New Reproductive Biotechnologies that Affect the Dairy Industry

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Abstract

The emerging biotechnologies will have a revolutionary effect upon domestic animal breeding. The current state of development for in vitro maturation of oocytes, their in vitro fertilization and "cloning" suggests that they will have a major impact on animal agriculture within 5-10 years.

Outline

I. Introduction
II. Effect of exogenous growth hormone
III. Embryo technologies
    1. Collection
    2. Gene injection
    3. Amplication via splitting
    4. Embryo transfer
    5. Mosaics
    6. Extra-specific embryo transfer
    7. Nuclear transplantation
IV. Summary

Literature Review

Progress has been made toward obtaining cow oocyte maturation in vitro (4, 28, 29, 32, 38, 40, 43, 44, 58). Fewer successful reports of in vitro fertilization of cow ova appear in the literature (6, 10, 13, 14, 32, 43). Even fewer reports couple in vitro oocyte maturation with in vitro fertilization. Three recent reviews devoted to these subjects in domestic animals are availabe (7, 12, 67). Only one calf has been born as the result of in vitro fertilization (13).

Mammalian oocytes, upon removal from antral follicles and culture in vitro, spontaneously undergo nuclear maturation (56). Sato et al. (59), Liebfried and First (40) and Fukui and Sakuma (29) reported that significantly more bovine oocytes invested with cumulus cells would mature to the 2nd metaphase than without cumulus cells. Cumulus cells are metabolically coupled to the oocyte via gap junctions conjoining the oolema with the cumulus cell processes that traverse the zona pellucida (2, 25, 26). FSH uncouples sheep oocytes from their cumulus cells (50,51). In vivo, uncoupling occurs near the time of ovulation (26) and this appears to be correlated with mucification (expansion) of the cumulus cells.

Although FSH may not be needed for nuclear maturation in vitro (28, 40, 58), it enhanced maturation (expansion) of the cumulus cells surrounding the oocytes. Ball et al. (4) reported that full expansion of the cumulus oophorus did not occur unless FSH was included in the culture medium. Hensleigh and Hunter (32) found that HCG was included in the degree of cumulus maturation.

Fukui et al. (28) found that the addition of HCG to a bovine oocyte maturation media produced no significant difference in the rate of nuclear maturation as compared to that of LH. Channing and Tsafriri (16) reported that LH overcame the inhibitory action of porcine follicular fluid on oocyte meiosis. However, Liebfried and First (41) reported that neither bovine follicular fluid nor granulosa cells affected the completion of the first meiotic division of bovine oocytes in vitro. However, they (42) found that LH induced resumption of meiosis in porcine oocytes in an in vitro culture system involving follicle wall and cumulus. Receptors for LH/HCG are present on both oocyte (36) and cumulus cells (1). In addition, Darga and Reichert (21) reported that fluid from bovine follicles of all sizes significantly inhibited FSH binding to bovine granulosa cells. Follicular fluid inhibits nuclear maturation and maintains the oocyte in a state of meiotic arrest (17, 24) probably via its effect on the cumulus cells (17). Follicular fluid contains sulfated glycoaminoglycans (5, 27) which can suppress FSH induced cumulus expansion (27). Whether HCG resulted in cumulus maturation via its FSH activity or LH activity is unknown.

Bedirian *et al.* (8) reported that an unexpanded cumulus may present a barrier to sperm penetration. Cross and Brinster (20) and Eppig (24) demonstrated that the presence of cumulus cells during mouse oocyte maturation was important for a high frequency of embryonic development. Ball et al. (6) showed that the in vitro induction of cumulus expansion by FSH prior to in vitro fertilization increased the incidence of bovine oocyte penetration and pronuclear formation by bovine epididymal spermatozoa. Testart et al. (61) found a higher proportion of human eggs cleaved after in vitro fertilization when cumulus expansion was complete. Hensleigh and Hunter (32) concluded that the best treatment for maturing cumulus cells involved adding 1 μg/ml FSH to the culture medium.

Bondioli and Wright (10) incubated ram sperm with sheep cumulus cells to capacitate them prior to in vitro fertilization and subsequently obtained a 12% production of 2-cell embryos from ovarian oocytes. They hypothesized that the cumulus cells aided in sperm capacitation. Mumford et al.

(52) detected aminopeptidase, endopeptidase, trypsin-like, and elastase-like activities in golden hamster cumulus cells. The expansion of the bovine cumulus is significantly, positively correlated with the production of the glycosaminoglycan hyaluronic acid (4) by the cumulus complex. Hyaluronic acid is highly effective at inducing acrosome reactions in bull spermatozoa in vitro (31). Although these compounds may play a role in ovulation, they might also function to capacitate sperm and/or prepare sperm for fertilization. Capacitation involves the removal of epididymal or seminal plasma components (coating proteins) from the sperm membrane thus allowing the acrosome reaction to occur (3, 34, 37). Hensleigh and Hunter (32) reported that in vitro matured oocytes when mixed with ejaculated bull sperm, cleaved to the 2-cell stage with a frequency of 15%.

Other workers have used caudal epididymal spermatozoa (6, 43) or have used earlier stages of fertilization (penetration, male and female pronuclei formation) to assay their in vitro matured bovine oocytes (6, 43, 59). Recently, Bondioli and Wright (10) collected oocytes from the oviduct of cows, superovulated with FSH, fertilized them in vitro with washed fresh or frozen bovine semen and reported that both resulted in 7-11% of the oocytes undergoing division.

Hensleigh and Hunter (32), Miller (48) and Brackett and co-workers (12, 13) reported individual bull differences in the ability to induce cleavage under in vitro conditions.

Thadini (62) and Markert (44) have injected dead sperm into oocytes and observed nuclear decondensation.

Two methods are available for cloning embryos, namely splitting embryos and nuclear transplantation. The first method was developed at ARS, Cambridge, England by Willadsen et al (65) and has found widespread acceptance. Reports using this method can be found in references 15, 39, 53, 63, 66.

The most useful method for cloning desirable animals would be by nuclear transplantation. However no adult nuclei have ever been cloned (47) but nuclei from early mouse embryos have yielded normal young (33, 35, 45, 46).

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