1 Introduction

Embryonic development is a fundamental and dynamic process that leads a single undifferentiated cell in to a highly organized individual with differentiated tissues and organs. However, using the in vitro production system, the success of this intricate pathway involving complex processes, despite its importance, has so far been restricted to below average levels.

In the past three decades, the introduction and wide use of in vitro embryo production has greatly advanced the basic understanding of embryo development and applications of this technology. Despite the dire desire to expand the field applications of in vitro production in cattle, the developmental competence has been limited to 30-40 % (Rizos et al. 2002a). As possible causes, different studies have identified various morphological (Merton 2002), chromosomal (Gianaroli et al. 2000) and transcript alterations (Niemann and Wrenzycki 2000) for the reduced success rate. The cumulative effects of these factors have been implicated in poor growth performance, with the greatest embryonic loss to occur during the preimplantation stage of development (Morris et al. 2001). As a result, the different studies converge to suggest that the underlying causes of abnormal development must be determined during the first seven days of development (Boerjan et al. 2000, Farin et al. 2001), if developmental competence and chances of implantation are to be improved.

It is widely accepted that embryonic development is controlled by genes that are expressed in a temporal and spatial manner (Lonergan et al. 2003). In line with this, the involvement of thousands of gene transcripts at the preimplantation stages of embryo development have been already observed (Ko et al. 2000, Niemann and Wrenzycki 2000), but little is accomplished so far in characterizing these genes, in spite of their importance (Mohan et al. 2002). This is mainly due to limitations in embryo sample volume, high cost of sample production, delicate nature of the samples for handling and processing (Mamo et al. 2003c) as well as scope limitations in the traditional analysis methods (Kozian and Kirchbaum 1999, Liang and Pardee 2003). Moreover, the comparatively low focus given to cattle has created further hindrance for gene characterization in this species. Consequently, most of the information for cattle have been limited to few genes initially studied in model organisms (Mohan et al. 2002). In

addition to these, the existing few data on cattle embryo gene expression were generated under different experimental conditions, creating further hindrance to understand and characterize this dynamic developmental process. Therefore, relevant study directed towards improving the performance is timely and still vital to understand the key players of developmental dynamics and their specific requirements at various stages.

Recently the methodologies have made a remarkable progress to fill the gap. Methodological possibilities to amplify RNA, assess large numbers of genes simultaneously, efficient handling and analysis of the generated data, large number of expressed sequence tags (ESTs) in the data bank, and possibilities of locating the identified genes on the chromosome with a better resolution for ordering genes have all enabled to ease the potential problem and detailed characterization.

Characterizing embryo developmental competence requires, among others, enough probe materials, appropriate analysis methods to identify the genes that show variations or similarity in type or patterns of expression under similar experimental conditions, and the variations that might be observed in level of expression. Further characterization by locating the position and order of genes will serve as a bench mark for comparative mapping purposes in subsequent studies and positional candidate gene identifications.

Therefore the objectives of this study were:-

1. To establish methodologies for the production of enough probe material from a single bovine embryo input, for the analysis of large number of gene transcripts,
2. To establish and apply cDNA microarray procedures for use in the global analysis and identification of transcripts contributing for embryo developmental competence,
3. To quantify some gene transcripts and monitor the transcript variations between different embryo qualities,
4. To map some important genes expressed in the preimplantation stages of embryo development, in order to contribute for the enrichment of the comparative mapping data.

2 Literature review

2.1 Developmental competence

Developmental competence is the ability of the oocyte to pass through different physiological procedures and develop into normal, viable and fertile offspring after fertilization (Sirard 2001, Hansen 2002) that has a potential to develop normally. Developmental competence is a difficult parameter to assess, since embryonic development may fail due to reasons independent of oocyte quality (Mayes 2002, Nusser et al. 2001). Nevertheless, it is usually expressed as the percentage of oocytes that can develop to the blastocyst stage, even if blastocyst stage does not guarantee that the embryo will develop to term (Trounson and Gardner 2000, Nusser et al. 2001). Mammalian oocytes must undergo a regulated program of growth and differentiation from primordial to pre-ovulatory stage in order to be capable of successful maturation, fertilization and preimplantation embryo development (Telfer 1998). However, the mechanisms governing this complex sequence of events are still not well understood but involve a developmentally regulated dialogue between the oocyte and somatic cells (Hashimoto et al. 1998, Telfer 1998). In this process, the role of cumulus cells is to provide nutrients to the oocytes during its growth, to participate in the zona formation, and following the leutinizing hormone (LH) surge, to synthesize the matrix composed of proteins and hyaluronic acid important in oviductal transport or in sperm trapping (Kim et al. 1996, Tesarik et al. 1988).

Oocyte maturation was the focus of myriads of studies in the past decades. Recognising the vital roles of oocyte maturation in development, in cattle alone, various studies (Wise 1987, Blondin and Sirard 1995, Hazelger et al. 1995, Sirard and Blondin 1996, Moor et al. 1998, Ginther et al. 2000, Calder et al. 2001, Mayes 2002, Boni et al. 2002, Beg et al. 2002, Zheng et al. 2003) have been carried. These and other studies have examined the developmental competence of oocytes from various perspectives and all concluded that the respective factors mentioned play significant roles towards achieving the developmental competence. The developmental competence of bovine oocytes has been evaluated, in vitro, in relation to the number and size of the follicles present in the ovary at the time of aspiration. Based on the study, it was found that follicular size is

positively correlated with developmental competence of oocytes in cattle (Pavlok et al. 1992). Oocytes retrieved from ovaries that have at least one follicle larger than 10 mm in diameter or with more than 10 follicles of 2 to 5 mm size have a high developmental potential. In contrast, oocytes retrieved from ovaries with fewer than 10 follicles of 2 to 5 mm or no follicle larger than 10 mm reached the blastocyst stage at a lower rate, and the blastocyst had lower cell numbers (Gandolfi et al. 1997, Blondin et al. 1997). Another study (Blondin and Sirard 1995) that examined the fate of individual oocytes according to the origin of different follicular sizes confirmed that developmental competence increases with size of source follicle. Oocytes from bovine follicles greater than 6 mm in diameter produced blastocysts in vitro at substantially greater rates than those from 2 to 6 mm size (Lonergan et al. 1994, Carolan et al. 1998) and follicles smaller than 2 mm yield oocytes capable of fertilization, but lack the ability to cleave beyond the 8-cells stage (Pavlock et al. 1992). Therefore, the follicle must reach a diameter of at least 2-3 mm before the oocyte reaches a satisfactory developmental competence (Mayes 2002). However, additional factors may also be critical for the oocyte developmental competence. Because some large size follicles contain developmentally incompetent oocytes, while some medium sized follicles contain competent oocytes (Blondin and Sirard 1995, Hyttel et al. 1997). Moreover, oocyte diameter was also related to developmental competence. In cows, oocytes appear to acquire full meiotic competence at a diameter of 115 μm and attain the competence for preimplantation embryo development at a diameter of 120 μm (Otoi et al. 1997).

In addition to the above, variations in the age of oocytes, growth stage, size and atresia grade of the corresponding follicle (Boni et al. 2002), cumulus cell density (Hashimoto et al. 1998, Liu et al. 1995), maturation media (Watson et al. 2000), water quality (Weimer et al. 1998), egg quality, maternal age, environmental exposures and even the stimulating hormones themselves (Schatten 2002) have been found to compromise success. All these factors attribute the variations in the developmental competence, which is reflected in the difference between quality and number of cells per embryo as well as the percentage of oocytes that reach to the blastocyst stage (Rizos et al. 2002b).

In this respect, in vitro production system has been utilized in the past three decades to assist the efforts of establishing optimum culture environment and marker selection procedures to improve developmental competence.

2.2 In vitro embryo production

2.2.1 Background and applications of in vitro embryo production

In vitro production (IVP) is a laboratory technique whereby oocytes taken from a female animal ovary (donor) passes through maturation procedures, fertilized with spermatozoa and the embryo allowed to grow until few cycles of cell division, outside the animal body, in an environment simulated to mimic events that occur naturally. The embryo can then be used to be transferred to the recipient animal, used for genetic selection or used for basic research. Early history of in vitro fertilization, specially the progress until early 1970's has been well reviewed (Bavister 2002b). Application of embryo transfer technology to the cattle industry began in the early 1970's when the European dual purpose breeds of cattle became popular in North America, Australia and New Zealand (Seidel and Seidel 1991). This market driven demand continues until 1977 and gave rise to the development of procedures for non surgical recovery and transfer of cattle embryos (Seidel and Seidel 1991). In vitro production of cattle embryos has its roots in this times and the first trial of in vitro fertilization (IVF) in cattle began in 1970's with the first normal calf born in 1981 (Brackett et al. 1982), three years later after the first IVF baby was born in 1978 (Steptoe and Edwards 1978). However, until the beginning of 1990's fewer than 150 calves were born and has not been used commercially at all in cattle (Seidel and Seidel 1991).

In vitro production practices are more useful in cattle because of low reproduction rate and long generation intervals in this species. It is useful to get multiple embryos from normal genetically superior animals by means of superovulation (Nicholas 1996) and also enables to obtain more offspring from genetically superior cows but became infertile due to disease, age or injury (Lewis 1996). Infertile cows due to anatomical and environmental causes leading to ovulatory failure, oviductal transport failure, disease of the uterus and non responsiveness to stimulatory hormones can also be productive by using in vitro production techniques (Martin and Dolores 2002). Potential applications for animal production, transgenesis, conservation of rare breeds and producing potential bank of genetic material have also been discussed (reviewed by Telfer 1998). Additional potential for producing cloned animals, sexed embryos, and to elucidate