SUMMARY

This work was carried out in IVF laboratory (International Livestock Management and Training Center, ILMTC) Sakha, Kafr El-Sheikh Governorate, belonging to Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture.

This study aimed to investigate the maturation, fertilization and development of rabbit oocytes *in vitro* as affected by season of the year, type of culture medium, source of oocytes and level of osmolarity. The present work comparised two main experiments. The first one dealt with *in vitro* maturation (IVM) with checking fetal calf serum (FCS) and bovine serum albumin (BSA) supplements, *in vitro* fertilization (IVF) and early embryonic development *in vitro* as affected by above mentioned factors. While the second one was designed to study IVF and early embryonic development for superovulated does slaughtered at two different periods.

Experiment I:

I. A. Some morphological measurements on the ovary:

In this study 14 virgin female New Zealand white rabbits aged 6-7 months old were used without any treatment (untreated does). Another 14 females were injected with Pregnant Mare Serum Gonadotrophin (PMSG) hormone (75 IU) and slaughtered after 60 hrs of treatment (PMSG-treated does). Ovaries were excised from both groups to determine the effect of season of the year on left and right ovaries weights, number of corpora lutea (CL), number of follicles and number of oocytes obtained/doe. Results obtained for this experiment could be summarized as follows:

1. Season of the year had a significant effect ($P<0.001$) on the left and right ovaries weights for the untreated group. But this effect was not significant for the PMSG-treated group.
2. Season of the year significantly ($P<0.05$) affected the number of CL on the left ovary, while this effect was not significant for CL presented on the right ovary in the untreated group. In PMSG-treated group, season had no significant effect on the number of CL presented on the both ovaries.
3. Season of the year had a significant effect ($P<0.01$) on the number of visible follicles presented on the left ovary of the untreated group, while this effect was not significant on the number of the follicles presented on the right ovaries in both groups and those presented on the left ovary of the PMSG-treated group.
4. Season of the year did not significantly affected the number of oocytes obtained from the ovaries of the untreated group, while this effect was significant ($P<0.05$) for the PMSG-treated group.

I. B. Oocytes *in vitro* maturation:

Oocytes recovered from untreated and PMSG-treated does at different seasons were selected, washed and cultured in tissue culture medium (TCM-199) supplemented with FCS or BSA, then incubated for 20 hrs in 5% CO2, 95% air. After 20 hrs of incubation all oocytes were examined for evidences of maturation. Results obtained could be summarized as follows:

1. No significant differences among seasons for the maturation rate were obtained.
2. Addition of FCS to TCM-199 medium elevated the percentage of oocytes matured *in vitro* than those of BSA (73.1% vs. 54.4%) for untreated does and (81.3 vs. 65.2%) for PMSG-treated does.
3. Irrespect to the effect of season and protein supplement, PMSG treatment increased significantly ($P<0.05$) the percentage of oocytes maturation (73.2%) compared to (63.7%) for untreated does.

I. C. *In vitro* fertilization of rabbit oocytes:

The oocytes matured *in vitro* pooled and incubated with capacitated spermatozoa *in vitro* for 24 hrs in two media (Brackett's or TCM-199) with varied osmolarity (285, 305 and 325 mOsm/kg) at different seasons. Results obtained could be summarized as follows:

1. No significant differences among seasons were obtained for the fertilization rate.
2. Brackett's medium elevated the percentage of fertilized oocytes *in vitro* compared with TCM-199 (31.0 vs. 20.7% for oocytes collected from untreated does and 32.2 vs. 24.3% for PMSG-treated does).
3. The fertilization rate was significantly ($P<0.05$) higher (41.3%) for the oocytes cultured in media of 305 mOsm/kg than those cultured in media of 285 or 325 mOsm/kg (24.7% and 10.7%, respectively) for untreated does, while these percentages were 46.8, 26.1 and 11.0%, in the respective order for PMSG-treated does.
4. Irrespect of the season effect, media and osmolarity levels, the fertilization percentage was nonsignificantly higher for the ooytes collected from PMSG-treated does than those collected from untreated does (28.3 vs. 25.9%).

I. D. *In vitro* development of rabbit embryos:

The viability of embryos cultured in the two media and assigned the three levels of osmolarity were observed carefully at 24 hrs intervals for progress of development to morula, early blastocyst and blastocyst stages up to six days and the medium was replaced daily with fresh one. Results obtained could be summarized as follows:

1. In TCM-199 culture medium, 20.8% and 28.9% of fertilized oocytes were developed to morula stage after 72-96 hrs of insemination for untreated and PMSG-treated groups,

respectively, while in Brackett's medium 13.9% and 23.1% of fertilized oocytes were developed to the same stage for the respective groups.

2. For untreated and PMSG-treated groups, 16.7% and 18.4%, respectively, of fertilized oocytes cultured in TCM-199 medium were developed to early blastocyst stage after 96-120 hrs of insemination, while these percentages were 5.6% and 13.5%, respectively, in Brackett's medium.
3. Medium of 305 mOsm/kg enhanced the percentage of fertilized oocytes to develop to morula and blastocyst stages, while fertilized oocytes cultured in media of 285 or 325 mOsm/kg failed to reach these stages of development.
4. Irrespect of the effect of media and osmolarity levels, the percentage of fertilized oocytes reached morula and blastocyst stages were 16.7% and 3.3% for those of untreated group, while these percentages were 25.8% and 8.9% for those of PMSG-treated group.

Experiment II:

II. A. Some morphological measurements on the ovary:

In this study, the female rabbits were injected with PMSG hormone (150 IU), 83 hrs later they were injected with HCG hormone (75 IU) to induce superovulation and these females were divided into two groups. The female rabbits in the first group were slaughtered after 10 hrs of HCG injection, while the female rabbits in the second group were slaughtered after 12 hrs of HCG injection, at different seasons. Results obtained could be summarized as follows:

1. Season of the year had a significant ($P<0.001$) effect on the left and right ovaries weights in the females of group 2, while this effect was not significant on the weight of left and right ovaries of the group 1.
2. Season of the year did not affect the number of CL presented on both ovaries in the two groups.
3. Season of the year significantly ($P<0.05$) affected the number of follicles presented on left and right ovaries, also, the total number of follicles on both ovaries of the group 1, while this effect was not significant for group 2.
4. Season of the year did not affected the number of oocytes obtained from the ovaries in the two groups.

II. B. *In vitro* fertilization of rabbit oocytes:

Rabbit ovarian follicular oocytes were collected from superovulated does that slaughtered after 10 hrs of HCG injection (10 hrs group). Also, rabbit oviductal ova and follicular oocytes were collected from superovulated does that slaughtered after 12 hrs of HCG injection (12 hrs group). These oviductal ova and follicular oocytes were

inseminated *in vitro* in a chemically defined media (Brackett's or TCM-199) with capacitated sperm *in vitro* and incubated together for 24 hrs. Results obtained could be summarized as follows:

1. In 10 hrs group, there were no significant differences in fertilization rate between Brackett's and TCM-199 media (49.4 vs. 42.7%).
2. In 12 hrs group, Brackett's medium improved significantly ($P<0.05$) fertilization rate than TCM-199 medium (56 vs. 46.6%).
3. Also, in 12 hrs group, the percentage of fertilization of oviductal ova was significantly ($P<0.05$) higher than those of ovarian follicular oocytes (61.6 vs. 47%).

II. C. *In vitro* development of rabbit embryos:

The fertilized ovarian follicular oocytes that collected from 10 hrs group, also the fertilized oviductal ova and ovarian follicular oocytes that collected from 12 hrs group were cultured in Brackett's and TCM-199 media up to six days after insemination and the medium was replaced daily with fresh medium. Results obtained could be summarized as follows:

1. In 10 hrs group, TCM-199 medium enhanced embryo development to morula, blastocyst and expanding blastocyst stages (44.7, 13.2 and 2.6%, respectively), while these percentages were 31.8, 4.5 and 2.3%, respectively, in Brackett's medium. However, the percentage of the embryos cultured in TCM-199 medium and developed to early blastocyst was significantly ($P<0.05$) higher than those cultured in Brackett's medium.
2. In 12 hrs group, the percentage of embryos developed to morula stage in Brackett's medium was slightly higher than those of TCM-199 medium (45.3 vs. 40.8%), while the percentages of embryos that developed to early blastocyst, blastocyst and expanding blastocyst in TCM-199 medium were slightly higher (25.4, 11.3 and 5.6%, respectively) than those in Brackett's medium (22.1, 9.3 and 3.5%, respectively).
3. Also, in 12 hrs group, the percentages of embryos from oviductal ova fertilized *in vitro* that developed to morula, early blastocyst and blastocyst stages were significantly ($P<0.05$) higher (61.4, 33.3 and 17.5%, respectively) than those from fertilized ovarian follicular oocytes (33, 18 and 6%, respectively). However, the difference between percentages of embryos that developed to expanding blastocyst were not significant (7% vs. 3%).