RESULTS

RESULTS

***Experiment I*:**

I-A. Ovarian measurements:

I.A.1. Untreated does:

Overall mean and least square means±S.E. for season effect on ovarian measurements of untreated does are shown in Table (3).

The overall mean±S.E. for the left and right ovaries weights were 0.251±0.01 and 0.256±0.01 gm, respectively. The highest weights of left and right ovaries were obtained during winter season (0.315±0.01and 0.325±0.01 gm, respectively), followed by those of spring season (0.243±0.02 and 0.240±0.02 gm, respectively) and the lowest weights were recorded during autumn season (0.194±0.02 and 0.202±0.02 gm, respectively). The differences among seasons were significant (P<0.001) as shown in Table (3).

The overall mean±S.E. of corpora lutea number on left and right ovaries were 1.0±0.2 and 1.9±0.3 CL, respectively. The differences among seasons for CL on the left ovary were significant (P<0.05), but were not significant for those on the right ovary (Table 3).

The overall mean±S.E. for the number of follicles presented on the left and right ovaries were 14.9±0.4 and 15.8±0.9 follicles, respectively, (Table 3). During winter season, number of follicles presented on left and right ovaries were 16.8±0.6 and 17.5±1.4 follicles, respectively, decreased to 15.3±0.7 and 14.3±1.7 follicles during spring, reached to 12.5±0.7 and 15.8±1.7 follicles during auteum season for the respective ovaries. The differences among seasons for number of follicles on the left ovary were significant (P<0.01), but were not significant for those on the right ovary (Table 3).

The overall mean±S.E. for total number of follicles presented on both left and right ovaries and total oocytes obtained from total number of follicles were 30.7±1.0 follicles and 27.5±1.1 oocytes, respectively. The highest total number of follicles and total oocytes aspirated from follicles were obtained during winter season (34.3±1.5 follicles and 30.2±1.6 oocytes) followed by spring season (29.5±1.8 follicles and 26.8±2.0 oocytes) and the lowest were obtained during autumn season (28.3±1.8 follicles and 25.5±2.0 oocytes), the differences among seasons for total number of follicles were significant (P<0.05), but were not significant for total oocytes obtained (Table 3).

Recovery rate was calculated as number of oocytes obtained/number of follicles presented on the two ovaries. The recovery rate was 91.0% (386 oocytes/424 follicles) in

Table 3. Least square means ± S.E. for ovarian measurements as affected by season for untreated does.

Items	No. of does	Left ovary weight (gm)	Right ovary weight (gm)	No. of corpus lutium on left ovary	No. of corpus lutium on right ovary	No. of follicles on left ovary	No. of follicles on right ovary	Total number of follicles/doe	Total oocytes obtained/doe
Overall mean	14	0.251±0.01	0.256±0.01	1.0±0.2	1.9±0.3	14.9±0.4	15.8±0.9	30.7±1.0	27.5±1.1
Season:									
Autumn	4	0.194±0.02	0.202±0.02	0.5±0.4	1.5±0.6	12.5±0.7	15.8±1.7	28.3±1.8	25.5±2.0
Winter	6	0.315±0.01	0.325±0.01	1.8±0.3	2.3±0.5	16.8±0.6	17.5±1.4	34.3±1.5	30.2±1.6
Spring	4	0.243±0.02	0.240±0.02	0.8±0.4	1.8±0.6	15.3±0.7	14.3±1.7	29.5±1.8	26.8±2.0

F-ratios and test of significant of least-squares analysis of variance for means presented in Table 3.

S.O.V.	d.f.	F - ratios							
Season	2	18.937***	17.210***	4.287*	0.594	10.991**	1.159	3.884*	1.863
Remainder d.f.	11								
Remainder MS.		0.00097	0.00115	0.598	1.553	2.053	11.181	13.553	15.871

* Significant (P< 0.05) .

** Significant (P< 0.01) .

*** Significant (P< 0.001) .

this group. Culture ova percentage was calculated as the number of ova cultured/ number of ova obtained from follicles on the two ovaries. The cultured ova percentage was 94.3% (364 /386 oocytes) in this group.

I.A.2. PMSG-treated does:

Overall mean and least square means±S.E. for the effect of season on ovarian measurements for PMSG-treated does are presented in Table (4).

The effect of season was significant (P<0.05) on total follicles on both left and right ovaries and total oocytes obtained from total follicles, but was not significant for the ovaries weights, number of corpora lutea and number of follicles on left and right ovaries (Table 4). Left and right ovaries weights were highest during winter season followed by spring, then autumn seasons, being 0.472±0.04 gm and 0.481±0.04 gm for the left and right ovaries during winter, 0.354±0.04 gm and 0.368±0.04 gm for the respective ovaries during spring and 0.305±0.07gm for left and 0.314±0.07gm for right ovaries. during autumn season. Concerning total follicles on both left and right ovaries, the highest mean was obtained during winter (45.7±1.7 follicles), followed by autumn (42.0±3.0 follicles) and the lowest was obtained during spring season (39.0±1.7 follicles). Similarly, the total oocytes obtained from total follicles were highest during winter season (40.7±1.6 oocytes) and were nearly equal (35.0±2.8 oocytes and 34.3±1.6 oocytes) during autumn and spring seasons, respectively.

The recovery rate and culture ova percentage in this group were 91.9 (524 oocytes/570 follicles) and 94.1% (493/524 oocytes) , respectively.

I.A.3. Untreated and PMSG-treated groups:

The results of untreated and PMSG-treated groups for ovary measurements are presented in Table (5). Least square means±S.E. for left and right ovaries weights were 0.249±0.02 and 0.253±0.02 gm for the untreated group, and were 0.380±0.02 and 0.391±0.02 gm for the treated group, respectively. The differences between groups were significant (P<0.001). The rate of change was 52.6 and 54.5% for left and right ovaries, respectively. The number of CL presented on left and right ovaries were 1.12±0.3 and 1.89±0.3 CL for untreated group and were 1.89±0.3 and 2.24±0.4 CL for PMSG-treated group, respectively, the differences between groups were not significant (Table 5). However, the rate of change was 68.8 and 18.5% for the number of CL on left and right ovaries, respectively. The least square mean±S.E. for the number of follicles observed on

Table 4 . Least square means ± S.E. for ovarian measurements as affected by season for PMSG-treated does.

Items	No. of does	Left ovary weight (gm)	Right ovary weight (gm)	No. of corpus lutium on left ovary	No. of corpus lutium on right ovary	No. of follicles on left ovary	No. of follicles on right ovary	Total number of follicles/doe	Total oocytes obtained/doe
Overall mean	14	0.377±0.03	0.387±0.03	2.00±0.42	2.33±0.40	20.1±0.7	22.2±0.9	42.2±1.3	36.7±1.2
Season:									
Autumn	2	0.305±0.07	0.314±0.07	2.00±0.98	2.50±0.92	19.5±1.6	22.5±2.2	42.0±3.0	35.0±2.8
Winter	6	0.472±0.04	0.481±0.04	1.50±0.56	2.33±0.53	21.8±0.9	23.8±1.3	45.7±1.7	40.7±1.6
Spring	6	0.354±0.04	0.368±0.04	2.50±0.56	2.17±0.53	18.8±0.9	20.2±1.3	39.0±1.7	34.3±1.6

F- ratios and test of significant of least-squares analysis of variance for means presented in Table 4.

S.O.V.	d.f.	F - ratios							
Season	2	3.266	3.341	0.786	0.056	2.760	2.152	3.799*	4.034*
Remainder d.f.	11								
Remainder Ms.		0.0094	0.0089	1.909	1.697	5.106	9.470	17.576	16.242

* Significant (P< 0.05).

** Significant (P< 0.01).

Table 5. Least square means ± S.E. for ovary measurements as affected by season and PMSG-treatment.

Items	No. of does	Left ovary weight (gm)	Right ovary weight (gm)	No.of corpus lutium on left ovary	No. of corpus lutium on right ovary	No. of follicles on left ovary	No. of follicles on right ovary	Total number of follicles/doe	Total oocytes obtained/doe
Overall mean	28	0.314±0.01	0.323±0.01	1.51±0.2	2.06±0.2	17.3±0.4	19.0±0.6	36.3±0.8	32.0±0.8
Season:									
Autumn	6	0.253±0.03	0.262±0.03	1.13±0.5	1.89±0.5	15.6±0.8	19.0±1.3	34.7±1.6	30.2±1.6
Winter	12	0.394±0.02	0.403±0.02	1.67±0.3	2.33±0.4	19.3±0.6	20.7±0.9	40.0±1.1	35.4±1.1
Spring	10	0.297±0.02	0.303±0.02	1.72±0.4	1.97±0.4	16.9±0.6	17.2±1.0	34.1±1.2	30.4±1.2
Group:									
Untreated	14	0.249±0.02	0.253±0.02	1.12±0.3	1.89±0.3	14.9±0.5	15.8±0.8	30.7±1.0	27.4±1.1
Treated	14	0.380±0.02	0.391±0.02	1.89±0.3	2.24±0.4	19.7±0.5	22.1±0.9	41.8±1.1	36.6±1.1

F-ratios and test of significant of least-squares analysis of variance for means presented in Table 5.

S.O.V.	d.f.	F - ratios							
Season	2	9.682 ***	10.294 ***	0.513	0.358	8.546 **	3.481 *	7.479 **	5.882 **
group	1	23.650 ***	26.859 ***	2.814	0.529	43.525 ***	27.866 ***	56.193 ***	38.016 ***
Remainder d.f.	24								
Remainder MS.		0.00491	0.00473	1.427	1.528	3.701	9.491	14.920	15.195

* Significant (P< 0.05).

** Significant (P< 0.01).

*** Significant (P< 0.001).

the left ovary were 14.9±0.5 and 19.7±0.5 follicles, for untreated and PMSG-treated groups, respectively, and these numbers were 15.8±0.8 and 22.1±0.9 follicles on the right ovary for the respective groups. The mean total numbers of follicles on both left and right ovaries were 30.7±1.0 follicles and 41.8±1.1 follicles for untreated and PMSG-treated groups, respectively, the differences between groups for number of follicles on left and right ovaries, also the total number of follicles were significant (P<0.001) as presented in Table (5).

Concerning total oocytes obtained, least square means±S.E. was 27.4±1.1 oocytes for untreated group, but this value was 36.6±1.1 oocytes for PMSG-treated group. These differences between the two groups were significant (P<0.001).

I-B. Oocytes maturation *in vitro*:

Oocytes were collected from 14 does in order to study the factors that influence rabbit oocytes maturation *in vitro*. This study was performed to examine the effect of season and effect of the use of TCM-199 medium supplemented with fetal calf serum (FCS) or bovine serum albumin (BSA) as a protein supplements on *in vitro* rabbit oocytes maturation.

I.B.1. Untreated does:

Data in Table (6) represent *in vitro* maturation of rabbit oocytes collected from untreated group, after 20 hours of incubation. Statistical analysis demonstrated no significant differences among seasons for the degenerated oocytes, germinal vesicle, germinal vesicle breakdown stages percentages and maturation rate. However, the maturation rate was highest during winter season (66.3%) than those of autumn and spring seasons (63.0 and 60.4%, respectively). Also, degenerated oocytes percentage increased from 5.5% during winter to 7.0 and 8.9% for autumn and spring seasons, respectively. The percentages of oocytes resumed germinal vesicle stage were 10.9, 12.3 and 14%, for spring, winter and autumn seasons, respectively. The percentages of oocytes resumed germinal vesicle breakdown were similar in autumn and winter seasons (16.0%), while this percentage was 19.8% for spring season.

The addition of FCS to TCM-199 medium elevated the percentage of oocytes matured *in vitro* than those of BSA, being 73.1 vs. 54.4%. The differences between FCS and BSA supplement were significant (P<0.05) as presented in Table (6) and Figure (1). Also, the addition of FCS decreased the percentage of degenerated oocytes to 3.3% compared to 10.4% for BSA, the differences were significant (P< 0.05). The same trend

was observed for germinal vesicle stage percentage (7.1 vs. 17.6% for FCS and BSA), also the differences were significant (P<0.05). No significant differences were observed for the percentage of germinal vesicle breakdown stage between FCS and BSA (16.5 and 17.6%, respectively).

I.B.2. PMSG-treated does:

The results in Table (7) indicated that season had no significant effect on all stages of maturation and the percentage of degenerated oocytes after 20 hours of incubation of rabbit oocytes.

Comparing the effect of adding of FCS or BSA to TCM-199 medium on rabbit oocytes maturation *in vitro*. The results in Table (7) and Figure (2) show that FCS increased the percentage of oocyte maturation to 81.3% compared with 65.2% for BSA. The differences between FCS and BSA supplements were significant (P< 0.05). FCS added to the TCM-199 medium decreased significantly (P<0.05) the percentage of degenerated oocytes to 1.6% compared with 4.5% for BSA. Also, FCS decreased germinal vesicle stage percentage significantly (P<0.05) to 4.9% compared with 13.4% for BSA. However, no significant differences were observed between FCS and BSA for GVBD stage percentage (12.2 and 17.0%, respectively).

I.B.3. Untreated and PMSG-treated groups:

Irrespect to the effect of season and FCS or BSA supplement, the comparison between the percentage of oocytes maturated *in vitro* collected from untreated and PMSG-treated does after 20 hours of incubation are presented in Table (8) and Figure (3). PMSG treatment increased significantly (P<0.05) the percentage of oocytes maturation (73.2%) compared with 63.7% for untreated females. The rate of change of maturation percentage due to hormonal treatment was 14.9%. On the other hand, PMSG treatment decreased degenerated oocytes percentage significantly (P<0.05) to 3.0% compared with 6.9% for the untreated group. No significant differences were observed between the two groups for GV and GVBD stages. However, untreated group had higher GV and GVBD stages (12.4 and 17.0%, respectively) than those of PMSG-treated group (9.1 and 14.6%, respectively).

Table 6. Effects of season and FCS or BSA supplement to TCM-199 medium on *in vitro* maturation of rabbit oocytes collected from untreated does.

Items	No. of oocytes	No. (%)[2] of oocytes at each stage			
		Degeneration	G.V.[3]	G.V.B.D.[4]	Maturation
Season:					
Autumn	100	7 (7.0)	14 (14.0)	16 (16.0)	63 (63.0)
Winter	163	9 (5.5)	20 (12.3)	26 (16.0)	108 (66.3)
Spring	101	9 (8.9)	11 (10.9)	20 (19.8)	61 (60.4)
TCM-199 plus:					
FCS	182	6 (03.3)[b]	13 (07.1)[b]	30 (16.5)	133 (73.1)[a]
BSA	182	19 (10.4)[a]	32 (17.6)[a]	32 (17.6)	99 (54.4)[b]
Total[1]	364	25 (6.9)	45 (12.4)	62 (17.0)	232 (63.7)

Table 7. Effects of season and FCS or BSA supplement to TCM -199 medium on *in vitro* maturation of rabbit oocytes collected from PMSG-treated does.

Items	No. of oocytes	No. (%)[2] of oocytes at each stage			
		Degeneration	G.V.[3]	G.V.B.D.[4]	Maturation
Season:					
Autumn	63	2 (3.2)	5 (7.9)	10 (15.9)	46 (73.0)
Winter	239	5 (2.1)	23 (9.6)	35 (14.6)	176 (73.6)
Spring	191	8 (4.2)	17 (8.9)	27 (14.1)	139 (72.8)
TCM-199 plus:					
FCS	246	4 (1.6)[b]	12 (04.9)[b]	30 (12.2)	200 (81.3)[a]
BSA	247	11 (4.5)[a]	33 (13.4)[a]	42 (17.0)	161 (65.2)[b]
Total[1]	493	15 (3.0)	45 (9.1)	72 (14.6)	361 (73.2)

[1] The total oocytes were derived from 14 does.

[2] As a proportion of cultured oocytes.

[3] Germinal vesicle stage.

[4] Germinal vesicle breakdown stage.

[a,b] Values with different superscript within a column for individual treatment are significantly different ($P< 0.05$).

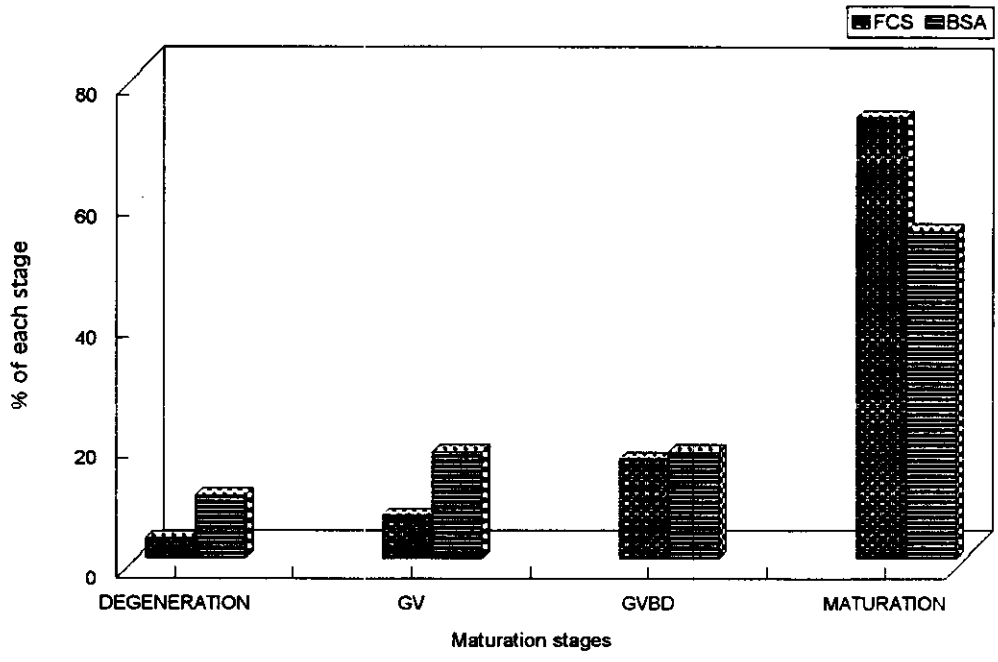

Figure 1. Effect of FCS and BSA on *in vitro* oocytes maturation of untreated does.

GV = Germinal vesicle. GVBD =Germinal vesicle breakdown.

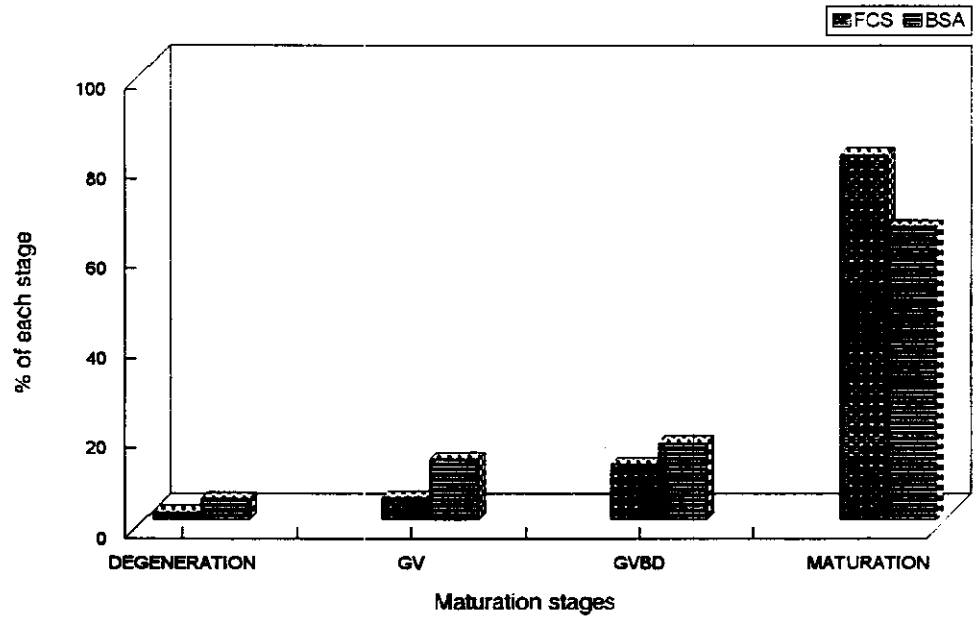

Figure 2. Effect of FCS and BSA on *in vitro* oocytes maturation of PMSG-treated does.

GV = Germinal vesicle. GVBD = Germinal vesicle breakdown.

Table 8. Effects of PMSG-treatment on rabbit oocytes maturated *in vitro*.

Group	No. of oocytes[1]	No. (%)[2] of oocytes at each stage			
		Degeneration	G.V.[3]	G.V.B.D.[4]	Maturation
Untreated*	364	25 (6.9)[a]	45 (12.4)	62 (17.0)	232 (63.7)[b]
Treated**	493	15 (3.0)[b]	45 (09.1)	72 (14.6)	361 (73.2)[a]

[1] The oocytes in each group were derived from 14 does.

[2] As a proportion of cultured oocytes.

[3] Germinal vesicle stage.

[4] Germinal vesicle breakdown stage.

[a,b] Values with different superscript within a column are significantly different ($P< 0.05$).

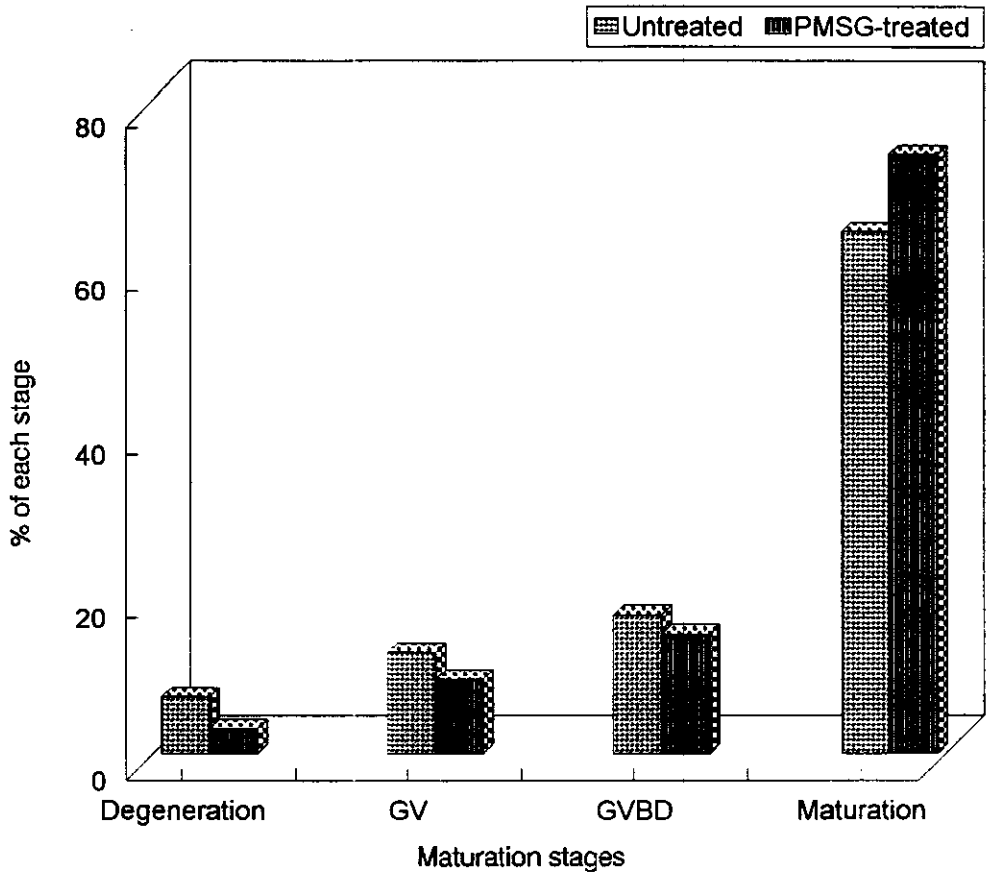

Figure 3. Comparison between untreated and PMSG-treated groups for oocytes maturation *in vitro*.

GV= Germinal vesicle. GVBD= Germinal vesicle breakdown.

I-C. Oocytes fertilization *in vitro*:

I.C.1. Untreated does:

In vitro fertilization of *in vitro* matured oocytes were performed in these studies to evaluate fertilizability and capacity for early embryonic development.

The present study was done to examine the effect of season, Brackett's or TCM-199 medium and three levels of osmolarity on fertilization rate after 24 hrs post-insemination.

Effect of season:

The results summarized in Table (9) show that, no significant differences among seasons were observed for the fertilization rate and developmental stages (1-cell, 2-cell, 4-cell and 8-cell) percentages.

Effect of media:

The results in Table (9) and Figure (4) showed that, Brackett's medium elevated the percentage of oocytes fertilized *in vitro* comparing with those of TCM-199 medium (31.0% and 20.7%), the differences between the two media were significant ($P<0.05$). In brackett's medium after 24 hrs post-insemination, 36 ova were fertilized, 3 (2.6%) of these were in the pronuclei, 20 (17.2%) in 2-cell and 13 (11.2%) in 4-cell stages, compared with 24 ova fertilized in TCM-199 medium, 3 (2.6%) in pronuclei, 11 (9.5%) in 2-cell, 9 (7.8%) in 4-cell and 1 (0.9%) in 8-cell stages.

Effect of osmolarity:

The fertilization rate of the oocytes cultured in media (Brackett's or TCM-199) of 305 mOsm/kg was higher (41.3%) than those cultured in media of 285 and 325 mOsm/kg (24.7% and 10.7%, respectively). The differences among varied osmolarity for fertilization rate, 2-cell and 4-cell stages percentages were significant ($P<0.05$). The percentages of fertilized oocytes cleaved to the 2-cell stage were (16.9%, 16.3% and 6.7%) for media of 285, 305 and 325 mOsm/kg, respectively, and those cleaved to the 4-cell stage were 5.2, 22.5 and 0.0% for the respective osmolarity. Fertilized oocytes cultured in media of 285 and 325 mOsm/kg failed to develop to 8-cell stage after 24 hrs post insemination , but 1.3% of fertilized oocytes cultured in media of 305 mOsm/kg were reached 8-cell stage. However, these differences were not significant. Also, osmoalrity levels had no significant effect on the 1-cell stage percentage (Table 9 and Figure 5).

Generally, the fertilized oocytes cultured in media of 285 mOsm/kg were developed to 2-cell and 4-cell stages after 24 hrs post-insemination, but these embryos were abnormal (swelling embryos). Also, media of 325 mOsm/kg developed abnormal

Table 9. Effects of season, media and osmolarity on *in vitro* fertilization rate of oocytes matured *in vitro* for untreated does.

Items	No. of oocytes[2]	No. (%)[3] Development stages 1-cell[4]	2-cell	4-cell	8-cell	Total fertilization
Season:						
Autumn	63	2 (3.2)	8 (12.7)	4 (06.3)	1 (1.6)	15 (23.8)
Winter	108	1 (0.9)	15 (13.9)	11 (10.2)	0 (0.0)	27 (25.0)
Spring	61	3 (4.9)	8 (13.1)	7 (11.5)	0 (0.0)	18 (29.5)
Media:						
Brackett's	116	3 (2.6)	20 (17.2)^{a}	13 (11.2)	0 (0.0)	36 (31.0)^{a}
TCM-199	116	3 (2.6)	11 (09.5)^{b}	9 (07.8)	1 (0.9)	24 (20.7)^{b}
Osmolarity:						
285 mOsm/kg	77	2 (2.6)	13 (16.9)^{a}	4 (05.2)^{b}	0 (0.0)	19 (24.7)^{b}
305 mOsm/kg	80	1 (1.3)	13 (16.3)^{a}	18 (22.5)^{a}	1 (1.3)	33 (41.3)^{a}
325 mOsm/kg	75	3 (4.0)	5 (06.7)^{b}	0 (00.0)^{c}	0 (0.0)	8 (10.7)^{c}
Total[1]	232	6 (2.6)	31 (13.4)	22 (09.5)	1 (0.4)	60 (25.9)

[1] The total oocytes were derived from 14 does.

[2] Number of inseminated oocytes.

[3] As a proportion of inseminated oocytes.

[4] One-cell stage means zygote or pronuclie formation.

[a,b,c] Values with different superscript within column for individual treatment are a significantly different ($P< 0.05$).

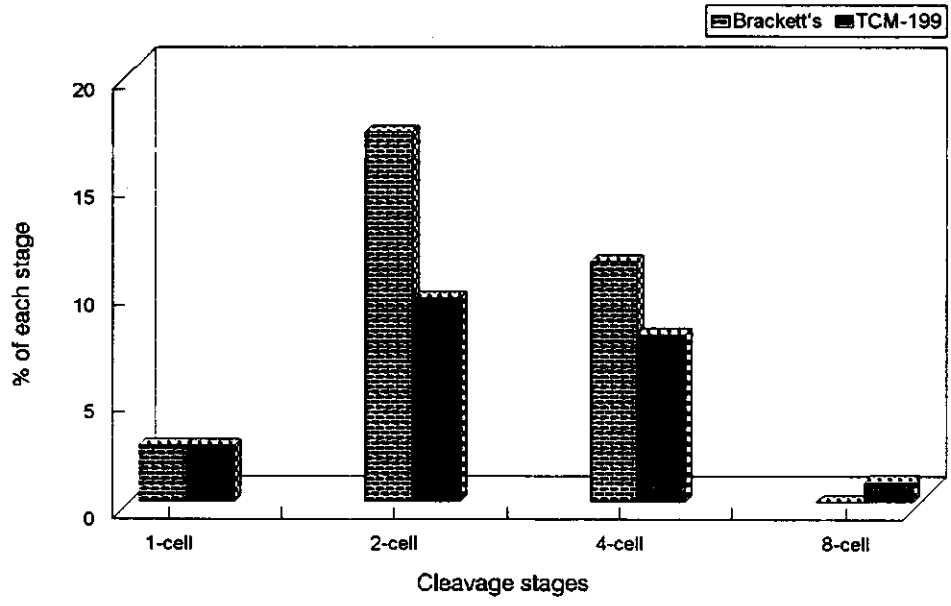

Figure 4. Effect of type of media on *in vitro* cleavage after 24 hrs post-insemination of untreated does.

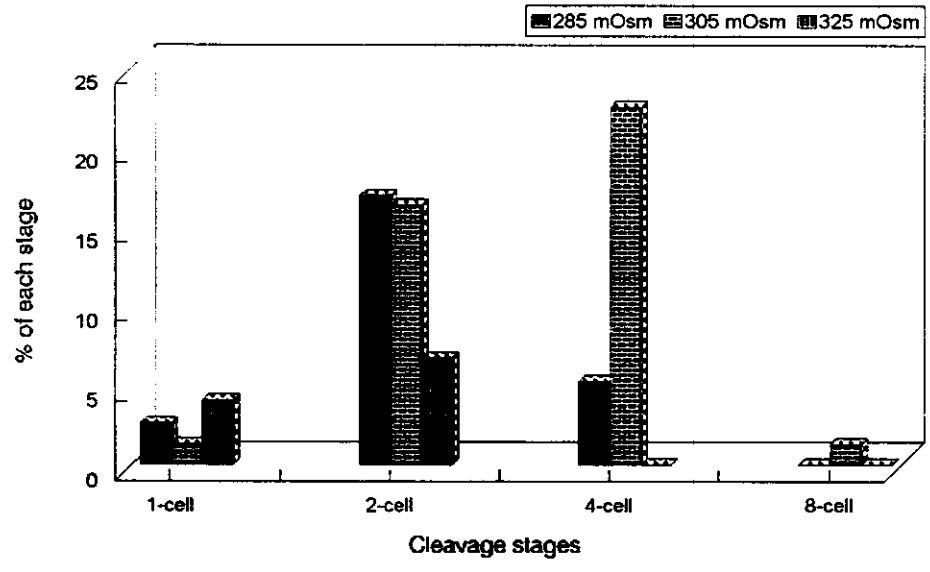

Figure 5. Effect of level ofosmolarity on *in vitro* cleavage after 24 hrs post-insemination of untreated does.

embryos (shrinking cytoplasm), but the fertilized oocytes were developed to 2-cell stage (6.7%). Normally embryos were obtained from fertilized oocytes cultured in the media of 305 mOsm/kg.

I.C.2. PMSG-treated does:

Results in Table (10) refer to the *in vitro* fertilization rate of matured oocytes collected from PMSG-treated does and developmental stages (1-cell, 2-cell, 4-cell and 8-cell) on the next day (after 24 hrs of insemination by capacitated sperm *in vitro*), as affected by season, media and osmolarity.

Effect of season:

It would be noticed that, no significant differences were observed among seasons for 1-cell, 2-cell, 4-cell and 8-cell stages. The fertilization rate was similar in autumn, winter and spring, respectively, (Table 10).

Effect of media:

The oocytes cultured and fertilized *in vitro* in Brackett's medium possessed better fertilization rate (32.2%) compared with oocytes cultured and fertilized *in vitro* in TCM-199 medium (24.3%), these differences were significant ($P<0.05$) as shown in Table (10). In Brackett's medium, of 58 fertilized ova, 4 (2.2%) were in the 1-cell, 20 (11.1%) in the 2-cell, 24 (13.3%) in the 4-cell and 10 (5.6%) in 8-cell stages compared 44 fertilized ova in TCM-199 medium, 3 (1.7%) in 1-cell, 19 (10.5%) in 2-cell, 17 (9.4%) in 4-cell and 5 (2.8%) in 8-cell stages. However, the differences between the two media for different stages of development were not significant (Table 10 and Figure 6).

Effect of osmolarity:

When the oocytes were cultured in media of 285, 305 and 325 mOsm/kg, the differences among the three levels of osmolarity were significant ($P<0.05$) for total fertilization rate, since media of 305 mOsm/kg revealed better level of osmolarity for total fertilization rate (46.8%), followed by media of 285 mOsm/kg (26.1%) and media of 325 mOsm/kg (11.0%) as shown in Table (10) and Figure (7). Thirty one fertilized oocytes cultured in the media of 285 mOsm/kg, 2 (1.7%) of these were in 1-cell, 13 (10.9%) in 2-cell, 12 (10.1%) in 4-cell and 4 (3.4%) in 8-cell stages. In media of 305 mOsm/kg, of 58 fertilized oocytes, 2 (1.6%) of these were in 1-cell, 17 (13.7%) in 2-cell, 28 (22.6%) in 4-cell and 4 (3.4%) in 8-cell stages. In media of 325 mOsm/kg, 13 ova were fertilized, 3 (2.5%) of these exhibited 1-cell, 9 (7.6%) in 2-cell and 1 (0.8%) in 4-cell stages (Table 10 and Figure 7). The differences among the three levels of osmolarity were not significant

Table 10. Effects of season, media and osmolarity on *in vitro* fertilization rate of oocytes matured *in vitro* for PMSG-treated does.

Items	No. of oocytes[2]	No. (%)[3] Development stages 1-cell[4]	2-cell	4-cell	8-cell	Total fertilization
Season:						
Autumn	46	1 (2.2)	6 (13.0)	4 (08.7)	2 (4.3)	13 (28.3)
Winter	176	5 (2.8)	17 (09.7)	20 (11.4)	8 (4.5)	50 (28.4)
Spring	139	1 (0.7)	16 (11.5)	17(12.2)	5 (3.6)	39 (28.1)
Media:						
Brackett's	180	4 (2.2)	20 (11.1)	24 (13.3)	10 (5.6)	58 (32.2)[a]
TCM-199	181	3 (1.7)	19 (10.5)	17 (09.4)	5 (2.8)	44 (24.3)[b]
Osmolarity:						
285 mOsm/kg	119	2 (1.7)	13 (10.9)	12 (10.1)[b]	4 (3.4)[b]	31 (26.1)[b]
305 mOsm/kg	124	2 (1.6)	17 (13.7)	28 (22.6)[a]	11 (8.9)[a]	58 (46.8)[a]
325 mOsm/kg	118	3 (2.5)	9 (07.6)	1 (00.8)[c]	0 (0.0)[c]	13 (11.0)[c]
Total[1]	361	7 (1.9)	39 (10.8)	41 (11.4)	15 (4.2)	102 (28.3)

[1] The total oocytes were derived from 14 does.

[2] Number of inseminated oocytes.

[3] As a proportion of inseminated oocytes.

[a,b,c] Values with different superscript within a column for individual treatment are significantly different ($P < 0.05$).

Table 11. Effects of PMSG-treatment on *in vitro* fertilization rate of oocytes matured *in vitro*.

Group[1]	No. of oocytes[2]	No. (%)[3] Development stages 1-cell[4]	2-cell	4-cell	8-cell	Total fertilization
Untreated	232	6 (2.6)	31 (13.4)	22 (09.5)	1 (0.4)[b]	60 (25.9)
Treated	361	7 (1.9)	39 (10.8)	41 (11.4)	15 (4.2)[a]	102 (28.3)

[1] The oocytes in each group were derived from 14 does.

[2] Number of inseminated oocytes.

[3] All stages were obtained after nearly 24 hrs of insemination as a proportion of inseminated oocytes.

[4] One-cell stage means zygote or pronuclei formation.

[a,b] Values with different superscript within a column are significantly different ($P < 0.05$).

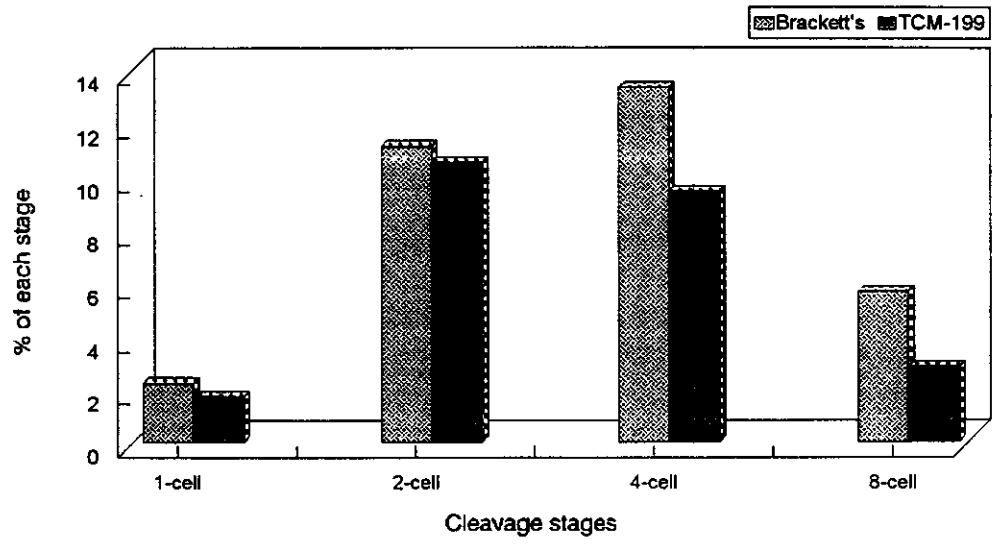

Figure 6. Effect of type of media on *in vitro* cleavage after 24 hrs post-insemination of PMSG-treated does.

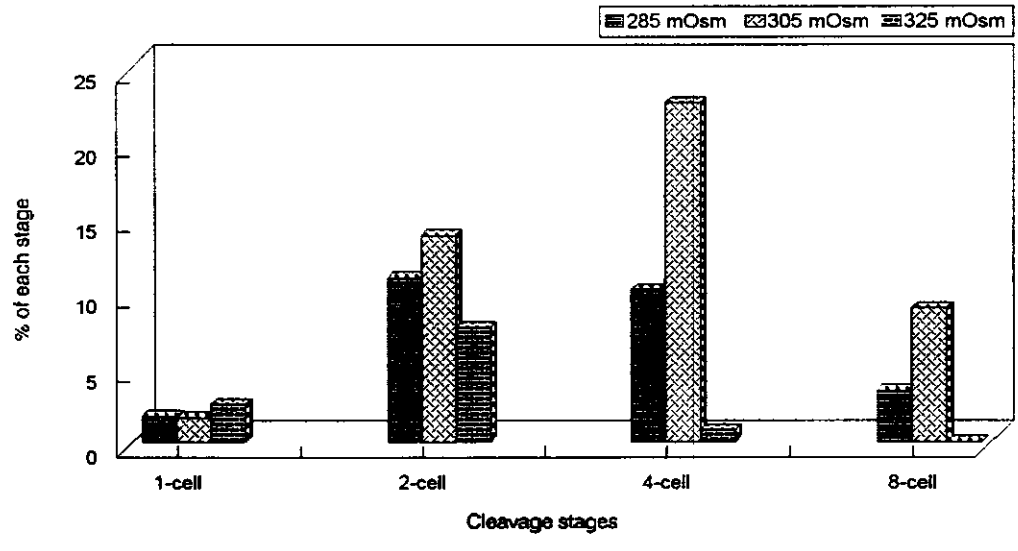

Figure 7. Effect of level of osmolarity on *in vitro* cleavage after 24 hrs post-insemination of PMSG-treated does.

for 1-cell and 2-cell stage, but significant ($P<0.05$) for 4-cell and 8-cell stages. The fertilized oocytes that cultured in media of 325 mOsm/kg developed abnormal embryos.

I.C.3. Untreated and PMSG-treated groups:

The effect of PMSG treatment on the cleavage stages percentages is presented in Table (11) and Figure (8).

PMSG treatment increased the percentage of fertilized oocytes 102 (28.3%) compared with 60 (25.9%) for oocytes collected from untreated does and the rate of change was 9.3%. However, the differences between the two groups were not significant (Table 11). 60 fertilized ova of untreated group, 6 (2.6%) of these were resumed 1-cell , 31 (13.4%) in 2-cell, 22 (9.5%) in 4-cell and 1 (0.4%) in 8-cell stages. 102 fertilized ova of PMSG-treated group, 7 (1.9%) of these were resumed 1-cell, 39 (10.8%) in 2-cell, 41 (11.4%) in 4-cell and 15 (4.2%) in 8-cell stages. However, the differences between the two groups were significant ($P<0.05$) at 8-cell stage.

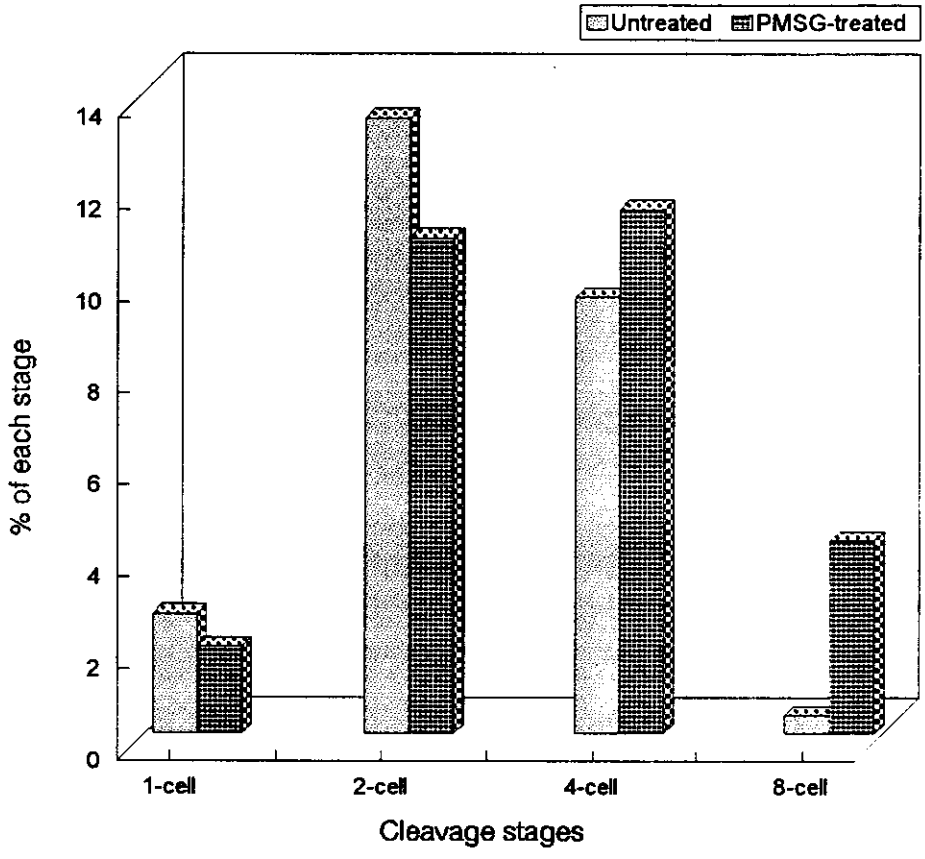

Figure 8. Comparison between untreated and PMSG-treated groups for *in vitro* cleavage of embryos after 24 hrs post-insemination.

I-D. *In vitro* development of fertilized oocytes:

I.D.1. Untreated does:

I.D.1.1. Fourty eight hours post-insemination:

Effect of media:

The viability of embryos cultured in two media (Brackett's and TCM-199) and three levels of osmolarity (285, 305 and 325 mOsm/kg) were measured as the stage of development reached 4-cell, 8-cell and 16-cell stages in addition the total cleavage percentages, also the percentage of degenerated embryos (Table 12). All percentages of development and total cleavage, also degenerated embryos were obtained as a proportion of inseminated oocytes.

Results in Table (12) indicated that Brackett's medium elevated total cleavage percentage (20.7%) of fertilized oocytes than those cultured in TCM-199 medium (12.1%). Significant differences ($P<0.05$) were observed between the two media for the total cleavage percentage. Greater cleavage rate of 4-cell stage was observed for inseminated oocytes that fertilized *in vitro* and cultured in Brackett's medium than those cultured in TCM-199 medium (12.1% vs. 3.4%), the differences were significant ($P<0.05$). Also, Brackett's medium enhanced the embryos to develop to 8-cell stage (8.6%) compared with TCM-199 medium (6.9%), however, the differences were not significant (Table 12). TCM-199 medium enhanced non-significantly the development of embryos to 16-cell stage, but Brackett's medium failed to develop embryos to this stage (1.7% vs. 0.0%).

Effect of osmolarity:

Table (12) shows that media of 305 mOsm/kg is the optimal osmolarity for total cleavage rate (32.5%), compared with media of 285 mOsm/kg (11.7%) and media of 325 mOsm/kg (4.0%). The differences among the three levels of osmolarity were significant ($P<0.05$). In media of 305 mOsm/kg, 10% of fertilized oocytes reached 4-cell stage after 48 hrs post-insemination, whereas, these percentages were 9.1 and 4.0% for those fertilized in media of 285 and 325 mOsm/kg, respectively. A percentage of inseminated oocytes that fertilized and cultured *in vitro* in media of 305 mOsm/kg reached 8-cell stage after 48 hrs of insemination was significantly ($P<0.05$) higher (20%) than 2.6 and 0.0% for media of 285 and 325 mOsm/kg, respectively. The media of 305 mOsm/kg enhanced 2.5% of inseminated oocytes that fertilized *in vitro* to develop to 16-cell stage, whereas, inseminated oocytes that fertilized *in vitro* and cultured in the media of 285 or 325 mOsm/kg failed to reach this stage of development (Table 12).

I.D.1.2. Seventy-two hours post-insemination:

The results in Table (13) show the developmental capacity of *in vitro* fertilized ova cultured in different media of varied osmolarity, collected from untreated does.

Effect of media:

Although, the degenerated embryos percentage of fertilized oocytes cultured in Brackett's medium was higher (5.2%) than those of TCM-199 medium (3.4%), Brackett's medium had higher percentage of total cleavage compared with those of TCM-199 medium (15.5 vs. 8.6%). The differences between the two media were not significant (Table 13).

The percentage of fertilized ova developed to 8-cell stage in Brackett's medium was significantly ($P<0.05$) higher than those cultured in TCM-199 medium (8.6 vs. 1.7%). No significant differences were observed between Brackett's and TCM-199 media for embryos developed to 16-cell stage (6.9 vs. 6.0%). TCM-199 medium enhanced 0.9% embryos to develop to ~32-cell stage, however, Brackett's medium failed to support embryos to reach this stage (Table 13).

Effect of osmolarity:

Fertilized ova cultured in media of 305 mOsm/kg reached to 16-cell and ~32-cell (morula) stages by 72 hrs post-insemination, however, media of 285 or 325 mOsm/kg did not enhance developmental capacity of *in vitro* fertilized oocytes. The differences among the three levels of osmolarity were significant ($P<0.05$) for total cleavage percentage (Table 13). Media of 285 and 325 mOsm/kg, delayed the cleavage of fertilized oocytes, by 72 hrs post-insemination the embryos reached 8-cell stage only. However, 8.8% of embryos were in 8-cell stage in media of 305 mOsm/kg.

I.D.1.3. Ninety six hours post-insemination:

The results in Table (14) show the developmental capacity of *in vitro* fertilized ova cultured in different media of varied osmolarity for untreated does.

Effect of media:

After 96 hrs post-insemination, 9.5% embryos in Brackett's medium cleaved, 4.3% were in 16-cell stage, 4.3% in ~32-cell stage and 0.9% in early blastocyst, but in TCM-199 medium, the respective percentages were 5.2%, 0.0%, 3.4% and 1.7% (Table 14). The differences between the two media for total cleavage percentage and at different stages of development except at 16-cell stage were not significant.

Table 12. Development of *in vitro* fertilized ova after 48 hrs of insemination cultured in different media and osmolarity for untreated does.

Items	No. of oocytes	No. (%) deg.embryos[2]	No. (%)[3] of oocytes developed to: 4-cell	8-cell	16-cell	Total cleavage
Media:[1]						
Brackett's	116	12 (10.3)	14 (12.1)[a]	10 (8.6)	0 (0.0)	24 (20.7)[a]
TCM-199	116	10 (08.6)	4 (03.4)[b]	8 (6.9)	2 (1.7)	14 (12.1)[b]
Osmolarity:						
285 mOsm/kg	77	10 (13.0)	7 (09.1)	2 (02.6)[b]	0 (0.0)	9 (11.7)[b]
305 mOsm/kg	80	7 (08.8)	8 (10.0)	16 (20.0)[a]	2 (2.5)	26 (32.5)[a]
325 mOsm/kg	75	5 (06.7)	3 (04.0)	0 (00.0)[b]	0 (0.0)	3 (04.0)[c]

Table 13. Development of *in vitro* fertilized ova after 72 hrs of insemination cultured in different media and osmolarity for untreated does.

Items	No. of oocytes	No. (%) deg.embryos[2]	No. (%)[3] of oocytes developed to: 8-cell	16-cell	~32-cell	Total cleavage
Media:[1]						
Brackett's	116	6 (5.2)	10 (8.6)[a]	8 (6.9)	0 (0.0)	18 (15.5)
TCM-199	116	4 (3.4)	2 (1.7)[b]	7 (6.0)	1 (0.9)	10 (08.6)
Osmolarity:						
285 mOsm/kg	77	5 (6.5)	4 (5.2)[ab]	0 (00.0)[b]	0 (0.0)	4 (05.2)[b]
305 mOsm/kg	80	3 (3.8)	7 (8.8)[a]	15 (18.8)[a]	1 (1.3)	23 (28.8)[a]
325 mOsm/kg	75	2 (2.7)	1 (1.3)[b]	0 (00.0)[b]	0 (0.0)	1 (01.3)[c]

[1] The oocytes cultured in both media were derived from 14 does.

[2] Degenerated embryos (calculated as: total cleavage of previous day minus total cleavage of the present day).

[3] As a proportion of inseminated oocytes.

[a,b,c] Values within columns for individual treatment with different superscripts differ significantly ($P< 0.05$).

Effect of osmolarity:

Only embryos cultured in media of 305 mOsm/kg were continued development to 16-cell, ~32-cell (morula) and early blastocyst stages (6.3, 11.3, and 3.8%, respectively), as shown in Table (14). All embryos cultured in media of 285 mOsm/kg were ruptured (may be due to the relatively lower salt concentration), while all embryos cultured in media of 325 mOsm/kg were shrunk (may be due to the higher concentration of the salt in the media).

I.D.1.4. One hundred and twenty hours post-insemination:

The developmental stages of fertilized oocytes *in vitro* (collected from untreated does, matured *in vitro*, inseminated with capacitated sperm *in vitro*) cultured in different media and varied osmolarity after 120 hrs of insemination are presented in Table (15).

Effect of media:

The differences between the two media for the total cleavage percentage and at different stages of development were not significant, however, the percentage of total cleavage was slightly higher in TCM-199 medium (2.6%) compared with (1.7%) for Brackett's medium. But the number and percentage of degenerated embryos were higher in Brackett's medium than TCM-199 medium (7.8 vs. 2.6%), as shown in Table (15). In Brackett's medium, only 1 embryo was developed to early blastocyst and another embryo was developed to blastocyst stage. In TCM-199 medium, 2 embryos were developed to early blastocyst and 1 embryo was developed to balstocyst stage.

Effect of osmolarity:

Only the media of 305 mOsm/kg were enhanced the embryos to develop to early blastocyst and blastocyst stages (3.8 and 2.5%, respectively) and total cleavage percentage was 6.3%. The degenerated embryos were increased to 15.0% after 120 hrs post-insemination (Table 15).

I.D.1.5. One hundred and fourty four hours post-insemination:

After 144 hrs post-insemination, it was noticed that all fertilized oocytes cultured in Brackett's or TCM-199 media of 305 mOsm/kg failed to develop beyond early blastocyst and blastocyst stages.

Table 14. Development of *in vitro* fertilized ova after 96 hrs of insemination cultured in different media and osmolarity for untreated does.

Items	No. of oocytes	No. (%) deg.embryos[2]	No. (%)[3] of oocytes developed to: 16-cell	~32-cell	E.B.[4]	Total cleavage
Media:[1]						
Brackett's	116	7 (6.0)	5 (4.3)[a]	5 (4.3)	1 (0.9)	11 (9.5)
TCM-199	116	4 (3.4)	0 (0.0)[b]	4 (3.4)	2 (1.7)	6 (5.2)
Osmolarity:						
285 mOsm/kg	77	4 (5.2)[ab]	0 (0.0)[b]	0 (00.0)[b]	0 (0.0)[b]	0 (00.0)[b]
305 mOsm/kg	80	6 (7.5)[a]	5 (6.3)[a]	9 (11.3)[a]	3 (3.8)[a]	17 (21.3)[a]
325 mOsm/kg	75	1 (1.3)[b]	0 (0.0)[b]	0 (00.0)[b]	0 (0.0)[b]	0 (00.0)[b]

Table 15. Development of *in vitro* fertilized ova after 120 hrs of insemination cultured in different media and osmolarity for untreated does.

Items	No. of oocytes	No. (%) deg.embryos[2]	No. (%)[3] of oocytes developed to: E.B.[4]	Blastocyst	Total cleavage
Media:[1]					
Brackett's	116	9 (7.8)[a]	1 (0.9)	1 (0.9)	2 (1.7)
TCM-199	116	3 (2.6)[b]	2 (1.7)	1 (0.9)	3 (2.6)
Osmolarity:					
285 mOsm/kg	77	0 (00.0)[b]	0 (0.0)[b]	0 (0.0)	0 (0.0)[b]
305 mOsm/kg	80	12 (15.0)[a]	3 (3.8)[a]	2 (2.5)	5 (6.3)[a]
325 mOsm/kg	75	0 (00.0)[b]	0 (0.0)[b]	0 (0.0)	0 (0.0)[b]

[1] The oocytes cultured in both media were derived from 14 does.

[2] Degenerated embryos (calculated as: total cleavage of previous day minus total cleavage of the present day).

[3] As a proportion of inseminated oocytes.

[4] Early blastocyst.

[a,b] Values within columns for individual treatment with different superscripts differ significantly ($P< 0.05$).

I.D.1.6. Developmental Capacity of untreated does:

Results summarized in Table (16) reveal the effect of media on *in vitro* fertilization rate and developmental stages (morula, early blastocyst and blastocyst) percentages during different periods of incubation. All percentages were calculated as a proportion of fertilized oocytes.

Effect of media:

Brackett's medium had a significantly ($P< 0.05$) higher fertilization rate than that of TCM-199 medium (31.0% vs. 20.7%). After 72-96 hrs of incubation, Brackett's and TCM-199 media developed 13.9% and 20.8% of fertilized oocytes to reach morula stage, respectively. After 96-120 hrs and at 120 hrs of incubation, the percentages of fertilized oocytes cultured in Brackett's medium and developed to early balstocyst and blastocyst stages were 5.6% and 2.8%, respectively, compared with 16.7% and 4.2%, respectively, for those cultured in TCM-199 medium. These differences between the two media were not significant (Table 16 and Figure 9). TCM-199 medium was superior to Brackett's medium for developmental capacity.

Effect of osmolarity:

A number of 33 out of 80 inseminated oocytes (41.3%) were fertilized in the media of 305 mOsm/kg, and the percentages developed to morula, early blastocyst and blastocyst were 30.3%, 18.2% and 6.1%, respectively, (Table 16). The other two levels of osmolarity (285 mOsm/kg and 325 mOsm/kg) failed to bring the fertilized oocytes to morula stage.

I.D.1.7. Developmental periods for untreated does:

The results summarized in Table (17) and Figure (10) show the total cleavage of fertilized oocytes (as a proportion of fertilized oocytes) from 48-144 hrs post-insemination, collected from untreated does, matured *in vitro*, inseminated with capacitated sperm *in vitro* and then cultured in different media (Brackett's and TCM-199).

The total cleavage rate was decreased by increasing the time of incubation in Brackett's or TCM-199 medium. The total cleavage percentage decreased from 66.7% after 48 hrs of insemination to 5.6% after 120 hrs of insemination in Brackett's medium, but these percentages were 58.3% and 12.5% at the same intervals in TCM-199 medium. Between 72 and 96 hrs post-insemination, Brackett's medium had higher degenerated embryos percentage than TCM-199 medium (19.4% vs. 16.7%, respectively). Similarly, between 96 and 120 hrs post-insemination, the degenerated embryos percentage reached 25.0% in Brackett's medium, but only 12.5% in TCM-199 medium (Table 17).

Table 16. Development of *in vitro* fertilized ova from morula to blastocyst stages cultured in different media of 305 mOsm/kg for untreated does.

Items	No. of oocytes inseminated	No. (%) oocytes fertilized	No. (%)[2] of oocytes developed to: Morula 72-96 hrs	Early blastocyst 96-120 hrs	Blastocyst 120 hrs
Media:[1]					
Brackett's	116	36 (31.0)[a]	5 (13.9)	2 (05.6)	1 (2.8)
TCM-199	116	24 (20.7)[b]	5 (20.8)	4 (16.7)	1 (4.2)
Osmolarity:					
305 mOsm/kg	80	33 (41.3)	10 (30.3)	6 (18.2)	2 (6.1)

[1] The oocytes in both media were derived from 14 does.

[2] As a proportion of fertilized oocytes.

[a,b] Values with different superscript within row are significantly different (P< 0.05).

Table 17. Development of fertilized ova cultured in different media ,collected from untreated does from 24-144 hrs after insemination as total cleavage.

Periods after insemination (hrs)	Brackett's No.	Total cleavage no.	(%)	TCM-199 No.	Total cleavage no.	(%)
24	116*	36	(31.0)[1 a]	116*	24	(20.7)[1 b]
48	36**	24	(66.7)[2]	24**	14	(58.3)[2]
72	36	18	(50.0)	24	10	(41.7)
96	36	11	(30.6)	24	06	(25.0)
120	36	02	(05.6)	24	03	(12.5)
144	36	00	(00.0)	24	00	(00.0)

* Number of inseminated oocytes (116 oocytes for each midium).

** Number of fertilized oocytes (36 oocytes in Brackett's and 24 oocytes in TCM-199 media).

[1] As a proportion of inseminated oocytes.

[2] As a proportion of fertilized oocytes.

[a,b] value within row with different superscripts differ significantly (P< 0.05).

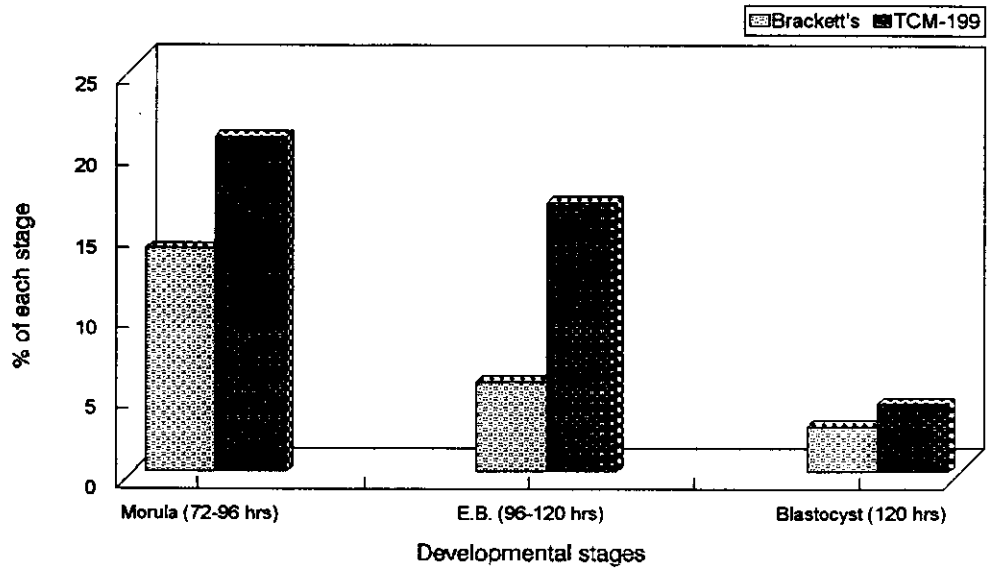

Figure 9. Effect of type of media on developmental capacity of *in vitro* matured and fertilized oocytes from 72-144 hrs post-insemination of untreated does.
E.B.=Early blastocyst.

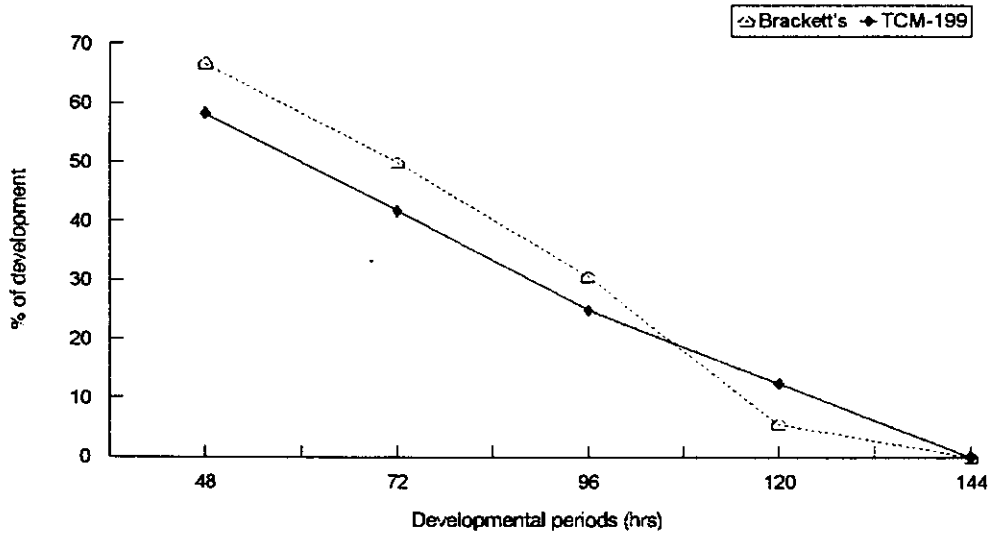

Figure 10. Effect of type of media on *in vitro* development of in vitro matured and fertilized rabbit oocytes from 48-144 hrs post-insemination of untreated does.

I.D.2. PMSG-treated does:

I.D.2.1. Fourty eight hours post-insemination:

In vitro development to 4-cell, 8-cell, 16-cell stages and beyond for rabbit follicular oocytes (PMSG-treated does) matured and fertilized *in vitro* after 48 hrs of insemination is given in Table (18). All percentages were calculated as a proportion of inseminated oocytes.

Effect of media:

After 48 hrs post-insemination (Table 18), Brackett's medium enhanced the fertilized oocytes to develop to different stages of development (4-cell, 8-cell and 16-cell), at a higher percentage (9.6, 10.3 and 5.1%, respectively) compared with those of TCM-199 medium (2.5, 8.9 and 3.2% , respectively), however, the differences were not significant. Total cleavage percentage was improved in Brackett's medium than those in TCM-199 medium (25.0% vs. 14.6%), the difference between the two media was significant ($P<0.05$). The percentages of degenerated embryos were 8.3% and 9.6% for Brackett's and TCM-199 media, respectively, (Table 18).

Effect of osmolarity:

Media of 305 mOsm/kg had the highest total cleavage percentage (37.0%) than those of 285 mOsm/kg and 325 mOsm/kg (18.4% and 2.9%, respectively), the differences between levels of osmolarity were significant ($P<0.05$) as shown in Table (18). In media of 285 mOsm/kg, of 19 (18.4%) cleaved ova, 10 (9.7%) were in 4-cell, 8 (7.8%) in 8-cell and 1 (1.0%) in 16-cell stages. In media of 305 mOsm/kg, of 40 (37.0%) cleaved ova, 6 (5.6%) were in 4-cell, 22 (20.4%) in 8-cell and 12 (11.1%) in 16-cell stages. Media of 325 mOsm/kg decreased the total cleavage percentage, and failed to support the fertilized oocytes to reach 8-cell stage. The percentage of degenerated embryos was nearly similar in the media of 305 and 325 mOsm/kg (8.3% and 8.8%, respectively), but was high in the media of 285 mOsm/kg (9.7%).

I.D.2.2. Seventy two hours post-insemination:

Results in Table (19) refer to the developmental stages for fertilized oocytes *in vitro*, total cleavage and degenerated embryos percentages after 72 hrs of insemination, cultured in two different media of varied osmolarity.

Effect of media:

Although, the degenerated embryos percentage was significantly ($P< 0.05$) higher in Brackett's medium than those in TCM-199 medium (10.9% vs. 3.8%), the total cleavage

Table 18. Development of *in vitro* fertilized ova after 48 hrs of insemination cultured in different media and osmolarity for PMSG-treated does.

Items	No. of oocytes	No. (%) deg.embryos[2]	No. (%)[3] of oocytes developed to: 4-cell	8-cell	16-cell	Total cleavage
Media:[1]						
Brackett's	156	13 (8.3)	15 (9.6)	16 (10.3)	8 (5.1)	39 (25.0)[a]
TCM-199	157	15 (9.6)	4 (2.5)	14 (08.9)	5 (3.2)	23 (14.6)[b]
Osmolarity:						
285 mOsm/kg	103	10 (9.7)	10 (09.7)[a]	8 (07.8)[b]	1 (01.0)[b]	19 (18.4)[b]
305 mOsm/kg	108	9 (8.3)	6 (05.6)[b]	22 (20.4)[a]	12 (11.1)[a]	40 (37.0)[a]
325 mOsm/kg	102	9 (8.8)	3 (02.9)[b]	0 (00.0)[c]	0 (00.0)[b]	3 (02.9)[c]

Table 19. Development of *in vitro* fertilized ova after 72 hrs of insemination cultured in different media and osmolarity for PMSG-treated does.

Items	No. of oocytes	No. (%) deg.embryos[2]	No. (%)[3] of oocytes developed to: 8-cell	16-cell	~32-cell	Total cleavage
Media:[1]						
Brackett's	156	17 (10.9)[a]	6 (3.8)	11 (7.1)	5 (3.2)	22 (14.1)
TCM-199	157	6 (03.8)[b]	2 (1.3)	10 (6.4)	5 (3.2)	17 (10.8)
Osmolarity:						
285 mOsm/kg	103	13 (12.6)[a]	4 (3.9)[a]	2 (01.9)[b]	0 (0.0)[b]	6 (05.8)[b]
305 mOsm/kg	108	7 (06.5)[ab]	4 (3.7)[a]	19 (17.6)[a]	10 (9.3)[a]	33 (30.6)[a]
325 mOsm/kg	102	3 (02.9)[b]	0 (0.0)[b]	0 (00.0)[c]	0 (0.0)[b]	0 (00.0)[c]

[1] The oocytes cultured in both media were derived from 12 does.

[2] Degenerated embryos (calculated as: total cleavage of previous day minus total cleavage of the present day).

[3] As a proportion of inseminated oocytes.

[a,b,c] Values within columns for individual treatment with different superscripts differ significantly ($P< 0.05$).

and developmental stages (8-cell and 16-cell) percentages of fertilized oocytes cultured in Brackett's medium was not significantly higher than those in TCM-199 medium, however, embryos developed to ~32-cell stage were similar for both media (3.2%) as presented in Table (19).

Effect of osmolarity:

After 72 hrs post-insemination, of 33 (30.6%) ova that were cleaved in media of 305 mOsm/kg; but in media of 285 mOsm/kg, the number and percentage were 6 (5.8%), as shown in Table (19). The differences between media of 285 mOsm/kg and 305 mOsm/kg were significant ($P<0.05$) at 16-cell, ~32-cell stages and total cleavage percentage. The embryos cultured in media of 285 mOsm/kg failed to reach ~32-cell stage. The percentage of degenerated embryos was highest in media of 285 mOsm /kg (12.6%) than those of 305 mOsm/kg (6.5%) and of 325 mOsm/kg (2.9%). After 72 hrs post-insemination, all embryos in media of 325 mOsm/kg were shrunk and degenerated (Table 19).

I.D.2.3. Ninety six hours post-insemination:

Results in Table (20) show the developmental stages of fertilized oocytes *in vitro*, total cleavage and degenerated embryos after 96 hrs post-insemination, cultured in two different media of varied osmolarity.

Effect of media:

Results summarized in Table (20) show that, no significant differences were observed between Brackett's and TCM-199 media after 96 hrs post-insemination for total cleavage percentage, developmental stages (16-cell, ~32-cell and early blastocyst) and the degenerated embryos percentage. In Brackett's medium, of 13 (8.3%) cleaved ova, 3 (1.9%) were in 16-cell, 7 (4.5%) in ~32-cell and 3 (1.9%) in early blastocyst stages, but these values in TCM-199 medium were 11 (7.0%), 1 (0.6%), 6 (3.8%) and 4 (2.5%), respectively.

Effect of osmolarity:

Only media of 305 mOsm/kg enhanced embryos to develop to 16-cell, ~32-cell and early blastocyst (3.7%, 12.0% and 6.5%, respectively). After 96 hrs post-insemination, all embryos cultured in media of 285 mOsm/kg were ruptured and failed to continue development (Table 20).

Table 20. Development of *in vitro* fertilized ova after 96 hrs of insemination, cultured in different media and osmolarity for PMSG-treated does.

Items	No. of oocytes	No. (%) degenerated embryos[2]	No. (%)[3] of oocytes developed to: 16-cell	~32-cell	Early bastocyst	Total cleavage
Media: [1]						
Brackett's	156	9 (5.8)	3 (1.9)	7 (4.5)	3 (1.9)	13 (8.3)
TCM-199	157	6 (3.8)	1 (0.6)	6 (3.8)	4 (2.5)	11 (7.0)
Osmolarity:						
285 mOsm/kg	103	6 (5.8)^{a}	0 (0.0)^{b}	0 (00.0)^{b}	0 (0.0)^{b}	0 (00.0)^{b}
305 mOsm/kg	108	9 (8.3)^{a}	4 (3.7)^{a}	13 (12.0)^{a}	7 (6.5)^{a}	24 (22.2)^{a}
325 mOsm/kg	102	0 (0.0)^{b}	0 (0.0)^{b}	0 (00.0)^{b}	0 (0.0)^{b}	0 (00.0)^{b}

[1] The oocytes cultured in both media were derived from 12 does.

[2] Calculated as: total cleavage of previous day minus total cleavage of present day.

[3] As a proportion of inseminated oocytes.

[a,b] Values within columns for individual treatment with different superscripts differ significantly ($P< 0.05$).

I.D.2.4. One hundred and twenty hours post-insemination:

Results in Table (21) reveal the developmental stages of fertilized oocytes *in vitro* (early blastocyst and blastocyst), total cleavage and degenerated embryos, cultured in two different media and varied osmolarity after 120 hrs post-insemination.

Effect of media:

The numbers and percentages of total cleavage were similar 6 (3.8%) for fertilized oocytes cultured in Brackett's or TCM-199 medium. Also, no significant differences were observed between media for the percentage of degenerated embryos (4.5% for Brackett's and 3.2% for TCM-199 media), as shown in Table (21). In Brackett's medium, of 6 (3.8%) total cleavage, 4 (2.6%) were in early blastocyst and 2 (1.3%) in blastocyst stages. Similarly, in TCM-199 medium of 6 (3.8%) total cleavage, 3 (1.9%) were in early blastocyst and 3 (1.9%) in blastocyst stages.

Effect of osmolarity:

Total cleavage percentage was decreased to 11.1% after 120 hrs post-insemination in the media of 305 mOsm/kg. While, the degenerated embryos percentage was increased to 11.1% (Table 21). The number and percentage of embryos developed to early blastocyst and blastocyst stages were 7 (6.5%) and 5 (4.6%). respectively.

I.D.2.5. One hundred and fourty four hours post-insemination:

In vitro development to blastocyst stage for rabbit follicular oocytes matured and fertilized *in vitro* after 144 hrs post-insemination are presented in Table (22).

Effect of media:

Only 0.6% of fertilized oocytes cultured up to 6 days in Brackett's medium were developed to blastocyst stage compared with 1.3% for TCM-199 medium, the differences were not significant. The degenerated embryos percentages were 3.2 and 2.5% in Brackett's and TCM-199 media, respectively, (Table 22).

Effect of osmolarity:

Irrespect to the media, fertilized oocytes continued the development to blastocyst stage (2.8%) in 305 mOsm/kg level as shown in Table (22).

I.D.2.6. Developmental capacity for PMSG-treated does:

Results summarized in Table (23) and Figure (11) reveal the effect of media on *in vitro* fertilization rate and developmental stages (morula, early blastocyst and blastocyst) percentages. All the percentages were calculated as a proportion of fertilized oocytes.

Table 21. Development of *in vitro* fertilized ova after 120 hrs of insemination cultured in different media and osmolarity for PMSG-treated does.

Items	No. of oocytes	No. (%) deg.embryos[2]	No. (%)[3] of oocytes developed to : E.B.[4]	Blastocyst	Total cleavage
Media:[1]					
Brackett's	156	7 (4.5)	4 (2.6)	2 (1.3)	6 (3.8)
TCM-199	157	5 (3.2)	3 (1.9)	3 (1.9)	6 (3.8)
Osmolarity:					
285 mOsm/kg	103	0 (00.0)^{b}	0 (0.0)^{b}	0 (0.0)^{b}	0 (00.0)^{b}
305 mOsm/kg	108	12 (11.1)^{a}	7 (6.5)^{a}	5 (4.6)^{a}	12 (11.1)^{a}
325 mOsm/kg	102	0 (00.0)^{b}	0 (0.0)^{b}	0 (0.0)^{b}	0 (00.0)^{b}

Table 22. Development of *in vitro* fertilized ova after 144 hrs of insemination cultured in different media and osmolarity for PMSG-treated does.

Items	No. of oocytes	No. (%) deg.embryos[2]	No. (%)[3] of oocytes developed to : Blastocyst	Total cleavage
Media:[1]				
Brackett's	156	5 (3.2)	1 (0.6)	1 (0.6)
TCM-199	157	4 (2.5)	2 (1.3)	2 (1.3)
Osmolarity:				
285 mOsm/kg	103	0 (0.0)^{b}	0 (0.0)^{b}	0 (0.0)^{b}
305 mOsm/kg	108	9 (8.3)^{a}	3 (2.8)^{a}	3 (2.8)^{a}
325 mOsm/kg	102	0 (0.0)^{b}	0 (0.0)^{b}	0 (0.0)^{b}

[1] The oocytes cultured in both media were derived from 12 does.

[2] Degenerated embryos (calculated as: total cleavage of previous day minus total cleavage of present day).

[3] As a proportion to inseminated oocytes.

[4] Early blastocyst.

[a,b] Values within columns for individual treatment with different superscripts differ significantly ($P< 0.05$).

Effect of media:

Brackett's medium had a significantly ($P<0.05$) higher fertilization percentage than that of TCM-199 medium (33.3% vs. 24.2%). After 72-96 hrs of *in vitro* insemination, Brackett's medium developed 23.1% of fertilized oocytes to reach morula stage, but TCM-199 medium enhanced higher percentage (28.9%), this difference was not significant. Also, after 96-120 and 120-144 hrs of *in vitro* insemination, TCM-199 medium enhanced fertilized oocytes to develop to early blastocyst and blastocyst stages higher than Brackett's medium (18.4 and 13.2% vs. 13.5 and 5.8%), these differences between the two media were not significant (Table 23).

Effect of osmolarity:

Irrespect to the type of media, a number of 49 out of 108 inseminated oocytes (45.4%) were fertilized in the media of 305 mOsm/kg, and the percentage of fertilized oocytes that developed to morula, early blastocyst and blastocyst were 46.9%, 28.6% and 16.3%, respectively, (Table 23). However, the other two levels of osmolarity (285 mOsm/kg and 325 mOsm/kg) failed to bring the fertilized oocytes to morula stage.

I.D.2.7. Developmental periods for PMSG-treated does:

The results summarized in Table (24) and Figure (12) show the total cleavage of fertilized oocytes, between 48-144 hrs post-insemination, collected from PMSG-treated does, matured *in vitro*, inseminated with capacitated sperm *in vitro* and then cultured in different media (Brackett's and TCM-199) of different levels of osmolarity.

The total cleavage percentages (as a proportion of fertilized oocytes) were decreased by increasing the time of incubation in Brackett's or TCM-199 medium. The percentage of total cleavage decreased from 75.0% after 48 hrs to 1.9% after 144 hrs post-insemination in Brackett's medium, but these percentages were 60.5% and 5.3%, respectively, in TCM-199 medium. Generally, after 48 hrs post-insemination, Brackett's medium had significantly higher total cleavage percentage than TCM-199 medium (75.0% vs. 60.5%).

In Brackett's medium the total cleavage decreased rapidly by 32.7% between 48 and 72 hrs post-insemination, but this percentage was 15.8% in TCM-199 medium. After 72 hrs post-insemination, total cleavage percentage in TCM-199 medium was slightly higher than those of Brackett's medium (44.7 vs. 42.3%). The same trend of superiority of TCM-199 to Brackett's medium was continued up to 6 days post-insemination (Table 24).

Table 23. Development of *in vitro* fertilized ova from morula to blastocyst stages cultured in different media of 305 mOsm/kg for PMSG-treated does.

Items	No. of oocytes inseminated	No. (%) oocytes fertilized	No. (%)[2] of oocytes developed to: Morula 72-96 hrs	Early blastocyst 96-120 hrs	Blastocyst 120-144 hrs
Media: [1]					
Brackett's	156	52 (33.3)[a]	12 (23.1)	7 (13.5)	3 (05.8)
TCM-199	157	38 (24.2)[b]	11 (28.9)	7 (18.4)	5 (13.2)
Osmolarity:					
305 mOsm/kg	108	49 (45.4)	23 (46.9)	14 (28.6)	8 (16.3)

[1] The oocytes cultured in both media were derived from 12 does.

[2] As a proportion of fertilized oocytes.

[a,b] Values within columns for individual treatment with different superscripts differ significantly ($P< 0.05$).

Table 24. Development of fertilized ova from 24-144 hrs after insemination as total cleavage for PMSG-treated does.

Periods after insemination (hrs)	Brackett's medium			TCM-199 medium		
	No.	Total cleavage no.	(%)	No.	Total cleavage no.	(%)
24	156*	52	(33.3)[1a]	157*	38	(24.2)[1b]
48	52**	39	(75.0)[2a]	38**	23	(60.5)[2b]
72	52	22	(42.3)	38	17	(44.7)
96	52	13	(25.0)	38	11	(28.9)
120	52	06	(11.5)	38	06	(15.8)
144	52	01	(01.9)	38	02	(05.3)

* Number of inseminated oocyte (156 in Brackett's and 157 in TCM-199 media).

** Number of fertilized oocyte (52 in Brackett's and 38 in TCM-199 media).

[1] As a proportion of inseminated oocytes.

[2] As a proportion of fertilized oocytes.

[a,b] Values with different superscript within row are significantly different ($P< 0.05$).

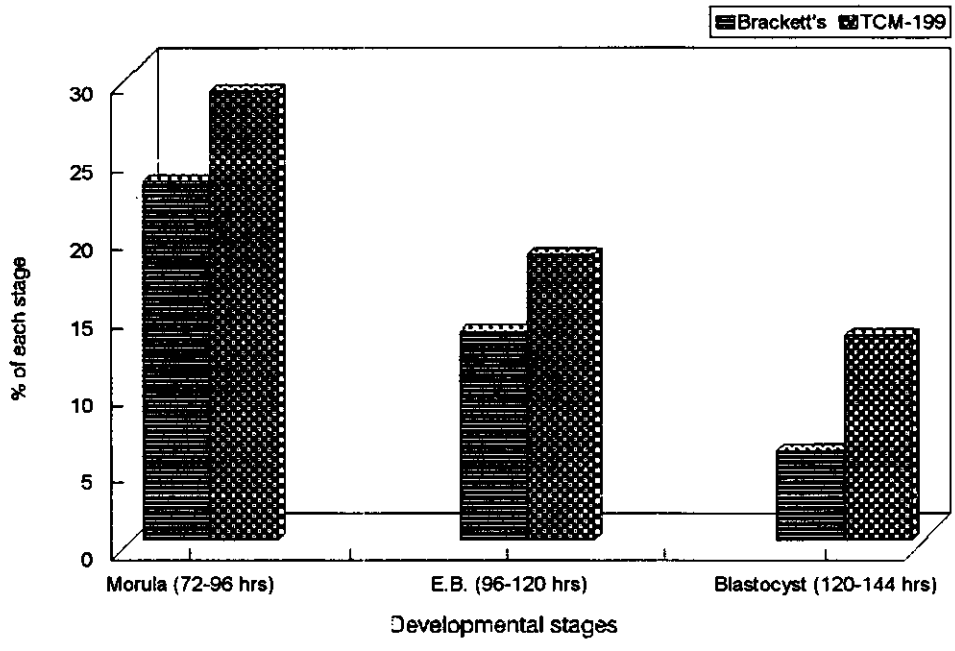

Figure11.Effect of type of media on developmental capacity of *in vitro* matured and fertilized oocytes from 72-144 hrs post-insemination of PMSG-treated does.
E.B. = Early blastocyst.

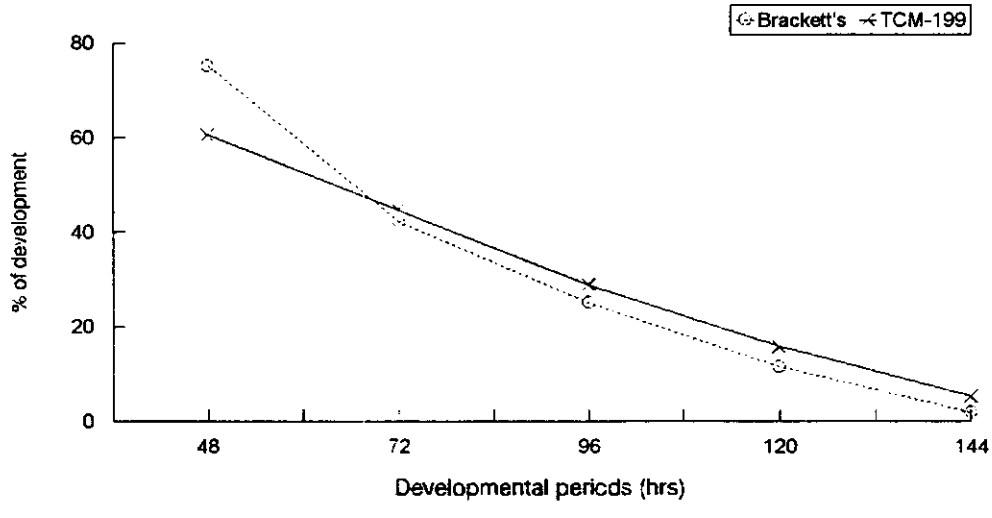

Figure 12. Effect of type of media on *in vitro* development of in vitro matured and fertilized rabbit oocytes from 48-144 post-insemination of PMSG-treated does.

D.3. Untreated and PMSG-treated groups:

I.D.3.1. Fourty eight hours post-insemination:

Results presented in Table (25) show the developmental stages of *in vitro* fertilized oocytes after 48 hrs post-insemination for untreated and PMSG-treated groups.

After 48 hrs of insemination and incubation, 38 (16.4%) fertilized oocytes out of 232 inseminated oocytes of untreated group and 62 (19.8%) out of 313 inseminated oocytes of PMSG-treated group were cleaved to 4-cell, 8-cell and 16-cell stages. In untreated group, of 38 (16.4%) cleaved ova, 18 (7.8%) were in 4-cell, 18 (7.8%) in 8-cell and 2 (0.9%) in 16-cell stages. These values were 62 (19.8%), 19 (6.1%), 30 (9.6%) and 13 (4.2%), respectively, for the PMSG-treated group (Table 25). Fertilized oocytes of PMSG-treated group maintained significantly ($P<0.05$) higher 16-cell stage percentage (4.2%) than those of untreated group (0.9%).

I.D.3.2. Seventy two hours post-insemination:

After 72 hrs of insemination and incubation, 28 (12.1%) fertilized oocytes out of 232 inseminated oocytes of untreated group and 39 (12.5%) out of 313 inseminated oocytes of PMSG-treated group were cleaved to 8-cell, 16-cell and ~32-cell stages. The percentage of fertilized oocytes of PMSG-treated group that developed to ~32-cell stage were significantly higher ($P< 0.05$) than those of untreated group (3.2% vs. 0.4%). Also PMSG-treated group had a significantly ($P<0.05$) higher percentage of degenerated embryos than untreated group (7.3% vs. 4.3%) as presented in Table (26).

I.D.3.3. Ninety six hours post-insemination:

After 96 hrs of insemination and incubation, the percentage of degenerated embryos (4.7 and 4.8%) and total cleavage percentage (7.3 and 7.7%) were nearly similar for fertilized oocytes in untreated and PMSG-treated groups (Table 27). In untreated group, of 17 (7.3%) total cleavage, 5 (2.2) reached 16-cell, 9 (3.9%) in ~32-cell and 3 (1.3%) in the early blastocyst stages, while these values were 24 (7.7%), 4 (1.3%), 13 (4.2%) and 7 (2.2%), respectively, in PMSG-treated group.

I.D.3.4. One hundred and twenty hours post-insemination:

Results in Table (28) show the non significant differences between untreated and PMSG-treated groups for degenerated embryos, developmental stages (early balstocyst and blastocyst) and total cleavage percentages after 120 hrs of insemination and incubation.

Table 25. Development of *in vitro* fertilized ova after 48 hrs of insemination for untreated and PMSG-treated groups.

Group	No. of oocytes	No. (%) deg.embryos[1]	No. (%)[2] of oocytes developed to: 4-cell	8-cell	16-cell	Total cleavage
Untreated*	232	22 (9.5)	18 (7.8)	18 (7.8)	2 (0.9)[b]	38 (16.4)
Treated **	313	28 (8.9)	19 (6.1)	30 (9.6)	13 (4.2)[a]	62 (19.8)

Table 26. Development of *in vitro* fertilized ova after 72 hrs of insemination for untreated and PMSG-treated groups.

Group	No. of oocytes	No. (%) deg.embryos[1]	No. (%)[2] of oocytes developed to: 8-cell	16-cell	~32-cell	Total cleavage
Untreated*	232	10 (4.3)[b]	12 (5.2)	15 (6.5)	1 (0.4)[b]	28 (12.1)
Treated **	313	23 (7.3)[a]	8 (2.6)	21 (6.7)	10 (3.2)[a]	39 (12.5)

Table 27. Development of *in vitro* fertilized ova after 96 hrs of insemination for untreated and PMSG-treated groups.

Group	No. of oocytes	No. (%) deg.embryos[1]	No. (%)[2] of oocytes developed to: 16-cell	32-cell	E.B.[3]	Total cleavage
Untreated*	232	11 (4.7)	5 (2.2)	9 (3.9)	3 (1.3)	17 (7.3)
Treated**	313	15 (4.8)	4 (1.3)	13 (4.2)	7 (2.2)	24 (7.7)

Table 28. Development of *in vitro* fertilized ova after 120 hrs of insemination for untreated and PMSG-treated groups.

Group	No. of oocytes	No. (%) deg.embryos[1]	No. (%)[2] of oocytes developed to: E.B.[3]	Blastocyst	Total cleavage
Untreated*	232	12 (5.2)	3 (1.3)	2 (0.9)	5 (2.2)
Treated**	313	12 (3.8)	7 (2.2)	5 (1.6)	12 (3.8)

* The oocytes in this group were derived from 14 does.

** The oocytes in this group were derived from 12 does.

[1] Degenerated embryos (calculated as: total cleavage of previous day minus total cleavage of present day).

[2] As a proportion of inseminated oocytes.

[3] Early blastocyst.

[a,b] Values with different superscripts within a column are significantly different ($P < 0.05$).

However, PMSG-treated group possessed higher cleavage percentage (3.8%) and lower degenerated embryos percentage (3.8%) than those of untreated group (2.2 and 5.2%, respectively).

I.D.3.5. One hundred and fourty four hrs post-insemination:

After 144 hrs post-insemination, it was noticed that all fertilized oocytes of untreated group failed to develop after 120 hrs post-insemination (degenerated embryos). However, 3 embryos were developed to blastocyst stage in PMSG-treated group after 144 hrs of insemination (See Table 22).

I.D.3.6. Developmental capacity:

Results summarized in Table (29) and Figure (13) show the comparison between untreated and PMSG-treated groups for fertilized oocytes developed to morula, early blastocyst and blastocyst stages from 72 to 144 hrs post-insemination. The results indicated that different developmental stages were higher in PMSG-treated group compared with untreated group. In untreated group, of 60 (25.9%) fertilized oocytes of 232 inseminated oocytes, 10 (16.7%) were in morula, 6 (10%) in early blastocyst and 2 (3.3%) in blastocyst stages. In PMSG-treated group, of 90 (28.8%) fertilized oocytes of 313 inseminated oocytes, 23 (25.6%) were in morula, 14 (15.6%) in early blastocyst and 8 (8.9%) in blastocyst stages (Table 29 and Figure 13).

I.D.3.7. Developmental periods:

The results in Table (30) and Figure (14) show the total cleavage of fertilized ova between 48-144 hrs post-insemination, collected from untreated and PMSG-treated groups.

The total cleavage percentages were decreased by increasing the time of incubation for untreated and PMSG-treated groups. The total cleavage percentage decreased from 63.3% after 48 hrs to 8.3% after 120 hrs post-insemination for untreated group , but these percentages were 68.9% and 13.3%, respectively, for PMSG-treated group. Also, the percentages were decreased by 16.7% and 25.6% between 48 and 72 hrs post-insemination for untreated and PMSG-treated groups, respectively. After 96 hrs post-insemination, the fertilized oocytes from PMSG-treated group were continued development to advanced stages at a higher percentages than those of untreated group. The total cleavage for untreated group after 120 hrs post-insemination was 8.3% and failed to develop , but this percentage was 13.3% for PMSG-treated group and 3.3% continued development till 144 hrs post-insemination.

Table 29. Development of *in vitro* fertilized ova from morula to blastocyst stages after 144 hrs of insemination for untreated and PMSG-treated groups.

Groups	No. of oocytes inseminated	No. (%)[1] oocytes fertilized	No. (%)[2] of oocytes developed to: Morula 72-96 hrs	Early blastocyst 96-120 hrs	Blastocyst 120-144 hrs
Untreated*	232	60 (25.9)	10 (16.7)	6 (10.0)	2 (3.3)
Treated**	313	90 (28.8)	23 (25.6)	14 (15.6)	8 (8.9)

* The oocytes in this group were derived from 14 does.

** The oocytes in this group were derived from 12 does.

[1] As a proportion of inseminated oocytes.

[2] As a proportion of fertilized oocytes.

Table 30. Development of fertilized ova from 24-144 hrs after insemination as total cleavage cultured in different media for untreated and PMSG-treated groups.

Periods after insemnation (hrs)	Untreated group No.	Total cleavage No.	(%)	PMSG-treated group No.	Total cleavage No.	(%)
24	232*	60	(25.9)[1]	313*	90	(28.8)[1]
48	60**	38	(63.3)[2]	90**	62	(68.9)[2]
72	60	28	(46.7)	90	39	(43.3)
96	60	17	(28.3)	90	24	(26.7)
120	60	5	(08.3)	90	12	(13.3)
144	60	0	(00.0)[b]	90	3	(03.3)[a]

* Number of inseminated oocytes (232 for untreated group and 313 for PMSG-treated groups).

** Number of fertilized oocytes (60 for untreated group and 90 for PMSG-treated groups).

[1] As a proportion to inseminated oocytes.

[2] As a proportion to fertilized oocytes.

[A,b] Values with different superscript within row are significantly different ($P< 0.05$).

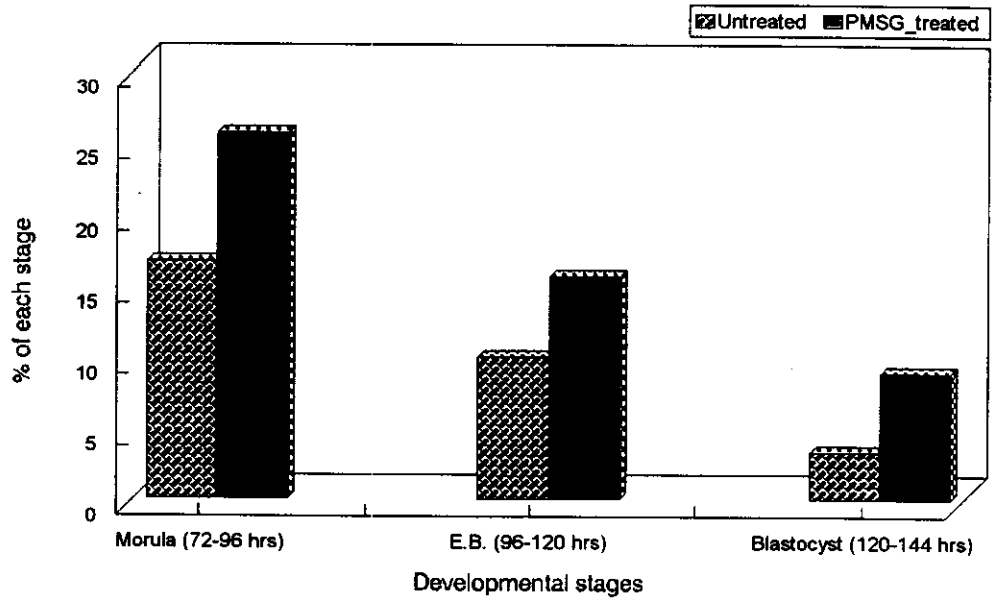

Figure 13. Comparison between untreated and PMSG-treared groups for developmental capacity of *in vitro* matured and fertilized oocytes 72-144 post-insemination.
E.B. = Early blastocyst.

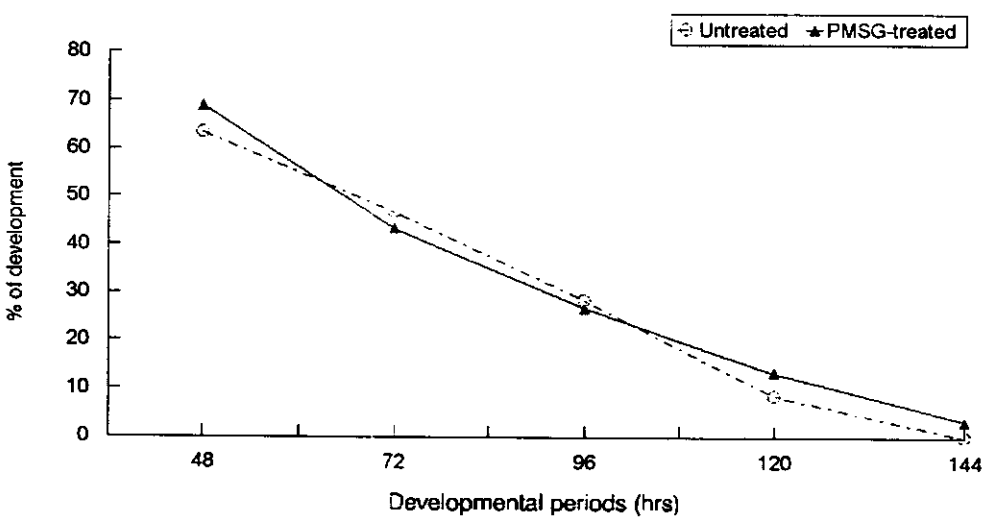

Figure 14. Comparison between untreated and PMSG-treated groups for *in vitro* development of *in vitro* matured and fertilized rabbit oocytes from 48-144 hrs post-insemination.

***Experiment II*:**

II-A. Ovarian measurements of superovulated does:

II.A.1. Superovulated does slaughtered after 10 hrs of HCG injection:

Overall mean and least square means±S.E. for season effect on ovarian measurements of superovulated does treated with PMSG and HCG , slaughtered after 10 hrs of HCG injection are shown in Table (31).

The overall mean±S.E. for the left and right ovaries weights were 0.640±0.05 and 0.659±0.05 gm, respectively. During winter season, left and right ovaries weights were non-significantly higher (0.745±0.07 and 0.763±0.07 gm, respectively) than those of spring season (0.535±0.06 and 0.555±0.06 gm) as presented in (Table 31).

The overall mean±S.E. for the number of corpora lutea on left and right ovaries were 2.1±0.5 and 2.9±0.6 CL, respectively. During winter season, the number of CL presented on left and right ovaries were 2.5±0.7 and 3.5±0.9, respectively, compared with 1.7±0.6 and 2.3±0.8 CL during spring on the respective ovaries. The differences among seasons were not significant (Table 31).

The overall mean±S.E. for the number of follicles presented on the left and right ovaries and total number of follicles on both ovaries were 20.1±0.8, 22.3±0.7 and 42.3±1.4 follicles, respectively. During winter season, these values were 22.5±1.2, 23.5±1.0 and 46.0±2.1 follicles, respectively, decreased to 17.7±1.0, 21.0±0.9 and 38.7±1.7 follicles during spring season, respectively. The differences among seasons for the number of follicles on the left ovary were significant ($P<0.05$), but the differences were not significant for the number of follicles on the right ovary and the total number of follicles presented on both ovaries (Table 31).

The overall mean±S.E. for the total number of oocytes obtained from the follicles presented on the two ovaries were 39.8±1.6. Generally, winter season had non-significantly higher number of oocytes obtained (44.0±2.4 oocytes), than those of spring season (35.7±2.0 oocytes) as presented in Table (31).

The recovery rate of the oocytes was 93.8% (195 oocytes/208 follicles) and the cultured ova percentage was 91.3% (178 oocytes cultured/195 oocytes obtained from follicles).

Table 31. Least square means ± S.E. for ovarian measurements as affected by season for superovulated does slaughtered after 10 hrs of HCG injection.

Items	No. of does	Left ovary weight (gm)	Right ovary weight (gm)	No.of corpora Lutea on left ovary	No.of corpora Lutea on right ovary	No. of follicles on left ovary	No. of follicles on right ovary	Total number of follicles/doe	Total oocytes obtained/doe
Overall mean	5	0.640±0.05	0.659±0.05	2.1±0.5	2.9±0.6	20.1±0.8	22.3±0.7	42.3±1.4	39.8±1.6
Season:									
Winter	2	0.745±0.07	0.763±0.07	2.5±0.7	3.5±0.9	22.5±1.2	23.5±1.0	46.0±2.1	44.0±2.4
Spring	3	0.535±0.06	0.555±0.06	1.7±0.6	2.3±0.8	17.7±1.0	21.0±0.9	38.7±1.7	35.7±2.0

F-ratios and test of significant of least-squares analysis of variance for means presented in Table 31.

S.O.V.	d.f.	F - ratios							
Season	1	0.052	0.052	0.833	1.633	28.03*	7.50	64.53	83.33
Remainder d.f.	3								
Remainder MS.		0.011	0.011	1.056	1.722	3.06	2.17	8.89	11.56

* Significant (P< 0.05).

II.A.2. Superovulated does slaughtered after 12 hrs of HCG injection:

The overall means and least square means±S.E. for season effect on ovarian measurements of superovulated does treated with PMSG and HCG, slaughtered after 12 hrs of HCG injection are shown in Table (32).

The overall mean±S.E. for the left and right ovaries weights were 0.575±0.02 and 0.585±0.02 gm, respectively. The highest weights of left and right ovaries were obtained during winter season followed by those of spring season and the lowest weights were recorded during summer season. The differences among seasons were significant ($P< 0.001$) as shown in Table (32).

The overall mean±S.E. number of corpora lutea on left and right ovaries were 4.8±0.4 and 5.7±0.6 CL, respectively. During winter season, these values were 5.5±0.8 and 6.0±1.3 CL, respectively, compared with 5.0±0.6 and 5.5±0.9 CL during spring, 4.0±0.6 and 5.5±0.9 CL during summer on the left and right ovaries. The differences among seasons were not significant (Table 32).

The overall mean±S.E. number of follicles presented on the left and right ovaries and total number of follicles on both ovaries were 14.8±0.5, 15.7±0.9 and 30.4±1.2 follicles, respectively, as shown in Table (32). During winter season, these values were 17.5±1.0, 18.0±1.8 and 35.5±2.6 follicles, respectively, decreased to 14.0±0.7, 15.0±1.3 and 29.0±1.8 follicles during spring, and decreased to 12.8±0.7, 14.0±1.3 and 26.8±1.8 follicles, respectively, during summer season. The differences among seasons for the number of follicles on the left ovary were significant ($P<0.05$), but were not significant for the number of follicles on the right ovary and total follicles on the both ovaries (Table 32).

The overall mean±S.E. number of oocytes obtained from ovaries (ovarian oocytes) and oviducts (oviductal ova) were 26.8±0.7 and 10.0±0.9 oocytes, respectively. Generally, winter season had the highest number of ovarian oocytes and oviductal ova obtained followed by those of spring season, then those of summer season. The differences among seasons were not significant for ovarian oocytes and oviductal ova obtained (Table 32).

The recovery rate of ovarian oocytes was 87.8% (258 oocytes/294 follicles) and for oviductal ova was 96.1% (99 ova/103 CL). The percentage of ovarian oocytes cultured for *in vitro* fertilization was 89.1% (230 oocytes cultured/258 oocytes obtained) and for oviductal ova cultured was 100.0% (99 ova cultured/99 ova obtained).

Table 32. Least square means ± S.E. for ovarian measurements as affected by season for superovulated does slaughtered after 12 hrs of HCG injection.

Items	No. of does	Left ovary weight (gm)	Right ovary weight (gm)	No. of corpora lutea on left ovary	No. of corpora lutea on right ovary	No. Of follicles on left ovary	No. of follicles on right ovary	Total number of follicles/doe	Total oocytes obtained from ovaries/doe	Total oocytes obtained from oviducts/doe
Overall mean	10	0.575±0.02	0.585±0.02	4.8±0.4	5.7±0.6	14.8±0.5	15.7±0.9	30.4±1.2	26.8±0.7	10.0±0.9
Season:										
Winter	2	0.765±0.05	0.775±0.05	5.5±0.8	6.0±1.3	17.5±1.0	18.0±1.8	35.5±2.6	31.5±2.5	10.5±2.0
Spring	4	0.595±0.03	0.603±0.04	5.0±0.6	5.5±0.9	14.0±0.7	15.0±1.3	29.0±1.8	26.0±1.8	10.0±1.4
Summer	4	0.366±0.03	0.376±0.04	4.0±0.6	5.5±0.9	12.8±0.7	14.0±1.3	26.8±1.8	22.8±1.8	9.5±1.4

F-ratios and test of significant of least-squares analysis of variance for means presented in Table 32.

S.O.V.	d.f.	F - ratios								
Season	2	24.146**	21.214**	1.482	0.058	6.966*	1.643	3.790	4.106	0.092
Remainder d.f.	7									
Remainder Ms.		0.005	0.006	1.214	2.429	2.18	6.57	13.61	12.46	7.64

* Significant (P< 0.05).

** Significant (P< 0.001).

II-B. Oocytes Fertilization *In vitro*:

II.B.1. Superovulated does (slaughtered after 10 hrs of HCG injection):

Five superovulated does were slaughtered after 10 hrs of HCG injection to study the effect of season and media on *in vitro* fertilization of rabbit oocytes (Table 33).

Effect of season:

Results summarized in Table (33) reveal the effect of season on *in vitro* fertilization rate, developmental stages (1-cell, 2-cell, 4-cell and 8-cell) on the next day (after 24 hrs) of *in vitro* insemination by capacitated sperm *in vitro*.

The total fertilization rate during spring season was non-significantly higher (47.4%) than those of winter season (44.4%). However, fertilized oocytes resumed 1-cell stage were non-significantly higher during winter season (4.9%) than spring season (3.1%). Fertilized oocytes cleaved to 2-cell, 4-cell and 8-cell stages were non-significantly higher during spring season (19.6, 17.5 and 7.2%, respectively) than winter season (17.3, 17.3 and 4.9%, respectively) as presented in (Table 33).

Effect of media:

Brackett's medium did not significantly improved fertilization rate (49.4%) than TCM-199 medium (42.7%). In Brackett's medium, of 44 (49.4%) fertilized oocytes, 3 (3.4%) were resumed 1-cell, 16 (18.0%) in 2-cell, 18 (20.2%) in 4-cell and 7 (7.9%) in 8-cell stages. In TCM-199 medium these values were 38 (42.7%), 4 (4.5%), 17 (19.1%), 13 (14.6%) and 4 (4.5%), respectively. However, the differences between the two media were not significant (Table 33) and (Figure 15).

II.B.2. Superovulated does (slaughtered after 12 hrs of HCG injection):

Ten superovulated does were slaughtered after 12 hrs of HCG injection to study the effect of season, source of oocytes (oviduct and ovary) and media (Brackett's and TCM-199) on *in vitro* rabbit oocytes fertilization.

Effect of season:

24 hrs later from the beginning of incubation, sperm-oocyte complex in Brackett's or TCM-199 media, all oocytes were examined for evidence of normal fertilization. The results summarized in Table (34) show that season had no significant effect on *in vitro* fertilization rate and developmental stages (1-cell, 2-cell, 4-cell and 8-cell percentages). However, the fertilization rate was highest during winter season (53.3%), than those of spring and summer seasons (51.8% and 49.6%, respectively). One-cell and 4-cell stage percentages were highest in winter than those of spring and summer seasons.

Table 33. Effects of season and media on *in vitro* fertilization rate of rabbit oocytes collected from superovulated does (slaughtered after 10 hrs of HCG injection).

Items	No. of oocytes	No. (%)[3] development stages				Total fertilization
		1-cell[2]	2-cell	4-cell	8-cell	
Season:						
Winter	81	4 (4.9)	14 (17.3)	14 (17.3)	4 (4.9)	36 (44.4)
Spring	97	3 (3.1)	19 (19.6)	17 (17.5)	7 (7.2)	46 (47.4)
Media:[1]						
Brackett's	89	3 (3.4)	16 (18.0)	18 (20.2)	7 (7.9)	44 (49.4)
TCM-199	89	4 (4.5)	17 (19.1)	13 (14.6)	4 (4.5)	38 (42.7)

[1] The oocytes cultured in both media were derived from five does.

[2] One-cell stage means zygote or pronuclei formation.

[3] As a proportion of inseminated oocytes.

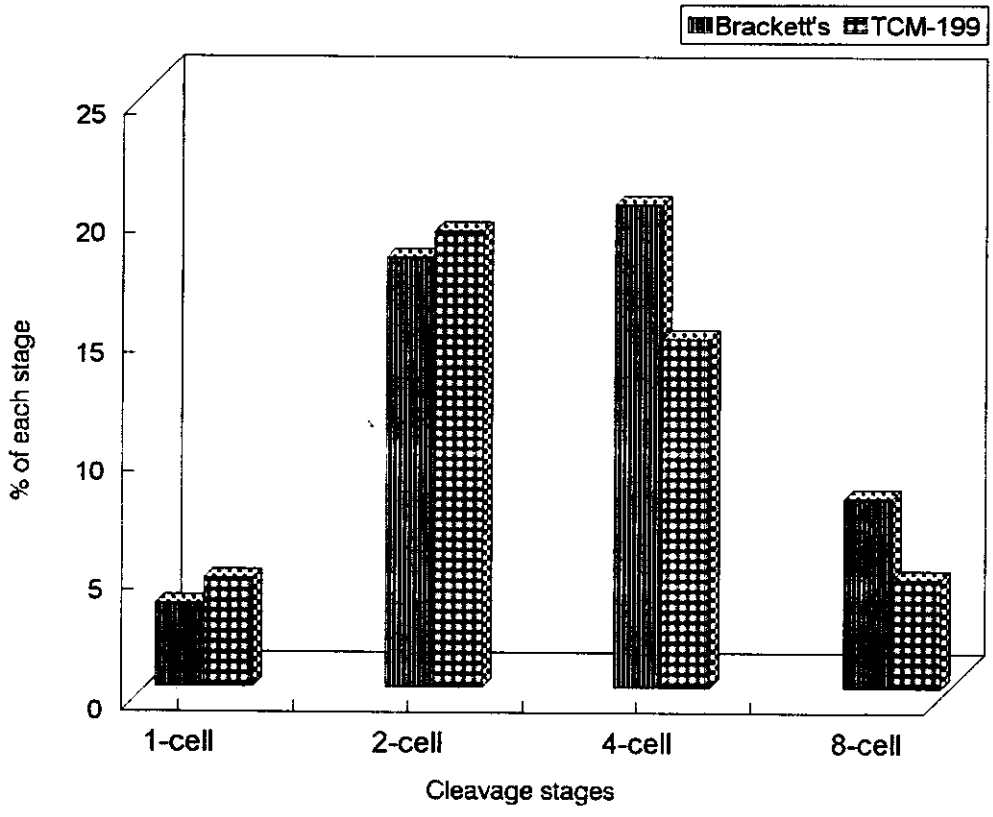

Figure 15. Effect of type of media on *in vitro* cleavage after 24 hrs post-insemination of superovulated does slaughtered after 10 hrs of HCG injection.

Table 34. Effects of season, source of oocytes and media after 24 hrs of insemination on *in vitro* fertilization rate of rabbit oocytes collected from superovulated does slaughtered after 12 hrs of HCG injection.

Items	No. of oocytes	No. (%)[2] development stages				Total fertilization
		1-cell[3]	2-cell	4-cell	8-cell	
Season:						
Winter	75	4 (5.3)	14 (18.7)	16 (21.3)	6 (08.0)	40 (53.3)
Spring	137	4 (2.9)	28 (20.4)	24 (17.5)	15 (10.9)	71 (51.8)
Summer	117	3 (2.6)	27 (23.1)	19 (16.2)	9 (07.7)	58 (49.6)
Oocytes from:						
Oviduct	99	0 (0.0)^{b}	21 (21.2)	27 (27.3)^{a}	13 (13.1)^{a}	61 (61.6)^{a}
Ovary	230	11 (4.8)^{a}	48 (20.9)	32 (13.9)^{b}	17 (07.4)^{b}	108 (47.0)^{b}
Source &Media						
Oviduct						
Brackett's	50	0 (0.0)	10 (20.0)	14 (28.0)	8 (16.0)	32 (64.0)
TCM-199	49	0 (0.0)	11 (22.4)	13 (26.5)	5 (10.2)	29 (59.2)
Ovary						
Brackett's	116	5 (4.3)	26 (22.4)	18 (15.5)	12 (10.3)^{a}	61 (52.6)^{a}
TCM-199	114	6 (5.3)	22 (19.3)	14 (12.3)	5 (04.4)^{b}	47 (41.2)^{b}
Media:[1]						
Brackett's	166	5 (3.0)	36 (21.7)	32 (19.3)	20 (12.0)^{a}	93 (56.0)^{a}
TCM-199	163	6 (3.7)	33 (20.2)	27 (16.6)	10 (06.1)^{b}	76 (46.6)^{b}

[1] The oocytes cultured in both media were derived from 10 does.

[2] As a proportion of inseminated oocytes.

[3] One-cell stage means zygte or pronuclie formation.

[a,b] Values within columns for individual treatment with different superscripts differ significantly ($P< 0.05$).

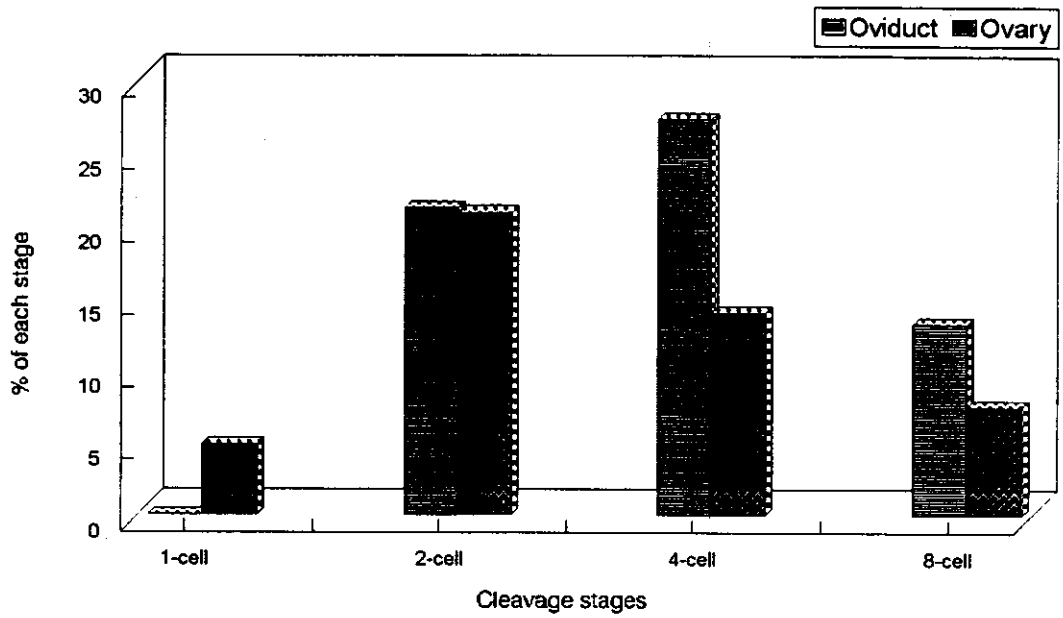

Figure 16. Effect of source of oocytes on *in vitro* cleavage after 24 hrs post-insemination of superovulated does slaughtered after 12 hrs of HCG injection.

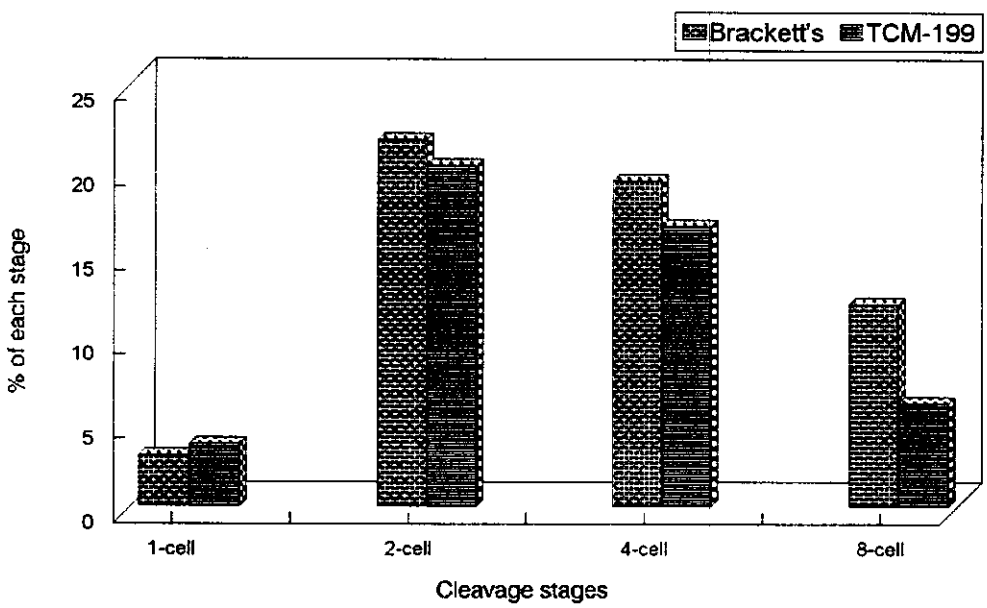

Figure 17. Effect of type of media on *in vitro* cleavage after 24 hrs post-insemination of superovulated does slaughtered after 12 hrs of HCG injection.

Table 35. Effect of slaughter time (10 and 12 hrs after HCG injection) of superovulated does on *in vitro* fertilization rate.

Slaughter time	No. of oocytes	No. (%)[2] development stages				Total fertilization
		1-cell[1]	2-cell	4-cell	8-cell	
After 10 hrs*	178	7 (3.9)	33 (18.5)	31 (17.4)	11 (6.2)	82 (46.1)
After 12 hrs**	329	11 (3.3)	69 (21.0)	59 (17.9)	30 (9.1)	169 (51.4)

* The oocytes cultured were derived from five does.

** The oocytes cultured were derived from ten does.

[1] One-cell stage means zygote or pronuclei formation.

[2] As a proportion of inseminated oocytes.

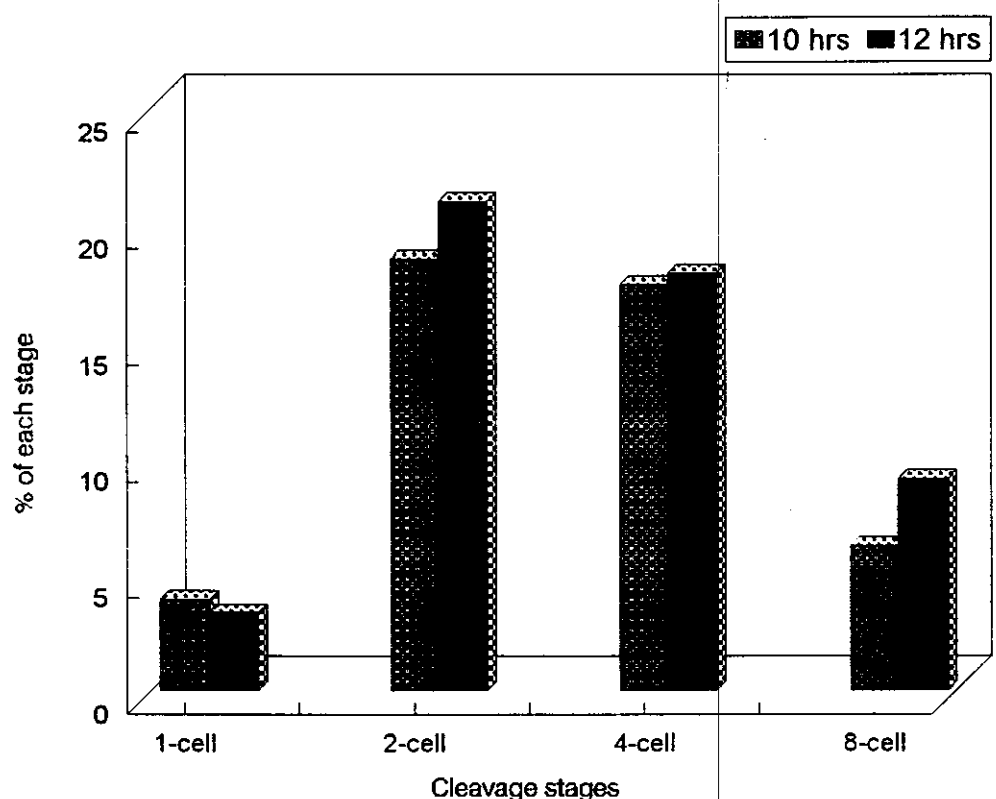

Figure 18. Effect of slaughter time (10 or 12 hrs) after HCG injection on *in vitro* cleavage after 24 hrs post-insemination.

II-C. *In vitro* Development of Fertilized Oocytes:

II.C.1. Oocytes collected after 10 hrs of HCG injection:

II.C.1.1. Fourty eight hours post-insemination:

Results present in Table (36) refer to the degenerated embryos, developmental stages (4-cell, 8-cell and 16-cell) and total cleavage percentages of fertilized oocytes *in vitro* after 48 hrs of insemination and incubation as affected by media (Brackett's and TCM-199). All percentages were calculated as a proportion of inseminated oocytes.

Brackett's medium enhanced 38.2% of inseminated oocytes to cleave compared with 34.8% in TCM-199 medium. Fertilized oocytes developed to 8-cell and 16-cell stages were non-significantly higher (16.9 and 7.9%) in Brackett's medium than 13.5 and 5.6% in TCM-199 medium. However, 15.7% of fertilized oocytes delayed development beyond 4-cell stage after 48 hrs post-insemination in TCM-199 medium and were non-significantly higher than 13.5% in Brackett's medium. The degenerated embryos percentage after 48 hrs post-insemination was non-significantly higher in Brackett's medium than those in TCM-199 medium (11.2 vs. 7.9%, respectively) as presented in Table (36).

II.C.1.2. Seventy two hours post-insemination:

Results summarized in Table (37) indicat that total cleavage percentage was similar (28.1%) in both Brackett's and TCM-199 media after 72 hrs post-insemination. The degenerated embryos percentage in Brackett's medium was higher by 3.4% than those degenerated in TCM-199 medium. However, TCM-199 medium developed non-significantly higher 8-cell stage percentage (13.5%) than in Brackett's medium (10.1%). On the contrary, the fertilized oocytes cultured in Brackett's medium were developed to 16-cell and ~32-cell stages at a non-significantly higher percentage (12.4 and 5.6%) than 10.1 and 4.5% in TCM-199 medium (Table 37).

II.C.1.3. Ninety six hours post-insemination:

Results presented in Table (38) show that the total cleavage percentage was non-significantly higher (22.5%) in TCM-199 medium than those cleaved (16.9%) in Brackett's medium. CM-199 medium had less degenerated embryos (5.6%) than 11.2% in Brackett's medium. TCM-199 medium supported the fertilized oocytes to develop to ~32-cell and early blastocyst at a non-significantly higher percentage (7.9 and 3.4%) than those in Brackett's medium (4.5 and 1.1%). However. the percentage of embryos that delayed to develop beyond 16-cell stage was similar (11.2%) in Brackett's and TCM-199 media.

Table 36. Development of *in vitro* fertilized ova after 48 hrs of insemination for superovulated does slaughtered after 10 hrs of HCG injection.

Media[1]	No. of oocytes	No. (%) deg.embryos[2]	No. (%)[3] of oocytes developed to: 4-cell	8-cell	16-cell	Total cleavage
Brackett's	89	10 (11.2)	12 (13.5)	15 (16.9)	7 (7.9)	34 (38.2)
TCM-199	89	7 (07.9)	14 (15.7)	12 (13.5)	5 (5.6)	31 (34.8)

Table 37. Development of *in vitro* fertilized ova after 72 hrs of insemination for superovulated does slaughtered after 10 hrs of HCG injection.

Media[1]	No. of oocytes	No. (%) deg.embryos[2]	No. (%)[3] of oocytes developed to: 8-cell	16-cell	~32-cell	Total cleavage
Brackett's	89	9 (10.1)	9 (10.1)	11 (12.4)	5 (5.6)	25 (28.1)
TCM-199	89	6 (06.7)	12 (13.5)	9 (10.1)	4 (4.5)	25 (28.1)

Table 38. Development of *in vitro* fertilized ova after 96 hrs of insemination for superovulated does slaughtered after 10 hrs of HCG injection.

Media[1]	No. of oocytes	No. (%) deg.embryos[2]	No. (%)[3] of oocytes developed to: 16-cell	~32-cell	E.B.[4]	Total cleavage
Brackett's	89	10 (11.2)	10 (11.2)	4 (4.5)	1 (1.1)	15 (16.9)
TCM-199	89	5 (05.6)	10 (11.2)	7 (7.9)	3 (3.4)	20 (22.5)

[1] The oocytes cultured in both media were derived from five does.

[2] Degenerated embryos (calculated as: total cleavage of previous day minus total cleavage of present day).

[3] As a proportion of inseminated oocytes.

[4] Early blastocyst.

II.C.1.4. One hundred and twenty hours post-insemination:

Results presented in Table (39) show the effect of media (Brackett's and TCM-199) on degenerated embryos, developmental stages (~32-cell, early blastocyst and blastocyst) and total cleavage percentages after 120 hrs post-insemination. All percentages were calculated as a proportion of inseminated oocytes.

No significant differences were determined between Brackett's and TCM-199 media for degenerated embryos, developmental stages and total cleavage percentages. However, TCM-199 medium supported more fertilized oocytes to develop to ~32-cell, early blastocyst and blastocyst stages (6.7, 6.7 and 2.2%, respectively) than Brackett's medium (5.6, 2.2 and 1.1%, respectively). Also, total cleavage percentage after 120 hrs post-insemination was higher (15.7%) in TCM-199 medium than (9.0%) in Brackett's medium. The degenerated embryos percentage was less (6.7%) in TCM-199 medium than 7.9% in Brackett's medium (Table 39).

II.C.1.5. One hundred and fourty four hours post-insemination:

Results in Table (40) show the effect of media (Brackett's and TCM-199) on the degenerated embryos, developmental stages (early blastocyst, blastocyst and expanding blastocyst) and total cleavage percentages after 144 hrs post-insemination. All percentages were calculated as a proportion of inseminated oocytes.

TCM-199 medium promoted 5.6% of the inseminated oocytes to continue cleavage and development after 144 hrs post-insemination than 2.2% in Brackett's medium. In TCM-199 medium 1.1% of inseminated oocytes were developed to early blastocyst, 3.4% to blastocyst and 1.1% to expanding blastocyst stages compared with 0.0, 1.1 and 1.1% in Brackett's medium, respectively. However, in TCM-199 medium after 144 hrs post-insemination, the degenerated embryos percentage was higher (10.1%) than (6.7%) in Brackett's medium (Table 40).

Table 39. Development of *in vitro* fertilized ova after 120 hrs of insemination for superovulated does slaughtered after 10 hrs of HCG injection.

Media[1]	No. of oocytes	No. (%) deg.embryos[2]	No. (%)[3] of oocytes developed to: ~32-cell	E.B.[3]	Blastocyst	Total cleavage
Brackett's	89	7 (7.9)	5 (5.6)	2 (2.2)	1 (1.1)	8 (09.0)
TCM-199	89	6 (6.7)	6 (6.7)	6 (6.7)	2 (2.2)	14 (15.7)

[1] The oocytes cultured in both media were derived from five does.

[2] Degenerated embryos (calculated as: total cleavage of previous day minus total cleavage of present day).

[3] As a proportion to inseminated oocytes.

[4] Early blastocyst.

Table 40. Development of *in vitro* fertilized ova after 144 hrs of insemination for superovulated does slaughtered after 10 hrs of HCG injection.

Media[1]	No. of oocytes	No. (%) deg.embryos[2]	No. (%)[3] of oocytes developed to: E.B.[4]	Blastocyst	Expan.B.[5]	Total cleavage
Brackett's	89	6 (06.7)	0 (0.0)	1 (1.1)	1 (1.1)	2 (2.2)
TCM-199	89	9 (10.1)	1 (1.1)	3 (3.4)	1 (1.1)	5 (5.6)

[1] The oocytes cultured in both media were derived from five does.

[2] Degenerated embryos (calculated as: total cleavage of previous day minus total cleavage of present day).

[3] As a proportion to inseminated oocytes.

[4] Early blastocyst.

[5] Expanding blastocyst.

II.C.1.6. Developmental capacity:

Results presented in Table (41) and Figure (19) reveal the developmental capacity (morula, early blastocyst, blastocyst and expanding blastocyst) of fertilized oocytes cultured in Brackett's and TCM-199 media up to 6 days post-insemination. All percentages were calculated as a proportion of fertilized oocytes.

TCM-199 medium was superior to Brackett's medium in the developmental capacity, that enhanced higher percentages for different stages of development. However, the differences between the two media were not significant except for early plastocyst stage ($P<0.05$) as shown in Table (41).

In TCM-199 medium, 38 (42.7%) out of 89 inseminated oocytes were fertilized. 17 (44.7%) of these were in morula stage by 72-120 hrs post-insemination, 10 (26.3%) in early blastocyst stage by 96-144 hrs post-insemination. 5 (13.2%) in blastocyst stage by 120-144 hrs post-insemination and 1 (2.6%) in expanding blastocyst stage after 144 hrs post-insemination. However, In Brackett's medium, 44 (49.4%) out of 89 inseminated oocytes were fertilized, 14 (31.8%) of these were in morula stage by 72-120 hrs post-insemination, 3 (6.8%) in early blastocyst stage by 96-144 hrs post-insemination, 2 (4.5%) in blastocyst stage by 120-144 hrs post-insemination and 1 (2.3%) in expanding blastocyst stage after 144 hrs post-insemination.

II.C.1.7. Developmental periods:

The results in Table (42) and Figure (20) represent the total cleavage of fertilized oocytes between 48-144 hrs post-insemination in Brackett's and TCM-199 media.

The total cleavage percentages (as a proportion of fertilized oocytes) were decreased by advancing incubation time in Brackett's or TCM-199 media. The percentage of total cleavage decreased from 77.3% after 48 hrs to 4.5% after 144 hrs post-insemination in Brackett's medium, but these percentages were 81.6% and 13.2% in TCM-199 medium, respectively.

Between 48-72 hrs post-insemination, 20.5% of embryos were degenerated in Brackett's medium, while this percentage was 15.8% in TCM-199 medium. Also, between 72-96 hrs post-insemination, 22.7% of embryos were degenerated in Brackett's medium compared with 13.2% in TCM-199 medium. After 96 hrs post-insemination, TCM-199 medium enhanced significantly ($P<0.05$) total cleavage percentage (52.6%) compared with 34.1% in Brackett's medium. After 120 hrs post-insemination, total cleavage percentage was significantly ($P<0.05$) greater in TCM-199 medium (36.8%) than (18.2%) in Brackett's medium (Table 42).

Table 41. Development of *in vitro* fertilized ova from morula to expanding blastocyst stages for superovulated does slaughtered after 10 hrs of HCG injection.

Media[1]	No. of oocytes inseminated	No. (%) oocytes fertilized	No. (%)[2] of oocytes developed to: Morula 72-120 hrs	E.B.[3] 96-144 hrs	Blastocyst 120-144 hrs	Expan.B.[4] 144 hrs
Brackett's	89	44 (49.4)	14 (31.8)	3 (06.8)[b]	2 (04.5)	1 (2.3)
TCM-199	89	38 (42.7)	17 (44.7)	10 (26.3)[a]	5 (13.2)	1 (2.6)

[1] The oocytes cultured in both media were derived from five does.

[2] As a proportion of fertilized oocytes.

[3] Early blastocyst.

[4] Expanding blastocyst.

[a,b] Values within columns with different superscripts differ significantly (P< 0.05).

Table 42. Development of *in vitro* fertilized ova from 24-144 hrs after insemination as total cleavage for superovulated does slaughtered after 10 hrs Of HCG injection.

Periods after insemination (hrs)	Brackett's medium No.	Brackett's medium Total cleavage no. (%)	TCM-199 medium No.	TCM-199 medium Total cleavage no. (%)
24	89*	44 (49.4)[1]	89*	38 (42.7)[1]
48	44**	34 (77.3)[2]	38**	31 (81.6)[2]
72	44	25 (56.8)	38	25 (65.8)
96	44	15 (34.1)[b]	38	20 (52.6)[a]
120	44	8 (18.2)[b]	38	14 (36.8)[a]
144	44	2 (04.5)	38	5 (13.2)

* Number of inseminated oocytes (89 in Brackett's and 89 in TCM-199 media).

** Number of fertilized oocytes (44 in Brackett's and 38 in TCM-199 media).

[1] As a proportion of inseminated oocytes.

[2] As a proportion of fertilized oocytes.

[a,b] Values within row with different superscripts differ significantly (P< 0.05).

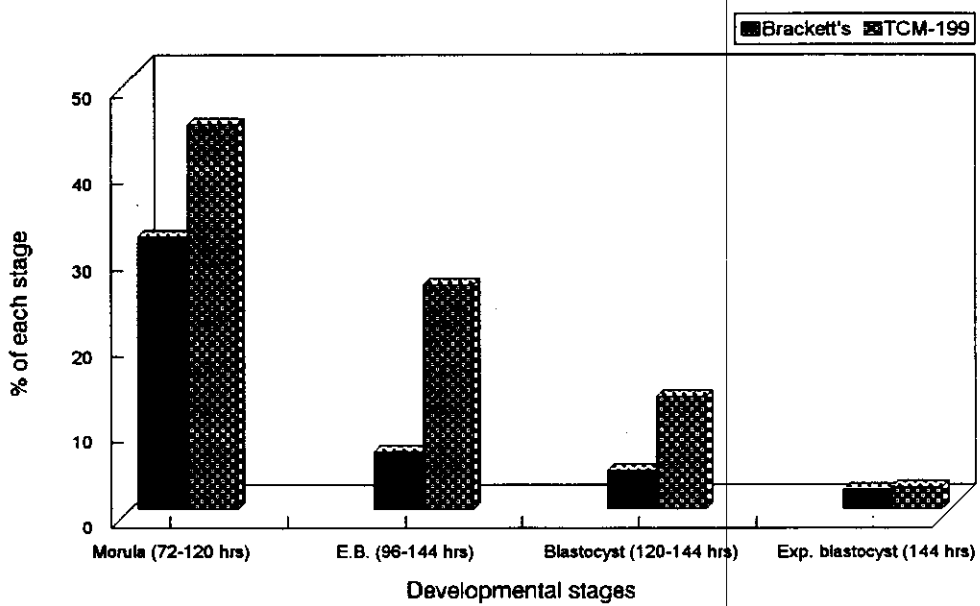

Figure 19. Effect of type of media on developmental capacity of *in vitro* fertilized oocytes from 72-144 hrs post-insemination of superovulated does slaughtered after 10 hrs of HCG injection.

E.B. = Early blastocyst. Exp. = Expanding.

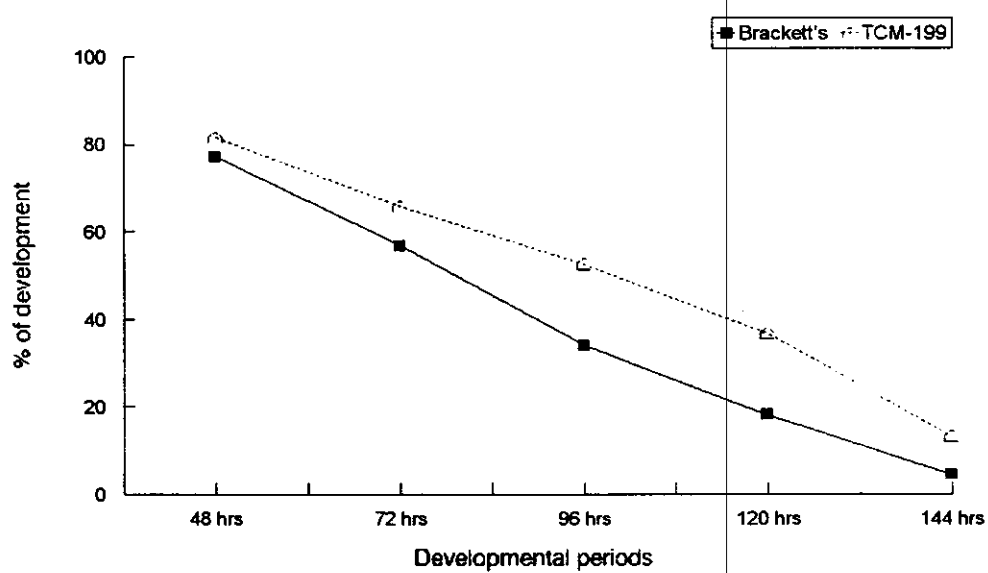

Figure 20. Effecct of type of media on *in vitro* development of *in vitro* fertilized rabbit oocytes from 48-144 hrs post-insemination of superovulated does slaughtered after 10 hrs of HCG injection.

II.C.2. Oocytes collected after 12 hrs of HCG injection:

II.C.2.1. Fourty eight hours post-insemination:

Results present in Table (43) show the degenerated embryos, developmental stages (4-cell, 8-cell and 16-cell) and total cleavage percentages of fertilized oocytes *in vitro* after 48 hrs of insemination and incubation as affected by the source of oocytes (oviducts or ovaries) and media (Brackett's and TCM-199). All percentages were calculated as a proportion of inseminated oocytes.

Effect of source of oocytes:

The oviductal ova fertilized *in vitro* maintained significantly higher ($P<0.05$) total cleavage percentage than those of ovarian oocytes fertilized *in vitro* (55.6% vs. 34.6%). Also, a significantly higher ($P<0.05$) percentage of fertilized oviductal ova were developed to 8-cell and 16-cell stages (25.6% and 15.6%, respectively) compared with those of fertilized ovarian oocytes (14.2% and 5.2%, respectively). Although, the percentage of degenerated embryos after 48 hrs of insemination and incubation was not differ significantly between fertilized oviductal ova and ovarian oocytes, the degenerated embryos percentage (fertilized ovarian oocytes) was higher than those of fertilized oviductal ova (12.8% vs. 7.8%). 4-cell stage percentage was non-significantly higher (15.2%) in fertilized ovarian oocytes than 14.4% in fertilized oviductal ova (Table 43).

Effect of media on fertilized oviductal ova and ovarian oocytes:

Although, no significant differences were observed between Brackett's and TCM-199 media on the total cleavage of fertilized oviductal ova and fertilized ovarian oocytes, Brackett's medium had a higher total cleavage percentage of oviductal ova and ovarian oocytes (58.7% and 39.6%, respectively) than those in TCM-199 medium (52.3% and 29.5%, respectively) after 48 hrs post-insemination. However, fertilized oviductal ova cultured in Brackett's medium developed to 16-cell stage at a significantly higher ($P<0.05$) percentage than those cultured in TCM-199 medium (21.7% vs. 9.1%) as shown in (Table 43).

Fertilized ovarian oocytes cultured in Brackett's medium were developed to 16-cell stage at a non-significantly higher percentage than those cultured in TCM-199 medium (7.5% vs. 2.9%).

Generally, the degenerated embryos percentages were not significantly differ between Brackett's and TCM-199 media for fertilized oviductal ova (6.5% vs. 9.1%) and fertilized ovarian oocytes (13.2% vs. 12.4%) as shown in Table (43).

Table 43. Development of *in vitro* fertilized ova after 48 hrs of insemination obtained from two sources and cultured in different media for superovulated does slaughtered after 12 hrs of HCG injection.

Items	No. of oocytes	No. (%) deg.embryos[2]	No. (%)[3] of oocytes developed to: 4-cell	8-cell	16-cell	Total cleavage
Oocytes from:						
Oviduct	90	7 (07.8)	13 (14.4)	23 (25.6)[a]	14 (15.6)[a]	50 (55.6)[a]
Ovary	211	27 (12.8)	32 (15.2)	30 (14.2)[b]	11 (05.2)[b]	73 (34.6)[b]
Source &media:						
Oviduct						
Brackett's	46	3 (6.5)	5 (10.9)	12 (26.1)	10 (21.7)[a]	27 (58.7)
TCM-199	44	4 (9.1)	8 (18.2)	11 (25.0)	4 (09.1)[b]	23 (52.3)
Ovary						
Brackett's	106	14 (13.2)	18 (17.0)	16 (15.1)	8 (07.5)	42 (39.6)
TCM-199	105	13 (12.4)	14 (13.3)	14 (13.3)	3 (02.9)	31 (29.5)
Media:[1]						
Brackett's	152	17 (11.2)	23 (15.1)	28 (18.4)	18 (11.8)[a]	69 (45.4)[a]
TCM-199	149	17 (11.4)	22 (14.8)	25 (16.8)	7 (04.7)[b]	54 (36.2)[b]

[1] The oocytes cultured in both media were derived from 9 does.

[2] Degenerated embryos (calculated as: total cleavage of previous day minus total cleavage of present day).

[3] As a proportion of inseminated oocytes.

[a,b] Values within columns for individual treatment with different superscripts differ significantly ($P< 0.05$).

Effect of media:

Generally, Brackett's medium significantly ($P<0.05$) improved the total cleavage percentage (45.4%) compared with (36.2%) for TCM-199 medium. The fertilized oocytes cultured in Brackett's medium had developed to 16-cell stage at a significantly higher ($P<0.05$) percentage (11.8%) than those cultured in TCM-199 medium (4.7%). The fertilized oocytes cultured in Brackett's medium, possessed a degeneration percentage nearly similar to those cultured in TCM-199 medium after 48 hrs of incubation (11.2% vs. 11.4%, respectively). Similarly, 4-cell and 8-cell stage percentages were non-significantly higher (15.1 and 18.4%) in Brackett's medium than 14.8 and 16.8%, respectively, in TCM-199 medium (Table 43).

II.C.2.2. Seventy two hours post-insemination:

Results present in Table (44) show the degenerated embryos, developmental stages (8-cell, 16-cell and ~32-cell) and total cleavage percentages of fertilized oocytes *in vitro* after 72 hrs of insemination and incubation as affected by the source of oocytes (oviducts and ovaries) and media (Brackett's and TCM-199). All percentages were calculated as a proportion of inseminated oocytes.

Effect of source of oocytes:

The total cleavage percentage was increased from 29.9% for fertilized ovarian oocytes to 48.9% for fertilized oviductal ova after 72 hrs of incubation, these differences were significant ($P<0.05$). However, the degenerated embryos percentage (fertilized oviductal ova) was non significantly higher than degenerated embryos from fertilized ovarian oocytes (6.7% vs. 4.7%). The oviductal ova fertilized *in vitro* were developed to 16-cell and ~32-cell stages at a significantly higher ($P<0.05$) percentage (22.2% and 11.1%) than those fertilized ovarian oocytes (12.8% and 3.8%). After 72 hrs post-insemination, a higher percentage of fertilized oviductal ova (15.6%) was delayed to develop beyond 8-cell stage compared with 13.3% for fertilized ovarian oocytes (Table 44).

Effect of media on fertilized oviductal ova and ovarian oocytes:

No significant differences were observed between Brackett's and TCM-199 media for the total cleavage (50.0% vs. 47.7%), 8-cell stage (10.9% vs. 20.5%) , 16-cell stage (23.9% vs. 20.5%) and ~32-cell stage (15.2% vs. 6.8%) for oviductal ova fertilized *in vitro*. However, a significantly ($P<0.05$) higher total cleavage percentage of ovarian oocytes fertilized *in vitro* was recorded in Brackett's medium (35.8%) than 23.8% for TCM-199

Table 44. Development of *in vitro* fertilized ova after 72 hrs of insemination obtained from two sources and cultured in different media for superovulated does slaughtered after 12 hrs of HCG injection.

Items	No. of oocytes	No. (%) deg.embryos[2]	No. (%)[3] of oocytes developed to: 8-cell	16-cell	~32-cell	Total cleavage
Oocytes from:						
Oviduct	90	6 (6.7)	14 (15.6)	20 (22.2)[a]	10 (11.1)[a]	44 (48.9)[a]
Ovary	211	10 (4.7)	28 (13.3)	27 (12.8)[b]	8 (03.8)[b]	63 (29.9)[b]
Source &media:						
Oviduct						
Brackett's	46	4 (8.7)	5 (10.9)	11 (23.9)	7 (15.2)	23 (50.0)
TCM-199	44	2 (4.5)	9 (20.5)	9 (20.5)	3 (06.8)	21 (47.7)
Ovary						
Brackett's	106	4 (3.8)	16 (15.1)	17 (16.0)	5 (04.7)	38 (35.8)[a]
TCM-199	105	6 (5.7)	12 (11.4)	10 (09.5)	3 (02.9)	25 (23.8)[b]
Media:[1]						
Brackett's	152	8 (5.3)	21 (13.8)	28 (18.4)	12 (07.9)	61 (40.1)[a]
TCM-199	149	8 (5.4)	21 (14.1)	19 (12.8)	6 (04.0)	46 (30.9)[b]

[1] The oocytes cultured in both media were derived from 9 does.

[2] Degenerated embryos (calculated as: total cleavage of previous day minus total cleavage of present day).

[3] As a proportion of inseminated oocytes.

[a,b] Values within columns for individual treatment with different superscripts differ significantly ($P< 0.05$).

medium. The degenerated embryos percentage of fertilized oviductal ova in Brackett's medium was greater (8.7%) than those in TCM-199 medium (4.5%). On the contrary, the degenerated embryos percentage of ovarian oocytes in TCM-199 medium was slightly higher (5.7%) than those (3.8%) in Brackett's medium (Table 44).

Effect of media:

The total cleavage percentage for the fertilized oocytes cultured and developed in Brackett's medium were significant ($P<0.05$) greater (40.1%) compared with 30.9% for those cultured and developed in TCM-199 medium. The percentage of different stages (8-cell, 16-cell and ~32-cell) of development for fertilized oocytes cultured in Brackett's medium (13.8, 18.4 and 7.9%, respectively) were higher except at 8-cell stage than those (14.1, 12.8 and 4.0%, respectively) of TCM-199 medium (Table 44). The degenerated embryos percentages were nearly similar in Brackett's and TCM-199 media (5.3% vs. 5.4%).

II.C.2.3. Ninety six hours post-insemination:

Results summarized in Table (45) show degenerated embryos, developmental stages and total cleavage percentages of fertilized oocytes *in vitro* after 96 hrs of insemination as affected by the source of oocytes and media.

Effect of source of oocytes:

The total cleavage percentage for oviductal ova fertilized *in vitro* were significantly ($P<0.05$) higher than those of ovarian oocytes fertilized *in vitro* (38.9% vs. 19.4%). Fertilized oviductal ova developed to ~32-cell stage at a significantly ($P<0.05$) higher percentage than those of fertilized ovarian oocytes (22.2% vs. 7.1%). However, the fertilized oviductal ova were developed to 16-cell and early blastocyst at a slightly higher percentages than those of fertilized ovarian oocytes (10.0 and 6.7% vs. 8.5 and 3.8%). After 96 hrs post-insemination, the degenerated embryos percentage of fertilized oviductal ova (10.0%) was nearly similar to those (10.4%) of fertilized ovarian oocytes (Table 45).

Effect of media on fertilized oviductal ova and ovarian oocytes:

No significant differences were observed between Brackett's and TCM-199 media for total cleavage, developmental stages and the degenerated embryos percentages of fertilized oviductal ova and ovarian oocytes. However, Brackett's medium had a higher degenerated embryos percentage than those of TCM-199 medium for fertilized oviductal ova (13.0% vs. 6.8%) and ovarian oocytes (13.2% vs. 7.6%). On the contrary, TCM-199 medium had greater total cleavage percentage of fertilized oviductal ova (40.9%) than

Table 45. Development of *in vitro* fertilized ova after 96 hrs of insemination obtained from two sources and cultured in different media for superovulated does slaughtered after 12 hrs of HCG injection.

Items	No. of oocytes	No. (%) deg.embryos[2]	No. (%)[3] of oocytes developed to: 16-cell	~32-cell	E.B.[4]	Total cleavage
Oocytes from:						
Oviduct	90	9 (10.0)	9 (10.0)	20 (22.2)[a]	6 (6.7)	35 (38.9)[a]
Ovary	211	22 (10.4)	18 (08.5)	15 (07.1)[b]	8 (3.8)	41 (19.4)[b]
Source &media:						
Oviduct						
Brackett's	46	6 (13.0)	3 (06.5)	10 (21.7)	4 (8.7)	17 (37.0)
TCM-199	44	3 (06.8)	6 (13.6)	10 (22.7)	2 (4.5)	18 (40.9)
Ovary						
Brackett's	106	14 (13.2)	10 (09.4)	9 (08.5)	5 (4.7)	24 (22.6)
TCM-199	105	8 (07.6)	8 (07.6)	6 (05.7)	3 (2.9)	17 (16.2)
Media:[1]						
Brackett's	152	20 (13.2)[a]	13 (08.6)	19 (12.5)	9 (5.9)	41 (27.0)
TCM-199	149	11 (07.4)[b]	14 (09.4)	16 (10.7)	5 (3.4)	35 (23.5)

[1] The oocytes cultured in both media were derived from 9 does.

[2] Degenerated embryos (calculated as: total cleavage of previous day minus total cleavage of present day).

[3] As a proportion of inseminated oocytes.

[4] Early blastocyst.

[a,b] Values within columns for individual treatment with different superscripts differ significantly ($P< 0.05$).

those in Brackett's medium (37.0%).

The total cleavage percentage for fertilized ovarian oocytes was higher (22.6%) in Brackett's medium than 16.2% in TCM-199 medium (Table 45).

Effect of media:

Generally, Brackett's medium enhanced the inseminated oocytes to continue cleavage and development slightly higher than TCM-199 medium (27.0% vs. 23.5%). Also, Brackett's medium was superior to TCM-199 medium in ~32-cell stage (12.5% vs. 10.7%) and early blastocyst stage (5.9% vs. 3.4%). Although, the 16-cell stage percentage was less in Brackett's medium than TCM-199 medium (8.6% vs. 9.4%), the degenerated embryos percentage in Brackett's medium was significantly ($P<0.05$) higher than those of TCM-199 medium (13.2% vs. 7.4%) as shown in (Table 45).

II.C.2.4. One hundred and twenty hours post-insemination:

After 120 hrs of insemination and incubation, Table (46) shows the effect of sources of oocytes and media on degenerated embryos, developmental stages (~32-cell, early blastocyst and blastocyst) and total cleavage percentages.

Effect of source of oocytes:

Total cleavage percentage of oviductal ova fertilized *in vitro* was significantly ($P<0.05$) higher than those of ovarian oocytes fertilized *in vitro* (24.4% vs. 10.9%). Similarly, fertilized oviductal ova were developed to early blastocyst stage at a significantly ($P<0.05$) higher percentage than fertilized ovarian oocytes (13.3% vs. 4.3%). Although, no significant differences were observed between fertilized oviductal ova and fertilized ovarian oocytes for degenerated embryos (14.4 % vs. 8.5%), ~32-cell (5.6% vs. 4.7%) and blastocyst (5.6% vs. 1.9%) stages percentages, fertilized oviductal ova had higher percentages of developmental stages than those of fertilized ovarian oocytes (Table 46).

Effect of media on fertilized oviductal ova and ovarian oocytes:

TCM-199 medium enhanced more fertilized oviductal ova to develop to ~32-cell, early blastocyst stages and total cleavage than Brackett's medium after 120 hrs post-insemination. However, the differences between Brackett's and TCM-199 media were not significant for fertilized oviductal ova, for degenerated embryos (13.0% vs. 15.9%), ~32-cell stage (4.3% vs. 6.8%), early blastocyst (13.0% vs. 13.6%) and blastocyst (6.5 vs. 4.5%) stages, and total cleavage (23.9% vs. 25.0%). On the other hand, Brackett's medium enhanced more fertilized ovarian oocytes to develop to ~32-cell and total cleavage

Table 46. Development of *in vitro* fertilized ova after 120 hrs of insemination obtained from two sources and cultured in different media for superovulated does slaughtered after 12 hrs of HCG injection.

Items	No. of oocytes	No. (%) deg.embryos[2]	No. (%)[3] of oocytes developed to: ~32-cell	E.B.[4]	Blastocyst	Total cleavage
Oocytes from:						
Oviduct	90	13 (14.4)	5 (5.6)	12 (13.3)[a]	5 (5.6)	22 (24.4)[a]
Ovary	211	18 (08.5)	10 (4.7)	9 (04.3)[b]	4 (1.9)	23 (10.9)[b]
Source &media:						
Oviduct						
Brackett's	46	6 (13.0)	2 (4.3)	6 (13.0)	3 (6.5)	11 (23.9)
TCM-199	44	7 (15.9)	3 (6.8)	6 (13.6)	2 (4.5)	11 (25.0)
Ovary						
Brackett's	106	12 (11.3)	6 (5.7)	4 (03.8)	2 (1.9)	12 (11.3)
TCM-199	105	6 (05.7)	4 (3.8)	5 (04.8)	2 (1.9)	11 (10.5)
Media:[1]						
Brackett's	152	18 (11.8)	8 (5.3)	10 (06.6)	5 (3.3)	23 (15.1)
TCM-199	149	13 (08.7)	7 (4.7)	11 (07.4)	4 (2.7)	22 (14.8)

[1] The oocytes cultured in both media were derived from 9 does.

[2] Degenerated embryos (calculated as: total cleavage of previous day minus total cleavage of present day).

[3] As a proportion of inseminated oocytes.

[4] Early blastocyst.

[a,b] Values within columns for individual treatment with different superscripts differ significantly ($P< 0.05$).

than TCM-199 medium after 120 hrs post-insemination. The differences between the two media were not significant for fertilized ovarian oocytes, for degenerated embryos (11.3% vs. 5.7%), ~32-cell stage (5.7% vs. 3.8%), early blastocyst (3.8% vs. 4.8%) and blastocyst (1.9 vs. 1.9%) stages, and total cleavage (11.3% vs. 10.5%) as shown in (Table 46).

Effect of media:

Brackett's and TCM-199 media were not differ significantly in degenerated embryos (11.8% vs. 8.7%), ~32-cell stage (5.3% vs. 4.7%), early blastocyst stage (6.6% vs. 7.4%), blastocyst stage (3.3% vs. 2.7%) and total cleavage (15.1% vs. 14.8%) percentages (Table 46).

II.C.2.5. One hundred and fourty four hours post-insemination:

After 144 hrs of insemination, results in Table (47) shows the effect of sources of oocytes and media on degenerated embryos, developmental stages (early blastocyst, blastocyst and expanding blastocyst) and total cleavage percentages.

Effect of source of oocytes:

The fertilized oviductal ova possessed a significantly ($P<0.05$) higher total cleavage percentage (11.1%) than fertilized ovarian oocytes (2.8%). The differences between the two sources of oocytes were not significant for degenerated embryos (13.3% vs. 8.1%), early blastocyst (1.1% vs. 0.5%), blastocyst (5.6% vs. 1.0) and expanding blastocyst (4.4% vs. 1.4%) stages, after 144 hrs of incubation (Table 47).

Effect of media on fertilized oviductal ova and ovarian oocytes:

After 144 hrs of insemination, fertilized oviductal ova cultured in TCM-199 medium had possessed higher cleavage percentage (13.6%) than those cultured in Brackett's medium (8.7%). In Brackett's medium, 4.3% fertilized oviductal ova were developed to blastocyst stage and 4.3% to expanding blastocyst, the corresponding values in TCM-199 medium were 6.8% and 4.5%, respectively as shown in Table (47).

For fertilized ovarian oocytes in Brackett's medium, 1.0% developed to blastocyst and 1.0% to expanding blastocyst with total cleavage of 1.9% compared with 1.0%, 1.9% and 3.8%, respectively, in TCM-199 medium (Table 47).

Effect of media:

TCM-199 medium was superior to Brackett's medium after 144 hrs of insemination in developing the fertilized oocytes to early blastocyst, blastocyst, expanding blastocyst and total cleavage percentage. However, the differences between Brackett's and TCM-199 media were not significant for blastocyst and expanding blastocyst (2.0 vs. 2.7%) stages.

Table 47. Development of *in vitro* fertilized ova after 144 hrs of insemination obtained from two sources and cultured in different media for superovulated does slaughtered after 12 hrs of HCG injection.

Items	No. of oocytes	No. (%) deg.embryos[2]	No. (%)[3] of oocytes developed to: E.B.[4]	Blastocyst	Expan.B.[5]	Total cleavage
Oocytes from:						
Oviduct	90	12 (13.3)	1 (1.1)	5 (5.6)	4 (4.4)	10 (11.1)[a]
Ovary	211	17 (08.1)	1 (0.5)	2 (1.0)	3 (1.4)	6 (02.8)[b]
Sources & media:						
Oviduct						
Brackett's	46	7 (15.2)	0 (0.0)	2 (4.3)	2 (4.3)	4 (08.7)
TCM-199	44	5 (11.4)	1 (2.3)	3 (6.8)	2 (4.5)	6 (13.6)
Ovary						
Brackett's	106	10 (09.4)	0 (0.0)	1 (1.0)	1 (1.0)	2 (01.9)
TCM-199	105	7 (06.7)	1 (1.0)	1 (1.0)	2 (1.9)	4 (03.8)
Media:[1]						
Brackett's	152	17 (11.2)	0 (0.0)	3 (2.0)	3 (2.0)	6 (03.9)
TCM-199	149	12 (08.1)	2 (1.3)	4 (2.7)	4 (2.7)	10 (06.7)

[1] The oocytes cultured in both media were derived from 9 does.

[2] Degenerated embryos (calculated as: total cleavage of previous day minus total cleavage of present day).

[3] As a proportion of inseminated oocytes.

[4] Early blastocyst.

[5] Expanding blastocyst.

[a,b] Values within columns for individual treatment with different superscripts differ significantly ($P< 0.05$).

II.C.2.6. Developmental capacity:

Results summarized in Table (48) reveal the effect of sources of oocytes (oviducts and ovaries) and media (Brackett's and TCM-199) on *in vitro* fertilization rate and developmental stages (morula, early blastocyst, blastocyst and expanding blastocyst) percentages during 6 days post-insemination. All percentages were calculated as a proportion to fertilized oocytes.

Effect of source of oocytes:

Fertilized oviductal ova (inseminated *in vitro)* was significantly (P<0.05) higher than ovarian oocytes inseminated *in vitro* (63.3% vs. 47.4%). Between 72-120 hrs of incubation, 61.4% of the fertilized oviductal ova reached the morula stage compared with 33.0% for fertilized ovarian oocytes, the differences were significant (P<0.05). The same trend was observed between 96-144 hrs of incubation, the percentage of fertilized oviductal ova developed to early blastocyst was significantly (P<0.05) higher than fertilized ovarian oocytes (33.3% vs. 18.0%). Also, between 120-144 hrs of incubation, 17.5% of fertilized oviductal ova reached blastocyst stage compared with 6.0% for fertilized ovarian oocytes. This difference was significant (P<0.05). After 144 hrs, 7.0% of fertilized oviductal ova reached expanding blastocyst compared with 3.0% for fertilized ovarian oocytes. However, this difference was not significant (Table 48 and (Figure 21).

Effect of media:

Brackett's medium had a significantly (P<0.05) higher fertilization percentage than TCM-199 medium (56.6% vs. 47.7%). Between 72-120 hrs of incubation, Brackett's medium non-significantly improved the development of fertilized oocytes to reach morula stage than TCM-199 medium (45.3% vs. 40.8%). Between 96-144, 120-144 and after 144 hrs of incubation, TCM-199 medium supported more fertilized oocytes to develop to early blastocyst, blastocyst and expanding blastocyst stages (25.4, 11.3 and 5.6%, respectively) compared with 22.1, 9.3 and 3.5%, respectively, for those cultured in Brackett's medium (Table 48 and Figure 22).

II.C.2.7. Developmental periods:

Results summarized in Table (49) and Figure (23) reveal the changes in total cleavage percentage (as a proportion of fertilized oocytes) during the incubation period from 48-144 hrs post-insemination by 24 hrs interval for two different sources of oocytes (oviduct and ovaries) incubated in two different media (Brackett's and TCM-199).

The total cleavage percentage for fertilized oviductal ova cultured in Brackett's medium decreased from 90.0% after 48 hrs to 13.3% after 144 hrs of insemination.

Table 48. Development of *in vitro* fertilized ova from morula to expanding blastocyst stages obtained from two sources and cultured in different media for superovulated does slaughtered after 12 hrs of HCG injection .

Items	No. of oocytes insem.[1]	No. (%)[2] oocytes Fertilized	No. (%)[3] of oocytes developed to: Morula 72-120 hrs	E.B.[4] 96-144 hrs	Blastocyst 120-144 hrs	Expan.B.[5] 144 hrs
Oocytes from:						
Oviduct	90	57 (63.3)^{a}	35 (61.4)^{a}	19 (33.3)^{a}	10 (17.5)^{a}	4 (7.0)
Ovary	211	100 (47.4)^{b}	33 (33.0)^{b}	18 (18.0)^{b}	6 (06.0)^{b}	3 (3.0)
Media:						
Brackett's	152	86 (56.6)^{a}	39 (45.3)	19 (22.1)	8 (09.3)	3 (3.5)
TCM-199	149	71 (47.7)^{b}	29 (40.8)	18 (25.4)	8 (11.3)	4 (5.6)

[1] Inseminated., (The oocytes in both sources were derived from 9 does).

[2] As a proportion of inseminated oocytes.

[3] As a proportion of fertilized oocytes.

[4] Early blastocyst.

[5] Expanding blastocyst.

[a,b] Values within columns for individual treatment with different superscripts differ (P< 0.05).

Table 49. Development of fertilized ova from 48 -144 hrs after insemination as a total cleavage for superovulated does slaughtered after 12 hrs of HCG injection.

Periods after insemination (hrs)	Oviduct				Ovary			
	Brackett's		TCM -199		Brackett's		TCM -199	
	No.	Total Cleavage no. (%)	No.	Total cleavage no. (%)	No.	Total cleavage no. (%)	No.	Total cleavage no. (%)
24	46*	30 (65.2)^{1a}	44*	27 (61.4)^{1ab}	106*	56 (52.8)^{1b}	105*	44 (41.9)^{1c}
48	30**	27 (90.0)^{2a}	27**	23 (85.2)^{2ab}	56**	42 (75.0)^{2bc}	44**	31 (70.5)^{2c}
72	30	23 (76.7)^{a}	27	21 (77.8)^{a}	56	38 (67.9)^{ab}	44	25 (56.8)^{b}
96	30	17 (56.7)^{ab}	27	18 (66.7)^{a}	56	24 (42.9)^{bc}	44	17 (38.6)^{c}
120	30	11 (36.7)^{ab}	27	11 (40.7)^{a}	56	12 (21.4)^{c}	44	11 (25.0)^{bc}
144	30	4 (13.3)^{ab}	27	6 (22.2)^{a}	56	2 (03.6)^{b}	44	4 (09.1)^{b}

* Number of oocyte inseminated (90 from oviducts in both media and 210 from ovaries in both media)

** Number of fertilized oocytes (57 from oviducts in both media and 100 from ovaries in both media).

[1] As a proportion of inseminated oocytes.

[2] As a proportion of fertilized oocytes.

[a,b] Values within row with different superscripts differ significantly (P< 0.05).

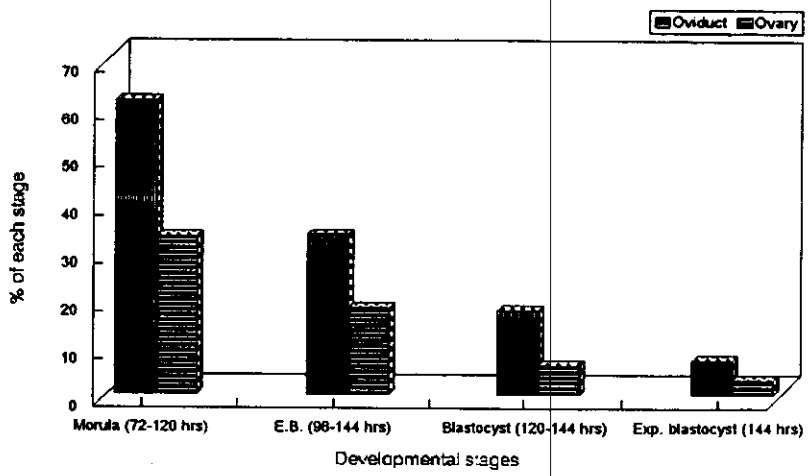

Figure 21. Effect of source of oocytes on developmental capacity of *in vitro* fertilized oocytes from 72-144 hrs post-insemination of superovulated does slaughtered after 12 hrs of HCG injection.

E.B.= Early blastocyst. Exp.= Expanding.

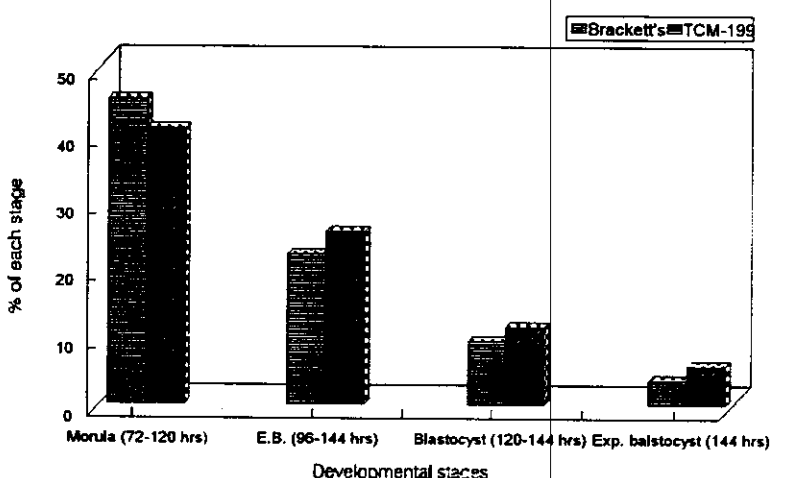

Figure 22. Effect of type of media on developmental capacity of *in vitro* fertilized oocytes from 72-144 hrs post-insemination of superovulated does slaughtered after 12 hrs of HCG injection.

E.B.= Early blastocyst. Exp.= Expanding.

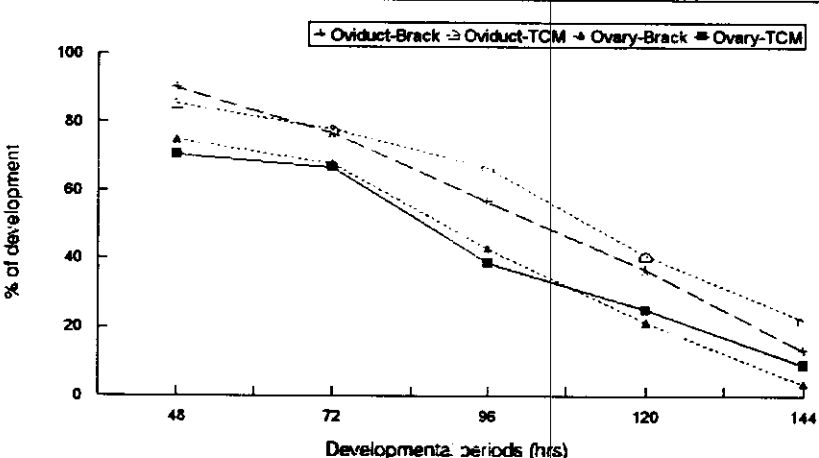

Figure 23. Effect of type of media on *in vitro* development of *in vitro* fertilized rabbit oviductal ova and ovarian oocytes from 48-144 hrs post-insemination for superovulated does slaughtered after 12 hrs of HCG injection.

II.C.3. Effect of slaughter periods:

II.C.3.1. Fourty eight hours post-insemination:

Results present in Table (50) show the degenerated embryos, developmental stages (4-cell, 8-cell and 16-cell) and total cleavage percentages of fertilized oocytes *in vitro* after 48 hrs of insemination and incubation as affected by the slaughter time (10 and 12 hrs after HCG injection). All percentages were calculated as a proportion fo inseminated oocytes.

Of 301 oocytes inseminated, collected from does slaughtered after 12 hrs of HCG injection, and incubated , 123 (40.9%) were cleaved, 54 (15.0%) of these were in 4-cell, 53 (17.6%) in 8-cell and 25 (8.3%) in 16-cell stages. For those collected from does slaughtered after 10 hrs of HCG injection, of 178 oocytes inseminated and incubated for 48 hrs, 65 (36.5%) were cleaved, 26 (14.6%) of these were in 4-cell, 27 (15.2%) in 8-cell and 12 (6.7%) in 16-cell stages. The degenerated embryos percentage was higher in 12 hrs (slaughter time) group (11.3%) than those in 10 hrs (slaughter time) group (9.6%).

II.C.3.2. Seventy two hours post-insemination:

Results present in Table (51) show the degenerated embryos, developmental stages (8-cell, 16-cell and ~32-cell) and total cleavage percentages of fertilized oocytes *in vitro* after 72 hrs of insemination and incubation as affected by slaughter time (10 or 12 hrs after HCG injection). All percentages were calculated as a proportion to inseminated oocytes.

The total cleavage, number of embryos in different developmental stages were non-significantly higher in the 12 hrs group than those of 10 hrs group. The degenerated embryos percentage was non-significantly higher (8.4%) in 10 hrs group than 5.3% in 12 hrs group.

II.C.3.3. Ninety six hours post-insemination:

Results summarized in Table (52) show degenerated embryos, developmental stages (16-cell, ~32-cell and early blastocyst) and total cleavage percentages of fertilized oocytes *in vitro* after 96 hrs of insemination and incubation as affected by slaughter time (10 and 12 hrs after HCG injection). All percentages were calculated as a proportion of inseminated oocytes.

Table 50. Development of *in vitro* fertilized ova after 48 hrs of insemination for superovulated does slaughtered after 10 and 12 hrs of HCG injection.

Time	No. of oocytes	No. (%) deg.embryos[1]	No. (%)[2] of oocytes developed to: 4-cell	8-cell	16-cell	Total cleavage
10 hrs*	178	17 (09.6)	26 (14.6)	27 (15.2)	12 (6.7)	65 (36.5)
12 hrs**	301	34 (11.3)	45 (15.0)	53 (17.6)	25 (8.3)	123 (40.9)

Table 51. Development of *in vitro* fertilized ova after 72 hrs of insemination for superovulated does slaughtered after 10 and 12 hrs of HCG injection.

Time	No. of oocytes	No. (%) deg.embryos[1]	No. (%)[2] of oocytes developed to: 8-cell	16-cell	~32-cell	Total cleavage
10 hrs*	178	15 (8.4)	21 (11.8)	20 (11.2)	9 (5.1)	50 (28.1)
12 hrs**	301	16 (5.3)	42 (14.0)	47 (15.6)	18 (6.0)	107 (35.5)

Table 52. Development of *in vitro* fertilized ova after 96 hrs of insemination for superovulated does slaughtered after 10 and 12 hrs of HCG injection.

Time	No. of oocytes	No. (%) deg.embryos[1]	No. (%)[2] of oocytes developed to: 16-cell	~32-cell	E.B.[3]	Total cleavage
10 hrs*	178	15 (08.4)	20 (11.2)	11 (06.2)	4 (2.2)	35 (19.7)
12 hrs**	301	31 (10.3)	27 (09.0)	35 (11.6)	14 (4.7)	76 (25.2)

* The oocytes cultured were derived from five does.

** The oocytes cultured were derived from nine does.

[1] Degenerated embryos (calculated as: total cleavage of previous day minus total cleavage of present day).

[2] As a proportion of inseminated oocytes.

[3] Early blastocyst.

After 96 hrs post-insemination, total cleavage percentage was higher (25.2%) in 12 hrs group than in 10 hrs group (19.7%). The fertilized oocytes from 12 hrs group developed more embryos to ~32-cell and early blastocyst stage percentage (11.6 and 4.7%) than those of 10 hrs group (6.2 and 2.2%, respectively). Therefore, 12 hrs group had non-significantly less 16-cell stage percentage (9.0%) compared with 11.2% for those in 10 hrs group (Table 52). The degenerated embryos percentage was higher (10.3%) in 12 hrs group than 8.4% in 10 hrs group.

II.C.3.4. One hundred and twenty hours post-insemination:

After 120 hrs of insemination and incubation, Table (53) shows the effect of slaughter time (10 and 12 hrs after HCG injection) on degenerated embryos, developmental stages (~32-cell, early blastocyst and blastocyst) and total cleavage percentages.

After 120 hrs post-insemination, more fertilized oocytes from 12 hrs group continued cleavage and development compared with those of 10 hrs group (15.0% vs. 12.4%). However, the degenerated embryos percentage was (7.3 and 10.3%, respectively) in 10 and 12 hrs groups.

II.C.3.5. One hundred and fourty four hours post-insemination:

After 144 hrs of insemination and incubation, results in (Table 54) shows the effect of slaughter time (10 and 12 hrs after HCG injection) on degenerated embryos, developmental stages (early blastocyst, blastocyst and expanding blastocyst) and total cleavage percentages. All percentages were calculated as a proportion to inseminated oocytes.

After 144 hrs post-insemination, total cleavage percentage was 5.3% for 12 hrs group compared with 3.9% for 10 hrs group. Fertilized oocytes from 12 hrs group maintained higher percentage of early blastocyst, blastocyst and expanding blastocyst (0.7, 2.3 and 2.3%) compared with 0.6, 2.2 and 1.1%, respectively, for those from 10 hrs group. The degenerated embryos percentage was higher (9.6%) in 12 hrs group than 8.4% in 10 hrs group (Table 54)

Table 53. Development of *in vitro* fertilized ova after 120 hrs of insemination for superovulated does slaughtered after 10 and 12 hrs of HCG injection.

Time	No. of oocytes	No. (%) deg.embryos[1]	No. (%)[2] of oocytes developed to: ~32-cell	E.B.[3]	Blastocyst	Total cleavage
10 hrs*	178	13 (07.3)	11 (6.2)	8 (4.5)	3 (1.7)	22 (12.4)
12 hrs**	301	31 (10.3)	15 (5.0)	21 (7.0)	9 (3.0)	45 (15.0)

* The oocytes cultured were derived from five does.

** The oocytes cultured were derived from nine does.

[1] Degenerated embryos (calculated as: total cleavage of previous day minus total cleavage of present day).

[2] As a proportion of fertilized oocytes.

[3] Early blastocyst.

Table 54. Development of *in vitro* fertilized ova after 144 hrs of insemination for superovulated does slaughtered after 10 and 12 hrs of HCG injection.

Time	No. of oocytes	No. (%) deg.embryos[1]	No. (%)[2] of oocytes developed to: E.B.[3]	Blastocyst	Expan.B.[4]	Total cleavage
10 hrs*	178	15 (8.4)	1 (0.6)	4 (2.2)	2 (1.1)	7 (3.9)
12 hrs**	301	29 (9.6)	2 (0.7)	7 (2.3)	7 (2.3)	16 (5.3)

* The oocytes cultured were derived from five does.

** The oocytes cultured were derived from nine does.

[1] Degenerated embryos (calculated as: total cleavage of previous day minus total cleavage of present day).

[2] As a proportion of fertilized oocytes.

[3] Early blastocyst.

[4] Expanding blastocyst.

II.C.3.6. Developmental capacity:

Results presented in Table (55) and Figure (24) reveal the effect of slaughter time (10 or 12 hrs after HCG injection) on *in vitro* fertilization rate and developmental stages (morula, early blastocyst, blastocyst and expanding blastocyst) percentages during 6 days post-insemination. All percentages were calculated as a proportion to fertilized oocytes.

The 12 hrs (slaughter time) group had non-significantly higher fertilization rate (52.2%) than 46.1% for those of 10 hrs (slaughter time) group. Between 72-120 hrs post-insemination, 43.3% of fertilized oocytes from (12 hrs group) were developed to morula compared with 37.8% for those from (10 hrs group). In (12 hrs and 10 hrs groups), 23.6 and 15.9% of fertilized oocytes developed to early blastocyst stage between 96-144 hrs post-insemination. Similarly, between 120-144 hrs post-insemination 10.2 and 8.5% of fertilized oocytes from (12 hrs and 10 hrs groups, respectively) developed to blastocyst stage. After 144 hrs post-insemination 4.5% of fertilized oocytes developed to expanding blastocyst in 12 hrs group compared with 2.4% for those in 10 hrs group.

II.C.3.7. Developmental periods:

The results summarized in Table (56) and Figure (25) show the total cleavage percentage of fertilized oocytes (as a proportion to fertilized oocytes) from 48-144 hrs post-insemination, collected from superovulated does, slaughtered after 10 hrs and 12 hrs of HCG injection.

Generally, the total cleavage rate was decreased by the advancing incubation time. In 10 hrs group, the total cleavage percentage decreased from 79.3% after 48 hrs to 8.5% after 144 hrs post-insemination, but these percentages were 78.3 and 10.2 at the same intervals in 12 hrs group. The rate of degeneration for the embryos between 48-72 hrs post-insemination was higher (18.3%) for 10 hrs group than 10.2% for those of 12 hrs group. However, the degenerated embryos percentage between 96-120 hrs post-insemination was less (15.9%) in 10 hrs group than 19.7% in those of 12 hrs group.

Table 55. Development of *in vitro* fertilized ova from morula to expanding blastocyst stages for superovulated does slaughtered after 10 and 12 hrs of HCG injection.

Time	No. of oocytes inseminated	No. (%)[1] oocytes fertilized	No. (%)[2] of oocytes developed to: Morula 72-120 hrs	E.B.[3] 96-144 hrs	Blastocyst 120-144 hrs	Expan.B.[4] 144 hrs
10 hrs*	178	82 (46.1)	31 (37.8)	13 (15.9)	7 (08.5)	2 (2.4)
12 hrs**	301	157 (52.2)	68 (43.3)	37 (23.6)	16 (10.2)	7 (4.5)

* The oocytes cultured were derived from five does.

** The oocytes cultured were derived from nine does.

[1] As a proportion of inseminated oocytes.

[2] As a proportion of fertilized oocytes.

[3] Early blastocyst.

[4] Expanding blastocyst.

Table 56. Development of fertilized ova from 48-144 hrs after insemination as total cleavage for superovulated does slaughtered after 10 and 12 hrs of HCG injection.

Periods after insemination (hrs)	Slaughtered after 10 hrs		Slaughter after 12 hrs	
	No.	Total cleavage no. (%)	No.	Total cleavage no. (%)
24	178*	82 (46.1)[1]	301*	157 (52.1)[1]
48	82**	65 (79.3)[2]	157**	123 (78.3)[2]
72	82	50 (61.0)	157	107 (68.2)
96	82	35 (42.7)	157	76 (48.4)
120	82	22 (26.8)	157	45 (28.7)
144	82	7 (08.5)	157	16 (10.2)

* Number of inseminated oocytes (178 in 10 hrs and 301 in 12 hrs groups).

** Number of fertilized oocytes (82 in 10 hrs and 157 in 12 hrs groups).

[1] As a proportion of inseminated oocytes.

[2] As a proportion of fertilized oocytes.

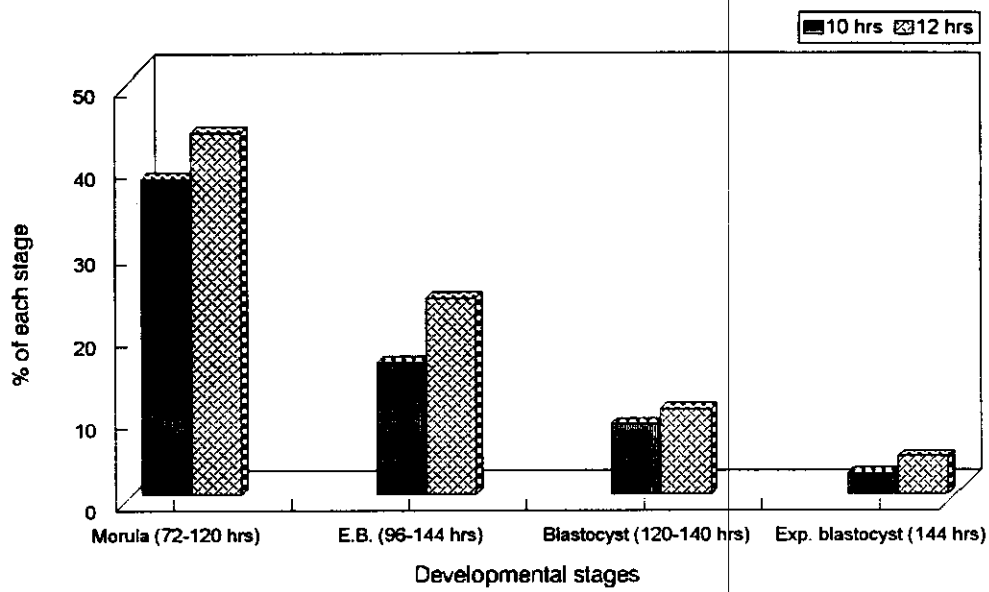

Figure 24. Effect of slaughter time after HCG injection on developmental capacity of *in vitro* fertilized oocytes from 72-144 hrs post-insemination.

E.B.= Early blastocyst. Exp.= Expanding.

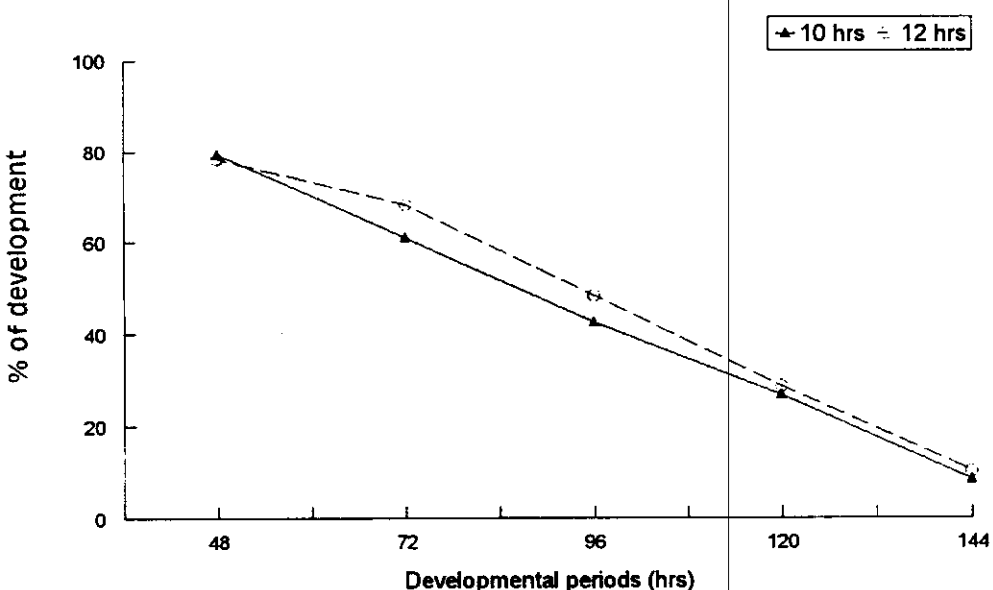

Figure 25. Effect of slaughter time after HCG injection on *in vitro* development of *in vitro* fertilzed rabbit oocytes from 48-144 hrs post-insemination.

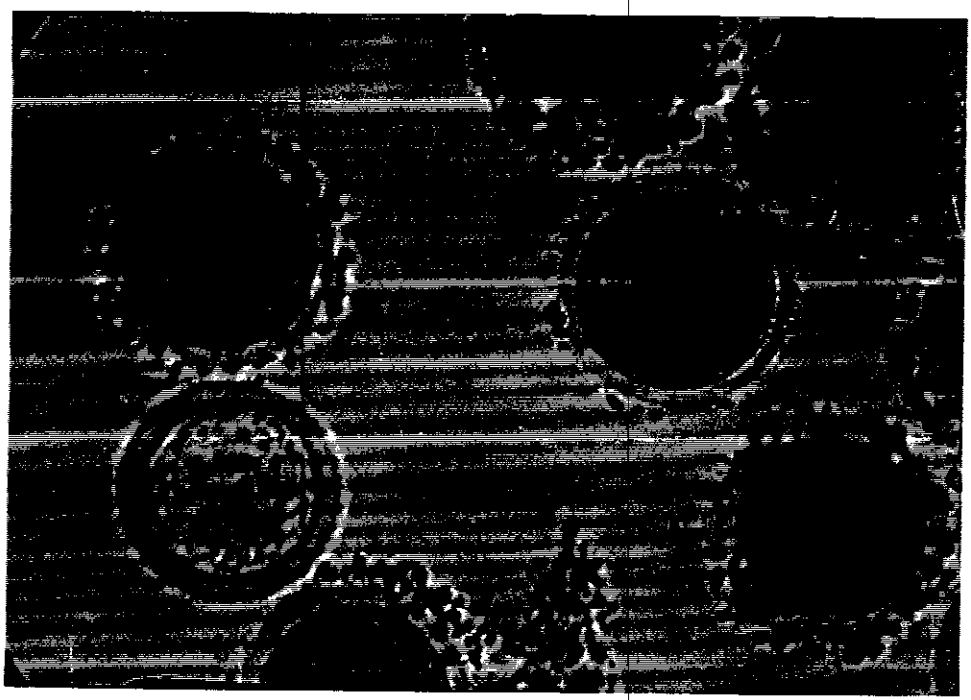

Plate (1). Immature rabbit oocytes with germinal vesicle.

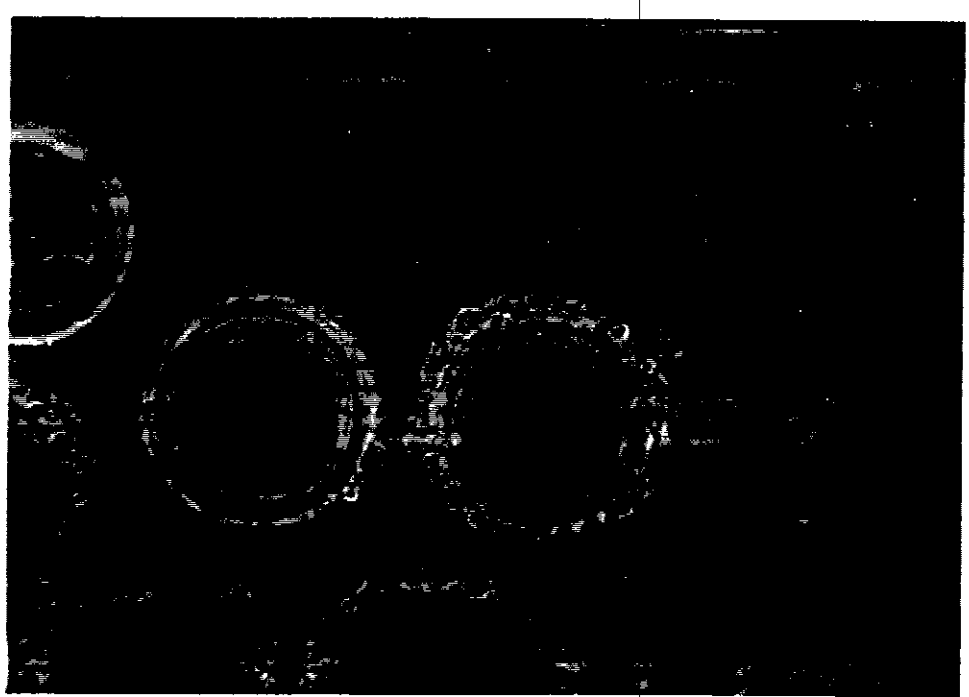

Plate (2). Immature rabbit oocytes initiating germinal vesicle breakdown.

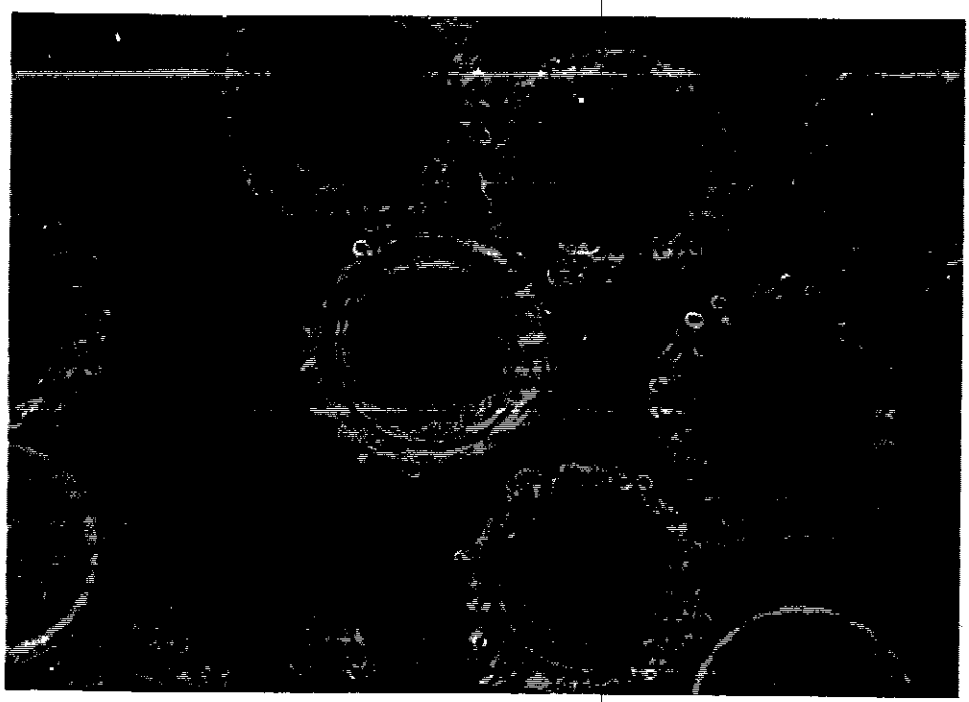

Plate (3). Mature rabbit oocytes derived from *in vitro* maturation after 20 hrs of incubation.

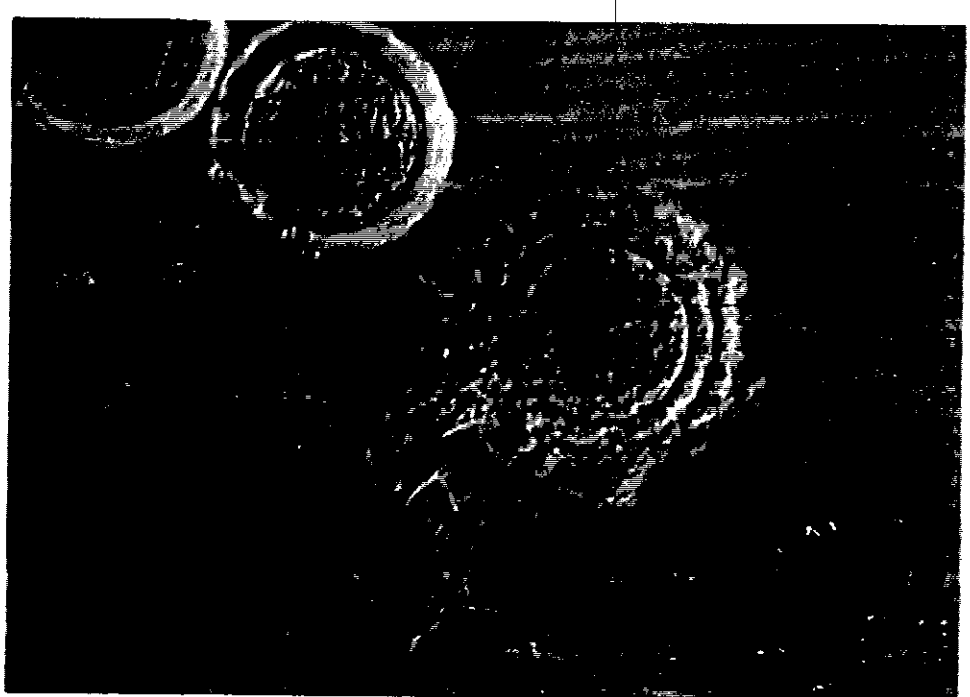

Plate (4). Mature rabbit oocytes derived from superovulated does.
a: denuded oocyte b: oocyte with cumulus cells.

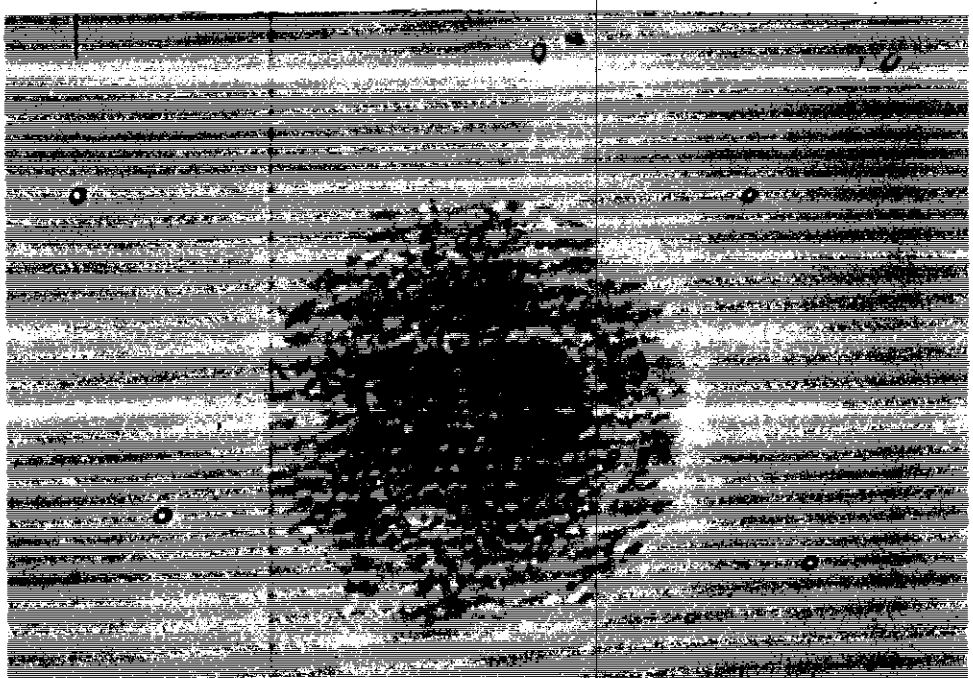

Plate (5). Oocyte in pronuclear stage derived from *in vitro* fertilized oocytes collected from superovulated doe (stained with orcein stain).

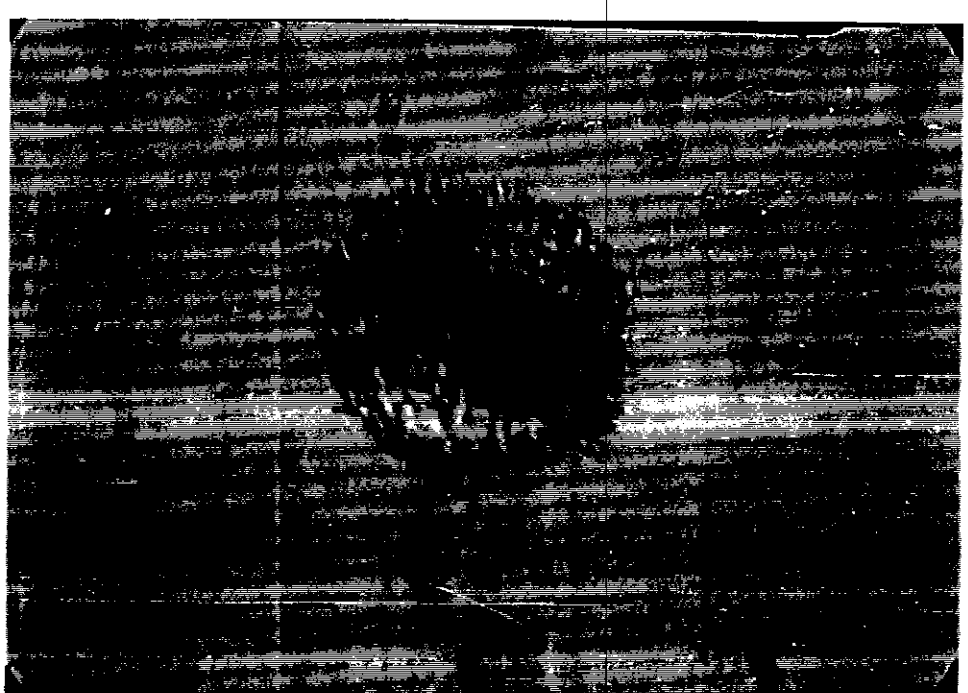

Plate (6). Two-cell embryo derived from *in vitro* matured and fertilized rabbit oocyte cultured in medium of 285 mOsm/kg.

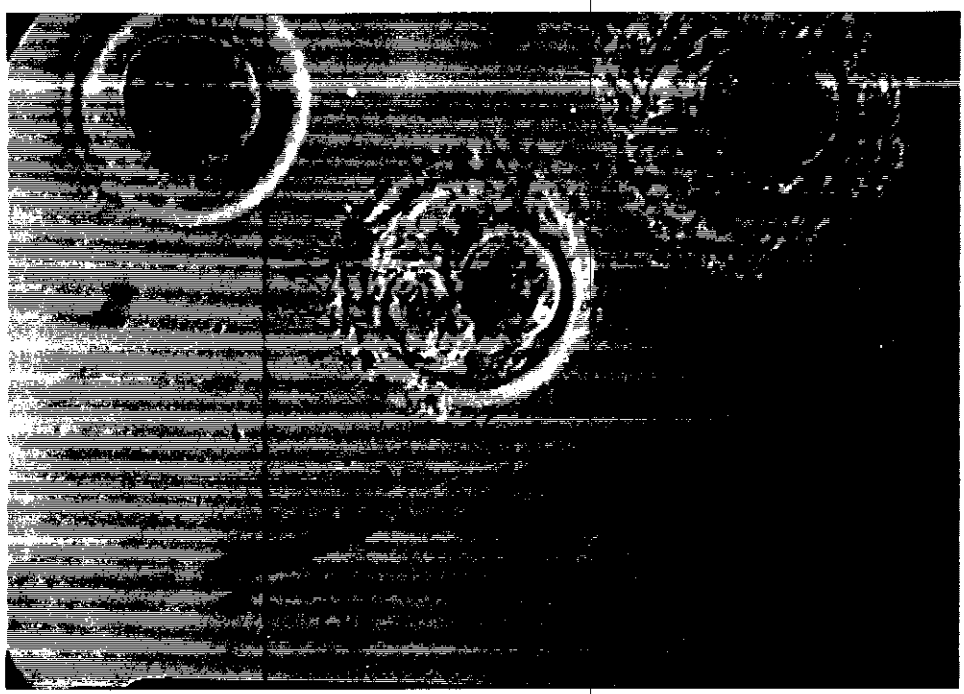

Plate (7). Two-cell embryo derived from *in vitro* matured and fertilized rabbit oocyte cultured in medium of 325 mOsm/kg. a: shrinking blastomeres.

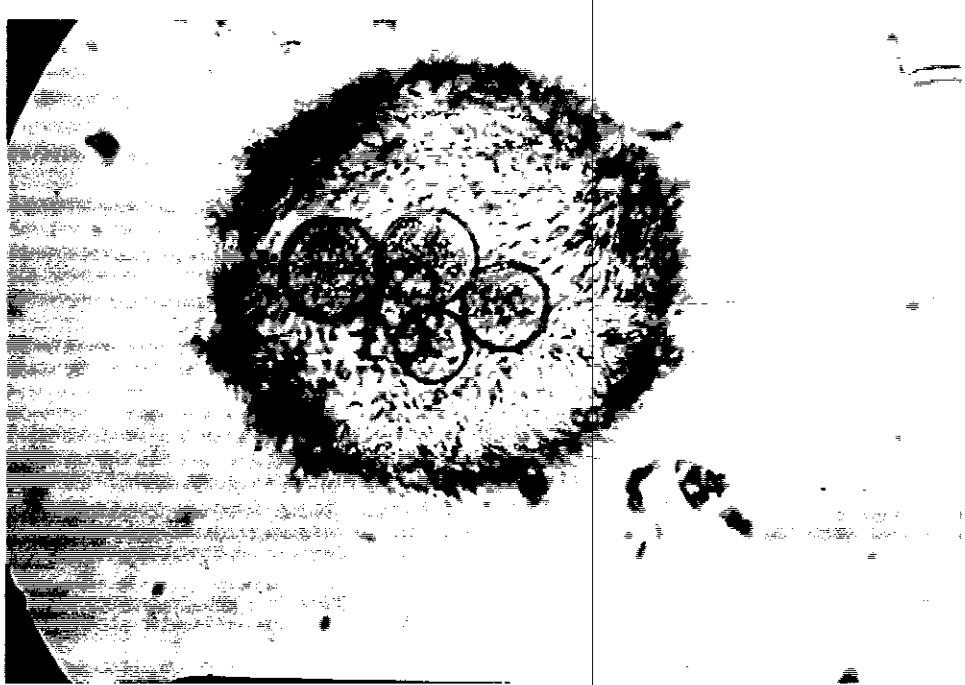

Plate (8). Four-cell stage derived from *in vitro* matured and fertilized rabbit oocyte after 48 hrs of incubation in medium of 285 mOsm/kg.

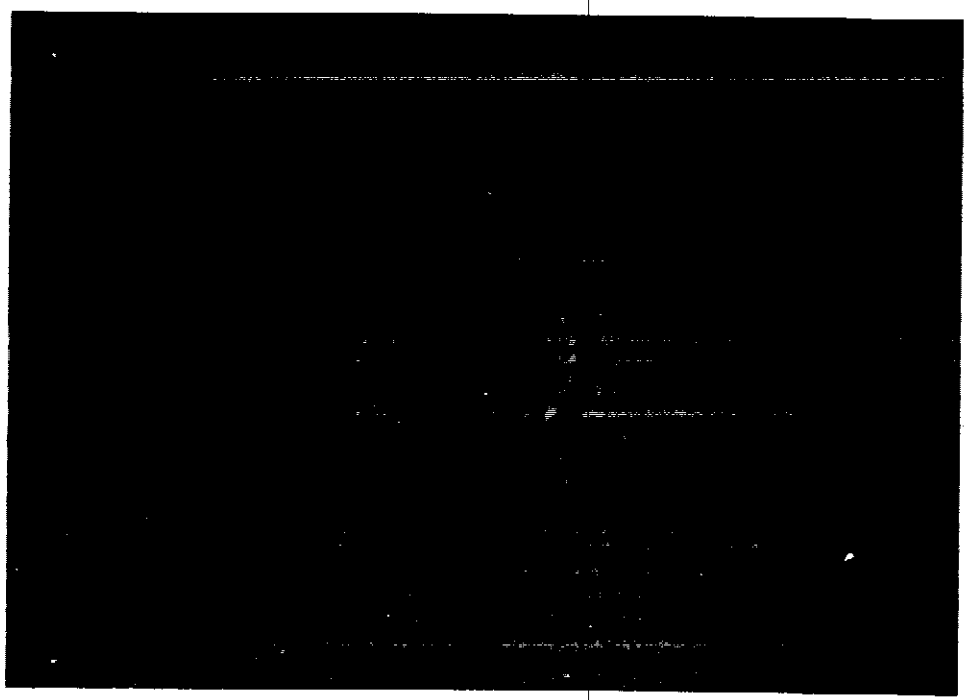

Plate (9). Four-cell stage derived from *in vitro* matured and fertilized rabbit oocyte after 48 hrs of incubation in medium of 325 mOsm/kg.

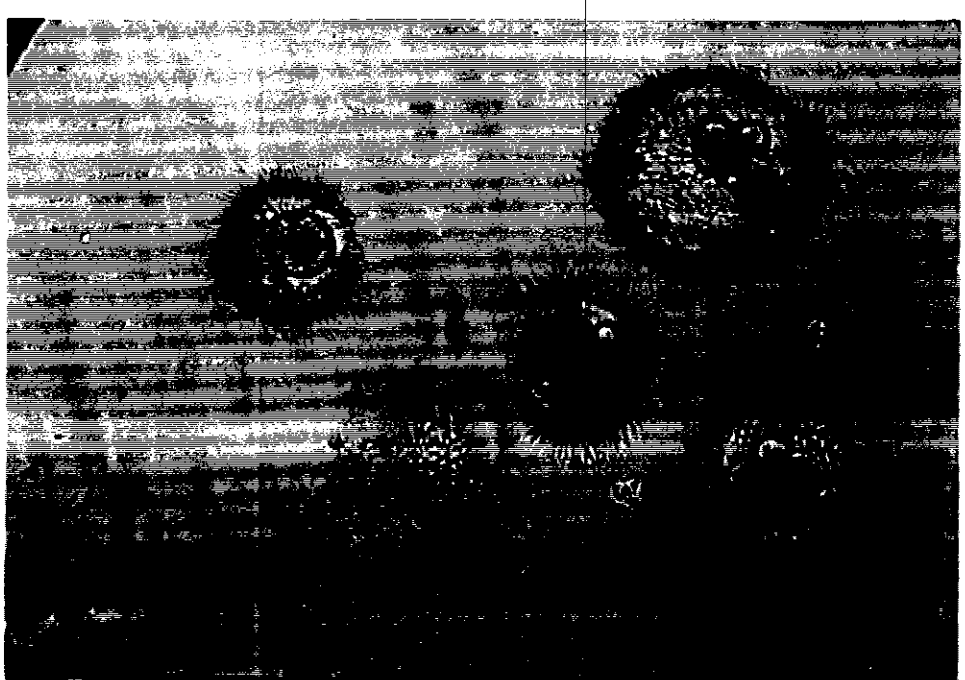

Plate (10). Some different developmental stages of rabbit embryos after 72 hrs of insemination in culture medium of 285 mOsm/kg. Note swelling embryos and unequal blastomeres.

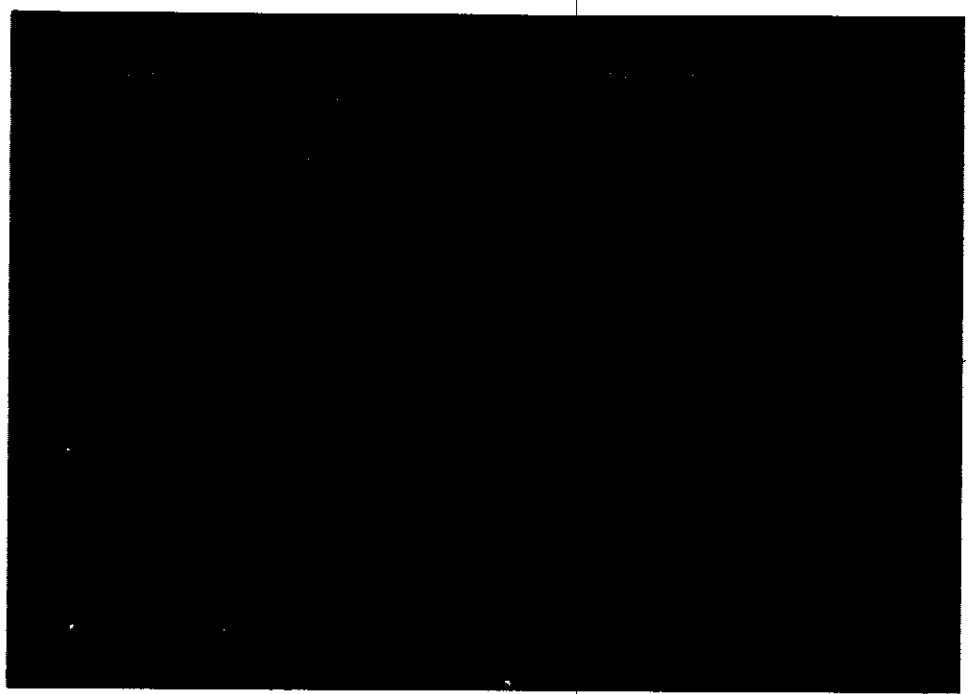

Plate (11). Two-cell stage rabbit embryo with 2nd polar body, recovered from ovarian follicle and exposed to capacitated spermatozoa *in vitro*, cultured in Brackett's medium of 305 mOsm/kg.

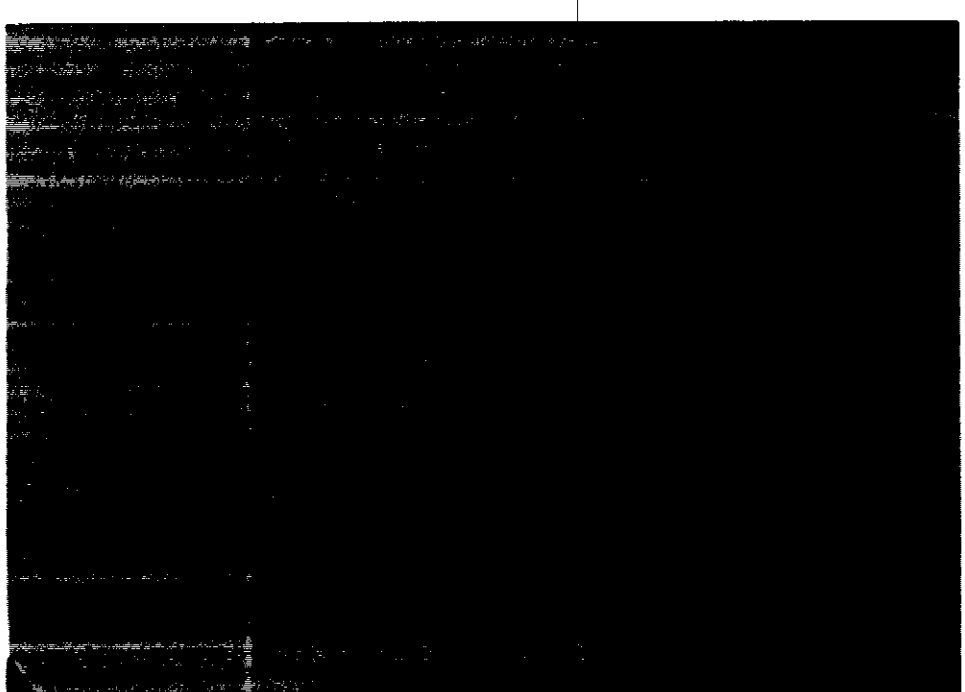

Plate (12). Four-cell stage rabbit embryo, recovered from oviduct and exposed to capacitated spermatozoa *in vitro*, cultured in Brackett,s medium.

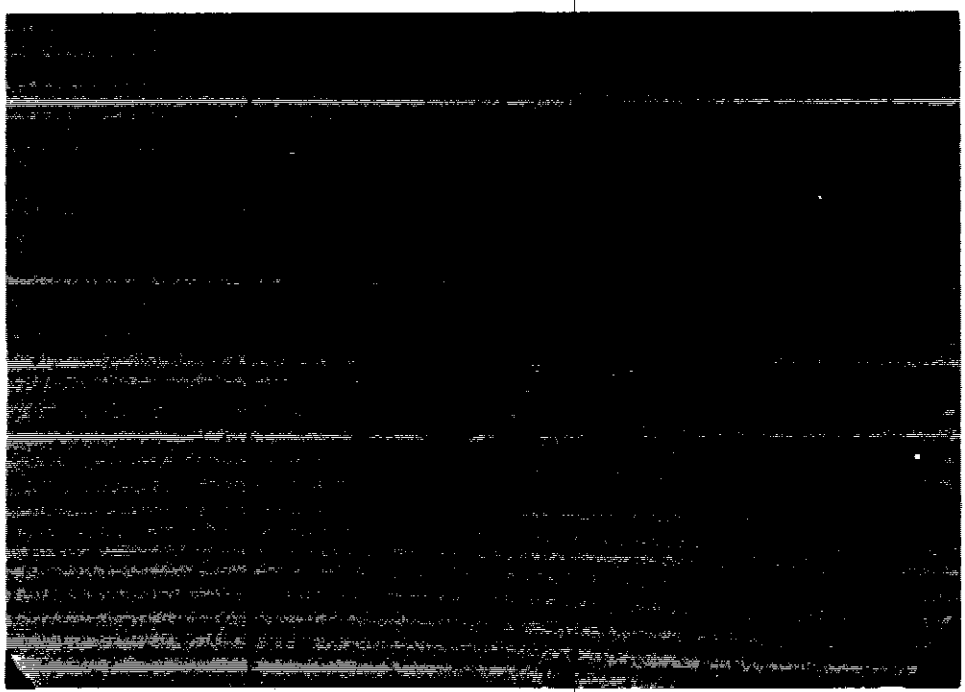

Plate (13). Eight-cell stage embryo derived from *in vitro* fertilized oocytes by capacitated spermatozoa *in vitro*, cultured in TCM-199 medium.

Plate (14). >16-cell stage rabbit embryo from oviductal ovum fertilized *in vitro* cultured in Brackett's medium.

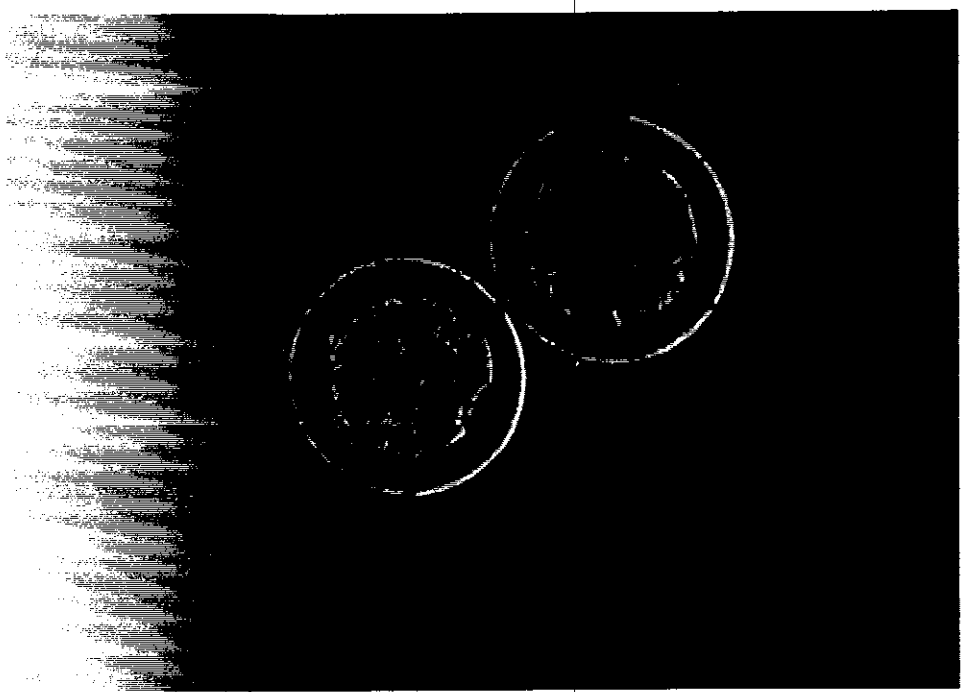

Plate (15). Morula compaction stage embryoes derived from *in vitro* matured and fertilized rabbit oocytes.

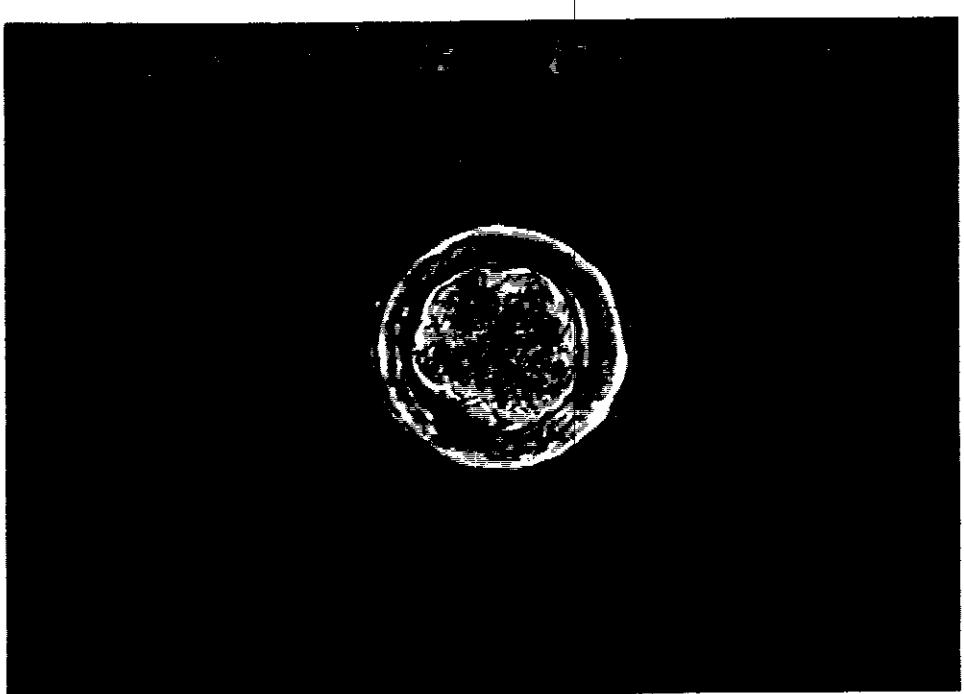

Plate (16). Embryo in late stage of morula after 96-120 hrs of insemination cultured in TCM-199 medium.

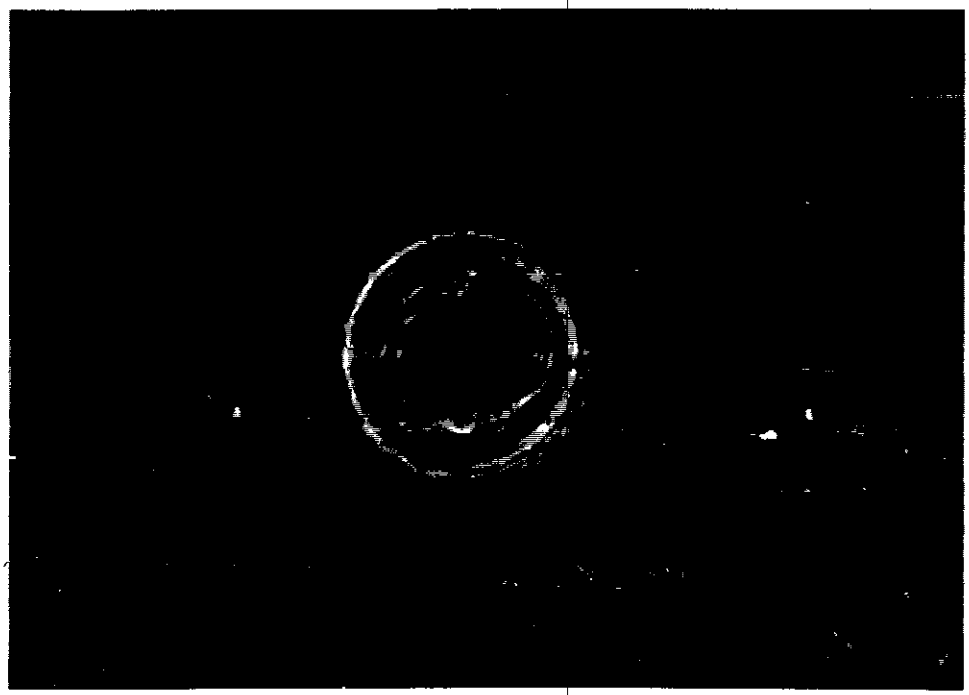

Plate (17). Early blastocyst stage rabbit embryo cultured *in vitro* following *in vitro* fertilization with capacitated spermatozoa *in vitro*.

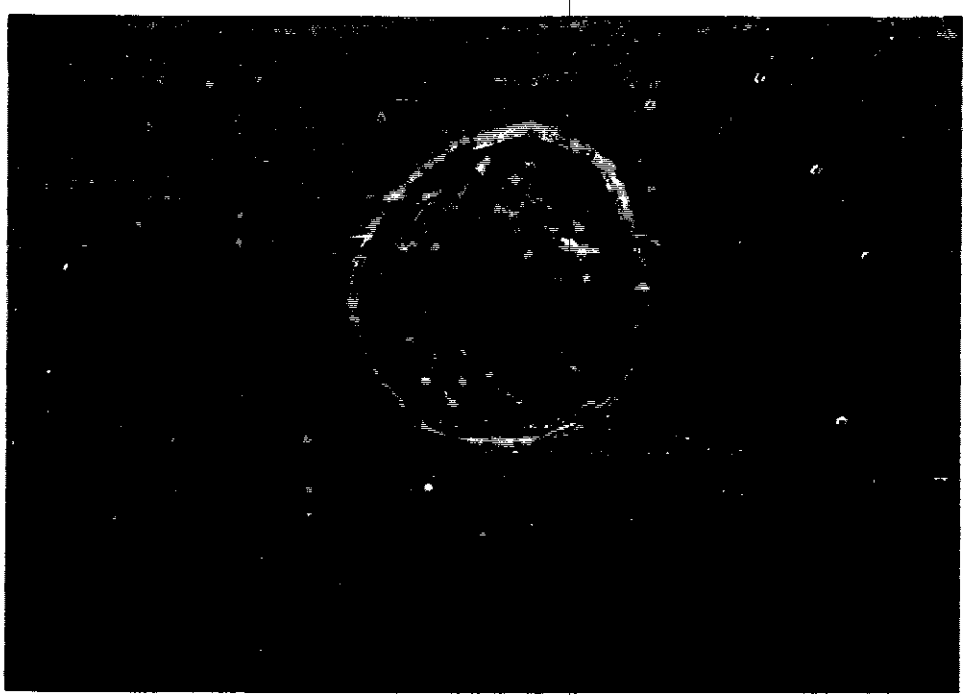

Plate (18). Rabbit expanding blastocyst stage, after approximately 6 days of insemination with *in vitro* capacitated spermatozoa, cultured in TCM-199 medium.