Endoscopy in Cattle Reproduction

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Abstract

Final follicle maturation, ovulation and early embryo development are highly dynamic processes which ultimately result in establishment of pregnancy and the birth of healthy offspring. Any intrinsic or extrinsic changes of the environmental conditions, in vivo and in vitro, including deviations caused by exogenous hormonal stimulation may have negative effects on conceptus development. To date, many technologies have provided important information contributing to our knowledge of early embryo development. Among these techniques, the application of endoscopy for the study of reproductive processes, characterised by a minimal invasive transvaginal entry into the peritoneal cavity, plays a significant role. Once established, endoscopy allows the direct visualisation of the surface of ovaries, oviducts and uterine horns in accordance to pathophysiological changes and enables the collection and transfer of oocytes and embryos at various developmental stages. This technology is particularly suitable for combining in vivo and in vitro embryo culture in order to pinpoint critical checkpoints on this process. This type of translocation from laboratory to the animal and back provides a unique chance to create novel designs and to increase understanding of early reproductive events.

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H. Niemann, C. Wrenzycki (eds.), *Animal Biotechnology 1*,
https://doi.org/10.1007/978-3-319-92327-7_9

9.1 Introduction

Embryo transfer has become an integral part of science and farm breeding management. Many decades of embryo transfer activities in different species historically attest to the impact of this reproductive technology. Apart from the routine application of embryo transfer technologies, continuous improvement of these techniques opens new avenues especially when combining different disciplines. Much work has been done in many species. The focus of this chapter is on the use of endoscopy as a tool to manipulate and to better understand early reproductive events, particularly in cattle. The use of endoscopy in the bovine species will be highlighted in this chapter.

Based on the current developmental progress in science and technology, much and detailed information can be obtained by subdividing this topic into *cells/embryos* and *animals/donor and recipient management*.

9.1.1 The Embryo

Embryo blastomeres represent targets for many diagnostic tools for genetic selection and reduction of the generation interval which significantly enhances efficacy and accelerates progress for research and commercial purposes. Besides the production of embryos via superovulation programmes, the in vitro culture of embryos allows visualisation of developmental steps from oocyte maturation and fertilisation to embryo cleavage up to the blastocyst stage and allows access to stages of early embryo development which are normally hidden and inaccessible when conventionally collecting embryos from the uterine horns. Although in vivo and in vitro methods are used to generate large numbers of embryos, the outcome of embryo culture is highly variable and ranges from early embryo death (Wiltbank et al. 2016) to normal development of the conceptus, implantation and birth of offspring. In order to understand the key mechanisms involved in early embryogenesis, many disciplines have been attracted, and major contributions have been made such as increasing visualisation by histological examinations (embryo structures, cell cleavage, degeneration, atresia; Abe and Hoshi 2003; Leidenfrost et al. 2011), facilitating and accelerating genetic evaluation using expression analyses (Gad et al. 2012), epigenetic approaches (Salilew-Wondim et al. 2015; Shojaei Saadi et al. 2014a), embryo genotyping (Thomasen et al. 2016; Shojaei Saadi et al. 2014b) and focusing on specified pathway analyses (Demant et al. 2015; Van Hoeck et al. 2011; Aardema et al. 2013).

9.1.2 Donor and Recipient Management

Many protocols have been experimentally proven to optimise embryo collection and to promote pregnancy following embryo transfer. To achieve this, detection strategies have been established for a better assessment of animal synchronisation, including precise estimation of heat and time of ovulation (Roelofs et al. 2010), development of the corpus luteum (Bollwein et al. 2013) and evaluation of the hormonal status and, in turn, the use of hormones for optimal synchronisation (Bó et al. 2010; Pereira et al. 2013). Besides slaughter, surgical collection allows the recovery of uterine as well as tubal-stage

embryos. However, it is an invasive procedure which requires particular facilities and expertise (Newcomb and Rowson 1975; Wolfe et al. 1990). Embryo transfer using the surgical route provides an unbiased visual access to the ovary and its corpus luteum, the oviduct and the uterine horns. Hence, this technique allows a controlled manipulation, i.e. the successful and precise transfer of embryos very close to the uterotubal junction. Overall, surgical manipulation has been described as being superior to non-surgical transfer (reviewed by Hasler 2006, Newcomb et al. 1980); however, due to the easy applicability, including ethical aspects, non-invasive techniques are the most preferred. The application of ultrasound is appropriate for detection of ovarian structures such as number and size of follicles and corpora lutea and for pregnancy detection. Surgery provides unimpeded optical access to reproductive organs, whereas ultrasound presents us with an echo modified by the density of the tissue, thus producing a depth effect. Consequently, ultrasonography is well accepted, being more reliable for differentiation of ovarian structures than palpation (Ginther 2014; Smith et al. 2014). Overall, monitoring cyclic activity by ultrasound images has a beneficial effect on optimization of the management of bovine recipients (Guimarães et al. 2015).

9.1.3 Endoscopy

The use of endoscopy provides more options in animal reproduction. It is a minimally invasive procedure, providing direct access to the reproductive organs. However, currently the frequency of routine application in cattle is limited only to a small number of teams (Reichenbach et al. 1994; Santl et al. 1998; Besenfelder and Brem 1998; Wirtu et al. 2010). Initially, there were some successive attempts to perform endoscopic access via the lumbar and mid-ventral area (Sirard and Lambert 1985; Fayrer-Hosken et al. 1989; Laurincík et al. 1991). But this route has been replaced by the transvaginal entry into the abdominal cavity (Reichenbach et al. 1993). In this respect, some groundbreaking work has been carried out in other species such as rabbits, swine and small ruminants (Besenfelder and Brem 1993; Besenfelder et al. 1994, 1997) all of which have contributed to the refinement of this technique in the bovine species.

There are some fundamental characteristics of this technique which make it an attractive method for cattle reproduction:

- The endoscope consists of an optical axis having a small diameter causing minimal lesions.
- Fibre optic for visualisation and illumination of organs.
- Extra channels and tubes for assisting manipulation.
- Minimal anaesthetic intervention.
- Unique in situ approach, avoiding displacement of organs.
- Prevention or minimising post-traumatic damages.
- Repetitive use of the same animal possible.
- Applicable for multiple purposes (OPU, in vivo culture, embryo collection, embryo transfer).

As endoscopy can be used to study final follicular growth, ovulation and early embryo development, it combines development of early embryos derived from

in vivo or in vitro for scientific as well as commercial purposes especially when looking at generating a high amount of developmentally competent embryos and, moreover, can be used as a tool to study the loss of embryos during the early stages of development (Lucy 2001; Diskin and Morris 2008).

9.2 Development of Laparoscopic Access to Ovaries and Oviducts

Attempts have been made to gain access to the oviducts of cattle in order to recover early stage embryos or ensure optimal culture conditions for in vitro matured and fertilised embryos. Initially, access to the bovine oviducts was performed surgically (Trounson et al. 1977) or through surgically prepared and cannulated fallopian tubes of a recipient cow (Jillella et al. 1977). Subsequently, the surgical procedure in cattle was replaced by the use of laparoscopy to minimise invasive access, manipulation stress and postsurgical complications and care. Laparoscopy provides a novel visual and manipulative approach to the bovine genital tract which serves as a basis for many applications such as recording physiological processes including changes during the oestrous cycle and pregnancy, recovery of oocytes from ovaries (Lambert et al. 1986; Sirard and Lambert 1985) and the recovery and transfer of early stage embryos via the oviducts. Fayrer-Hosken et al. (1989) developed a technique for transferring embryos into the oviduct using a bronchoscope. The manipulation of oviducts, ligaments and adjacent organs was done using a Semm's atraumatic forceps. The laparoscope and forceps were placed in the right paralumbar fossa. Mesovarium and fimbria of the oviduct ipsilateral to the ovulation were grasped by atraumatic forceps, and a Tom Cat catheter loaded with embryos was inserted into the oviduct. The transfer of two- to four-cell stage bovine embryos into the oviducts of four synchronised cows resulted in the birth of one healthy calf. Later, Reichenbach et al. (1993, 1994) described a simplified method for repeated laparoscopic exploration of reproductive organs and for aspirating oocytes from follicles of cows and heifers. The most important improvements they suggested were based on the transvaginal entry into the peritoneal cavity. A universal tube together with a blunt trocar was placed via the vagina in the middorsal area of the fornix. The blunt trocar was replaced by a traumatic trocar and introduced through the vaginal wall into the peritoneal cavity. When the traumatic trocar was pulled out of the universal tube, the slight peritoneal negative pressure caused suction of air into the cavity, necessary to have sufficient space for further manipulation under optical control. The bi-tubular system bearing the endoscope and the aspiration line was inserted into the universal tube. The ovaries were presented and navigated by slowly twisting in front of the endoscope via rectal manipulation. This procedure allowed the determination of the ovarian status and the correct positioning of follicles for ovum pickup. Reichenbach and co-workers concluded that this technique was suitable for repeated oocyte recovery without affecting animal fertility.

When using this transvaginal procedure for accessing uterine horns and ovaries, Besenfelder and Brem (1998) showed that it was possible to access the oviducts as well. In order to maximise the benefit from the oviductal impact on the developing embryo, special attention was paid to the preparation of animals. Animals were

deprived of feed for 12 h and restrained in a crush. An epidural anaesthesia using procaine hydrochloride guaranteed rectal manipulation by suppressing rectal contractions. After introduction of the universal tube into the cavity, the amount of air which inflates the abdomen was gradually regulated to avoid the formation of a voluminous peritoneal space which has been considered to negatively affect careful manipulation of reproductive organs. The ultimate objective was to act in situ, to accurately determine the ovarian response to hormonal treatment and to avoid any kind of disproportional manipulation causing hyperaemia or bleeding (see Fig. 9.1). After manipulation, the air from the peritoneal cavity was released using a vacuum

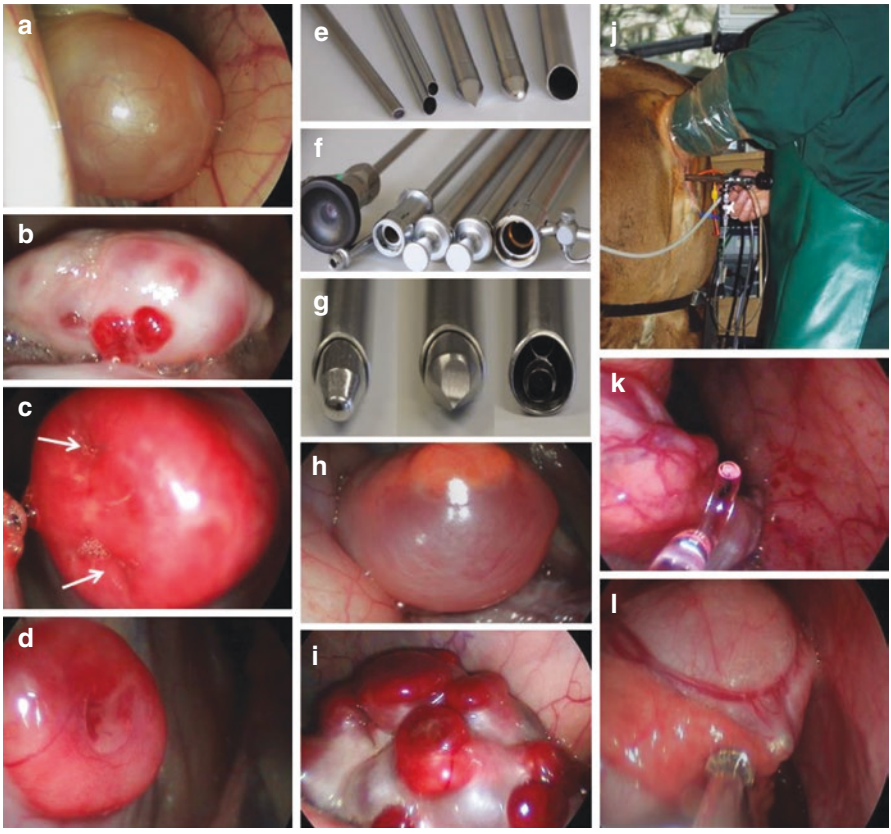

Fig. 9.1 Representative images of transvaginal endoscopy in cattle: (**a**) Graafian follicle showing luteinisation at the follicular basis; (**b**) ovary with a double ovulation at Day 1 and (**c**) at Day 3; (**d**) crown area of a Day 5 corpus luteum where the pulsation of blood vessels in the central dent could be observed; (**e**) tip and (**f**) rear of the endoscopic equipment (endoscope, bi-tubular working channel, traumatic and blunt trocar and universal tube); (**g**) universal tube plus an inserted blunt trocar, universal tube with an inserted traumatic trocar, universal tube and an inserted bi-tubular channel bearing the endoscope; (**h**) follicle 12 h before the expected time of ovulation and a regressing corpus luteum next to each other; (**i**) superstimulated ovary displaying Day 3 corpora lutea; (**j**) view of the donor animal during flushing showing the endoscope plus the uterine flushing catheter; (**k**) transfer glass capillary positioned parallel with the ampulla and (**l**) metal flushing catheter during introduction into the ampulla

pump, and the metal tube containing the endoscope was pulled out. Any further therapeutic treatment was not necessary (Besenfelder and Brem 1998; Besenfelder et al. 2001).

9.3 Transfer of Single Embryos into the Oviduct

Our first studies focused on the feasibility of transvaginal endoscopy as a method enabling the transfer of early stages of in vitro produced bovine embryos into the oviducts (Besenfelder and Brem 1998). The manipulation system consisted of a 1 ml syringe connected to a perfusor tube and a curved glass capillary. This system was entirely filled with medium and the embryos were loaded in the glass capillary. After insertion of the endoscope into the abdominal cavity, the side of ovulation was identified, and the quality of the corpus luteum (morphology and age) was estimated. The glass capillary was inserted via the infundibulum about 5 cm deep into the ipsilateral oviduct, and embryos were deposited in about 50 µl of medium in the ampulla. After overcoming initial problems, the duration of endoscopic manipulation now takes about 10 min.

First experiments describing the transfer of early stages of in vitro produced embryos in 24 animals resulted in the birth of eight calves. All of the short-term in vitro cultured and transferred embryos delivered to term with calves having a normal birth weight. In contrast, one embryo which had been cultured to the morula stage before transfer yielded an oversized calf which had to be delivered by caesarean section. These first data confirmed that the minimal invasive access to oviduct used is suitable for successful transfer of early tubal-stage embryos into a physiological environment (Besenfelder and Brem 1998).

9.4 Recovery of Early Embryo Stages from the Oviduct

Based upon the success of these first transfer experiments, the next target pursued aimed at collecting early stage bovine embryos from heifers by endoscopic flushing (Besenfelder et al. 2001).

For this purpose, the manipulation system was slightly adapted in order to allow oviduct flushing. The flushing system consisted of a 20 ml syringe, a perfusor tube and a curved metal tube with an olive in the front acting as palpation marker during fixation in the oviduct. After introduction of the metal flushing tube into the ampulla, the tube was fixed inside by slight pressure of thumb and forefinger around the olive. Later, the metal tube has been modified in order to enable flushing without digital fixation. For that purpose, numerous lateral holes were drilled and covered by a silicon tube. Consequently, increasing flushing pressure resulted in the inflation of the silicone tube similar to form a balloon which hermetically seals the oviduct and avoids reflux of flushing medium during the oviductal flushing process.

The embryos were flushed orthograde into the tip of the uterine horns where a uterine embryo flushing catheter was fixed as is normally done in MOET

programmes for collection of Day 7 embryos. The oviducts were flushed with 40–60 ml of medium via the uterotubal junction in the direction of the tip of uterine horn. Flushing medium with embryos passed into the uterine embryo flushing catheter connected to an embryo filter. First signs of successful tubal flushing were obtained when a medium flow from the uterine horns into the embryo filter could be observed. Additionally, the uterine horns were flushed with 300–500 ml medium each to ensure that a maximal number of embryos located close to the tip of uterine horns were recovered.

The oviductal flushing has been developed stepwise (Besenfelder et al. 2001). First, single-ovulating animals were flushed following unilateral flushing of superovulated donors. The procedure became successful through continuous refinement and tuning of the system and practice. Ultimately, the oviducts of superovulated animals were flushed bilaterally. In total, it was shown that nearly all oocytes and embryos could be recovered from oviduct flushing. In some cases, mainly depending on the success of hormonal treatment, it was difficult to record the exact numbers of those corpora lutea which were close together on the surface of the ovary forming one big confluent luteal area.

Once developed and established, this flushing method has been used for studies examining the effect of different hormonal treatments, developmental kinetics and repeated collection on embryo recovery (Besenfelder et al. 2008). In a study in which 119 superovulated animals using either FSH or eCG were bilaterally flushed, more than 1400 oocytes/embryos at various stages were collected. The flushing of all these animals illustrated the correlation between hormones and ovarian responses, including different sizes and appearances of follicles and corpora lutea and embryo cleavage during the first days of development. There was no negative effect of repeated flushing which confirmed the usefulness and applicability of this method.

9.5 In Vivo Culture of Bovine Embryos

During the last two decades, the production of bovine embryos for commercial purposes significantly increased. According to the data collated by the International Embryo Transfer Society (www.iets.com), more than one million bovine embryos are now produced annually. Over 600,000 transferable in vivo-derived embryos were collected in 2014, and another almost 600,000 bovine embryos were produced in vitro (Perry 2015). The increase in the in vitro production of bovine embryos has been most dramatic in the last 10 years, mainly due to an exponential increase in the use of the technology in Brazil, which now accounts for most of the commercial in vitro embryo transfer activity. Moreover, in the context of genomic selection in animal breeding, there is a boom awaited in the field of IVP mainly to reduce generation interval and increase selection pressure (Ponsart et al. 2013).

Prerequisites for successful and efficient production of embryos derived in vitro are the collection of high numbers of COCs and developmentally competent embryos leading to pregnancies and the birth of healthy calves. Rizos et al. (2002b) emphasised that the intrinsic quality of the oocyte is the main factor affecting

blastocyst yields, while the conditions of embryo culture play a pivotal role in determining blastocyst quality.

It has been well accepted that the in vitro environment markedly differs from the physiological milieu provided by oviducts and uterus, each of them capable of precisely responding to various changing demands such as the dynamic metabolic requirements according to each embryonic stage (Killian 2004).

The overall objective will always accomplish IVP broadly similar to oviductal performance by mimicking temporal changes in embryo requirements and oviductal fluid composition (Felmer et al. 2011; Wydooghe et al. 2014), using conditioned media (Lopera-Vásquez et al. 2016) or coculture systems with various cell types (Schmaltz-Panneau et al. 2015). Thirty years of research in this field have led to much progress, however, and from the current point of view, it seems to be an almost insurmountable challenge to copy in vitro the multitasking feature of the oviducts since regulatory key mechanisms are still not understood. Consequently, in vitro produced embryos qualitatively lag behind their in vivo counterparts which can be seen in many details such as morphology (Rizos et al. 2002a), altered gene expression (Tesfaye et al. 2004), embryo metabolism (De Souza et al. 2015), increasing cryo-sensitivity (Pollard and Leibo 1994) and embryo/foetal development after transfer and calves after birth (Young et al. 2001).

Being aware of these high demands, it is advisable to directly use tubal features for the improvement of embryo quality by transferring in vitro-derived embryos into the oviducts of temporary recipients.

In first studies attempting to benefit from the fallopian tube, an interspecies transfer was conducted. Bovine embryos were transferred into rabbit oviducts and temporarily cultured in vivo (Rowson and Adams 1972; Sirard et al. 1985). Other researchers cultured in vitro matured/fertilised bovine embryos in the oviducts of ewes (Sirard et al. 1988; Enright et al. 2000). The ewes were hormonally synchronised and prepared by an intravaginal progestogen-releasing device. Early stages of bovine embryos embedded in agar chips or without agar were surgically transferred into the oviducts prior to the ligation close to the uterotubal junction. The embryos were recovered 4–5 days later by the same surgical procedure (reviewed by Lazzari et al. 2010). The practical relevance of in vivo cultured bovine embryos in surrogate sheep oviducts was described by Galli et al. (2001, 2003). It was shown that this technology could be very efficient for the production of large numbers of embryos for commercial purposes. Moreover, the produced embryos were comparable to MOET embryos especially when they had been frozen-thawed before transfer (Galli et al. 2001, 2003). Nevertheless, the use of progestogen-supplemented, ligated heterologous sheep oviducts for in vivo culture of bovine embryos actually does not provide the basic scientific approach necessary to reveal species-specific particularities for bovine reproduction.

This in vivo approach has been accomplished in the bovine species by merging the endoscopic transfer with embryo collection procedure (Havlicek et al. 2005a). Unlike the hormonal treatment of sheep, bovine recipients are synchronised according to the developmental stage of the embryos. Embryos are transferred during the early growth of a corpus luteum which involves changes and modifications of the

tubal epithelium to maximally meet the needs of the embryo. Hence, embryos are placed preferentially ipsilateral to ovulation.

In our first experiments, about 2500 embryos were transferred in groups of 10–50 embryos each after 1–3 days of in vitro culture into the oviducts of synchronised heifers. Recollection was performed 4–6 days later. The recovery rate of embryos at Day 7 revealed the different migration of embryos from oviducts in uterine horns. After solely flushing of uterine horns followed by a second flush using a combined flushing of both oviducts and uterine horns, only about half of embryos were found in the uterus, whereas the other half of the embryos remained in the oviducts. Hence, combined flushing of oviducts and uterine horns was recommended for further and effective embryo recollection after in vivo culture in the oviduct (Havlicek et al. 2005a, b).

In the following studies, Wetscher et al. (2005a, b) examined factors such as temperature, embryo structure, developmental stage, gamete co-incubation and in vitro maturation influencing in vivo culture efficiency:

1. Our first in vivo culture results revealed variable success reflecting the high demand of embryos at a very sensitive stage. Changes of medium for the transfer, a short-term decrease of temperature of the medium and long duration in which the embryos are kept outside the incubator prior to transfer usually decreased blastocyst rates.
2. Tubal migration of transferred embryos is affected by their morphology. The recollection rate increased with the size of a solid matrix around the embryo. Therefore, the best recovery rates were obtained when zygotes were embedded in sodium alginate or transferred in cumulus cells. In contrast, a lower proportion of denuded zygotes or embryos in medium containing 6 mg/ml hyaluronan were recollected, probably due to disturbed migration caused by disoriented beating activity of the ciliated cells in the oviduct. There is much evidence that embryos were expelled into the abdominal cavity.
3. Embryo transfers on Day 1 and 2 resulted in a lower recovery rates on Day 7 compared to transfers on Days 3 and 4. In the periovulation period, the oviducts appear to be hyperactive compared to Days 3 and later (Ruckebusch and Bayard 1975).
4. Blastocyst rates correlate with the stage of transferred oocytes and embryos to synchronous recipient animals. The transfer of more advanced embryonic stages into the oviduct resulted in significantly higher blastocyst rates compared to the transfer of very early stages (Havlicek et al. 2005b; Wetscher et al. 2005b).
5. Gamete intrafallopian transfer (GIFT) seems not to work very well in cattle for starting in vivo culture. The transfer of a mix of in vitro matured COCs with capacitated spermatozoa did not result in an acceptable amount of blastocysts. However, when COCs and spermatozoa were co-incubated for at least 3–4 h before transfer, there was a significant success of blastocyst development.
6. In vitro matured oocytes are not compatible with those matured in vivo, since only a few blastocysts were obtained after the transfer of in vitro matured oocytes into the oviduct of inseminated heifers. Most of the oocytes did not show any

sign of fertilisation. Compared to the high numbers and concentration of spermatozoa necessary to successfully accomplish in vitro fertilisation of in vitro matured oocytes, it seems most likely that failures in fertilisation were caused by the low number of spermatozoa in the oviduct available for fertilisation of in vitro matured oocytes (Ward et al. 2002). Moreover, there is also evidence that initiation of zona hardening occurs immediately when oocytes are in contact with the epithelial cells and are exposed to the oviduct-specific glycoprotein (Coy and Avilés 2010).

7. The zona pellucida undergoes physical changes not only during fertilisation but also during the oviductal passage. Mertens et al. (2007) showed that embryos which migrated through the oviducts into the uterine horns and developed into morulae and blastocysts had a thicker ZP compared to in vitro cultured embryos. Histological examinations revealed an increase in the reticular part, the pores in the ZP were smaller in size and the surface was covered by granules. In contrast, the ZP of in vitro produced embryos showed signs of degeneration (Mertens et al. 2007). Besides the fact that the ZP texture reflects active molecule transportation between the embryo and its environment, this structure also plays a role in the context of reducing sanitary risks when transferring embryos (Van Soom et al. 2010).

Further studies benefited from these first trials and aimed at examining the most critical developmental stages during the early embryo culture period, in vivo culture of embryos in heifers and cows (dried-off vs. milking) and embryo development under superovulation conditions (see Table 9.1).

In a large-scale study, numerous embryos at various stages were produced in vitro and then cultured in vivo for the remaining time until blastocyst stage at Day 7 and vice versa embryos at different stages were collected from the oviducts and in vitro cultured until the blastocyst stage. Using expression profile analyses, it was shown that the most critical developmental steps were found to be around fertilisation, during embryo genome activation around the 8-cell stage and during blastocyst formation. The source of oocyte collection and maturation had detrimental effect on embryo quantity (Gad et al. 2012).

In order to examine the influence of lactation on early embryo development, about 2800 in vitro-derived embryos were transferred to heifers, dairy milking cows and cows which were dried-off immediately after parturition. Embryos were recovered on Day 7. It was demonstrated that the reproductive tract of post-partum dairy cows was less capable of providing the adequate environment for an optimal embryo development compared to heifers (Rizos et al. 2010) and dry Holstein cows (Maillo et al. 2012). Moreover, even the superstimulated reproductive tract significantly affected embryo development during the first 7 days compared to in vivo culture of embryos derived from superovulation donors and transfer into non-superovulated (mono-ovulatory) animals (Gad et al. 2011). Overall, it has been shown that early developing embryos respond rapidly to even small environmental changes which in turn provide a useful indicator system for inadequate culture conditions.

Table 9.1 Recovery and blastocyst development of bovine embryos after in vivo culture in cattle

Transferred embryos			No. of transferred embryos	No. of recovered embryos at Day 7 (%)	No. of blastocysts (%)	Authors
Origin	Stage	Day of transfer/ recipients				
In vitro	2–16-cell	D1–4/ heifers	100	31 uterus/34 oviduct	17 (26.2)	Havlicek et al. (2005a)
In vitro	2–4-cell	D1–2/ heifers	162	75 (46.3)	10 (13.3)	
In vitro	4–8-cell	D3/heifers	199	68 (34.2)	25 (36.8)	
In vitro	1–8-cell	D1–3/ heifers	1358	390 (28.7): recovery from the uterus	48 (12.3)	Havlicek et al. (2005b)
In vitro	1–8-cell	D1–3/ heifers	671	390 (58.1): recovery from the oviduct and uterus	105 (26.9)	
In vitro	COCs	D1/heifers	456	348 (76.3)	3 (0.9)	Wetscher et al. (2005b)
In vitro	GIFT	D1/heifers	514	351 (68.3)	70 (19.9)	
In vitro	4–8-cell	D3/heifers	682	545 (79.9)	304 (43.3)	
In vitro	GIFT	D1/heifers	425	315 (74.1)	114 (36.2)	Havlicek et al. (2010)
In vitro	4–8-cell	D2–3/ heifers	441	264 (59.9)	108 (40.9)	
In vitro	2–4-cell	D2/heifers	1000	790 (79.0)	273 (35.5)	Rizos et al. (2010)
In vitro	2–4-cell	D2/lactating cows	800	458 (57.2)	73 (15.9)	
In vitro	2–4-cell	D2/heifers	2004	1629 (81.3)	953 (58.5)	Carter et al. (2010)
In vitro	2–4-cell	D2/heifers high P4	1673	1240 (75.7)	742 (59.8)	
In vivo	2–4-cell	D2/heifers	164	146 (89.0)	76 (52.1)	Gad et al. (2011)
In vitro	4-cell	D2/heifers	642	642 (88.5)	223 (39.3)	Gad et al. (2012)
In vitro	16-cell	D4/heifers	811	811 (76.4)	350 (56.5)	
In vitro	2–4-cell	D2/lactating cows	435	289 (65.6)	97 (32.6)	Maillo et al. (2012)
In vitro	2–4-cell	D2/ non-lactating cows	627	403 (63.9)	203 (49.3)	

Conclusion

The application of endoscopy to embryo collection and transfer provides unrivalled access to the reproductive tract as well as facilitating the collection and manipulation of various different oocyte and embryo types resulting in increased knowledge and understanding of embryo development under optimal environmental conditions. While the use of endoscopy requires particular expertise and experience, this technique can be easily combined with other routine reproductive technique in order to generate large numbers of developmentally competent embryos for both science and commercial application.

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