36.3638	66.9280	.00	100.00
cell8	Grade A	4	40.3575	29.92955	14.96477	-7.2671	87.9821	.00	71.43
	Grade B	4	21.5375	24.99578	12.49789	-18.2364	61.3114	.00	46.15
	Grade C	2	.0000	.00000	.00000	.0000	.0000	.00	.00
	Mixed	12	37.1900	24.60184	7.10194	21.5587	52.8213	.00	75.00
	Total	22	31.5391	26.09804	5.56412	19.9679	43.1103	.00	75.00

morula	Grade A	4	16.0725	23.60016	11.80008	-21.4806	53.6256	.00	50.00
	Grade B	4	3.8450	7.69000	3.84500	-8.3915	16.0815	.00	15.38
	Grade C	2	.0000	.00000	.00000	.0000	.0000	.00	.00
	Mixed	12	7.1500	9.70661	2.80206	.9827	13.3173	.00	33.33
	Total	22	7.5214	12.62284	2.69120	1.9247	13.1180	.00	50.00
blastocyst	Grade A	4	.0000	.00000	.00000	.0000	.0000	.00	.00
	Grade B	4	.0000	.00000	.00000	.0000	.0000	.00	.00
	Grade C	2	.0000	.00000	.00000	.0000	.0000	.00	.00
	Mixed	12	.2775	.96129	.27750	-.3333	.8883	.00	3.33
	Total	22	.1514	.70996	.15136	-.1634	.4661	.00	3.33

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
cell2	Between Groups	1760.484	3	586.828	.424	.738
	Within Groups	24916.846	18	1384.269		
	Total	26677.331	21			
cell4	Between Groups	487.099	3	162.366	.119	.947
	Within Groups	24461.342	18	1358.963		
	Total	24948.441	21			
cell8	Between Groups	3083.806	3	1027.935	1.649	.213
	Within Groups	11219.455	18	623.303		
	Total	14303.262	21			
morula	Between Groups	461.347	3	153.782	.960	.433
	Within Groups	2884.712	18	160.262		
	Total	3346.059	21			
blastocyst	Between Groups	.420	3	.140	.248	.862
	Within Groups	10.165	18	.565		
	Total	10.585	21			

cell2

Duncan[a,,b]

VAR00001	N	Subset for alpha = 0.05
		1
Grade B	4	50.0000
Grade C	2	50.0000
Mixed	12	63.8050
Grade A	4	76.6675
Sig.		.384

cell4

Duncan[a,,b]

VAR00001	N	Subset for alpha = 0.05
		1
Grade C	2	37.5000
Grade B	4	50.0000
Grade A	4	53.7500
Mixed	12	53.8508
Sig.		.588

cell8

Duncan[a,,b]

VAR00001	N	Subset for alpha = 0.05
		1
Grade C	2	.0000
Grade B	4	21.5375
Mixed	12	37.1900
Grade A	4	40.3575
Sig.		.057

morula

Duncan[a,,b]

VAR00001	N	Subset for alpha = 0.05
		1
Grade C	2	.0000
Grade B	4	3.8450
Mixed	12	7.1500
Grade A	4	16.0725
Sig.		.129

blastocyst

Duncan[a,,b]

VAR00001	N	Subset for alpha = 0.05
		1
Grade A	4	.0000
Grade B	4	.0000
Grade C	2	.0000
Mixed	12	.2775
Sig.		.651

Appendix Table 2.33: Cleavage rate of bovine oocyte in parthenogenetic activation with different oocyte gradings

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
GradeA	2cell	4	76.6675	29.05894	14.52947	30.4282	122.9068	40.00	100.00
	4cell	4	53.7500	43.46934	21.73467	-15.4194	122.9194	.00	100.00
	8cell	4	40.3575	29.92955	14.96477	-7.2671	87.9821	.00	71.43
	morula	4	16.0725	23.60016	11.80008	-21.4806	53.6256	.00	50.00
	blastocyst	4	.0000	.00000	.00000	.0000	.0000	.00	.00
	Total	20	37.3695	37.86871	8.46770	19.6464	55.0926	.00	100.00
GradeB	2cell	4	50.0000	57.73503	28.86751	-41.8693	141.8693	.00	100.00
	4cell	4	50.0000	57.73503	28.86751	-41.8693	141.8693	.00	100.00
	8cell	4	21.5375	24.99578	12.49789	-18.2364	61.3114	.00	46.15
	morula	4	3.8450	7.69000	3.84500	-8.3915	16.0815	.00	15.38
	blastocyst	4	.0000	.00000	.00000	.0000	.0000	.00	.00

	Total	20	25.0765	40.64594	9.08871	6.0536	44.0994	.00	100.00
GradeC	2cell	2	50.0000	70.71068	50.00000	-585.3102	685.3102	.00	100.00
	4cell	2	37.5000	53.03301	37.50000	-438.9827	513.9827	.00	75.00
	8cell	2	.0000	.00000	.00000	.0000	.0000	.00	.00
	morula	2	.0000	.00000	.00000	.0000	.0000	.00	.00
	blastocyst	2	.0000	.00000	.00000	.0000	.0000	.00	.00
	Total	10	17.5000	37.36085	11.81454	-9.2263	44.2263	.00	100.00
Mixed	2cell	12	63.8050	25.90819	7.47905	47.3437	80.2663	22.73	100.00
	4cell	12	53.8508	23.31619	6.73080	39.0364	68.6652	13.64	83.33
	8cell	12	37.1900	24.60184	7.10194	21.5587	52.8213	.00	75.00
	morula	12	7.1500	9.70661	2.80206	.9827	13.3173	.00	33.33
	blastocyst	12	.2775	.96129	.27750	-.3333	.8883	.00	3.33
	Total	60	32.4547	31.55117	4.07324	24.3041	40.6052	.00	100.00

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
GradeA	Between Groups	14686.494	4	3671.623	4.385	.015
	Within Groups	12560.253	15	837.350		
	Total	27246.747	19			
GradeB	Between Groups	9337.975	4	2334.494	1.588	.229
	Within Groups	22051.775	15	1470.118		
	Total	31389.750	19			
GradeC	Between Groups	4750.000	4	1187.500	.760	.593
	Within Groups	7812.500	5	1562.500		
	Total	12562.500	9			
Mixed	Between Groups	37665.107	4	9416.277	24.582	.000
	Within Groups	21067.992	55	383.054		
	Total	58733.099	59			

GradeA

Duncan[a]

VAR00001	N	Subset for alpha = 0.05		
		1	2	3
blastocyst	4	.0000		
morula	4	16.0725	16.0725	
8cell	4	40.3575	40.3575	40.3575
4cell	4		53.7500	53.7500
2cell	4			76.6675
Sig.		.080	.100	.112

GradeB

Duncan[a]

VAR00001	N	Subset for alpha = 0.05
		1
blastocyst	4	.0000
morula	4	3.8450
8cell	4	21.5375
2cell	4	50.0000
4cell	4	50.0000
Sig.		.115

GradeC

Duncan[a]

VAR00001	N	Subset for alpha = 0.05
		1
8cell	2	.0000
morula	2	.0000
blastocyst	2	.0000
4cell	2	37.5000
2cell	2	50.0000
Sig.		.273

Mixed

Duncan[a]

VAR00001	N	Subset for alpha = 0.05		
		1	2	3
blastocyst	12	.2775		
morula	12	7.1500		
8cell	12		37.1900	
4cell	12			53.8508
2cell	12			63.8050
Sig.		.393	1.000	.218

Appendix Table 2.34: Number of oocytes per ovary in caprine parthenogenetic activation

Descriptives

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
GradeA	14	1.2679	1.21165	.32383	.5683	1.9674	.00	3.00
GradeB	14	1.4779	1.12862	.30164	.8262	2.1295	.00	3.00
GradeC	14	1.6564	1.76991	.47303	.6345	2.6783	.00	6.00
Total	42	1.4674	1.37422	.21205	1.0391	1.8956	.00	6.00

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.059	2	.530	.270	.764
Within Groups	76.368	39	1.958		
Total	77.427	41			

Duncan[a]

VAR00001	N	Subset for alpha = 0.05
		1
GradeA	14	1.2679
GradeB	14	1.4779
GradeC	14	1.6564
Sig.		.495

Appendix Table 2.35: Percent distribution of caprine oocyte in parthenogenetic activation

Descriptives

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
GradeA	11	32.3973	29.37615	8.85724	12.6621	52.1324	.00	100.00
GradeB	11	41.5864	30.23546	9.11633	21.2739	61.8988	14.29	100.00
GradeC	11	53.2900	31.21628	9.41206	32.3186	74.2614	.00	100.00
Total	33	42.4245	30.58203	5.32365	31.5806	53.2685	.00	100.00

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2412.375	2	1206.188	1.315	.283
Within Groups	27515.970	30	917.199		
Total	29928.346	32			

Duncan[a]

		Subset for alpha = 0.05
VAR00001	N	1
GradeA	11	32.3973
GradeB	11	41.5864
GradeC	11	53.2900
Sig.		.136

Appendix Table 2.36: Maturation rate of caprine oocyte in parthenogenetic activation

Descriptives

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Grade A	9	70.5722	25.47604	8.49201	50.9896	90.1548	33.33	100.00
Grade B	11	67.1327	35.29889	10.64302	43.4186	90.8468	.00	100.00
Grade C	10	37.2620	29.91753	9.46075	15.8603	58.6637	.00	100.00
Mixed	2	43.8100	19.75656	13.97000	-133.6957	221.3157	29.84	57.78
Total	32	57.3078	32.68927	5.77870	45.5221	69.0936	.00	100.00

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7028.047	3	2342.682	2.513	.079
Within Groups	26098.194	28	932.078		
Total	33126.242	31			

Duncan[a,,b]

		Subset for alpha = 0.05
VAR00001	N	1
Grade C	10	37.2620
Mixed	2	43.8100
Grade B	11	67.1327
Grade A	9	70.5722
Sig.		.125

Appendix Table 2.37: Cleavage rate of caprine oocyte in parthenogenetic activation in different embryo developmental stages

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
cell2	Grade A	9	15.5556	23.51123	7.83708	-2.5168	33.6279	.00	50.00
	Grade B	11	30.9091	45.92484	13.84686	.0564	61.7618	.00	100.00
	Grade C	10	8.3330	18.00154	5.69259	-4.5445	21.2105	.00	50.00
	Mixed	2	45.5800	60.64148	42.88000	-499.2621	590.4221	2.70	88.46
	Total	32	20.4528	34.19922	6.04563	8.1227	32.7829	.00	100.00
cell4	Grade A	9	7.7778	17.15938	5.71979	-5.4121	20.9676	.00	50.00
	Grade B	11	21.8182	40.45199	12.19673	-5.3578	48.9942	.00	100.00
	Grade C	10	8.3330	18.00154	5.69259	-4.5445	21.2105	.00	50.00
	Mixed	2	37.8900	49.76618	35.19000	-409.2413	485.0213	2.70	73.08
	Total	32	14.6597	29.28275	5.17651	4.1021	25.2172	.00	100.00
cell8	Grade A	9	2.2222	6.66667	2.22222	-2.9022	7.3467	.00	20.00
	Grade B	11	2.2727	7.53778	2.27273	-2.7912	7.3367	.00	25.00
	Grade C	10	.0000	.00000	.00000	.0000	.0000	.00	.00
	Mixed	2	11.5400	16.32002	11.54000	-135.0896	158.1696	.00	23.08
	Total	32	2.1275	6.75098	1.19342	-.3065	4.5615	.00	25.00

morula	Grade A	9	2.2222	6.66667	2.22222	-2.9022	7.3467	.00	20.00
	Grade B	11	.0000	.00000	.00000	.0000	.0000	.00	.00
	Grade C	10	.0000	.00000	.00000	.0000	.0000	.00	.00
	Mixed	2	1.9250	2.72236	1.92500	-22.5344	26.3844	.00	3.85
	Total	32	.7453	3.57882	.63265	-.5450	2.0356	.00	20.00
blastocyst	Grade A	9	.0000	.00000	.00000	.0000	.0000	.00	.00
	Grade B	11	.0000	.00000	.00000	.0000	.0000	.00	.00
	Grade C	10	.0000	.00000	.00000	.0000	.0000	.00	.00
	Mixed	2	1.9250	2.72236	1.92500	-22.5344	26.3844	.00	3.85
	Total	32	.1203	.68059	.12031	-.1251	.3657	.00	3.85

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
cell2	Between Groups	4150.169	3	1383.390	1.206	.326
	Within Groups	32107.020	28	1146.679		
	Total	36257.189	31			
cell4	Between Groups	2469.495	3	823.165	.956	.427
	Within Groups	24112.364	28	861.156		
	Total	26581.859	31			
cell8	Between Groups	222.766	3	74.255	1.747	.180
	Within Groups	1190.081	28	42.503		
	Total	1412.846	31			
morula	Between Groups	34.080	3	11.360	.876	.465
	Within Groups	362.967	28	12.963		
	Total	397.047	31			
blastocyst	Between Groups	6.948	3	2.316	8.750	.000
	Within Groups	7.411	28	.265		
	Total	14.359	31			

cell2

Duncan[a,,b]

VAR00001	N	Subset for alpha = 0.05
		1
Grade C	10	8.3330
Grade A	9	15.5556
Grade B	11	30.9091
Mixed	2	45.5800
Sig.		.122

cell4

Duncan[a,,b]

VAR00001	N	Subset for alpha = 0.05
		1
Grade A	9	7.7778
Grade C	10	8.3330
Grade B	11	21.8182
Mixed	2	37.8900
Sig.		.149

cell8

Duncan[a,,b]

VAR00001	N	Subset for alpha = 0.05	
		1	2
Grade C	10	.0000	
Grade A	9	2.2222	
Grade B	11	2.2727	
Mixed	2		11.5400
Sig.		.609	1.000

morula

Duncan[a,,b]

VAR00001	N	Subset for alpha = 0.05
		1
Grade B	11	.0000
Grade C	10	.0000
Mixed	2	1.9250
Grade A	9	2.2222
Sig.		.383

blastocyst

Duncan[a,,b]

VAR00001	N	Subset for alpha = 0.05	
		1	2
Grade A	9	.0000	
Grade B	11	.0000	
Grade C	10	.0000	
Mixed	2		1.9250
Sig.		1.000	1.000

Appendix Table 2.38: Cleavage rate of caprine oocyte in parthenogenetic activation with different oocyte gradings

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
GradeA	2cell	9	15.5556	23.51123	7.83708	-2.5168	33.6279	.00	50.00
	4cell	9	7.7778	17.15938	5.71979	-5.4121	20.9676	.00	50.00
	8cell	9	2.2222	6.66667	2.22222	-2.9022	7.3467	.00	20.00
	morula	9	2.2222	6.66667	2.22222	-2.9022	7.3467	.00	20.00
	blastocyst	9	.0000	.00000	.00000	.0000	.0000	.00	.00
	Total	45	5.5556	14.23114	2.12145	1.2800	9.8311	.00	50.00
GradeB	2cell	11	30.9091	45.92484	13.84686	.0564	61.7618	.00	100.00
	4cell	11	21.8182	40.45199	12.19673	-5.3578	48.9942	.00	100.00
	8cell	11	2.2727	7.53778	2.27273	-2.7912	7.3367	.00	25.00
	morula	11	.0000	.00000	.00000	.0000	.0000	.00	.00
	blastocyst	11	.0000	.00000	.00000	.0000	.0000	.00	.00
	Total	55	11.0000	29.55535	3.98524	3.0101	18.9899	.00	100.00
GradeC	2cell	10	8.3330	18.00154	5.69259	-4.5445	21.2105	.00	50.00
	4cell	10	8.3330	18.00154	5.69259	-4.5445	21.2105	.00	50.00

	8cell	10	.0000	.00000	.00000	.0000	.0000	.00	.00
	morula	10	.0000	.00000	.00000	.0000	.0000	.00	.00
	blastocyst	10	.0000	.00000	.00000	.0000	.0000	.00	.00
	Total	50	3.3332	11.66389	1.64952	.0184	6.6480	.00	50.00
Mixed	2cell	2	45.5800	60.64148	42.88000	-499.2621	590.4221	2.70	88.46
	4cell	2	37.8900	49.76618	35.19000	-409.2413	485.0213	2.70	73.08
	8cell	2	11.5400	16.32002	11.54000	-135.0896	158.1696	.00	23.08
	morula	2	1.9250	2.72236	1.92500	-22.5344	26.3844	.00	3.85
	blastocyst	2	1.9250	2.72236	1.92500	-22.5344	26.3844	.00	3.85
	Total	10	19.7720	33.05458	10.45278	-3.8738	43.4178	.00	88.46

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
GradeA	Between Groups	1422.222	4	355.556	1.899	.129
	Within Groups	7488.889	40	187.222		
	Total	8911.111	44			
GradeB	Between Groups	9147.273	4	2286.818	3.007	.027
	Within Groups	38022.727	50	760.455		
	Total	47170.000	54			
GradeC	Between Groups	833.267	4	208.317	1.607	.189
	Within Groups	5833.000	45	129.622		
	Total	6666.267	49			
Mixed	Between Groups	3398.223	4	849.556	.660	.646
	Within Groups	6435.227	5	1287.045		
	Total	9833.450	9			

GradeA

Duncan[a]

VAR00001	N	Subset for alpha = 0.05	
		1	2
blastocyst	9	.0000	
8cell	9	2.2222	2.2222
morula	9	2.2222	2.2222
4cell	9	7.7778	7.7778
2cell	9		15.5556
Sig.		.280	.065

GradeB

Duncan[a]

VAR00001	N	Subset for alpha = 0.05	
		1	2
morula	11	.0000	
blastocyst	11	.0000	
8cell	11	2.2727	
4cell	11	21.8182	21.8182
2cell	11		30.9091
Sig.		.096	.443

GradeC

Duncan[a]

VAR00001	N	Subset for alpha = 0.05
		1
8cell	10	.0000
morula	10	.0000
blastocyst	10	.0000
2cell	10	8.3330
4cell	10	8.3330
Sig.		.152

Mixed

Duncan[a]

VAR00001	N	Subset for alpha = 0.05
		1
morula	2	1.9250
blastocyst	2	1.9250
8cell	2	11.5400
4cell	2	37.8900
2cell	2	45.5800
Sig.		.289

Appendix Table 2.39: Cleavage rate of oocyte in parthenogenetic activation in different species in different embryo developmental stages

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
cell2	bovine	16	63.8956	29.20273	7.30068	48.3346	79.4567	16.67	100.00
	caprine	16	26.7687	39.01486	9.75371	5.9792	47.5583	.00	100.00
	Total	32	45.3322	38.79292	6.85768	31.3458	59.3185	.00	100.00
cell4	bovine	16	52.9688	29.80655	7.45164	37.0860	68.8515	.00	100.00
	caprine	16	17.8319	30.99315	7.74829	1.3168	34.3470	.00	100.00
	Total	32	35.4003	34.83227	6.15753	22.8419	47.9587	.00	100.00
cell8	bovine	16	34.5613	24.33058	6.08265	21.5964	47.5261	.00	75.00
	caprine	16	3.8381	8.56223	2.14056	-.7244	8.4006	.00	25.00
	Total	32	19.1997	23.78032	4.20381	10.6260	27.7734	.00	75.00
morula	bovine	16	7.2494	9.18294	2.29574	2.3561	12.1426	.00	33.33
	caprine	16	1.0738	3.40651	.85163	-.7414	2.8889	.00	13.33
	Total	32	4.1616	7.50068	1.32595	1.4573	6.8658	.00	33.33
blastocyst	bovine	16	.2081	.83250	.20813	-.2355	.6517	.00	3.33
	caprine	16	.2406	.96250	.24063	-.2723	.7535	.00	3.85
	Total	32	.2244	.88537	.15651	-.0948	.5436	.00	3.85

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
cell2	Between Groups	11027.239	1	11027.239	9.286	.005
	Within Groups	35624.376	30	1187.479		
	Total	46651.615	31			
cell4	Between Groups	9876.800	1	9876.800	10.683	.003
	Within Groups	27735.090	30	924.503		
	Total	37611.889	31			
cell8	Between Groups	7551.283	1	7551.283	22.701	.000
	Within Groups	9979.333	30	332.644		
	Total	17530.617	31			
morula	Between Groups	305.107	1	305.107	6.361	.017
	Within Groups	1438.960	30	47.965		
	Total	1744.067	31			
blastocyst	Between Groups	.008	1	.008	.010	.919
	Within Groups	24.292	30	.810		
	Total	24.300	31			

Appendix Table 2.40: Cleavage rate of oocyte in parthenogenetic activation for different species

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
bovine	2cell	16	63.8956	29.20273	7.30068	48.3346	79.4567	16.67	100.00
	4cell	16	52.9688	29.80655	7.45164	37.0860	68.8515	.00	100.00
	8cell	16	34.5613	24.33058	6.08265	21.5964	47.5261	.00	75.00
	morula	16	7.2494	9.18294	2.29574	2.3561	12.1426	.00	33.33
	blastocyst	16	.2081	.83250	.20813	-.2355	.6517	.00	3.33
	Total	80	31.7766	32.92872	3.68154	24.4487	39.1046	.00	100.00
caprine	2cell	16	26.7687	39.01486	9.75371	5.9792	47.5583	.00	100.00
	4cell	16	17.8319	30.99315	7.74829	1.3168	34.3470	.00	100.00
	8cell	16	3.8381	8.56223	2.14056	-.7244	8.4006	.00	25.00
	morula	16	1.0738	3.40651	.85163	-.7414	2.8889	.00	13.33
	blastocyst	16	.2406	.96250	.24063	-.2723	.7535	.00	3.85
	Total	80	9.9506	24.49882	2.73905	4.4987	15.4026	.00	100.00

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
bovine	Between Groups	49386.346	4	12346.587	25.528	.000
	Within Groups	36273.397	75	483.645		
	Total	85659.743	79			
caprine	Between Groups	8886.546	4	2221.636	4.325	.003
	Within Groups	38528.655	75	513.715		
	Total	47415.200	79			

bovine

Duncan[a]

		Subset for alpha = 0.05		
VAR00001	N	1	2	3
blastocyst	16	.2081		
morula	16	7.2494		
8cell	16		34.5613	
4cell	16			52.9688
2cell	16			63.8956
Sig.		.368	1.000	.164

caprine

Duncan[a]

		Subset for alpha = 0.05		
VAR00001	N	1	2	3
blastocyst	16	.2406		
morula	16	1.0738	1.0738	
8cell	16	3.8381	3.8381	
4cell	16		17.8319	17.8319
2cell	16			26.7687
Sig.		.676	.051	.268

Appendix Table 2.41: Cleavage rate of Grade A oocyte in parthenogenetic activation for different species in different embryo developmental stages

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
cell2	bovine	4	76.6675	29.05894	14.52947	30.4282	122.9068	40.00	100.00
	caprine	9	15.5556	23.51123	7.83708	-2.5168	33.6279	.00	50.00
	Total	13	34.3592	37.96671	10.53007	11.4162	57.3023	.00	100.00
cell4	bovine	4	53.7500	43.46934	21.73467	-15.4194	122.9194	.00	100.00
	caprine	9	7.7778	17.15938	5.71979	-5.4121	20.9676	.00	50.00
	Total	13	21.9231	34.00603	9.43158	1.3734	42.4727	.00	100.00
cell8	bovine	4	40.3575	29.92955	14.96477	-7.2671	87.9821	.00	71.43
	caprine	9	2.2222	6.66667	2.22222	-2.9022	7.3467	.00	20.00
	Total	13	13.9562	24.27306	6.73214	-.7119	28.6242	.00	71.43
morula	bovine	4	16.0725	23.60016	11.80008	-21.4806	53.6256	.00	50.00
	caprine	9	2.2222	6.66667	2.22222	-2.9022	7.3467	.00	20.00
	Total	13	6.4838	14.59932	4.04912	-2.3384	15.3061	.00	50.00

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
cell2	Between Groups	10342.162	1	10342.162	16.356	.002
	Within Groups	6955.489	11	632.317		
	Total	17297.651	12			
cell4	Between Groups	5852.618	1	5852.618	8.023	.016
	Within Groups	8024.306	11	729.482		
	Total	13876.923	12			
cell8	Between Groups	4027.291	1	4027.291	14.559	.003
	Within Groups	3042.889	11	276.626		
	Total	7070.180	12			
morula	Between Groups	531.222	1	531.222	2.884	.118
	Within Groups	2026.459	11	184.224		
	Total	2557.681	12			

Appendix Table 2.42: Cleavage rate of Grade A oocyte in parthenogenetic activation for different embryo developmental stages in different species

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
bovine	2cell	4	76.6675	29.05894	14.52947	30.4282	122.9068	40.00	100.00
	4cell	4	53.7500	43.46934	21.73467	-15.4194	122.9194	.00	100.00
	8cell	4	40.3575	29.92955	14.96477	-7.2671	87.9821	.00	71.43
	morula	4	16.0725	23.60016	11.80008	-21.4806	53.6256	.00	50.00
	Total	16	46.7119	36.75536	9.18884	27.1263	66.2974	.00	100.00
caprine	2cell	9	15.5556	23.51123	7.83708	-2.5168	33.6279	.00	50.00
	4cell	9	7.7778	17.15938	5.71979	-5.4121	20.9676	.00	50.00
	8cell	9	2.2222	6.66667	2.22222	-2.9022	7.3467	.00	20.00
	morula	9	2.2222	6.66667	2.22222	-2.9022	7.3467	.00	20.00
	Total	36	6.9444	15.64233	2.60706	1.6518	12.2370	.00	50.00

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
bovine	Between Groups	7704.096	3	2568.032	2.453	.113
	Within Groups	12560.253	12	1046.688		
	Total	20264.350	15			
caprine	Between Groups	1075.000	3	358.333	1.531	.225
	Within Groups	7488.889	32	234.028		
	Total	8563.889	35			

bovine

Duncan[a]

		Subset for alpha = 0.05	
VAR00001	N	1	2
morula	4	16.0725	
8cell	4	40.3575	40.3575
4cell	4	53.7500	53.7500
2cell	4		76.6675
Sig.		.143	.156

caprine

Duncan[a]

		Subset for alpha = 0.05
VAR00001	N	1
8cell	9	2.2222
morula	9	2.2222
4cell	9	7.7778
2cell	9	15.5556
Sig.		.100

Appendix Table 2.43: Cleavage rate of Grade B oocyte in parthenogenetic activation for different species in different embryo developmental stages

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
cell2	bovine	4	50.0000	57.73503	28.86751	-41.8693	141.8693	.00	100.00
	caprine	11	30.9091	45.92484	13.84686	.0564	61.7618	.00	100.00
	Total	15	36.0000	47.92852	12.37509	9.4581	62.5419	.00	100.00
cell4	bovine	4	50.0000	57.73503	28.86751	-41.8693	141.8693	.00	100.00
	caprine	11	21.8182	40.45199	12.19673	-5.3578	48.9942	.00	100.00
	Total	15	29.3333	45.27167	11.68909	4.2627	54.4039	.00	100.00
cell8	bovine	4	21.5375	24.99578	12.49789	-18.2364	61.3114	.00	46.15
	caprine	11	2.2727	7.53778	2.27273	-2.7912	7.3367	.00	25.00
	Total	15	7.4100	15.88171	4.10064	-1.3850	16.2050	.00	46.15
morula	bovine	4	3.8450	7.69000	3.84500	-8.3915	16.0815	.00	15.38
	caprine	11	.0000	.00000	.00000	.0000	.0000	.00	.00
	Total	15	1.0253	3.97110	1.02533	-1.1738	3.2245	.00	15.38

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
cell2	Between Groups	1069.091	1	1069.091	.447	.515
	Within Groups	31090.909	13	2391.608		
	Total	32160.000	14			
cell4	Between Groups	2329.697	1	2329.697	1.149	.303
	Within Groups	26363.636	13	2027.972		
	Total	28693.333	14			
cell8	Between Groups	1088.652	1	1088.652	5.794	.032
	Within Groups	2442.549	13	187.888		
	Total	3531.201	14			
morula	Between Groups	43.366	1	43.366	3.178	.098
	Within Groups	177.408	13	13.647		
	Total	220.775	14			

Appendix Table 2.44: Cleavage rate of Grade B oocyte in parthenogenetic activation for different embryo developmental stages in different species

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
bovine	2cell	4	50.0000	57.73503	28.86751	-41.8693	141.8693	.00	100.00
	4cell	4	50.0000	57.73503	28.86751	-41.8693	141.8693	.00	100.00
	8cell	4	21.5375	24.99578	12.49789	-18.2364	61.3114	.00	46.15
	morula	4	3.8450	7.69000	3.84500	-8.3915	16.0815	.00	15.38
	Total	16	31.3456	43.39401	10.84850	8.2226	54.4687	.00	100.00
caprine	2cell	11	30.9091	45.92484	13.84686	.0564	61.7618	.00	100.00
	4cell	11	21.8182	40.45199	12.19673	-5.3578	48.9942	.00	100.00
	8cell	11	2.2727	7.53778	2.27273	-2.7912	7.3367	.00	25.00
	morula	11	.0000	.00000	.00000	.0000	.0000	.00	.00
	Total	44	13.7500	32.53129	4.90428	3.8596	23.6404	.00	100.00

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
bovine	Between Groups	6193.820	3	2064.607	1.124	.378
	Within Groups	22051.775	12	1837.648		
	Total	28245.596	15			
caprine	Between Groups	7483.523	3	2494.508	2.624	.064
	Within Groups	38022.727	40	950.568		
	Total	45506.250	43			

bovine

Duncan[a]

VAR00001	N	Subset for alpha = 0.05
		1
morula	4	3.8450
8cell	4	21.5375
2cell	4	50.0000
4cell	4	50.0000
Sig.		.183

caprine

Duncan[a]

VAR00001	N	Subset for alpha = 0.05	
		1	2
morula	11	.0000	
8cell	11	2.2727	
4cell	11	21.8182	21.8182
2cell	11		30.9091
Sig.		.124	.493

Appendix Table 2.45: Cleavage rate of Grade C oocyte in parthenogenetic activation for different species in different embryo developmental stages

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
cell2	bovine	2	50.0000	70.71068	50.00000	-585.3102	685.3102	.00	100.00
	caprine	10	8.3330	18.00154	5.69259	-4.5445	21.2105	.00	50.00
	Total	12	15.2775	31.34858	9.04955	-4.6404	35.1954	.00	100.00
cell4	bovine	2	37.5000	53.03301	37.50000	-438.9827	513.9827	.00	75.00
	caprine	10	8.3330	18.00154	5.69259	-4.5445	21.2105	.00	50.00
	Total	12	13.1942	25.48949	7.35818	-3.0011	29.3894	.00	75.00

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
cell2	Between Groups	2893.565	1	2893.565	3.655	.085
	Within Groups	7916.500	10	791.650		
	Total	10810.065	11			
cell4	Between Groups	1417.856	1	1417.856	2.475	.147
	Within Groups	5729.000	10	572.900		
	Total	7146.856	11			

Appendix Table 2.46: Cleavage rate of Grade C oocyte in parthenogenetic activation for different embryo developmental stages in different species

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
bovine	2cell	2	50.0000	70.71068	50.00000	-585.3102	685.3102	.00	100.00
	4cell	2	37.5000	53.03301	37.50000	-438.9827	513.9827	.00	75.00
	Total	4	43.7500	51.53882	25.76941	-38.2598	125.7598	.00	100.00
caprine	2cell	10	8.3330	18.00154	5.69259	-4.5445	21.2105	.00	50.00
	4cell	10	8.3330	18.00154	5.69259	-4.5445	21.2105	.00	50.00
	Total	20	8.3330	17.52142	3.91791	.1327	16.5333	.00	50.00

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
bovine	Between Groups	156.250	1	156.250	.040	.860
	Within Groups	7812.500	2	3906.250		
	Total	7968.750	3			
caprine	Between Groups	.000	1	.000	.000	1.000
	Within Groups	5833.000	18	324.056		
	Total	5833.000	19			

Appendix Table 2.47: Cleavage rate of mixed grades oocyte in parthenogenetic activation for different species in different embryo developmental stages

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
cell2	bovine	12	63.8050	25.90819	7.47905	47.3437	80.2663	22.73	100.00
	caprine	2	45.5800	60.64148	42.88000	-499.2621	590.4221	2.70	88.46
	Total	14	61.2014	29.91060	7.99394	43.9316	78.4713	2.70	100.00
cell4	bovine	12	53.8508	23.31619	6.73080	39.0364	68.6652	13.64	83.33
	caprine	2	37.8900	49.76618	35.19000	-409.2413	485.0213	2.70	73.08
	Total	14	51.5707	26.15556	6.99037	36.4689	66.6725	2.70	83.33
cell8	bovine	12	37.1900	24.60184	7.10194	21.5587	52.8213	.00	75.00
	caprine	2	11.5400	16.32002	11.54000	-135.0896	158.1696	.00	23.08
	Total	14	33.5257	24.88739	6.65143	19.1562	47.8953	.00	75.00
morula	bovine	12	7.1500	9.70661	2.80206	.9827	13.3173	.00	33.33
	caprine	2	1.9250	2.72236	1.92500	-22.5344	26.3844	.00	3.85
	Total	14	6.4036	9.15933	2.44793	1.1151	11.6920	.00	33.33
blastocyst	bovine	12	.2775	.96129	.27750	-.3333	.8883	.00	3.33
	caprine	2	1.9250	2.72236	1.92500	-22.5344	26.3844	.00	3.85

	Total	14	.5129	1.30764	.34948	-.2422	1.2679	.00	3.85

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
cell2	Between Groups	569.401	1	569.401	.618	.447
	Within Groups	11060.968	12	921.747		
	Total	11630.370	13			
cell4	Between Groups	436.711	1	436.711	.620	.446
	Within Groups	8456.764	12	704.730		
	Total	8893.476	13			
cell8	Between Groups	1127.867	1	1127.867	1.955	.187
	Within Groups	6924.098	12	577.008		
	Total	8051.965	13			
morula	Between Groups	46.801	1	46.801	.538	.477
	Within Groups	1043.812	12	86.984		
	Total	1090.613	13			
blastocyst	Between Groups	4.653	1	4.653	3.177	.100
	Within Groups	17.576	12	1.465		
	Total	22.229	13			

Appendix Table 2.48: Cleavage rate of mixed grades oocyte in parthenogenetic activation for different embryo developmental stages in different species

Descriptives

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
bovine	2cell	12	63.8050	25.90819	7.47905	47.3437	80.2663	22.73	100.00
	4cell	12	53.8508	23.31619	6.73080	39.0364	68.6652	13.64	83.33
	8cell	12	37.1900	24.60184	7.10194	21.5587	52.8213	.00	75.00
	morula	12	7.1500	9.70661	2.80206	.9827	13.3173	.00	33.33
	blastocyst	12	.2775	.96129	.27750	-.3333	.8883	.00	3.33
	Total	60	32.4547	31.55117	4.07324	24.3041	40.6052	.00	100.00
caprine	2cell	2	45.5800	60.64148	42.88000	-499.2621	590.4221	2.70	88.46
	4cell	2	37.8900	49.76618	35.19000	-409.2413	485.0213	2.70	73.08
	8cell	2	11.5400	16.32002	11.54000	-135.0896	158.1696	.00	23.08
	morula	2	1.9250	2.72236	1.92500	-22.5344	26.3844	.00	3.85
	blastocyst	2	1.9250	2.72236	1.92500	-22.5344	26.3844	.00	3.85
	Total	10	19.7720	33.05458	10.45278	-3.8738	43.4178	.00	88.46

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
bovine	Between Groups	37665.107	4	9416.277	24.582	.000
	Within Groups	21067.992	55	383.054		
	Total	58733.099	59			
caprine	Between Groups	3398.223	4	849.556	.660	.646
	Within Groups	6435.227	5	1287.045		
	Total	9833.450	9			

bovine

Duncan[a]

		Subset for alpha = 0.05		
VAR00001	N	1	2	3
blastocyst	12	.2775		
morula	12	7.1500		
8cell	12		37.1900	
4cell	12			53.8508
2cell	12			63.8050
Sig.		.393	1.000	.218

caprine

Duncan[a]

		Subset for alpha = 0.05
VAR00001	N	1
morula	2	1.9250
blastocyst	2	1.9250
8cell	2	11.5400
4cell	2	37.8900
2cell	2	45.5800
Sig.		.289

APPENDIX 3: SELECTED PUBLISHED ARTICLE

Effect of Sperm Insemination Duration on In Vitro Fertilisation (IVF) Performance in Goats

Tan, W.L., Wan Khadijah, W.E. and Abdullah, R.B.

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EFFECT OF SPERM INSEMINATION DURATION ON *IN VITRO* FERTILISATION (IVF) PERFORMANCE IN GOATS

Tan, W.L., Wan Khadijah, W.E. and Abdullah*, R.B.

Animal Biotechnology-Embryo Laboratory (ABEL), Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia
*ramli@um.edu.my (Corresponding Author)
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ABSTRACT The objective of this study was to evaluate the effect of sperm insemination duration during *in vitro* fertilisation (IVF) on the cleavage rate of goat embryos. Goat oocytes were retrieved through the slicing of ovaries obtained from abattoir and laparoscopic oocyte pick-up (LOPU). For *in vitro* maturation (IVM), the collected cumulus oocyte complexes (COCs) were matured and then incubated overnight in CO_2 (5%) incubator at 38.5°C for 24-27 hr (abattoir source) or 18-21 hr (LOPU source). Oocytes were partially denuded and co-incubated with post-thawed sperm with concentration of $1x10^6$ sperm/ml. After 8-14 hr or 18-24 hr of insemination, *in vitro* culture (IVC) of presumptive zygotes were further incubated in CO_2 (5%) incubator at 38.5°C for preimplantation embryonic development. The cleavage of the embryos was observed and recorded daily under inverted microscope. The fertilisation rate with insemination duration of 8-14 hr (39.82%) was higher than that with 18-24 hr (33.59%); however, there was no significant difference ($P>0.05$) between 2 groups of insemination durations. There was significant ($P<0.05$) decrease as the embryo developmental stages increased. Generally, the embryo developmental rates of all stages for insemination durations of 8-14 hr were higher than that of 18-24 hr, which were 49.36 vs. 27.92%; 33.77 vs. 22.29%; 7.32 vs. 2.08% and 0.56 vs. 0.00% for 2-, 4-, 8-cell and morula, respectively. In conclusion, shorter insemination duration may be needed to improve the IVF performance in goats.

ABSTRAK Objektif kajian ini adalah untuk menilai kesan tempoh inseminasi sperma semasa persenyawaan *in vitro* (IVF) ke atas kadar pembelahan embrio kambing. Oosit kambing diperolehi melalui penghirisan ovari yang diperolehi dari rumah sembelihan dan *laparoscopic oocyte pick-up* (LOPU). Bagi pematangan *in vitro* (IVM), kompleks oosit kumulus (COCs) dimatangkan dan kemudian dieram semalaman dalam inkubator CO_2 (5%) pada 38.5ºC selama 24-27 jam (sumber rumah sembelihan) atau 18-21 jam (sumber LOPU). Oosit ditanggal kumulus sebahagiannya dan dieram bersama dengan sperma nyahsejukbeku dengan kepekatan $1x10^6$ sperma/ml. Selepas 8-14 jam atau 18-24 jam diinseminasi, kultur *in vitro* (IVC) zigot andaian dieram seterusnya dalam inkubator CO_2 (5%) pada 38.5ºC bagi perkembangan embrio prapenempelan. Pembelahan embrio diperhati dan direkodkan setiap hari di bawah mikroskop *inverted*. Kadar persenyawaan dengan tempoh inseminasi 8-14 jam (39.82%) adalah lebih tinggi daripada 18-24 jam (33.59%); walau bagaimanapun, tiada perbezaan signifikan ($P>0.05$) ditemui antara 2 kumpulan tempoh inseminasi. Terdapat penurunan yang signifikan semasa setiap peningkatan peringkat perkembangan embrio. Secara amnya, kadar perkembangan embrio bagi semua peringkat perkembangan embrio untuk tempoh inseminasi 8-14 jam adalah lebih tinggi daripada 18-24 jam, di mana 49.36 vs. 27.92%; 33.77 vs. 22.29%; 7.32 vs. 2.08% and 0.56 vs. 0.00% bagi 2-, 4-, 8-sel dan morula, masing-masing. Kesimpulannya, kadar inseminasi yang lebih pendek mungkin diperlukan untuk memajukan prestasi IVF dalam kambing.

(**Keywords**: IVF, goat, insemination duration, embryo)

INTRODUCTION

In vitro embryo production (IVP) technology has been widely used to increase the population of superior genetic merit of livestock animals including goats. To achieve success optimally, sufficient number of oocytes is the prerequisite for various reproductive techniques related to IVP procedures. In order to obtain abundant source of oocytes for large-scale production of embryos by IVP procedure, getting ovaries from abattoir is one of the ways [1]. However, in Malaysia, the number of female goats slaughtered is few and consequently it is quite difficult to carry out proper experiments in goat biotechnologies. Another source of oocytes could be alternatively obtained through laparoscopic oocyte pick-up (LOPU). It has been shown that the ovaries could be hyperstimulated several times and followed by aspiration of oocytes for various embryo techniques [2].

There are 3 main steps involved in the method of IVP of embryos, namely maturation of primary oocytes from large antral follicles, fertilisation of matured secondary oocytes with frozen-thawed semen and *in vitro* culture (IVC) of the putative embryos for up to 7 days until the formation of blastocysts. Conventionally, IVP in goats involves the superstimulation procedure in matured female animals either for flushing of embryos and oocytes or LOPU. Various gonadotrophins such as follicle stimulating hormone (FSH), pregnant mare's serum gonadotrophin (PMSG), equine chorionic gonadotrophin (eCG) and human chorionic gonadotrophin (hCG) have been introduced to the hormone regime for superovulation and superstimulation procedures.

A reduction of the period of sperm-oocyte exposure has been suggested to increase the incidence of monospermy [3, 4]. In fact, most of the current IVF systems use a 5-6 hr sperm-oocyte insemination duration [5, 6-9], compared to 12-18 hr insemination duration used in the original porcine IVF systems [10-12]. Such information is not readily available in the literature and this issue is still controversial.

In the present study, we evaluated the efficiency of IVF performance for different insemination durations (co-incubation durations) during IVF procedure in goats.

MATERIALS AND METHODS

Ovary source and animal preparation

The sources of ovaries were from abattoir and LOPU. In order to prepare goats for LOPU, oestrus synchronisation and hyperstimulation of goats is necessary. Oestrous cycle of a donor goat was synchronised using insertion of a Controlled Intravaginal Drug Release device (CIDR, 0.3 g progesterone) into the vagina for 14 days (at 0900 hours on day 0) using a sterile CIDR applicator with water-based lubricant (K-Y Jelly). At 36 hr prior to CIDR removal, a luteolytic treatment of Estrumate® (125 µg cloprostenol) was administered intramuscularly (i.m.; at 0900 hours on day 13) to regress corpus luteum that facilitated initiation of pro-oestrus and eventually resulted in oestrogen surge for the onset of heat (oestrus). PMSG (1500 IU) was administered (i.m.; at 1600 hours on day 14). Upon the removal of CIDR (at 2100 hours on day 14), Ovidrel® (250 IU chorionic gonadotrophin) was administered (i.m.; at 2100 hours on day 14) to stimulate multiple follicular development prior to oocyte-retrieval surgery (at 0800 hours on day 17).

Oocytes recovery

For the abattoir source, the ovary was placed in a Petri dish (90 mm) containing TL-Hepes working solution (5 ml) on a heating stage (38.5ºC). The ovary was held with the help of forceps and each ovary was sliced individually. Checker-board incisions were made along the whole ovarian surface using a quarter section of a stainless steel razor blade held with a sterile haemostat. The COCs in the Petri dish were then examined under a stereomicroscope.

For LOPU source, the donor goat was sedated and anaesthetised and a small incision was made on the disinfected abdominal area and a trocar connected to a CO_2 tank via the CO_2 insufflator was inserted into the incision to insufflate the abdominal cavity with CO_2. Three small incisions (3-5 mm) were made once the peritoneum cavity was expanded. One of the incisions was made near the umbilicus to facilitate insertion of trocar for passing the light probe, one on the right side of lower-ventral abdomen to insert the trocar for passing the grasping forceps and one on the left side of lower-ventral abdomen for passing oocyte retrieval needle.

Using the grasper, the uterine horns were gently manipulated to allow visualisation of each stimulated ovary. Once an ovary was identified, the ovarian ligament was grasped to stabilise the ovary for follicular puncture. The follicular contents were aspirated from all follicles visible on the surface of the ovaries by puncturing follicles with the aspiration needle. The collection tube containing aspirated fluid (3-5 ml) with COCs was dispensed

into a sterile Petri dish (90 mm) for COCs searching under a stereomicroscope.

In vitro maturation (IVM)

A Petri dish (35 mm) with IVM droplets (100 µl) which were overlaid with equilibrated silicone oil was prepared overnight. The dish was kept in CO_2 incubator (5%). Under the stereomicroscope, the COCs were identified and picked up, using a hand-controlled pipette. The COCs from LOPU were then washed in three droplets of flushing medium followed by three droplets of IVM medium, while COCs from ovary slicing were washed with TL-Hepes working solution followed by three droplets of IVM medium. The collected COCs were then matured and incubated overnight in CO_2 (5%) incubator at 38.5°C for 24-27 hr (abattoir source) or 18-21 hr (LOPU source).

In vitro fertilisation (IVF)

Oocytes were partially denuded by sucking in and out from the pipette. The oocytes left with one or two layers of cumulus cells were washed thrice with droplets of IVF medium (100 µl) before being transferred to the insemination culture dish and kept in the CO_2 incubator (5%) to maintain its temperature (38.5ºC) and gas equilibration until required for insemination.

A frozen straw of goat sperm was pre-thawed at room temperature (25ºC, 1 min) followed by thawing in a water bath (37ºC, 3 min). Using a pair of sterile scissors, the straw was cut at both ends and the sperm was slowly released at the bottom of the swim-up tube (15 ml). Without agitating the mixture of sperm and medium, the swim-up tube was carefully held at 60º to the horizontal surface in the CO_2 incubator (5%) and incubated at 38.5ºC in humidified air (45 min) to allow sperm swim-up. The upper layer of the medium containing highly motile sperm (sperm suspension) was aspirated (1 ml) from the swim-up tube and placed in a sterile conical tube (15 ml) and sperm-SOF medium (2 ml) was added to the sperm suspension to make a total volume of 3 ml. With the tube tightly capped, the tube was centrifuged twice for 5 min (200x *g*) and the supernatant was discarded. While conducting the assessment of sperm concentration, the capacitation-treated sperm were incubated in CO_2 incubator (5%). After the sperm concentration has been determined by haemacytometer, sufficient volume of sperm suspension was added to the fertilisation droplets (30 µl) containing 5-10 matured oocytes to yield final concentration $1.0x10^6$ sperm/ml.

In vitro culture (IVC)

The dish containing IVC droplets under silicone oil were equilibrated overnight at 38.5ºC in CO_2 incubator (5%). The culture medium used for *in vitro* embryo culture was KSOM. After 8-14 hr or 18-24 hr sperm insemination duration, all the presumptive zygotes were removed from the insemination droplets and washed, subsequently were transferred to new IVC droplets (100 µl) for culture (38.5ºC, 5% CO_2). Each culture droplet (100 µl) contained 5-10 presumptive zygotes.

Statistical analysis

Data were analysed by analysis of variance (ANOVA) and Duncan's multiple range tests (D-MRT), using the SPSS statistical software package version 17. A probability of $P<0.05$ was considered significant for all statistical tests. Values were presented as mean±SEM.

RESULTS AND DISCUSSION

The fertilisation rate of insemination duration of 8-14 hr (39.82%) was relatively higher than that of 18-24 hr (33.59%) (Table 1). The co-incubation of oocytes with sperm for 17 hr was originally established for practical reasons and corresponds to the time for observation of pronuclei. As reported in goat IVF system [13], there was only 10.00% of oocytes fertilised following 1 hr co-incubation with sperm; the cleavage rates were 71.00 vs. 78.00% (3 vs. 17 hr co-incubation) and blastocyst rates were 50.00 vs. 53.00% (3- vs. 17 hr co-incubation), respectively. The cleavage rates in their reports were relatively higher than that of the present study (49.36 vs. 29.32% for insemination durations of 8-14 vs. 18-24 hr, respectively) (Table 2). The 6 hr delay in gamete interaction indicated that this duration is probably necessary for sperm to cross the cumulus or that differences in sperm capacitation kinetics exist between IVF systems.

It was suggested that reducing the oocyte-sperm insemination duration lowers oocyte penetration and fertilisation during bovine IVF [14], but resulted in similar oocyte cleavage rates [15], and either similar [15] or reduced [16] rates of blastocyst formation. Shortening the gamete insemination duration during human IVF increases normal fertilisation [17, 18], embryo development [17, 19 and 20], increased embryo morphology [20-23], implantation and pregnancy rates [21 and 23].

According to [24 and 25], penetration of IVM porcine oocytes as well as polyspermic fertilisation occur as early as 2 hr post-insemination. Moreover, it is known that the incidence of acrosome-reacted

living sperm, under capacitating conditions, remains constant after 2, 4, and 6 hr of co-incubation with oocytes [26]. It was suggested that by reducing the sperm-oocyte insemination duration from 12-18 to 8 hr [27] as well as the removal of adherent sperm from the zona pellucida after 6-8 hr of insemination duration [28], decrease the incidence of polyspermy. [29] proposed to decrease the exposure duration of matured oocytes to sperm from 5 hr to 10 min and maintain the oocytes with the zona-bound sperm in a fresh IVF medium drop without sperm for an additional 5 hr incubation; and this modification of the IVF conditions resulted in a higher penetration rate (57.00 vs. 80.00%) and blastocyst development (8.00 vs. 30.00%) as compared to the conventional procedure of gametes co-incubated together for 5 hr. Frozen-thawed sperm cells generate increased amounts of reactive oxygen species (ROS) as by-products of metabolism and cell death [30, 31] which may be related to longer insemination duration. Several studies have shown that ROS plays an important role in mediating sperm-zona interactions as it increases DNA fragmentation [32], modifies the cytoskeleton [33], and produces a loss of fluidity, integrity and competence of the sperm membrane to participate in the membrane events associated with fertilisation [34-36].

Table 1: Fertilisation rate (%, mean±SEM) of goat oocytes for different insemination durations in *in vitro* fertilisation

Insemination duration	No. of matured oocytes	Fertilisation rate (n)
8-14 hr	134	39.82 ± 8.18^{a} (n=68)
18-24 hr	60	33.59 ± 10.97^{a} (n=17)

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

Table 2: Cleavage rate (%, mean±SEM) of goat oocytes for different insemination durations in *in vitro* fertilisation

Insemination duration	Cleavage rate (n)			
	2-cell	4-cell	8-cell	Morula
8-14 hr	$49.36\pm10.41^{a,z}$ (n=46)	$33.77\pm8.76^{a,z}$ (n=28)	$7.32\pm5.06^{a,y}$ (n=10)	$0.56\pm0.56^{a,y}$ (n=2)
18-24 hr	$27.92\pm10.87^{a,z}$ (n=10)	$22.29\pm9.26^{a,yz}$ (n=7)	$2.08\pm2.08^{a,xy}$ (n=1)	$0.00\pm0.00^{a,x}$ (n=0)

[a]Mean value within a column with same superscript was not significantly different (P>0.05)
[yz]Mean values within a row with different superscripts were significantly different (P<0.05)

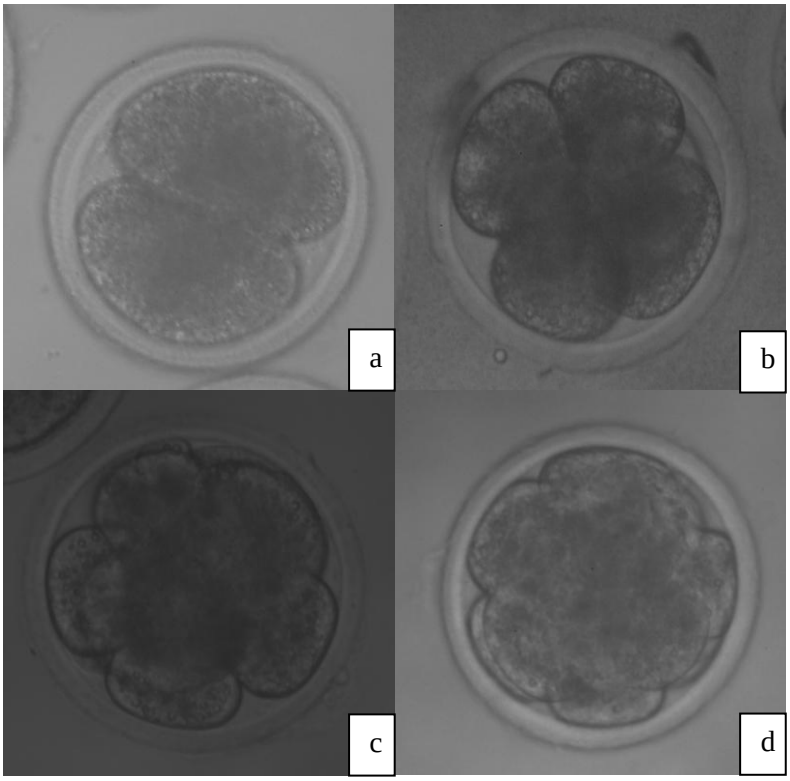

Figure 1(a-d): Embryos at 2- (a), 4- (b), 8-cell (c) and morula (d) obtained from goat IVF.

CONCLUSION

Many factors are involved in the IVF performance in goat including internal and external factors. This research indicates that insemination duration may play important role in the cleavage of goat embryo. It is suggested that shorter insemination durations of 8-14 hr may be better than that of 18-24 hr as consistently shown in our data that the former was higher in the cleavage rates.

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