Reproductive physiology and assessment of early pregnancy

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Introduction

The physiological processes which control or influence the reproductive cycle are diverse. Endocrine factors essential for the release of mature ova and successful implantation and development of the early conceptus are quite complex. They control the cyclical changes in the levels of steroids and gonadotrophic hormones necessary as part of the reproductive cycle.

Conventional 2D/3D transvaginal (TVS) ultrasound is unable to provide quantitative measurement of physiological change unlike serum biochemistry. However, improvements in TVS ultrasound technology continue to increase the ability to recognise and understand anatomical factors, particularly those relating to the ovaries and uterus, which reflect the above.

The ovary is an extremely dynamic, constantly changing organ during the reproductive life span. Its basic functions are to produce mature eggs for fertilisation as well as generate specific steroid hormones namely oestrogens and progesterones. TVS imaging demonstrates the considerable morphological changes within the ovary associated with ovulatory status, fertility and early pregnancy development. In addition, it provides detailed examination of the endometrium, the target organ particularly for oestrogens and progesterones secreted by the ovary, as well as a gland responsible for supporting and facilitating the implantation process. TVS recognises characteristic ultrasound appearances of the endometrial stroma and associated changes at given stages of the menstrual/reproductive cycle.

The ability of ultrasound to gauge physiological changes is further enhanced by advances in colour Doppler imaging, particularly SMI (Superb Microvascular Imaging). SMI identifies vascular changes in tissues which reflect hormonal activity. It highlights angiogenesis as part of endometrial proliferation and placentation. Studies confirm peripheral vascularity of the peri-ovulatory follicle as shown by SMI technology accurately reflects hormonal function of the ovary.

Modern TVS ultrasound technologies, which include high-resolution 2D and 3D grey-scale and SMI imaging modalities, have a crucial role in assessing key elements of the reproductive cycle. They provide important clinical information relating to the ovulatory cycle and fertility, recognition of ovulatory disorders and evaluation of early pregnancy progress and associated complications. In addition, they are able to recognise functional issues, particularly those emanating from the ovaries and involving hormonal activity. Functional disorders remain a major cause of gynaecological symptoms such as abnormal uterine bleeding and/or pelvic pain.

Ovaries

The ovary basically consists of 3 anatomical components: the ovarian capsule, ovarian cortex and ovarian medulla. In ultrasound, the cortex and medulla are collectively termed the ovarian stroma. The ovarian cortex remains the main focus of ultrasound examination – it contains an enormous number of oocyte-containing follicles.

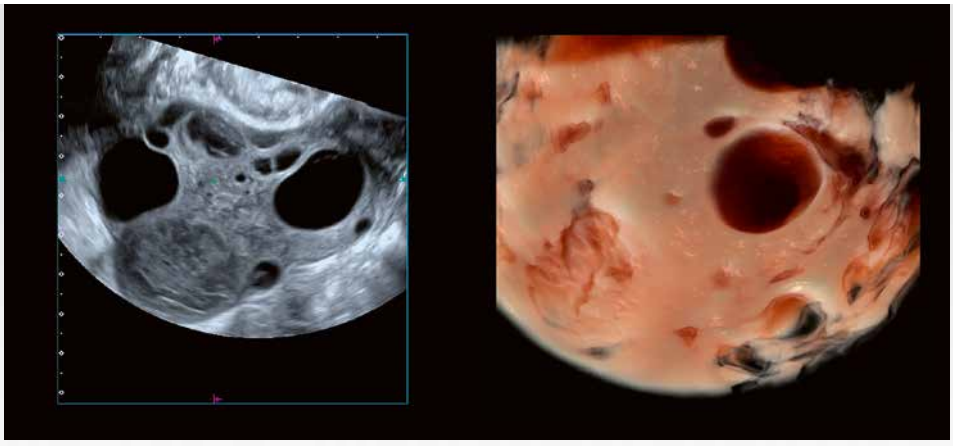

Fig. 1: Detailed imaging of ovarian morphology utilising 3D grey-scale reconstruction and Shadow Glass rendering respectively. Advanced 3D TVS imaging demonstrates a persistent, clot-filled luteal cyst, immature pre-ovulatory follicles, antral follicles as well as atretic follicles and remnants of a previous ovulated follicle.

During any cycle between menarche and menopause the ovarian cortex supports follicles at varying stages of development. Those visible to ultrasound range from multiple antral follicles to a number of immature follicles to the usually single dominant pre-ovulatory (Graafian) follicle (Figs. 1 + 3).

The ultrasound appearances of the ovary vary significantly corresponding to the morphological changes which occur relative to age and parity. The anatomical impressions gained from an extensive observational study are illustrated in Figure 2. 10,000 patients aged 15–60 years were examined utilising 3D (volumetric) TVS imaging. The findings relate to ultrasound features present within the mid-proliferative phase of the menstrual cycle.

The ovaries are characteristically "multifollicular" in nature following the onset of puberty and typically remain so until pregnancy occurs or the woman enters middle age. There appears to be a trend of increasing follicle size with corresponding decrease in follicle numbers noted for this stage of the ovulatory cycle which continues throughout the reproductive life span. This variation in follicle patterns is seen to be accompanied by simultaneous development of the ovarian stroma. The changes illustrated in Fig. 2 appear to correspond well with fluctuations in levels of serum FSH and AMH, particularly within the later age groups.

Variation in the follicle/stromal pattern described can often be associated with clinical issues. The ultrasound characteristics of the so-called PCO ovary is well documented but can be associated with other forms of ovulatory/menstrual problems. Indeed "normal" multifollicular changes present within the teenage-early twenties age group appear to fit the ultrasound definition of PCO as stated by the ESHRE/ASRM Rotterdam Consensus guidelines! Multifollicular ovaries with reduced stromal activity is often present in cases of hypothalamic-pituitary dysfunction or failure and typically linked to the use of oral contraception or even life style factors. Multifollicular ovaries particularly within the later stage of reproductive life span are frequently found to be associated with menorrhagia and other menstrual problems often involving "oestrogen dominance". Understanding morphological changes within the ovary in relation to ovarian (endocrine) function is essential in the investigation of gynaecological disorders, as well as in the field of reproductive medicine.

The ovarian capsule envelops the ovarian stroma. 3D (volumetric) TVS provides accurate delineation of the capsule and uniformity of thickness. It typically appears as a thin, regular interface. Histologically it is composed of germinal epithelium with an overlying layer of connective tissue (tunica albuginea). There is no significant change in appearances during the cycle. Increased thickening has been shown to be associated with PCOS. Localised irregularity can be caused by pathological changes such as endometriosis, ovarian malignancy etc.

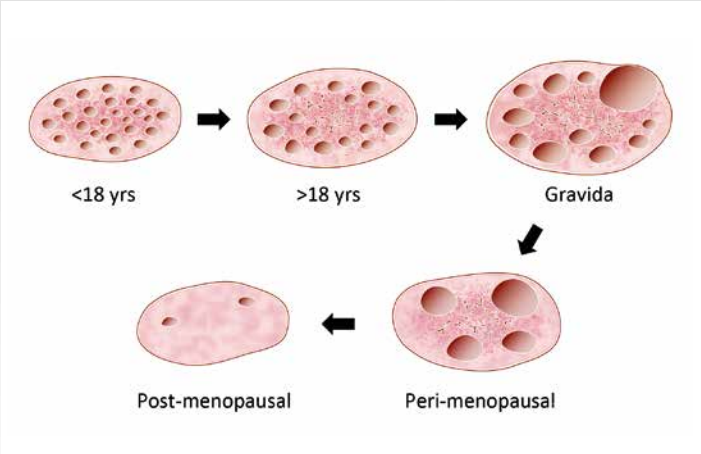

Fig. 2: Changes in the ovarian follicle – stromal pattern with age and parity. Impressions from a large study utilizing 3D (volumetric) TVS.

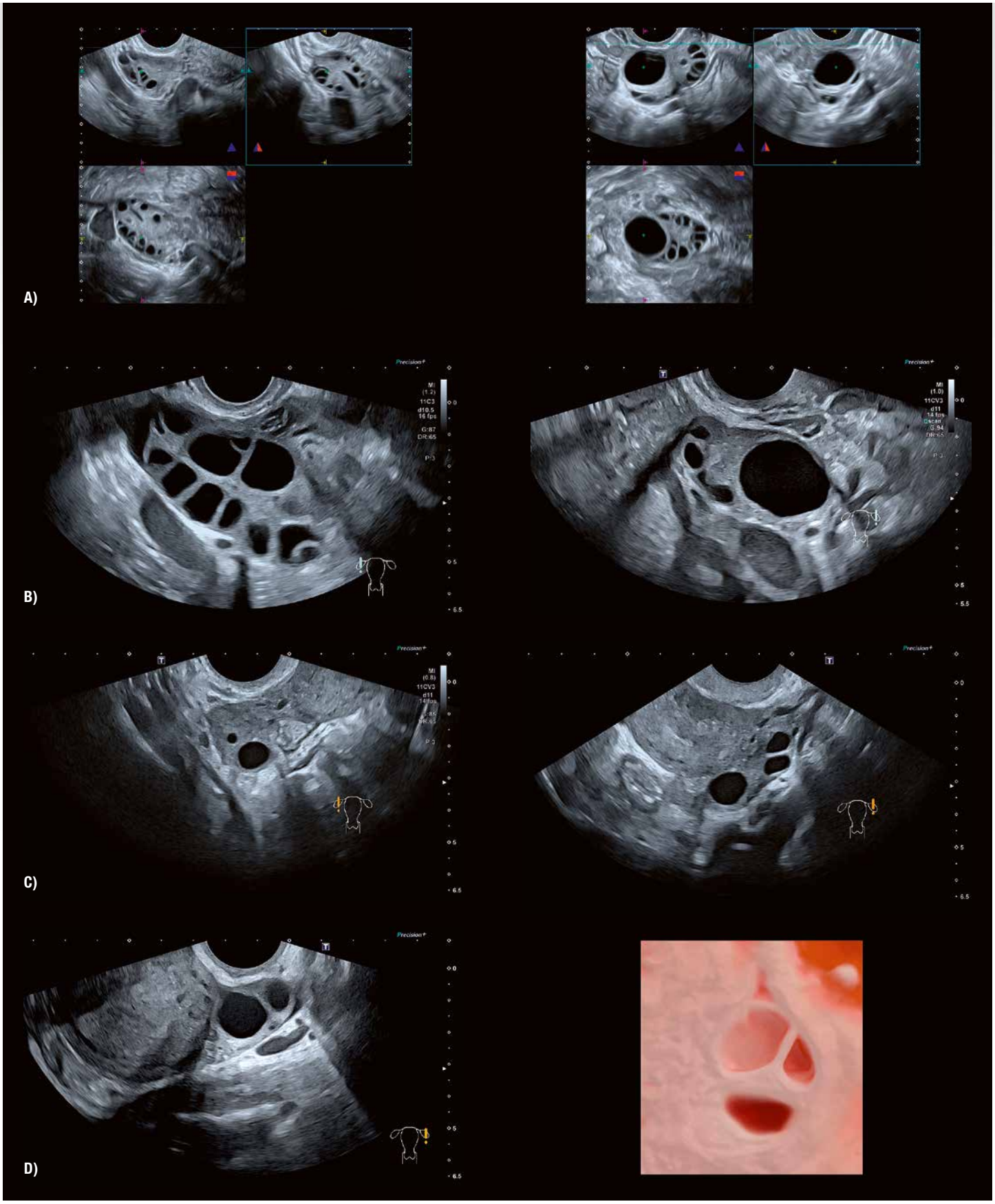

Fig. 3 A: 3D anatomical planes show the typical multifollicular nature of the ovarian follicle pattern in the early to mid-late teens age group. Fig. 3 B demonstrates the changing follicle-stromal characteristics for women, such as those in their mid-twenties and thirties, particularly following childbirth. Fig. 3 C: Typical appearances for the forties age group. Fig. 3 D: 2D and 3D (Luminance) images delineate follicles of a women in the mid-late forties age group. The rendered image clearly shows the follicle outline comprising the granulosa and theca cell layers.

The medulla forms the inner, central part of the ovarian stroma. It consists largely of connective tissue containing the essential neurovascular network of the ovary. Vessels and nerves enter the medullary stroma at the hilum of the ovary. SMI visualisation of the hilar vessels (Fig. 4) can often assist in identifying and localising the ovary. This applies to visualisation of inactive ovaries particularly in the case of poor ovarian function or in post-menopausal patients.

The cortex contains the primordial follicles and supports their transition to the pre-antral stage of development as part of follicle growth and maturation. The follicle at this stage consists of a primitive, or primordial, oocyte surrounded by granulosa cells encapsulated by a collar of cells termed theca cells. Increased thickening of the granulosa and theca cells occurs with formation of a central fluid collection. These developments result in the formation of the antral follicle. The "cystic" appearance enables visualisation of the antral follicles from 2 to 3 mm size. Antral follicle size ranges between 2 and 8 mm. Antral follicle counts, carried out prior to IVF treatments or to estimate ovarian reserve in peri-menopausal patients, are accurately gauged using 3D TVS and carried out around cycle days 3–5 following onset of menstruation.

The ultrasound measurement and monitoring of follicle growth during a natural or ART-related cycle has long been established. Advances in TVS imaging technology have significantly increased the ability to evaluate follicle maturation and predict the onset of ovulation (Fig. 5). High-definition grey-scale scanning demonstrates the thickening of the follicle wall due to increasing development of the granulosa and theca cells. Internal shedding of the granulosa cells can be identified within the peri-ovulatory follicle. Fly Thru 3D imaging often appears to recognise the cumulus mass immediately prior to follicle collapse at ovulation (Fig. 6). Follicle size at the onset of ovulation can vary anywhere between 1.8 to 3.0 cm. Note: Follicle size alone is not always indicative of follicle maturation.

SMI outlines increasing peripheral vascularity associated with (Graafian) follicle maturation. It also offers accurate gauging of the timing of ovulation within a monitored cycle. Increased vascularity has been shown to be associated with ovulatory status and development of a favourable peri-ovulatory endometrium (Figs. 5 + 6). Studies have shown a good correlation between peripheral angiogenesis of the peri-ovulatory and post-ovulatory follicle with serum oestrogen and progesterone levels respectively. SMI provides a reliable means to assess luteal phase function as part of fertility monitoring and assessment of very early pregnancy (Figs. 9 + 13).

The post-ovulatory follicle, or corpus luteum, can vary tremendously in its appearances and can mimic pathological lesions. Peripheral blood flow around the lesion is very evident certainly within the early-mid luteal phase. It reduces in non-conception cycles within the week leading up to menstruation with gradual collapse of the lesion. This corresponds to reducing levels of progesterone provided by the ovary resulting in menses approximately 14 days after ovulation in a typical cycle. If pregnancy occurs, the corpus luteum remains intact and active.

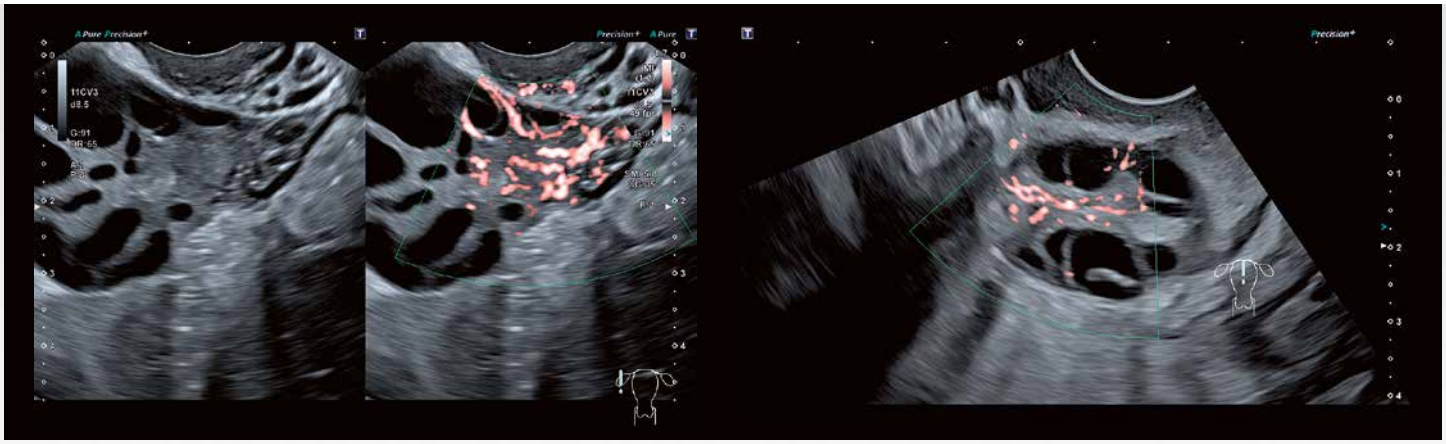

Fig. 4: SMI clearly demonstrates the ovarian hilum and normal distribution of the ovarian vessels within the medullary stroma of the ovary in separate patients.

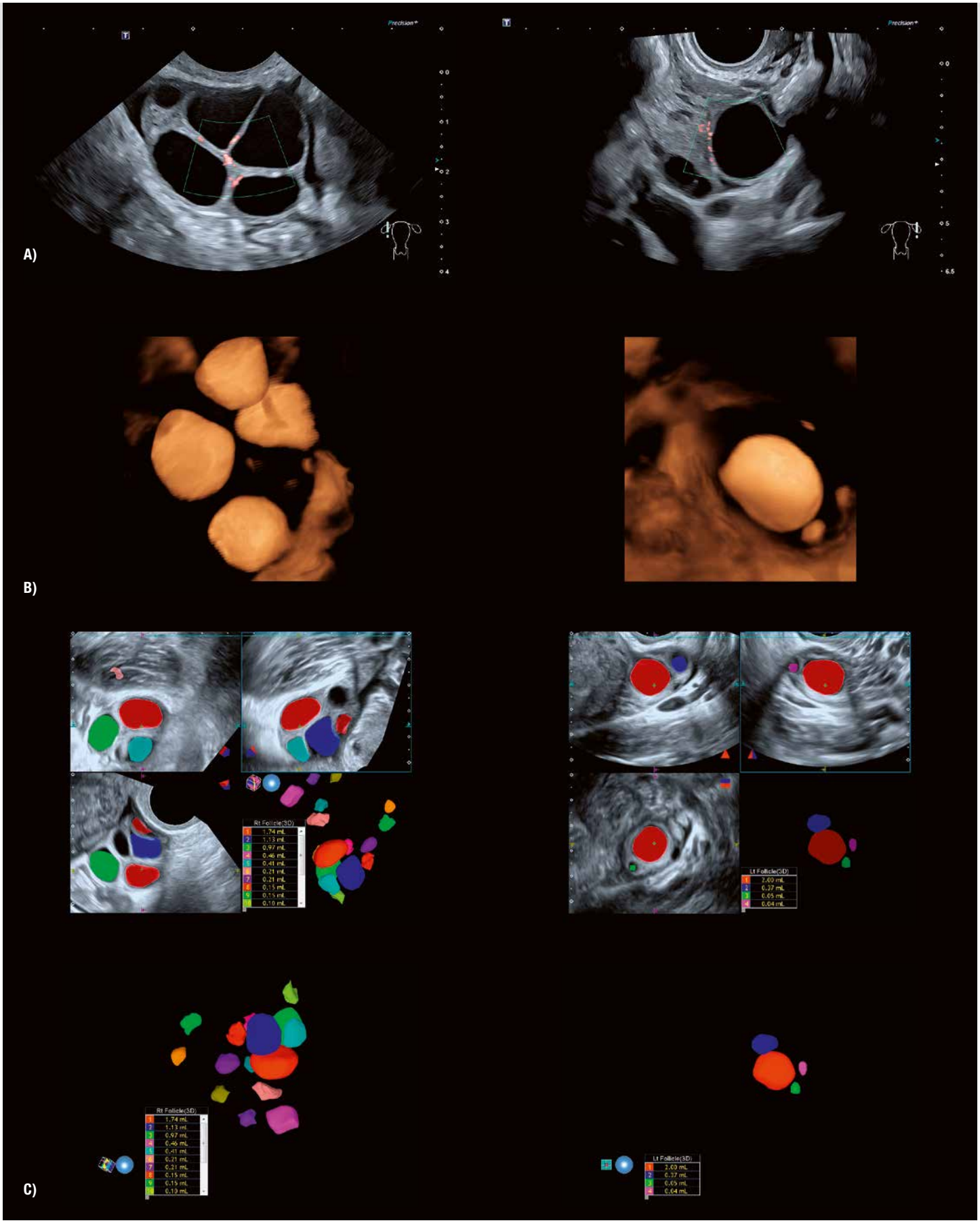

Fig. 5 A: SMI identifies peripheral angiogenesis associated with maturing follicles following ovarian stimulation. Fig. 5 B: 3D surface rendering clearly delineates the follicles on both ovaries. Fig. 5 C: Automatic follicle volume measurement with colour coding function is of obvious benefit in terms of monitoring ART cycles – it provides very effective evaluation of follicle numbers as well as follicle growth.

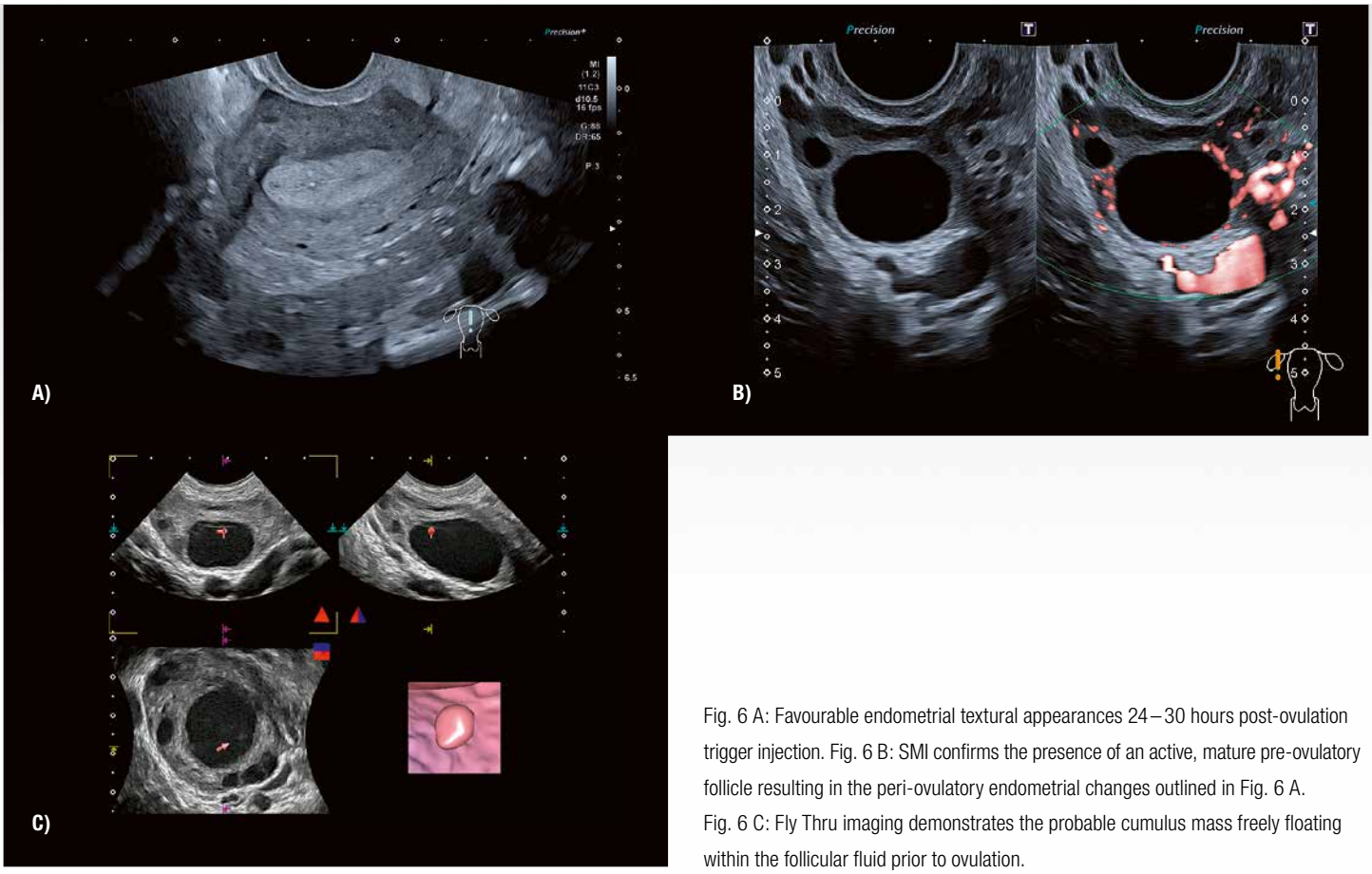

Fig. 6 A: Favourable endometrial textural appearances 24–30 hours post-ovulation trigger injection. Fig. 6 B: SMI confirms the presence of an active, mature pre-ovulatory follicle resulting in the peri-ovulatory endometrial changes outlined in Fig. 6 A.
Fig. 6 C: Fly Thru imaging demonstrates the probable cumulus mass freely floating within the follicular fluid prior to ovulation.

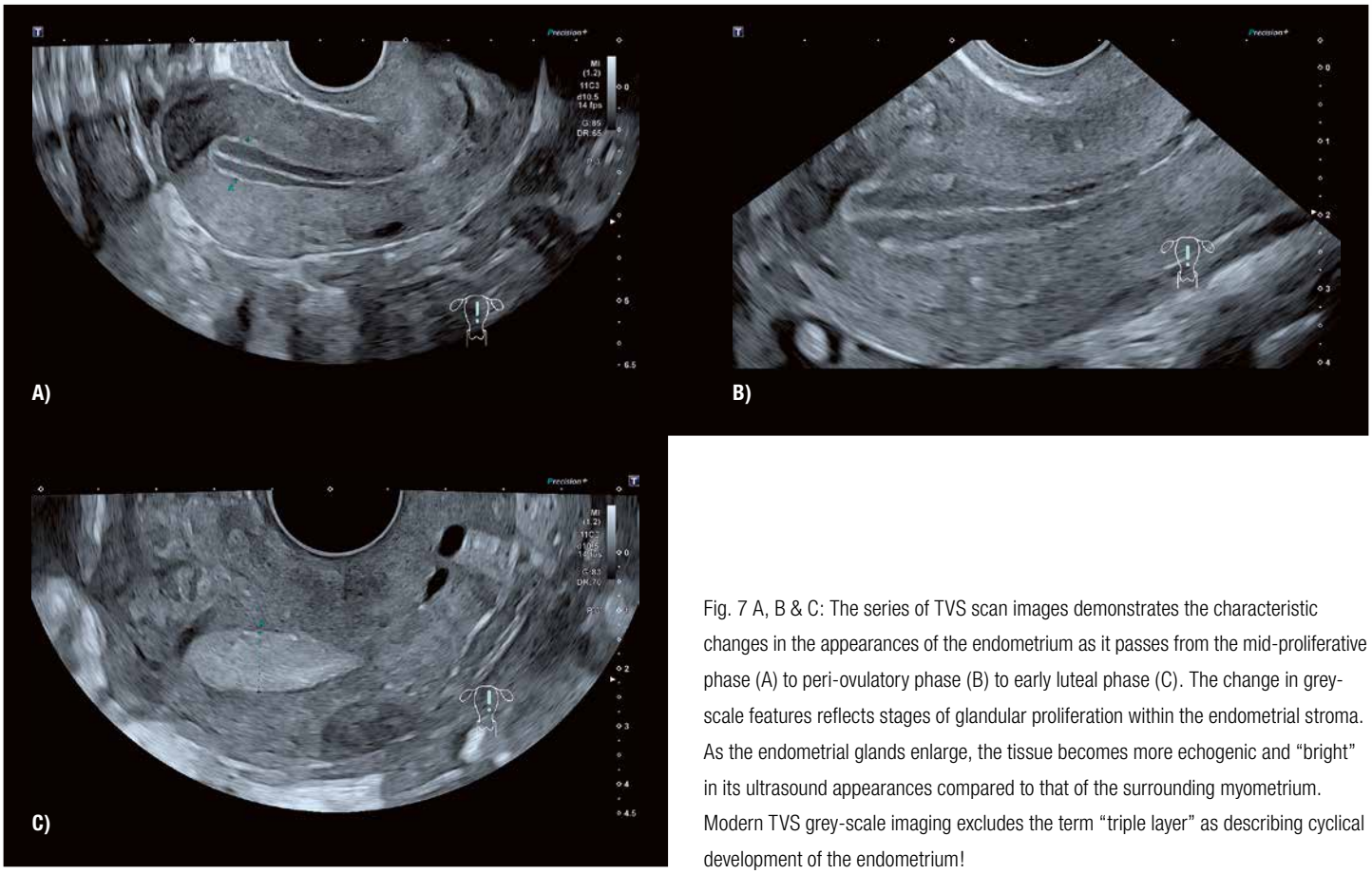

Fig. 7 A, B & C: The series of TVS scan images demonstrates the characteristic changes in the appearances of the endometrium as it passes from the mid-proliferative phase (A) to peri-ovulatory phase (B) to early luteal phase (C). The change in grey-scale features reflects stages of glandular proliferation within the endometrial stroma. As the endometrial glands enlarge, the tissue becomes more echogenic and "bright" in its ultrasound appearances compared to that of the surrounding myometrium. Modern TVS grey-scale imaging excludes the term "triple layer" as describing cyclical development of the endometrium!

Uterus

Both the myometrium and, in particular, the endometrium are extremely sensitive to ovarian steroids, namely oestrogens and progesterones. Oestrogens have a direct effect on the smooth muscle fibres of the myometrium. The changes in the appearances of the myometrium caused by fluctuating levels of oestrogen tend to be relatively gradual (except in the case of pregnancy), certainly compared to its influence on the endometrium. They are reflected ultrasonically in terms of uterine size and myometrial thickness, textural (grey-scale) and glandular appearances as well as myometrial vascularity. In comparison, the endometrium, composed of glandular epithelium, undergoes striking microscopic changes in its glandular structure and function. This occurs on a cyclical basis and provides the physiological basis of the ovulatory/menstrual cycle.

During the pre-ovulatory stage (i. e. proliferative or follicular phase) of the menstrual cycle, the endometrium proliferates profusely under the influence of oestrogen. The endometrial glands rapidly develop and create what is termed the endometrial stroma. It is the enlargement and elongation of the endometrial glands, which emanate from the basal region and grow towards the superficial tissue layers that produce the cyclical changes and characteristic ultrasound (grey-scale) appearances of the endometrium (Fig. 7). Modern high-definition TVS demonstrates the glandular development in detail as the pre-ovulatory stage approaches. Simultaneously, SMI identifies the spiral arteries growing between the glands. Full and appropriate assessment of the endometrium involves evaluation of thickness, texture and vascularity (Fig. 8).

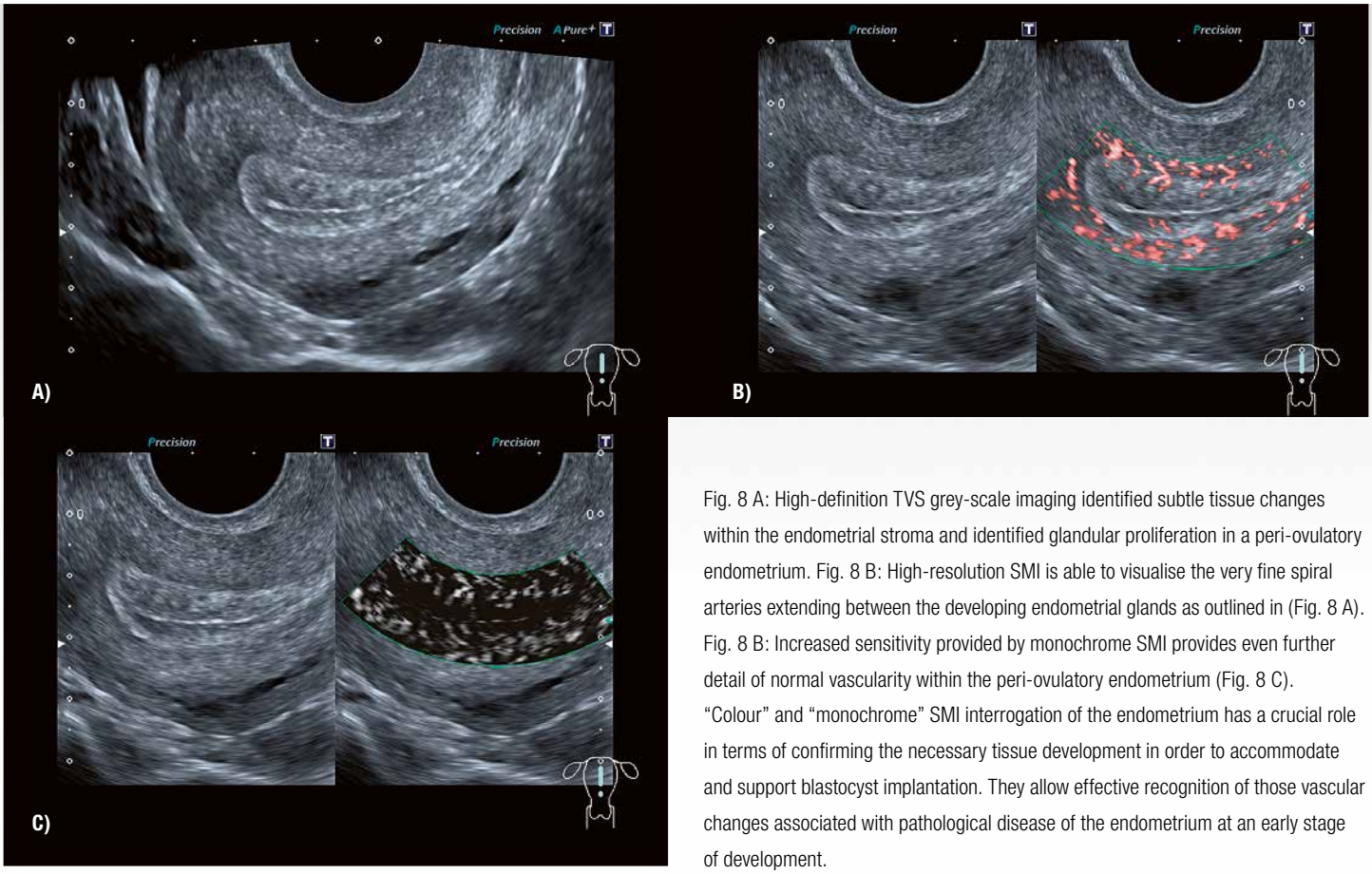

Fig. 8 A: High-definition TVS grey-scale imaging identified subtle tissue changes within the endometrial stroma and identified glandular proliferation in a peri-ovulatory endometrium. Fig. 8 B: High-resolution SMI is able to visualise the very fine spiral arteries extending between the developing endometrial glands as outlined in (Fig. 8 A). Fig. 8 B: Increased sensitivity provided by monochrome SMI provides even further detail of normal vascularity within the peri-ovulatory endometrium (Fig. 8 C). "Colour" and "monochrome" SMI interrogation of the endometrium has a crucial role in terms of confirming the necessary tissue development in order to accommodate and support blastocyst implantation. They allow effective recognition of those vascular changes associated with pathological disease of the endometrium at an early stage of development.

The length of the follicular phase, i. e. from cessation of menses to ovulation in natural cycles, is typically 10 to 12 days but can vary tremendously in a number of cases. Endometrial thickness usually ranges from 7.5 to 10.0 mm at ovulation but again significant variation can occur. Note: Thickness measurement alone does not necessarily reflect what can be regarded as adequate peri-ovulatory endometrial development.

In most normal cycles, ovulation occurs around 13 to 16 days from the first day of the preceding menstrual period. Increasing levels of oestrogen trigger a surge in luteinising hormone secreted from the anterior pituitary gland which in turn promotes ovulation within 30 to 40 hours. Rapid changes occur in the textural appearances of the endometrium around ovulation. The endometrium appears uniformly very echogenic compared to the grey-scale nature of the myometrium (Fig. 9).

This is the result of rapid changes within the glandular epithelium and associated vascularity. Note: SMI is not used from ovulation onwards due to concerns regarding colour Doppler bio-effects. The ovulatory cycle now enters the secretory or luteal phase. The features described are the direct result of ovarian follicle maturation. The hormonal environment within the uterus and particularly that influencing the endometrium becomes progesterone-dominant and relies on continuing hormonal activity of the corpus luteum following ovulation (Fig. 9).

The principal function of progesterone is to stimulate the secretory endometrium to create a receptive environment for implantation to occur and support the development of the early conceptus. Concurrent with increasing glandular changes at the post-ovulation stage are marked alterations within the stromal cells themselves. Increased metabolic activity occurs within the cells which become very eosinophilic. These changes occurring at a very cellular level promote what is termed decidualisation if conception occurs. If pregnancy does not occur within a given menstrual cycle, progesterone provided by the corpus luteum starts to reduce during the latter stage of the secretory phase and stops by days 10–12 post ovulation. The endometrium undergoes ischemic necrosis and breaks down eventually shedding as menstrual debris accompanied by bleeding (Fig. 10).
In the presence of pregnancy the endometrium, termed the decidua, continues to develop under the influence of oestrogens and maintained by secretion of progesterone from an intact, active corpus luteum (Fig. 13). TVS grey-scale imaging confirms continuing proliferation of the endometrium and SMI confirms the presence of a persistent corpus luteum. Breakdown of endometrial tissue and/or failure of the corpus luteum can be identified

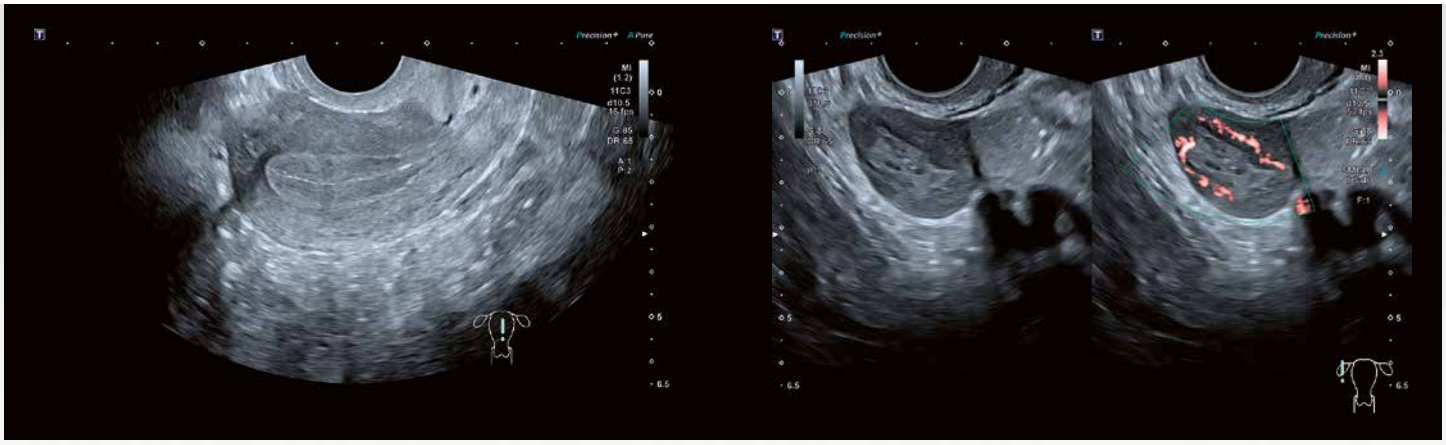

Fig. 9: Typical appearances of the endometrium and recently collapsed (ovulated) corpus luteum approx. 40 hours following the (urinary) LH surge. SMI demonstrates the continuing activity of the corpus luteum in order to maintain the endometrium into the luteal phase of the cycle.

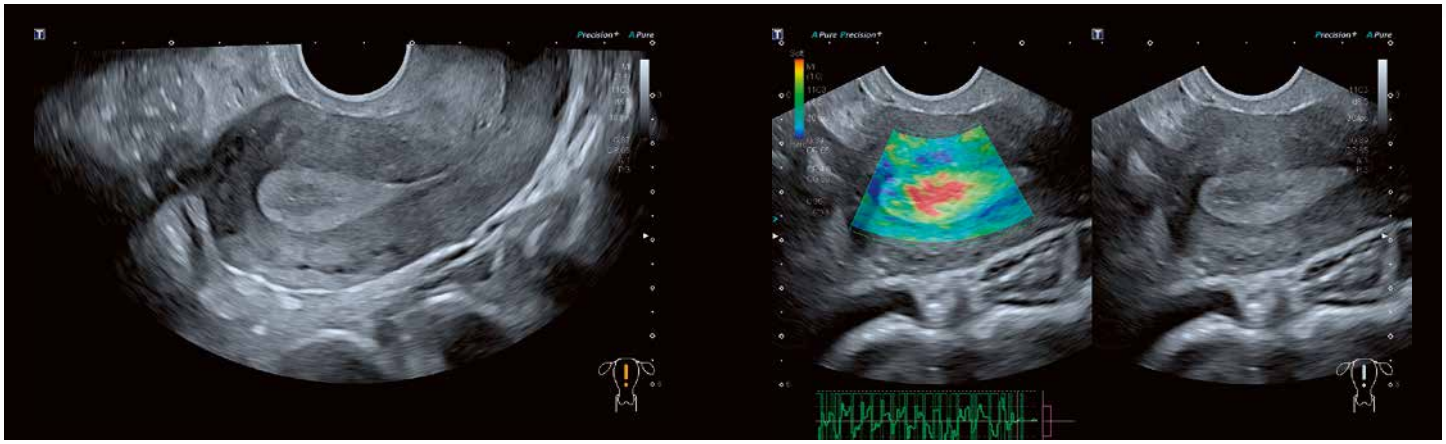

Fig. 10: TVS grey-scale and realtime elastography (RTE) examination of the endometrium. RTE highlights mid-luteal phase breakdown of tissue, which emanates from the superficial layers of the endometrium, in a non-conception cycle.

relatively early within the secretory phase. The ability to visualise these changes by modern TVS techniques enables ultrasound to identify cases presenting with luteal phase (progesterone) deficiency. Ultrasound remains a crucial element not only as part of fertility studies but also monitoring of early pregnancy progress and investigation of recurrent miscarriage.

Early pregnancy

Fertilisation takes place within the Fallopian tube. Cilia, part of the columnar epithelium lining the lumen of the tube, provide wavelike motions in order to transport the non-mobile fertilised egg towards the uterine cavity. A failure involving this mechanism can result in implantation within the tube itself and presence of an ectopic pregnancy. At fertilisation the nuclei of the sperm and oocyte fuse to produce the zygote. Rapid cell division occurs to form a solid cluster of cells, the morula. Separation of internal cells result in the formation of a fluid-filled space surrounded by the outer cells. This process is completed within approx. 5 days following fertilisation. The resulting organism is called the blastocyst. The first stages of implantation within the uterine cavity occurs approximately a day following blastocyst formation. At this point the outer cell layers of the blastocyst form the trophoblast. These cells will form the placenta and other extraembryonic structures. An inner cell mass, the embryonic disc, forms within the enclosed fluid space – these cells will form the embryo.

As part of the implantation process, the trophoblastic cells further differentiate into two layers. The outer layer (syncytiotrophoblast) is active in the production of placental hormones to include hCG (human chorionic gonadotropin) and is responsible for nutrient transport from mother to the early fetus. The inner layer (cytotrophoblast) is involved in the invasive process essential for successful implantation and ultimately formation of the placenta.

Concurrent with implantation of the blastocyst, the endometrium undergoes dramatic biochemical and morphological change, i. e. decidualisation. The decidualised cells rapidly proliferate and spread to completely envelope the early conceptus. The endometrium, now termed the decidua, continues to rely on progesterone secreted by the existing corpus luteum of the ovary. HCG produced by the developing trophoblast promotes corpus luteal activity which is essential during the first few months of pregnancy. A failure in this crucial interrelationship between the ovary and the endometrium is regarded by many as a major cause of early miscarriage.

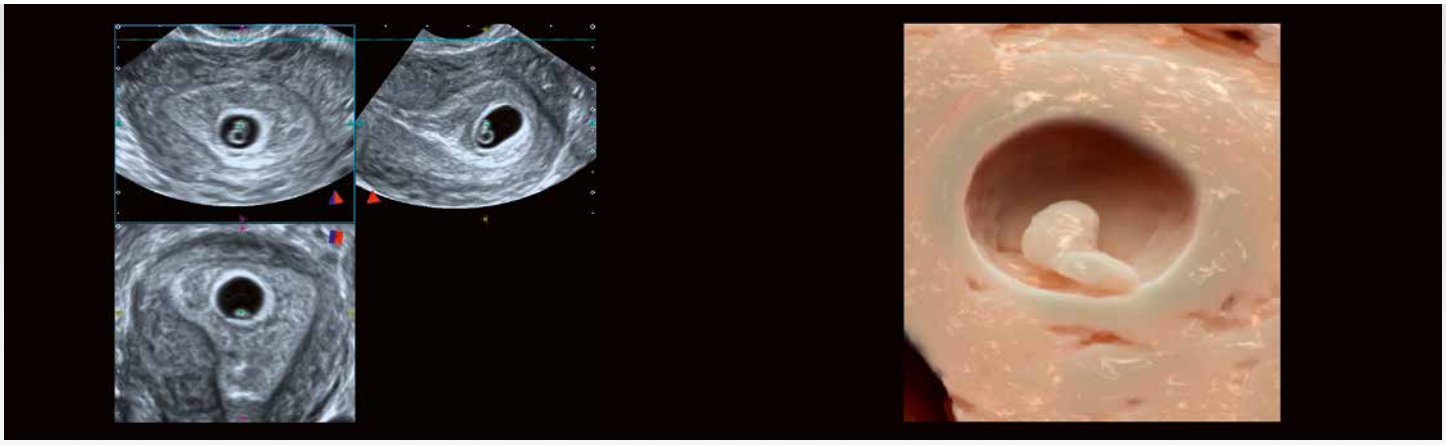

Fig. 11: 3D TVS assessment of an early pregnancy (ovulation + 26/7). Multisectional 3D reconstruction demonstrates the correct location and uniformity of the pregnancy sac as well as favourable ultrasound appearances of the decidual response. The rendered image clearly shows the fetal CRL approaching the same size as the yolk sac suggesting normal early embryological development at that stage. Very fine detail provided by post-processing of the rendered image is able to recognise uniform thickening of the pregnancy sac wall resulting from rapid trophoblastic changes.

TVS confirms continuing endometrial thickening and evidence of associated glandular development within the first few weeks of pregnancy. SMI outlines peripheral vascularity around the corpus luteum. The pregnancy sac is usually identified by 3 weeks post conception, i. e. 5 weeks clinical gestation. Uniform thickening of the early sac wall is indicative of a normal trophoblastic response and remains a useful marker in evaluating pregnancy progress prior to the stage when fetal cardiac activity can be demonstrated. SMI of trophoblastic activity is not carried out in the case of viable pregnancy. However, it is a useful indication of the degree of attachment of the sac in cases of confirmed early pregnancy failure. The vascularity of the trophoblastic tissue associated with extra-uterine implantation as demonstrated by SMI is shown to be a reliable indication of pregnancy status and an important factor regarding clinical management.

The fetus itself and evidence of fetal cardiac activity is normally identified by 4 weeks post conception, i. e. 6 weeks clinical gestation. At this stage the diameter of the yolk sac is approximately the same as the fetal CRL measurement. The pregnancy sac remains uniform in outline with the trophoblastic layers very evident. Localised increase in the thickness of the trophoblastic layer results from differential changes as part of normal placentation and becomes evident by 7 weeks clinical gestation. The endometrium remains proliferative in terms of its ultrasound appearances (Fig. 12). Visualisation of CL cyst activity remains crucial at this stage from a prognostic point of view (Fig. 13).

Conclusion

Advances in TVS ultrasound imaging have provided a greater understanding of the physiological process associated with ovulation and early conception. Physiological changes particularly within the uterus and ovary can be gauged by detailed ultrasound visualisation of relevant anatomical features. SMI outlines various changes which reflect the activity of key hormones. Modern ultrasound is established as an integral part of fertility assessments and ART management. It is a key element in the investigation and treatment of recurrent miscarriage. The developments in ultrasound technology outlined have greatly contributed to the realisation that functional issues rather than pathological disease remain a major cause of so-called gynaecological symptoms.

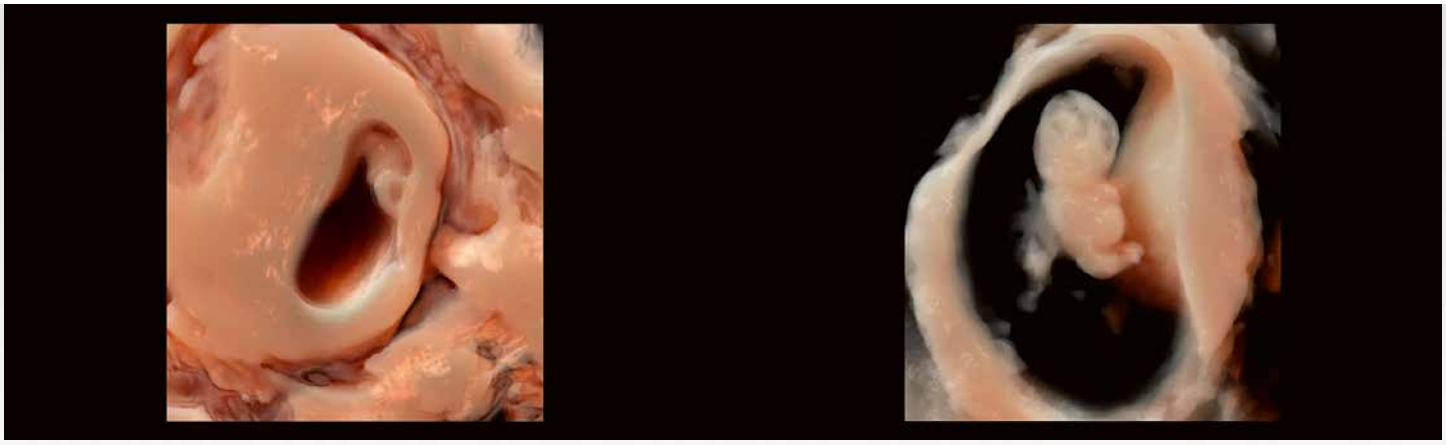

Fig. 12: Shadow Glass imaging showing the developing conceptus (ovulation + 26/7) and fetal growth (ovulation + 39/7) respectively. Developing placental tissue could be clearly seen in the latter.

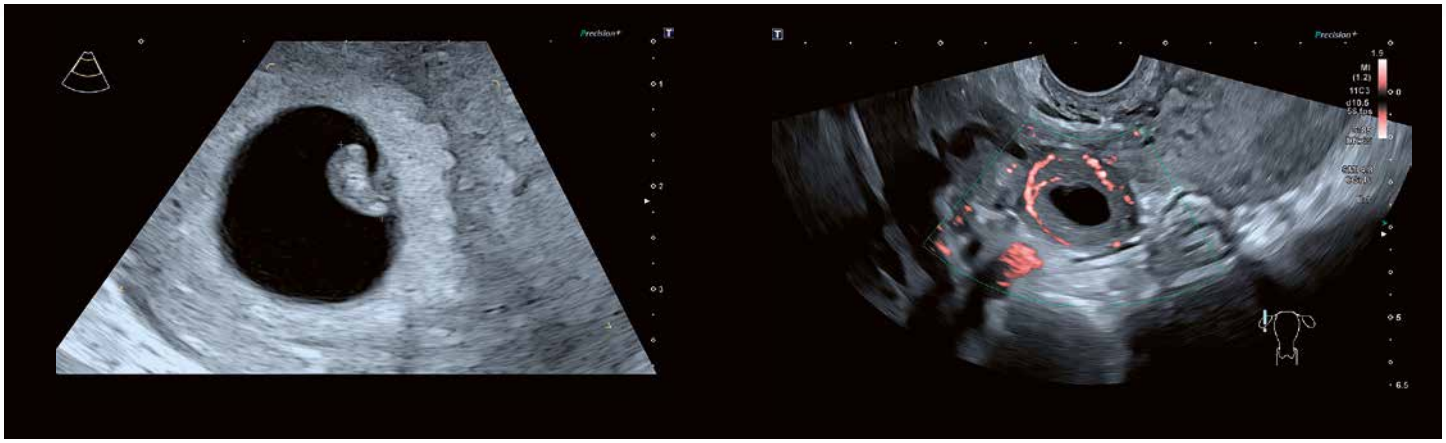

Fig. 13: Normal fetal development at ovulation + 32/7 and SMI assessment of corresponding corpus luteum activity. The grey-scale image demonstrates the area of increased trophoblastic (cytotrophoblast) thickening as part of early placentation.

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