CHAPTER 5

DISCUSSION

The retrieved bovine oocytes by n-OPU technique significantly higher for the FSH-treated cows than the untreated ones. Similar finding was also reported by Lonergan et al. (1993). Consequently, treatment with FSH increases the number of follicles available for aspiration as compared with those of non-treatment heifers aspirated once a week (Goodhand et al., 1999), as reported in studies on stimulation with FSH (Looney et al., 1994; Stubbings et al., 1995) or with PMSG (Pieterse, 1988; Pieterse et al., 1992).

The good quality oocytes trended to be high from FSH treatment donor cows. Exogenous FSH could be improvement of oocyte quality associated with development and function in follicles (Spicer et al., 1994). Thus, a possible involvement of IGF-I receptor density increasing and decreases the amount of IGF-binding-protein II in bovine follicles (Echternkamp, 1992). The percentage of denuded oocytes significantl higher from non-FSH treatment cows than FSH treatment cows. The retrieved oocytes could be collected from antral follicles of different sizes. These oocytes represented a heterogenous population, since they could retrive from both atretic and non-atretic follicles. Increased rates of abnormal fertilization have been seen in denuded from small follicles and oocytes from follicles smaller than 2-3 mm will have an impaired developmental ability (Leibfried-Rutledge, 1999).

From our study, while the percentage of cleaved embryos of FSH-treated cows were higher than those of untreated cows, they did not differ in the developmental competence following IVF to morulae and blastocysts. This was confirmed by Gibbons et al.(1994) who observed no effect of FSH treatment on the percentage of oocytes developing to morulae and blastocysts. But Goodhand et al.(1999), on the contrary, observed the highest embryo production rate (39.0± 1.0%) from the oocytes of FSH treated donors, rather than non-treated ones. This embryo production rate is comparable to that observed in pregnant cows (Meintjes et al., 1993) and problem breeders (Looney et a., 1994), and may be due to a beneficial effect of FSH which synchronizes the follicle population, advancing their development and initiating oocyte maturation in vivo before recovery (Gibbons et a., 1994). A number of researchers have reported that oocyte quality or developmental competency increase with follicle size (Arlotto et al., 1996; Lonergan et al., 1992 and 1993). Not all studies, however, have agreed with this proposal (Blondin et al., 1994 and 1995) and it has been postulated that hormonal stimulation may uncouple oocyte and follicle maturation (Blondin et al., 1996 and de Loos et al., 1991) and cause asynchrony between the nuclear and cytoplasmic components of oocyte maturation (Bousquet et al., 1995), leading to a disturbance in oocyte development. Other reported that FSH impaired oocyte quality in superovulation protocols, possibly by altering the length of the follicular phase (Greve et al., 1995). In this respect, it is worth nothing that FSH treatment has been suggested to be more beneficial in poorly responding donor cows in OPU procedures (Armstrong et al., 1993; Looney et al., 1994; Gong et al., 1996).

The effect of FSH treatment on oocyte yields and developmental competence may depend on the mode of administration. Multiple doses of FSH have been found

to be more effective in these respects than a single administration of FSH (Goodhand et al., 1999; Stubbings et al., 1993). Combined FSH stimulation and anti-inhibin immunization in unilaterally ovariectomized cows did not improve oocyte retrieved compared with that of intact, untreated cows (Rocha et al., 1996), whereas anti-inhibin immunization alone was found to increase the number of oocytes collected from intact cattle (Konishi et al., 1996). Improvement in vitro embryo development using in vivo matured oocytes from heifers or cows superovulated with a controlled proovulatory LH surge (van de Leemput et al., 1999). In vivo matured oocytes were aspirated from preovulatory follicles in eCG/PG/anti-eCG-superovulated heifers 22 hours after a fixed time GnRH-induced LH surge; endogenous release of the LH surge was suppressed by a Norgestmet ear implant. In the vitro developmental potential of in vivo matured oocytes was twice as high as that of in vitro matured oocytes, with blastocyst formation and hatching rates. It is concluded that IVM is a major factor limiting in the in vitro production of viable embryos, although factors such as the lack of normal preovulatory development of IVM oocytes contributed to the observed differences. Two studies (Bugartz et al., 1995; Gibbons et al., 1994) have found that twice weekly collection could not be improved upon by administration of FSH, possibly because the increased frequency of aspiration resulted in donors having elevated endogenous levels of FSH.

In both gonadotrophin-stimulated and untreated ovaries, the proportion of competent oocytes rise when follicles become larger than 8 mm. This might be due to either 1) a positive selection of follicles with competent oocyte proceeding to the >8 mm stage, 2) a lower rate of atresia, or 3) a further advanced follicular development (Hendriksen et al., 2000). Theoretically, oocytes of 3-7 mm follicles might have a

lower competence than oocytes of > 8 mm follicles. The increase in proportion of competent oocytes in large follicles is probably due to differentiation taking place at the more advanced stages of follicular development. Several changes have been reported in follicles of >8 mm in diameter such as expression of LH receptors by granulosa cells, decrease of IGF- binding proteins and increase of IGF-I within the follicular fluid and increasing expression of growth factors, such as TGF-β, activin and inbibin (Webb et al., 1999). Reis et al.(2002), suggested that intrafollicular regulators of oocyte competence are more important determinants in controlling oocyte competence than peripherally detectable shifts in some endocrine parameters. Indeed, there has been growing evidence that a range of growth factors, binding proteins and metabolites help to orchestrate intraovarian folliculogenesis and oocyte's acquisition of developmental competence (Mc Evoy, 1999 and Webb et al., 1999). The competent oocytes originate from growing follicles reaching a minimum of 3 to 4 mm in size in order to acquire the capacity to response to developmental signals. This capacity increases the percentage of oocytes capable of responding as the follicle reaches a plateau phase or when growth resulting either from dominance or early atresia (both occur during FSH starvation) is decreased. The triggering signal is provided by follicular conditions around the time of the LH surge. Follicular atresia mimics some of the post LH changes occuring in follicle (Sirard et al., 1999), like the rapid decrease in estradiol or the rise in androgen, progesterone and PGE_2. In addition, these is also decreased free radical protection and the occurance of inflammatory-type conditions (Espey L.L., 1994). As the oocytes grow within a follicle, a number of factors influence their health and developmental competence. These factors induce follicle size, day of estrous cycle, level of atresia and influence

of other follicles such as the dominant follicle (Haemann LJ, 1999). Both meiotic and developmental competence increase with the size of the follicle from which the oocytes are removed and with the oocyte diameter.

The study of effect of repeated n-OPU technique on function of bovine ovaries (Kurykin et al., 2002) found that the cyclicity of oocyte donors did not change due to aspiration, the mean length of estrous cycle was 22.05±0.87 days (analyzed by progesterone concentration). For histological examination of bovine ovaries on day 6 following the last puncture session revealed the presence of connective tissue cells in the cortex and hardening of stroma. On day 12, small strips to connective tissue under cortex and mind leukocytes infiltration in stroma were found. These similar to Hill (1995) that all cows maintained regular estrous cycles during the aspiration periods and no adhesion detectable, few indications of ovarian intrusion after slaughtered within 14 days of the last puncture sessions. From our previous study (unpublished data) it has been shown that n-OPU can be repeated in the same animal for at least 5 months without any side effects on the animal. It has also been demonstrated that at the end of the n-OPU treatment, experimental animals are able to return to multiple ovulations and be used in embryo transfer programs. The studied animals could naturally become pregnant and deliver calves. The n-OPU can be used on young or old animals when tertiary follicles are present. The procedure can be performed with or without hormone treatment, and can be used in problem animals that are not detected standing heat or infertile cows.

Conclusion

The results of this study indicated that combination of non-ultrasound guided transvaginal ovum pick-up with FSH follicle stimulation clearly increases the number of retrieved oocytes per cow per session. Eventhough the number of productive embryos from both groups were not different, that demonstrated ovarian stimulation alone to increase follicular size is not sufficient for oocytes to acquire developmetal competence.